Molecular testing for myeloproliferative disease

Part A – Polycythaemia vera, essential thrombocythaemia and primary myelofibrosis

Part B - Systemic mast cell disease, hypereosinophilic syndrome and chronic eosinophilic leukaemia

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The Medical Services Advisory Committee (MSAC) is an independent committee which has been established to provide advice to the Minister for Health and Ageing on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost-effectiveness. This advice will help to inform government decisions about which medical services should attract funding under Medicare.

MSAC recommendations do not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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Contents

Part A – Polycythaemia vera, essential thrombocythaemia and primary myelofibrosis

Part B – systemic mast cell disease, hypereosinophilic syndrome and chronic eosinophilic leukaemia

Tables

Boxes

Figures

Executive summary

A rigorous assessment of evidence is the basis of decision-making when funding is sought under Medicare. A team from Adelaide Health Technology Assessment (AHTA), University of Adelaide, was engaged to conduct a systematic review of the literature and an economic evaluation of molecular testing in myeloproliferative disorders (MPDs). An Advisory Panel with expertise in this area provided assistance to AHTA in this assessment of the safety, effectiveness and cost-effectiveness of molecular testing in MPDs.

The test

Molecular testing for the diagnosis of polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF) can establish the presence or absence of specific mutations known to occur in patients with these disorders. In particular, the *JAK2* V617F mutation is known to occur in 90–95% of patients with PV and approximately 50% of patients with ET and PMF. Other clinically relevant mutations include *JAK2* exon 12 mutations and *MPL* W515K/L.

Molecular testing alone does not provide a diagnosis for these disorders. The results of such analysis need to be considered in addition to other clinical and laboratory information in order to provide or exclude a diagnosis of PV, ET or PMF.

For patients with suspected PV, molecular testing would occur in addition to the ascertainment of serum erythropoietin levels. For some patients in whom these results have been equivocal, bone marrow (BM) biopsy may also be required. For patients suspected of ET, molecular testing will aid in informing the decision as to whether a patient also requires BM biopsy. For patients suspected of PMF, molecular testing will be performed in addition to BM biopsy.

The comparator test strategy for this assessment includes all available clinical and laboratory information required to make a diagnosis. For patients with suspected PV, this will include serum erythropoietin levels and BM biopsy; and for patients suspected of ET and PMF it will include BM biopsy.

Assessment of molecular testing for the diagnosis of myeloproliferative disorders

Clinical need

Polycythaemia vera

Few data are available regarding the prevalence of PV in Australia. In 2003 the incidence of PV was reported as 1.6 cases per 100,000 population and the number of PV-related hospitalisations was approximately 2,300 (AIHW 2008; AIHW & AACR 2007).

The expert opinion of the Advisory Panel estimates that 3,000 investigations following a suspicion of PV are likely to be undertaken per year.

Essential thrombocythaemia

Specific data for the incidence and prevalence of ET in Australia were unavailable. The reported incidence of MPDs other than PV was 1.5 cases per 100,000 population. The numbers of hospital separations associated with ET were approximately 180 and 260 for the periods 2003–04 and 2006–07 respectively (AIHW 2008). These numbers are indicative of the asymptomatic nature of ET and also the relatively normal life span of ET patients compared with the general population.

The expert opinion of the Advisory Panel estimates that the number of investigations for suspected ET will be 4,500 per year.

Primary myelofibrosis

In the absence of specific data regarding the incidence and prevalence of PMF in Australia, the Advisory Panel has estimated that approximately 175 investigations following a suspicion of PMF would be performed per year.

Safety

Polycythaemia vera, essential thrombocythaemia and primary myelofibrosis

Adverse events related to molecular testing will generally be associated with sample collection of either peripheral blood or a BM biopsy.

Although no data were available regarding the safety of molecular testing in the investigation of suspected PV, the risks associated with sampling peripheral blood are low. Additionally, although there are some risks associated with BM biopsy including pain and discomfort, the risk of serious adverse events associated with this procedure would be considered small.

In the absence of data, it can be argued that the avoidance of BM biopsy in the vast majority of patients suspected of PV would result in a reduction of adverse events. Consequently, molecular testing in the investigation of suspected PV is likely to be safer than the comparator test strategy, which includes BM biopsy.

As BM biopsy is likely to be avoided in approximately 30% of patients suspected of ET, it can be argued that the use of a diagnostic strategy that includes molecular testing is potentially safer than the comparator test strategy.

Patients suspected of PMF are unlikely to avoid BM biopsy; consequently, the addition of molecular testing would be expected to be at least as safe as the comparator.

Effectiveness

No direct evidence of the relative effectiveness of molecular testing in the diagnosis of PV, ET or PMF was identified; therefore, a linked evidence approach, which considered the diagnostic accuracy, change in management and change in patient health outcomes associated with the use of molecular testing, was undertaken for all three indications.

The linked evidence approach was complicated with respect to diagnostic accuracy due to the imperfect nature of the reference standard. The reference standard in this assessment is unlikely to correctly classify all patients; consequently, the results of diagnostic accuracy studies are unlikely to be an accurate reflection of the true diagnostic

performance of the addition of molecular testing to the investigation of MPDs. In the one study that cross-classified patients suspected of MPDs using the 2001 WHO diagnostic criteria (which excludes molecular testing) as a reference standard, the authors reported that there was a change in diagnosis of some patients after molecular analysis despite the results of testing with the reference standard.

In the case of an imperfect reference standard, direct evidence is required to fully inform the question regarding comparative effectiveness. Such evidence is unlikely to become available, as the 2008 WHO diagnostic criteria, which includes molecular testing, is now considered to be the new reference standard.

Polycythaemia vera

With regard to diagnostic accuracy, low-level evidence provided limited information concerning the addition of *JAK2* V617F analysis in the investigation of suspected PV. Although no comparative data were available, diagnostic yield ranged from 9% to 43% and was likely to vary as a result of patient selection.

The diagnostic accuracy of analysing *JAK2* exon 12 mutations in patients who are V617F-negative remains uncertain as the evidence-base was complicated by small study numbers, variation in molecular methods and the use of an imperfect reference standard. Sensitivity and specificity ranged from 0–83% and 73–100%, respectively; however, it is likely that little weight can be given to these results.

Limited evidence of a change in management following the use of molecular analysis in the diagnosis of PV was available. One small study indicated that more accurate diagnosis would result in a change in management of patients diagnosed with the addition of molecular testing.

As there was no evidence of earlier treatment or a change in treatment following diagnosis with molecular testing, treatment effectiveness was not assessed.

Essential thrombocythaemia

No comparative data regarding diagnostic accuracy were available for patients with suspected ET. One study reported the diagnostic yield of ET in patients with suspected MPDs as 65%.

No evidence was available of a change in management subsequent to a diagnosis ascertained with the addition of molecular testing. However, if the 2008 WHO criteria are superior in diagnosing patients with ET, it would be expected that a change in management would occur as a result of more accurate diagnosis.

With no comparative data available and the presence of a mutation occurring in only 50% of patients with ET, the overall benefit of molecular testing in patients with suspected ET remains uncertain.

Primary myelofibrosis

Similar to molecular testing for the diagnosis of ET, no comparative data were identified for molecular testing of patients suspected of PMF. The available evidence was limited to a single study reporting a diagnostic yield of 8%.

Further, no evidence was available to establish a change in management following diagnosis with molecular testing.

The absence of evidence in the diagnosis of PMF with the addition of molecular analysis prevents any conclusion regarding the comparative effectiveness of this testing strategy.

As disease-relevant mutations are only present in approximately 50% of patients with PMF, conclusions regarding the overall benefit of the addition of molecular testing to the investigation of PMF remain uncertain.

Economic considerations

Insufficient evidence was available to establish the comparative safety and effectiveness of molecular testing in the diagnosis of PV, ET and PMF, and to support an economic evaluation. However, as there may be cost savings realised as a result of the avoidance of BM biopsy in patients suspected of PV and ET, an indicative economic evaluation was performed for the PV and ET scenarios. The aim was to determine the diagnostic accuracy that would be required of the molecular tests in order to realise such cost savings. In addition, the cost implications to the Australian healthcare system, Medicare Benefits Schedule (MBS) and the states/territories have been estimated for all indications.

Polycythaemia vera

For an estimated 1,500 investigations per year, it is expected that the cost implications to the Australian healthcare system overall, for the addition of molecular testing to the testing strategy, would be between $355,000 and $568,000. The cost of the comparator test strategy for the same number of investigations is estimated to be $2,178,000. Cost savings for the Australian health system overall are expected to range from $1,610,000 to $1,823,000. In the private healthcare system the estimated cost to the MBS would range between $80,000 and $257,000 for the 1,200 patients expected to be investigated. In comparison, the alternative test strategy is expected to incur a cost of $451,000 per year to the MBS. The states/territories would be expected to save between $322,000 and $365,000 per year for the investigation of 300 patients in the public health sector.

These cost savings are primarily associated with the avoidance of BM biopsy in patients investigated for suspected PV. The extent of savings will depend on both the diagnostic accuracy of molecular testing and serum erythropoietin determination, and the prevalence of disease in the population tested. Analysis of the number of BM biopsies that might be avoided has indicated that the diagnostic accuracy of molecular testing plus serum erythropoietin determination will result in cost savings between $445 and $1,175 per patient. A sensitivity analysis to estimate the effect of a change in disease prevalence was not considered to be necessary for this indication as the assumption in the base case was considered to be appropriate.

Essential thrombocythaemia

It is expected that 4,500 patients would be investigated per year as a result of suspicion of ET. Of these, approximately 80% (3,600) would be investigated in the private health sector.

The cost of the molecular testing strategy for the investigation of ET would result in a financial burden of between $4,684,000 and $5,325,000 per year to the Australian

healthcare system, which compares favourably with the cost of the comparator test strategy ($6,087,000). For those patients investigated in the private healthcare sector, molecular testing would result in a cost saving of between $195,000 and $337,000 per year relative to the comparator strategy. The states/territories would also benefit from a cost saving of between $152,000 and $281,000 per year relative to the comparator, depending on the molecular methods used.

The savings described are expected to depend on the diagnostic accuracy of *JAK2* analysis and the prevalence of the mutation within the population tested. The base case analysis of the impact of the diagnostic accuracy of molecular testing and the prevalence of the mutation indicates that cost savings are likely to be realised as a result of avoided BM biopsy regardless of the diagnostic accuracy of molecular testing. This is also likely to be the case if the population tested were expanded to reflect a more clinically relevant scenario (i.e. the prevalence of the mutation was decreased to ensure that as few cases of ET were missed as possible). In this case the sensitivity and specificity required to be a cost-saving testing strategy would need to increase to at least 80–85%.

Primary myelofibrosis

The financial implications of the addition of molecular testing to the investigation of PMF are based on the assumption that approximately 175 patients would be investigated per year.

Savings are not expected to be realised as a result of avoiding BM biopsy in this group of patients.

The financial impact to the Australian healthcare system overall of molecular testing in the investigation of PMF would be an additional cost of $16,000 and $41,000 per year. This includes an estimated cost of investigating 140 patients in the private sector of between $4,000 and $25,000 that would be borne by the Commonwealth as a result of the Medicare rebate. The states/territories are expected to incur a cost of between $3,000 and $8,000 per year for the 35 patients expected to be investigated for PMF in the public healthcare system.

Glossary and abbreviations

±	with/without
AHTA	Adelaide Health Technology Assessment
Allele burden	the ratio of mutant DNA to wild-type (normal) DNA
AS-PCR	allele specific PCR
BCR-ABL fusion gene	a chromosomal translocation t(9;22)(q34;q11) associated with chronic myeloid leukaemia—also called the Philadelphia chromosome
BSCH	British Committee for Standards in Haematology
CML	chronic myeloid leukaemia
CMPD	chronic myeloproliferative disorder/disease
ddNTP	dideoxynucleotide triphosphates
EPO	erythropoietin, a hormone that stimulates erythrocyte production
Erythrocytes	red blood cells
ET	essential thrombocythaemia
FBE	full blood examination
Haematocrit	the ratio of volume occupied by packed red blood cells to the volume of the whole blood
Haematopoiesis	the production of blood cells from haematopoietic stem cells
Haemoglobin	a molecule in red blood cells that carries oxygen
Haemorrhage	loss of blood from a blood vessel
IE	idiopathic erythrocytosis
JAK2	gene encoding for JAK2 tyrosine kinase
MBS	Medicare Benefits Schedule
MDS	myelodysplastic syndromes, a group of diseases that affect normal blood cell production in the bone marrow—the bone marrow produces abnormal, immature blood cells that fail to develop properly and are dysfunctional

Megakaryocyte	large cell generally found in the bone marrow from which fragments form platelets
MPD	myeloproliferative disorder/disease
MPL	a gene that encodes for the thrombopoietin receptor
MSAC	Medical Services Advisory Committee
Mutation	a change in the genetic sequence that results in altered qualities or form of the product of the sequence
NHMRC	National Health and Medical Research Council
PCR	polymerase chain reaction
PMF	primary myelofibrosis
PV	polycythaemia vera
PVSG	Polycythaemia Vera Study Group
Red cell mass studies	an assay using a very weak radioactive dye, to determine if there is an absolute erythrocytosis (raised red cell mass, normal plasma volume) or an apparent erythrocytosis (normal red cell mass, reduced plasma volume)
RFLP	restriction fragment length polymorphism
SE	secondary erythrocytosis
TGA	Therapeutic Goods Administration
Thrombocytosis	increased levels of platelets
Venesection	removing blood from a vein as a therapeutic treatment
Venous thrombosis	a blood clot that forms within a vein
Wild-type DNA	DNA that is considered to not have a mutation

Introduction

Adelaide Health Technology Assessment (AHTA), with input and advice from an appropriately constituted Advisory Panel of experts (see Appendix A), has reviewed the current evidence for molecular testing for the diagnosis of *BCR-ABL*-negative chronic myeloproliferative disorders (MPDs) and, in particular, the diagnosis of polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF). This assessment report is intended for the Medical Services Advisory Committee (MSAC). The MSAC evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Schedule in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. The MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

The MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, surgery, internal medicine and general practice, clinical epidemiology, health economics, consumer health and health administration.

Background

Molecular testing for the diagnosis of myeloproliferative disorders—PV, ET and PMF

Mutations in *BCR-ABL*-negative myeloproliferative disorders

JAK2 V617F testing detects the point mutation of guanine to thymine in the JH2 pseudokinase region of the *JAK2* gene (Tefferi & Pardanani 2006). This mutation encodes for a valine to phenylalanine substitution in the subsequent tyrosine kinase. Such a substitution provides unregulated activity in the corresponding JAK-STAT pathway, which is involved in the control of cellular proliferation, differentiation and cellular survival (Antonioli et al 2008; James et al 2005; Levine et al 2005).

The V617F is a somatic mutation in exon 14 of the *JAK2* gene, that is an acquired mutation that occurs in haematopoietic stem cells and is reported to be present in 95% of polycythaemia vera (PV) cases and 50% of patients with essential thrombocythaemia (ET) and primary myelofibrosis (PMF) (Tefferi 2008; Tefferi et al 2007). The V617F mutation is the only exon 14 mutation that is clinically relevant in diagnosing PV, ET or PMF. The acquired nature of the somatic mutation indicates that it is not inherited or passed on to offspring.

Other mutations that may be of clinical importance include *JAK2* exon 12 mutations in PV and *MPL* mutations (W515L or W515K) associated with ET and PMF (Pardanani et al 2006; Pikman et al 2006; Pikman & Levine 2007).

The *MPL* gene encodes for the thrombopoietin receptor and plays a role in regulating megakaryocyte formation and proliferation (Beer et al 2008). W515L or W515K mutations within this gene result in constitutive activation of the thrombopoietin receptor and JAK-STAT pathway (Beer et al 2008; Pikman & Levine 2007). Studies have shown that *MPL* mutations are prevalent in 5% and 1% of patients with PMF and ET respectively (Guglielmelli et al 2007). Patients with PMF and the W515L/K mutation appear to present with more severe anaemia and are likely to have a greater dependence on transfusion therapy than those without (Guglielmelli et al 2007).

A number of somatic mutations in *JAK2* exon 12 have been characterised in patients with *JAK2* V617F-negative PV or idiopathic erythrocytosis (Koppikar & Levine 2008). It has been suggested that patients with these mutations may have a specific clinical phenotype that is characterised by erythrocytosis with leukocytosis of varying severity and without an associated thrombocytosis (Koppikar & Levine 2008).

Phenotypic expression and allele burden

Expression of the *JAK2* V617F mutation can result in one of three different clinical disease states or phenotypes. This has led to the question of whether PV, ET and PMF are in fact part of the same disease continuum (Moliterno et al 2008).

The presence of the V617F mutation in ET is associated with higher haemoglobin levels and white cell counts, and lower platelet counts, serum ferritin and erythropoietin levels.

Hence, the V617F mutation may give patients a more 'polycythaemic' phenotype (Campbell et al 2005; Rudzki et al 2007).

A number of studies have measured allele burden and its impact on disease phenotype, particularly in patients with ET. According to Larsen et al (2007), very few patients with ET are homozygous for the V617F mutation and, in fact, the allele burden in this disease is significantly less than that of PV and PMF.

A study published by Antonioli et al (2008) reported that, in ET, patients with an increased allele burden were at higher risk of splenomegaly and microvessel symptoms, as well as arterial thrombosis at diagnosis. In patients with PV, an association between allele burden and the patient's response to hydroxyurea therapy has also been reported (Sirhan et al 2008).

The test

Detection of the V617F mutation requires molecular testing of peripheral blood granulocytes or bone marrow (BM) biopsy. The principle behind testing involves using a polymerase chain reaction (PCR) that enables detection of specific DNA sequences. PCR exploits the process of DNA replication by using an enzyme (DNA polymerase) capable of replicating DNA to produce multiple copies of specific sequences (Watson et al 1992). Specificity is provided by the use of small single-stranded DNA sequences (primers) that bind to the target DNA sequence and allow initiation of replication (primer extension) by the DNA polymerase (Appendix D).

Given that there are no diagnostic kits available for the detection of *JAK2* mutations in Australia, molecular pathology laboratories employ in-house methods of detection. A number of different detection techniques are used to ascertain the presence of the mutation, including DNA sequencing, allele-specific PCR, melting curve analysis and restriction fragment length polymorphism (RFLP) analysis. These different techniques can provide either qualitative or semi-quantitative results. A non-exclusive list of the common techniques is provided below.

DNA sequencing

DNA sequencing reactions are similar to PCR reactions in that they require template DNA, specific primers, DNA polymerase and deoxynucleotides (dNTPs). In addition, sequencing also requires dideoxynucleotide triphosphates (ddNTPs). When a ddNTP is incorporated into the DNA strand, further elongation is prevented due to their inability to form phosphodiester bonds with subsequent nucleotides. The random nature of incorporating ddNTPs into the extending DNA allows termination to occur at various positions along the target DNA sequence, resulting in DNA fragments of varying length. Each fragment can then be separated by electrophoresis according to its length and size. Fluorescent labelling of ddNTPs enables determination of the DNA sequence by detection of the specific fluorescent label that corresponds to a particular ddNTP (Watson et al 1992; Steensma 2006).

The V617F mutation can be detected to levels of 20% mutant DNA, which has significant implications in the context of *JAK2* V617F testing (Steensma 2006). This is particularly the case for patients with ET, who have a significantly lower allele burden than patients with PV or PMF.

Allele-specific PCR

Allele-specific PCR (AS-PCR), also called amplification refractory mutation system PCR, works on the principle that DNA primers bind to complementary DNA sequences. DNA polymerases will only extend the primers if there are no mismatches between the DNA template and the primer (Newton et al 1989). If the sequence of the primer is altered to reflect the specific point mutation of interest, there will only be PCR amplification if the mutant allele is present (Steensma 2006).

It is likely that, in some instances, there will be PCR amplification in the presence of one base-pair mismatch, that is amplification of the wild-type allele in the presence of the mutant primers. Therefore, the introduction of an additional mismatch will increase the specificity of the PCR reaction to amplify only the mutant allele (Newton et al 1989).

AS-PCR has an advantage over DNA sequencing in its ability to detect low-level single point mutations in a background of wild-type DNA. However, the disadvantage of AS-PCR is that it will only detect specific mutations; therefore, if there are variant mutant alleles, numerous specific primer sets are required.

Restriction fragment length polymorphism

Restriction enzymes (endonucleases) cleave DNA into fragments at specific sequence sites, and the subsequent DNA fragments can be separated according to size by gel electrophoresis. The presence or absence of fragments of specific size can confirm the presence of a mutation.

The *JAK2* mutation of V617F does not create any additional sites for enzyme cleavage; however, the presence of the mutation does remove the recognition site for the enzyme BsaXI and thereby prevents cleavage in the presence of the mutation (Steensma 2006).

For detection of the V617F mutation, the exon 14 region of the *JAK2* gene is amplified by PCR and then digested with BsaXI. If the wild-type allele is present, three DNA fragments will be produced. If the mutant allele is present, there will be no cleavage by the enzyme, leaving a single, larger fragment (Campbell et al 2006).

Advantages of this technique are that it is simple and inexpensive; however, a relative lack of sensitivity (20% mutant DNA in a wild-type background) may limit its usefulness in a clinical setting (Steensma 2006).

Quantitative (real-time) PCR

Quantitative PCR enables the amplification and quantification of a specific DNA sequence in a single reaction vessel. Detection of the amplified product can be achieved through use of either DNA dyes that bind non-specifically within the amplified product, or through hybridisation of a labelled DNA probe that is specific for a DNA sequence within the amplified product. The degree of fluorescence is proportional to the amount of amplified product, which itself is proportional to the amount of original DNA template (Steensma 2006).

Melting curve analysis

This technique can be performed in conjunction with quantitative PCR. It relies on the strength of binding of two single strands of DNA being dependent on their length, guanine–cytosine content and the complementarity between the strands (Steensma 2006).

Typically, two DNA hybridisation probes, which bind to the amplified target DNA sequence, are used in melting curve analysis. One probe has an excitation dye incorporated and the other has a receptor dye. When the two dyes are within close range, fluorescence is emitted due to the process of fluorescence resonance energy transfer (Murugesan et al 2006).

When heated, the DNA probe will be denatured from the target DNA sequence at its respective melting point temperature. Such denaturation will result in a loss of fluorescence. If the probe is bound tightly to the target sequence, the melting point temperature will be relatively high due to the complementarity between the probe and target DNA. If there is a mismatch between the probe and target DNA, the melting point temperature will be relatively low (Figure 1).

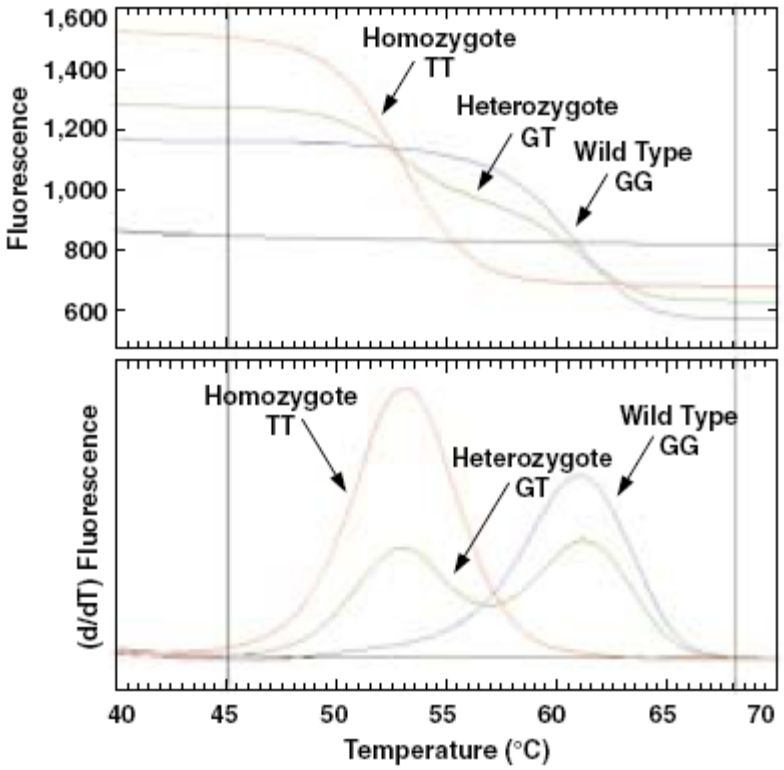


Figure 1 Melting curves and melting peaks for wild-type and mutant DNA
Note: TT, GT and GG are base pairs in target DNA sequence
Figure published with permission (Murugesan et al 2006) © 2006 American Society for Clinical Pathology

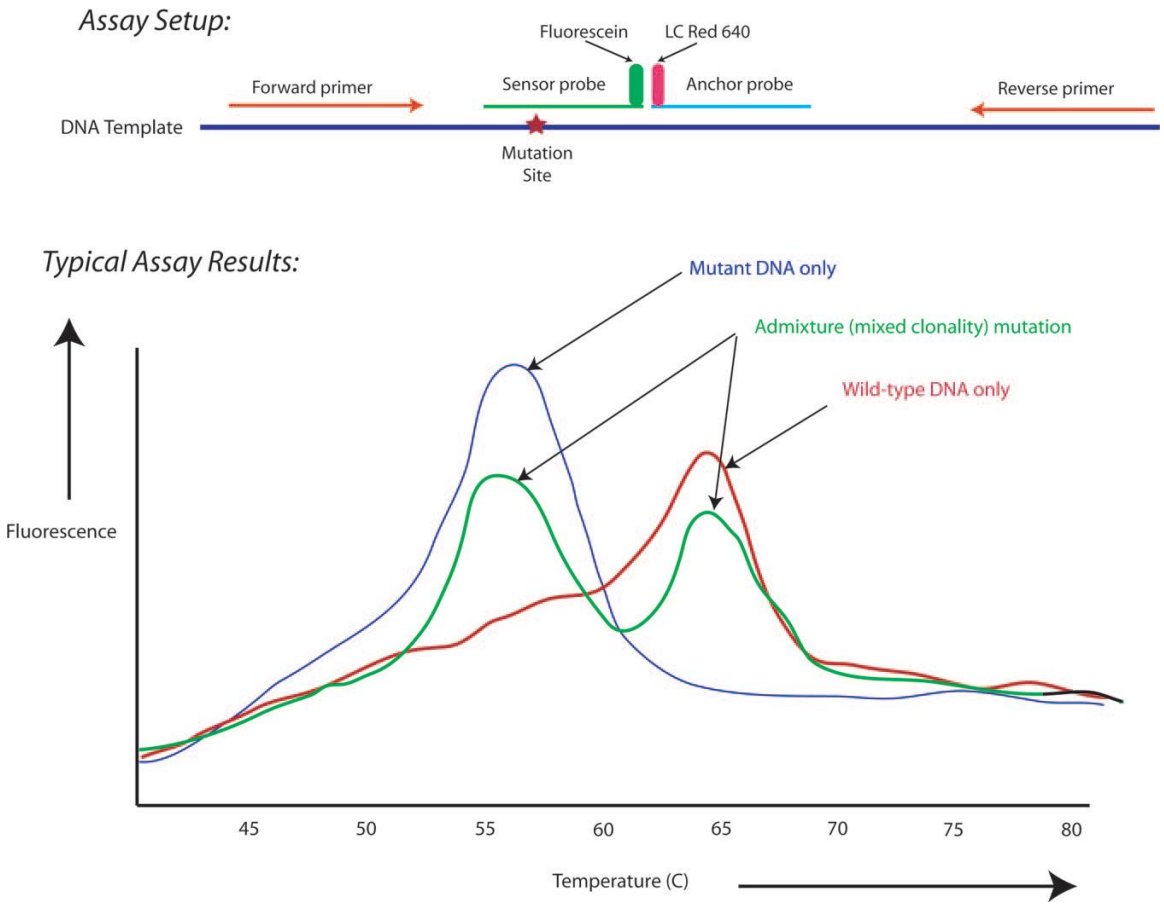


Figure 2 Melting curve analysis

Reprinted from Steensma (2006) with permission from the American Society for Investigative Pathology.

In a method described by McClure et al (2006), a base-pair mismatch can result in a 10 °C discrepancy in melting point temperatures. This difference allows for easy differentiation between wild-type and mutant DNA. The analytical sensitivity of melting curve analysis in the detection of the *JAK2* V617F mutation has been reported to be between 1% and 10% of mutant DNA (McClure et al 2006).

With the use of different techniques and their associated analytical sensitivities, comparison of diagnostic results across techniques will also require consideration of their respective assay sensitivities.

Equipment requirements are dependent on the particular assay being performed. Assays that use quantitative techniques such as melting curve analysis require more specialised equipment. In contrast, qualitative detection of the mutation requires relatively simple thermocycler and gel-based detection systems. Each particular technique also requires commercially available reagents and DNA primers specific for that technique.

Intended purpose

JAK2 mutation testing is used in patients suspected of having an MPD on the basis of a full blood examination, or due to presentation with splenomegaly or a history of haemorrhage or venous thromboses. Patients who have an MPD may also be asymptomatic.

Where there is clinical suspicion of an MPD, patients will undergo a full blood examination to demonstrate:

- elevated levels of erythrocytes, which may suggest PV, or
- persistently elevated levels of platelets, which may suggest ET, or
- anaemia associated with teardrop poikilocytes and a leukoerythroblastic blood picture, which may suggest PMF.

In addition, patients suspected of having ET will undergo testing to determine their erythrocyte sedimentation rate, and C-reactive protein and serum ferritin levels, as well as iron studies to rule out an inflammatory or reactive process.

Patients with suspected ET or PMF are likely to undergo testing for the presence of the *BCR-ABL* mutation to exclude chronic myeloid leukaemia.

The presence of clinically relevant mutations, for example *JAK2* V617F, or mutations of *JAK2* exon 12 or *MPL* do not provide a diagnosis of the specific pathology; rather, this information indicates the likely presence of an MPD. Thus, molecular testing must be used in conjunction with other relevant information (including prior tests) in order to provide a disease-specific diagnosis. The additional information required for diagnosis of PV, ET and PMF is outlined in the clinical pathways for these diseases (Figures 3–5).

Clinical need

Data regarding the prevalence of *BCR-ABL*-negative MPDs in Australia are scarce. Similarly, there are minimal data regarding the incidence of these disorders, and specific data are only available for PV.

In 2003 the incidence of PV in Australia was 1.6 cases per 100,000 population (AIHW & AACR 2007). The reported incidence of other MPDs, incorporating ICD-10 codes D47.1 (chronic MPDs) with D47.3 (ET), was 1.5 cases per 100,000 population (AIHW & AACR 2007).

The number of hospital separations for patients with PV was 2,320 in 2003–04 and 3,059 in 2006–07 (AIHW 2008). For patients with ET, the numbers of separations in 2003–04 and 2006–07 were 185 and 261 respectively, and the numbers of separations for other MPDs were 2,507 and 2,343 respectively (AIHW 2008). These figures may reflect multiple admissions and possibly day separations also. The substantially lower number of separations for ET may reflect the fact that many patients with ET may never require hospitalisation.

Survival in patients with MPDs varies greatly depending on the disease and its phenotype. The life expectancy of patients with PV is less than that of the general population (Cervantes et al 2008). According to reported results from a large study in Italy, mortality in patients with PV is 60% higher than that of the general population. In patients with ET, no difference in mortality compared with the general population has been reported (Passamonti et al 2004).

In contrast, the survival of patients with PMF is significantly shortened. The reported median survival of patients following diagnosis ranges from 4 to 5.5 years (Cervantes et

al 2008). Comparison with age- and sex-matched controls indicates that patients with PMF have a 31% reduction in life expectancy (Rozman et al 1991).

Use of *JAK2* V617F testing has been estimated to be 1,700 tests per year based on a survey of genetic testing conducted in 2006 (Royal College of Pathologists of Australasia 2008). The expert opinion of the Advisory Panel indicates that this number is unlikely to truly reflect the number of tests that would be performed in Australia. The Advisory Panel estimates that approximately 12,000 samples would be analysed for the *JAK2* V617F mutation per year. This estimate is likely to include some retrospective testing of patients already diagnosed with *BCR-ABL*-negative MPDs. However, it is possible that, if public funding of molecular testing for *JAK2* mutations is supported, access to this test will be increased and the number of tests required for incident cases may remain stable.

Should molecular testing for chronic MPDs be incorporated into the Pathology Services Table, there is the potential for over-ordering to occur in the primary care setting. However, similar concerns were raised with the listing of *BCR-ABL* testing for chronic myeloid leukaemia, and no increase in ordering by general practitioners has eventuated.

Additionally, leakage is likely to occur if the potential for targeted therapy (in the form of tyrosine kinase inhibitors) is realised and there is a requirement for *JAK2* mutation testing for the monitoring of residual disease.

Existing tests

Diagnosis of *BCR-ABL*-negative MPDs requires the use of all available clinical and laboratory information. In the case of suspected ET or PMF, diagnosis relies heavily on the exclusion of other diseases and on the causes of thrombocytosis or fibrosis respectively.

PV

Diagnosis of PV requires demonstration of erythrocytosis in the form of increased haematocrit or haemoglobin levels. For diagnosis, causes of secondary or reactive erythrocytosis must be excluded, generally through testing of oxygen saturation levels or demonstration of normal or low serum erythropoietin levels (Tefferi et al 2007).

Bone marrow histology may indicate an increase in cellularity, predominantly of the erythroid, granulocytic and megakaryocytic lineages (Cao et al 2006).

Red cell mass studies may be performed to exclude a secondary or reactive erythrocytosis. However, the availability of this assay is limited to a few centres in Australia and therefore may not be used routinely in the investigation of PV.

Abdominal ultrasound is used to assess spleen size, which may be an indicator of PV when the spleen is enlarged.

Patients with Budd-Chiari syndrome range from being asymptomatic to presenting with hepatomegaly caused by thrombosis or obstruction of the splanchnic (portal, splenic and superior mesenteric) veins (Aydinli & Bayraktar 2007). Clinical features of Budd-Chiari syndrome such as haemodilution, occult bleeding and hypersplenism may obscure changes to blood cell counts, which would normally be used to identify patients with MPDs (Primignani et al 2006).

ET

A diagnosis of ET relies primarily on the exclusion of other causes of thrombocytosis. It is characterised by a persistent increase in platelets, platelet anisocytosis, and BM histology that shows proliferation of large, mature megakaryocytes without a noticeable increase in granulocyte or erythrocyte production (Tefferi et al 2007).

Causes of reactive thrombocytosis, including iron deficiency, haemolytic anaemia, surgery, infection, chronic inflammatory disorders, lymphoproliferative disorders, metastatic cancer and drug reactions, must also be excluded (Briere 2007; Sanchez & Ewton 2006; Tefferi et al 2007).

Additionally, there should be no evidence of PV, PMF, chronic myeloid leukaemia or myelodysplastic syndromes. These diseases can be excluded on the basis of a full blood examination, BM histology including cytogenetic analysis, and clinical judgement (Tefferi et al 2007).

PMF

Similar to ET, the diagnosis of PMF is heavily reliant on the exclusion of other causes of symptoms and laboratory findings. Classic findings of PMF are a leukoerythroblastic blood film with demonstration of teardrop poikilocytes, and BM histology indicating varying degrees of fibrosis and atypical megakaryocytes (Ahmed & Chang 2006).

The findings of BM histology in both the prefibrotic and fibrotic stages of primary myelofibrosis are outlined in Table 1.

Table 1 Morphological findings in prefibrotic and fibrotic stages of PMF

Findings	Prefibrotic stage	Fibrotic stage
Peripheral blood	No or mild leukoerythroblastosis No or mild red blood cell poikilocytosis Few if any (teardrop) dacrocytes	Leukoerythroblastosis Prominent red blood cell poikilocytosis Prominent dacrocytes
Bone marrow	Increased cellularity Neutrophilic proliferation Megakaryocyte proliferation with atypia Minimal or absent reticulin fibrosis	Decreased cellularity Neutrophilic proliferation Prominent megakaryocyte proliferation with atypia Reticulin and/or collagen fibrosis Dilated marrow sinuses with intrasinusoidal haematopoiesis Osteosclerosis (new bone formation)

Adapted from Ahmed & Chang (2006); Tefferi et al (2007)

The morphological findings associated with PMF can be quite variable depending on the stage of disease (Ahmed & Chang 2006). Misdiagnosis of ET can occur in the prefibrotic stage due to the lack of leukoerythroblastic blood film but the presence of pronounced thrombocytosis (Ahmed & Chang 2006).

Treatment for chronic myeloproliferative disorders

PV

The primary aim of therapy in patients with PV is to minimise the risk of both vascular complications and transformation to a secondary neoplasm such as myelofibrosis or acute myeloid leukaemia (McMullin et al 2005; Penninga & Bjerrum 2006).

Venesection is recommended in patients to help maintain a haematocrit less than 45% (Finazzi & Barbui 2007; McMullin et al 2005; Penninga & Bjerrum 2006; Tefferi 2008). Low-dose aspirin (40–100 mg/day) has also been shown to reduce the risk of thrombotic events, although it is contraindicated in patients with a platelet count greater than 1,000 x 10^9/L due to the risk of acquired von Willebrand syndrome (Finazzi & Barbui 2007; McMullin et al 2005; Penninga & Bjerrum 2006; Tefferi 2008).

Cytoreductive therapy can be considered in patients who are intolerant to venesection, develop thrombocytosis greater than 1,000 x 10^9/L, have a history of thrombosis or have cardiovascular risk factors (eg obesity, smoking, hypertension, hypercholesterolaemia, diabetes mellitus and coronary artery disease). Hydroxyurea would be considered as first-line cytoreductive therapy in most patients (Campbell & Green 2005; Penninga & Bjerrum 2006).

ET

Similar to PV, the aim of therapy in patients with ET is to lower the risk of thrombohaemorrhagic events. This is done by lowering the platelet count (Penninga & Bjerrum 2006). Therapy is provided that is appropriate to the risk of thrombosis and haemorrhage in each individual patient (Table 2).

Table 2 Risk stratification of patients with ET

Low risk	High risk
Age < 60 years	Age ≥ 60 years
No history of thrombosis	and/or history of thrombosis
Platelet count < 1,500 x 10^9/L	and/or platelet count > 1,500 x 10^9/L
No cardiovascular risk factors	

Adapted from Briere (2007); Penninga & Bjerrum (2006)

It is recommended that all patients with ET attempt to reduce reversible risk factors for cardiovascular disease. These risk factors include smoking, obesity, hypertension and hypercholesterolaemia (Campbell & Green 2005; Harrison 2005).

Anti-thrombotic therapy in the form of low-dose aspirin is recommended for all patients except those with a platelet count greater than 1,500 x 10^9/L (Penninga & Bjerrum 2006). Low-risk patients would receive low-dose aspirin therapy (unless contraindicated) without additional cytoreductive therapy.

For patients at high risk, it is recommended that they receive cytoreductive therapy to reduce their platelet count. Hydroxyurea is generally considered a first-line drug for lowering the number of platelets; however, there is still some concern as to whether it increases the risk of transformation to leukaemia (Briere 2007; Penninga & Bjerrum 2006). An alternative to hydroxyurea is anagrelide, which inhibits megakaryocyte maturation and, subsequently, platelet count. Although anagrelide is not known to

increase the risk of leukaemic transformation, there are significant adverse effects associated with the drug including palpitations, tachycardia, headaches and gastrointestinal symptoms (Penninga & Bjerrum 2006). In patients with a very high platelet count (> 1,500 x 10^9/L), low-dose aspirin therapy might be used once a reduction in platelets has been achieved (Penninga & Bjerrum 2006). Younger patients may receive interferon-α to minimise any potential risk of progression to leukaemia from using other cytoreductive agents (Finazzi & Barbui 2008). Additionally, interferon-α is the only therapy known to be safe to use during pregnancy (Birgegard 2009). Side effects may include influenza-like symptoms, nausea, diarrhoea, myalgia and depression. Most patients experience these at the onset of treatment and symptoms will generally ease (Birgegard 2009).

PMF

Of the *BCR-ABL*-negative MPDs, PMF is associated with the poorest prognosis (Arana-Yi et al 2006), with risk factors including marked anaemia, age, white cell count, cytogenetic abnormalities and circulating blast cells (Barosi et al 2007; Cervantes 2007). As there is variation in the clinical course of the disease, risk assessment of patients with PMF enables appropriate therapies to be individually targeted.

For patients who are asymptomatic at diagnosis, no treatment is recommended until symptoms or other signs of disease progression become apparent. For these patients regular follow-up is required to detect further signs of disease (Arana-Yi et al 2006).

Patients with one risk factor present are treated with the intention of reducing symptoms and improving quality of life. A number of different symptoms of the disease can be treated, including splenomegaly and constitutional symptoms such as fever, fatigue and night sweats. Clinical features of PMF include extramedullary haematopoiesis, anaemia and cellular proliferation (Mesa et al 2006). Treatment options for both symptoms and clinical features are outlined in Table 3.

Table 3 Treatment options for patients with PMF

Anaemia	Cellular proliferation	Splenomegaly	Extramedullary haemopoiesis	Constitutional symptoms
Corticosteroids	Hydroxyurea	Hydroxyurea	Radiation therapy	Thalidomide
Erythropoietin	Interferon-α	Thalidomide	Surgery	Etanercept
Thalidomide	Anagrelide	Splenic irradiation		Corticosteroids
Transfusion	Busulfan	Splenectomy		
Chelation therapy				

Adapted from Arana-Yi et al (2006); Mesa et al (2006)

Based on their risk profile, patients can be considered for more aggressive therapy. Allogeneic stem cell transplant is the only therapy with the potential for curing PMF. Use of myeloablative stem cell transplant should be considered in patients less than 45 years of age. For patients aged between 45 years and 65 years, the decision should be made on an individual basis depending on the patient's suitability for myeloablative stem cell transplantation (Arana-Yi et al 2006; Tefferi 2008).

Potential impact of the test

The introduction of *JAK2* V617F testing is likely to simplify the diagnostic algorithm for PV by eliminating the need to exclude an apparent or secondary cause of erythrocytosis.

Therefore, oxygen saturation and red cell mass studies will no longer be required. In addition, a positive *JAK2* V617F test in association with low serum erythropoietin will enable a diagnosis of PV without the need for BM biopsy or other relevant investigation, unless indicated by unusual or atypical features in the full blood examination.

Many patients with suspected ET will still require a BM biopsy as part of the diagnostic pathway. There will, however, be a proportion (those who are positive for the *JAK2* mutation and who are without any atypical features) who may be diagnosed without the need for this procedure.

For PMF the diagnostic algorithm is unlikely to be significantly altered by the introduction of *JAK2* V617F testing. Patients will still require a BM biopsy in addition to the *JAK2* V617F test. This is primarily due to the prevalence of the mutation occurring in only 50% of patients with the disease. In those cases where the mutation is present, the diagnostic certainty will be increased above that of using BM biopsy alone.

In addition, *JAK2* V617F mutation testing is likely to improve access for patients who are suspected of having an MPD. Red cell mass studies have limited availability and can only be accessed at specialised centres. The same applies to BM biopsy. The patient is sedated prior to a biopsy and, as a consequence, some patients require care similar to that of a day stay procedure, which limits the number of centres that are able to perform this procedure. Such limited access particularly impacts on people in remote and rural areas. In contrast, *JAK2* testing requires a simple blood sample and is therefore likely to be readily available to such people.

The potential for targeted therapy in the form of tyrosine kinase inhibitors is yet to be realised. There are clinical trials currently being conducted that are evaluating the effectiveness of particular inhibitors. Development of a tyrosine kinase inhibitor, which is successful in treating PV, ET and/or PMF, is likely to see testing for the *JAK2* V617F mutation having a significant impact on patient management (Mesa 2008; Tefferi 2008).

Marketing status of the technology

Commercially available detection systems do exist for *JAK2* V617F detection; however, these are for research purposes only and are not used in a routine diagnostic setting. Consequently, testing for clinically relevant mutations using assays developed in house is being conducted at a number of centres in Australia for patients who are suspected of having an MPD.

The use of in-house techniques does not require approval from the Therapeutic Goods Administration (TGA). If diagnostic commercial kits were to become available, these would require approval by the TGA.

Current reimbursement arrangement

Currently, there is no listing on the Medicare Benefits Schedule (MBS) for any test that detects the *JAK2* V617F mutation or other clinically relevant mutations.

There are, however, MBS items that allow reimbursement for molecular tests that detect specific genetic mutations and/or monitor patients with disease (Table 4). The range of MBS fees associated with these items are indicative of the range of molecular

methodologies used to detect the relevant mutations. Quantitative or semi-quantitative assays will incur greater costs than methods that are simply qualitative.

Table 4 MBS items related to detection of genetic mutations

Item 73308	Characterisation of the genotype of a patient for Factor V Leiden gene mutation, or detection of the other relevant mutations in the investigation of proven venous thrombosis or pulmonary embolism - 1 or more tests Fee: $36.70
Item 73317	Detection of the C282Y genetic mutation of the HFE gene and, if performed, detection of other mutations for haemochromatosis where: (a) the patient has an elevated transferrin saturation or elevated serum ferritin on testing of repeated specimens; or (b) the patient has a first degree relative with haemochromatosis; or (c) the patient has a first degree relative with homozygosity for the C282Y genetic mutation, or with compound heterozygosity for recognised genetic mutations for haemochromatosis (Item is subject to rule 20) Fee: $36.70
Item 73320	Detection of HLA-B27 by nucleic acid amplification includes a service described in 71147 unless the service in item 73320 is rendered as a pathologist determinable service. (Item is subject to rule 27) Fee: $40.80
Item 73314	Characterisation of gene rearrangement or the identification of mutations within a known gene rearrangement, in the diagnosis and monitoring of patients with laboratory evidence of: (a) acute myeloid leukaemia; or (b) acute promyelocytic leukaemia; or (c) acute lymphoid leukaemia; or (d) chronic myeloid leukaemia; Fee: $232.50

Source: Department of Health and Ageing (2009)

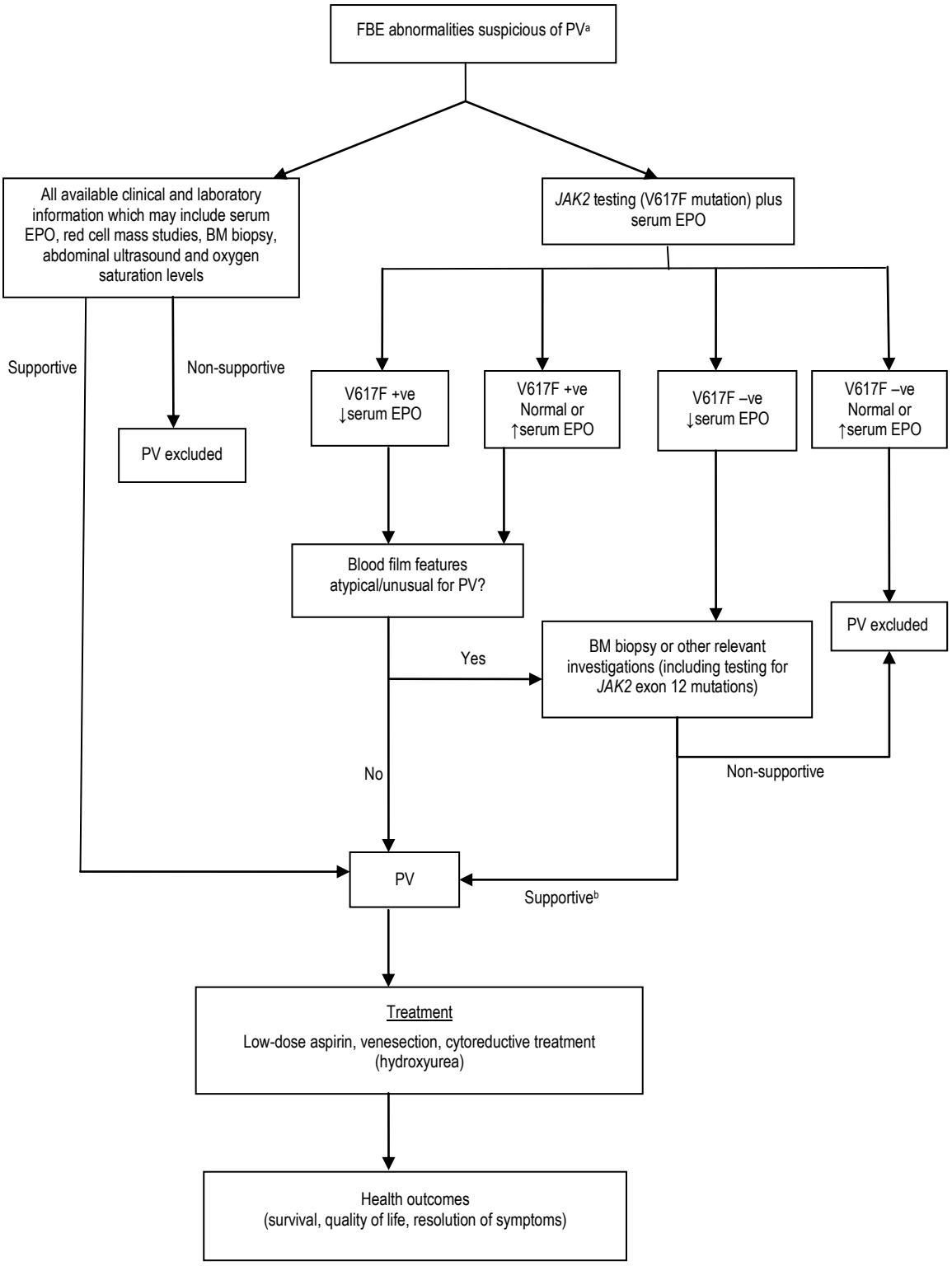
Approach to assessment

Objective

The objective of this assessment is to determine whether there is sufficient evidence in relation to clinical need, safety, effectiveness and cost-effectiveness to recommend public funding for molecular testing in the diagnosis of MPDs, in particular in regard to molecular testing for the diagnosis of polycythaemia vera (PV), essential thrombocythaemia (ET) or primary myelofibrosis (PMF).

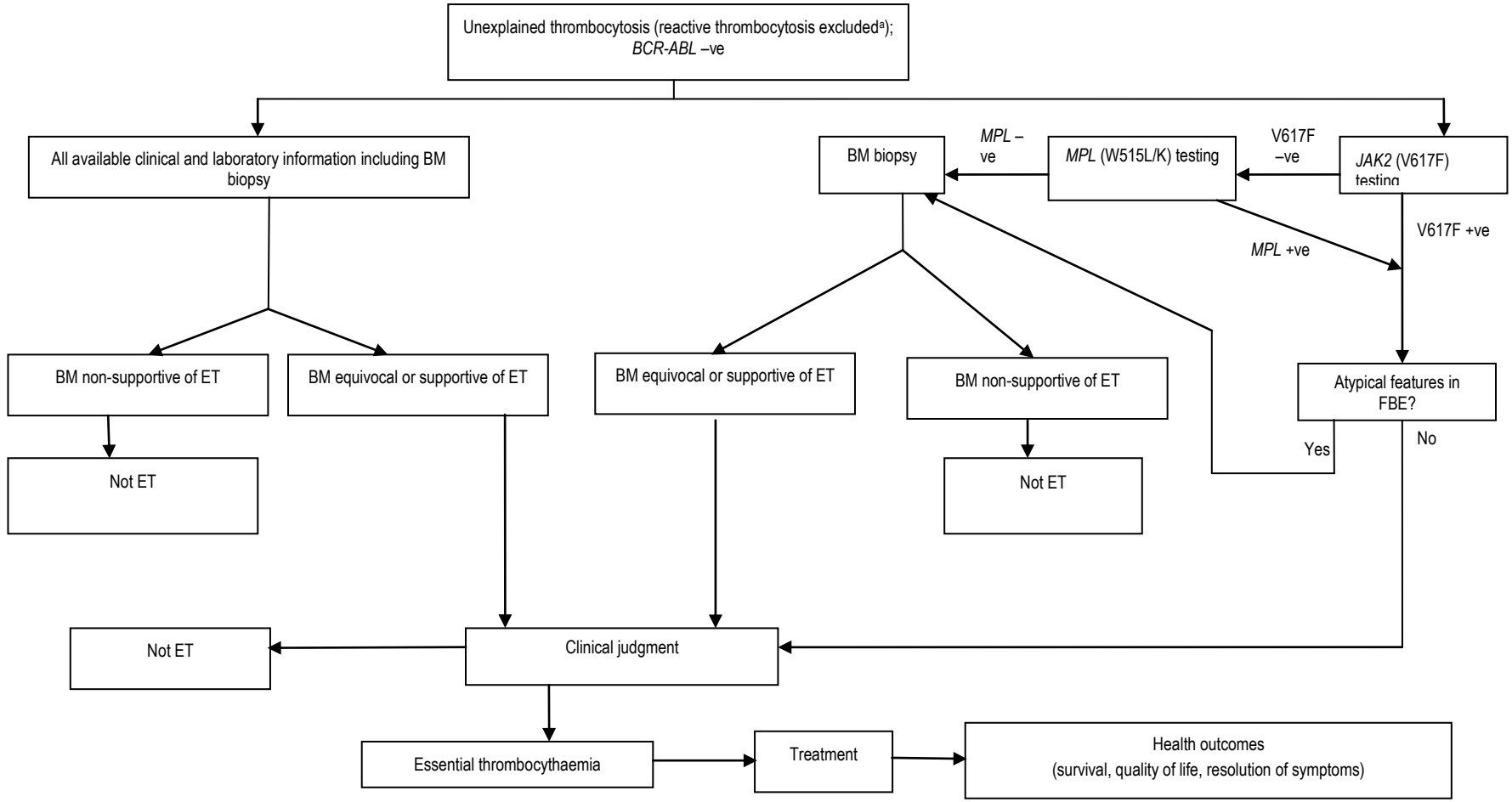
Clinical decision pathway

The clinical pathways for the diagnosis of PV, ET and PMF are shown in Figure 3, Figure 4 and Figure 5.



FBE = full blood examination; BM = bone marrow; PV = polycythaemia vera; EPO = erythropoietin
[a] As a consequence of myeloid proliferation, up to 20% of patients will require testing for the *BCR-ABL* rearrangement prior to entering the pathway;
[b] A BM biopsy which is supportive of a PV diagnosis would demonstrate hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic and megakaryocytic proliferation.

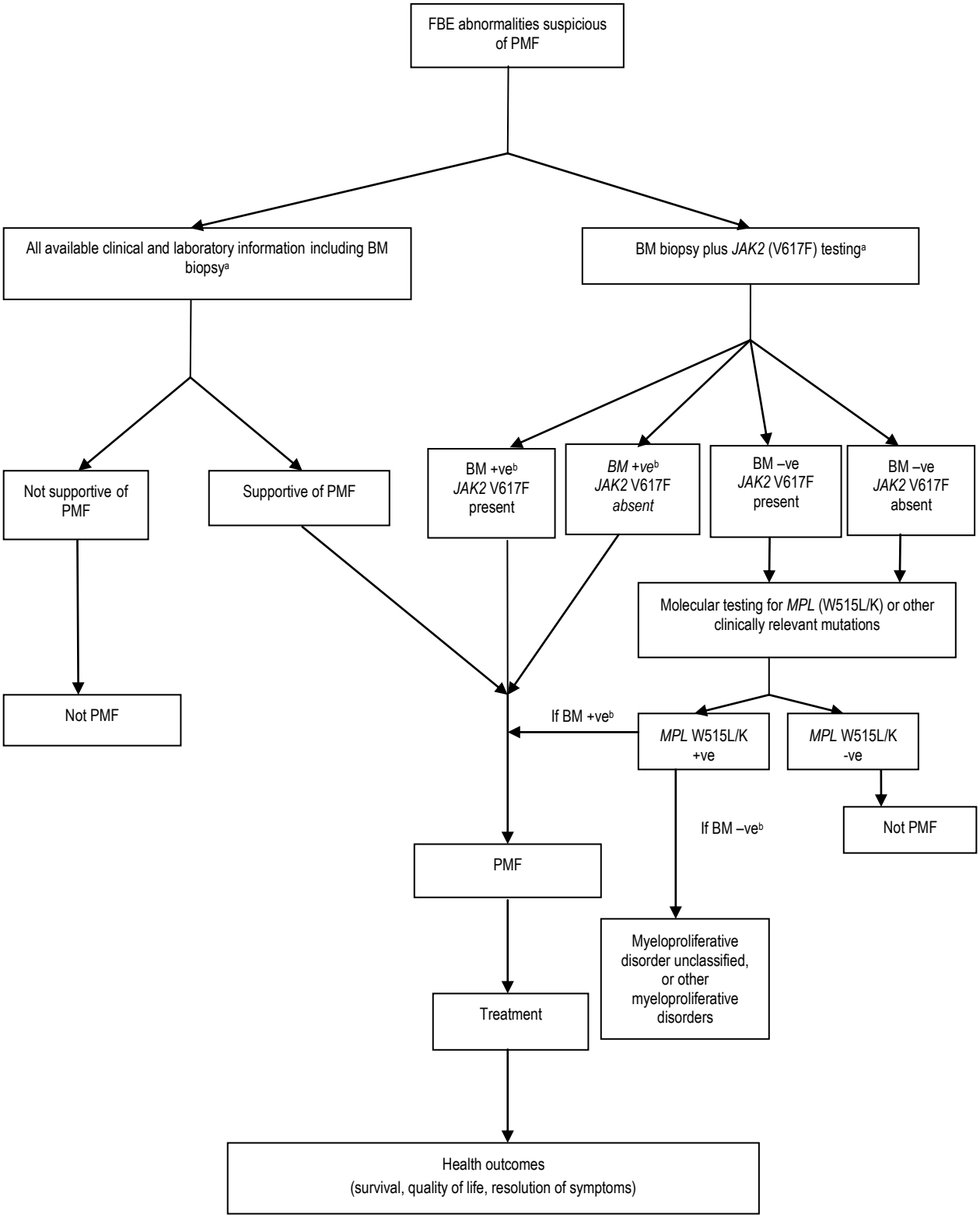
Figure 3 Clinical pathway for the diagnosis of PV



BM = bone marrow; *JAK2* = gene encoding for JAK2 tyrosine kinase; megakaryocyte proliferation ligand (MPL) = gene encoding for thrombopoietin receptor; ET = essential thrombocythaemia ; FBE = full blood examination

[a] The presence of a condition associated with reactive thrombocytosis does not exclude the possibility of ET if all other criteria have been met.

Figure 4 Clinical pathway for the diagnosis of ET



FBE = full blood examination; BM = bone marrow; PMF = primary myelofibrosis; JAK2 = gene encoding for JAK2 tyrosine kinase; megakaryocyte proliferation ligand (MPL) = gene encoding for thrombopoietin receptor
[a] exclude PV haematocrit and haemoglobin levels, exclude chronic myeloid leukaemia by the absence of *BCR-ABL* fusion gene (As a consequence of granulocyte proliferation, 10–20% of patients will require testing for the *BCR-ABL* rearrangement prior to entering the pathway), exclude myelodysplastic syndrome by absence of dyserythropoiesis and dysgranulopoiesis; [b] BM biopsy is supportive of a PMF diagnosis.

Figure 5 Clinical pathway for the diagnosis of PMF

Comparator

The comparator for molecular testing in the diagnosis of PV, ET and PMF is all available clinical and laboratory information required to make a diagnosis.

For PV, this may include:

- serum erythropoietin levels
- bone marrow biopsy
- abdominal ultrasound
- red cell mass studies
- erythroid colony formation studies.

For ET and PMF this will also include BM biopsy.

The reference standard

Diagnostic criteria for *BCR-ABL*-negative MPDs have recently been updated by the World Health Organization (WHO) to incorporate relevant information regarding the molecular pathogenesis of these diseases (Table 5) (Tefferi & Vardiman 2008).

Table 5 2008 WHO criteria for diagnosis of *BCR-ABL*-negative myeloproliferative disorders

Criteria for diagnosis of PV[a]	
Major	Hb >18.5 g/dL in men, 16.5 g/dL in women, or other evidence of increased red cell volume (eg Hb or haematocrit greater than 99th percentile of method-specific reference range for age, sex, altitude of residence; or Hb greater than 17 g/dL in men, 15 g/dL in women, if associated with a documented and sustained increase of at least 2 g/dL from an individual's baseline value that cannot be attributed to correction of iron deficiency, or elevated red cell mass greater than 25% above mean normal predicted value)
	Presence of *JAK2* V617F or other functionally similar mutation such as *JAK2* exon 12 mutation
Minor	Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
	Serum erythropoietin level below the reference range for normal
	Endogenous erythroid colony formation in vitro
Criteria for diagnosis of ET[b]	
	Sustained platelet count ≥ 450 x 10^9/L
	Megakaryocyte proliferation with large and mature morphology. No or little granulocyte or erythroid proliferation
	Not meeting WHO criteria for PV, PMF, chronic myelogenous leukaemia, myelodysplastic syndromes or other myeloid neoplasm
	Demonstration of *JAK2* V617F mutation or other clonal marker, or, in the absence of a clonal marker, no evidence of reactive thrombocytosis
Criteria for diagnosis of PMF[c]	
Major	Presence of megakaryocyte proliferation and atypia, usually accompanied by either reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterised by granulocytic proliferation and often decreased erythropoiesis (ie prefibrotic cellular-phase disease)
	Not meeting WHO criteria for PV, PMF, chronic myelogenous leukaemia, myelodysplastic syndromes or other myeloid neoplasm.
	Demonstration of *JAK2* V617F mutation or other clonal marker, or, in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases.
Minor	Leukoerythroblastosis
	Increase in serum lactate dehydrogenase level
	Anaemia
	Palpable splenomegaly

Source: Tefferi & Vardiman (2008)
Hb = haemoglobin; [a] Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria; [b] diagnosis requires meeting all four criteria; [c] diagnosis requires meeting all three major criteria and two minor criteria.; PV = primary myelofibrosis; ET = essential thrombocythaemia ; PMF = primary myelofibrosis

As stated, these criteria would be inappropriate reference standards for diagnosis of MPDs as they incorporate the presence of the *JAK2* V617F mutation as a marker of disease. However, the absence of the *JAK2* V617F mutation does not rule out disease, and therefore the 2008 WHO criteria, with the exclusion of *JAK2* V617F status, may still be useful to make a diagnosis.

As such, the reference standard for diagnosis of PV, ET or PMF is all relevant clinical and laboratory information, other than *JAK2* V617F status, that is required to make a diagnosis. For all intents and purposes, the reference standards for PV, ET and PMF are consistent with the 2001 WHO diagnostic criteria, which are the same as the 2008 criteria with the exception that molecular testing is not included.

Research questions

A number of research questions have been developed to determine the safety and effectiveness of molecular testing in the diagnosis of MPDs. These are described below according to patient indication:

PV

1. Is the use of *JAK2* V617F mutation testing in conjunction with serum erythropoietin testing, or testing for *JAK2* exon 12 mutations or other clinically relevant mutations, as safe as, or safer than, usual clinical and laboratory diagnosis for patients with suspected PV?

2. Is the testing for the *JAK2* V617F mutation in conjunction with determination of serum erythropoietin levels as effective as, or more effective than, usual clinical and laboratory diagnostic testing at improving the health outcomes of patients with suspected PV?

3. Is the testing for *JAK2* exon 12 mutations or any other clinically relevant mutations as effective as, or more effective than, usual and clinical and laboratory diagnostic testing at improving the health outcomes of patients with suspected PV?

4. What is the cost-effectiveness of molecular analysis in addition to serum erythropoietin testing relative to usual clinical and laboratory diagnosis for patients with suspected PV?

ET

5. Is the use of *JAK2* V617F mutation testing with or without (±) bone marrow (BM) biopsy, as well as testing for *MPL* mutations or any other clinically relevant mutations, as safe as, or safer than, usual clinical and laboratory diagnostic testing for patients with suspected ET?

6. Is testing for the *JAK2* V617F mutation ± BM biopsy as effective as, or more effective than, usual clinical and laboratory diagnostic testing at improving the health outcomes of patients with suspected ET?

7. Is testing for *MPL* mutations or any other clinically relevant mutations as effective as, or more effective than, usual clinical and laboratory diagnostic testing at improving the health outcomes of patients with suspected ET?

8. What is the cost-effectiveness of molecular analysis ± BM biopsy relative to usual clinical and laboratory diagnosis for patients with suspected ET?

PMF

9. Is the use of *JAK2* V617F mutation testing plus BM biopsy, as well as testing for *MPL* mutations or any other clinically relevant mutations, as safe as, or safer than, usual clinical and laboratory diagnostic testing for patients with suspected PMF?

10. Is testing for the *JAK2* V617F mutation plus BM biopsy as effective as, or more effective than, usual clinical and laboratory diagnostic testing at improving the health outcomes of patients with suspected PMF?

11. Is testing for *MPL* mutations or any other clinically relevant mutations as effective as, or more effective than, usual clinical and laboratory diagnostic testing at improving the health outcomes of patients with suspected PMF?

12. What is the cost-effectiveness of molecular analysis in addition to BM biopsy relative to usual clinical and laboratory diagnosis for patients with suspected PMF?

Diagnostic assessment framework

In order to assess the effectiveness of a diagnostic strategy, there needs to be consideration of its diagnostic accuracy (in comparison to a reference standard), its impact on the clinical management of the patient, and its ultimate impact on the health outcomes of the patient. The primary goal of this assessment was to find ***direct evidence*** of the effectiveness of using molecular testing in the diagnosis of MPDs on health outcomes. That is, one group of patients with suspected MPDs would receive molecular testing ± serum erythropoietin testing ± BM biopsy (index test strategy), and treatment and follow-up. This group would be compared to another group receiving BM biopsy ± serum erythropoietin testing ± red cell mass studies (comparator test strategy), and treatment and follow-up, for a period of time until the impact on health outcomes (ie survival) could be evaluated. No direct evidence of the impact of including molecular testing in the diagnostic strategy on final health outcomes was identified; thus, a ***linked evidence*** approach was undertaken.

In some situations it is appropriate to narratively link evidence from studies that report on the following factors to infer the effect of the diagnostic test on patient health outcomes:

- diagnostic test performance (diagnostic accuracy)—sensitivity, specificity and accuracy
- impact on clinical decision making—does clinical decision-making (patient management) change as a result of the test?
- impact of the treatment of diagnosed patients on health outcomes—do patients receiving a change in management benefit in terms of health outcomes?

Using a linked evidence approach requires either an improvement in relative diagnostic accuracy or a change in patient management that results in better patient outcomes, to imply a benefit from the diagnostic test or strategy for patients. As no change in management is anticipated as a result of incorporating molecular testing into the diagnostic strategies for PV, ET or PMF, evidence of an improvement in diagnostic accuracy is required to indicate a benefit in patient outcomes.

Review of the literature

Literature sources and search strategies

The medical literature was searched to identify relevant studies and reviews for the period 2005–09. Appendix B describes the electronic databases that were used for this search and the other sources of evidence that were investigated.

The search terms used to identify literature in electronic databases on the safety and effectiveness of molecular testing in the diagnosis of PV, ET and PMF are also presented in Appendix B.

Selection criteria

Criteria for the selection of studies relevant for an assessment of the safety and effectiveness of molecular testing for the diagnosis of PV, ET and PMF are described in Box 1 – Box 5 in the 'Results' section of this report. These criteria are applicable for an assessment using either a direct or a linked evidence approach.

The criteria for including articles in this report varied depending on the type of research question being addressed. Often a study was assessed more than once because it addressed more than one research question. One researcher applied the inclusion criteria to the collated literature. If there was any doubt concerning inclusion of papers, this was resolved by group consensus between members of the evaluation team to ensure that all potentially relevant studies were captured. In general, studies were excluded if they:

- did not address the research question;
- did not provide information on the pre-specified target population;
- did not include the pre-specified intervention;
- did not compare results with the pre-specified comparators;
- did not address one of the pre-specified outcomes and/or provided inadequate data on these outcomes (in some instances, a study was included to assess one or more outcomes but had to be excluded for other outcomes due to data inadequacies);
- were in other languages and were of a lower level of evidence than that available in English; or
- did not have the study design specified in the review protocol.

Specific selection criteria relating to each research question can be found in the 'Results' section of this report.

Studies that reported on the use of *JAK2* V617F analysis in the diagnosis of 'latent' MPDs were also included in this assessment. Studies that included subjects with splanchnic vein thromboses, hepatic vein thromboses, portal vein thrombosis and/or Budd-Chiari syndrome were included in the assessment. If studies included only subjects with venous thromboembolism without providing the location of the thromboses, or deep vein thrombosis (DVT) or pulmonary embolism (PE), they were excluded.

Search results

The process of study selection for this report went through six phases:

1. All reference citations from all literature sources were collated into an Endnote X1 database.
2. Duplicate references were removed.

3. Studies were excluded, on the basis of the citation information, if it was obvious that they did not meet the pre-specified inclusion criteria. Citations were assessed independently by one reviewer. Studies marked as requiring further evaluation were retrieved for full-text assessment.

4. Studies were included to address the research questions if they met the pre-specified criteria, again independently applied by one reviewer to the full-text articles. Those articles meeting the criteria formed part of the evidence-base. The remainder provided background information.

5. The reference lists of the included articles were pearled for additional relevant studies. These were retrieved and assessed according to phase 4.

6. The evidence-base consisted of articles from phases 4 and 5 that met the inclusion criteria.

Any doubt concerning inclusions at Phase 4 was resolved by consensus between members of the evaluation team. The results of the process of study selection are provided in Figure 6.

Quorum flowchart

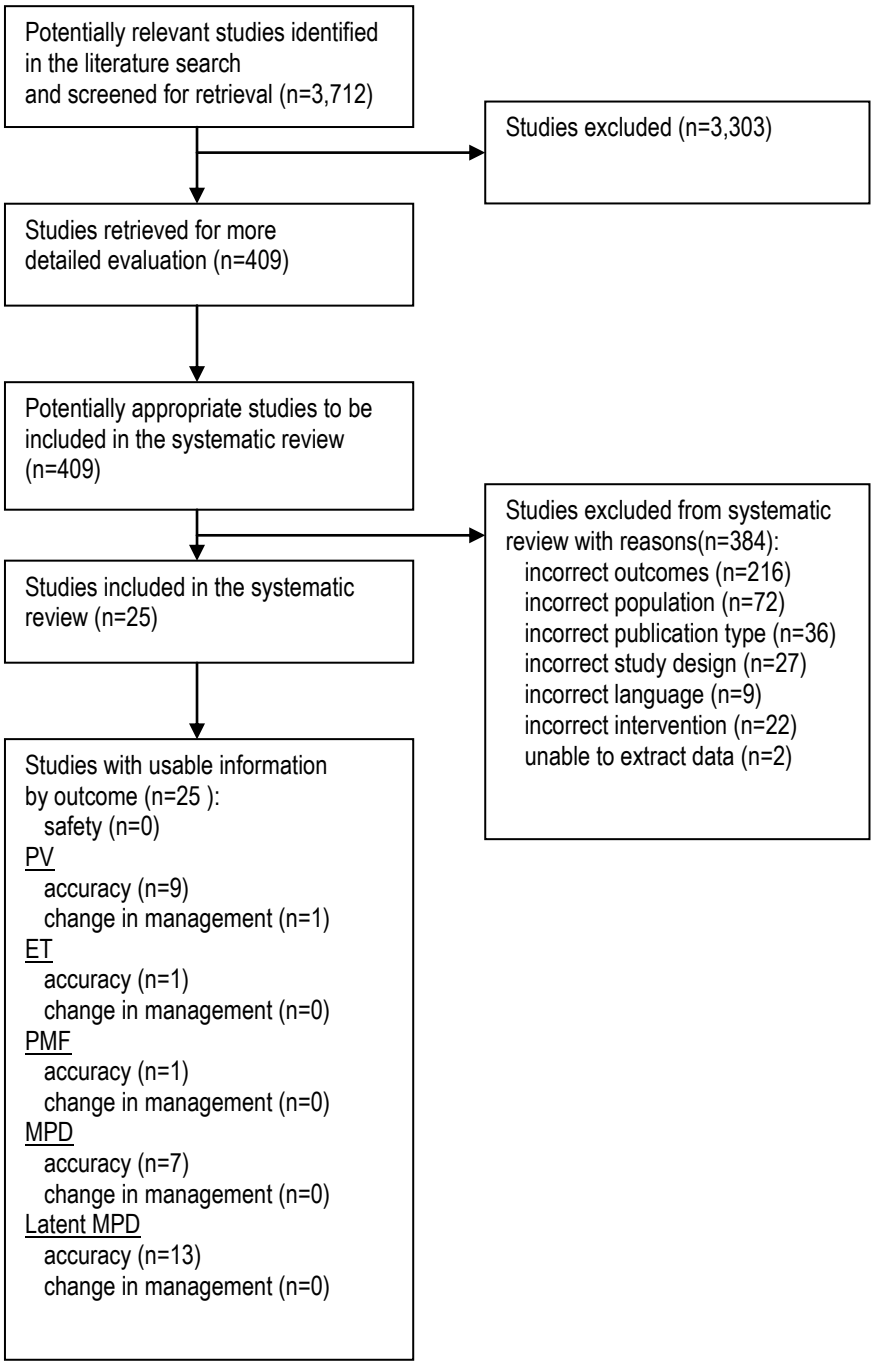


Figure 6 Summary of the process used to identify and select studies for the review
Adapted from Moher et al (1999); some of the included studies were able to report outcomes for multiple indications.

Data extraction and analysis

A profile of key characteristics including study design and location, level and quality of evidence, population, intervention and outcomes was developed for each study selected for this report (Appendix C).

In studies with diagnostic case-control design, studies were excluded if the controls consisted only of healthy volunteers. If there was a mix of healthy subjects and patients who did not have MPDs, these studies were included. Normal controls were included as they may have initially been suspected of MPD but were subsequently found to be normal.

Subgroup analysis was conducted on studies that used molecular testing to diagnose MPD or latent MPD in patients with Budd-Chiari syndrome, portal vein thrombosis or splanchnic vein thromboses.

Assessing diagnostic accuracy

To assess the diagnostic accuracy of each of the tests for dichotomous outcomes, calculations of sensitivity, specificity, negative and positive predictive value of the tests, and 95% confidence intervals, were undertaken where possible. Data were extracted using the classic 2 x 2 table, whereby the results of the index diagnostic test are cross-classified against the results of the reference standard (Armitage et al 2002; Deeks 2001), and Bayes' Theorem was applied:

		Reference standard *All relevant clinical and laboratory information*		
		Disease +	*Disease –*	
Index test strategy *Molecular testing ± serum EPO ± BM biopsy*	*Test +*	True positive	False positive	Total test positive
	Test –	False negative	True negative	Total test negative
		Total MPD +	Total MPD –	Total tested

The sensitivity of the index test (molecular testing ± serum EPO ± BM biopsy) was calculated as the proportion of people who met the 2008 WHO diagnostic criteria for MPD as confirmed by the 2001 WHO diagnostic criteria:

Sensitivity (true positive rate, %) = number of true positives / total with MPD+ * 100

The specificity of the index test strategy (molecular testing ± serum EPO ± BM biopsy) was calculated as the proportion of people who did not meet the 2008 WHO diagnostic criteria for MPD as confirmed by the 2001 WHO diagnostic criteria:

Specificity (true negative rate, %) = number of true negatives / total MPD– * 100

The positive predictive value was calculated as the proportion of positive results using the index test strategy (molecular testing ± serum EPO ± BM biopsy) as confirmed by the 2001 WHO diagnostic criteria for MPD.

Positive predictive value (PPV, %) = number of true positive results / total test positives * 100

The negative predictive value was calculated as the proportion of negative results using the index test strategy (molecular testing ± serum EPO ± BM biopsy) as confirmed by the 2001 WHO diagnostic criteria for MPD.

Negative predictive value (NPV, %) = number of true negative results / total test negatives * 100

When a 95% confidence interval was not provided in the relevant study, it was calculated using exact binomial methods.

Appraisal of the evidence

Appraisal of the evidence was conducted in three stages:

Stage 1: Appraisal of the applicability and quality of individual studies included in the review.

Stage 2: Appraisal of the precision, size and clinical importance of the primary outcomes used to determine the safety and effectiveness of the intervention.

Stage 3: Integration of this evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

Validity assessment of individual studies

The evidence presented in the selected studies was assessed and classified using the dimensions of evidence defined by the National Health and Medical Research Council (NHMRC 2000b).

These dimensions (Table 6) consider important aspects of the evidence supporting a particular intervention and include three main domains: strength of the evidence, size of the effect and relevance of the evidence. The first domain is derived directly from the literature identified as informing a particular intervention. The last two each requires expert clinical input as part of its determination.

Table 6 Evidence dimensions

Type of evidence	Definition
Strength of the evidence	
level	The study design used, as an indicator of the degree to which bias has been eliminated by design.[a]
quality	The methods used by investigators to minimise bias within a study design.
statistical precision	The p-value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect.
Size of effect	The distance of the study estimate from the 'null' value and the inclusion of only clinically important effects in the confidence interval.
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used.

[a] See Table 7

Strength of the evidence

The three subdomains (level, quality and statistical precision) are collectively a measure of the strength of the evidence.

Level

The 'level of evidence' reflects the effectiveness of a study design to answer a particular research question. Effectiveness is based on the probability that the design of the study has reduced or eliminated the impact of bias on the results.

The NHMRC evidence hierarchy provides a ranking of various study designs ('levels of evidence') by the type of research question being addressed (see Table 7).

Table 7 Designations of levels of evidence according to type of research question (including table notes)

Level	Intervention[a]	Diagnostic accuracy[b]
I[c]	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard[d], among consecutive persons with a defined clinical presentation[e]
III-1	A pseudo randomised controlled trial (ie alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard[d], among non-consecutive persons with a defined clinical presentation[e]
III-2	A comparative study with concurrent controls: – non-randomised, experimental trial[f] – cohort study – case-control study – interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for level II and III-1 evidence
III-3	A comparative study without concurrent controls: – historical control study – two or more single-arm studies[g] – interrupted time series without a parallel control group	Diagnostic case-control study[e]
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard)[h]

Sources: Merlin et al (2009); NHMRC (2008a)

Table notes

[a] Definitions of these study designs are provided in NHMRC (2000b); pp 7–8

[b] The dimensions of evidence apply only to studies of diagnostic accuracy. To assess the effectiveness of a diagnostic test there also needs to be a consideration of the impact of the test on patient management and health outcomes (MSAC 2005; Sackett & Haynes 2002).

[c] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence. Systematic reviews of level II evidence provide more data than the individual studies and any meta-analyses will increase the precision of the overall results, reducing the likelihood that the results are affected by chance. Systematic reviews of lower level evidence present results of likely poor internal validity and thus are rated on the likelihood that the results have been affected by bias, rather than whether the systematic review itself is of good quality. Systematic review quality should be assessed separately. A systematic review should consist of at least two studies. In systematic reviews that include different study designs, the overall level of evidence should relate to each individual outcome/result, as different studies (and study designs) might contribute to each different outcome.

[d] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its/their timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study (Whiting et al 2003).

[e] Well-designed population based case-control studies (eg screening studies where test accuracy is assessed on all cases, with a random sample of controls) capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease are compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease, are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias or spectrum effect because the spectrum of study participants will not be representative of patients seen in practice (Mulherin & Miller 2002).

[f] This also includes controlled before-and-after (pre-test/post-test) studies, as well as adjusted indirect comparisons (ie using A vs B and B vs C to determine A vs C, with statistical adjustment for B).

[g] Comparing single-arm studies, ie case series from two studies. This would also include unadjusted indirect comparisons (ie using A vs B and B vs C to determine A vs C, with no statistical adjustment for B).

[h] Studies of diagnostic yield provide the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard. These may be the only alternative when there is no reliable reference standard.

Note A: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms are rare and cannot feasibly be captured within randomised controlled trials; physical harms and psychological harms may need to be addressed by different study designs. Harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note B: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question, eg level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence.

Source: Hierarchies adapted and modified from: Bandolier (1999; Lijmer et al (1999); NHMRC (1999); Phillips et al (2001).

Individual studies assessing diagnostic effectiveness were graded according to pre-specified quality and applicability criteria (MSAC 2005), as shown in Table 8.

Table 8 Grading system used to rank included studies

Validity criteria	Description	Grading system
Appropriate comparison	Did the study evaluate a direct comparison of the index test strategy with the comparator test strategy?	C1 direct comparison CX other comparison
Applicable population	Did the study evaluate the index test in a population that is representative of the subject characteristics (age and sex) and clinical setting (disease prevalence, disease severity, referral filter and sequence of tests) for the clinical indication of interest?	P1 applicable P2 limited P3 different population
Quality of study	Was the study designed and to avoid bias? High quality = no potential for bias based on pre-defined key quality criteria Medium quality = some potential for bias in areas other than those pre-specified as key criteria Poor quality = poor reference standard and/or potential for bias based on key pre-specified criteria	Q1 high quality Q2 medium Q3 poor reference standard poor quality or insufficient information

Quality

The appraisal of intervention studies pertaining to treatment safety and effectiveness was undertaken using a checklist developed by the NHMRC (2000a). This checklist was used for trials and cohort studies. Uncontrolled before-and-after case series are a poorer level of evidence with which to assess effectiveness. The quality of this type of study design was assessed according to a checklist developed by the UK National Health Service (NHS) Centre for Reviews and Dissemination (Khan et al 2001). Studies of diagnostic accuracy were assessed using the QUADAS quality assessment tool (Whiting et al 2003).

Statistical precision

Statistical precision was determined using statistical principles. Small confidence intervals and p-values give an indication as to the probability that the reported effect is real and not attributable to chance (NHMRC 2000b). Studies need to be appropriately powered to ensure that a real difference between groups will be detected in the statistical analysis.

Size of effect

For change in management studies following molecular testing, it would have been important to assess whether statistically significant differences between the comparators were also clinically important. The size of the effect would need to be determined, as well as whether the 95% confidence interval included only clinically important effects. Data from the one change in management study that was identified did not report an effect size and, as a consequence, the study was discussed narratively.

Relevance of evidence

The outcomes being measured in this report should be appropriate and clinically relevant. Inadequately validated (predictive) surrogate measures of a clinically relevant outcome should be avoided (NHMRC 2000b).

Assessment of the body of evidence

Appraisal of the body of evidence was conducted along the lines suggested by the NHMRC in their guidance on clinical practice guideline development (NHMRC 2008b; NHMRC 2008a). Five components are considered essential by the NHMRC when judging the body of evidence:

- the evidence-base—which includes the number of studies sorted by their methodological quality and relevance to patients
- the consistency of the study results—whether the better quality studies had results of a similar magnitude and in the same direction, that is homogenous or heterogeneous findings
- the potential clinical impact—appraisal of the precision, size and clinical importance or relevance of the primary outcomes used to determine the safety and effectiveness of the test
- the generalisability of the evidence to the target population
- the applicability of the evidence—integration of the evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

A matrix for assessing the body of evidence for each research question, according to the components above, was used for this assessment (Table 9) (NHMRC 2008b).

Table 9 Body of evidence assessment matrix

Component	A	B	C	D
	Excellent	**Good**	**Satisfactory**	**Poor**
Evidence-base[a]	Several level I or II studies with low risk of bias	One or two level II studies with low risk of bias, or a SR/multiple level III studies with low risk of bias	Level III studies with low risk of bias, or level I or II studies with moderate risk of bias	Level IV studies, or level I to III studies with high risk of bias
Consistency[b]	All studies consistent	Most studies consistent and inconsistency may be explained	Some inconsistency reflecting genuine uncertainty around clinical question	Evidence is inconsistent
Clinical impact	Very large	Substantial	Moderate	Slight or restricted
Generalisability	Population(s) studied in body of evidence are the same as the target population for the guideline	Population(s) studied in the body of evidence are similar to the target population for the guideline	Population(s) studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population[c]	Population(s) studied in body of evidence differ to target population and it is hard to judge whether it is sensible to generalise to target population

Applicability	Directly applicable to Australian healthcare context	Applicable to Australian healthcare context with few caveats	Probably applicable to Australian healthcare context with some caveats	Not applicable to Australian healthcare context

Adapted from NHMRC (2008b)

[a] Level of evidence determined from the NHMRC evidence hierarchy

[b] If there is only one study, rank this component as 'not applicable'.

[c] For example, results in adults that are clinically sensible to apply to children OR psychosocial outcomes for one cancer that may be applicable to patients with another cancer

Expert advice

An Advisory Panel was established to provide guidance to the Evaluators to ensure that the assessment is clinically relevant and takes into account consumer interests. In selecting members for advisory panels, the MSAC's practice is to approach the appropriate medical colleges, specialist societies and associations, and consumer bodies for nominees. The panel had expertise in haematology, oncology, molecular pathology and consumer issues. Membership of the Advisory Panel is provided at Appendix A.

Results of assessment

Is it safe?

Molecular testing requires sampling of the patient's blood, generally from veins in the upper limbs. Venesection may rarely be associated with physical harms such as pain, bruising, nerve damage, arterial puncture or infection of the puncture site (Lavery & Ingram 2005; Scales 2008). Molecular testing for MPDs may also be performed on BM specimens. Although considered rare, complications associated with obtaining these samples may include pain and haemorrhage (Riley et al 2004).

This assessment of safety considered any physical harms related to obtaining a sample necessary for molecular testing in the diagnosis of PV, ET or PMF. Relevant studies were assessed by applying the inclusion criteria outlined in Box 1.

Box 1 Inclusion criteria for identification of studies relevant to assessment of the safety of molecular testing for PV, ET and PMF

Research question	
Is molecular testing for clinically relevant mutations in addition to usual diagnostic assessment as safe as, or safer than, usual clinical and laboratory diagnosis for patients with suspected PV, ET or PMF?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected PV or ET (including patients presenting with venous thromboses) or PMF
Intervention	Molecular testing for clinically relevant mutations, ie *JAK2* or *MPL* ± BM biopsy ± serum erythropoietin levels
Comparator(s)	Clinical and laboratory diagnosis using all available information required for diagnosis
Outcomes	Physical harms from testing
Search period	2005 – February 2009
Language	Non-English language articles will be excluded unless they appear to provide a higher level of evidence than the English language articles identified

PV = polycythaemia vera; ET = essential thrombocythaemia; PMF = primary myelofibrosis; BM = bone marrow

No studies were identified that reported safety outcomes related to molecular testing for the diagnosis of PV, ET or PMF. Similarly, no case reports reported any adverse outcomes associated with the use of molecular testing in the diagnosis of MPD.

Possible adverse events that can be associated with obtaining samples for use in molecular testing are examined in the 'Discussion' section of this report.

Summary of safety

No studies were identified that could inform an assessment of the safety of molecular testing in the diagnosis of PV, ET or PMF.

Is it effective?

Direct evidence

In this assessment direct evidence would evaluate whether there is a change to patient health outcomes following the use of molecular testing for diagnosing patients suspected of MPDs. The inclusion criteria to identify such evidence are outlined in Box 2.

Box 2 Inclusion criteria for identification of studies relevant to assessment of direct evidence of the effectiveness of molecular testing for PV, ET and PMF

Research question	
Is molecular testing for clinically relevant mutations in addition to usual diagnostic assessment as effective as, or more effective than, usual clinical and laboratory diagnosis in improving the health outcomes of patients with suspected PV, ET or PMF?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected PV or ET (including patients presenting with venous thromboses) or PMF
Intervention	Molecular testing for clinically relevant mutations, ie *JAK2* or *MPL* ± BM biopsy ± serum erythropoietin levels
Comparator(s)	Clinical and laboratory diagnosis using all available information required for diagnosis
Outcomes	Primary: mortality; quality of life; reduction in symptoms or life-threatening events, including thrombotic or haemorrhagic events, stroke or heart attack, psychosocial outcomes (eg anxiety); avoidance of unnecessary treatments Secondary: rate of blood transfusion, venesection
Search period	2005 – February 2009
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified

PV = polycythaemia vera; ET = essential thrombocythaemia; PMF = primary myelofibrosis; BM = bone marrow

No direct evidence was identified that reported a change in patient health outcomes following molecular testing in addition to usual clinical and laboratory diagnosis in patients suspected of MPDs.

Linked evidence

In the absence of direct evidence, a linked evidence approach was attempted, where evidence of diagnostic accuracy, change in clinical management and treatment effectiveness were linked to provide an assessment of the effectiveness of using molecular testing in the diagnosis of chronic MPDs. The inclusion criteria for such an assessment are outlined in Box 3 to Box 5. The linked evidence has been presented according to the specific type of MPD.

Box 3 Inclusion criteria for identification of studies relevant to assessment of the diagnostic accuracy of molecular testing for PV, ET and PMF

Research question	
Is molecular testing for clinically relevant mutations in addition to usual diagnostic assessment as accurate as, or more accurate than, usual clinical and laboratory diagnosis in diagnosing patients with suspected PV, ET or PMF?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected PV or ET (including patients presenting with venous thromboses) or PMF
Intervention	Molecular testing for clinically relevant mutations, ie *JAK2* or *MPL* ± BM biopsy ± serum erythropoietin levels
Comparator(s)	Clinical and laboratory diagnosis using all available information required for diagnosis
Outcomes	Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values, diagnostic odds ratios, receiver operator characteristic curves, area under the curve, accuracy
Search period	2005 – February 2009
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified

PV = polycythaemia vera; ET = essential thrombocythaemia; PMF = primary myelofibrosis; BM = bone marrow

Box 4 Inclusion criteria for identification of studies relevant to assessment of a change in patient management as a result of molecular testing for PV, ET and PMF

Research question	
Does molecular testing for clinically relevant mutations in addition to usual diagnostic assessment change patient management compared with usual clinical and laboratory diagnosis in improving the health outcomes of patients with suspected PV, ET or PMF?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected PV or ET (including patients presenting with venous thromboses) or PMF
Intervention	Molecular testing for clinically relevant mutations, ie *JAK2* or *MPL* ± BM biopsy ± serum erythropoietin levels
Comparator(s)	Clinical and laboratory diagnosis using all available information required for diagnosis
Outcomes	Rates of treatment, method of treatment, rates of referral, type of referral, hospitalisation, rates of consultation
Search period	2005 – February 2009
Language	Non-English language articles were excluded unless they appear to provide a higher level of evidence than the English language articles identified

PV = polycythaemia vera; ET = essential thrombocythaemia; PMF = primary myelofibrosis; BM = bone marrow

Box 5 Inclusion criteria for identification of studies relevant to assessment of treatment effectiveness following a change in patient management as a result of molecular testing for PV, ET and PMF

Research question	
Is molecular testing for clinically relevant mutations in addition to usual diagnostic assessment as effective as, or more effective than, usual clinical and laboratory diagnosis in improving the health outcomes of patients with suspected PV, ET or PMF?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected PV or ET (including patients presenting with venous thromboses) or PMF
Intervention	Molecular testing for clinically relevant mutations, ie *JAK2* or *MPL* ± BM biopsy ± serum erythropoietin levels
Comparator(s)	Clinical and laboratory diagnosis using all available information required for diagnosis
Outcomes	Primary: mortality; quality of life; reduction in symptoms or life-threatening events, including thrombotic or haemorrhagic events, stroke or heart attack, psychosocial outcomes (eg anxiety); avoidance of unnecessary treatments Secondary: rate of blood transfusion, venesection
Search period	2005 – February 2009
Language	Non-English language articles were excluded unless they appear to provide a higher level of evidence than the English language articles identified

PV = polycythaemia vera; ET = essential thrombocythaemia; PMF = primary myelofibrosis; BM = bone marrow

Polycythaemia vera

Is it accurate?

JAK2 exon 14 mutations

Three studies met the inclusion criteria and reported on the analysis of *JAK2* exon 14 mutations in the diagnosis of PV. In the absence of diagnostic accuracy outcomes, diagnostic yield was assessed.

Extensive variation is noted in the results between the level IV studies of diagnostic yield (Table 10). The high-quality study conducted by Rumi et al (2009) reported the yield of PV, secondary erythrocytosis (SE) and idiopathic erythrocytosis (IE) from a group of patients who presented with isolated erythrocytosis. Using the 2008 WHO criteria to provide the diagnosis for these patients, the yield of PV was 14%, and 51% and 35% for SE and IE respectively.

The fair-quality study by Girodon et al (2007) assessed the diagnostic yield of molecular testing used in addition to the 2001 WHO criteria. The patients included in this study were being investigated for an elevated haematocrit and, although little information is given regarding the setting for this study, it is assumed that the patients are all attending the same clinic. In comparison, the study by Ammatuna et al (2007) indicates that patient samples were sent from regional hospitals to their reference laboratory for *JAK2* analysis and, consequently, may be subject to different referral patterns. It is possible that this may, in part, explain the heterogeneity in diagnostic yield.

Table 10 Diagnostic yield of molecular testing for *JAK2* V617F in the diagnosis of PV

Study	Study quality[a]	Population	Diagnostic criteria used for PV	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield
(Rumi et al 2009)	Level IV diagnostic evidence CX P2 Q1	91 patients with isolated erythrocytosis	2008 WHO criteria	AS-qPCR	Not reported	PV: 13/91 (14%)[b] SE: 46/91 (51%)[b] IE: 32/91 (35%)[b]
(Girodon et al 2007)	Level IV diagnostic evidence CX P1 Q2	168 patients undergoing investigation of elevated Hct	2001 WHO criteria for diagnosis of PV plus molecular testing	qPCR PB	Not reported	72/168 (43%)
(Ammatuna et al 2007)	Level IV diagnostic evidence CX P2 Q3	92 patients suspected of MPD	2001 WHO criteria for diagnosis of MPD plus molecular testing	Multiplex PCR to detect *BCR-ABL* and *JAK2* V617F RNA extracted from PB or BM	1% of heterozygous V617F DNA in *BCR-ABL*-+ve DNA and vice versa	8/92 (9%)

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] This study reported the post-test classification for all patients; PCR = polymerase chain reaction; PB = peripheral blood; PV = polycythaemia vera; RNA = ribonucleic acid; AS-qPCR = allele-specific quantitative PCR; SE = secondary erythrocytosis; IE = idiopathic erythrocytosis; BM = bone marrow; Hct = haematocrit; MPD = myeloproliferative disorder; DNA = deoxyribonucleic acid

Known *JAK2* exon 12 mutations

Six studies reported on the use of *JAK2* exon 12 mutation analysis in the diagnosis of PV (Table 11 and Table 12). Three studies were diagnostic case-control studies (level III-3 diagnostic evidence) and the remaining studies reported diagnostic yield (level IV diagnostic evidence).

The studies by Pardanani et al (2007) and Martinez-Aviles et al (2007) reported the effectiveness of exon 12 mutation analyses in PV cases and IE controls (level III-3 diagnostic evidence). The results of these studies were quite disparate but this may be attributable to the method of mutation detection and the diagnostic criteria used in each study. In the medium-quality study by Pardanani et al (2007), detection of the exon 12 mutations was by allele-specific PCR, and cases and controls were defined by the 2001 WHO criteria. The sensitivity and specificity of exon 12 mutation analysis in this population were 83% and 100% respectively. In comparison, the poor-quality study by Martinez-Aviles et al (2007) used cases and controls defined by the Polycythaemia Vera Study Group (PVSG) criteria, which provide a more select population than that of the WHO criteria. Additionally, the investigators used a direct sequencing method to analyse the presence of exon 12 mutations. The analytic sensitivity of sequencing methods is substantially less than that of allele-specific PCR methods. As a consequence of these methodological differences, the calculated diagnostic accuracy between the two studies is markedly different. The study by Martinez-Aviles et al (2007) reported very low to fair specificity and sensitivity at 11% and 73% respectively.

In the poor-quality study (level III-3 diagnostic evidence) by Kondo et al (2008), the presence of exon 12 mutations was determined in *JAK2* V617F-negative patients with PV or IE with the 2008 WHO criteria defining the cases (PV) and controls (IE). Small

numbers in this study are likely to limit the inferences that can be drawn; however, when considering these results, it must be remembered that the reference standard, that is all relevant and clinical information without the use of *JAK2* exon 12 molecular testing, is likely to be imperfect. Consequently, some cases of exon 12-positive PV may be missed and misclassified as IE by the reference standard. This would result in some patients in whom an exon 12 mutation was detected being considered as false positives and therefore reducing the specificity of the index test strategy. As is the case in Kondo et al (2008), one patient who had been diagnosed with IE by the reference standard was reclassified as PV following the detection of an exon 12 mutation. Additionally, the low prevalence of known exon 12 mutations in patients with PV is likely to result in poor sensitivity, as demonstrated by the level III-3 evidence (Table 11). This shows very poor test sensitivity because the absence of a known exon 12 mutation is unable to rule out the presence of PV.

The three poor-quality studies of diagnostic yield (level IV diagnostic evidence) reported dissimilar rates of diagnosis based on the analysis of *JAK2* exon 12 mutations (Table 12). Based on the information reported, it is difficult to ascertain the reason for this disparity, which resulted in diagnostic yields ranging from 8% to 38%.

Table 11 Diagnostic accuracy of molecular analysis of *JAK2* exon 12 in the diagnosis of PV

Study	Study quality[a]	Population and comparator	Molecular test technique and sample type	Diagnostic test accuracy			
				Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
(Pardanani et al 2007)	Level III-3 diagnostic evidence CX P1 Q2	12 patients diagnosed with PV or IE Cases: PV (n=6) Controls: IE (n=6) ***Reference standard*** 2001 WHO criteria	Sequencing and AS-PCR PBMC, granulocytes or BM	83% [35.9, 99.6]	100% [54.1, 100.0]	100% [47.8, 100.0]	86% [42.1, 99.6]
(Kondo et al 2008)[b]	Level III-3 diagnostic evidence C1 P2 Q3	19 patients with erythrocytosis Cases: PV n=3 Controls: IE n=16 ***Reference standard*** 2001 WHO criteria	PCR followed by sequencing (sensitivity = 20% of homozygous HEL cell line) PB	0% [0.0, 0.71]	94% [69.8, 99.8]	0% [0.0, 98.5]	83% [58.6, 96.4]
(Martinez-Aviles et al 2007)	Level III-3 diagnostic evidence CX P2 Q3	20 patients who were *JAK2* V617F-negative Cases: PV n=9 Controls: IE n=11 ***Reference standard*** PVSG criteria for PV and BCSH for IE	Direct sequencing	11% [0.3, 48.3]	73% [39.0, 94.0]	25% [0.6, 80.6]	50% [24.7, 75.4]

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] This study also reported results of *JAK2* V617F analysis (Table 16); PV = polycythaemia vera; IE = idiopathic erythrocytosis; WHO = World Health Organization; Sn = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; PCR = polymerase chain reaction; AS-PCR = allele specific PCR; PBMC = peripheral blood mononuclear cells; BM = bone marrow; PVSG = Polycythaemia Vera Study Group; HEL = human erythroleukaemia; CI = confidence intervals; PB = peripheral blood; BCSH = British Committee for Standards in Haematology

Table 12 Diagnostic yield of molecular testing for *JAK2* exon 12 mutations in the diagnosis of PV

Study	Study quality	Population	Diagnostic criteria used for PV	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield
(Percy et al 2007)	Level IV diagnostic evidence CX P1 Q3	58 patients with IE and normal to low serum EPO	Not reported	AS-PCR assays to detect K539L; N542-E543del; F537-K539delinsL; and H538QK539L mutations. PB or purified granulocyte DNA	Not reported	8/58 (14%)
(Jones et al 2008)	Level IV diagnostic evidence CX P2 Q3	50 patients with erythrocytosis	2008 WHO criteria	HRM	Not reported	4/50 (8%)
(Kouroupi et al 2008)	Level IV diagnostic evidence CX P2 Q3	21 patients with IE	2008 WHO criteria	AS-PCR assays to detect K539L; N542-E543del; F537-K539delinsL; and H538QK539L mutations PB	Not reported	8/21 (38%)

IE = idiopathic erythrocytosis; AS-PCR = allele specific polymerase chain reaction; PB = peripheral blood; HRM = high-resolution melting curve analysis; EPO = erythropoietin; DNA = deoxyribonucleic acid; WHO = World Health Organization

Does it change patient management?

Minimal evidence was identified regarding a change in patient management following diagnosis of MPD using molecular testing. One study reported the impact on patient management in terms of a change in diagnosis and subsequent patient management of *JAK2* V617F testing alone (Means 2008). This medium-quality study described the impact of *JAK2* V617F testing in small academic practices. Using retrospective chart review, 16 patients who attended five clinics at the University of Kentucky or Lexington Veteran Affairs centres were identified as having a diagnosis of PV or suspected PV. The 2001 WHO criteria were used to make the original diagnosis of PV. The study reported nine patients who underwent *JAK2* V617F analysis, mostly soon after diagnosis. Those who did not undergo analysis comprised five patients who had met WHO criteria for PV and two patients who had stable disease, of which one was undergoing observation only.

Of the nine patients tested for the presence of the V617F mutation, seven tested positive, five of which were newly diagnosed patients. Of all seven patients tested, the diagnosis and subsequent management of two changed as a result of the molecular test results. One patient who had tested positive for the mutation had not met the 2001 WHO criteria for PV; however, with the presence of the V617F mutations detected, the diagnosis was altered to that of PV along with management. The patient had previously been undergoing phlebotomy with a target relevant to a patient with secondary

polycythaemia; however, this was changed to a phlebotomy target relevant to a patient with PV. Conversely, one patient who had previously been suspected of PV was undergoing phlebotomy with a target relevant to PV; however, the presence of wild-type *JAK2* resulted in revision of the diagnosis to secondary polycythaemia along with a change in the phlebotomy target.

Does change in management improve patient outcomes?

As there was no comparative evidence that the type of treatment would change or that earlier treatment would occur as a result of molecular testing, a systematic review of the impact of a change in management on patient outcomes was not required. A description of the established treatment of PV has been provided.

Evidence-based guidelines endorsed by the British Committee for Standards in Haematology (BCSH) were published in 2005 and are based on a systematic review of literature published between 1966 and June 2004.

These guidelines recommend the management of PV through venesection to maintain the patient's haematocrit below 45%. Additionally, all patients should receive low-dose aspirin (75 mg/mL) daily unless otherwise indicated. The guidelines also recommend that cytoreductive therapy should be considered when there is poor tolerance to venesection, evidence of disease progression or thrombocytosis. The particular choice of cytoreductive therapy should be based on the age of the patient, as outlined in Table 13. These recommendations are grade C, using evidence from expert committee reports or opinions, and are an indication of an absence of directly applicable clinical studies of good quality that assess treatment effect (McMullin et al 2005).

Table 13 Recommended cytoreductive therapy for patients with PV

Age of patient	First-line therapy	Second-line therapy
< 40 years	Interferon-α	Hydroxyurea or anagrelide
40–75 years	Hydroxyurea	Interferon-α or anagrelide
> 75 years	Hydroxyurea	^{32}P or low-dose busulphan

Source: McMullin et al (2005)

Essential thrombocythaemia

Is it accurate?

No studies reported the diagnostic accuracy of testing for *JAK2* mutations in those suspected of ET.

Only one study reported the diagnostic yield of molecular testing in the diagnosis of ET (Table 14). This poor-quality study by Ammatuna et al (2007) (level IV diagnostic evidence) had the primary aim of simplifying the molecular analysis of *JAK2* mutations and *BCR-ABL* rearrangements by developing a multiplex PCR. Patient samples were referred from regional hospitals to the reference laboratory in Rome, Italy, and therefore the population characteristics of this study are dependent on the referral patterns in regional hospitals near Rome. In this study 60 of 92 (65%) samples received were given a diagnosis of ET using molecular analysis in conjunction with the 2001 WHO criteria.

Table 14 Diagnostic yield of molecular testing for the diagnosis of ET

Study	Study quality[a]	Population	Diagnostic criteria used for ET	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield
(Ammatuna et al 2007)[b]	Level IV diagnostic evidence CX P2 Q3	92 patients suspected of MPD 50 patients with ≥ 2 elevated myeloid cell lines 42 patients with isolated thrombocythaemia with main causes of reactive disease excluded	2001 WHO criteria for diagnosis of MPD plus molecular testing	Multiplex PCR to detect *BCR-ABL* and *JAK2* V617F from RNA extracted from PB or BM	1% of heterozygous V617F DNA in *BCR-ABL*-+ve DNA and vice versa	60/92 = 65%

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] This study also reported results for the diagnosis of PV (Table 10) and PMF (Table 15); ET = essential thrombocythaemia; MPD = myeloproliferative disease; WHO = World Health Organization; PCR = polymerase chain reaction; PB = peripheral blood; BM = bone marrow; DNA = deoxyribonucleic acid; RNA = ribonucleic acid

Does it change patient management?

No studies were identified that assessed a change in management of patients with ET following diagnosis with molecular testing. Subsequently, a systematic review of the impact on patient outcomes following a change in management was not required.

Does change in management improve patient outcomes?

Consensus guidelines, based on a systematic review published in Canada in 2008, provide recommendations regarding the management of malignant thrombocytosis in *BCR-ABL*-negative MPD (Matthews et al 2008). These recommendations are consistent with many other non-systematic reviews that discuss the treatment of ET (Campbell & Green 2005; Harrison 2005; Tefferi 2008).

The major recommendations of these guidelines suggest that all patients with ET should decrease their thrombotic risk by receiving low-dose aspirin therapy unless contraindicated by a platelet count greater than 1,500 x 10^9/L (Penninga & Bjerrum 2006). Cytoreductive therapy is not recommended for asymptomatic patients but should be considered in patients with a high risk of thrombosis, that is a previous history of thrombosis or thrombocytosis. Hydroxyurea is recommended as the preferred cytoreductive agent and should be used to maintain a platelet count of less than 600 x 10^9/L. If hydroxyurea is not appropriate, anagrelide or interferon-α should be considered.

Primary myelofibrosis

Is it accurate?

Similar to the assessment of diagnostic accuracy in ET, there was only one study identified that reported the diagnostic yield of molecular analysis for PMF (Table 15). As discussed earlier, the study by Ammatuna et al (2007) (level IV diagnostic evidence) reported the diagnostic yield of *JAK2* analysis in 92 patients suspected of MPD. The diagnostic yield of PMF was 7 of 92 (8%) patients.

Table 15 Diagnostic yield of molecular testing for the diagnosis of PMF

Study	Study quality[a]	Population	Diagnostic criteria used for PMF	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield
(Ammatuna et al 2007)[b]	Level IV diagnostic evidence CX P2 Q3	92 patients suspected of MPD	2001 WHO criteria for diagnosis of MPD plus molecular testing	Multiplex PCR to detect *BCR-ABL* and *JAK2* V617F from RNA extracted from PB or BM	1% of heterozygous V617F DNA in *BCR-ABL*-+ve DNA and vice versa	7/92 = 8%

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] This study also reported results for the diagnosis of PV (Table 10) and ET (Table 14); MPD = myeloproliferative disease; WHO = World Health Organization; PCR = polymerase chain reaction; PB = peripheral blood; PMF = primary myelofibrosis; BM = bone marrow; DNA = deoxyribonucleic acid; RNA = ribonucleic acid

Does it change patient management?

No studies were identified that reported a change in patient management subsequent to diagnosis of PMF with molecular testing.

Does change in management improve patient outcomes?

An absence of evidence regarding change in management outcomes meant that it was not possible to determine if clinical management was altered following diagnosis with additional molecular analysis. Subsequently, systematic assessment of treatment effectiveness was not required. Established methods for the management of PMF are outlined below.

Evidence-based guidelines for the management of primary myelofibrosis are unavailable. Experts recommend surveillance for low-risk asymptomatic patients with PMF. These patients should be monitored every 3–6 months for signs of progression or transformation (Arana-Yi et al 2006). Hydroxyurea is used for patients with splenomegaly or cellular proliferation, and androgens or corticosteroids are recommended for symptomatic anaemia (Arana-Yi et al 2006; Tefferi 2008). Patients with more than one risk factor for a poor prognosis may undergo allogeneic stem cell transplant; however, this would not be considered common in clinical practice in Australia.

Myeloproliferative disorders

A number of studies that reported analysis of *JAK2* mutations did not report the diagnostic accuracy or yield of PV, ET or PMF specifically; rather, they reported the yield of MPD diagnoses. The following section describes the studies that reported on the use of molecular testing to diagnose both overt and latent forms of MPD. All but one study provided low-levels of evidence and reported diagnostic yields between 0% and 53%, depending on the population and specific mutation analysed.

Is it accurate?

JAK2 exon 14 mutations

Diagnostic yield of overt MPD was reported in three studies of patients presenting with venous thromboses of the splanchnic veins (Table 17). Diagnosis in these studies relied on the addition of molecular analysis, in particular *JAK2* V617F analysis, to laboratory and clinical information.

The medium-quality study described by Kondo et al (2008) (level III-2 diagnostic evidence) reported the diagnostic accuracy of the 2008 WHO criteria for the diagnosis of MPD, compared with the preceding 2001 WHO criteria (Table 16). The reported sensitivity of the 2008 WHO criteria was very high, at 100% [95% CI: 93.7, 100.0], but the specificity was lower, at 89%, with greater uncertainty surrounding the point estimate [95%CI: 65.3, 98.6]. This uncertainty can be attributed to the high prevalence of disease, and the subsequent low number of MPD-negative subjects, in this study. Additionally, this study had the potential for selection bias to overestimate the positive predictive values as a proportion of the subjects had previously been diagnosed with MPD. This is likely to have inflated the prevalence of disease and have a direct impact on the positive predictive value, although the extent is unknown.

Table 16 Diagnostic accuracy of molecular testing in the diagnosis of myeloproliferative disease

Study	Study quality[a]	Population and comparator	Molecular test technique and sample type	Diagnostic test accuracy				
				Prevalence of MPD in study	Sn [95% CI]	Sp [95% CI]	PPV [95% CI]	NPV [95% CI]
(Kondo et al 2008)	Level III-2 diagnostic evidence C1 P2 Q3	75 patients with or suspected of MPD ***Comparator*** 2001 WHO criteria for diagnosis of MPD	PCR followed by sequencing (sensitivity = 20% of homozygous HEL cell line) PB (n=73) BM (n=2)	76%	100% [93.7, 100.0]	89% [65.3, 98.6]	97% [88.3, 99.6]	100% [79.4, 100.0]

[a] A description of study quality characteristics is provided in Table 7 and Table 8; MPD = myeloproliferative disease; PCR = polymerase chain reaction; BM = bone marrow; PB = peripheral blood; Sn = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; WHO = World Health Organization; CI = confidence interval; HEL = human erthroleukaemic

Reported diagnostic yield of MPD varied substantially between studies (from 11% to 44%). This variation is difficult to explain based on the information provided in the published reports. All studies had relatively unselected populations and included patients with provoking circumstances such as surgery, oral contraception and trauma. In addition, the studies had been conducted in tertiary thrombosis centres, indicating that the baseline risk of patients was likely to be similar. However, the differences in diagnostic criteria may be sufficient to explain the variation in diagnostic yield.

The results of the studies were also inconsistent based on the site of thrombosis, as the medium-quality study by Kiladjian et al (2008) reported a higher diagnostic yield (37% vs 14%) for portal vein thrombosis than the poor-quality study by De Stefano et al (2007). Although the yields reported in patients with Budd-Chiari syndrome were not vastly different, there was overlap of patients between these studies, which may explain their consistency (Kiladjian et al 2008; Smalberg et al 2006).

Table 17 Diagnostic yield of molecular testing for *JAK2* exon 14 mutations in the diagnosis of myeloproliferative disease

Study	Study quality[a]	Population	Diagnostic criteria used for MPD	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield of MPD
(Kiladjian et al 2008) *Likely overlap of patients with Smalberg et al (2006)*	Level IV diagnostic evidence CX P2 Q2	241 patients with BCS (n=104) or PVT (n=137)	Not reported Diagnosis was based on BM biopsy findings and molecular testing	SNP genotyping of *JAK2* exon 14 by real-time PCR Archival or fresh PB	0.5–1% of HEL cell line DNA in non-mutated DNA 2–4% of a homozygous patient's DNA in normal DNA	Overall = 44% BCS = 53% PVT = 37%
(Smalberg et al 2006) *Likely overlap of patients with Kiladjian et al (2008)*	Level IV diagnostic evidence CX P2 Q2	29 patients with BCS	2001 WHO criteria ± molecular testing for *JAK2* V617F	PCR Not reported	Not reported	BCS = 11/29 (38%)[b]
(De Stefano et al 2007a)	Level IV diagnostic evidence CX P2 Q3	76 patients with MVT or PVT	Conventional criteria[c] plus molecular testing for *JAK2* V617F	Not reported	Not reported	Overall = 11% MVT = 0/17 (0%) PVT = 8/59 (14%)[d]

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] These patients were also analysed for the presence of *JAK2* exon 12 and *MPL* mutations see Table 18 and Table 19 respectively; [c] A description of conventional criteria was not provided in the article; [d] The diagnostic yield for latent MPD is reported in Table 20; BCS = Budd-Chiari syndrome; PVT = portal vein thrombosis; SNP = single nucleotide polymorphism; PCR = polymerase chain reaction; PB = peripheral blood; MPD = myeloproliferative disease; DNA = deoxyribonucleic acid; HEL = human erythroleukaemic; MVT = mesenteric vein thrombosis; PVT = portal vein thrombosis; SVT = splanchnic vein thrombosis; AS-qPCR = allele-specific quantitative PCR; WHO = World Health Organization

JAK2 exon 12 mutations

Two studies reported on the use of *JAK2* exon 12 mutation analysis in the diagnosis of MPD (Table 18).

The medium-quality study by Kiladjian et al (2008) (level IV diagnostic evidence) analysed the *JAK2* gene for the presence of exon 12 mutations. No mutations were detected in the 123 patients with Budd-Chiari syndrome who had previously tested negative for the *JAK2* V617F mutation.

Bergamaschi et al (2008) reported a poor-quality study (level IV diagnostic evidence) that was prone to selection bias due to the inclusion of two subjects who had previously been diagnosed with ET based on full blood examination and BM biopsy. These two patients were also positive for the *MPL* W515K mutation.

Table 18 Diagnostic yield of molecular analysis of *JAK2* exon 12 in the diagnosis of myeloproliferative disease

Study	Study quality[a]	Population	Criteria used for diagnosis of MPD	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield of MPD
(Kiladjian et al 2008)	Level IV diagnostic evidence CX P2 Q2	123 patients with BCS (n=104) or PVT (n=137) and who were *JAK2* V617F—ve	Not reported Diagnosis was based on BM biopsy findings and molecular testing	Direct sequencing of *JAK2* exon 12 Archival or fresh PB	Not reported	Overall = 0/123 (0%)[b]
(Bergamaschi et al 2008)	Level IV diagnostic evidence CX P2 Q3	93 patients with SVT	Not reported	Direct sequencing PB	Not reported	3/93 (3%)[c]

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] This study also reported the diagnostic yield of *JAK2* exon 14 and *MPL* analysis in the diagnosis of MPD (see Table 17 and Table 19 respectively); [c] The patients in this study were also reported in the study by Primignani et al (2006) (Table 20); PB = peripheral blood; PVT = portal vein thrombosis; SVT = splanchnic vein thrombosis; BCS = Budd-Chiari syndrome

MPL mutations

A single study reported MPD diagnosis following molecular analysis of *MPL* mutations in patients suspicious of MPD. The medium-quality study provided low-level evidence (level IV diagnostic evidence) and reported a diagnostic yield of 0% in 212 patients presenting with Budd-Chiari syndrome or portal vein thromboses and who were analysed for the presence of *MPL* mutations (Kiladjian et al 2008).

Table 19 Diagnostic yield of molecular testing for *MPL* mutations in the diagnosis of MPD

Study	Study quality[a]	Population	Diagnostic criteria used for MPD	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield of MPD
(Kiladjian et al 2008)	Level IV diagnostic evidence CX P2 Q2	212 patients with BCS (n=104) or PVT (n=137)	Not reported Diagnosis was based on BM biopsy findings and molecular testing	Direct sequencing of *MPL* exon 10 Archival or fresh PB	5–10%	Overall = 0% (n=212)[b]

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] Results of *JAK2* exon 12 and 14 analysis were also reported (see Table 19 and Table 17 respectively); BCS = Budd-Chiari syndrome; PVT = portal vein thrombosis; BM = bone marrow; PB = peripheral blood

Latent MPD

If the WHO or PVSG criteria for the diagnosis of an MPD are not met, the presence of the *JAK2* V617F mutation may provide a diagnosis of latent or occult MPD. Data from studies that reported on the diagnosis of latent MPD in patients presenting with venous thromboses are provided in Table 20.

It should be noted that, in some of these studies, a proportion of patients were known to already have MPD prior to presenting with venous thromboses. Those patients were therefore not included in the denominator for diagnostic yield. However, if the patients were not known to have MPD when presenting with venous thromboses, but were subsequently diagnosed with overt MPD at the same time as others were diagnosed with latent MPD, they were included in the denominator (but not the numerator).

Twelve studies reported the diagnosis of latent MPD after molecular analysis of *JAK2* exon 14. These studies all provided low-level evidence (level IV diagnostic evidence) that ranged from good to poor quality. Diagnostic yield varied from 2% in patients investigated for hypercoagulability (Abel et al 2008) to 74% in patients with hepatic or portal vein thromboses (Goulding et al 2008).

Goulding et al (2008) reported a high diagnostic yield of latent MPD in a good-quality study. This is likely to be due to the restrictive inclusion criteria for this study. The majority of other studies determined the yield of latent MPD as part of, or after, a thrombophilia investigation. Goulding et al determined the yield of latent MPD only in patients for whom a thrombophilic condition had been ruled out, therefore potentially increasing the pre-test probability of the patients. Interestingly, in this study the follow-up of patients showed that 13 of 14 (93%) patients who were diagnosed with latent MPD went on to develop overt MPD after a median of 38 months (range 2–96). None of the five patients who were *JAK2* V617F-negative developed overt MPD after a median of 39 months (range 9–60).

Patient selection may also be the cause of the high diagnostic yield seen in the medium-quality study by Patel et al (2006), where only patients with idiopathic Budd-Chiari syndrome were included. Again, this is likely to increase the pre-test probability of the patients and subsequently increase the diagnostic yield of latent MPD.

Table 20 Diagnostic yield of molecular testing for the diagnosis of latent MPD

Study	Study quality[a]	Population	Diagnostic criteria used for MPD	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield of MPD
(Goulding et al 2008)	Level IV diagnostic evidence CX P1 Q1	19 patients with HVT or PVT	Not reported	AS-PCR and RFLP performed independently PB	Not reported	14/19 = 74%
(Regina et al 2007)	Level IV diagnostic evidence CX P1 Q2	88 patients with idiopathic SVT or DVT of the lower limb PVT (n=42) HVT (n=2) Spontaneous DVT (n=44)	Not reported	AS-PCR and RFLP PB	Not reported	Overall = 9% SVT = 8/44 (18%) DVT = 0/44 (0%)
(Patel et al 2006)	Level IV diagnostic evidence CX P1 Q2	41 patients with idiopathic BCS	2001 WHO criteria	AS-PCR followed by sequencing in *JAK2* V617F-+ve samples Archival BM = 35 Fresh PB = 6	Not reported	24/41 (59%)
(Abel et al 2008)	Level IV diagnostic evidence CX P2 Q2	66 patients investigated for hypercoagulability	Not reported	AS-PCR PB	> 5% mutated alleles	Overall = 1/66 (2% [95% CI 1.4, 4.5]) CVA/stroke = 0/10 DVT/PE = 0/45 SVT = 1/7 (14%) Other arterial thromboses = 0/4
(Bayraktar et al 2008)	Level IV diagnostic evidence CX P2 Q2	25 chronic non-cirrhotic PVT patients	2001 WHO criteria	AS-PCR PB	Not reported	PVT = 5/19 (26%)[b]
(Colaizzo et al 2007)	Level IV diagnostic evidence CX P2 Q2	99 consecutive patients with PMVT	2001 WHO criteria	RFLP PB	Not reported	PMVT = 10/92 (11%)[c]
(McMahon et al 2007)	Level IV diagnostic evidence CX P2 Q2	42 patients with catastrophic IAT 3/42 patients were previously known or suspected of having MPD[d]	Not reported	AS-PCR PB	Not reported	IAT = 4/39 (10%)

(Smalberg et al 2006)	Level IV diagnostic evidence CX P2 Q2	29 patients with BCS	2001 WHO criteria ± molecular testing for *JAK2* V617F	PCR Not reported	Not reported	BCS = 2/29 (7%)[e]
(Colaizzo et al 2008)	Level IV diagnostic evidence CX P2 Q3	33 patients presenting with BCS, 8 of which were previously diagnosed with MPD	2001 WHO criteria	RFLP PB	Not reported	BCS = 3/25 (12%)[f]
(De Stefano et al 2007a)	Level IV diagnostic evidence CX P2 Q3	76 patients with MVT (n=17) or PVT (n=59)	Conventional criteria[g] plus molecular testing for *JAK2* V617F	Not reported	Not reported	PVT = 16/59 (27%)[e]
(De Stefano et al 2007b)	Level IV diagnostic evidence CX P2 Q2	139 patients with venous thromboses 19/139 patients were known to have overt MPD[f]	Updated PVSG criteria	AS-PCR	Not reported	Overall = 17/79 (22%)[h] HVT = 1/11 (9%) PVT = 16/50 (32%) MVT = 0/16 (0%) SVT = 0/2 (0%) CVT = 2/41 (5%)
(Boissinot et al 2006)	Level IV diagnostic evidence CX P2 Q3	49 patients with SVT with low or normal Hct or platelet counts	Not reported Diagnosis of latent MPD required presence of *JAK2* V617F and/or EEC/EMC	AS-qPCR	Not reported	SVT = 17/49 (35%)
(Primignani et al 2006)	Level IV diagnostic evidence CX P2 Q3	93 consecutive patients with BCS (n=20) or EHPVO (n=73)	Not reported	AS-PCR with positive samples confirmed by RFLP PB	5-10%	Overall = 34/93 (37%) EHPVO = 26/73 (36%) BCS = 8/20 (40%)

[a] A description of study quality characteristics is provided in Table 7 and Table 8; [b] 6 patients were already diagnosed with MPD and therefore can not be included in the denominator; [c] 7 patients were already known to have MPD and therefore can not be included in the denominator; [d] Patients with overt MPD have not been included in the denominator for the calculation of diagnostic yield; [e] Diagnostic yield of overt MPD are reported in Table 19; [f] 8 patients were already diagnosed with MPD and therefore can not be included in the denominator; [g] A description of conventional criteria was not provided in the article; [h] excluding CVT; BCS = Budd-Chiari syndrome; MPD = myeloproliferative disease; PB = peripheral blood; RFLP = restriction fragment length polymorphism; EHPVO = extrahepatic portal vein obstruction; SVT = splanchnic vein thromboses (PVT plus HVT); IAT = intra-abdominal thromboses; PVSG = Polycythaemia Vera Study Group; EEC/EMC = endogenous erythrocyte/megakaryocyte colonies; PMVT = portal and mesenteric vein thrombosis; CVT = cerebral vein thrombosis; BM = bone marrow; HVT = hepatic vein thrombosis; PVT = portal vein thrombosis; DVT = deep vein thrombosis; AS-PCR = allele-specific polymerase chain reaction; AS-qPCR = allele-specific quantitative polymerase chain reaction; CVA = cerebrovascular accident; Hct = haematocrit; WHO = World Health Organization

Does it change patient management?

No studies were identified that reported on change in management for patients diagnosed with (latent) MPD following diagnosis with molecular analysis of *JAK2* mutations.

Does change in management improve patient outcomes?

As no evidence was available that reported change in management outcomes for patients diagnosed with MPD using molecular methods, an assessment of treatment effectiveness was not conducted. Established methods of management of patients with PV, ET or PMF have been discussed in the prior sections.

Summary of effectiveness

No studies were identified that provided direct evidence of a change in patient health outcomes as a result of molecular testing in the diagnosis of PV, ET and PMF.

A linked evidence approach was incorporated into the assessment, which considered the diagnostic accuracy, change in management and change in patient outcomes following a change in management. This approach was considered for each of the specific indications of PV, ET, PMF, and for MPD in general.

PV

Three studies reported the diagnostic yield of the addition of exon 14 molecular analysis to the diagnostic strategy for the diagnosis of PV (level IV diagnostic evidence). Substantial variation in the diagnostic yield is apparent between these three studies and is possibly due to different referral patterns and variation in patient population. The reported diagnostic yields ranged from 9% to 43% across the three studies (Ammatuna et al 2007; Girodon et al 2007; Rumi et al 2009).

Substantial heterogeneity was reported with regard to the diagnostic accuracy of *JAK2* exon 12 analysis in the diagnosis of PV. Three diagnostic case-control studies were identified that reported sensitivity ranging from 0% to 83% and specificity from 73% to 100%. All three studies had small numbers of patients, which may have directly contributed to such variation. The positive and negative predictive values were also associated with marked variation, ranging from 0% to 100%, and 50% to 86% respectively. It is difficult to give much weight to these results with such a lack of consistency across studies. Furthermore, the imperfect nature of the reference standard and the low prevalence of exon 12 mutations in patients with PV are likely to result in poor test accuracy due to the potential for misclassification by the reference standard and because the absence of the mutation is unable to rule out disease.

No studies were identified that reported change in management in patients suspected of having PV diagnosed with the use of molecular analysis.

ET

Diagnosis of ET with the use of molecular methods was reported in only one poor-quality study, which provided low-level evidence. This study reported the diagnostic yield of ET in a population suspected of MPD (Ammatuna et al 2007). Using molecular analysis of *JAK2* exon 14, the diagnostic yield of ET was 65% in patients suspected of MPD. Little information was provided regarding the selection criteria of patients in the study, and therefore it is uncertain whether selection bias may have had an impact on these results.

No studies were identified that reported change in management of patients diagnosed with ET

using molecular methods.

PMF

Again, one poor-quality study was identified that reported the diagnostic yield of PMF in patients suspected of MPD. This study (level IV diagnostic evidence) reported a diagnosis of PMF in 8% of patients who presented with suspected MPD.

No studies were identified that reported a change in management for patients following diagnosis of PMF with the use of molecular methods.

MPDs

Only one study (level III-2 diagnostic evidence) compared the diagnostic accuracy of *JAK2* exon 14 molecular testing, in addition to all available clinical and laboratory information, with the reference standard of all relevant clinical and laboratory information. In this case the reference standard was the preceding 2001 WHO criteria for the diagnosis of PV.

The sensitivity and specificity of this diagnostic strategy, which included molecular testing, were 100% [95% CI: 93.7, 100.0] and 89% [95%CI: 65.3, 98.6] respectively (Kondo et al 2008). The poorer specificity is likely to be attributable to the imperfect reference standard, which is unable to diagnose disease on the basis of molecular status. The positive and negative predictive values were 97% [95% CI: 88.3, 99.6] and 100% [95%CI: 79.4, 100.0] respectively. These results should be considered with some caution as there is evidence of selection bias relating to the inclusion of subjects who were known to have MPD, although the extent of this bias is unknown.

Reporting of the diagnosis of MPD also occurred in studies of patients presenting with venous thromboses. These studies used molecular testing with or without other clinical and laboratory information to diagnose overt or latent MPD respectively.

Diagnostic yield of overt MPD was reported in five studies, of which one reported the diagnostic yield following testing for *JAK2* exon 14, *JAK2* exon 12 and *MPL* mutations. In this medium-quality study by Kiladjian et al (2008) (level IV diagnostic evidence), molecular testing for *JAK2* exon 14 mutations yielded an MPD diagnosis in 44% of patients with Budd-Chiari syndrome or portal vein thromboses. Subsequent molecular testing of *JAK2* exon 12 and *MPL* mutations did not provide any additional MPD diagnoses.

From the three studies (level IV diagnostic evidence) that reported overt MPD diagnoses after *JAK2* exon 14 analysis, the diagnostic yields ranged from 11% to 44%. This variation may be explained by differences in the molecular analytical methodology and the populations analysed.

Diagnosis of latent MPD was reported in 12 studies of diagnostic yield (level IV diagnostic evidence) with yields ranging from 2% to 74%. Again, this variation is most likely explained by the different testing methodologies and populations analysed. The lowest yield was in a medium-quality study by Abel et al (2008), which reported a diagnostic yield of 2% in a population of patients presenting with hypercoagulability that included patients with stroke, and deep vein and other arterial thromboses. The highest yield of 74% was reported in the good-quality study by Goulding et al (2008) in a population of patients with hepatic or portal vein

thromboses.

To summarise, the vast majority of studies included in the assessment of molecular testing in the diagnosis of PV, ET and PMF provided low-level evidence. Of these 23 low-level studies, only one was of good quality, with many results likely to have been influenced by selection bias. Without comparison to the reference standard or comparator test strategies, the value of the information provided by level IV diagnostic evidence is very limited.

Minimal evidence was identified that was able to inform of the diagnostic accuracy of molecular testing in the diagnosis of PV, ET and PMF. Only one study reported on diagnostic outcomes for ET and PMF. This medium-quality study reported diagnostic accuracy outcomes of the 2008 WHO diagnostic criteria, compared with the 2001 WHO diagnostic criteria, in patients suspicious of PV. This study is limited, however, by a potentially inferior reference standard—because it does not incorporate molecular status, there is a real possibility that patients with PV may be misdiagnosed by the reference standard. This explains the poorer diagnostic accuracy of the intervention, particularly when analysing *JAK2* exon 12 mutations.

Other relevant considerations

In addition to the relative safety and effectiveness of molecular testing, there are other issues not identified by the systematic review that may impact on the assessment and the decision to support public funding.

World Health Organization classification of myeloproliferative disorders

The diagnosis of ET and PMF has previously been somewhat problematic due to the difficulty in excluding reactive causes, and in differentiating between PV and ET, and PV and PMF, on BM histology. Therefore, the addition of molecular testing to the diagnostic algorithm is likely to significantly simplify diagnosis. The presence of a *JAK2* or *MPL* mutation would provide evidence of the presence of an MPD, which avoids the requirement for further tests to rule out reactive or secondary causes.

Prior to the 2008 WHO classification of MPDs, diagnosis relied heavily on excluding other causes of myeloid proliferation. The rapid uptake by clinicians and laboratories to incorporate molecular testing, particularly *JAK2* V617F testing, into the evaluation of patients suspicious of PV, ET and PMF indicates that physicians appreciate the utility of this test. Additionally, for those patients who carry the mutations, the ability to provide a specific and certain diagnosis with relative ease may provide considerable satisfaction for both patient and doctor.

A recently published study by Girodon et al (2009) reported the impact of the 2008 WHO diagnostic criteria in a population of ET patients in France. Using a well-established registry of haematological malignancies and retrospective *JAK2* V617F and exon 12 testing of samples in 2005, the investigators compared the incidence of ET in 2005–07 with the period 1980–2004 (when diagnosis was first made according to the PVSG criteria), and then after 2000 according to the 2001 WHO criteria. The incidence of ET in 2005–07 (when the 2008 WHO criteria were applied) was 3.0 per 100,000 population per year, which was more than twice the incidence in the same population using the 2001 WHO criteria prior to 2005—an incidence of 1.2 per 100,000 population per year. When the period after 2005 is analysed according to the 2001 WHO criteria, there is no statistical difference in the incidence of ET compared with the period before 2005 (Girodon et al 2009). The authors attribute this difference to two factors—the decreased threshold of platelets at which ET would be suspected, and the use of *JAK2* mutation analysis. The authors note that, at onset of *JAK2* analysis in the region, a number of patients with idiopathic thrombocytosis were tested, which would have contributed to an artificial increase in incidence (Girodon et al 2009). The mean platelet count in patients diagnosed after 2005 was clinically significantly lower than in patients diagnosed before 2005, indicating that the new diagnostic criteria may be leading to patients being diagnosed earlier or that the lower threshold allows more people to be diagnosed (Girodon et al 2009).

Quality assurance and molecular methodologies

The Australian Genetic Testing Survey (2006) reported that, of eight laboratories performing *JAK2* mutation testing, three were not accredited by the National Association of Testing Authorities (NATA) for that specific test. Non-accreditation in this instance referred to: a laboratory having NATA accreditation but with a scope that did not include *JAK2* testing; a laboratory with no NATA accreditation; or a laboratory with

NATA accreditation in genetics but the *JAK2* test had yet to be validated (Royal College of Pathologists of Australasia 2008).

Previously, there has not been a quality assurance program to monitor the performance of laboratories providing molecular testing for *JAK2*. However, in 2008 the Royal College of Pathologists of Australia (RCPA) Quality Assurance Program (QAP) has included *JAK2* testing into its molecular diagnostics program (RCPA Quality Assurance Programs Pty Ltd 2009). As there are no commercially available diagnostic kits for the detection of *JAK2* or *MPL* mutations, a number of different methodologies are employed to analyse for their presence. This lack of standardisation has resulted in methodologies being developed and validated in house, that is within each individual laboratory. Additionally, development of new methodologies and technologies to detect molecular mutations are possible. Each different methodology will be associated with its own specific analytical sensitivity and specificity. The initiation of the RCPA QAP will enable monitoring of assay performance within and between laboratories to ensure that the performance of assays and laboratories is optimal.

Access and equity of tests and procedures

In determining the recommendation for a health technology to receive public funding, the MSAC also considers access and equity of provision of the intervention.

Expert opinion and experience has suggested that a number of patients who would be suitable for molecular testing based on their clinical features would not currently undergo such analysis due to the cost of the investigation. If public funding for molecular analysis was supported, a significant proportion of these patients would be able to receive this investigation.

In addition, access to BM biopsy can be limited in some regions of Australia, particularly those areas outside of metropolitan centres. If molecular testing were to replace the need for a proportion of BM biopsies, fewer patients would need to attend higher level facilities to undergo BM biopsy. As molecular testing can be performed on peripheral blood samples, patients would only be required to attend a sample collection facility, allowing greater access for those in rural and regional areas.

Targeted therapies

The success of imatinib mesylate in the treatment of *BCR-ABL*-positive chronic myeloid leukaemia (CML) has demonstrated that selective therapies targeted at tyrosine kinases are achievable, and are comparatively safer and more effective than antineoplastic drugs (Ghoreschi et al 2009).

The potential for a safe and effective protein tyrosine kinase inhibitor targeted to the *JAK2* V617F mutation is yet to be fully realised and would benefit only those patients with the mutation. There are a number of clinical trials that are currently assessing the safety and effectiveness of tyrosine kinase inhibitors including molecules specifically targeted to *JAK2*, although not specifically the V617F mutation, which would benefit all patients with MPD (Ghoreschi et al 2009; U.S. National Institutes of Health 2009). These studies have not assessed patient health outcomes specifically but have looked at the impact of treatment on outcomes such as spleen size and quality of life.

Prognosis and monitoring

The role of *JAK2* mutation analysis in the prognosis of PV, ET or PMF is yet to be fully determined, but it is thought that these mutations may play a role in disease progression and course (Goulding et al 2008; Passamonti 2009). However, at this time *JAK2* mutation status is not used for prognostic purposes in Australia.

According to the expert opinion of the Advisory Panel, monitoring of disease by the assessment of allele burden is also not currently in use in Australian clinical practice. The relevance of allele burden is still to be defined but it is thought that it may have an impact on clinical disease (Passamonti & Rumi 2009). Should the potential for targeted therapy be realised, monitoring of allele burden is likely to also be incorporated into clinical practice for those patients known to carry the relevant mutations.

Referral and ordering

Due to the low prevalence of MPDs and the difficulty in diagnosis, the expert opinion of the Advisory Panel recommends that investigation and diagnosis are carried out by physicians (eg haematologists) who have undergone extensive training in the diagnosis and treatment of haematological disorders. As such, the ordering of molecular tests to determine the mutation status of MPDs should ideally be undertaken by haematologists. Should general practitioners order molecular testing for patients suspected of MPD, it would be recommended that this be done in consultation with a haematologist. Ideally, patients would be referred on to a haematologist.

Consumer perspective

The advantages of molecular testing for MPD from a consumer perspective are the potential avoidance of BM biopsy and the obtainment of a definitive diagnosis. The potential to avoid BM biopsy is appealing for a number of reasons, including averting potential pain and discomfort from the procedure, and avoiding issues of access to the procedure, particularly for those patients who reside in regional areas. Opportunity costs for avoiding the need to undergo BM biopsy include those associated with time off work and the need for a carer after the procedure.

As has been noted before, there can be difficulties in diagnosing MPD; molecular status would enable increased diagnostic certainty for those patients with or without a mutation present. The simplification of the diagnostic algorithm is likely to reduce anxiety associated with the investigation of a potential haematological malignancy. Again, it should be noted that the absence of relevant molecular mutations would not necessarily rule out disease.

Molecular and genetic testing is associated with a number of ethical considerations (Bradley 2005). However, with the mutations associated with MPD being of a somatic nature, consumers should be reassured that this information would not be used in a predictive sense except, it might be argued, in the case of diagnosing latent MPD. However, even in this case the arguments may be blurred as patients could be considered to be symptomatic due to the presence of thrombotic complications despite not meeting accepted criteria for diagnosis.

What are the economic considerations?

The purpose of economic evaluation is to assist decision-makers in ensuring that society's ultimately scarce resources are allocated to those activities from which we will get the most value. That is, it seeks to enhance economic efficiency. To determine whether further economic evaluation is required, the comparative safety and effectiveness of the intervention must first be determined.

The decision of whether to perform an economic evaluation has been based on evidence of relative safety and effectiveness compared with the comparator. If the evidence indicates that the intervention is likely to be no worse in terms of safety and effectiveness outcomes, an economic evaluation should be considered. The type of economic evaluation is determined by the net benefits and harms associated with the intervention relative to the comparator (Table 21).

Table 21 Type of economic evaluation that should be presented for various classifications of a service under MSAC consideration

Classification	Type of economic evaluation
The service is more effective than the appropriate comparator and is associated with improved safety	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
The service is more effective than the appropriate comparator and is no worse than the comparator in terms of safety	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
The service is more effective than the appropriate comparator but is associated with reduced safety:	
(i) Overall, there are net benefits to patients as the benefits from improved effectiveness outweigh the harms from reduced safety and/or changed risk profile	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
(ii) Overall, the service is no worse than the comparator because the benefits from improved effectiveness at least offset the harms from reduced safety and/or changed risk profile	Cost–consequences, cost-effectiveness. (which may be reducible to cost-minimisation, ie presentation of an incremental cost-effectiveness for the base case may be inappropriate if net clinical benefits are assumed to be zero)
(iii) Overall, there are net harms to patients as the harms from reduced safety and/or changed risk profile outweigh the benefits from improved effectiveness	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service
The service is no worse than the comparator in terms of effectiveness but is associated with improved safety	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
The service is indisputably demonstrated to be no worse than the comparator in terms of both effectiveness and safety	Cost-minimisation. In the case where there is any uncertainty around the conclusion that the service is no worse than the comparator in terms of effectiveness and safety, cost–consequences, cost-effectiveness and/or cost–utility analyses should be provided
The service is no worse than the comparator in terms of effectiveness but is associated with reduced safety.	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service.
The service is less effective than the comparator but is associated with improved safety:	
(i) Overall, there are net benefits to patients as the benefits from improved safety and/or changed risk profile outweigh the harms from reduced effectiveness	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
(ii) Overall, the proposed service is no worse than the comparator because the benefits from improved safety at least offset the harms from reduced effectiveness and/or changed risk profile	Cost–consequences, cost-effectiveness (which may be reducible to cost-minimisation, ie presentation of an incremental cost-effectiveness for the base case may be inappropriate if net clinical benefits are assumed to be zero)
(iii) Overall, there are net harms to patients as the harms from reduced effectiveness outweigh the benefits from improved safety and/or changed risk profile	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service
The proposed service is less effective than the comparator and is no worse than the comparator in terms of safety	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service
The proposed service is less effective than the comparator and is associated with reduced safety compared with the comparator	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service

PV

With regard to the diagnosis of PV, no evidence was identified that reported the safety of molecular testing for relevant mutations plus serum erythropoietin levels either alone or compared with normal diagnosis using serum erythropoietin levels plus BM biopsy. Despite this, it can be assumed that the use of molecular testing that requires the taking of blood is likely to be no worse in terms of safety compared with the comparator investigations. In fact, it may be argued that the avoidance of BM biopsy would provide

the intervention with improved safety outcomes for patients. However, no evidence in this population was identified in order to support these outcomes.

Due to the imperfect nature of the reference standard, direct evidence of a change in patient health outcomes is required to establish the comparative effectiveness of the addition of molecular testing to the diagnostic strategy for PV. Lack of such evidence makes it difficult to draw conclusions regarding the effectiveness of molecular testing in the diagnosis of PV.

With a paucity of evidence available, it is inappropriate to proceed with an economic evaluation; hence, a cost comparison of diagnostic strategies for the diagnosis of PV is provided.

ET

As in the case of PV, no evidence was available to determine the safety of molecular testing in the diagnostic strategy for ET. However, expert opinion indicates that a proportion of patients investigated for ET would no longer be required to undergo BM biopsy. It would therefore be appropriate to assume that there would be an overall benefit in terms of safety for patients being investigated for ET.

With regard to effectiveness, the benefit of molecular testing is uncertain. No comparative evidence is available regarding the diagnostic accuracy of molecular testing in the diagnosis of ET. Logically, with the *JAK2* V617F mutation present in only 50% of patients with ET, the benefit of molecular testing would be apparent only in those patients with the mutation, as its absence would not rule out disease. For those patients who carry the mutation, the imperfect reference standard may not be adequate to accurately determine those with disease and those without. Therefore, direct evidence of an improvement in patient outcomes is required to establish the comparative effectiveness of molecular testing in the diagnostic strategy of ET. As no such evidence is available, it would be inappropriate to proceed with an economic evaluation. Consequently, a cost comparison of diagnosis of ET with the intervention and comparator has been conducted.

PMF

With no evidence regarding the safety of molecular testing in the diagnosis of PMF, it is not possible to determine the comparative safety of this diagnostic strategy. However, as the diagnostic algorithm is unlikely to change with the introduction of molecular testing, patients will still require a BM biopsy; and therefore, it may be argued that diagnosis with molecular testing is likely to be as safe as the comparator.

The effectiveness of molecular testing in the diagnosis of PMF has been informed by a single poor-quality study of diagnostic yield. Without comparative evidence, it is not possible to determine the effectiveness of the addition of molecular testing to the diagnostic strategy for PMF relative to the comparator; subsequently, an economic evaluation could not be conducted.

As in the case of ET, with the benefit of molecular testing uncertain due to the prevalence of the mutation in patients with PMF being 50% and an imperfect reference standard, it would be inappropriate to proceed with an economic evaluation without direct evidence of benefit to patient outcomes. Consequently, a cost comparison has

been provided to determine the relative costs of the addition of molecular testing to the diagnostic strategy for PMF.

Comparator

The comparator for this assessment is all available clinical and laboratory information for the diagnosis of PV, ET or PMF. For the purposes of determining the financial implications of diagnosing PV, it will be assumed that an investigation of a patient suspicious of PV by the comparator will require analysis of serum erythropoietin levels and histological examination of a BM aspirate and trephine biopsy. Use of red cell mass studies is likely to depend on whether serum erythropoietin levels and BM biopsy results are conflicting, as well as the availability of red cell mass studies. The majority of patients will also require abdominal ultrasound to assess the spleen.

The comparator for ET and PMF will also include BM biopsy.

Financial analysis

PV

Likely number of investigations per year

As indicated earlier in this report, the incidence of PV in Australia in 2003 was 1.6 cases per 100,000 population (AIHW & AACR 2007). A survey conducted on behalf of the Royal College of Pathologists of Australia (2008) reported that the number of *JAK2* V617F tests conducted in the surveyed laboratories was 700 in 2006. The expert opinion of the Advisory Panel suggests that this is an underestimate and that the actual number of tests would differ substantially from this survey. For the purposes of this analysis, 1,500 patients with suspected PV per year will be considered as the base case in Australia. Assuming that the number of investigations for this indication does not change, it would be estimated that there will be 1,500 investigations performed per year for the investigation of PV. Sensitivity analysis will consider that this estimate may range from 320 to 3,000 investigations per year.

There is the potential for leakage, initially with a proportion of molecular tests being conducted retrospectively. Furthermore, should the potential for targeted therapies be realised, the issue of monitoring disease is likely to have a substantial impact on the ordering of *JAK2* molecular analysis.

Unit costs of index test strategy and the comparator

The index test strategy for PV involves first-line analysis of serum erythropoietin levels and *JAK2* V617F mutation status. According to expert opinion, if there is still a suspicion of PV after first-line testing, patients are likely to undergo BM biopsy as *JAK2* exon 12 analysis is only known to be available in South Australia. It has been assumed that the proportion of patients who would not require BM biopsy is likely to be 90% of patients who test positive for the *JAK2* V617F mutation.

Both index and comparator test strategies will incur costs associated with consultation with specialist physicians. This analysis assumes that the same number of physician consultations will be required regardless of the diagnostic strategy followed, except for follow-up consultations associated with BM biopsy. Consequently, only physician

consultations associated with BM biopsies will be considered in the estimate of the financial implications.

A proportion of patients receiving both the index and comparator test strategies will also require abdominal ultrasound to assess the spleen. The expert opinion of the Advisory Panel indicates that with the addition of molecular testing to the investigation, this proportion is expected to decrease from 80% to 10%.

The comparator includes determination of serum erythropoietin levels, and BM biopsy and red cell mass studies in some patients. As serum erythropoietin levels will be determined in all patients regardless of whether they receive the index or comparator diagnostic strategy, the cost of this item is not needed to inform of the incremental costs in this analysis. As different proportions of patients will receive BM biopsy depending on the diagnostic strategy they receive, this item will be included in the analysis.

In addition, a proportion of patients would also undergo lung function and oxygen saturation tests as part of the comparator test strategy; however, these tests would be avoided by using the index test strategy. It should be noted that, although these items have not been included in this financial analysis (due to uncertainty surrounding the number of patients who would receive these tests), their avoidance would result in further savings to the Australian healthcare system.

Expert opinion indicates that the MBS schedule fees for BM aspirate and trephine biopsy (MBS items 30084 and 30087) do not accurately reflect the true cost of these procedures. As a consequence, this financial analysis has used the cost of AR-DRG R61C as a comparable procedure.

It is likely that a small proportion of patients will receive red cell mass studies; however, this will be dependent on the availability of the assay and on whether results of serum erythropoietin and BM biopsy are conflicting. As such, this analysis assumes that the cost of the comparator will be determined by the cost of BM biopsy for all patients and red cell mass studies in 5% of patients.

Table 22 outlines the incremental costs associated with the index test strategy. Of note in this table is the cost of *JAK2* molecular analysis being the cost associated with a qualitative assay. If a quantitative assay were incorporated into the testing strategy, the maximum cost of diagnosis would increase to $1,627. Also noteworthy is that the cost of a qualitative assay is more than that of a comparable assay listed on the MBS schedule. The expert opinion of the Advisory Panel has indicated that a more accurate cost of a qualitative assay would range from $80 to $100. This analysis will assume that the cost of qualitative *JAK2* V617F analysis will be $90. These estimates do not include the costs of serum erythropoietin determinations as all patients would receive this assay regardless of the test strategy followed. Importantly, these costs include the cost of BM biopsy and examination but, as discussed earlier, not all patients would receive this procedure.

Table 22 Unit costs of items associated with the diagnosis of PV for patients undergoing the index test strategy

Item	Source of estimate	Unit cost ($)[a]
First-line test strategy		
JAK2 V617F molecular analysis	Expert opinion (MBS item 73314)	90 (233)[b]
Abdominal ultrasound	MBS item 55036	111
Second-line test strategy		
Consultation with anaesthetist	MBS item 1761	40
Initiation of anaesthesia/sedation	MBS item 21112	73
Physician consult—follow-up	MBS item 116	70
BM biopsy	AR-DRG R61C Lymphoma/N-A Leukaemia+Sameday Version 5.1 National Hospital Cost Data Collection Cost Report Round 11 (2006-07)[c]	1,006
BM pathology	MBS item 65084	164

[a] Costs are rounded to the nearest dollar; [b] Unit cost of a quantitative molecular assay (MBS item 73314); [c] Source: Department of Health & Ageing; MBS = Medicare Benefits Schedule (Department of Health & Ageing 2009); AR-DRG = Australian Refined – Diagnosis Related Groups; BM = bone marrow

As previously explained, both test strategies use serum erythropoietin levels for diagnosis; consequently, this item does not need to be considered in the financial analysis. Table 23 outlines the costs associated with diagnosis using the comparator test strategy.

Table 23 Unit costs of items associated with the diagnosis of PV for patients undergoing the comparator test strategy

Item	Source of estimate	Unit cost ($)
Abdominal ultrasound	MBS item 55036	111
Consultation with anaesthetist	MBS item 1761	40
Initiation of anaesthesia/sedation	MBS item 21112	73
Physician consult—follow-up	MBS item 116	70
BM biopsy	AR-DRG R61C Lymphoma/N-A Leukaemia+Sameday Version 5.1 National Hospital Cost Data Collection Cost Report Round 11 (2006-07)[a]	1,006
BM pathology	MBS item 65084	164
Red cell mass studies	MBS item 12500	200

[a] Source: Department of Health & Ageing; MBS = Medicare Benefits Schedule (Department of Health & Ageing 2009); AR-DRG = Australian Refined – Diagnosis Related Groups; BM = bone marrow

It should be noted that this financial analysis has not considered the financial implications of BM biopsies that result in a dry tap.

Costs to the Australian healthcare system overall

Total healthcare costs incorporate all direct costs associated with the diagnosis of PV, regardless of the person or agency who incurs them. To determine the costs of the index test strategy to the Australian healthcare system overall, the cost of using both qualitative and quantitative molecular methods has been calculated (Table 24). The minimum cost of the use of molecular testing is estimated at $354,600 for 1,500 patients being investigated for PV, and also includes the cost of 150 patients undergoing BM biopsy and 150 patients who would also undergo abdominal ultrasound. The maximum cost of

investigating these patients using quantitative molecular methods is estimated to be $568,300.

In contrast, assuming that the 75 patients undergoing the comparator test strategy would also undergo red cell mass studies, the estimated cost to the Australian healthcare system overall is $2,178,000. This includes the cost of an additional 1,350 patients who would require BM biopsy and 1,050 patients who would require abdominal ultrasound.

Table 24 Costs to the Australian healthcare system for the diagnosis of PV

Item	Cost of index test strategy with qualitative molecular methods	Cost of index test strategy with quantitative molecular methods	Cost of comparator test strategy (unit cost x number)
JAK2 V617F analysis	$90 x 1,500	$233 x 1,500	n/a
Abdominal ultrasound	$111 x 150	$111 x 150	$111 x 1,200
Anaesthesia[a]	$113 x 150	$113 x 150	$113 x 1,500
Physician consult[b]	$70 x 150	$70 x 150	$70 x 1,500
BM biopsy	$1,006 x 150	$1,006 x 150	$1,006 x 1,500
BM pathology	$164 x 150	$164 x 150	$164 x 1,500
Red cell mass studies	n/a	n/a	$200 x 75
Total	**$354,593**	**$568,343**	**$2,177,535**

[a] Incorporating costs of consultation (MBS item 1761) and anaesthesia/sedation (MBS item 21112); [b] prior to BM biopsy procedure; BM = bone marrow; n/a = not applicable

Therefore, the addition of molecular testing to the diagnostic strategy for PV is likely to provide a cost saving to the Australian healthcare system overall of between $1,609,000 and $1,823,000 per year.

Sensitivity analysis around the likely number of investigations per year would see the cost to the Australian healthcare system overall range from $75,600 to $1,136,700 for the index test strategy (depending on the molecular methodology used) and $465,000 to $4,355,000 for the comparator test strategy (Table 25).

Table 25 Impact of test strategy use on costs to the Australian healthcare system

Number of likely investigations (per year)	Cost of index test strategy with qualitative molecular methods	Cost of index test strategy with quantitative molecular methods	Cost of comparator test strategy	Incremental cost / saving relative to the comparator (range)
320	$75,646	$121,246	$464,541	**$343,300–$388,900**
1,500	$354,593	$568,343	$2,177,535	**$1,609,200–$1,822,900**
3,000	$709,185	$1,136,685	$4,355,070	**$3,218,400–$3,645,900**

Bold type denotes a cost saving relative to the comparator.

Costs to the Medical Benefits Schedule

The Australian Government is responsible for payment of the rebate on items from the MBS. As patients will be investigated for PV as out-patients, the rebate for a private patient on items in the MBS would be 75% of the scheduled fee.

Expert opinion suggests that the publicly funded to MBS-funded split of patients for suspected of PV is 20% to 80%. Therefore, 80% of all patients undergoing investigations for PV would be eligible for MBS reimbursement, with the remaining 20% coming under the Australian Healthcare Agreements between the states/territories and the

Commonwealth. As it has been assumed that 1,500 patients would be investigated for PV per year, approximately 1,200 patients would be eligible for MBS reimbursement. Of these 1,200 patients, 1,080 would avoid BM biopsy under the index test strategy. In contrast, all 1,200 patients undergoing the comparator test strategy would still require BM biopsy and, in addition, 60 patients would also require red cell mass studies. Furthermore, of the 960 patients who would require abdominal ultrasound when investigated with the comparator test strategy, only 120 patients would require this procedure with the index test strategy.

A comparison of the 75% rebate on MBS items that would be eligible is provided in Table 26. As patients would undergo these investigations as out-patients, the Commonwealth would incur the cost of the 75% rebate on these items.

Table 26 Comparison of MBS items for index and comparator test strategies

MBS item	Index test strategy using qualitative molecular methods (75% rebate x number of units)	Index test strategy using quantitative molecular methods (75% rebate x number of units)	Comparator test strategy (75% rebate x number of units)
JAK2 V617F analysis	$28 x 1,200	$176 x 1,200	n/a
Abdominal ultrasound	$84 x 120	$84 x 120	$84 x 960
Consultation with anaesthetist	$30 x 120	$30 x 120	$30 x 1,200
Initiation of anaesthesia/sedation	$55 x 120	$55 x 120	$55 x 1,200
Physician consult[a]	$52 x 120	$52 x 120	$52 x 1,200
BM biopsy	$41 x 120	$41 x 120	$96 x 1,200
BM pathology	$123 x 120	$123 x 120	$123 x 1,200
Red cell mass studies	n/a	n/a	$150 x 60
Total	**$79,746**	**$257,346**	**$450,540**

[a] prior to BM biopsy procedure; BM = bone marrow; n/a = not applicable

The cost to the Commonwealth in terms of the Medicare rebate for 1,200 patients to undergo investigation for PV is estimated to be $80,000 and $257,300 for the index test strategy with quantitative and qualitative molecular methods, respectively, and $450,500 for the comparator test strategy, per year. This will result in a saving to the Commonwealth of $371,000 per year if qualitative *JAK2* V617F analysis is performed and $193,200 per year if quantitative methods were employed.

The impact of uncertainty surrounding the number of investigations per year for PV is described in Table 27. This sensitivity analysis considers the impact of test strategy use ranging from 320 to 3,000 investigations per year.

Table 27 Impact of test strategy use on costs incurred by the MBS

Number of likely investigations (per year)	Cost of index test strategy with qualitative molecular methods	Cost of index test strategy with quantitative molecular methods	Cost of comparator test strategy	Incremental cost / saving relative to the comparator (range)
320	$15,672	$53,560	$82,714	**$41,200–$79,000**
1,500	$73,464	$251,064	$387,720	**$193,200–$370,800**
3,000	$146,928	$502,128	$775,440	**$386,400–$741,600**

MBS = Medicare Benefits Schedule; **bold** type denotes a cost saving relative to the comparator.

Costs to the state/territory health systems

Under the current Australian Healthcare Agreements, the states/territories fund in-patient procedures on public patients in public hospitals, as well as public patients in an out-patient facility. To estimate the costs to the states/territories, two assumptions have been made—that the unit costs of the test strategies are the same for a public patient as they are for a private patient, and that 300 patients would be investigated for PV in the public health system.

Table 28 shows that, for 300 investigations, 30 patients undergoing the index test strategy would also require a BM biopsy and/or abdominal ultrasound. Therefore, the estimate of cost of the index test strategy using qualitative molecular methods is $70,900 to the states/territories; using quantitative molecular methods would increase this cost to $113,700. In contrast, the cost to the states/territories of the comparator test strategy, including the cost of 15 patients undergoing red cell mass studies and 240 patients requiring abdominal ultrasound, is $435,500.

Therefore, the incorporation of molecular testing into the investigation of PV would provide a cost *saving* of between $322,000 and $364,600 per year to the states/territories. This saving is primarily attributed to a large proportion of patients avoiding the need for BM biopsies and abdominal ultrasounds in the index test strategy.

Table 28 Financial implications of investigating PV to the states/territories

Number of likely investigations (per year)	Cost of index test strategy with qualitative molecular methods	Cost of index test strategy with quantitative molecular methods	Cost of comparator test strategy	Incremental cost / saving relative to the comparator (range)
320	$14,683	$23,803	$88,444	**$68,700–$77,800**
1,500	$68,826	$111,576	$414,582	**$321,800–$364,600**
3,000	$137,652	$223,152	$829,164	**$643,700 $729,200**

Bold type denotes a cost saving relative to the comparator.

Table 28 also demonstrates the financial impact of variation in the number of investigations per year. The minimum estimated saving would be between $68,700 and $77,800 per year. The upper estimate of the financial impact of investigating patients with suspected PV shows a cost saving of between $643,700 and $729,200 per year for the states/territories.

ET and PMF

Likely number of investigations per year

There are no specific data available to indicate the likely incidence of ET or PMF in Australia. Expert opinion indicates that the likely number of investigations per year for ET and PMF will be 4,500 and 175 tests respectively.

Again, there is the potential for leakage, initially with a proportion of diagnosed patients being tested for the mutation retrospectively. However, the greatest impact on the number of tests being ordered would be seen with the development of targeted therapies to the mutation and the subsequent need for monitoring.

Sensitivity analysis will be conducted to determine the impact of a change in the number of investigations performed per year. This will consider the number of investigations for

ET ranging from 950 to 9,000 per year. As the incidence of PMF is notably lower than that of ET, sensitivity analysis concerning the number of investigations for this indication will reflect use, increasing up to 2,000 investigations per year.

Unit costs of index test strategy and the comparator

In this financial analysis of the cost implications of ET, it has been assumed that patients who test negative for the *JAK2* V617F mutation in the index test strategy will not be analysed for *MPL* mutations as the availability of this assay in Australia is likely to be extremely limited. Additionally, it has been assumed that 10% of patients who are *JAK2* V617F-positive will also have atypical features that require BM biopsy and clinical judgement to determine the diagnosis. Therefore, under the index test strategy, 3,164 patients would undergo BM biopsy compared with 4,500 patients in the comparator test strategy.

Both index and comparator test strategies will incur costs associated with consultation with specialist physicians. This analysis assumes that the same number of physician consultations will be required regardless of the diagnostic strategy followed. Consequently, these items do not need to be considered in the estimate of the financial implications.

With regard to PMF, it has also been assumed that *MPL* analysis is not readily available in Australia, and therefore patients undergoing the index test strategy will not have access to this information. As patients will undergo BM biopsy regardless of the test strategy they receive, it is not necessary to include this item in this financial analysis. Therefore, the cost implications of the index test strategy for the diagnosis of PMF will consist simply of the cost of *JAK2* V617F mutation analysis.

Table 29 outlines the costs associated with the diagnosis of ET and PMF using the index test strategies and the comparator test strategy for ET. As before, it should be noted that expert opinion indicates that the cost associated with performing BM biopsy is unlikely to be accurately reflected by the schedule fee listed in the MBS; consequently, a comparable diagnosis related group (DRG) has been used in the unit cost comparisons. Furthermore, the schedule fee for the cost of a comparable qualitative molecular testing is considered by the Advisory Panel to inaccurately reflect the true cost associated with this assay. The informed opinion of the Advisory Panel suggests that $90 is a more accurate estimate for this assay.

Table 29 Unit costs of items associated with the diagnosis of ET and PMF

Item	Source of estimate	Index test strategy using quantitative molecular methods for ET and PMF	Index test strategy using qualitative molecular methods for ET and PMF	Comparator test strategy for ET
JAK2 V617F analysis	Expert opinion and MBS item 73314	$90	$233	n/a
Physician consult[a]	MBS item 116	$70[c]	$70[c]	$70[c]
BM biopsy	AR-DRG R61C Lymphoma/N-A Leukaemia+Sameday Version 5.1 National Hospital Cost Data Collection Cost Report Round 11 (2006–07)[b]	$1,006[c]	$1,006[c]	$1,006[c]
BM pathology	MBS item 65084	$164	$164[b]	$164

[a] prior to BM biopsy procedure; [b] Source: Department of Health & Ageing; [c] These items have not been included in the analysis of the financial implications of investigating PMF; MBS = Medicare Benefits Schedule (Department of Health & Ageing 2009); AR-DRG = Australian Refined – Diagnosis Related Groups; BM = bone marrow; ET = essential thrombocythaemia; PMF = primary myelofibrosis

Costs to the Australian healthcare system overall

To calculate the cost to the Australian healthcare system overall for the incorporation of molecular analysis into the diagnostic strategies of ET and PMF, all direct costs associated with their diagnosis have been considered, regardless of the agency or person that incurs them (Table 30).

The estimated cost to the Australian healthcare system of investigation of ET in 4,500 patients with the index test strategy ranges from approximately $4,684,000 to $5,325,000, which includes the cost of BM biopsy in 3,164 patients. In comparison, a cost of $6,087,000 will be incurred by the Australian healthcare system by 4,500 patients undergoing investigation of ET by the comparator test strategy.

Table 30 Costs to the Australian healthcare system for the diagnosis of ET

Item	Cost of index test strategy with qualitative molecular methods for ET (unit cost x number of units)	Cost of index test strategy with quantitative molecular methods for ET (unit cost x number of units)	Cost of comparator test strategy (unit cost x number of units)
JAK2 V617F analysis	$90 x 4,500	$235 x 4,500	n/a
Anaesthetic consultation	$40 x 3,164	$40 x 3,164	$40 x 4,500
Initiation of anaesthetic management	$73 x 3,164	$73 x 3,164	$73 x 4,500
Physician consult[a]	$70 x 3,164	$70 x 3,164	$70 x 4,500
BM biopsy	$1,006 x 3,164	$1,006 x 3,164	$1,006 x 4,500
BM pathology	$164 x 3164	$164 x 3,164	$164 x 4,500
Total	**$4,684,108**	**$5,325,358**	**$6,086,925**

[a] prior to BM biopsy procedure; ET = essential thrombocythaemia; BM = bone marrow

Sensitivity analysis regarding the impact of a change in the number of investigations performed per year would see a significant impact on the financial implications to the Australian healthcare system (Table 31).

Table 31 Sensitivity analysis regarding the impact of a change in use of investigation of ET

Use (investigations per year)	Cost of index test strategy for ET with qualitative molecular methods	Cost of index test strategy for ET with quantitative molecular methods	Cost of comparator test strategy for ET	Incremental cost / saving relative to the comparator (range)
950	$988,867	$1,124,242	$1,285,018	**$160,775–$296,150**
4,500	$4,684,108	$5,325,358	$6,086,925	**$761,567–$1,402,817**
9,000	$9,368,217	$10,650,717	$12,173,850	**$1,523,133–$2,805,633**

ET = essential thrombocythaemia; **bold** type denotes a cost saving relative to the comparator.

With regard to diagnosing PMF, the Australian healthcare system will incur costs of between $15,800 and $40,700 for the diagnosis of 175 patients using the index test strategy. These costs will be in addition to those of the comparator.

An increase in the number of investigations performed each year would see this estimate climb to an additional $180,000 and $465,000 per year for qualitative and quantitative molecular methods, respectively, if 2,000 investigations were performed per year.

Costs to the Medical Benefits Schedule

According to expert opinion, the public to private split of patients being investigated for ET and PMF will be the same as that for patients being investigated for PV. Therefore, 80% of all patients undergoing investigations for ET or PMF would be eligible for MBS reimbursement, with the remaining 20% coming under the Australian Healthcare Agreements between the states/territories and the Commonwealth. As it has been assumed that 4,500 patients would be investigated for ET per year, approximately 3,600 patients would be eligible for MBS reimbursement. Of these 3,600 patients, approximately 1,069 would avoid BM biopsy under the index test strategy. The comparison of the reimbursement of BM biopsy has considered the MBS items associated with this procedure; however, these figures are unlikely to reflect the true cost associated with this process (Table 32). As patients would undergo these investigations as out-patients, the Commonwealth would incur the cost of the 75% rebate on these items.

Table 32 Comparison of MBS items for index and comparator test strategies for ET

MBS item	Index test strategy using qualitative molecular methods	Index test strategy using quantitative molecular methods	Comparator test strategy
JAK2 V617F analysis	$28 x 3,600	$176 x 3,600	n/a
Anaesthetic consultation	$30 x 2,531	$30 x 2,531	$30 x 3,600
Initiation of anaesthetic management	$55 x 2,531	$55 x 2,531	$55 x 3,600
Physician consult[a]	$52 x 2,531	$52 x 2531	$52 x 3,600
BM biopsy	$41 x 2,531	$41 x 2,531	$41 x 3,600
BM pathology	$123 x 2,531	$123 x 2,531	$123 x 3,600
Total	**$888,765**	**$1,421,565**	**$1,084,140**

[a] prior to BM biopsy procedure; BM = bone marrow

The total cost to the MBS of 3,600 patients receiving the index test strategy for ET in the private health sector is estimated at $888,800 using qualitative molecular methods and $1,421,600 using quantitative molecular methods. The comparator test strategy would result in a total cost to the MBS of $1,084,140 per year. Therefore, the cost or saving realised by the Commonwealth should the index test strategy be reimbursed for ET

would depend on the molecular methods used. A cost *saving* of $195,375 per year would be estimated if only qualitative molecular methods were used compared with a *cost* of $337,425 per year if only quantitative methods were used.

The impact to the MBS of a change in use of ET investigations is shown in Table 33. If the maximum expected number of investigations for ET were performed, an estimated saving of $156,700 per year to the MBS could be expected if qualitative molecular methods were used in the index test strategy. If quantitative molecular methods were employed, the MBS would incur a cost, relative to the comparator test strategy, of $184,300 per year.

Table 33 Sensitivity analysis regarding the impact of a change in use of investigation of ET

Utilisation (investigations per year)	Cost of index test strategy for ET with qualitative molecular methods	Cost of index test strategy for ET with quantitative molecular methods	Cost of comparator test strategy for ET	Incremental cost / saving relative to the comparator (range)
950	$187,628	$300,108	$228,874	**$71,234–$41,246**
4,500	$888,765	$1,421,565	$1,084,140	**$195,375–**$337,425
9,000	$1,777,529	$2,843,129	$2,168,280	**$390,751–**$674,849

ET = essential thrombocythaemia; **bold** type denotes a cost saving relative to the comparator.

The MBS reimbursement associated with the index test strategy for PMF will be over and above the comparator test strategy as molecular analysis will be performed in addition to the comparator test strategy. Hence, the costs incurred by the Commonwealth if the index test strategy is adopted are estimated to be $3,900 and $24,600 per year for qualitative and quantitative molecular methods, respectively, if 140 patients are investigated in the private sector.

If the estimated number of patients investigated per year for PMF were to increase to 2,000, it would be expected that 1,600 of these patients would be Medicare-billed. This would result in an additional cost to the MBS of between $44,800 and $281,600 per year depending on the molecular methods used.

Costs to the state/territory health systems

The estimate of the costs to the states/territories has been made using three assumptions—that the unit costs of the test strategies are the same for a public patient as they are for a private patient; that 900 and 35 patients would be investigated for ET and PMF, respectively, in the public health system per year; and that, of the patients being investigated for ET by the index test strategy, 267 would avoid BM biopsy.

Investigating ET in the public health system would result in a cost to the states/territories of between $936,800 and $1,065,000 depending on the method of molecular analysis. The comparator test strategy is estimated to cost $1,217,400 per year, resulting in a cost saving of between $152,300 and $280,600 per year if the index test strategy was adopted. This saving arises from the reduction in the number of BM biopsies required with the index test strategy. Sensitivity analysis suggests that this saving could vary to be as little as $32,155–$59,230 or as large as $304,600–$561,127 per year depending on the molecular methods used.

Again, the costs to the states/territories of the index test strategy for PMF will be incurred over and above those of the comparator test strategy as a result of the addition

of molecular analysis to the test strategy. Consequently, the cost of investigating patients suspicious of PMF in the public health sector will result in additional costs of $3,200 and $8,100 for qualitative and quantitative molecular methods, respectively, to the states/territories. Sensitivity analysis of the number of investigations per year indicates this estimate may increase to $36,000–$93,000 per year in addition to the cost of the comparator test strategy, depending on the molecular methods used.

Indicative economic evaluation

Although it has been argued that there is insufficient evidence to support an economic evaluation of the use of molecular testing in the investigation of PV or ET, it is possible that cost savings will be realised as a result of the avoidance of BM biopsies. Hence, an indicative evaluation has been conducted for the respective indications of PV and ET to determine the diagnostic accuracy of molecular testing that would be required to realise cost savings to the Australian healthcare system overall.

As the diagnostic accuracy of the addition of molecular testing to the investigation of PV and ET has not been established in the evidence-base, there may be value in knowing the threshold diagnostic accuracy of the index test strategy at which the costs associated with the addition of molecular testing are offset by the savings associated with the avoidance of BM biopsy.

The post-test probability or positive predictive value of the index test strategy determines the requirement for further testing in the form of a BM biopsy. That is, if the positive predictive value of molecular testing is sufficiently high, the clinician will no longer need the additional information provided by BM biopsy as there will be sufficient certainty in the result of the index test strategy.

PV

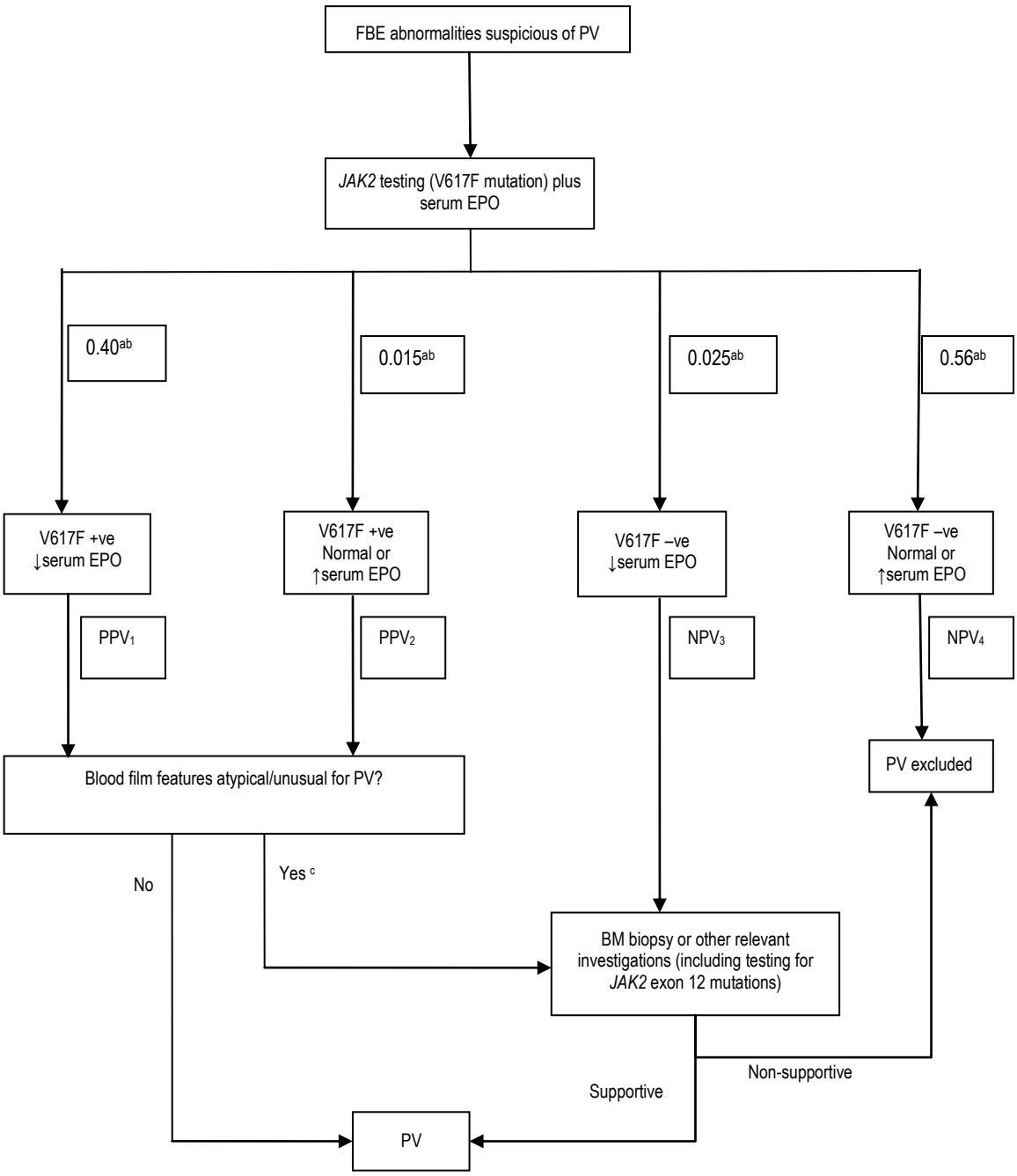
To estimate the threshold diagnostic accuracy of molecular testing that is required for the investigation of suspected PV to be cost saving as a result of the avoidance of BM biopsies, some assumptions have been required.

1. To calculate the positive predictive value, it has been assumed that the possible outcomes of *JAK2* V617F analysis and serum erythropoietin determination, and the respective proportion of patients with these outcomes, are as described in Figure 7. These assumptions have been based on an overall diagnostic yield for the addition of molecular testing to the investigation of suspected PV being 43%, as reported by Girodon et al (2007). In this study adults with an haematocrit greater than 50% in males or 48% in females were investigated for PV using the 2008 WHO criteria.

2. The referral rate for BM biopsy for patients with a positive *JAK2* V617F result has been assumed to be 1–positive predictive value (or negative predictive value), where the positive predictive value is the proportion of positive results for the combination of *JAK2* molecular testing plus serum erythropoietin levels, which are true PV.

3. The proportion of *JAK2* analyses performed by qualitative analysis is 33%, and it is assumed that the remainder are performed by quantitative molecular methods. This assumption is based on limited information collected in a survey of 21 Australian laboratories, as well as the expert opinion of the Advisory Panel (Royal College of Pathologists of Australasia 2008).

4. Test sensitivity and specificity of *JAK2* analysis plus serum erythropoietin levels are equivalent.



[a] The proportion of outcomes investigated for PV; [b] A diagnostic yield for PV of 43%, as reported by Girodon et al (2007), was used to estimate the proportions for each of the possible results of *JAK2* V617F and serum erythropoietin testing. Girodon et al (2007) used the 2008 WHO diagnostic criteria (including serum erythropoietin levels) to determine the diagnostic yield of PV; [c] Equivalent to negative predictive value; PV = polycythaemia vera; BM = bone marrow; EPO = erythropoietin; PPV_1 and PPV_2 = positive predictive value (to be calculated); NPV_1 and NPV_2 = negative predictive value (to be calculated)

Figure 7 Pathway for diagnosis of PV with molecular testing

Using Bayes' Theorem[1] it is possible to calculate the positive and negative predictive values of having the *JAK2* V617F mutation present and, subsequently, given the assumptions outlined previously, the likelihood of the patient requiring BM biopsy. It should be noted that Bayes' Theorem requires knowledge of the prevalence of disease in order to calculate the positive (or negative) predictive value. With regard to the clinical pathway for the diagnosis of PV, if the results of the tests are not able to provide a diagnosis, the prevalence of disease is not an appropriate indicator of pre-test probability. This can be demonstrated by calculating PPV_1 and PPV_2 (Figure 7) using the prevalence of PV in the population to be investigated. This would give the same result, which does not seem reasonable, as it would be expected that a *JAK2*-positive and low serum erythropoietin test result would have a higher probability of disease than a *JAK2*-positive and normal or low serum erythropoietin result. Consequently, the proportion of the population that would have each test combination outcome has been used as the pre-test probability of disease. These proportions may alter if the prevalence of disease were to change.

Table 34 shows that a decrease in diagnostic accuracy of *JAK2* analysis reduces the proportion of BM biopsies that would be avoided. Subsequently, as test accuracy decreases, so too do the cost savings realised through the avoidance of BM biopsy. Despite this, the negative and positive predictive values are sufficient to avoid enough BM biopsies to ensure that the investigation of 1,500 people with molecular analysis is likely to provide cost savings compared to the comparator test strategy, even if the diagnostic accuracy of the index test strategy were no better than chance.

Table 34 Cost savings from avoided BM biopsies in 1,500 patients with suspected PV

Sensitivity (%)	Specificity (%)	BM biopsies avoided[a]	Cost savings per patient
100	100	1,500	**–$1,175**
99	99	1,471	**–$1,150**
90	90	1,290	**–$996**
80	80	1,112	**–$843**
75	75	1,028	**–$771**
60	60	792	**–$569**
50	50	647	**–$445**

[a] Positive and negative predictive values were calculated to determine the number of bone marrow (BM) biopsies that would be required based on the results of *JAK2* and serum erythropoietin testing (Appendix E), and these were then used to calculate the number of BM biopsies that would be avoided; **bold** typeface denotes a cost-saving scenario.

Sensitivity analysis to determine whether different proportions for the possible test outcomes have an impact on the number of BM biopsies avoided is believed to be unnecessary given that a normal clinical scenario would see a broader population tested in order to capture as many patients with disease as possible, and that the base case (56% of patients tested are *JAK2*-negative with normal to high serum erythropoietin levels) represents a relatively conservative scenario.

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[1] Bayes' Theorem:

Positive predictive value = (prevalence)(sensitivity) / (prevalence)(sensitivity) + (1–prevalence)(1–specificity)

Negative predictive value = (1–prevalence)(specificity) / (prevalence)(1–sensitivity) + (1–prevalence)(specificity)

ET

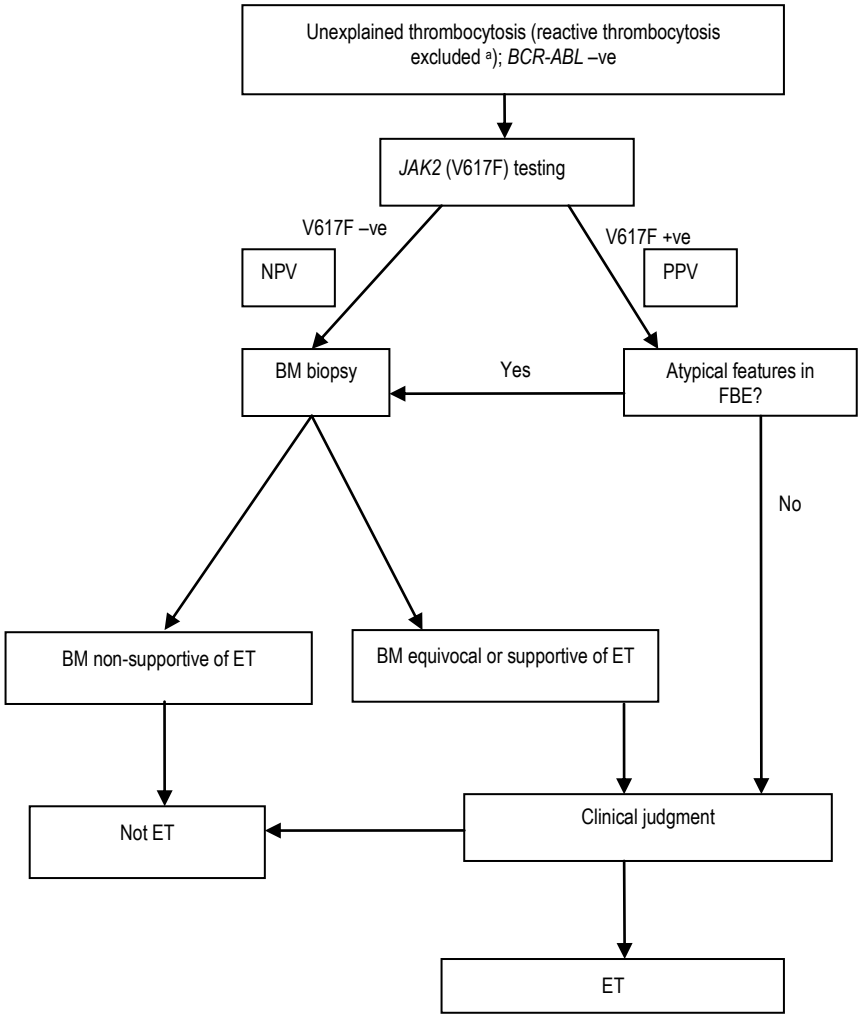
The assumptions made when conducting the analysis for ET are similar to those for PV.

1. The proportion of patients who would truly have ET is 65%. This assumption is based on a diagnostic yield for ET of 65%, as reported in the poor-quality study of Ammatuna et al (2007). As a consequence of this disease prevalence, it is expected that approximately 33% of patients tested will be *JAK2* V617F-positive.

2. The proportion of *JAK2* V617F analyses conducted by qualitative methods is 33% and it is assumed that the remainder are conducted by quantitative molecular methods. This assumption is based on limited information collected in a survey of 21 Australian laboratories and the expert opinion of the Advisory Panel (Royal College of Pathologists of Australasia 2008).

3. The sensitivity and specificity of *JAK2* analysis for suspected ET are equal.

Although patients who are found to be *JAK2* V617F-negative will undergo BM biopsy, a proportion of mutation-positive patients will also be referred for this procedure. The positive predictive value of the *JAK2* V617F-positive result will determine the need for BM biopsy in this group of patients (Figure 8). Therefore, it is unnecessary to calculate the negative predictive value of molecular testing as those patients who test negative are unable to avoid BM biopsy regardless of the diagnostic accuracy of the test.



PPV = positive predictive value to be calculated
NPV = negative predictive value to be calculated
FBE = full blood examination
ET = essential thrombocythaemia

Figure 8 Pathway for diagnosis of ET with molecular testing

The positive predictive value of *JAK2* analysis can be calculated using Bayes' Theorem. Similar to PV, a positive molecular test may not be sufficient to provide a diagnosis of ET; consequently, the prevalence of disease should not be used in the calculation of positive predictive value.

Table 35 shows the impact of diagnostic accuracy of *JAK2* analysis on the cost implications of investigating ET. At a test sensitivity and specificity of 60%, the investigation is no longer cost saving as a result of the avoidance of BM biopsies.

Table 35 Cost savings from avoided bone marrow biopsies in 4,500 patients with suspected ET

Sensitivity (%)	Specificity (%)	Bone marrow biopsies avoided[a]	Cost savings per patient
100	100	1,485	**–$238**
99	99	1,455	**–$229**
90	90	1,212	**–$160**
80	80	985	**–$95**
75	75	886	**–$67**
60	60	631	$6
50	50	490	$46

[a] Positive and negative predictive values were calculated to determine the number of bone marrow (BM) biopsies that would be required based on the results of *JAK2* testing (Appendix E), and these were then used to calculate the number of BM biopsies that would be avoided; **bold** typeface denotes a cost saving

The diagnostic yield of ET reported by Ammatuna et al (2007) is expected to have been influenced by selection bias, and therefore it is appropriate to perform a sensitivity analysis to determine the effect of this variable on the number of BM biopsies avoided.

In a more likely clinical scenario to maximise the capture of all patients with ET, a diagnostic yield of 40–50% might be reasonably expected. These scenarios substantially diminish the cost savings realised per patient, as shown in Table 36.

Table 36 Sensitivity analysis of diagnostic yield in investigating 4,500 patients with suspected ET

Prevalence of *JAK2* mutation (%)	Sensitivity (%)	Specificity (%)	Bone marrow biopsies avoided	Cost savings per patient
20	100	100	900	**–$71**
	99	99	653	**–$61**
	90	90	623	$8
	80	80	450	$58
	75	75	386	$76
	60	60	245	$116
	50	50	180	$134
25	100	100	1,125	**–$135**
	99	99	1092	**–$126**
	90	90	844	**–$55**
	80	80	643	$3
	75	75	563	$25
	60	60	375	$79
	50	50	281	$106

Bold typeface denotes a cost saving

Limitations of indicative evaluation

This evaluation was dependent on a number of assumptions that are likely to introduce uncertainty into the results of the analysis.

A lack of data regarding the prevalence of ET and PV in the population of patients investigated in Australia meant that the base case relied on the report of diagnostic yield in medium- and low-quality studies conducted in Europe as a proxy for the prevalence of these diseases. Should the prevalence of disease alter from the base case, the expected

proportions of each test result are also likely to differ. This would be expected to impact on the likely cost savings of molecular testing.

The estimated proportion of *JAK2* testing conducted by qualitative and quantitative methods is largely based on expert opinion of reported testing in a sample of 21 Australian laboratories that conduct molecular testing.

Finally, as a result of a lack of data, the assumption that the sensitivity and specificity of molecular testing with or without serum erythropoietin levels are equivalent was used to indicate the likely relationship between the two diagnostic accuracy outcomes.

Discussion

Is it safe?

Although no reports of adverse events associated with molecular testing in the diagnosis of PV, ET or PMF were identified, it is recognised that there are some risks associated with BM aspiration and biopsy, and the taking of blood (venepuncture) (Bain 2005; Riley et al 2004). Potential complications associated with venepuncture include bruising, pain, nerve damage and arterial puncture (Lavery & Ingram 2005).

Adverse events that have been reported from BM biopsy include needle breakage, haemorrhage, pain and infection (Bain 2005; Riley et al 2004). In addition to this, death has been reported in at least one patient who suffered from multiple myeloma (Gupta et al 1992). In 2003 a national audit conducted by the British Society for Haematology reported adverse events associated with BM biopsy procedures. From the 63 hospitals that provided data, 19,259 procedures were performed including 13,147 combined procedures (aspirate and biopsy). Sixteen (0.08%) adverse events were reported as a result of these procedures. The adverse events reported were haemorrhage (11), infection (2), persistent pain (2) and a serous leak (Bain 2005). As with any invasive procedure requiring anaesthesia, there are risks involved. However, it would appear from this data that these risks are a rare occurrence.

Is it effective?

Ideally, this assessment would identify evidence that reported a direct impact on patient health outcomes; however, no direct evidence was available. In the absence of such evidence, a linked evidence approach was undertaken to determine the relative diagnostic accuracy of the testing; whether there was a change in management as a result of the test; and whether patient outcomes altered as a result of a change in management.

In general, the study designs included in the assessment of diagnostic accuracy were weakened by the lack of standardisation of diagnostic criteria for MPD. With a number of different diagnostic criteria available to diagnose MPD, heterogeneity of results was expected. Additionally, lack of standardised methods for molecular analysis in subjects has also led to variation in results, making comparison between studies difficult. Heterogeneity will have been introduced between studies as a result of differences in the analytical sensitivity (the lowest level of mutant DNA that can be reliably detected) of different assay methods and, additionally, from differences in the diagnostic threshold (the level of mutant DNA that indicates a diseased state) used to designate a positive or negative test result. Analytical sensitivity was poorly reported in the studies included in this assessment. Those studies that did provide this information indicated that the analytical sensitivity of the methods used ranged from 0.5% to 10% of mutant DNA in wild-type DNA.

Assays that used higher diagnostic thresholds, that is the ratio of mutant DNA to wild-type DNA, are more likely to introduce a greater proportion of false negative results, as patients would require higher levels of *JAK2* V617F in order to be diagnosed with a MPD. Assays that use lower diagnostic thresholds are more likely to introduce a greater proportion of false positives, as patients would only require a small amount of mutant DNA present to be diagnosed with an MPD. Consensus regarding the appropriate

methodology for the detection of *JAK2* V617F and a clinically relevant diagnostic threshold is essential to ensure generalisability between studies.

The use of all relevant clinical and laboratory information, except for molecular status, to make a diagnosis of MPD is likely to act as an imperfect reference standard due to the lack of consideration of molecular status in patients. Without this information there is a real possibility of patients being misdiagnosed, in particular to be falsely diagnosed as not having MPD. This argument is supported by the fact that some patients underwent a change in diagnosis after being informed by their molecular status (Means 2008). Further in support of this argument, change in the WHO diagnostic criteria for MPD to incorporate the molecular status of a patient indicates that the addition of molecular testing will provide a more accurate diagnosis.

The imperfect nature of the reference standard is likely to be most relevant for patients suspected of PV, as the prevalence of *JAK2* mutations in this population is greater than 90%. However, for patients being investigated for ET or PMF, where the prevalence of the mutation in those with disease is approximately 50%, a negative result for a relevant molecular mutation will not rule out disease.

Due to the imperfect nature of the reference standard, direct evidence of a change in patient outcomes is required to inform of the comparative effectiveness of the addition of molecular testing in the investigation of MPD (MSAC 2005). However, as the 2008 WHO criteria is now considered to be the gold standard for diagnosing PV, ET and PMF, it is unlikely that such direct evidence will become available.

Overall, regardless of the indication for molecular testing, there was a constant theme of weakness in the evidence identified. In general, the evidence was of the lowest level and mostly of a medium to poor quality, with all studies having flaws to weaken the data and conclusions. To determine the comparative effectiveness of molecular testing compared with normal diagnosis, studies that cross-classified only patients suspected of MPD with both the 2001 and 2008 WHO diagnostic criteria would be able to inform this question. However, due to the rapid uptake of molecular testing into clinical practice, such studies are unlikely to occur.

PV

The question of the relative effectiveness of *JAK2* exon 14 molecular testing in the diagnostic strategy of PV was poorly informed by three studies of diagnostic yield (level IV diagnostic evidence). The diagnostic yield of PV diagnoses in these studies ranged from 9% to 43%. The results of these studies were inconsistent, primarily due to likely differences between the populations tested.

The evidence regarding the effectiveness of *JAK2* exon 12 mutation analysis in the assessment included two diagnostic case-control studies that defined their cases and controls using different diagnostic criteria. PVSG criteria are considered to be more restrictive and may therefore produce some false negative results. As a result, reports using these criteria should be considered carefully. As is the case with the poor-quality study reported by Martinez-Aviles et al (2007) (level III-3 diagnostic evidence), the diagnostic accuracy outcomes showed that molecular analysis of *JAK2* exon 12 was less accurate than chance in determining patients with *JAK2* V617F-negative PV or IE. By comparison, the study by Pardanani et al (2007) reported improved diagnostic accuracy against the 2001 WHO diagnostic criteria. The results of this study are weakened by the

small numbers included but, as discussed earlier, the imperfect reference standard is likely to reflect poorly on the true diagnostic accuracy of molecular analysis of *JAK2* exon 12 mutations.

Minimal evidence was identified that reported change in patient management following diagnosis using molecular analysis. Change in management was reported in one small study as a consequence of a change in diagnosis (Means 2008). These changes in diagnosis were due to both detection and absence of the mutation, clearly indicating the utility of *JAK2* V617F analysis in the diagnosis of PV.

A linked evidence approach attempts to link evidence of diagnostic accuracy, change in management and patient outcomes following a change in management. However, as there was no evidence of earlier diagnosis or a change in treatment based on the results of diagnosis with molecular testing, a systematic evaluation of treatment effectiveness following such a change in management was not required. An assessment of the body of evidence relating to PV is provided in Table 37.

Table 37 Completed body of evidence matrix relating to the assessment for PV

Component	A	B	C	D
	Excellent	**Good**	**Satisfactory**	**Poor**
Evidence-base[a]				Level IV studies, or level I to III studies with high risk of bias
Consistency[b]			Some inconsistency reflecting genuine uncertainty around clinical question	
Clinical impact			Moderate	
Generalisability		Population(s) studied in the body of evidence are similar to the target population for the guideline		
Applicability		Applicable to Australian healthcare context with few caveats		

Adapted from NHMRC (2008b)
[a] Level of evidence determined from the NHMRC evidence hierarchy
[b] If there is only one study, rank this component as 'not applicable'.

ET and PMF

Insufficient evidence was available to inform of the diagnostic accuracy of using molecular testing in the diagnosis of ET and PMF. One poor-quality study (level IV diagnostic evidence) was identified that reported diagnostic yields for ET and PMF of 65% and 8% respectively (Ammatuna et al 2007).

When considering the utility of *JAK2* V617F analysis in the diagnosis of ET and PMF, it should be remembered that this mutation is only prevalent in 50% of patients with

disease. As such, the added benefit in the diagnostic strategy is likely to be less than that in the investigation of patients suspected of PV.

Assessments of the body of evidence relating to ET and PMF are provided in Table 38 and Table 39 respectively.

Table 38 Completed body of evidence matrix relating to the assessment for ET

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence-base[a]				Level IV studies, or level I to III studies with high risk of bias
Consistency			Some inconsistency reflecting genuine uncertainty around clinical question[b]	
Clinical impact				Slight or restricted
Generalisability		Population(s) studied in the body of evidence are similar to the target population for the guideline		
Applicability		Applicable to Australian healthcare context with few caveats		

Adapted from NHMRC (2008b)

[a] Level of evidence determined from the NHMRC evidence hierarchy

[b] If there is only one study, rank this component as 'not applicable'. The evidence regarding ET was limited to one study although studies of MPDs included patients diagnosed with ET. However, there remains a genuine and substantial uncertainty surrounding the clinical question.

Table 39 Completed body of evidence matrix relating to the assessment of PMF

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence-base[a]				Level IV studies, or level I to III studies with high risk of bias
Consistency			Some inconsistency reflecting genuine uncertainty around clinical question[b]	
Clinical impact				Slight or restricted
Generalisability		Population(s) studied in the body of evidence are similar to the target population for the guideline		

Applicability		Applicable to Australian healthcare context with few caveats		

Adapted from NHMRC (2008b)

[a] Level of evidence determined from the NHMRC evidence hierarchy

[b] If there is only one study, rank this component as 'not applicable'. The evidence regarding PMF was limited to one study however, studies of MPDs may have included patients diagnosed with PMF. However, there remains a genuine and substantial uncertainty surrounding the clinical question.

MPDs

The highest level of evidence identified in this assessment reported a cross-classification study (level III-2 diagnostic evidence) that compared the 2001 WHO diagnostic criteria with the 2008 WHO diagnostic criteria for MPD. This study, which was limited by selection bias, reported diagnostic sensitivity and specificity of 100% and 89%, respectively, indicating that the 2008 WHO diagnostic criteria were able to detect all patients classified with disease by the 2001 WHO criteria. It also indicates that, according to the 2001 criteria, some patients classified by the 2008 criteria as having MPD did not have disease. However, due to the imperfect nature of the reference standard, these 'false positives' did, in fact, have MPD.

Interestingly, in the study by Kondo et al (2008) the negative and positive predictive values were 100% and 97% respectively. This is more likely a reflection of the imperfect reference standard rather than the fact that these were a mixture of patients with suspected PV, ET and PMF; therefore, a significant proportion of them may not have carried the *JAK2* V617F mutation despite having disease.

Overt MPD

A number of studies reported on the diagnostic yield of patients with MPD in populations who are at high risk of having MPD, specifically patients with Budd-Chiari syndrome or portal, hepatic, mesenteric or splanchnic vein thromboses. Diagnostic yields ranged from 11% to 44%, with the variation mostly explained by differences in molecular methodologies and populations analysed. It is possible that the sites of thromboses are associated with different risks of MPD; for example, patients with mesenteric venous thromboses were unlikely to meet the necessary criteria for a diagnosis of MPD.

The addition of *JAK2* exon 12 and *MPL* analysis to patients with venous thromboses who had tested negative for *JAK2* V617F did not add to the number of patients diagnosed in the one study that tested for mutations in all three loci in patients with Budd-Chiari syndrome or portal vein thromboses (Kiladjian et al 2008).

Again, due to variations in these subgroups of patients suspected of MPD and molecular methodologies, it is difficult to make judgements regarding the effectiveness of molecular testing in the diagnosis of MPD.

Latent MPD

In general, the use of molecular testing in the diagnosis of MPD has been discussed as an addition to other tests. However, there are some patients who present with venous thromboses who do not meet the criteria for MPD as a result of either occult bleeding or

haemodilution and hypersplenism stemming from portal hypertension (Bayraktar et al 2008; De Stefano et al 2007b; Primignani et al 2006).

The detection of relevant molecular mutations may indicate underlying disease that is yet to meet diagnostic criteria. Consequently, a number of studies attempted to determine the use of molecular testing in diagnosing latent MPD. These were effectively studies of the prevalence of the *JAK2* V617F mutation in patients presenting with particular venous thromboses, and provided only low-level evidence. The diagnostic yields of these studies ranged from 2% in a population presenting with hypercoagulability to 74% in patients with hepatic and portal vein thromboses. Again, the heterogeneity in patient populations and molecular methodologies have ensured substantial variation in the diagnostic yields presented. In addition to the low level of evidence identified, the quality of the evidence ensures that judgements regarding the effectiveness of molecular testing in the diagnosis of latent MPD are problematic.

While this report has included a number of studies with the aim of detecting latent forms of MPD, it should be noted that this assessment did not attempt to evaluate whether these patients underwent a change in management subsequent to a diagnosis of latent MPD, or went on to develop overt MPD.

What are the economic considerations?

The incorporation of molecular testing into the investigation of PV and ET is likely to result in savings to the Australian healthcare system. These savings can be attributed to the avoidance of BM biopsy in the majority of patients suspected of PV and in a proportion of patients suspected of ET. The investigation of PMF using molecular testing is estimated to result in a cost to the Australian healthcare system as this will be conducted in addition to the comparator test strategy.

An indicative evaluation to model the likely cost savings associated with the avoidance of BM biopsy was conducted for a range of test sensitivities and specificities for patients suspected of PV or ET.

When considering the financial analysis in this assessment, it is important to remember that the estimates of resource use were heavily reliant on expert opinion and, therefore, the calculated financial implications are limited by substantial uncertainty.

PV

Due to a lack of data regarding the comparative safety and effectiveness of molecular analysis in the diagnosis of PV and subsequent uncertainty regarding a net benefit to patients, it was considered inappropriate to conduct a formal economic evaluation of the test strategy.

A financial analysis of the costs associated with the index test strategy relative to the comparator test strategy has been considered from a healthcare perspective. This analysis has been based on the assumption that 1,500 patients will be investigated per year due to a suspicion of PV, of which 1,200 will be in the private health sector. Of the 1,500 patients, 420 will require BM biopsy if investigated by the index test strategy, compared with all 1,500 patients requiring the procedure if investigated by the comparator test strategy.

With regard to the financial implications to the Commonwealth in terms of the Medicare rebate on private patients, the index test strategy is estimated to provide a cost saving to the Commonwealth of between $193,200 and $370,800 per year depending on the molecular methods used.

Of the 300 patients who will be investigated for PV in the public health sector, the financial implications of the index test strategy to the states/territories is expected to result in a saving of between $321,800 and $364,600 per year depending on the molecular methods used.

Depending on the methods used for molecular analysis, the financial implications to the Australian healthcare system overall of the index test strategy compared with the comparator test strategy is estimated to result in a saving of between $1,609,000 and $1,823,000 per year. This saving is primarily attributable to avoidance of BM biopsy in the majority of patients undergoing the index test strategy.

It should be noted that this financial analysis has not considered the costs associated with the need for lung function and oxygen saturation tests in the comparator test strategy. As these tests would be avoided by the index test strategy, there would be further savings to the Australian healthcare system as a result of incorporating the index test strategy.

The diagnostic accuracy of *JAK2* analysis and its associated positive and negative predictive values has limited influence over the cost savings realised from avoiding BM biopsy.

The investigation of suspected PV is expected to become cost neutral when the diagnostic accuracy of *JAK2* analysis and serum erythropoietin determination is less than 50%. When varying the diagnostic accuracy between 50% and 100%, the estimated cost savings range from $445 to $1,175 per patient.

ET

Again, a lack of data regarding the comparative safety and effectiveness of molecular analysis in the diagnosis of ET has meant that an economic evaluation would not be appropriate. As the *JAK2* V617F mutation is prevalent in only 50% of patients with ET, there is significant uncertainty surrounding the net benefit of the index test strategy. Without direct evidence of an improvement in patient outcomes, an economic evaluation would be inappropriate.

The financial analysis of the index test strategy requires consideration of the avoidance of BM biopsy in approximately 30% of patients undergoing investigation. The analysis has been based on two assumptions: that 4,500 patients will be investigated per year for ET and that 3,600 of these investigations will occur in the private sector; and that, of the 4,500 patients being investigated, the index test strategy will result in the avoidance of BM biopsy in 1,336 patients.

The implications of this, and the additional costs of molecular analysis, result in a saving to the states/territories of between $152,300 and $280,600 relative to the comparator, depending on the methodology used for mutation analysis. The relative implications to the Commonwealth in terms of the Medicare rebate range from a *cost* of $337,400 to a *saving* of $195,400 per year, again depending on the methodology used in the molecular analysis.

The overall cost to the Australian healthcare system of incorporating molecular analysis into the diagnostic strategy of ET is estimated to provide a saving of between $761,600 and $1,402,800 per year relative to the comparator strategy.

Savings from the avoidance of BM biopsy may also be realised in the investigation of suspected ET. These savings are more sensitive to the diagnostic accuracy of *JAK2* molecular testing, particularly the prevalence of the V617F mutation, than in the investigation of PV. Analysis of the base-case scenario indicates that the investigation of suspected ET is expected to become cost neutral when the sensitivity and specificity of *JAK2* analysis are below 65%. The likely testing of a broader population (than the base-case scenario) would see a further reduction in cost savings. It would also result in the index test strategy becoming cost neutral if the sensitivity and specificity of *JAK2* analysis were between 80% and 90% and the prevalence of the *JAK2* V617F mutation was 20–25%.

PMF

Due to the prevalence of relevant *JAK2* mutations being approximately 50% in patients with PMF, there is uncertainty surrounding the benefit of molecular analysis in the diagnostic strategy. In addition, the lack of an adequate reference standard requires direct evidence to inform of the relative benefits of the index test strategy to patients.

As no evidence was available to inform of the comparative safety and effectiveness of molecular analysis in the diagnosis of PMF, using either a direct or linked evidence approach, an economic evaluation could not be justified.

The financial analysis of the index test strategy in the diagnosis of PMF has considered the cost of molecular analysis in addition to that of the comparator test strategy in 175 patients being investigated per year. As BM biopsy will not be avoided in patients undergoing the index test strategy, only the cost of the molecular testing has been considered, as these costs will be in addition to those of the comparator.

The additional cost to the Commonwealth in terms of the Medicare rebate for 35 investigations in the private health sector is estimated to be between $3,900 and $24,600 per year depending on the molecular methods used. The states/territories are estimated to incur an additional cost of between $3,200 and $8,100 per year for qualitative and quantitative molecular methods respectively.

The overall cost implications to the Australian healthcare system will result in an additional cost of between $15,800 and $40,700 per year for the investigation of PMF by the index test strategy.

Conclusions

Safety

The question of the comparative safety of the index test strategy was not informed by the available evidence. No studies reported safety outcomes associated with the use of molecular analysis in the diagnosis of PV, ET or PMF.

Despite a lack of evidence, it is unlikely that the taking of blood samples for molecular analysis would result in any significant safety issues to patients. Although venesection is associated with a small risk of bruising, discomfort and nerve damage (rarely), this risk would apply to all patients undergoing testing requiring blood and/or serum samples. In fact, patients undergoing investigation of PV, ET and PMF would have already undergone a full blood examination before entering the diagnostic pathway for MPD.

Molecular analysis of BM biopsies may be associated with a small risk of adverse events including pain, haemorrhage, infection and risks associated with sedation. However, evidence of adverse outcomes associated with BM biopsy indicates that these are likely to be rare occurrences.

Overall, molecular analysis in the diagnosis of PV, ET and PMF is likely to be a safe procedure and would be expected to be at least as safe as the relevant comparator strategies for these indications. With the expected avoidance of BM biopsy in most patients suspected of PV and a proportion of patients with suspected ET, the index test strategy is likely to be safer than the comparator in these patients.

Effectiveness

The comparative effectiveness of molecular analysis in the diagnosis of PV, ET and PMF was poorly informed by the available evidence. No direct evidence was identified that compared the diagnosis of PV, ET or PMF using molecular analysis with the comparator test strategy with regard to patient outcomes.

The subsequent attempt to link evidence of diagnostic accuracy, change in management and treatment effectiveness to a change in patient health outcomes was complicated by an imperfect reference standard, which has the potential to misdiagnose patients compared with the index test strategy. As a consequence, direct evidence is required to fully inform of any patient benefits that may be associated with the addition of molecular analysis to the diagnostic strategies of PV, ET and PMF. However, due to the rapid uptake of molecular analysis in the diagnosis of PV, ET and PMF into clinical practice, it is unlikely that such evidence will be provided.

PV

Evidence of diagnostic accuracy with regard to the addition of molecular analysis in the diagnosis of PV was limited, and related to both the analysis of *JAK2* exon 14 and exon 12 mutations.

Level IV diagnostic evidence indicates that the diagnostic yield of molecular analysis of *JAK2* V617F in addition to other available clinical and laboratory data varied between 9% and 43%. This inconsistency is likely explained by variation in study populations.

Some uncertainty surrounds the available estimates of diagnostic accuracy of *JAK2* exon 12 analysis as a result of small patient numbers. The level III-3 diagnostic evidence indicates sensitivity and specificity in the range 0–83% and 73–100% respectively. These results were complicated by variation in study population, different molecular methods and the use of an imperfect reference standard.

Evidence of a change in management following diagnosis of PV using molecular methods was restricted to one low-level study (level IV intervention evidence) indicating that change in management occurred as a result of a change in diagnosis.

As no evidence was identified that indicated that diagnosis of PV using molecular methods would be made earlier or that treatment would change, systematic review of treatment effectiveness was not required.

Given that the reference standard is unlikely to be *more* accurate than a diagnostic strategy that includes molecular analysis, and that molecular analysis will be performed in addition to other available clinical and laboratory tests, it is reasonable to suggest that the index test strategy will be at least as accurate as the comparator test strategy. The realisation of potential targeted therapies is likely to increase any benefit to patients who carry the mutation.

ET

There was insufficient evidence to determine the accuracy of the addition of molecular analysis to the diagnostic strategy for ET. Level IV diagnostic evidence was limited to one study indicating a diagnostic yield of 65% in patients suspected of MPD.

No evidence was available that indicated a change in management following analysis of relevant molecular mutations. As a consequence, a review of the treatment effectiveness was not performed.

Based on the limited evidence available, the benefit of molecular analysis in patients suspected of ET remains uncertain. Any improvement to diagnostic accuracy due to molecular analysis will only benefit those patients who carry the relevant mutation, as the absence of a relevant mutation would not rule out ET. Consequently, the question regarding the change in patient outcomes following diagnosis using molecular analysis remains unanswered.

PMF

The evidence regarding molecular analysis in the diagnosis of PMF was insufficient to determine its benefit relative to the comparator test strategy.

Low-level evidence (level IV diagnostic evidence) indicated that the diagnostic yield in patients suspected of MPD was 8%. No additional evidence was available to indicate any change in management as a consequence of applying the index test strategy. As a result, systematic review of treatment effectiveness was not performed.

As the prevalence of the *JAK2* V617F mutation is approximately 50% in patients with PMF, the benefit of molecular analysis with regard to diagnostic accuracy and overall patient outcomes is uncertain. Improved diagnosis is likely to benefit only those patients who carry the mutation.

MPDs

The highest level of evidence regarding the diagnostic accuracy of using molecular analysis in the diagnosis of MPD in general reported a cross-classification study comparing the 2008 WHO diagnostic criteria with the 2001 WHO diagnostic criteria. Although weakened by selection bias, this study indicated that the sensitivity of the 2008 WHO criteria was as good as the 2001 WHO criteria, and that the specificity was 89% compared with the 2001 criteria. Due to the inability of the 2001 criteria to make a diagnosis based on molecular status, it is possible that the false positives reported by the 2008 criteria were, in fact, patients with MPD.

The imperfect nature of the reference standard (2001 WHO diagnostic criteria) requires direct evidence of an impact on patient health outcomes to determine the comparative effectiveness of the addition of molecular testing to the investigation of MPDs. However, as the 2008 WHO diagnostic criteria, which includes molecular analysis, is now accepted as the new reference standard for diagnosing MPDs, such evidence is unlikely to become available.

Given the potentially greater accuracy of the addition of molecular analysis to the test strategy in the diagnosis of MPDs, it is likely that the index test strategy is at least as accurate as the comparator for PV; however, the benefit in the diagnosis of ET and PMF remains uncertain.

Economic considerations

Lack of appropriate data and uncertainty surrounding the net benefit in patients suspected of ET and PMF prevented a formal economic evaluation being conducted.

As a consequence, a financial analysis of the expenditures associated with molecular analysis in the diagnostic strategies of PV, ET and PMF has been conducted. These estimates have been limited by the extensive use of expert opinion to estimate resource use.

An indicative evaluation to model the likely cost savings associated with the avoidance of BM biopsy was conducted for a range of test sensitivities and specificities for patients suspected of PV or ET.

PV

The expected uptake of the index test strategy is estimated at 1,500 patients per year. Of these, it is expected that 1,350 patients will avoid the need for BM biopsy when undergoing the index test strategy.

The total cost to the MBS for the comparator test strategy is $450,500 per year. The total cost to the MBS for the addition of molecular analysis to the diagnostic strategy of PV is estimated to be between $79,700 and $257,300 per year depending on the molecular

methods used. This represents a saving to the Commonwealth of between $193,200 and $370,800 per year.

The total cost to the Australian healthcare system including the MBS for the index test strategy is estimated to be between $354,600 and $568,300 per year. Compared with a total cost of $2,178,000 for the comparator, the index test strategy will provide a saving to the Australian healthcare system of between $1,609,000 and $1,823,000 per year.

Savings as a result of the avoidance of BM biopsy are likely to be realised regardless of the diagnostic accuracy of molecular testing plus the determination of serum erythropoietin levels. Additional savings may also be realised from the avoidance of lung function and oxygen saturation tests.

ET

The expected uptake of the index test strategy is estimated at 4,500 patients per year. Of these, it is expected that 1,336 patients will avoid the need for BM biopsy when undergoing the index test strategy.

The total cost to the MBS for the comparator test strategy is $1,084,000 per year compared with between $888,800 and $1,421,600 for the index test strategy depending on the method of molecular analysis.

The total cost to the Australian healthcare system for the index test strategy is estimated to be between $4,684,000 and $5,325,000 compared with a cost of $6,087,000 for the comparator test strategy. This will result in a saving to the Australian healthcare system overall of between $761,600 and $1,403,000 per year for the diagnosis of ET with the use of molecular methods. Savings from the avoidance of BM biopsy are likely to be realised if molecular testing has a sensitivity and specificity greater than 60%. The selection of the population to be investigated for ET may also have an impact on the cost savings realised.

PMF

The expected uptake of the index test strategy, which includes molecular analysis, in the diagnosis of PMF is 175 investigations per year. The addition of molecular analysis to the diagnostic strategy will not reduce the need for BM biopsy.

The total cost to the MBS for the addition of molecular analysis to the diagnostic strategy will be between $3,900 and $24,600 over and above the yearly cost of the comparator strategy.

The total cost to the Australian healthcare system for the index test strategy is estimated to be between $15,800 and $40,700 in addition to the costs for the comparator test strategy.

The estimated costs and/or savings of the index test strategy relative to the comparator are summarised in Table 40 according to the agency that incurs the cost.

Table 40 Summary of incremental costs of the index test strategy relative to the comparator

	Estimated number of investigations per year	Index test strategy (qualitative)	Index test strategy (quantitative)
PV			
Australian healthcare system	1,500	**–$1,728,780**	**–$1,515,030**
MBS	1,200	**–$314,256**	**–$136,656**
States/territories	300	**–$345,756**	**–$303,006**
ET			
Australian healthcare system	4,500	**–$1,309,596**	**–$668,346**
MBS	3,600	**–$165,217**	$49,208
States/territories	900	**–$186,565**	**–$133,669**
PMF			
Australian healthcare system	175	$15,750	$40,688
MBS	140	$3,920	$24,640
States/territories	35	$3,150	$8,138

'–' sign and **bold** type denotes a cost saving

In addition to the costs summarised in Table 40, the incorporation of the index test strategy would realise savings for the individual patient, particularly due to the avoidance of BM biopsy procedures. Such savings have not been calculated in the financial analysis in this assessment but would include savings from avoiding lost time at work and the need for a carer following the biopsy procedure.

Appendix A Advisory Panel and Evaluators

Advisory Panel – Application 1125 – Molecular testing for myeloproliferative disorders

Member	Nomination / Expertise or affiliation
Professor Richard Fox (Chair)	Member of MSAC Haematology/Oncology
Dr Kwun Fong	Member of MSAC Thoracic/Sleep medicine
Dr Rosemary Harrup	The Medical Oncology Group nominee Haematology/Oncology
Dr Robert Lindeman	Royal College of Pathologists nominee Haematology
Mr Russell McGowan	Consumer Health Forum nominee Consumer health
Dr Ian Prosser	Member of MSAC Haematology
Dr Zbigniew (Barney) Rudzki	Co-opted Molecular Pathology
Dr David Westerman	Haematology Society of Australia and New Zealand nominee Haematology
Dr Bronwyn Williams	Royal College of Pathologists nominee Haematology/Transfusion medicine

Evaluators

Name	Organisation
Ms Liz Buckley	Research officer, Adelaide Health Technology Assessment
Ms Tracy Merlin	Manager, Adelaide Health Technology Assessment

Appendix B Search strategies

Literature sources

Electronic bibliographic databases were searched to find relevant studies (those meeting the inclusion criteria) addressing each of the research questions developed for this MSAC assessment. These databases are described in Table 41. Molecular testing for the diagnosis of PV, ET or PMF appears in the literature only since 2005, so the search period was restricted to 2005 or, if inception of the database was later, from that date until February 2009.

Table 41 Electronic databases searched for relevant literature

Database	Period covered
CINAHL	2005 – 02/2009
Cochrane Library – including, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Database, the NHS Economic Evaluation Database	2005 – 02/2009
Current Contents	2005 – 02/2009
Embase.com (including Embase and Medline)	2005 – 02/2009
Pre-Medline	2005 – 02/2009
Web of Science – Science Citation Index Expanded	2005 – 02/2009
EconLit	2005 – 02/2009

Search terms for identifying literature within these bibliographic databases are given below (Table 42).

Table 42 Search terms used to identify potential studies

Element of clinical question	Suggested search terms
Population	myeloproliferative disorders [MeSH] OR myeloproliferative dis* OR polycythemia vera [MeSH] OR polycythemia [MeSH] OR polycythaemia [text] OR polycythemia [text] OR thrombocythemia, hemorrhagic [MeSH] OR thrombocythaemia [text] OR thrombocythemia [text] OR myelofibrosis [MeSH] OR myelofibrosis [text] OR erythrocytosis [MeSH] OR erythrocytosis [text] OR thrombocytosis [MeSH] OR thrombocytosis [text] OR anemia [MeSH] OR anaemia [text] OR anemia [text] OR splenomegaly [MeSH] OR splenomegaly [text] OR budd-chiari syndrome [MeSH] or budd-chiari syndrome [text] OR thrombosis [MeSH] OR thromb* [text] OR hemorrhage [MeSH] OR hemorrhag* [text] OR haemorrhag* [text] OR myeloid metaplasia [MeSH] OR myeloid metaplasia [text] OR agnogenic myeloid metaplasia [text] OR AMM [text] OR myeloid neoplasms [text] OR pancytopaenia [text] OR pancytopenia [text] OR pancytopenia [MeSH]
Intervention/test	exon12 [text] OR Receptors, Thrombopoietin/genetics[MeSH] OR MPL [text] or W515K [text] OR W515L [text]
Comparator (if applicable)	n/a
Outcomes (if applicable)	n/a
Limits	Human

Additional sources of literature—peer-reviewed or grey literature—were sought from the sources listed in Table 43 and from the HTA agency websites listed in Table 44.

Table 43 Additional sources of literature

Source	Location
Internet	
Australian Clinical Trials Registry	http://www.actr.org.au
NHMRC- National Health and Medical Research Council (Australia)	http://www.health.gov.au/nhmrc/
US Department of Health and Human Services (reports and publications)	http://www.os.dhhs.gov/
New York Academy of Medicine Grey Literature Report	http://www.nyam.org/library/greylit/index.shtml
Trip database	http://www.tripdatabase.com
Current Controlled Trials metaRegister	http://controlled-trials.com/
National Library of Medicine Health Services/Technology Assessment Text	http://text.nlm.nih.gov/
U.K. National Research Register	http://www.update-software.com/National/
Google Scholar	http://scholar.google.com/
Hand searching (journals 2007–09)	
Blood	Library or electronic access
Leukemia research	Library or electronic access
Leukemia	Library or electronic access
Best practice & research clinical haematology	Library or electronic access
American Journal of Hematology	Library or electronic access
European Journal of Haematology	Library or electronic access
Haematologica	Library or electronic access
Journal of Molecular Diagnostics	Library or electronic access
British Journal of Haematology	Library or electronic access
New England Journal of Medicine	Library or electronic access
American Journal of Clinical Pathology	Library or electronic access
Expert clinicians	
Studies other than those found in regular searches	MSAC Advisory Panel
Speciality websites	
American Society of Hematology	www.hematology.org
MPD Foundation	www.mpdfoundation.org
National Cancer Institute	www.cancer.gov/cancertopics/pdq/treatment/myeloproliferative/patient
The Myeloproliferative Disorders Research Consortium	www.mpd-rc.org/home.php
MPD Online Resource	www.mpdinfo.org/index.html
The Leukaemia Foundation	www.leukaemia.org.au/web/aboutdiseases/related_index.php
The British Society for Haematology	www.b-s-h.org.uk/
The Leukemia and Lymphoma Society	www.leukemia.org/hm_lls
Haematology Society of Australia and New Zealand	www.hsanz.org.au
International Society of Laboratory Hematology	www.islh.org
Royal College of Pathologists of Australasia	www.rcpa.edu.au
International Society of Haematology, Asian-Pacific Division	www.ishapd.org
Australasian Leukaemia and Lymphoma Group	www.petermac.org/allg/
Association of Cancer Online Resources	www.acor.org
MPD Support Group	http://members.aol.com/mpdsupport/
Pearling	
Reference lists of all included articles were searched for additional relevant source material	

Table 44 Health Technology Assessment Agency websites

AUSTRALIA	
Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP-S)	http://www.surgeons.org/open/asernip-s.htm
Centre for Clinical Effectiveness, Monash University	http://www.mihsr.monash.org/cce
Centre for Health Economics, Monash University	http://www.buseco.monash.edu.au/che/
AUSTRIA	
Institute of Technology Assessment / HTA unit	http://www.oeaw.ac.at/ita/e1-3.htm
CANADA	
Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (AETMIS)	http://www.aetmis.gouv.qc.ca/site/home.php/
The Canadian Agency for Drugs And Technologies in Health (CADTH)	http://www.cadth.ca/index.php/en/
Centre for Health Economics and Policy Analysis (CHEPA), McMaster University	http://www.chepa.org
Centre for Health Services and Policy Research (CHSPR), University of British Columbia	http://www.chspr.ubc.ca
Health Utilities Index (HUI)	http://www.fhs.mcmaster.ca/hug/index.htm
Institute for Clinical and Evaluative Studies (ICES)	http://www.ices.on.ca
Institute of Health Economics	http://www.ihe.ca
Saskatchewan Health Quality Council (Canada)	http://www.hqc.sk.ca
DENMARK	
Danish Centre for Evaluation and Health Technology Assessment (DACEHTA)	www.sst.dk/Planlaegning_og_behandling/Medicinsk_teknologivurdering.aspx?lang=en
Danish Institute for Health Services Research (DSI)	http://www.dsi.dk/engelsk.html
FINLAND	
Finnish Office for Health Technology Assessment (FINOHTA)	http://www.stakes.fi/EN/index.htm
FRANCE	
L'Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES)	http://www.anaes.fr/
GERMANY	
German Institute for Medical Documentation and Information (DIMDI) / HTA	http://www.dimdi.de/static/en
THE NETHERLANDS	
Health Council of the Netherlands Gezondheidsraad	http://www.gr.nl/index.php
Institute for Medical Technology Assessment (Netherlands)	http://www.imta.nl/
NEW ZEALAND	
New Zealand Health Technology Assessment (NZHTA)	http://nzhta.chmeds.ac.nz/
NORWAY	
Norwegian Knowledge Centre for Health Services	http://www.nokc.no/About+us
SPAIN	
Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III"l/Health Technology Assessment Agency (AETS)	http://www.isciii.es/htdocs/en/investigacion/Agencia_quees.jsp
Andalusian Agency for Health Technology Assessment (Spain)	http://www.juntadeandalucia.es/salud/orgdep/AETSA/default.asp?V=EN
Catalan Agency for Health Technology Assessment (CAHTA)	http://www.aatrm.net/html/en/Du8/index.html
SWEDEN	
Center for Medical Health Technology Assessment	http://www.cmt.liu.se/english/publications
Swedish Council on Technology Assessment in Health Care (SBU)	http://www.sbu.se/www/index.asp
SWITZERLAND	

Swiss Network on Health Technology Assessment (SNHTA)	http://www.snhta.ch/
UNITED KINGDOM	
National Health Service Health Technology Assessment (UK) / National Coordinating Centre for Health Technology Assessment (NCCHTA)	http://www.hta.nhsweb.nhs.uk/
NHS Quality Improvement Scotland	http://www.nhshealthquality.org/
National Institute for Clinical Excellence (NICE)	http://www.nice.org.uk/
The European Information Network on New and Changing Health Technologies	http://www.euroscan.bham.ac.uk/
University of York NHS Centre for Reviews and Dissemination (NHS CRD)	http://www.york.ac.uk/inst/crd/
UNITED STATES	
Agency for Healthcare Research and Quality (AHRQ)	http://www.ahrq.gov/clinic/techix.htm
Harvard School of Public Health – Cost-Utility Analysis Registry	http://www.tufts-nemc.org/cearegistry/
Institute for Clinical Systems Improvement (ICSI)	http://www.icsi.org
Minnesota Department of Health (US)	http://www.health.state.mn.us/
National Information Centre of Health Services Research and Health Care Technology (US)	http://www.nlm.nih.gov/hsrph.html
Oregon Health Resources Commission (US)	http://egov.oregon.gov/DAS/OHPPR/HRC/about_us.shtml
U.S. Blue Cross/ Blue Shield Association Technology Evaluation Center (Tec)	http://www.bcbs.com/consumertec/index.html
Veteran's Affairs Research and Development Technology Assessment Program (US)	http://www.va.gov/resdev

Appendix C Studies included in the review

Study profiles of included studies in the assessment of the effectiveness of molecular testing for the diagnosis of PV, ET and PMF

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Abel et al 2008) General Hospital, Boston, USA	Case series Level IV diagnostic evidence CX P2 Q2	66 patients who were investigated for Factor V Leiden and Prothrombin 2010 mutations	*Inclusion:* Investigated for Factor V Leiden and Prothrombin mutations and were negative *Exclusion:* Patients without documented thrombosis. Patients with malignancy, MPD or heparin-induced thrombocytopaenia	Molecular testing for *JAK2* V617F	Diagnostic yield	n/a
(Ammatuna et al 2007) University of Tor Vergata, Rome, Italy	Case series Level IV diagnostic evidence CX P2 Q3	92 patients suspected of MPD 50 patients with ≥ 2 elevated myeloid cell lines 42 patients with isolated thrombocythaemia with main causes of reactive disease excluded	Not reported	Molecular testing plus other clinical and laboratory information required to make a diagnosis according to 2001 WHO criteria	Diagnostic yield in PV, ET and PMF	n/a
(Bayraktar et al 2008) Hacettepe University Hospital, Turkey	Retrospective case series Level IV diagnostic evidence CX P2 Q2	25 chronic non-cirrhotic PVT patients Females = 16 Mean age = 44.9 (range 24–73) years	*Inclusion:* Available DNA samples and a completed thrombophilia investigation Exclusion: Acute or sub-acute	2001 WHO diagnostic criteria for MPD Molecular testing for *JAK2* V617F for latent MPD	Diagnostic yield	n/a

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
			(< 3 months) PVT, cirrhosis or liver malignancy			
(Bergamaschi et al 2008) Italy	Case series Level IV diagnostic evidence CX P2 Q3	93 patients with SVT Some of these patients had already tested positive for *JAK2* V617F as well as being diagnosed with MPD	Not reported	AS-PCR for *MPL* mutations and sequencing for exon 12 mutations	Diagnostic yield	n/a
(Boissinot et al 2006) France	Case series Level IV diagnostic evidence CX P2 Q3	49 patients with SVT	Not reported	Molecular testing plus other clinical and laboratory information required to make a diagnosis of latent MPD, including serum EPO, EEC/EMC ± BM biopsy	Diagnostic yield	n/a
(Colaizzo et al 2008) A. Cardarelli Hospital, Naples, Italy	Case series Level IV diagnostic evidence CX P2 Q3	33 patients presenting with BCS 8 patients had previously been diagnosed with MPD Females = 23 (70%) Median age = 35 (range 14–66) years	Not reported	MPD was diagnosed according to 2001 WHO criteria Investigations included *JAK2* V617F testing, screening for Factor V Leiden and Prothrombin mutations, coagulation studies	Diagnostic yield	n/a
(Colaizzo et al 2007) A. Cardarelli Hospital, Naples, Italy	Case series Level IV diagnostic evidence CX P2 Q2	99 consecutive patients with PMVT Nine patients were known to have MPD	*Inclusion*: Not reported *Exclusion*:Liver cirrhosis, hepatocellular carcinoma or BCS	Molecular testing for *JAK2* V617F MPD was diagnosed according to 2001 WHO criteria	Diagnostic yield	n/a
(De Stefano et al	Case series	76 patients with MVT (n=17)	Not reported	Molecular testing for *JAK2*	Diagnostic yield	n/a

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
2007a) Italy *Likely overlap of patients with De Stefano et al 2007*	Level IV diagnostic evidence CX P2 Q3	or PVT (n=59)		V617F		
(De Stefano et al 2007b) Italy *Likely overlap of patients with De Stefano et al 2007*	Retrospective case series Level IV diagnostic evidence CX P2 Q2	139 adult patients with SVT or CVT 19/139 patients were known to have overt MPD *With overt MPD*: HVT n=4 PVT n=8 SVT n=3 CVT n=4 *Without overt MPD:* HVT n=11 PVT n=50 MVT n=16 SVT n=2 CVT n=41	Not reported	Molecular testing for *JAK2* V617F MPD diagnosed with updated PVSG criteria	Diagnostic yield	n/a
(Girodon et al 2007) France	Case series Level IV diagnostic evidence CX P1 Q2	168 patient investigated for an elevated Hct	Increased Hct > 50% in males > 48% in females	Serum EPO +*JAK2* V617F testing ± EEC ± RCM ± BM histology	Diagnostic yield of PV	n/a
(Goulding et al 2008) London, United Kingdom	Retrospective case series Level IV diagnostic evidence CX P1 Q1	19 patients with HVT or PVT Females = 9 Median age = 41 (range 24–76) years	*Exclusion*: Identifiable thrombophilia – protein C, protein S and antithrombin III deficiencies, Prothrombin and Factor V Leiden	Molecular testing for *JAK2* V617F	Diagnostic yield	Range 2–96 months

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
			mutations; lupus anticoagulant or anti-phospholipid antibodies or other hypercoagulable state including sepsis, malignancy, pregnancy or oral contraceptive use, or pre-existing cirrhosis. Also, patients known to have an MPD or who were diagnosed with an MPD were excluded			
(Jones et al 2008) United Kingdom	Case series Level IV diagnostic evidence CX P1 Q3	50 patients with erythrocytosis who had tested negative for *JAK2* V617F mutation	Not reported	HRM assay to detect exon 12 mutations	Diagnostic yield	n/a
(Kiladjian et al 2008) European Network for Vascular Disorders of the Liver – France, Spain and The Netherlands *Likely overlap of patients with Smalberg et al (2006)*	Retrospective case series Level IV diagnostic evidence CX P2 Q2	241 patients with BCS (n=104) or PVT (n=137) BCS: median age = 36 years (IQR = 27–46) Females = 66% PVT: median age = 42 years (IQR = 30–57) Females = 44%	*Inclusion*: Diagnosis of BCS or PVT Exclusion of associated malignancy or cirrhosis DNA available for molecular testing Patient-informed consent obtained with the Declaration of Helsinki	*JAK2* V617F (n=241) *MPL* W515K/L (n=212) *JAK2* exon 12 (n=123) Serum EPO (n=142) EEC (n=125) Red cell mass (n=121) Clinical, haematological and liver function tests (n=241)	Diagnostic yield	n/a

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Kondo et al 2008) Japan	Cross-classification Level III-2 diagnostic evidence C1 P2 Q3	75 patients with or suspected of MPD	Not reported	Application of 2008 WHO criteria for the diagnosis of MPD ***Reference Standard*** Application of 2001 WHO criteria for the diagnosis of MPD	Diagnostic accuracy – sensitivity, specificity, PPV, NPV	n/a
	Case series Level IV diagnostic evidence CX P2 Q3	19 patients with or suspected of MPD, and who have tested negative for *JAK2* V617F	Not reported	*JAK2* exon 12 genotyping	Diagnostic yield	n/a
(Kouroupi et al 2008) France	Case series Level IV diagnostic evidence CX P1 Q3	21 patients with *JAK2* V617F-negative IE	Not reported	2008 WHO criteria for the diagnosis of PV	Diagnostic yield	n/a
(Martinez-Aviles et al 2007) Barcelona, Spain	Diagnostic case-control Level III-3 diagnostic evidence CX P2 Q3	20 patients who were *JAK2* V617F-negative Cases: PV (n=9) Controls: IE (n=11)	Not reported	***Reference standard*** PVSG criteria for PV diagnosis BCSH criteria for IE diagnosis	Diagnostic accuracy – sensitivity, specificity, PPV, NPV	n/a
(McMahon et al 2007) USA	Retrospective case series Level IV diagnostic evidence CX	42 patients with catastrophic intra-abdominal thromboses requiring small intestinal or multivisceral transplants 3 patients had previously	Inclusion: Available DNA samples	Molecular testing for *JAK2* V617F	Diagnostic yield	n/a

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	P2 Q2	diagnosed or recognised MPD				
(Pardanani et al 2007) Mayo Clinic, USA	Diagnostic case-control Level III-3 diagnostic evidence CX P1 Q2	12 patients diagnosed with PV or IE Cases: PV (n=6) *JAK2* V617F-negative (n=6) Controls: IE or UE (n=6) *JAK2* V617F-negative (n=6)	Not reported	***Reference standard*** 2001 WHO criteria for the diagnosis of PV	Diagnostic accuracy – sensitivity, specificity, PPV, NPV	n/a
(Patel et al 2006) King's College Hospital, London, UK	Case series Level IV diagnostic evidence CX P1 Q2	41 patients with idiopathic BCS Female n=26 Mean age = 35.5 years (SD = 13.3 years)	*Inclusion*: Not reported *Exclusion*: Patients with secondary BCS	Molecular testing for *JAK2* V617F 2001 WHO criteria for the diagnosis of MPD	Diagnostic yield	n/a
(Percy et al 2007) Ireland	Case series Level IV diagnostic evidence CX P1 Q3	58 patients with IE and normal to low serum EPO	Not reported	Not reported	Diagnostic yield	n/a
(Primignani et al 2006) Thrombosis Center of the Maggiore Hospital Foundation of Milan, Italy	Case series Level IV diagnostic evidence CX P2 Q3	93 consecutive patients with BCS (n=20) or extrahepatic portal vein obstruction (n=73)	Not reported	Molecular testing for *JAK2* V617F	Diagnostic yield	n/a
(Regina et al 2007) France	Case series Level IV diagnostic evidence	88 patients with idiopathic SVT or DVT of the lower limb Female n=42	Not reported	Molecular testing for *JAK2* V617F	Diagnostic yield	n/a

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	CX P1 Q2	PVT (n=42) HVT (n=2) Spontaneous DVT (n=44)				
(Rumi et al 2009) Italy	Case series Level IV diagnostic evidence CX P2 Q1	91 consecutive patients with isolated erythrocytosis	*Inclusion:* Absolute erythrocytosis and normal oxygen saturation	2008 WHO criteria for diagnosis of PV	Diagnostic yield	n/a
(Smalberg et al 2006) Rotterdam, The Netherlands *Likely overlap of patients with Kiladjian et al (2008)*	Case series Level IV diagnostic evidence CX P2 Q2	29 patients with primary, non-malignant BCS who underwent investigation for MPD	Not reported	2001 WHO criteria for diagnosis of MPD ± molecular testing for relevant mutations	Diagnostic yield	n/a

BCS = Budd-Chiari syndrome; PVT = portal vein thrombosis; IQR = interquartile range; MPD = myeloproliferative disease; WHO = World Health Organization; PV = polycythaemia vera; ET = essential thrombocythaemia; PMF = primary myelofibrosis; IE = idiopathic erythrocytosis; PPV = positive predictive value; NPV = negative predictive value; HVT = hepatic vein thrombosis; SVT = splanchnic vein thrombosis; MVT = mesenteric vein thrombosis; CVT = cerebral vein thrombosis; DVT = deep vein thrombosis; PVSG = Polycythemia Vera Study Group; EPO = erythropoietin; EEC/EMC = endogenous erythrocyte / megakaryocyte colonies; BM = bone marrow; Hct = haematocrit; RCM = red cell mass studies; UE = unexplained erythrocytosis; PMVT = portal or mesenteric vein thrombosis; SD = standard deviation

Appendix D Polymerase chain reaction diagram

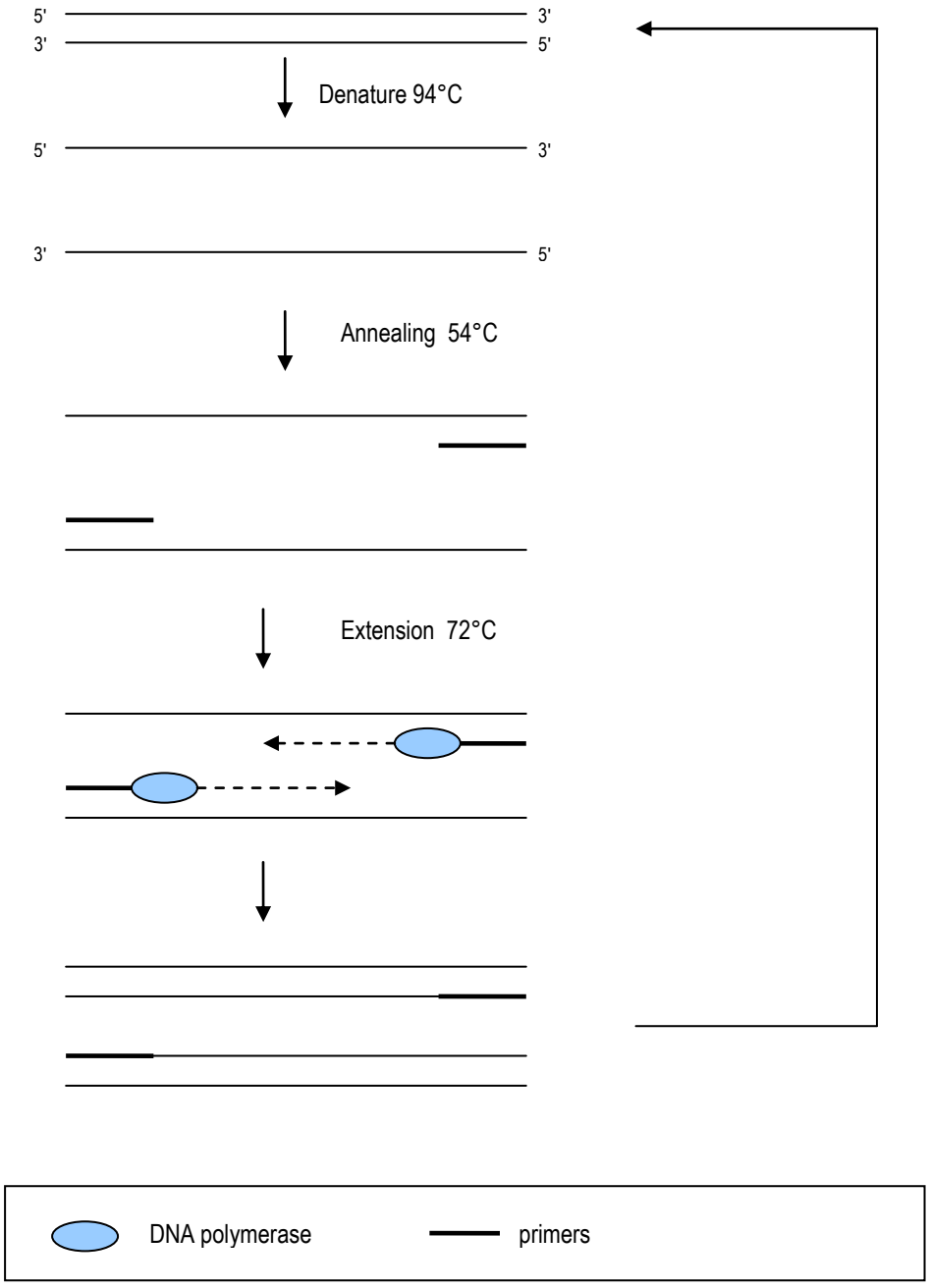


Figure 9 **Schematic diagram of polymerase chain reaction**

Appendix E Indicative evaluation

Table 45 Positive and negative predictive values of molecular testing and serum erythropoietin levels in the investigation of suspected PV

Sensitivity (%)	Specificity (%)	PPV_1 (%)	PPV_2 (%)	NPV_3 (%)	NPV_4 (%)
100	100	100	100	100	100
99	99	99	60	100	99
95	95	93	22	100	94
90	90	86	12	100	88
85	85	79	8	100	82
80	80	73	6	99	76
75	75	67	4	99	70
70	70	61	3	99	65
65	65	55	3	99	59
60	60	50	2	98	54
55	55	45	2	98	49
50	50	40	2	98	44

PPV_1 = positive predictive value of a *JAK2* V617F-positive result and low serum erythropoietin levels; PPV_2 = positive predictive value of a *JAK2* V617F-positive result and normal or increased serum erythropoietin levels; NPV_3 = negative predictive value of a *JAK2* V617F-negative result and low serum erythropoietin levels; NPV_4 = negative predictive value of a *JAK2* V617F-negative result and normal or increased serum erythropoietin levels

Table 46 Positive and negative predictive values of molecular testing in the investigation of suspected ET

Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
100	100	100	100
99	99	98	98
95	95	90	90
90	90	82	82
85	85	74	74
80	80	66	66
75	75	60	60
70	70	53	53
65	65	48	48
60	60	42	42
55	55	38	38
50	50	33	33

PPV = positive predictive value of a *JAK2* V617F-positive result; NPV = negative predictive value of a *JAK2* V617F-negative result

Appendix F Excluded studies

Incorrect intervention

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Part B - Molecular testing for the diagnosis of systemic mast cell disease, hypereosinophilic syndromes and chronic eosinophilic leukaemia

December 2009

MSAC application 1125B

Assessment report

Executive summary

A rigorous assessment of evidence is the basis of decision-making when funding is sought under Medicare. A team from Adelaide Health Technology Assessment (AHTA), University of Adelaide, was engaged to conduct a systematic review of the literature and an economic evaluation of molecular testing in myeloproliferative disorders (MPDs). An Advisory Panel with expertise in this area provided assistance to AHTA in this assessment of the safety, effectiveness and cost-effectiveness of molecular testing in MPDs.

The test

Systemic mast cell disease

Molecular testing in patients suspected of systemic mast cell disease (SMCD) enables detection of relevant mutations known to occur in patients with this disease. Specific mutations include the *KIT* D816V mutation and, although other *KIT* mutations are known to occur in SMCD, the D816V alteration is the most prevalent.

In a small subset of patients with SMCD who also present with eosinophilia, the presence of the *FIP1L1-PDGFRA* rearrangement predicts a response to imatinib mesylate therapy. In contrast, the presence of the *KIT* D816V mutation in patients with SMCD can indicate that patients will be refractory to imatinib mesylate.

Molecular testing alone is not sufficient in the diagnosis of SMCD; rather, it is used in addition to bone marrow (BM) biopsy, serum tryptase levels and flow cytometry. The methodology required to determine the presence of a relevant genetic alteration is mostly dependent on the type of mutation being considered. For *KIT* mutations, qualitative polymerase chain reaction (PCR)-based methods are adequate to reliably detect the presence of a mutation. Due to the low level of mast cells in the peripheral circulation, it is appropriate to conduct this analysis on genetic material obtained from BM biopsy.

The detection of the *FIP1L1-PDGFRA* fusion gene or other genetic rearrangements requires more complex methods such as reverse transcriptase PCR or fluorescent in-situ hybridisation to detect a deletion of genetic material that includes the *CHIC2* gene, and results in the fusion of the *FIP1L1* and *PDGFRA* genes.

The comparator test strategy, against which molecular testing for the investigation of suspected SMCD is assessed, is all available clinical and laboratory information, which can include BM biopsy, serum tryptase levels and flow cytometry.

Hypereosinophilic syndrome and chronic eosinophilic leukaemia

Evidence of clonal eosinophilia, either the presence of a relevant genetic alteration or otherwise, enables a diagnosis of chronic eosinophilic leukaemia (CEL); the absence of such evidence allows a diagnosis of hypereosinophilic syndrome (HES). Molecular testing in patients with persistent eosinophilia can provide evidence of a clonal eosinophilic disorder. In addition, the presence of the *FIP1L1-PDGFRA* or other *PDGFR* rearrangements can predict the patient's successful treatment by imatinib mesylate.

Patients with HES associated with abnormal lymphocytes would undergo further molecular analysis of the *FGFR1* gene, and the presence of this gene would exclude a diagnosis of T-cell-associated HES.

As is the case for SMCD, molecular testing for a genetic rearrangement is insufficient for a diagnosis of HES or CEL. Molecular analysis, determination of serum tryptase levels and echocardiography are also required.

Assessment of molecular testing for the diagnosis of myeloproliferative disorders

Clinical need

Systemic mast cell disease

There are no readily available data regarding the clinical need for molecular testing in the diagnosis of SMCD in Australia. Expert opinion suggests that this disorder is very rare in clinical practice and that less than 150 people would be investigated per year for SMCD.

Hypereosinophilic syndrome and chronic eosinophilic leukaemia

Again, there is an absence of data regarding the clinical need and burden of disease of HES or CEL in Australia. The expert opinion of the Advisory Panel indicated that these disorders were likely to be rarer than SMCD in clinical practice and estimated that up to 50 investigations for HES or CEL would be required per year in Australia.

Safety

Systemic mast cell disease

No evidence was identified in the literature searches that reported safety outcomes associated with the addition of molecular analysis to the investigation of SMCD.

The assessment of *KIT* mutations and genetic arrangements of *PDGFRA*, *PDGFRB* or *FGFR1* requires the extraction of genetic material from peripheral blood or BM biopsy. The known risks involved with obtaining peripheral blood are slight although there is a greater risk of adverse events associated with BM biopsy. Nonetheless, the overall risk associated with sample collection would be considered small.

As patients would provide a peripheral blood sample and BM biopsy regardless of the testing strategy they underwent, genetic material could be obtained from these without the need for further sample collection. As such, the safety of the addition of molecular analysis to the investigation of SMCD or HES and CEL would be considered to be as safe as an investigation without molecular analysis.

Hypereosinophilic syndrome and chronic eosinophilic leukaemia

No evidence regarding the safety of molecular analysis in the investigation of HES or CEL was identified.

As suggested for SMCD, the genetic material required for molecular analysis can be obtained from BM biopsy or peripheral blood samples, both of which would be required

prior to undergoing molecular testing. Consequently, the addition of molecular analysis to the testing strategy would require no additional sample collection and would therefore be at least as safe as the comparator test strategy.

Effectiveness

Systemic mast cell disease

Although no direct evidence comparing the investigation of suspected SMCD with and without the addition of molecular analysis was available, one study provided weak evidence that additional molecular testing improved the health outcomes of the small subset of patients with SMCD associated with eosinophilia.

Due to the limited nature of the direct evidence, a linked evidence approach was undertaken in an attempt to link evidence of diagnostic accuracy and change in management to a change in patient health outcomes. Studies of diagnostic accuracy reported only on the analysis of *KIT* mutations in patients with and without SMCD. Excellent test specificity was reported, indicating that molecular analysis of *KIT* mutations is unlikely to result in a false positive result. However, sensitivity of 88–99% suggests that the absence of a *KIT* mutation is unable to rule out the presence of SMCD. The potential for false negative results with regard to *KIT* mutations increases the risk of inappropriate treatment, as the presence of the *KIT* D816V mutation indicates that a patient would not be responsive to imatinib mesylate.

No studies were available that reported a change in management subsequent to patients undergoing molecular analysis; however, the direct evidence included in this assessment implies that patients identified as having the *FIP1L1-PDGFRA* rearrangement would receive imatinib mesylate therapy. Additionally, studies in other populations with primary eosinophilia have reported that the *FIP1L1-PDGFRA* rearrangement is a target for imatinib mesylate therapy, providing further argument that the addition of molecular analysis to the testing strategy for patients with suspected SMCD associated with eosinophilia is likely to result in a change of management.

As only patients with SMCD associated with eosinophilia are likely to undergo a change in management, treatment effectiveness was assessed in these patients. Low-level evidence, which was further limited by small patient numbers, indicated that patients with the *CHIC2* deletion (a surrogate marker of the *FIP1L1-PDGFRA* rearrangement) would benefit considerably from imatinib mesylate therapy compared with patients who carried the *KIT* D816V mutation.

Overall, the linked evidence approach consisted of low-level evidence that was often limited by small patient numbers. With further evidence unlikely to become available due to the very low prevalence of SMCD, it is expected that investigation of patients with SMCD with the addition of molecular analysis is likely to be at least as effective as the comparator test strategy (without molecular analysis). In patients with suspected SMCD associated with a persistent eosinophilia, the addition of molecular analysis to the test strategy is likely to be more effective than the comparator in providing improved patient outcomes. It should be noted that molecular analysis in patients with suspected SMCD should consist of both *KIT* and *FIP1L1-PDGFRA* analysis, as the absence of a relevant *KIT* mutation does not necessarily indicate the presence of the *FIP1L1-PDGFRA* or, subsequently, predict a response to imatinib mesylate.

Hypereosinophilic syndrome and chronic eosinophilic leukaemia

No studies were available that considered the use of molecular analysis of the *FGFR1* gene in the diagnosis of T-cell-associated HES.

Direct evidence of effectiveness of molecular testing in the diagnosis of HES and CEL was limited to a small case series that provided weak evidence of a benefit to patients diagnosed with CEL with the addition of molecular analysis and, subsequently, receiving treatment with imatinib mesylate. However, no comparative evidence was available that compared the health consequences of diagnosis with molecular analysis to diagnosis without.

Consequently, a linked evidence approach was undertaken. As the use of molecular analysis will not change patient management in this group[2], it is necessary to establish that it provides improved diagnostic accuracy and, subsequently, improved patient outcomes from more accurate diagnosis.

Only low-level evidence was available to assess test accuracy and results were inconsistent. In the absence of comparative data, it is not possible to establish the accuracy of diagnosis with molecular analysis; however, as it would be used in addition to the comparator, it is likely that it would be at least as accurate as diagnosis without molecular analysis.

Economic considerations

Systemic mast cell disease

In the absence of suitable data and some uncertainty regarding the extent of any net benefit of molecular analysis in the diagnosis of SMCD, a cost-effectiveness analysis was not conducted. Rather, a financial analysis of the cost implications associated with the addition of molecular analysis to the diagnostic strategy for this population was undertaken.

With an expected 134 investigations required per year, it is estimated that 80% of investigations would be eligible for Medicare reimbursement. Consequently, the cost implications of the addition of molecular analysis of both *KIT* and *FIP1L1-PDGFRA* to the diagnostic strategy would result in a cost of $22,000 per year.

The cost to the Australian healthcare system overall, including the cost of treatment with imatinib mesylate in patients with SMCD associated with eosinophilia and the *FIP1L1-PDGFRA* rearrangement, would result in an additional cost of $234,000 per year. The majority of this can be attributed to the cost of imatinib mesylate therapy.

Hypereosinophilic syndrome and chronic eosinophilic leukaemia

The absence of comparative evidence evaluating the effectiveness of molecular analysis in the diagnosis of HES and CEL prevented a formal economic evaluation being conducted. Consequently, the direct costs of the addition of molecular analysis in the diagnosis of HES and CEL were considered with respect to the Australian healthcare

[2] It has already been established that imatinib mesylate is an effective therapy for patients with CEL.

system overall and to the Commonwealth as a consequence of the Medicare rebate for private patients.

Due to the complete absence of data regarding the use of *FGFR1* analysis in the diagnosis of HES, testing for this rearrangement was not considered in the financial analysis.

The expert opinion of the Advisory Panel estimated that up to 50 investigations of suspected HES or CEL would be required per year, of which 80% would be eligible for Medicare reimbursement. Overall, the addition of molecular analysis would result in an additional burden of $11,800 to the Australian healthcare system per year. The cost to the Commonwealth as a consequence of the Medicare rebate would be $7,000 per year.

Glossary and abbreviations

±	with/without
AHNMD	associated haematological (clonal) non-mast cell lineage disease
AHTA	Adelaide Health Technology Assessment
Amplicons	double stranded DNA fragments produced by PCR amplification of a segment of DNA using specific PCR primers
Apoptosis	the process of programmed cell death that occurs as a part of normal development
BCR-ABL fusion gene	a chromosomal translocation t(9;22)(q34;q11) associated with chronic myeloid leukaemia— also called the Philadelphia chromosome
CD	cluster designation marker
CEL	chronic eosinophilic leukaemia
CML	chronic myeloid leukaemia
CMPD	chronic myeloproliferative disorders
DNA	deoxyribonucleic acid
EMS	8p11 myeloproliferative disorder
Eosinophil	a white blood cell which contains granules that are involved in allergic inflammatory reactions
FBE	full blood examination
FGFR1	gene encoding for fibroblast growth factor receptor 1
FIP1L1-PDGFRA	gene rearrangement resulting from the deletion of part of the long arm of chromosome 4
FISH	fluorescent in-situ hybridisation—a cytogenetic technique used to detect the presence or absence of specific DNA sequences on chromosomes, particularly if they have been involved in a translocation; fluorescent probes bind to only those parts of the chromosome with which they show a high degree of sequence similarity
Flow cytometry	a technique used to identify and sort cells using fluorescent dyes—fluorescent emitted light and direction of light scatter (forward scatter indicates cell volume and side scatter indicates inner complexity of cell) enable identification of cells
HES	hypereosinophilic syndrome
Interstitial deletion	an internal deletion of a chromosome of varying size that might not be identified by traditional G-banding techniques

KIT	gene encoding for a tyrosine kinase receptor (CD117) for stem cell factor—CD117 is found on the surface of haematopoietic stem cells
Lymphadenopathy	swollen lymph nodes throughout the body
Mast cell	cells found in several types of tissue and containing granules rich in histamine and heparin—mast cells play an important role in allergy and anaphylaxis
MBS	Medicare Benefits Schedule
MPD	myeloproliferative disorder/disease
mRNA	messenger RNA
MSAC	Medical Services Advisory Committee
NHMRC	National Health and Medical Research Council
PBS	Pharmaceutical Benefits Scheme
PCR	polymerase chain reaction
PDGFRA	gene encoding for platelet-derived growth factor α
PDGFRB	gene encoding for platelet-derived growth factor β
PNA	peptide nucleic acid
RFLP	restriction fragment length polymorphism
RT-PCR	reverse transcriptase PCR *Note: The abbreviation RT-PCR may also be used for real-time PCR; however, in this report it will only refer to reverse transcriptase PCR.*
SMCD	systemic mast cell disease
SNP	single nucleotide polymorphism—a variation in DNA sequence that occurs when a single nucleotide in a genome is altered
TGA	Therapeutic Goods Administration
Translocation	the movement or reciprocal exchange of large chromosomal segments, typically between two different chromosomes
WHO	World Health Organization

Introduction

Adelaide Health Technology Assessment (AHTA), with input and advice from an appropriately constituted Advisory Panel of experts (see Appendix G), has reviewed the current evidence for molecular testing for the diagnosis of *BCR-ABL*-negative chronic myeloproliferative disorders (MPDs) and, in particular, of systemic mast cell disease (SMCD), hypereosinophilic syndrome (HES) and chronic eosinophilic leukaemia (CEL). This assessment report is intended for the Medical Services Advisory Committee (MSAC). The MSAC evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Schedule (MBS) in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. The MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

The MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, surgery, internal medicine and general practice, clinical epidemiology, health economics, consumer health and health administration.

Background

Molecular testing for the diagnosis of myeloproliferative disorders—SMCD, HES and CEL

Mutations in SMCD

The *KIT* gene is located on chromosome 4 and encodes for a cell surface receptor known as a receptor tyrosine kinase. The specific molecule that binds to this receptor is stem cell factor (SCF), which, when bound, results in activation of cellular proliferation and activation pathways (Tan et al 2006). The *KIT* receptor tyrosine kinase (also known as CD117) is expressed on mast cells, haematopoietic stem cells, germ cells, melanocytes and some cells of the gastrointestinal tract (Pardanani et al 2006a).

Somatic point mutations, or single nucleotide polymorphisms (SNPs) in *KIT* have been reported in patients with all subtypes of systemic mast cell disease (SMCD) (Lim et al 2008). In particular, the mutation at codon 816, which results in an aspartic acid to valine substitution, has been associated with patients with SMCD. There is some uncertainty with regard to the prevalence of this mutation in patients with SMCD, possibly attributable to attempts to detect the mutation in different cell types or the use of different assays for detection (Lim et al 2008). However, the likely frequency of this mutation is between 70% and 90% (Valent et al 2007). Although the D816V mutation is the predominant SNP detected in patients with SMCD, other variants at codon 816 have also been associated with SMCD, including D816Y, D816F and D816H mutations (Horny et al 2007).

Approximately half of the patients who have a subtype of SMCD associated with eosinophilia will have the *FIP1L1-PDGFRA* gene rearrangement. The *KIT* mutation and the *FIP1L1-PDGFRA* rearrangement appear to be mutually exclusive (Pardanani et al 2006a). *FIP1L1-PDGFRA* results from a deletion of genetic material at chromosome 4q12 (ie region 12 on the long arm of chromosome 4) that is not apparent in normal karyotyping techniques (Pardanani et al 2006a). Within this deletion of genetic material is the *CHIC2* gene, whose absence may be used as a surrogate marker for the *FIP1L1-PDGFRA* fusion gene (Fink et al 2009).

Mutations in HES and CEL

As in SMCD, the *FIP1L1-PDGFRA* fusion gene is present in many patients with hypereosinophilia, and is indicative of a clonal origin of the eosinophils (Gotlib 2005). The *FIP1L1-PDGFRA* fusion gene results in deregulation of platelet derived growth factor α (PDGFRA) activity, and prevents activation by its ligand (Gotlib 2005). Although the fusion gene is characterised by an interstitial deletion at 4q12, there is a degree of heterogeneity with regard to the breakpoints in the respective partner gene that must be considered when using molecular techniques to identify the rearrangement (Gotlib 2005).

Rearrangements of the platelet-derived growth factor β (*PDGFRB)* gene involve translocations of 5q31-33 with various partner genes (Table 47) (Macdonald & Cross 2007). As this rearrangement involves the transfer of genetic material, as opposed to the interstitial deletion seen in the *FIP1L1-PDGFRA* fusion gene, it may be evident on

cytogenetic analysis of the bone marrow (BM) (Macdonald & Cross 2007). The *PDGFRB* fusion gene not only results in a constitutive activation of its receptor protein tyrosine kinase, but it may also be influenced by the genetic regulatory elements associated with the partner gene. As a consequence, there may be abnormal expression of *PDGFRB* (Macdonald & Cross 2007).

Table 47 Potential fusion genes associated with myeloproliferative disorders

Receptor protein tyrosine kinase gene	Possible partner genes	
PDGFRA	*FIP1L1*	*BCR*
	KIF5B	*ETV6*
	CDK5RAP2	
PDGFRB	*PDE4PIP*	*NIN*
	WDR48	*KIAA1509*
	GOLGA4	*CEV14*
	ETV6	*HCMOGT1*
	HIP1	*RABEP1*
	H4	*TP53FP1*
FGFR1	*ZNF198*	*CEP1*
	BCR	*TIP1*
	HERV-K	*FGFR1OP1*
	MYO18A	*FGFR1OP2*

Adapted from Macdonald & Cross (2007); *PDGFRA* = platelet-derived growth factor α; *PDGFRB* = platelet derived growth factor β; *FGFR1* = fibroblast growth factor 1

FGFR1 fusion genes result in constitutive activation of fibroblast growth factor receptor 1 (FGFR1), a receptor tyrosine kinase involved in activation of pathways associated with cellular proliferation and apoptosis. The most frequent translocation usually involves *ZNF198* at chromosome 13q12; however, a number of partner genes are associated with reciprocal translocations with chromosome 8p11 (Table 47) (Cross & Reiter 2008). Translocations involving this locus are generally associated with a phenotype that resembles T-cell lymphoblastic lymphoma, BM myeloid hyperplasia and marked eosinophilia (Gotlib et al 2006).

Methods for molecular testing in SMCD, HES and CEL

Detection of *KIT* mutations

Detection of the *KIT* D816V or other SNPs of clinical relevance in SMCD is achieved through the use of methods based on the polymerase chain reaction (PCR). Commercially produced kits are not available for the detection of *KIT* mutations in the diagnosis of mast cell disease. Consequently, in-house protocols have been developed for use in molecular pathology laboratories in Australia.

The number of mast cells present in a patient's sample will impact on the assay sensitivity required to detect the D816V mutation, which, in turn, may also be influenced by the severity of disease. As such, it is recommended that diagnostic assays are performed on either BM aspirates or mononuclear cell-enriched samples (Lim et al 2008; Valent et al 2007).

Consensus from the Working Conference on Mastocytosis (2005) recommends the use of either reverse transcriptase PCR with restriction fragment length polymorphism

analysis; peptide nucleic acid mediated PCR; or allele-specific PCR for the detection of *KIT* mutations (Valent et al 2007).

Restriction fragment length polymorphism (RFLP)

Restriction enzymes cleave DNA into fragments at specific sequence sites, and the subsequent DNA fragments can be separated by gel electrophoresis according to their size. The presence or absence of fragments of specific size can confirm the presence of a mutation.

Advantages of this technique are that it is simple and inexpensive; however, a relative lack of analytical sensitivity (20% mutant DNA in a wild-type background) may limit its usefulness in a clinical setting (Steensma 2006).

Peptide nucleic acid (PNA) PCR

Peptide nucleic acids (PNAs) are synthetic DNA molecules with a unique chemical structure that provides the molecule with properties that allow high affinity binding with DNA or RNA molecules (Pellestor et al 2005). PNAs by themselves do not interact with DNA polymerase; however, a PNA–DNA complex will be recognised by DNA polymerase and can subsequently be used as a primer in PCR reactions.

SNP detection occurs through competition for primer sites between specific PNA and DNA. If the template DNA does not contain any mismatches, the PNA will have a higher affinity for binding than the DNA primer and will therefore block binding of the primer and subsequent amplification (Orum 2000). This method is often called PNA directed PCR clamping (Figure 10).

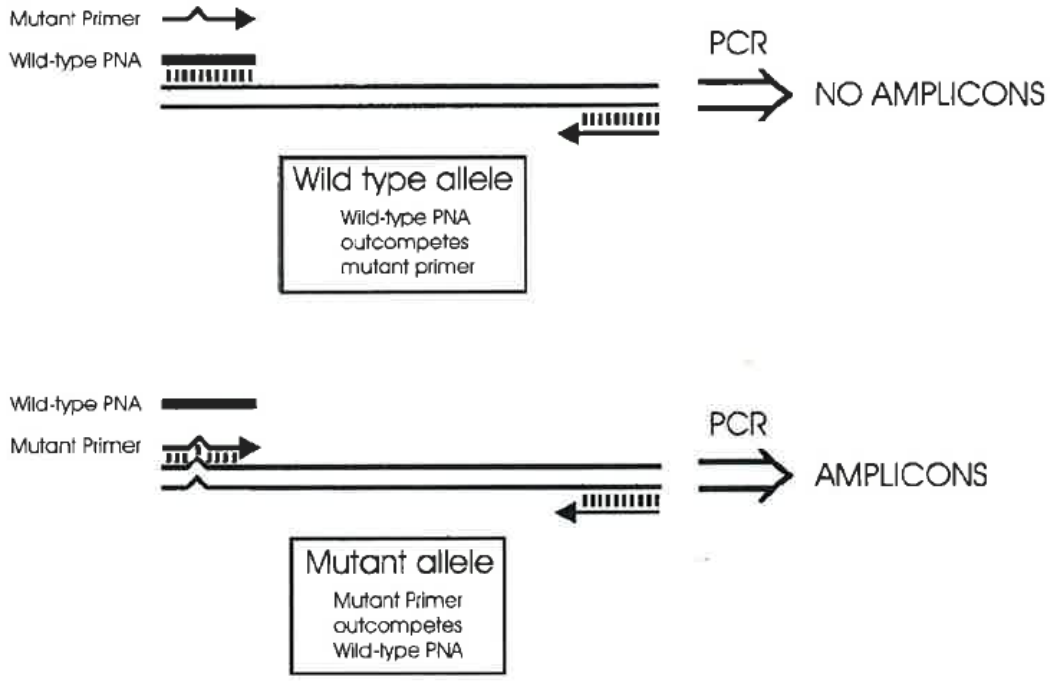


Figure 10 Schematic diagram of PNA-PCR
Figure reproduced with permission (Orum 2000)

Allele-specific PCR

Allele-specific PCR works on the principle that DNA primers, which bind to complementary DNA sequences, will only be extended by DNA polymerase if there are no mismatches between the DNA template and the primer (Newton et al 1989). If the sequence of the primer is altered to reflect the specific point mutation of interest, there will only be PCR amplification if the mutant allele is present (Steensma 2006).

In practice, an additional mismatch is often required to increase the specificity of the PCR reaction to amplify only the mutant allele (Newton et al 1989).

Detection of molecular rearrangements

Genomic DNA comprises regions called introns and exons. Exons provide the coding region of DNA, and are transcribed into RNA and then translated into proteins. Introns are the non-coding regions of DNA and are removed during transcription by mRNA splicing. Introns can vary in size from 100 base pairs to over 100,000 base pairs (Degos et al 2005).

The majority of chromosomal translocations have breakpoints in introns but the location of these breakpoints can vary widely between patients. As such, the amplification of the fusion products from DNA is unreliable with a standard set of primers (Degos et al 2005).

Reverse transcriptase PCR (RT-PCR)

Removal of introns during messenger RNA (mRNA) splicing generally results in a consistent fusion product which can then be reliably amplified using a standard set of primers (Degos et al 2005). Similar to PCR, which amplifies specific segments of DNA, RT-PCR amplifies specific segments of RNA. This requires reverse transcription of the RNA segment into the complementary DNA strand, which is then amplified by PCR. RT-PCR is a highly sensitive method for the detection of specific mRNA, with the ability to detect and amplify the mRNA from a single cell (O'Connell 2002).

Fluorescent in-situ hybridisation (FISH)

FISH enables visualisation of genetic rearrangements through the use of fluorescently tagged single strand DNA probes. The probes are specific for a particular locus or gene and bind to their respective complementary chromosomal DNA. This makes it possible to determine the location of the gene and whether or not there has been a genetic rearrangement (Figure 11).

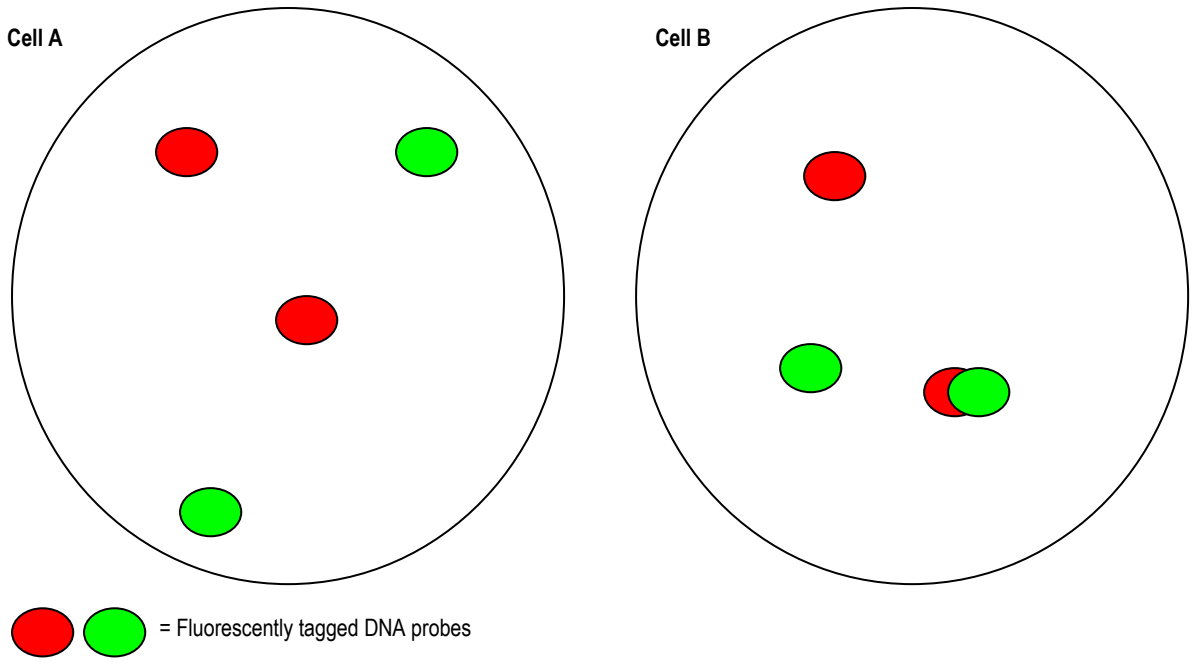


Figure 11 Schematic diagram of fluorescent in-situ hybridisation demonstrating normal and fusion genes

Cell A represents a normal cell without genetic rearrangement; Cell B demonstrates a fusion gene with overlap of red and green tagged probes.

Intended purpose

Molecular testing for *KIT* mutations or *PDGFRA, PDGFRB* or *FGFR1* rearrangements will be performed when patients are suspected of having SMCD, HES or CEL. In addition, molecular testing may be used to distinguish SMCD from cutaneous mast cell disease.

SMCD

Patients suspected of SMCD may present with varying symptoms or, possibly, be asymptomatic. Symptomatic patients are likely to experience the effects of mast cell mediators, which may include pruritus, tachycardia, hypotension, headache, and gastrointestinal symptoms such as nausea, vomiting and diarrhoea (Butterfield 2006).

Patients who have SMCD associated with another clonal haematological disorder will also experience symptoms related to the non-mast cell haematological disorder. Patients with aggressive SMCD are likely to present with symptoms related to compromised organ function as a result of mast cell infiltration (Butterfield 2006).

Further investigations can include a BM biopsy including immunohistochemical staining, serum tryptase levels and flow cytometry. Molecular testing will be performed to determine the presence of a clinically relevant *KIT* mutation. If the patient has also presented with eosinophilia, testing for rearrangements of *PDGFRA* or *PDGFRB* genes will also be performed.

Patients for whom there is a suspicion of urticaria pigmentosa or cutaneous mast cell disease may be tested for the presence of *KIT* mutations to enable SMCD to be ruled out.

HES and CEL

Primary eosinophilia will be associated with symptoms related to the damage of organs and tissues following infiltration by eosinophils. These symptoms are variable and depend on the organs or tissues involved. The organs most commonly targeted by eosinophils include the skin, heart, lungs, gastrointestinal system and central nervous system (Roufosse et al 2007).

Full blood examinations demonstrating a persistent eosinophilia over a period of at least 6 months will result in the strong suspicion of HES or CEL, once reactive causes of eosinophilia have been excluded.

Patients with suspected HES or CEL will be further investigated by echocardiography, determination of serum tryptase levels and molecular testing to determine whether genetic rearrangements of *PDGFRA* (including *FIP1L1-PDGFRA*) or *PDGFRB* are present. The presence of these molecular rearrangements will establish clonality of the eosinophils and provide a diagnosis of CEL; its absence, along with no other signs of clonality, will provide a diagnosis of HES. For patients with HES associated with an abnormal T-cell population, further investigation by lymph node biopsy and molecular testing for the presence of the *FGFR1* rearrangement would be performed. The presence of *FGFR1* rearrangements would rule out a diagnosis of T-cell-associated HES and provide a diagnosis of 8p11 myeloproliferative syndrome.

Clinical need

Data on the clinical need and burden of disease regarding SMCD, HES and CEL in Australia are scant. Specific information relating to the burden of these diseases in Australia is not readily available. The expert opinion of the Advisory Panel indicates that these disorders are very rare in clinical practice.

A survey of 56 public, academic and private laboratories in Australia who provide molecular testing services indicated that less than 200 assays each for *FGFR1*, *FIP1L1-PDGFRA* and *KIT* mutations were being performed per year (Royal College of Pathologists of Australasia 2008). The rate of testing for *FGFR1*, *FIP1L1-PDGFRA* and *KIT* mutations was 8.2, 5.3 and 8.3 tests per 1 million population respectively (Royal College of Pathologists of Australasia 2008).

The number of assays performed for these mutations are substantially less than those for testing for the *BCR-ABL* rearrangement and *JAK2* mutations (Figure 12).

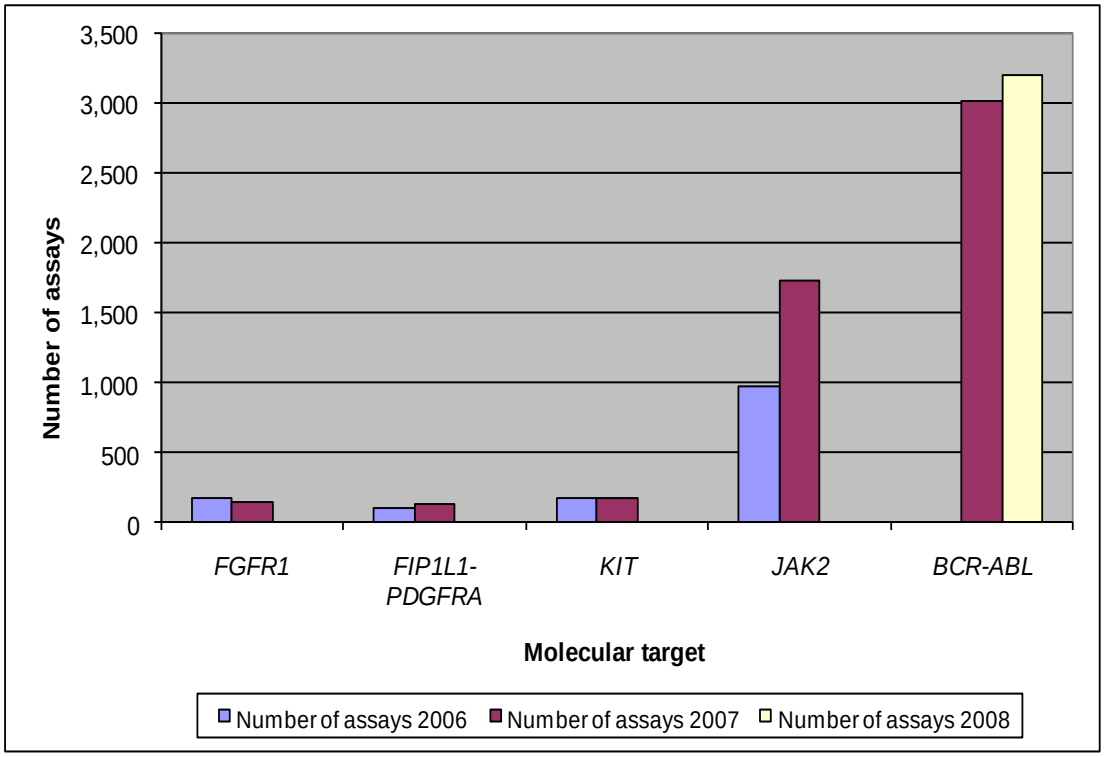


Figure 12 Molecular testing in the diagnosis of myeloproliferative neoplasms in Australia

Source: Department of Health & Ageing 2009; Royal College of Pathologists of Australasia (2008)
Note: Molecular testing for *KIT* mutations may also include testing for the diagnosis of gastrointestinal stromal tumours.

Existing tests

Molecular testing for SMCD, HES or CEL would be used in addition to the comparator, which comprises all available clinical and laboratory information required for diagnosis other than molecular analysis.

In patients with suspected SMCD, this includes BM biopsy including immunohistochemical staining and testing of serum tryptase levels. For a diagnosis of SMCD, BM biopsy should demonstrate characteristic mast cell lesions and immunohistochemical staining should indicate the presence of CD25 and CD117 on the surface of mast cells (Valent et al 2007).

For HES and CEL, diagnosis may include the determination of serum tryptase levels and echocardiography and BM biopsy. Elevated serum tryptase levels would be indicative of

SMCD associated with eosinophilia, and echocardiography will determine the presence and extent of cardiac involvement and any related damage or abnormalities (Ommen et al 2000; Roufosse et al 2007).

A diagnosis of CEL requires evidence of clonality, which may not be apparent upon cytogenetic analysis. Clinical features such as hepatomegaly and/or splenomegaly, along with an increase in blast cells in the peripheral blood or BM, may be sufficient for a diagnosis of CEL (Bain 2004).

Treatment for SMCD, HES and CEL

SMCD

As patients often have widely varying symptoms, it is important that treatment be directed towards those symptoms (Horny et al 2007). All patients who experience mast cell mediated symptoms such as pruritus or itching, anaphylaxis or hypotension would be advised to avoid triggers for mast cell degranulation and, in addition, would be prescribed corticosteroids (Valent et al 2007).

Patients with SMCD with an associated clonal haematological non-mast cell lineage disease (AHNMD) will be treated independently for both the SMCD and the AHNMD component. Those who have clinical progression will require cytoreductive therapy in the form of interferon-α or cladribine (Horny et al 2007).

Recent evidence suggests that patients who present with eosinophilia associated with the SMCD may carry a known target of imatinib mesylate in the form of the *FIP1L1-PDGFRA* rearrangement (Horny et al 2007). It is recommended that if conventional therapy in the form of corticosteroids and interferon-α is unsuccessful, imatinib mesylate should be administered.

HES and CEL

The primary indication for treatment of HES is evidence of eosinophil-mediated tissue injury (Tefferi 2005). First-line therapy for patients involves immunosuppressive corticosteroids and hydroxyurea to reduce the eosinophil count (Roufosse et al 2007). If this proves unsuccessful, interferon-α may be used as a second-line treatment (Tefferi 2005).

Evidence of clonal eosinophilia in the form of the *FIP1L1-PDGFRA* rearrangement or other *PDGFRA* or *PDGFRB* rearrangements indicates that patients would be responsive to imatinib mesylate (Tefferi 2005). If evidence of clonality other than an imatinib mesylate-responsive molecular rearrangement is present (eg increased blast cells in the BM), a trial of imatinib mesylate is recommended. If this is not successful in reducing the eosinophil count, conventional therapy such as corticosteroid and hydroxyurea is recommended (Roufosse et al 2007).

Potential impact of the test

The potential impact of molecular testing in the diagnosis of SMCD, HES or CEL is likely to be significant. With respect to SMCD diagnosis, the presence of a *KIT* mutation is likely to simplify the diagnostic process as well as have an impact on the management of patients with SMCD. The presence of the *KIT* mutation provides an indication that

the patient will be resistant to imatinib mesylate therapy. In contrast, for patients with SMCD associated with eosinophilia, the presence of the *FIP1L1-PDGFRA* rearrangement suggests that the patient will respond successfully to treatment with imatinib mesylate.

The differential diagnosis of HES and CEL will be made significantly easier with the addition of molecular testing. CEL is defined as a clonal disorder of eosinophils, and the presence of rearrangements of *PDGFRA* or *PDGFRB* provide evidence of such clonality. In cases of eosinophilia and an abnormal T-cell population, the presence of an *FGFR1* rearrangement will differentiate between 8p11 myeloproliferative syndrome and T-cell-associated hypereosinophilia.

Marketing status of molecular testing for myeloproliferative disorders

Testing for these mutations and gene rearrangements, using assays developed in house, is available at a limited number of centres in Australia.

The use of in-house techniques does not require approval from the Therapeutic Goods Administration (TGA). If a diagnostic commercial kit were to become available, it would require approval by the TGA.

Current reimbursement arrangements

Currently, there are no items on the MBS that allow reimbursement for molecular testing used in the diagnosis of SMCD, HES or CEL. Reimbursement is available for molecular testing for other unrelated indications as outlined in Table 48.

The disparity in the schedule fees in Table 48 is related to the methodologies used to detect molecular abnormalities. Detection of simple genetic mutations, requiring a relatively simple molecular assay such as for the C282Y mutation, are associated with a substantially smaller schedule fee compared with the detection of gene rearrangements such as *BCR-ABL*, which require a more complex assay.

Table 48 MBS items covering the detection of genetic mutations

Item 73308	Characterisation of the genotype of a patient for Factor V Leiden gene mutation, or detection of the other relevant mutations in the investigation of proven venous thrombosis or pulmonary embolism - 1 or more tests Fee: $36.70
Item 73317	Detection of the C282Y genetic mutation of the HFE gene and, if performed, detection of other mutations for haemochromatosis where: (a) the patient has an elevated transferrin saturation or elevated serum ferritin on testing of repeated specimens; or (b) the patient has a first degree relative with haemochromatosis; or (c) the patient has a first degree relative with homozygosity for the C282Y genetic mutation, or with compound heterozygosity for recognised genetic mutations for haemochromatosis (Item is subject to rule 20) Fee: $36.70
Item 73320	Detection of HLA-B27 by nucleic acid amplification includes a service described in 71147 unless the service in item 73320 is rendered as a pathologist determinable service. (Item is subject to rule 27) Fee: $40.80
Item 73314	Characterisation of gene rearrangement or the identification of mutations within a known gene rearrangement, in the diagnosis and monitoring of patients with laboratory evidence of: (a) acute myeloid leukaemia; or (b) acute promyelocytic leukaemia; or (c) acute lymphoid leukaemia; or (d) chronic myeloid leukaemia. Fee: $232.50

Source: Department of Health and Ageing (2009)

Approach to assessment

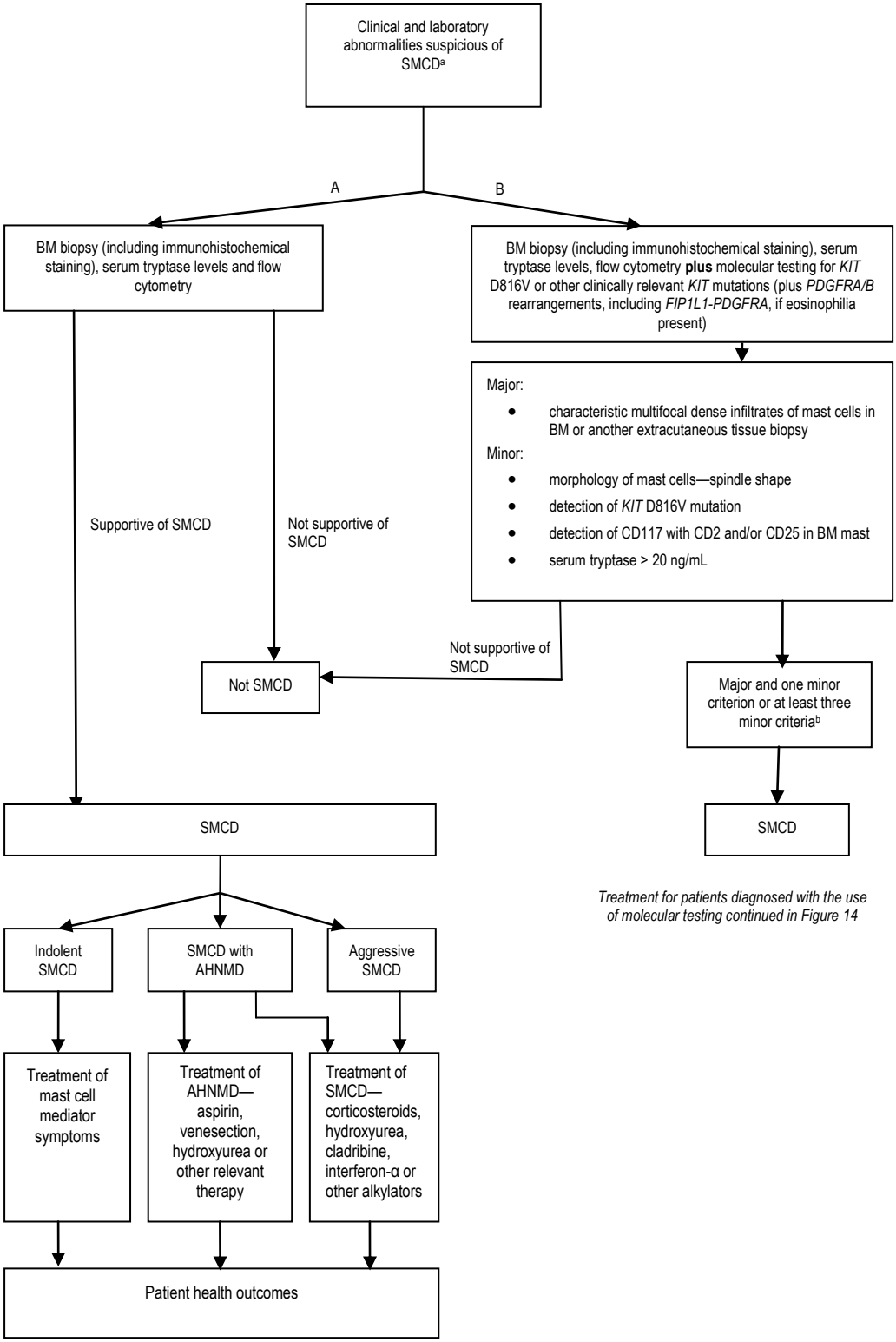
Objective

The objective of this assessment is to determine whether there is sufficient evidence in relation to clinical need, safety, effectiveness and cost-effectiveness to recommend public funding for molecular testing in the diagnosis of MPDs, in particular, in regard to systemic mast cell disease (SMCD), hypereosinophilic syndrome (HES) and chronic eosinophilic leukaemia (CEL).

Clinical decision pathway

The clinical pathway for the diagnosis of SMCD is shown in Figure 13. This figure also outlines the treatment options for patients diagnosed by the comparator (ie without molecular testing). Figure 14 shows the treatment pathway for patients diagnosed with SMCD by molecular testing.

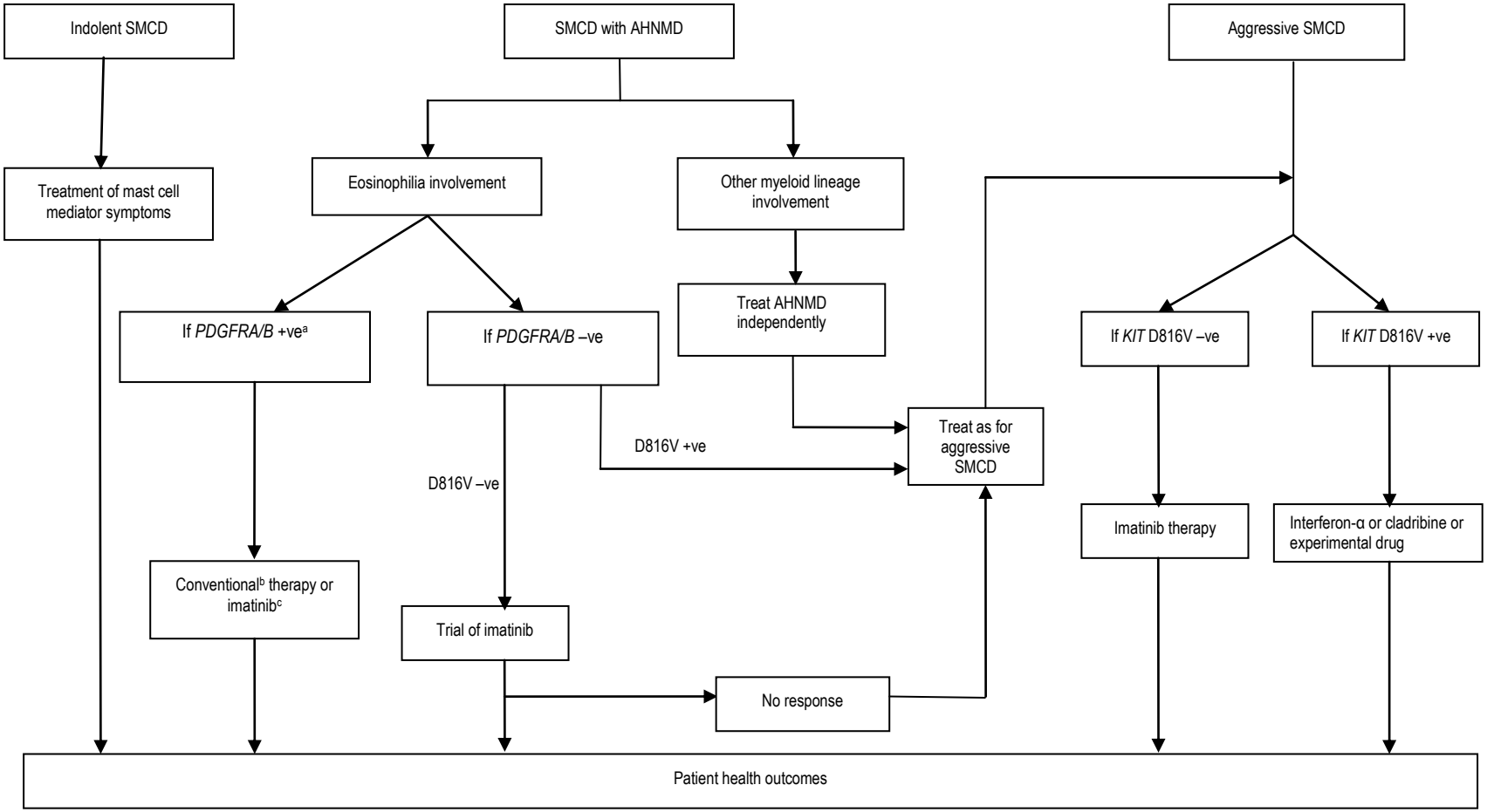
The clinical pathways for the diagnosis and treatment of HES and CEL are shown in Figure 15 and Figure 16.



A = pathway without molecular testing; B = pathway with molecular testing; SMCD = systemic mast cell disorder; BM = bone marrow; AHNMD = associated haematological clonal non-mast cell lineage disease

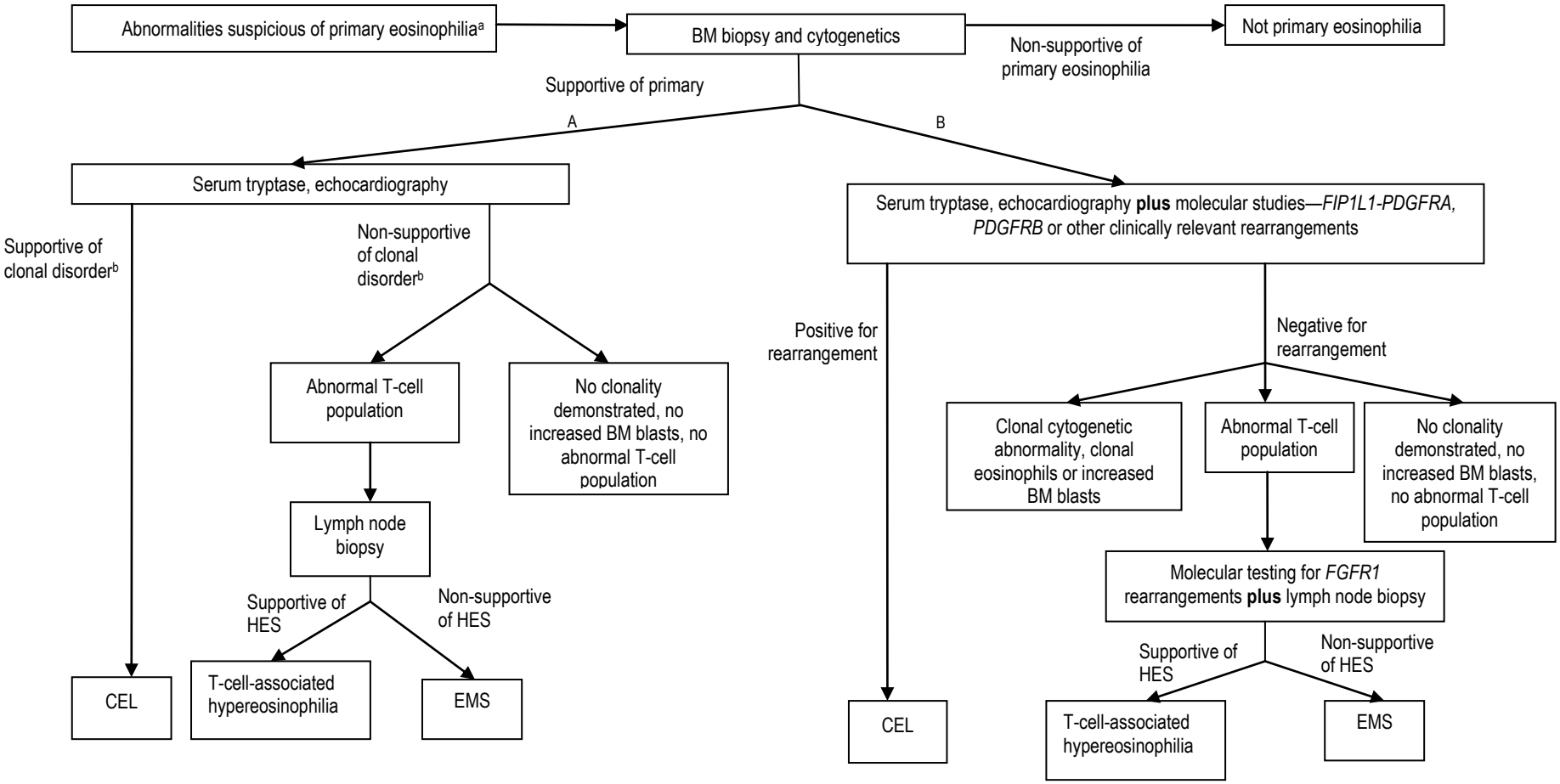
[a] This may include patients who present with urticaria pigmentosa or cutaneous mast cell disorder; [b] World Health Organization criteria for diagnosis of SMCD (Pardanani et al 2006a).

Figure 13 Clinical decision pathway for the diagnosis of SMCD



[a] *FIP1L1-PDGFRA* and D816V mutations appear to be mutually exclusive (Pardanani et al 2006a); [b] Conventional therapy may include corticosteroids, hydroxyurea or cladribine. If conventional therapy fails, patients would proceed to imatinib therapy; [c] Imatinib as a first line therapy is likely to occur as part of a clinical trial or user-pays situation; SMCD = systemic mast cell disease; AHNMD = associated haematological clonal non-mast cell lineage disease.

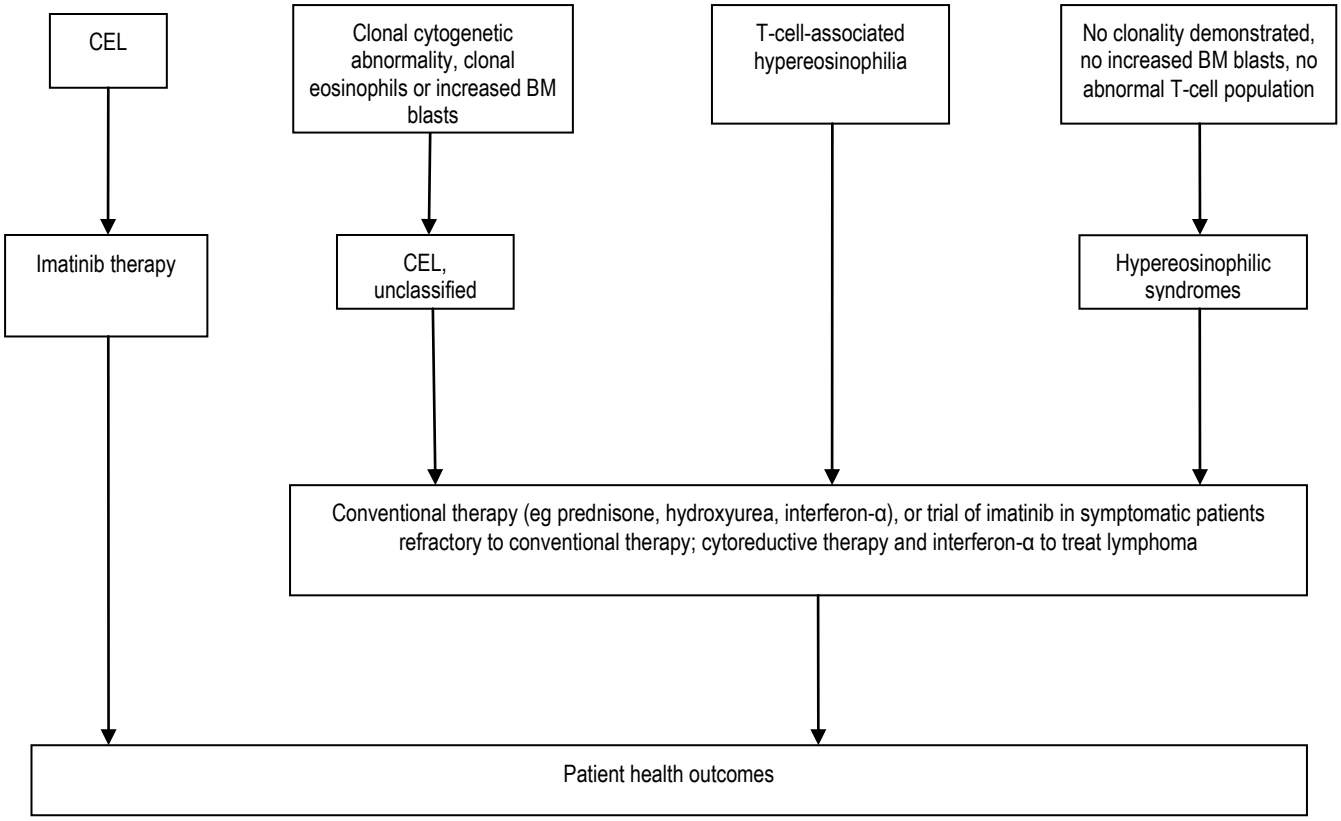
Figure 14 Clinical pathway for the treatment of SMCD following diagnosis with the addition of molecular testing



A = pathway without molecular testing; B = pathway with molecular testing; BM = bone marrow; CEL = chronic eosinophilic leukaemia; HES = hypereosinophilic syndrome; EMS = 8p11 myeloproliferative syndrome; [a] Causes of secondary eosinophilia must be excluded; [b] Eosinophilic clonal disorder requires exclusion of all neoplastic disorders with secondary eosinophilia, exclusion of other neoplastic disorders in which eosinophils are part of the neoplastic clone and exclusion of T-cell population with unusual phenotype and abnormal cytokine population (Gotlib et al 2006).

Treatment continued in Figure 16

Figure 15 Clinical pathway for the diagnosis of HES and CEL



BM = bone marrow; CEL = chronic eosinophilic leukaemia; EMS = 8p11 myeloproliferative syndrome; [a] Causes of secondary eosinophilia must be excluded; [b] Eosinophilic clonal disorder requires exclusion of all neoplastic disorders with secondary eosinophilia, exclusion of other neoplastic disorders in which eosinophils are part of the neoplastic clone and exclusion of T-cell population with unusual phenotype and abnormal cytokine population (Gotlib et al 2006).

Figure 16 Clinical pathway for the treatment of HES and CEL

Comparator

The comparator for molecular testing in the diagnosis of SMCD and HES or CEL is all available clinical and laboratory information required to make a diagnosis.

For SMCD this may include:

- bone marrow biopsy (including immunohistochemical staining)
- serum tryptase levels
- flow cytometry.

For HES or CEL this may also include:

- serum tryptase level
- echocardiography

- lymph node biopsy.

The reference standard

The myeloid neoplasm classification scheme is shown in Appendix I. The World Health Organization (WHO) criteria for the classification of SMCD, HES and CEL are outlined in Table 49.

Table 49 2001 WHO classification of SMCD, HES and CEL

Criteria for the diagnosis of SMCD
Major criteria: Multifocal, dense infiltrates of mast cells (15 or more cells in aggregates) detected in sections of BM and/or other extracutaneous organs, and confirmed by tryptase immunohistochemistry or other special stains
Minor criteria: • In biopsy sections of BM or other extracutaneous organs, more than 25% of the mast cells in the infiltrate are spindle shaped or have atypical morphology; or, of all mast cells in BM aspirate smears, more than 25% are immature or atypical mast cells • Detection of *KIT* mutations at codon 816 in BM, blood or other extracutaneous organs[a] • Mast cells in BM, blood or other extracutaneous organs that co-express CD117 with CD2 and/or CD25 • Serum total tryptase persistently > 20 ng/mL (unless there is an associated clonal myeloid disorder)
A diagnosis of SMCD is appropriate if one major and one minor criterion, or if three minor criteria, are present.
Criteria for the diagnosis of HES and CEL
Persistent eosinophilia ≥ 1.5 x 10^9/L in blood, increased number of BM eosinophils and myeloblasts < 20% in blood or marrow
Exclude all causes of reactive eosinophilia including: • allergy • parasitic disease • infectious disease • pulmonary disease • collagen vascular disease. Exclude all neoplastic disorders with secondary, reactive eosinophilia: • T-cell lymphomas • Hodgkin's lymphoma • acute lymphoblastic leukaemia/lymphoma • systemic mast cell disease. Exclude other neoplastic disorders in which eosinophilia are part of the neoplastic clone: • chronic myeloid leukaemia • acute myeloid leukaemia • other myeloproliferative diseases (PV, ET and PMF) • myelodysplastic syndromes. Exclude T-cell population with aberrant phenotype and abnormal cytokine production.
If there is no demonstrable disease that could cause eosinophilia, no abnormal T-cell population and no evidence of a clonal myeloid disorder, a diagnosis of HES is appropriate.
If all requirements are met, and if the myeloid cells demonstrate a clonal chromosomal abnormality or are shown to be clonal by other means, or if blast cells are present in the peripheral blood (> 2%) or are increased in the BM (> 5% but < 19% or nucleated BM cells), a diagnosis of CEL is appropriate.

[a] This includes other relevant mutations at codon 816; PV = polycythaemia vera; ET = essential thrombocythaemia; PMF = primary myelofibrosis; HES = hypereosinophilic syndrome; SMCD = systemic mast cell disease; CEL = chronic eosinophilic leukaemia; BM = bone marrow
Source: Jaffe et al (2001)

As stated, these criteria would be inappropriate reference standards for the diagnosis of SMCD, HES or CEL as they incorporate molecular analysis as an indicator of disease. However, as the absence of a molecular mutation or rearrangement does not rule out disease, the WHO criteria may be useful to make a diagnosis.

As such, the reference standard for diagnosis of SMCD, HES and CEL is all relevant clinical and laboratory information, other than molecular status, that is required to make a diagnosis.

Research questions

The research questions developed to assess the safety and effectiveness of including molecular testing in the diagnostic strategies for SMCD, HES and CEL are listed below according to their relevant patient population.

SMCD

1. Is molecular testing for clinically relevant *KIT* mutations with/without (±) *PDGFRA/B* rearrangements in addition to usual diagnostic assessment as safe as, or safer than, usual clinical and laboratory diagnosis for patients with suspected SMCD?

2. Is molecular testing for clinically relevant *KIT* mutations ± *PDGFRA/B* rearrangements in addition to usual diagnostic assessment as effective as, or more effective than, usual clinical and laboratory diagnosis at improving the health outcomes of patients with suspected SMCD?

3. What is the cost-effectiveness of molecular testing for clinically relevant *KIT* mutations ± *PDGFRA/B* rearrangements in addition to usual diagnostic assessment, relative to usual clinical and laboratory diagnosis, for patients with suspected SMCD?

HES and CEL

1. Is molecular testing for *PDGFRA/B* or *FGFR1* gene rearrangements in addition to usual diagnostic assessment as safe as, or safer than, usual clinical and laboratory diagnosis for patients with suspected HES or CEL?

2. Is the detection of *PDGFRA/B* gene rearrangements in addition to usual diagnostic assessment as effective as, or more effective than, usual clinical and laboratory diagnosis at improving the health outcomes of patients with suspected HES or CEL?

3. Is molecular testing for *FGFR1* gene rearrangements in addition to lymph node biopsy as effective as, or more effective than, usual clinical and laboratory diagnosis for patients with suspected HES associated with an abnormal T-cell population?

4. What is the cost-effectiveness of *PDGFRA/B* gene rearrangements plus usual diagnostic assessment, relative to usual clinical and laboratory diagnosis, for patients with suspected HES or CEL?

Diagnostic assessment framework

In order to assess the effectiveness of a diagnostic strategy, there needs to be consideration of its diagnostic accuracy (in comparison to a reference standard), its impact on the clinical management of the patient, and its ultimate impact on the health outcomes of the patient. The primary goal of this assessment was to find *direct evidence* of the effectiveness of using molecular testing in the diagnosis of MPDs on health outcomes. That is, one group of patients with suspected SMCD or primary

eosinophilia would receive molecular testing + serum tryptase testing ± BM biopsy ± echocardiography (index test strategy), and treatment and follow-up. This group would be compared with another group receiving serum tryptase testing ± BM biopsy ± echocardiography (comparator test strategy), and treatment and follow-up, for a period of time until the impact on health outcomes (ie survival) could be evaluated. As limited and non-comparative direct evidence of the impact of including molecular testing in the diagnostic strategy on final health outcomes was identified, a *linked evidence* approach was undertaken.

In some situations it is appropriate to narratively link evidence from studies that report on the following factors to infer the effect of the diagnostic test on patient health outcomes:

- diagnostic test performance (diagnostic accuracy)—sensitivity, specificity and accuracy
- impact on clinical decision-making—does clinical decision-making (patient management) change as a result of the test?
- impact of the treatment of diagnosed patients on health outcomes—do patients receiving a change in management benefit in terms of health outcomes?

Using a linked evidence approach requires either an improvement in relative diagnostic accuracy or a change in patient management that results in better patient outcomes, to imply a benefit from the diagnostic test or strategy for patients.

Review of the literature

Literature sources and search strategies

The medical literature was searched to identify relevant studies and reviews for the period 1992–2009. Appendix H describes the electronic databases that were used for this search and the other sources of evidence that were investigated.

The search terms used to identify literature in electronic databases on the safety and effectiveness of molecular testing in the diagnosis of SMCD, HES and CEL are also presented in Appendix H.

Selection criteria

Criteria for studies relevant to an assessment of the safety and effectiveness of molecular testing for the diagnosis of SMCD, HES or CEL are described in Box 6 – Box 10 in the 'Results' section of this report. These criteria are applicable for an assessment using either a direct or a linked evidence approach.

The criteria for including articles in this report varied depending on the type of research question being addressed. Often a study was assessed more than once because it addressed more than one research question. One researcher applied the inclusion criteria to the collated literature. If there was any doubt concerning inclusion of papers, this was resolved by group consensus between members of the evaluation team to ensure that all potentially relevant studies were captured. In general, studies were excluded if they:

- did not address the research question;

- did not provide information on the pre-specified target population;
- did not include the pre-specified intervention;
- did not compare results with the pre-specified comparators;
- did not address one of the pre-specified outcomes and/or provided inadequate data on these outcomes (in some instances, a study was included to assess one or more outcomes but had to be excluded for other outcomes due to data inadequacies);
- were in other languages and were of a lower level of evidence than that available in English; or
- did not have the study design specified in the review protocol.

Specific selection criteria relating to each research question can be found in the 'Results' section of this report.

Search results

The process of study selection for this report went through six phases:

7. All reference citations from all literature sources were collated into an Endnote X1 database.
8. Duplicate references were removed.
9. Studies were excluded, on the basis of the citation information, if it was obvious that they did not meet the pre-specified inclusion criteria. Citations were assessed independently by one reviewer. Studies marked as requiring further evaluation were retrieved for full-text assessment.
10. Studies were included to address the research questions if they met the pre-specified criteria, again independently applied by one reviewer to the full-text articles. Those articles meeting the criteria formed part of the evidence-base. The remainder provided background information.
11. The reference lists of the included articles were pearled for additional relevant studies. These were retrieved and assessed according to phase 4.
12. The evidence-base consisted of articles from phases 4 and 5 that met the inclusion criteria.

Any doubt concerning inclusions at Phase 4 was resolved by consensus between members of the evaluation team. The results of the process of study selection are provided in Figure 17.

Quorum flowchart

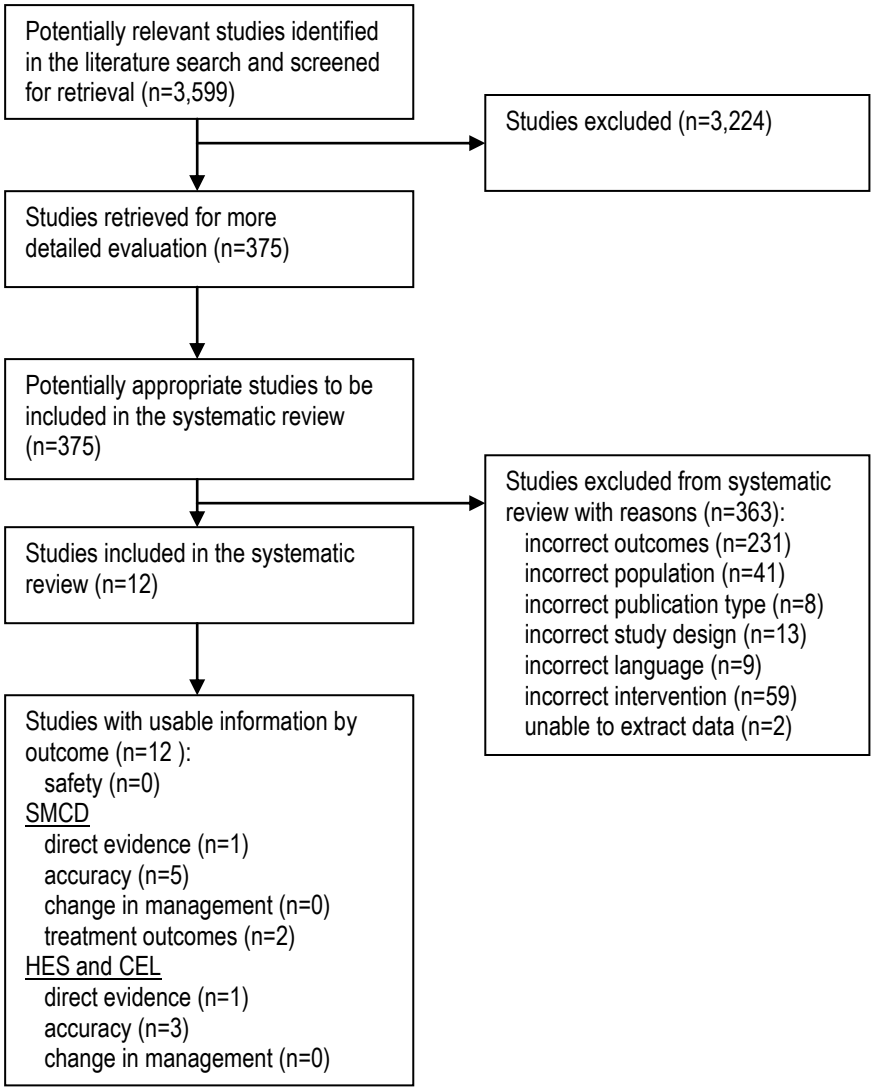


Figure 17 Summary of the process used to identify and select studies for the review
Adapted from Moher et al (1999)

Data extraction and analysis

A profile of key characteristics including study design and location, level and quality of evidence, population, intervention and outcomes was developed for each study selected for this report (Appendix J).

In studies with diagnostic case-control design, studies were excluded if the controls consisted only of healthy volunteers. If there was a mix of healthy subjects and patients who did not have SMCD, these studies were included.

Assessing diagnostic accuracy

To assess the diagnostic accuracy of each of the tests for dichotomous outcomes, calculations of sensitivity, specificity, negative and positive predictive value of the tests, and 95% confidence intervals, were undertaken where possible. Data were extracted using the classic 2 x 2 table, whereby the results of the index diagnostic test were cross-

classified against the results of the reference standard (Armitage et al 2002; Deeks 2001), and Bayes' Theorem[3] was applied:

		Reference standard *All relevant clinical and laboratory information*		
		Disease +	*Disease –*	
Index test strategy	*Test +*	True positive	False positive	Total test positive
Molecular testing for *KIT* D816V	*Test –*	False negative	True negative	Total test negative
		Total SMCD +	Total SMCD –	Total tested

In diagnostic case-control studies the presence of relevant molecular mutations was compared in cases, and controls were determined by the 2001 WHO criteria for SMCD without the use of molecular analysis.

The sensitivity of the index test strategy for SMCD (molecular testing) was calculated as the proportion of people who tested positive for the *KIT* D816V mutation as confirmed by the 2001 WHO diagnostic criteria without the use of molecular testing:

Sensitivity (true positive rate, %) = number of true positives / total SMCD+ * 100

The specificity of the index test strategy for SMCD (molecular testing) was calculated as the proportion of people who tested negative for the *KIT* D816V mutation as confirmed by the 2001 WHO diagnostic criteria without the use of molecular testing:

Specificity (true negative rate, %) = number of true negatives / total SMCD– * 100

The positive predictive value was calculated as the proportion of positive results for the *KIT* D816V mutation as confirmed by the 2001 WHO diagnostic criteria for SMCD.

Positive predictive value (PPV, %) = number of true positive results / total test positives * 100

The negative predictive value was calculated as the proportion of negative results for the *KIT* D816V mutation as confirmed by the 2001 WHO diagnostic criteria for SMCD.

Negative predictive value (NPV, %) = number of true negative results / total test negatives * 100

When a 95% confidence interval was not provided in the relevant study, it was calculated using exact binomial methods.

---

[3] Bayes Theorem:

Positive predictive value = (prevalence)(sensitivity) / (prevalence)(sensitivity) + (1–prevalence)(1–specificity)

Negative predictive value = (1–prevalence)(specificity) / (prevalence)(1–sensitivity) + (1–prevalence)(specificity)

Appraisal of the evidence

Appraisal of the evidence was conducted in three stages:

Stage 1: Appraisal of the applicability and quality of individual studies included in the review.

Stage 2: Appraisal of the precision, size and clinical importance of the primary outcomes used to determine the safety and effectiveness of the intervention.

Stage 3: Integration of this evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

Validity assessment of individual studies

The evidence presented in the selected studies was assessed and classified using the dimensions of evidence defined by the National Health and Medical Research Council (NHMRC 2000b).

These dimensions (Table 50) consider important aspects of the evidence supporting a particular intervention and include three main domains: strength of the evidence, size of the effect and relevance of the evidence. The first domain is derived directly from the literature identified as informing a particular intervention. The last two each requires expert clinical input as part of its determination.

Table 50 Evidence dimensions

Type of evidence	Definition
Strength of the evidence:	
level	The study design used, as an indicator of the degree to which bias has been eliminated by design.[a]
quality	The methods used by investigators to minimise bias within a study design.
statistical precision	The p-value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect.
Size of effect	The distance of the study estimate from the 'null' value and the inclusion of only clinically important effects in the confidence interval.
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used.

[a] See Table 51

Strength of the evidence

The three subdomains (level, quality and statistical precision) are collectively a measure of the strength of the evidence.

Level

The 'level of evidence' reflects the effectiveness of a study design to answer a particular research question. Effectiveness is based on the probability that the design of the study has reduced or eliminated the impact of bias on the results.

The NHMRC evidence hierarchy provides a ranking of various study designs ('levels of evidence') by the type of research question being addressed (see Table 51).

Table 51 Designation of levels of evidence according to type of research question (including tablenotes)

Level	Intervention[a]	Diagnostic accuracy[b]
I[c]	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard[d], among consecutive persons with a defined clinical presentation[e]
III-1	A pseudo randomised controlled trial (ie alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard[d], among non-consecutive persons with a defined clinical presentation[e]
III-2	A comparative study with concurrent controls: – non-randomised, experimental trial[f] – cohort study – case-control study – interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for level II and III-1 evidence
III-3	A comparative study without concurrent controls: – historical control study – two or more single-arm studies[g] – interrupted time series without a parallel control group	Diagnostic case-control study[e]
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard)[h]

Source: Merlin et al (2009)

Table notes

[a] Definitions of these study designs are provided in NHMRC (2000b); pp. 7–8.

[b] The dimensions of evidence apply only to studies of diagnostic accuracy. To assess the effectiveness of a diagnostic test there also needs to be a consideration of the impact of the test on patient management and health outcomes (MSAC 2005; Sackett & Haynes 2002).

[c] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence. Systematic reviews of level II evidence provide more data than the individual studies and any meta-analyses will increase the precision of the overall results, reducing the likelihood that the results are affected by chance. Systematic reviews of lower level evidence present results of likely poor internal validity, and thus are rated on the likelihood that the results have been affected by bias rather than whether the systematic review itself is of good quality. Systematic review quality should be assessed separately. A systematic review should consist of at least two studies. In systematic reviews that include different study designs, the overall level of evidence should relate to each individual outcome/result, as different studies (and study designs) might contribute to each different outcome.

[d] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its/their timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study (Whiting et al 2003).

[e] Well-designed population-based case-control studies (eg population based screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease are compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease, are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias or spectrum effect because the spectrum of study participants will not be representative of patients seen in practice (Mulherin & Miller 2002).

[f] This also includes controlled before-and-after (pre-test/post-test) studies, as well as adjusted indirect comparisons (ie using A vs B and B vs C to determine A vs C, with statistical adjustment for B).

[g] Comparing single-arm studies, ie case series from two studies. This would also include unadjusted indirect comparisons (ie using A vs B and B vs C to determine A vs C, but with no statistical adjustment for B).

[h] Studies of diagnostic yield provide the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard. These may be the only alternatives when there is no reliable reference standard.

Note A: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms are rare and cannot feasibly be captured within randomised controlled trials; physical harms and psychological harms may need to be addressed by different study designs. Harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note B: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question, eg level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence.

Source: Hierarchies adapted and modified from: Bandolier (1999; Lijmer et al (1999); NHMRC (1999); Phillips et al (2001).

Individual studies assessing diagnostic effectiveness were graded according to pre-specified quality and applicability criteria (MSAC 2005), as shown in Table 52.

Table 52 Grading system used to rank included studies

Validity criteria	Description	Grading system
Appropriate comparison	Did the study evaluate a direct comparison of the index test strategy with the comparator test strategy?	C1 direct comparison CX other comparison
Applicable population	Did the study evaluate the index test in a population that is representative of the subject characteristics (age and sex) and clinical setting (disease prevalence, disease severity, referral filter and sequence of tests) for the clinical indication of interest?	P1 applicable P2 limited P3 different population
Quality of study	Was the study designed and to avoid bias? High quality = no potential for bias based on pre-defined key quality criteria Medium quality = some potential for bias in areas other than those pre-specified as key criteria Poor quality = poor reference standard and/or potential for bias based on key pre-specified criteria	Q1 high quality Q2 medium Q3 poor reference standard poor quality or insufficient information

Quality

The appraisal of intervention studies pertaining to treatment safety and effectiveness was undertaken using a checklist developed by the NHMRC (2000a). This checklist was used for trials and cohort studies. Uncontrolled before-and-after case series are a poorer level of evidence with which to assess effectiveness. The quality of this type of study design was assessed according to a checklist developed by the UK National Health Service (NHS) Centre for Reviews and Dissemination (Khan et al 2001). Studies of diagnostic accuracy were assessed using the QUADAS quality assessment tool (Whiting et al 2003).

Statistical precision

Statistical precision was determined using statistical principles. Small confidence intervals and p-values give an indication as to the probability that the reported effect is real and not attributable to chance (NHMRC 2000b). Studies need to be appropriately powered to ensure that a real difference between groups will be detected in the statistical analysis.

Size of effect

For intervention studies of molecular testing in the diagnosis of SMCD or HES or CEL, it was important to assess whether statistically significant differences between the comparators were also clinically important. The size of the effect needed to be determined, as well as whether the 95% confidence interval included only clinically important effects.

Relevance of evidence

The outcomes being measured in this report should be appropriate and clinically relevant. Inadequately validated (predictive) surrogate measures of a clinically relevant outcome should be avoided (NHMRC 2000b).

Assessment of the body of evidence

Appraisal of the body of evidence was conducted along the lines suggested by the NHMRC in their guidance on clinical practice guideline development (NHMRC 2008). Five components are considered essential by the NHMRC when judging the body of evidence:

- the evidence-base—which includes the number of studies sorted by their methodological quality and relevance to patients
- the consistency of the study results—whether the better quality studies had results of a similar magnitude and in the same direction, that is homogenous or heterogenous findings
- the potential clinical impact—appraisal of the precision, size and clinical importance or relevance of the primary outcomes used to determine the safety and effectiveness of the test
- the generalisability of the evidence to the target population
- the applicability of the evidence—integration of the evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

A matrix for assessing the body of evidence for each research question, according to the components above, was used for this assessment (Table 53) (NHMRC 2008).

Table 53 Body of evidence assessment matrix

Component	A	B	C	D
	Excellent	**Good**	**Satisfactory**	**Poor**
Evidence-base[a]	Several level I or II studies with low risk of bias	One or two level II studies with low risk of bias, or a SR/multiple level III studies with low risk of bias	Level III studies with low risk of bias, or level I or II studies with moderate risk of bias	Level IV studies, or level I to III studies with high risk of bias
Consistency[b]	All studies consistent	Most studies consistent and inconsistency may be explained	Some inconsistency reflecting genuine uncertainty around clinical question	Evidence is inconsistent
Clinical impact	Very large	Substantial	Moderate	Slight or restricted
Generalisability	Population(s) studied in body of evidence are the same as the target population for the guideline	Population(s) studied in the body of evidence are similar to the target population for the guideline	Population(s) studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population[c]	Population(s) studied in body of evidence differ to target population and it is hard to judge whether it is sensible to generalise to target population

Applicability	Directly applicable to Australian healthcare context	Applicable to Australian healthcare context with few caveats	Probably applicable to Australian healthcare context with some caveats	Not applicable to Australian healthcare context

Adapted from NHMRC (2008)

[a] Level of evidence determined from the NHMRC evidence hierarchy

[b] If there is only one study, rank this component as 'not applicable'.

[c] For example, results in adults that are clinically sensible to apply to children OR psychosocial outcomes for one cancer that may be applicable to patients with another cancer

Expert advice

An Advisory Panel was established to provide guidance to the Evaluators to ensure that the assessment is clinically relevant and takes into account consumer interests. In selecting members for advisory panels, the MSAC's practice is to approach the appropriate medical colleges, specialist societies and associations, and consumer bodies for nominees. The panel had expertise in haematology, oncology, molecular pathology and consumer issues. Membership of the Advisory Panel is provided at Appendix G.

Results of assessment

Is it safe?

Molecular testing uses genetic material from cell types involved in the disease process. Harms from molecular testing may result from injuries obtained during the sample-taking process. For the diagnosis of SMCD, HES or CEL, cells from peripheral blood or BM may be used to obtain the necessary genetic material.

Box 6 Inclusion criteria for identification of studies relevant to assessment of the safety of molecular testing for SMCD, HES and CEL

Research question	
Is molecular testing for clinically relevant mutations and/or gene rearrangements in addition to usual diagnostic assessment as safe as, or safer than, usual clinical and laboratory diagnosis for patients with suspected SMCD, HES or CEL?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected SMCD (including patients presenting with associated eosinophilia), HES or CEL
Intervention	All available clinical and laboratory information in addition to molecular testing for clinically relevant mutations and/or gene rearrangements, including *KIT* D816V ± *PDGFRA/B* for SMCD; *PDGFRA/B* ± *FGFR1* for HES or CEL
Comparator(s)	Clinical and laboratory diagnosis using all available information required for diagnosis
Outcomes	Physical harms from testing
Search period	1992 – January 2009
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified.

SMCD = systemic mast cell disease; HES = hypereosinophilic syndrome; CEL = chronic eosinophilic leukaemia

No studies were identified that reported safety outcomes related to molecular testing for the diagnosis of SMCD, HES or CEL. Similarly, no case reports reported any adverse outcomes associated with the use of molecular testing in the diagnosis of these MPDs.

Potential harms associated with molecular testing are discussed in the 'Other relevant considerations' section of this report.

Summary of safety

No studies were identified that reported physical harms associated with molecular testing in the diagnosis of SMCD, HES or CEL.

Is it effective?

Direct evidence

Box 7 Inclusion criteria for identification of studies relevant to assessment of the effectiveness of molecular testing for SMCD, HES or CEL

Research question	
Is molecular testing for clinically relevant mutations including *KIT* mutations and *PDGFRA/B* or *FGFR1* rearrangements in addition to usual diagnostic assessment as effective as, or more effective than, usual clinical and laboratory diagnosis at improving the health outcomes of patients with suspected SMCD, HES or CEL?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected SMCD, HES or CEL
Intervention	All available clinical and laboratory information in addition to molecular testing for relevant mutations and/or gene rearrangement[a]
Comparator(s)	All available clinical and laboratory information other than that provided by molecular testing for disease-relevant mutations and/or gene rearrangement[a]
Outcomes	Primary: mortality; quality of life; reduction or resolution of symptoms; psychosocial outcomes (eg anxiety); avoidance of unnecessary treatments Secondary: haematological response to treatment; hospitalisation
Search period	1992 – January 2009
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified.

SMCD = systemic mast cell disease; HES = hypereosinophilic syndrome; CEL = chronic eosinophilic leukaemia; [a] D816V or other clinically relevant *KIT* mutations ± *PDGFRA/B* rearrangements for SMCD, *PDGFRA/B* ± *FGFR1* or other clinically relevant rearrangements for HES or CEL.

SMCD

Limited evidence of a change in the health outcomes of patients with SMCD, diagnosed with the aid of molecular testing, was identified. This benefit was seen in the subgroup of patients with SMCD associated with eosinophilia who also carry the *FIP1L1-PDGFRA* fusion gene as characterised by a *CHIC2* deletion. Improved health outcomes were seen as a consequence of the targeted treatment of these patients with imatinib mesylate.

Pardanani et al (2006b) reported a medium-quality study (level IV intervention evidence) that assessed patients presenting to a referral centre with moderate to severe eosinophilia. Bone marrow histology and other relevant laboratory and clinical information were used to diagnose patients in accordance with the 2001 WHO classification system for haematopoietic tumours. Additionally, molecular analysis of the *KIT* and *CHIC2* loci using DNA sequencing and two FISH strategies, respectively, enabled detection of the *KIT* D816V mutation and deletion or translocation of the *CHIC2* allele, and the subsequent formation of the *FIP1L1-PDGFRA* fusion gene.

From 830 patients included in this study, 32 (4%) were reported as carrying the *FIP1L1-PDGFRA* fusion gene. Of these patients, 10 with SMCD associated with eosinophilia and one with CEL were known to be treated with imatinib mesylate. The remaining 21 patients were presumably treated as usual by their referring physician. All 11 patients who received imatinib mesylate had a complete response to treatment, which included normalisation of the eosinophil count and complete resolution of clinical symptoms.

HES and CEL

The medium-quality study by Loules et al (2009) reported limited direct evidence of the diagnosis and treatment of patients presenting with eosinophilia (level IV intervention evidence). This study reported on 15 patients who were investigated for primary eosinophilia by molecular analysis for the *FIP1L1-PDGFRA* fusion gene, as well as cytogenetics and flow cytometry for lymphocyte-mediated HES. Serum vitamin B12 and IL-5 and mast cell tryptase levels were not determined. Diagnosis of SMCD, HES or CEL was made on the basis of WHO criteria or on the results of molecular analysis. The diagnostic yield of the investigations was five (33%) patients diagnosed with primary eosinophilia including CEL (n=2), idiopathic HES (n=2) and SMCD with eosinophilia (n=1). All five patients diagnosed with primary eosinophilia received imatinib mesylate and all but one obtained a complete haematological response. Two patients, both with CEL, also obtained clinical and molecular remission with imatinib therapy. The patient who had no response to therapy had been diagnosed with SMCD and was known to not carry either the *KIT* D816V mutation or the *FIP1L1-PDGFRA* fusion gene.

As a change in management following diagnosis of CEL and HES is not expected (ie the use of imatinib mesylate to treat CEL is already established in Australia), the benefit of molecular analysis must come from either earlier diagnosis or more accurate diagnosis. The study by Loules et al (2009) indicated that the detection of the *FIP1L1-PDGFRA* fusion gene allowed earlier diagnosis of CEL in one patient and had a substantial impact on their clinical outcome. However, in the absence of a comparison with patients diagnosed without the addition of molecular analysis, it is not known if such a benefit can be attributed to the use of molecular methods.

Linked evidence

The limited nature of the direct evidence in this assessment made it appropriate to pursue a linked evidence approach. As such, evidence of diagnostic accuracy, change in management, and treatment effectiveness subsequent to a change in management were assessed and reported according to their relevant indication. The inclusion criteria for such an assessment are outlined in Box 8 to Box 10.

Box 8 Inclusion criteria for identification of studies relevant to assessment of the diagnostic accuracy of molecular testing for SMCD, HES or CEL (linked evidence)

Research question	
What is the diagnostic accuracy of molecular testing in addition to all available clinical and laboratory information, relative to all available clinical and laboratory information alone, in patients suspected of SMCD, HES or CEL?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected SMCD, HES or CEL
Intervention	All available clinical and laboratory information in addition to molecular testing for relevant mutations and/or gene rearrangement[a]
Comparator(s)	All available clinical and laboratory information other than that provided by molecular testing for disease-relevant mutations and/or gene rearrangement[a]
Outcomes	Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values, diagnostic odds ratios, receiver operator characteristic curves, area under the curve, accuracy
Search period	1992 – January 2009
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified.

SMCD = systemic mast cell disease; HES = hypereosinophilic syndrome; CEL = chronic eosinophilic leukaemia; [a] D816V or other clinically relevant *KIT* mutations ± *PDGFRA/B* rearrangements for SMCD, *PDGFRA/B* ± *FGFR1* or other clinically relevant rearrangements for HES or CEL.

Box 9 Inclusion criteria for identification of studies relevant to assessment of a change in management following molecular testing for SMCD, HES or CEL (linked evidence)

Research question	
What is the change in management of patients following diagnosis by molecular testing in addition to all available clinical and laboratory information, relative to all available clinical and laboratory information alone, of SMCD, HES or CEL?	
Selection criteria	**Inclusion criteria**
Population	Patients with suspected SMCD, HES or CEL
Intervention	All available clinical and laboratory information in addition to molecular testing for relevant mutations and/or gene rearrangement[a]
Comparator(s)	All available clinical and laboratory information other than that provided by molecular testing for disease-relevant mutations and/or gene rearrangement[a]
Outcomes	Rates of treatment, method of treatment, rates of referral, type of referral, hospitalisation, rates of consultation
Search period	1992 – January 2009
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified.

SMCD = systemic mast cell disease; HES = hypereosinophilic syndrome; CEL = chronic eosinophilic leukaemia; [a] D816V or other clinically relevant *KIT* mutations ± *PDGFRA/B* rearrangements for SMCD, *PDGFRA/B* ± *FGFR1* or other clinically relevant rearrangements for HES or CEL.

Box 10 Inclusion criteria for identification of studies relevant to assessment of treatment effectiveness following a change in management in patients with SMCD (linked evidence)

Research question	
Is there a clinical benefit for patients with SMCD following a change in management?	
Selection criteria	**Inclusion criteria**
Population	Patients diagnosed with SMCD
Intervention	• imatinib therapy following failed conventional therapy (which may include hydroxyurea, corticosteroids or cladribine)—second-line therapy, or • imatinib used as a first-line therapy, or • earlier treatment than would otherwise occur
Comparator(s)	Conventional therapy or timing of treatment
Outcomes	Primary: mortality; quality of life; reduction or resolution of symptoms; psychosocial outcomes (eg anxiety); avoidance of unnecessary treatments Secondary: haematological response to treatment; hospitalisation
Search period	1992 – January 2009
Language	Non-English language articles were excluded unless they appeared to provide a higher level of evidence than the English language articles identified.

SMCD = systemic mast cell disease

SMCD

Is it accurate?

KIT D816V

Five studies were identified that reported on the diagnosis of SMCD with molecular analysis; two were diagnostic case-control studies (level III-3 diagnostic intervention) and the others reported diagnostic yield (level IV diagnostic evidence).

The medium-quality diagnostic case-control studies are likely to have substantial overlap of patients between them as they were conducted in the same centre (Table 54). In spite of this, the sensitivity and negative predictive values reported by the studies show some inconsistency (Krokowski et al 2005; Sotlar et al 2004). Based on the published reports, it is difficult to determine the source of the variation but, as the molecular methods were likely the same, the discrepancy may be attributable to differences in population.

Despite the variation, both studies reported excellent specificity as the *KIT* D816V mutation was not found in any of the controls. The sensitivity in the studies ranged from 88% to 99%, suggesting that the mutation is not detectable in all patients with disease, and therefore its absence would not exclude a diagnosis of SMCD.

Table 54 Diagnostic accuracy of molecular testing in the diagnosis of SMCD

Study	Study quality[a]	Population	Molecular test technique and sample type	Diagnostic test accuracy			
			Mutation tested	**Sn [95% CI]**	**Sp [95% CI]**	**PPV [95% CI]**	**NPV [95% CI]**
(Krokowski et al 2005) *Likely overlap of patients with Sotlar et al (2004)*	Level III-3 diagnostic evidence CX P2 Q2	88 patients with either SMCD or MCH *Cases:* SMCD (n=57) *Controls:* MCH (n=31)	PNA-PCR and melting point analysis *KIT* D816V Not reported	88% [76.3,94.9]	100% [88.8, 100.0]	100% [92.9, 100.0]	82% [65.7, 92.3]
(Sotlar et al 2004) *Likely overlap of patients with Krokowski et al (2005)*	Level III-3 diagnostic evidence CX P2 Q2	148 patients with either SMCD or MCH *Cases:* SMCD (n=73) *Controls:* MCH (n=75)	PNA-PCR plus MCA of PCR products if mutation present. If negative, then nested PCR and MCA of microdissected mast cells or RT-PCR and *HinfI* digestion of isolated mononuclear cells	99% [92.60, 99.97]	100% [95.2, 100.0]	100% [95.0, 100.0]	99% [92.9, 99.97]

[a] A description of study quality characteristics is provided in Table 51 and Table 52; SMCD = systemic mast cell disease; RT-PCR = reverse transcriptase polymerase chain reaction; PCR = polymerase chain reaction; MCA = melting curve analysis; PNA = peptide nucleic acid; Sp = specificity; CI = confidence interval; Sn = sensitivity; PPV = positive predictive value; NPV = negative predictive value; MCH = mast cell hyperplasia

Reports of diagnostic yield are seen in Table 55 (level IV diagnostic evidence) and show substantial variation, with diagnosis of SMCD being made in 23–90% of patients. It would be expected that this variation is due to the populations being analysed, particularly as the populations in the studies by Sonneck et al (2007) and Johnson et al (2009) are highly selected—which has likely overestimated the true diagnostic yield.

The medium-quality study by Tan et al (2006) is significant because of its applicability to the Australian healthcare context. Although primarily a study of newly developed molecular methodologies, the diagnostic yield of a subgroup of patients at the Peter MacCallum Cancer Centre who were suspected of having SMCD or who did not completely meet the WHO criteria was reported to be 23%.

Table 55 Diagnostic yield of molecular testing (index test) in the diagnosis of SMCD

Study	Study quality[a]	Population	Diagnostic criteria used for SMCD	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield of index test strategy
(Johnson et al 2009)	Level IV diagnostic evidence CX P1 Q2	59 patients with a high clinical suspicion of SMCD	2001 WHO criteria for diagnosis of SMCD	Various techniques including pyrosequencing, Sanger sequencing and AS-PCR BM	Not reported	53/59 (90%)
(Sonneck et al 2007)	Level IV diagnostic evidence CX P1 Q2	6 patients with hypotension following insect stings	2001 WHO criteria for diagnosis of SMCD	RT-PCR and RFLP BM	Not reported	4/6 (67%)
(Tan et al 2006)	Level IV diagnostic evidence CX P1 Q2	13 patients with clinical suspicion of SMCD	2001 WHO criteria for diagnosis of SMCD	ESMA and ACB-PCR Not reported	ESMA: 1–3% of HMC-1 cell line[a] ACB-PCR: 0.1% of HMC-1 cell line[b]	3/13 (23%)

[a] A description of study quality characteristics is provided in Table 51 and Table 52; [b] HMC-1 is a human cell line with heterozygous *KIT* D816V mutation; SMCD = systemic mast cell disease; ESMA = enriched sequencing of mutant allele; ACB-PCR = allele-specific competitive blocker polymerase chain reaction; BM = bone marrow; AS-PCR = allele-specific polymerase chain reaction; RFLP = restriction fragment length polymorphism; RT-PCR = reverse transcriptase polymerase chain reaction

Does it change patient management and improve patient outcomes?

No studies were identified that met the inclusion criteria for a change in management as a consequence of incorporating molecular testing into the diagnostic strategy for SMCD.

Does change in management improve patient outcomes?

Two studies (level IV intervention evidence) were identified that reported the treatment effectiveness of imatinib mesylate in patients with SMCD associated with eosinophilia (Table 56).

Both good-quality studies reported outcomes in patients with SMCD who received imatinib mesylate therapy. The study by Droogendijk et al (2006) reported the effect of imatinib treatment in a small group of patients with SMCD whose *KIT* D816V and *FIP1L1-PDGFRA* status had been determined. Although the primary outcomes of this study were not relevant to this assessment, the secondary outcomes included 5 of 9

(56%) patients having a reduction in skin lesions; a reduction in hepatosplenomegaly in 3 of 6 (50%) patients; and a decrease in symptoms in 8 of 13 (62%) patients. Interestingly, the one patient with the *FIP1L1-PDGFRA* rearrangement reported a complete response following imatinib mesylate therapy. Furthermore, 36% of patients with the *KIT* D816V mutation reported a major response to treatment (> 50% reduction in serum tryptase levels, urinary N-methylhistamine excretion or skin lesions).

The good-quality study by Pardanani et al (2003) reported the outcome of imatinib mesylate therapy in five patients with SMCD associated with eosinophilia. Three of these patients were known to have the *CHIC2* deletion, a surrogate marker of the *FIP1L1-PDGFRA* rearrangement. Although a definition of a complete response was not provided, the investigators reported a complete response to imatinib mesylate therapy in all patients with the *CHIC2* deletion. In contrast, neither patient with the *KIT* D816V mutation reported a complete response; indeed, these two patients were refractory to imatinib mesylate.

Table 56 Treatment effectiveness of imatinib mesylate in patients with SMCD associated with eosinophilia

Study	Study quality[a]	Population	Treatment	Treatment response
(Droogendijk et al 2006)	Case series CX P1 Q1	14 patients with SMCD D816V-positive n=11 *FIP1L1-PDGFRA*-positive n=1 No mutations detected n=2	400 mg/day of imatinib mesylate (orally)	D816V-positive: Reduction in > 2 endpoints[b] = 11 (100%) Major response[c] = 4/11 (36%) *FIP1L1-PDGFRA*-positive: CR[d] = 1 (100%) No mutations: Major response[c] = 1 (50%)
(Pardanani et al 2003)	Case series CX P1 Q1	5 patients with SMCD associated with eosinophilia *CHIC2* deletion-positive n=3 *KIT* D816V-positive n=2	100–400 mg/day of imatinib mesylate	*CHIC2* deletion-positive: CR=3 (100%)[e] NR=0 (0%) D816V-positive: CR=0 (0%)[e] NR=2 (100%)

[a] A description of study quality characteristics is provided in Table 51 and Table 52; [b] Primary end points were reductions in serum tryptase activity and urinary N-methylhistamine excretion. Secondary end points were reduction in skin lesions, percentage of mast cells in bone marrow (BM) sections, hepatomegaly and/or splenomegaly and symptoms; [c] Major response was a reduction of > 50% in serum tryptase levels, urinary N-methylhistamine excretion, or skin lesions. BM aspirate showing < 10% mast cells and the absence of progression of SMCD; [d] Complete response was the absence of symptoms, < 5% mast cells in BM aspirate, the complete disappearance of skin lesions, and the normalisation of serum tryptase levels and urinary N-methylhistamine excretion; [e] Definition of complete response was not reported in this study; CR = complete response; NR = no response.

HES and CEL

Is it accurate?

Three studies were identified that reported the diagnostic yield of CEL in patients with persistent hypereosinophilia (level IV diagnostic evidence). No studies were identified that considered the use of molecular analysis of the *FGFR1* gene. The range of CEL diagnoses in these patients was between 5% and 33% (Table 57).

The medium-quality study by Metzgeroth et al (2007) (level IV diagnostic evidence) reported the number of patients diagnosed with CEL after molecular analysis of the *FIP1L1-PDGFRA* fusion gene. This study was primarily focused on the presence of *FIP1L1-PDGFRA* in patients with acute myeloid leukaemia and lymphoblastic T-cell non-Hodgkins lymphoma; however, the yield of patients with CEL was also reported in all the patients presenting with persistent unexplained hypereosinophilia. The patients included in this study had samples referred to the laboratory from throughout Germany;

therefore, the inclusion criteria for patients in the study is reliant on the referral patterns of physicians throughout Germany. Additionally, only patients who tested positive for the fusion gene appeared to have their clinical history and other available laboratory test results examined, suggesting a potential for verification bias in the study. Of the 580 patients whose peripheral blood or BM biopsies were analysed, 29 (5%) were diagnosed with CEL.

Table 57 Diagnostic yield of molecular testing (index test) in the diagnosis of CEL

Study	Study quality[a]	Population	Diagnostic criteria used for HES or CEL	Molecular test technique and sample type	Detection limit of molecular test	Diagnostic yield of CEL using index test strategy
(Wang et al 2008)	Level IV diagnostic evidence CX P1 Q2	24 patients with persistent hypereosinophilia	2001 WHO criteria for the classification of HES and CEL plus molecular testing	Nested RT-PCR and FISH	0.01% of mutant cDNA	8/24 = 33%
(Metzgeroth et al 2007)	Level IV diagnostic evidence CX P1 Q2	580 patients with persistent eosinophilia	All available laboratory tests and patient history	Nested RT-PCR in PB or BM	Not reported	29/580 (5%)
(Bacher et al 2006)	Level IV diagnostic evidence CX P1 Q2	40 patients with unexplained and persistent eosinophilia	Not reported	RT-PCR and FISH	Not reported	8/40 (20%)

[a] A description of study quality characteristics is provided in Table 51 and Table 52; HES = hypereosinophilic syndrome; CEL = chronic eosinophilic leukaemia; RT-PCR = reverse transcriptase polymerase chain reaction; FISH = fluorescent in-situ hybridisation; PB = peripheral blood; BM = bone marrow; cDNA = complementary deoxyribonucleic acid

Does it change patient management?

No studies were identified that reported outcomes related to a change in management following a diagnostic strategy incorporating molecular analysis of clinically relevant mutations and/or gene rearrangements.

Does change in management improve patient outcomes?

As no change in management has been established for patients with HES or CEL following molecular analysis, a systematic review of subsequent patient outcomes has not been conducted.

Established treatment for patients with CEL is based on the significant findings regarding treatment of *FIP1L1-PDGFRA*-positive patients with imatinib mesylate, which identified the *FIP1L1-PDGFRA* rearrangement as an effective target of imatinib mesylate (Cools et al 2003). In patients with HES, or patients with CEL unclassified who have not responded to imatinib mesylate, conventional therapy such as prednisolone and hydroxyurea or interferon-α is administered. For patients with HES associated with an abnormal T-cell population, cytoreductive therapy and interferon-α is recommended.

Summary of effectiveness

Direct evidence—SMCD

No direct evidence was identified in patients diagnosed with the addition of molecular analysis to the diagnostic strategy, relative to a strategy without molecular analysis; therefore, the comparative effectiveness of molecular analysis in the diagnostic strategy cannot be determined.

Low-level direct evidence (level IV intervention evidence) suggests that there are improved patient outcomes for those who are identified with the *FIP1L1-PDGFRA* rearrangement as part of the diagnostic strategy for patients with suspected SMCD associated with eosinophilia.

No direct evidence regarding the molecular analysis of the *KIT* gene on patient outcomes was identified.

Linked evidence—SMCD

Evidence of the diagnostic accuracy of including molecular analysis for patients with suspected SMCD was limited to two diagnostic case-control studies (level III-3 diagnostic evidence) and three studies of diagnostic yield (level IV diagnostic evidence). All five studies analysed the presence of the *KIT* D816V mutation.

It is highly likely that there was substantial overlap of patients in the diagnostic case-control studies that reported sensitivity in the range 88%–99% and specificity of 100%. These results indicate that molecular analysis did not produce any false positive results, but that the absence of *KIT* D816V was not able to rule out SMCD.

Evidence of the diagnostic yield of a test strategy that incorporated molecular analysis was available in three medium-quality studies (level IV diagnostic evidence). Substantial variation was evident in the reported yields (range 23– 90%) and it is probable that the populations selected in the studies by Johnson et al (2009) and Sonneck et al (2007) have overestimated the true yield that could be expected from patients with suspected SMCD. The study by Tan et al (2006) is notable for its applicability to the Australian setting. This study, conducted in Melbourne, Victoria, reported the diagnosis of SMCD in 23% of patients who were either suspected of SMCD or had not been able to meet the WHO criteria for diagnosis.

No studies were identified that reported a change in management following diagnosis of SMCD using molecular analysis.

Treatment with imatinib mesylate in patients with SMCD associated with eosinophilia appeared to be effective in those identified with the *FIP1L1-PDGFRA* rearrangement. Although low-level evidence (level IV intervention evidence) in a very small number of patients, all of those identified with the rearrangement achieved a complete response after imatinib mesylate therapy (Droogendijk et al 2006; Pardanani et al 2003). The study by Droogendijk et al (2006) also indicates that imatinib mesylate may have some effectiveness in improving patient outcomes in patients carrying the *KIT* D816V mutation.

Direct evidence—CEL and HES

Direct evidence of a change in patient outcomes after diagnosis of CEL and HES using molecular analysis of the *FIP1L1-PDGFRA* fusion gene was limited to one study of patients presenting with eosinophilia (level IV intervention evidence) (Loules et al 2009). Diagnosis in five patients included SMCD (1), HES (2) and CEL (2), and all five patients went on to receive imatinib mesylate. The authors indicated that earlier diagnosis was achieved in one patient with CEL and that this had a significant impact on the patient's health outcomes. Both patients with CEL achieved a complete response to imatinib mesylate, while both patients with HES achieved only a complete haematological response. The patient with SMCD, and who was not known to carry any relevant molecular mutations, was refractory to imatinib mesylate.

Linked evidence—CEL and HES

No evidence was available that reported on the diagnosis of HES or CEL with molecular testing, compared with diagnosis without molecular analysis.

Evidence of diagnostic accuracy was limited to studies of diagnostic yield (level IV diagnostic evidence) in three studies that reported yields ranging from 5% to 33%. These studies all considered the analysis of the *FIP1L1-PDGFRA* rearrangement, and no studies were identified that reported the diagnostic accuracy of *PDGFRB* or *FGFR1* rearrangements.

The medium-quality study that reported the lowest diagnostic yield was limited by the referral patterns of the physicians in the various regions of Germany, and was focused primarily on the presence of the *FIP1L1-PDGFRA* rearrangement in acute myeloid leukaemia and lymphoma (Metzgeroth et al 2007). The other two medium-quality studies reported similar diagnostic yields (33% and 20%) in patients presenting with persistent eosinophilia in single-centre studies.

No evidence of change in management, particularly with regard to earlier diagnosis, was identified in patients suspected of primary eosinophilia as a consequence of diagnosis with molecular analysis.

A systematic review of treatment effectiveness in patients with HES or CEL was not required as a change in management has not been established.

Other relevant considerations

In addition to the relative safety and effectiveness of molecular testing, there are other issues not identified by the systematic review that may impact on the assessment and the decision to support public funding.

World Health Organization classification of myeloproliferative disorders

Recent changes to the WHO classification of MPDs saw significant modification to the classification of SMCD, HES and CEL. The 2008 criteria introduced a new category of neoplasm—myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* and *FGFR1*—which specifically classifies myeloid neoplasms on a molecular basis. This category would now include CEL and SMCD associated with eosinophilia, and 8p11 myeloproliferative syndrome.

These modifications have significant impacts of both a diagnostic and therapeutic nature. As already indicated, the diseases are now classified by their molecular status and, consequently, molecular analysis would be required in the diagnostic strategies for these diseases. Furthermore, classification by this system implies the therapeutic consequences of the molecular rearrangements. The presence of *PDGFR* rearrangements are known to indicate responsiveness to imatinib mesylate, while the *FGFR1* rearrangement is known to be refractory to the same treatment (Tefferi et al 2009).

Quality assurance and molecular methodologies

According to the report of the Australian Genetic Testing Survey (2008), two laboratories within Australia provide assays for the detection of *KIT* mutations and the *FIP1L1-PDGFRA* rearrangement. Currently, there is no quality assurance program being conducted by the Royal College of Pathologists of Australasia Quality Assurance Program Pty Ltd (RCPA QAP) to specifically monitor the performance of assays that detect *KIT* D816V or *PDGFRA* rearrangements. However, the expert opinion of the Advisory Panel indicates that a quality assurance program that covers testing based on single nucleotide polymorphisms (SNPs) would be sufficient to monitor the performance of assays and laboratories that provided molecular analysis for *KIT* mutations. Furthermore, quality assurance for FISH assays for the detection of genetic rearrangements would be covered by the Australian Society of Cytogeneticists and, similarly, would provide quality assurance for the methodology used in the assay rather than for an assay specifically for the *FIP1L1-PDGFRA* rearrangement.

The importance of such monitoring relates to the use of different assays developed in house within pathology laboratories. The lack of standardisation of molecular methodologies used to detect these genetic alterations may lead to variation in the ability to not only detect the mutation or rearrangement, but to subsequently diagnose disease.

Access and equity of test procedures

As discussed in Part A of this assessment, the expert opinion of the Advisory Panel indicates that a proportion of patients suspected of MPD are unable to access molecular testing due to the cost of the molecular assays, particularly assays that require more complex methodology, such as quantitative PCR or FISH. Should public funding of molecular testing in the diagnosis of MPD be supported, this will provide access to these investigations for a greater proportion of patients. For the small number of patients

presenting with eosinophilia, availability of specific molecular analysis may have a significant impact on their management if it is determined that they carry a relevant *PDGFR* rearrangement.

Monitoring

Monitoring the presence and/or level of the *FIP1L1-PDGFRA* rearrangement may occur in patients for whom there is evidence of the rearrangement and who are receiving imatinib mesylate therapy. As the *FIP1L1-PDGFRA* rearrangement is a target of imatinib mesylate, response to therapy can be assessed using molecular analysis to determine the presence and/or level of the malignant clone in patients who are receiving imatinib mesylate therapy. Monitoring therapeutic response to other therapies in patients who are *FIP1L1-PDGFRA*-negative is more likely to occur through the patient's eosinophil count.

Consumer perspective

The provision of a definitive diagnosis is of significant benefit from a consumer perspective, particularly in the case of CEL where, without molecular analysis of relevant rearrangements, it has previously been difficult to establish evidence of clonality. As a result, a number of patients were misdiagnosed with HES and would not have received access to the appropriate therapy, that is imatinib mesylate.

Concerns regarding the genetic information generated by this analysis should be dispelled by the somatic nature of these mutations and rearrangements, which are present as part of the pathogenesis of disease. Detection of these genetic alterations is not used to predict future disease, nor would they be inherited or passed on to children.

Referral and ordering

As discussed previously in Part A of this assessment, the expert opinion of the Advisory Panel recommends that the investigation and diagnosis of MPD should ideally be conducted by a specialist physician (eg a haematologist). This is particularly important considering the extremely low incidence and prevalence of the disease in the community. Few general practitioners in Australia would have much experience in diagnosing and managing patients with SMCD, HES or CEL; it would therefore be recommended by the Advisory Panel for diagnosis and treatment to be provided by a haematologist.

Reimbursement of treatment

Treatment with imatinib mesylate of patients with SMCD associated with eosinophilia or CEL is indicated only in those patients with the *FIP1L1-PDGFRA* fusion gene or other relevant rearrangement. Accordingly, Pharmaceutical Benefits Scheme (PBS) reimbursement of imatinib therapy in patients with SMCD with eosinophilia or CEL is restricted to patients with the *FIP1L1-PDGFRA* rearrangement. Evidence of the rearrangement in the form of a pathology report is required in order to be eligible for reimbursement by the PBS.

What are the economic considerations?

The purpose of economic evaluation is to assist decision-makers in ensuring that society's ultimately scarce resources are allocated to those activities from which we will get the most value. That is, it seeks to enhance economic efficiency. To determine whether an economic evaluation is required, the comparative safety and effectiveness of the intervention must first be determined.

The decision of whether to perform an economic evaluation has been based on evidence of relative safety and effectiveness compared with the comparator. If the evidence indicates that the intervention is likely to be no worse in terms of safety and effectiveness outcomes, an economic evaluation should be considered. The type of economic evaluation is determined by the net benefits and harms associated with the intervention relative to the comparator (Table 58).

Table 58 Type of economic evaluation that should be presented for various classifications of a service under MSAC consideration

Classification	Type of economic evaluation
The service is more effective than the appropriate comparator and is associated with improved safety	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
The service is more effective than the appropriate comparator and is no worse than the comparator in terms of safety	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
The service is more effective than the appropriate comparator but is associated with reduced safety:	
(i) Overall, there are net benefits to patients as the benefits from improved effectiveness outweigh the harms from reduced safety and/or changed risk profile	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
(ii) Overall, the service is no worse than the comparator because the benefits from improved effectiveness at least offset the harms from reduced safety and/or changed risk profile	Cost–consequences, cost-effectiveness (which may be reducible to cost-minimisation, ie presentation of an incremental cost-effectiveness for the base case may be inappropriate if net clinical benefits are assumed to be zero)
(iii) Overall, there are net harms to patients as the harms from reduced safety and/or changed risk profile outweigh the benefits from improved effectiveness	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service
The service is no worse than the comparator in terms of effectiveness but is associated with improved safety	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
The service is indisputably demonstrated to be no worse than the comparator in terms of both effectiveness and safety	Cost-minimisation. In the case where there is any uncertainty around the conclusion that the service is no worse than the comparator in terms of effectiveness and safety, cost–consequences, cost-effectiveness and/or cost–utility analyses should be provided
The service is no worse than the comparator in terms of effectiveness but is associated with reduced safety.	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service
The service is less effective than the comparator but is associated with improved safety:	
(i) Overall, there are net benefits to patients as the benefits from improved safety and/or changed risk profile outweigh the harms from reduced effectiveness	Cost–consequences, cost-effectiveness, cost–utility, cost–benefit
(ii) Overall, the proposed service is no worse than the comparator because the benefits from improved safety at least offset the harms from reduced effectiveness and/or changed risk profile	Cost–consequences, cost-effectiveness (which may be reducible to cost-minimisation, ie presentation of an incremental cost-effectiveness for the base case may be inappropriate if net clinical benefits are assumed to be zero)
(iii) Overall, there are net harms to patients as the harms from reduced effectiveness outweigh the benefits from improved safety and/or changed risk profile	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service
The proposed service is less effective than the comparator and is no worse than the comparator in terms of safety	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service
The proposed service is less effective than the comparator and is associated with reduced safety compared with the comparator	No economic evaluation needs to be presented; MSAC is unlikely to recommend government subsidy of this service

SMCD

No evidence was available that informed the question of the safety of molecular testing in the diagnosis of SMCD. It is recommended that molecular analysis for *KIT* mutations is performed on BM samples, and therefore there is unlikely to be a need for a peripheral blood sample to be taken. As BM samples are required for diagnosis of SMCD regardless of whether or not molecular analysis is to be performed, no additional sample collection would be required. Analysis of *FIP1L1-PDGFRA* rearrangements can, however, be

performed on peripheral blood as there will be an elevated eosinophil count in patients with suspected SMCD associated with eosinophilia. As it is unlikely that collection of peripheral blood will result in serious adverse events, it would be expected that the addition of molecular analysis to the diagnostic strategy would be as safe as diagnosis without molecular analysis; however, there is no evidence, comparative or otherwise, to support this statement.

Direct evidence of a benefit to patients following a diagnostic strategy that incorporated molecular testing related only to patients who were diagnosed with SMCD associated with eosinophilia. Pardanani et al (2006b) (level IV intervention evidence) reported a complete response in 100% of patients diagnosed with SMCD associated with eosinophilia and who were known to have the *FIP1L1-PDGFRA* rearrangement. Due to the very low prevalence of this SMCD subtype, the study was limited by both small numbers of patients and a lack of diagnostic and treatment comparison. Additionally, as the subgroup of patients with SMCD associated with eosinophilia and the *FIP1L1-PDGFRA* rearrangement is a very small proportion of the population of patients diagnosed with SMCD, the overall benefit seen by a change in management is likely to be very small.

Furthermore, the linked evidence approach did not adequately support an improvement in patient outcomes relative to the comparator with regard to diagnostic accuracy, change in management and treatment effectiveness. Two diagnostic case-control studies (level III-3 diagnostic evidence) were identified that indicated very good sensitivity and excellent specificity from incorporating *KIT* analysis into the diagnostic strategy for suspected SMCD. However, no studies reported the diagnostic accuracy of incorporating *FIP1L1-PDGFRA* or *CHIC2* analysis into the investigative strategy for SMCD. Despite no explicit evidence of a change in management in patients with SMCD associated with eosinophilia who are also positive for the *FIP1L1-PDGFRA* rearrangement, treatment with imatinib mesylate was very successful. The value of this evidence is limited by the lack of comparison with patients who have SMCD associated with eosinophilia but for which the *FIP1L1-PDGFRA* status has not been determined.

With this in mind, this assessment does not provide sufficient evidence, either direct or linked, to indicate an improvement in the comparative effectiveness of molecular testing in patients suspected of SMCD. Consequently, a formal economic evaluation in this population would be inappropriate; however, a financial analysis of the costs associated with diagnosis and treatment of patients with SMCD has been provided.

HES and CEL

Again, no evidence of the safety, comparative or otherwise, regarding molecular analysis as part of the diagnostic strategy of HES and CEL was identified. Molecular analysis may be performed on genetic material obtained from BM biopsy or peripheral blood; however, as all patients would have undergone BM biopsy to establish primary eosinophilia, it is unlikely that there would be a need to obtain additional BM material for the purpose of molecular analysis. Again, collection of peripheral blood is unlikely to result in serious adverse outcomes, so it may be argued that the addition of molecular analysis to the diagnostic strategy for HES and CEL is likely to be as safe as the comparator strategy.

Direct evidence of comparative effectiveness of the addition of molecular analysis in the diagnosis of HES and CEL, relative to a strategy without molecular analysis, was

unavailable. As it has been established that the best therapy for patients with CEL is provided by imatinib mesylate, a change in management that provides benefit to patients must indicate that diagnosis is made earlier than the comparator strategy. The study by Loules et al (2009) primarily reported on patient outcomes following diagnosis with molecular methods and treatment with imatinib mesylate in a small group of patients with primary eosinophilia (level IV intervention evidence). The authors did indicate that detection of the *FIP1L1-PDGFRA* rearrangement provided earlier diagnosis of CEL in one patient, which had a substantial impact on the patient's outcomes. However, without comparison with diagnosis without molecular analysis, it is unclear whether this improvement can be attributed to the use of molecular methods.

Linked evidence for HES and CEL was restricted to three studies of diagnostic yield (level IV diagnostic evidence), which contribute limited evidence to the question of diagnostic accuracy. Without comparative evidence, it cannot be determined whether the addition of molecular analysis to the diagnostic strategy for patients with persistent eosinophilia will improve diagnostic accuracy. No evidence of change in management was identified to indicate that patients with CEL may be diagnosed earlier with the use of molecular methods in the diagnostic strategy.

There was a complete absence of evidence regarding molecular analysis of the *FGFR1* gene in diagnosis of T-cell-associated hypereosinophilia. Due to the extremely low prevalence of this disease, and lack of data regarding the clinical need and diagnostic yield of using molecular analysis in diagnosis, it would be inappropriate to estimate the financial implications to the Australian healthcare system. Therefore, molecular analysis of *FGFR1* has not been included in the financial analysis of the costs associated with diagnosing HES and CEL.

In the absence of any comparative evidence, it is not possible to establish the comparative safety and effectiveness of molecular methods in the diagnosis of HES or CEL. With this in mind it would be inappropriate to perform an economic evaluation; consequently, an analysis of the financial implications of the index test strategy has been provided.

Comparator

The comparator for the assessment of SMCD, HES and CEL is all available clinical and laboratory information, which for SMCD may include BM biopsy (including immunohistochemical staining) and serum tryptase levels; for HES and CEL this is likely to include serum tryptase levels and echocardiography.

Financial analysis

SMCD

Likely number of investigations per year

There are no specific data that indicate the likely incidence of SMCD within Australia. A survey conducted on behalf of the Royal College of Pathologists of Australia (2008) indicates that the number of assays to detect *KIT* mutations performed in 2006 was 178 and the number of assays for the *FIP1L1-PDGFRA* fusion gene was 134. Because the detection of *KIT* mutations may also be used to diagnose gastrointestinal stromal tumours, the number of assays for *KIT* mutations may not necessarily reflect the number

of investigations for SMCD. Therefore, it will be assumed that there will be 134 investigations for SMCD per year.

Unit costs of index test strategy and the comparator

The index test strategy for the diagnosis of SMCD will include BM biopsy (aspirate and trephine) and immunohistochemical analysis of the BM specimen. In addition, serum tryptase levels will be determined as well as molecular analysis of relevant *KIT* mutations and the *FIP1L1-PDGFRA* fusion gene. The expert opinion of the Advisory Panel indicates that analysis of *PDGFRB* rearrangements is currently not available in Australia. In addition, it would be expected that a qualitative method of detection would be used for *KIT* mutations and that reverse transcriptase PCR or FISH would be used to detect the fusion gene. It is assumed that all molecular analysis would be conducted on BM biopsies.

The comparator test strategy includes BM biopsy (including immunohistochemical staining) and determination of serum tryptase levels. As these are also used in the index test strategy, it will not be necessary to include them in the analysis as these costs will be associated with diagnosis of SMCD regardless of whether or not molecular analysis is used.

With regard to therapy for patients with SMCD and the likely change in management that may be seen following molecular analysis, Figure 18 outlines the number of patients and the therapy they would receive following diagnosis of SMCD with molecular testing. It has been assumed that the diagnostic accuracy of the index test strategy is the same as the comparator test strategy.

As there would be no change in management following diagnosis with indolent or aggressive SMCD, the treatment pathways for these patients will not be included in this cost analysis. Only those patients who are diagnosed with SMCD associated with eosinophilia are likely to undergo a change in management and, therefore, only the treatment of these patients will be included in the analysis. As these patients would receive imatinib mesylate therapy only after they had failed conventional therapy, the cost of conventional therapy is not required in the cost analysis as this would also be provided to patients who were diagnosed using the comparator test strategy. Additionally, it has been assumed that all patients with SMCD associated with eosinophilia and the *FIP1L1-PDGFRA* rearrangement will fail conventional therapy.

For the purposes of this analysis, the cost of treatment with imatinib mesylate for patients with SMCD associated with eosinophilia will be estimated for 12 months; however, it should be noted that therapy for these patients is likely to be an ongoing requirement.

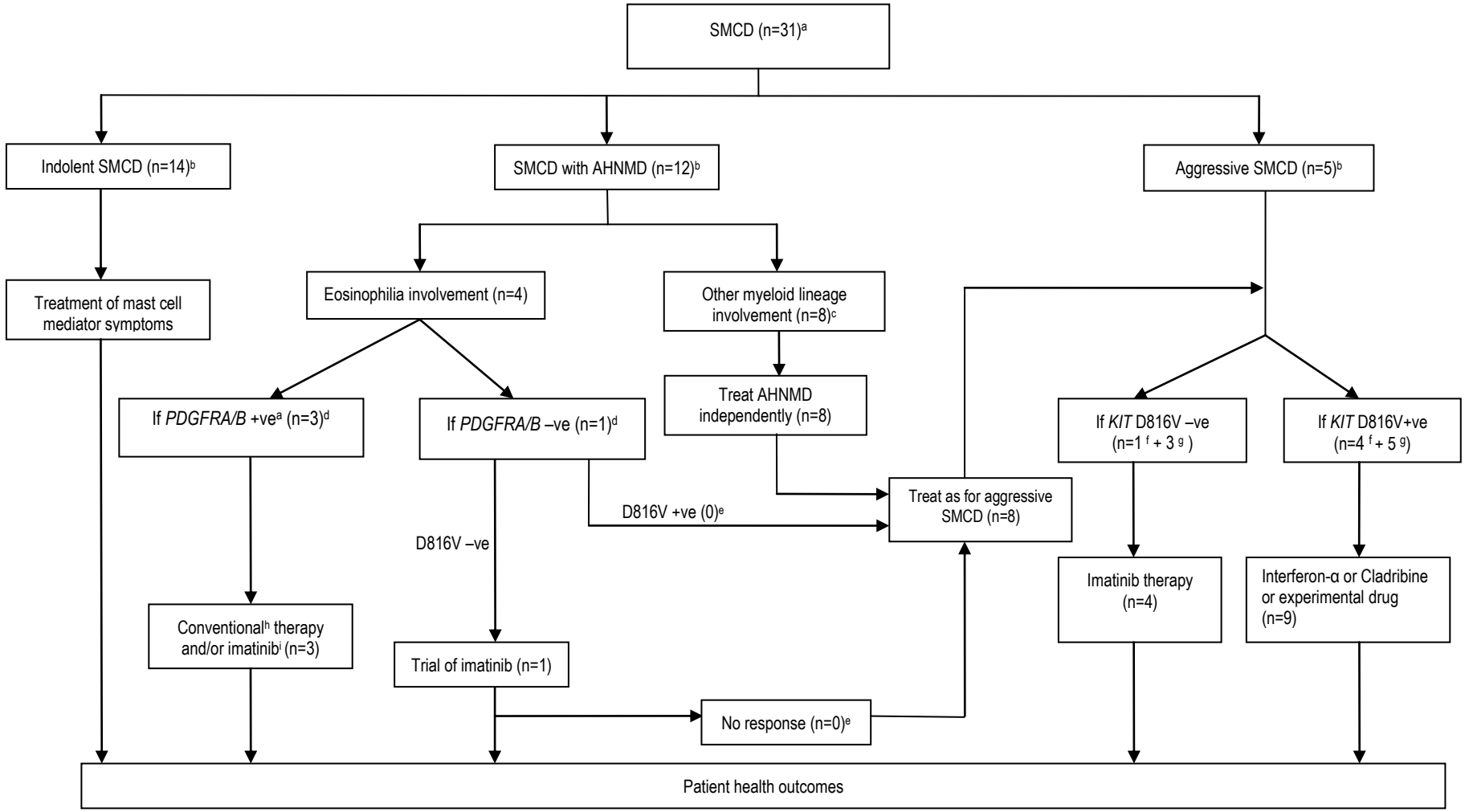


Figure 18 Treatment options for patients diagnosed with SMCD using molecular methods

Notes
[a] Diagnostic yield of 23% from Tan et al (2006) based on 134 investigations
[b] Proportions of SMCD subclassifications: indolent SMCD = 46%, SMCD with SHNMD = 40%, aggressive SMCD (including MCL) = 14% (Lim et al 2009)
[c] Proportion of patients with SMCD-AHNMD with eosinophilia = 31% (Lim et al 2009)
[d] Proportion of patients with SMCD-AHNMD with eosinophilia who are *FIP1L1-PDGFRA*-positive = 60% (Pardanani et al 2003). For the purposes of this financial analysis it has been assumed that 3 of 4 patients with SMCD-AHNMD with eosinophilia would carry the *FIP1L1-PDGFRA* fusion gene.
[e] It has been assumed that 1 of 4 patients with SMCD-AHNMD with eosinophilia would not carry any *PDGFRA/B* mutations and that they would be *KIT* D816V-negative. In addition, this patient would respond to a trial of imatinib.
[f] The proportion of *KIT* D816V-positive patients with aggressive SMCD = 82% (Lim et al 2009).
[g] The proportion of *KIT* D816V-positive patients with SMCD-AHNMD with eosinophilia = 60% (Lim et al 2009).
[h] Conventional therapy may include corticosteroids, hydroxyurea or cladribine. If conventional therapy fails, patients would proceed to imatinib therapy
[i] Imatinib as a first-line therapy is likely to occur as part of a clinical trial or user-pays situation.
SMCD = systemic mast cell disease; AHNMD = associated haematological clonal non-mast cell lineage disease.

Table 59 outlines the additional cost of items associated with the index test strategy compared with the comparator test strategy. The additional cost of adding molecular analysis to the diagnostic strategy for SMCD is $323 per patient. The discrepancy in the cost of molecular analysis of *KIT* mutations and the *FIP1L1-PDGFRA* rearrangement results from the different methodologies required to detect these genetic alterations.

The additional cost of treatment for patients with SMCD associated with eosinophilia who receive imatinib mesylate (400 mg/day) after failing conventional therapy is approximately $47,700/patient per year. If a patient with SMCD associated with eosinophilia were to receive imatinib mesylate at a dose of 100 mg/day, the unit cost for 12 months would be reduced to approximately $12,400.

Table 59 Unit costs of additional items in the diagnosis of SMCD for patients undergoing the index test strategy

Item	Source of estimate	Unit cost
Molecular analysis of *KIT* mutations	Expert opinion of the Advisory Panel	$90
Molecular analysis of *FIP1L1-PDGFRA* fusion gene	MBS item 73314	$233
Imatinib mesylate therapy (400 mg/day) per 12 months	PBS item 9175X	$47,672[a]

[a] This is the unit cost per 12 months of therapy ; MBS = Medicare Benefits Schedule; PBS = Pharmaceutical Benefits Scheme

Costs to the Australian healthcare system overall

Total healthcare costs incorporate all direct costs associated with the diagnosis and treatment of SMCD, regardless of the person or agency who incurs them. In this analysis these costs include the additional cost associated with molecular analysis in 134 patients suspected of SMCD and the cost of imatinib mesylate therapy, as a second-line therapy, in four patients with SMCD associated with eosinophilia (Table 60). The majority of the cost of treatment as a second-line therapy will be borne by the PBS, with the patient contributing the cost of the co-payment.

The estimated total cost to the Australian healthcare system overall for diagnosis and treatment (for 12 months with 400 mg/day imatinib mesylate) is an additional $234,000. If the dose of imatinib mesylate were to be 100 mg/day, this figure would be reduced to an additional cost of $92,700. These figures do not include the cost of ongoing therapy.

Table 60 Additional costs to the Australian healthcare system for the diagnosis and treatment of SMCD

Item	Unit cost	Usage	Total cost
Molecular analysis of *KIT* mutations	$90	134	$12,060
Molecular analysis of *FIP1L1-PDGFRA* fusion gene	$233	134	$31,222
Imatinib mesylate therapy (400 mg/day) per 12 months	$47,672	4	$190,688
Total			**$233,970**[a]

[a] Any discrepancy in total cost is due to rounding.

Costs to the Medical Benefits Schedule

The Australian Government is responsible for payment of the rebate on items from the Medicare Benefits Schedule (MBS). As patients will be investigated for SMCD as out-patients, the rebate for a private patient on items in the MBS would be 75% of the scheduled fee.

Expert opinion suggests that the publicly funded to MBS-funded split of patients suspected of having SMCD is 20% to 80%. Therefore, 80% of all patients undergoing investigations for SMCD would be eligible for MBS reimbursement, with the remaining 20% coming under the Australian Healthcare Agreements between the states/territories and the Commonwealth. As 134 patients are expected to be investigated per year, 107 patients are likely to be eligible for a rebate on Medicare items.

A comparison of the MBS items that would be eligible for the rebate is provided in Table 61. As patients would undergo these investigations as out-patients, the Commonwealth would incur the cost of the 75% rebate on these items. The cost of incorporating molecular analysis into the diagnostic strategy for suspected SMCD will incur an additional cost to the MBS of $22,000 per year for the investigation of 107 patients in the private healthcare sector.

Table 61 Additional MBS items in the index test strategy for diagnosis of SMCD

Item	Cost to the MBS (75% rebate)	Usage	Total cost
Molecular analysis of *KIT* mutations	$28	107	$2,996
Molecular analysis of *FIP1L1-PDGFRA* fusion gene	$176	107	$18,832
Total			**$21,828**[a]

[a] Any discrepancy in total cost is due to rounding.

Costs to the state/territory health systems

Under the current Australian Healthcare Agreements, the states/territories fund in-patient procedures on public patients in public hospitals, as well as procedures on public patients in an out-patient facility. To estimate the costs to the states/territories, two assumptions have been made—that the unit costs of the test strategies are the same for a public patient as they are for a private patient, and that 27 patients would be investigated for SMCD in the public health system.

As treatment will be provided to out-patients, the public healthcare sector will not incur the additional cost of treatment in patients.

Incorporating molecular analysis into the diagnostic strategy of 27 patients suspected of SMCD will result in an additional cost to the states/territories of approximately $8,600 (Table 62).

Table 62 Cost to the states/territories of the addition of molecular analysis to the testing strategy for SMCD

Item	Unit cost	Usage	Total cost
Molecular analysis of *KIT* mutations	$90	27	$2,430
Molecular analysis of *FIP1L1-PDGFRA* fusion gene	$233	27	$6,291
Total			**$8,643**[a]

[a] Any discrepancy in total cost is due to rounding.

CEL and HES

Likely number of investigations per year

There are no specific data that indicate the likely incidence of CEL or HES within Australia. In the absence of any relevant data, the expert opinion of the Advisory Panel has indicated that up to 50 investigations per year in Australia would be a reasonable estimate of the use of this testing strategy.

Unit costs of index test strategy and the comparator

The comparator test strategy for patients with persistent eosinophilia includes determination of serum tryptase levels and echocardiography. This analysis assumes that molecular evidence of clonality of eosinophils will be provided by the analysis of the *FIP1L1-PDGFRA* fusion gene and not any other molecular rearrangement.

As the index test strategy incorporates the addition of molecular analysis into the comparator test strategy, only the incremental cost of molecular analysis needs to be considered in this analysis. Furthermore, as there is no evidence of improved diagnostic accuracy or change in management, the costs of treatment for patients diagnosed with HES or CEL are not considered.

The additional costs of diagnosis of patients with persistent eosinophilia are outlined in Table 63 and are the result of the addition of molecular analysis to the diagnostic strategy.

Table 63 Unit costs of additional items for patients with persistent eosinophilia undergoing the index test strategy

Item	Source of estimate	Unit cost
Molecular analysis of *FIP1L1-PDGFRA* fusion gene	MBS item 73314	$233

MBS = Medicare Benefits Schedule

Costs to the Australian healthcare system overall

Total healthcare costs incorporate all direct costs associated with the diagnosis of HES or CEL, regardless of the person or agency who incurs them. In this analysis these costs include the incremental cost associated with molecular analysis of the *FIP1L1-PDGFRA*

fusion gene. For 50 patients to be investigated per year, the Australian healthcare system overall will incur an additional cost of $11,800 per year.

Costs to the Medical Benefits Schedule

As indicated previously, the Australian Government is responsible for payment of the rebate on items from the MBS. As patients will be investigated for persistent eosinophilia as out-patients, the rebate for a private patient on items in the MBS would be 75% of the scheduled fee.

Again, expert opinion has suggested that the public to private split of patients for suspected HES or CEL is 20% to 80%. Therefore, 80% of all patients undergoing investigations for persistent eosinophilia would be eligible for MBS reimbursement, with the remaining 20% coming under the Australian Healthcare Agreements between the states/territories and the Commonwealth. As up to 50 patients are expected to be investigated per year, at most 40 patients will be eligible for a rebate on Medicare items.

The cost of incorporating molecular analysis into the diagnostic strategy of HES and CEL for the MBS is estimated to be an additional $7,000 per year for 40 patients undergoing investigations.

Costs to the states/territories health systems

Under the current Australian Healthcare Agreements, the states/territories fund in-patient procedures on public patients in public hospitals, as well as public patients in an out-patient facility. To estimate the costs to the states/territories, two assumptions have been made—that the unit costs of the test strategies are the same for a public patient as they are for a private patient; and that 10 patients (50 patients overall minus 40 funded privately) would be investigated for HES or CEL in the public health system.

The expected cost to the states/territories for the incorporation of molecular analysis into the investigation of persistent eosinophilia is not likely to be greater than $2,300 per year.

Discussion

Is it safe?

No evidence was identified that reported adverse events as a consequence of molecular analysis of *KIT*, *PDGFR* or *FGFR1* rearrangements; however, there is potential for adverse events to occur as a result of sample collection.

The recommended diagnostic standard for SMCD is to analyse relevant *KIT* mutations in BM samples (Valent et al 2007). Potential risks associated with BM biopsy include haemorrhage, pain and infection (Bain 2005). As discussed in Part A of this assessment, an audit of BM biopsy procedures in a selection of hospitals in the United Kingdom reported an adverse event rate of 0.08%. Sixteen adverse events were reported as a consequence of 19,259 BM biopsies, of which 13,147 were a combination of aspirate and biopsy. The majority of adverse events were due to haemorrhage (11), with infection (2), persistent pain (2) and a serous leak also being reported (Bain 2005).

With regard to diagnosis of HES and CEL, molecular analysis can be performed on either peripheral blood or BM specimens (Roufosse et al 2007). Potential adverse events that could occur as a result of peripheral blood collection include bruising, pain, nerve damage and arterial puncture (Lavery & Ingram 2005). These events are not specific to sample collection for the diagnosis of SMCD, HES or CEL but, rather, can occur as a result of venepuncture conducted for any purpose.

As molecular analysis of relevant mutations and rearrangements will be performed in addition to the comparator test strategy, any adverse events that occur as a consequence of molecular analysis will be in addition to events associated with the comparator. However, it is likely that the risk of adverse events from sample collection for molecular analysis is very small.

Is it effective?

SMCD

Direct evidence of the effectiveness of the addition of molecular analysis of relevant mutations or rearrangements to the diagnostic strategy for SMCD was limited to a single case series (level IV intervention evidence). This medium-quality study provides evidence of a significant benefit in outcomes for patients who are known to have the *CHIC2* deletion, a surrogate marker of the *FIP1L1-PDGFRA* rearrangement (Pardanani et al 2006b).

Pardanani et al (2006b) showed that patients with SMCD associated with eosinophilia who were also *FIP1L1-PDGFRA*-positive were successfully treated with imatinib mesylate. All 11 (100%) patients who were *FIP1L1-PDGFRA*-positive (including one patient with CEL) and for whom treatment and response data were available reported complete remission.

This study incorporated patients who were previously reported in Pardanani et al (2004). The 2004 study, although limited by small numbers, reported seven patients who were diagnosed with SMCD with eosinophilia and were known to have the *CHIC2* deletion.

Other patients in the study also received imatinib mesylate, including another 5 with SMCD associated eosinophilia but without evidence of the *CHIC2* deletion, 10 with HES and 3 with CEL. All seven patients with SMCD associated with eosinophilia with the *CHIC2* deletion reported a complete response to imatinib mesylate. The only other complete response was seen in one patient with CEL who carried the *CHIC2* deletion. Partial responses were seen in 4 of 10 (40%) patients with HES.

Although the reports by Pardanani et al do not provide comparative evidence of effectiveness with the addition of molecular testing to the diagnostic strategy for SMCD, they do provide limited evidence of benefit to patients with SMCD associated with eosinophilia who are identified by molecular analysis as having the *FIP1L1-PDGFRA* fusion gene.

The nature of the *FIP1L1-PDGFRA* rearrangement, an interstitial deletion at chromosome 4q12, results in its inability to be detected by cytogenetic analysis. Subsequently, without molecular analysis of the relevant gene region, it would not be possible to identify those patients who would be successfully treated with imatinib mesylate.

With regard to linked evidence, two studies provided evidence of diagnostic accuracy in terms of diagnostic case-control studies (level III-3 diagnostic evidence). Both studies compared the detection of *KIT* D816V mutations in patients with SMCD and mast cell hyperplasia. Both studies, for which there was probable overlap of patients, reported excellent specificity (100%), but the sensitivity (88–99%) indicated that not all patients with SMCD had a detectable *KIT* D816V mutation.

False negative results, such as those seen in Krokowski et al (2005), could potentially result in patients receiving inappropriate treatment. Patients with the *KIT* D816V mutation are known to be resistant to imatinib (Patnaik et al 2007). If molecular analysis was unable to detect a mutation that was present, there is a risk that the patient may be considered a suitable candidate for imatinib therapy. Consequently, patients may receive a treatment that may not only be ineffective, but is also associated with significant costs and some adverse effects.

No studies specifically reporting a change in management were identified, particularly in patients diagnosed with SMCD associated with eosinophilia. The direct evidence of Pardanani et al (2006) reports that patients would receive imatinib mesylate therapy following detection of relevant gene rearrangements. However, the study design does not provide evidence of a change in management based on the addition of molecular analysis to the diagnostic strategy for SMCD, as other patients in whom the *CHIC2* deletion was not detected were also treated with imatinib mesylate. The expert opinion of the Advisory Panel confirms that a change in management would occur with the introduction of molecular analysis into the diagnostic strategy.

Further evidence of treatment effectiveness following a change in management in patients with SMCD associated with eosinophilia was provided by two good-quality studies (level IV intervention evidence). The study by Droogendijk et al (2006) reported the effect of imatinib mesylate in a small group of patients with SMCD who had been analysed for the *KIT* D816V mutation and the *FIP1L1-PDGFRA* rearrangement. Although the reported primary outcomes were not relevant to this assessment, the secondary outcomes were patient relevant. A complete response to treatment was seen in the one patient with a *FIP1L1-PDGFRA* rearrangement. Interestingly, all patients

reported some improvement (which may have included improvement in surrogate outcomes), including patients with the *KIT* D816V mutation.

The study by Pardanani et al (2003) reported the outcomes of imatinib mesylate therapy in a small group of five patients with SMCD associated with eosinophilia, three of whom were carrying the *CHIC2* deletion and two who were *KIT* D816V-positive. A complete response was reported in all patients with the *CHIC2* deletion, and all patients with the *KIT* mutation were shown to be refractory to imatinib mesylate. Despite the results of this study being limited by the small numbers studied and a lack of definition regarding a complete response, Pardanani et al (2003) provide evidence that a real benefit is likely in patients with SMCD associated with eosinophilia that are positive for the *FIP1L1-PDGFRA* rearrangement when treated with imatinib mesylate.

An evaluation of the body of evidence considered in this assessment is provided in Table 64.

Table 64 Completed body of evidence assessment matrix for the assessment of SMCD

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence-base[a]				Level IV studies, or level I to III studies with high risk of bias
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact			Moderate	
Generalisability		Population(s) studied in the body of evidence are similar to the target population for the guideline		
Applicability		Applicable to Australian healthcare context with few caveats		

Adapted from NHMRC (2008)
[a] Level of evidence determined from the NHMRC evidence hierarchy
[b] If there is only one study, rank this component as 'not applicable'.

HES and CEL

No studies included in this assessment analysed for the presence of rearrangements involving the *FGFR1* gene.

One study provided direct evidence of benefit to patients following the use of molecular analysis in the investigation of eosinophilia (Loules et al 2009). This study (level IV intervention evidence) reported the diagnostic yield of molecular analysis in addition to cytogenetic and flow cytometry. Diagnoses were made according to the 2001 WHO diagnostic criteria, and those patients diagnosed with primary eosinophilia (ie CEL, HES

and SMCD with eosinophilia) were treated with imatinib mesylate. The only patient who did not respond to imatinib mesylate had been diagnosed with SMCD and was known to not carry either the *KIT* D816V mutation or any rearrangement that would indicate a susceptibility to imatinib mesylate. The remaining patients with CEL and HES all achieved a haematological response to therapy, with both patients with CEL achieving a complete response.

Again, the study by Loules et al (2009) provides weak evidence of a direct benefit to patients who undergo investigation of eosinophilia with molecular analysis. The study consists of very small numbers and does not provide evidence relative to an investigation of eosinophilia without molecular analysis. Despite these limitations, the study indicates that there is some benefit to those patients who are diagnosed with primary eosinophilia with the use of molecular analysis, particularly if they are identified as having the *FIP1L1-PDGFRA* rearrangement.

The linked evidence approach only identified studies that provided low-level evidence of diagnostic accuracy (level IV diagnostic evidence). Diagnostic yield ranged from 5% to 33% in patients presenting with persistent hypereosinophilia. The study that reported a yield of 5% may be explained by different referral patterns in the regions that supplied this reference laboratory with samples. The other two studies were conducted in single centres and would not have been as reliant on the referral patterns of surrounding regions. It is therefore more likely that the true diagnostic yield of CEL in patients with persistent hypereosinophilia is between 20% and 33%.

No evidence was identified that specifically reported a change in management. As the effectiveness of treatment with imatinib mesylate is established for patients with CEL, a change in management would require evidence of earlier treatment as a consequence of diagnosis with molecular analysis. As treatment for CEL is established, a systematic review of treatment effectiveness was not conducted.

The benefit of molecular analysis in the investigation of persistent eosinophilia is expected to result from improved diagnostic accuracy—without molecular evidence of clonality, it has often been difficult to diagnose CEL (Bain 2004; Gotlib et al 2004). Furthermore, improved diagnostic accuracy would enable a proportion of patients to avoid further testing in order to determine a diagnosis. The benefit of molecular analysis in providing evidence of clonality should not be underestimated. However, studies of diagnostic accuracy that compare a diagnostic strategy using molecular analysis against all other relevant clinical and laboratory information are unlikely to show an improved diagnostic accuracy. This would be due to the imperfect nature of the reference standard, which is unable to accurately diagnose CEL owing to the difficulties in establishing a clonal origin of the eosinophils. In these circumstances it is necessary to use direct evidence of an improvement in patient health outcomes. The limited nature of the direct evidence identified in this assessment makes it difficult to draw conclusions regarding the benefit of molecular testing in the diagnosis of primary eosinophilia. Due to the scarcity of CEL and HES in the community and the recognised effectiveness of therapy based on molecular status, it is unlikely that significant studies will be published in the future.

An assessment of the body of evidence included in this assessment is provided in Table 65.

Table 65 Completed body of evidence assessment matrix for the assessment of HES and CEL

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence-base[a]				Level IV studies, or level I to III studies with high risk of bias
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact				Slight or restricted[c]
Generalisability		Population(s) studied in the body of evidence are similar to the target population for the guideline		
Applicability		Applicable to Australian healthcare context with few caveats		

Adapted from NHMRC (2008)
[a] Level of evidence determined from the NHMRC evidence hierarchy
[b] If there is only one study, rank this component as 'not applicable'.
[c] It is expected that, without molecular analysis, patients would require additional testing to determine their diagnosis.

Detection of the *FIP1L1-PDGFRA* fusion gene or other relevant rearrangements identifies patients who may be responsive to imatinib treatment. It is important that such patients are appropriately identified, as treatment with imatinib can be associated with considerable side effects as well as significant financial implications. Molecular testing in the diagnosis of these patients is not only required to prevent empirical treatment, but evidence of the relevant rearrangements is required to ensure reimbursement of imatinib treatment through the PBS.

What are the economic considerations?

SMCD

A lack of evidence regarding the safety, and limited evidence of the effectiveness, of molecular analysis in the diagnosis of SMCD compared with current test strategies prevent an economic evaluation being conducted.

A financial analysis of the costs associated with the index test strategy relative to the comparator test strategy has been considered from a healthcare perspective. This analysis has been based on the assumption that 134 patients would be investigated for suspected SMCD per year and that 107 of these investigations would be conducted in the private healthcare sector. Furthermore, it has been assumed that four patients with SMCD associated with eosinophilia would be suitable for imatinib mesylate therapy after failing conventional therapy. This analysis has only considered the incremental costs of the test strategy and the costs of imatinib mesylate therapy for 12 months. Further downstream costs of therapy have not been considered.

The financial implications to the Australian healthcare system overall for the addition of molecular analysis to the test strategy are estimated to be an additional $234,000 per year. The financial implications to the Commonwealth in terms of the Medicare rebate for 107 private patients are estimated to be an additional $22,000 per year.

The greater proportion of the costs to the Australian healthcare system is the cost of imatinib mesylate therapy, of which the majority will be borne by the PBS. This cost is likely to be associated with greater improvement in health outcomes for patients receiving imatinib mesylate; however, as this subgroup is a very small proportion of all patients with SMCD, the overall benefit to the whole population is likely to be small.

HES and CEL

Again, the absence of evidence regarding the safety of molecular analysis and uncertainty surrounding the benefit to patient health outcomes has prevented a formal economic evaluation being conducted.

Due to extensive uncertainty surrounding the likely need for *FGFR1* analysis, as well as a complete absence of evidence regarding diagnostic yield, assessment of the financial implications of molecular analysis of this rearrangement has not been considered.

The financial analysis conducted has been based on a number of assumptions: the number of likely investigations per year; that the molecular analysis will include only the *FIP1L1-PDGFRA* fusion gene; that the public to private split of patients will be 20% to 80%; and that the index test strategy is as effective as the comparator in terms of diagnostic accuracy.

Expert opinion indicated that the number of likely investigations of persistent eosinophilia would not exceed 50 per year.

The financial implications of the addition of molecular testing to the diagnostic strategy for HES and CEL to the Australian healthcare system overall is a cost of $11,800 per year for 50 patients.

The cost to the MBS in terms of the 75% rebate for private patients being investigated as out-patients would be an additional $7,000 per year for 40 patients.

Conclusions

Safety

No evidence was identified that could inform the research question regarding the comparative safety of molecular analysis in the diagnosis of SMCD, HES or CEL.

For the analysis of *KIT* mutations, it is recommended that DNA material be obtained from BM samples (Valent et al 2007). As patients suspected of SMCD would require BM biopsy regardless of whether or not molecular analysis was used in the diagnostic test strategy, it can be concluded that molecular analysis for suspected SMCD is likely to be as safe as the comparator diagnostic strategy without molecular testing.

For patients with persistent eosinophilia, DNA material for molecular analysis can be obtained from either peripheral blood or BM biopsy. Patients will have undergone BM biopsy prior to entering this clinical pathway. Consequently, genetic material may be obtained from the BM biopsy done previously or from collection of peripheral blood. The known risks associated with venepuncture in general are slight; so it would be reasonable to assume that the addition of *FIP1L1-PDGFRA* analysis to the diagnostic strategy of persistent eosinophilia would be as safe as a diagnostic test strategy without molecular analysis.

Effectiveness

SMCD—direct evidence

Weak evidence of an impact on patient outcomes informed the research question regarding the effectiveness of molecular testing in the diagnosis of SMCD. The evidence that was identified indicated that, after diagnosis of SMCD with eosinophilia and detection of the *FIP1L1-PDGFRA* rearrangement, treatment with imatinib mesylate would provide a significant benefit to patients. However, there was no direct evidence that reported the effectiveness of a diagnostic strategy that included molecular analysis relative to a diagnostic strategy without such analysis.

The benefit from imatinib mesylate therapy will be seen in only a small proportion of patients with SMCD. The model used to determine the financial implications of molecular analysis in the diagnosis of SMCD estimates that only 13% of all patients diagnosed with SMCD will have associated eosinophilia and the *FIP1L1-PDGFRA* rearrangement.

The lack of comparative data regarding effectiveness and the small proportion of patients who would benefit from imatinib mesylate therapy makes it difficult to conclude that the addition of molecular testing would be as or more effective than diagnosis of SMCD without molecular analysis based on direct evidence alone. However, due to the interstitial nature of the 4q12 deletion, which results in the *FIP1L1-PDGFRA* fusion gene, the comparator test strategy is unable to detect this rearrangement, and thus would not be able to identify patients who would benefit from imatinib mesylate.

SMCD—linked evidence

Diagnostic accuracy

The limited studies of diagnostic accuracy were weakened by their diagnostic case-control study design. Nevertheless, they indicated that molecular analysis of *KIT* mutations provided very good sensitivity (88–99%) and excellent specificity (100%) compared with the reference standard. This would indicate that the probability of false positive results is very low but that a negative result for the presence of a *KIT* mutation would not necessarily exclude SMCD. As it is proposed that molecular analysis be used in addition to BM biopsy and serum tryptase levels, it would be expected that the benefit of *KIT* analysis would be in addition to the current diagnostic strategy.

Ideally, it would be preferable to have evidence of diagnostic accuracy based on a cross-classification study; however, it is unlikely that such data would become available. With this in mind, it could be concluded that the benefit of molecular analysis of *KIT* mutations in the diagnosis of SMCD would be in addition to that of the current comparator test strategy. Therefore, molecular analysis in the diagnosis of SMCD is likely to be at least as accurate as the comparator test strategy.

Impact on patient management

No studies were identified that explicitly reported outcomes of change in patient management as a result of the addition of molecular analysis to the diagnostic test strategy. However, as studies in other populations have firmly established that the *FIP1L1-PDGFRA* fusion gene is a target of imatinib mesylate, it is unlikely that further data will become available to indicate a change in management in patients with SMCD associated with eosinophilia.

Furthermore, the direct evidence included in this assessment implies that a change in patient management occurred in patients who were identified as having the *FIP1L1-PDGFRA* rearrangement. Subsequently, it can be concluded that molecular analysis of the *FIP1L1-PDGFRA* rearrangement is likely to result in a change in management of the small subgroup of patients who have SMCD associated with eosinophilia.

Impact on health outcomes

The only patients who are likely to benefit from a change in patient management are those with SMCD associated with eosinophilia and who have the *FIP1L1-PDGFRA* rearrangement. Two very small case series reported that such patients who received imatinib mesylate therapy would achieve a complete response to therapy that included resolution of clinical and haematological signs of disease. No comparative evidence was available to indicate the effectiveness of conventional therapy in these patients; however, as imatinib mesylate would be given as a second-line therapy after the failure of conventional therapy, it may be concluded that imatinib mesylate is likely to be at least as effective as conventional therapy in patients with SMCD associated with eosinophilia and the *FIP1L1-PDGFRA* rearrangement.

Overall, the evidence that informed the research questions for the assessment of SMCD was scant and weak. However, due to the low disease prevalence, it is unlikely that any comparative data will become available in the future. Based on the available evidence and conservative argument, it may be concluded that the addition of molecular analysis to the

diagnostic strategy for SMCD is likely to be at least as effective as BM biopsy (including immunohistochemical staining) and determination of serum tryptase levels alone.

HES and CEL—direct evidence

A small case series provided weak evidence of an improvement in patient outcomes after diagnosis with molecular analysis of the *FIP1L1-PDGFRA* rearrangement. No comparative data were available for patients who were diagnosed with primary eosinophilia without molecular analysis.

As there are no comparative data available, the direct evidence identified is not able to answer the research question regarding the comparative effectiveness of the addition of molecular analysis to the diagnostic strategy for HES and CEL.

HES and CEL—linked evidence

The treatment for CEL and HES is well established and will not alter as a consequence of molecular analysis; therefore, evidence of improved diagnostic accuracy compared with diagnosis without molecular testing is required to indicate that the addition of molecular analysis in the investigation of patients with persistent eosinophilia is more effective than a strategy without. No such comparative evidence was identified.

As a consequence, there is some uncertainty surrounding the comparative effectiveness of molecular analysis in the diagnosis of HES and CEL. Again, the very low prevalence of the disease will make comparative data difficult and unlikely to be produced. As clonal eosinophilia is now classified according to the presence and type of molecular rearrangement present, and considering the imperfect nature of the reference standard due to the absence of molecular analysis, the probability of comparative evidence becoming available is further reduced.

Economic considerations

Lack of appropriate data and uncertainty surrounding the net benefit in patients suspected of SMCD, HES and CEL prevented a formal economic evaluation being conducted.

As a consequence, a financial analysis of the expenditures associated with molecular analysis in the diagnostic strategies of SMCD, HES and CEL has been conducted.

SMCD

The estimated number of investigations for suspected SMCD is 134 per year. It is expected that 107 of these will be conducted in the private healthcare sector. Furthermore, it is expected that four patients who are diagnosed with SMCD associated with eosinophilia and the *FIP1L1-PDGFRA* rearrangement will require additional treatment with imatinib mesylate. This analysis included the cost of therapy for 12 months only although it should be noted that ongoing treatment is likely.

The total cost to the MBS for the addition of molecular analysis to the diagnostic strategy for SMCD is estimated to be an additional $22,000 per year.

The total cost to the Australian healthcare system, including the MBS and PBS, for the addition of molecular analysis to the diagnostic strategy for SMCD is estimated to be an additional $234,000 per year. If the dose of imatinib mesylate is lowered from 400 mg/day to 100 mg/day, this cost is likely to be an additional $92,700 per year. The greater proportion of this cost relates to the treatment of the small number of patients with SMCD associated with eosinophilia and the *FIP1L1-PDGFRA* rearrangement.

HES and CEL

The financial analysis of expenditures related to the diagnosis of HES and CEL is surrounded by extensive uncertainty. Due to the paucity of data available, a number of assumptions were required.

It is estimated that the number of investigations for persistent eosinophilia is unlikely to exceed 50 per year. Additionally, it is assumed that 40 patients will be investigated in the private healthcare sector per year.

The total cost to the MBS for the addition of molecular analysis to the diagnostic strategy for HES and CEL is estimated to be an additional $7,000 per year.

The total cost to the Australian healthcare system including MBS for the addition of molecular analysis to the diagnostic strategy for HES and CEL is estimated to be an additional $11,800 per year.

The estimated costs and/or savings of the index test strategy relative to the comparator are summarised in Table 66 according to the agency that incurs the cost.

Table 66 Summary of incremental costs of the index test strategy relative to the comparator

	Estimated number of investigations per year	Index test strategy costs
SMCD		
Australian healthcare system	134	$233,904
MBS	107	$21,649
States/territories	27	$119,900
HES, CEL		
Australian healthcare system	≤ 50	$11,625
MBS	≤ 40	$ 6,976
States/territories	≤ 10	$2,325

MBS = Medicare Benefits Schedule
Note: All costs are in addition to the current testing strategy.

Appendix G Advisory Panel and Evaluators

Advisory Panel – Application 1125 – Molecular testing for myeloproliferative disorders

Member	Nomination / Expertise or affiliation
Professor Richard Fox (Chair)	Member of MSAC Haematology/Oncology
Dr Kwun Fong	Member of MSAC Thoracic/Sleep medicine
Dr Rosemary Harrup	The Medical Oncology Group nominee Haematology/Oncology
Dr Robert Lindeman	Royal College of Pathologists nominee Haematology
Mr Russell McGowan	Consumer Health Forum nominee Consumer health
Dr Ian Prosser	Member of MSAC Haematology
Dr Zbigniew (Barney) Rudzki	Co-opted Molecular Pathology
Dr David Westerman	Haematology Society of Australia and New Zealand nominee Haematology
Dr Bronwyn Williams	Royal College of Pathologists nominee Haematology/Transfusion medicine

Evaluators

Name	Organisation
Ms Liz Buckley	Research officer, Adelaide Health Technology Assessment
Ms Tracy Merlin	Manager, Adelaide Health Technology Assessment

Appendix H Search strategies

Electronic bibliographic databases were searched to find relevant studies (those meeting the inclusion criteria) addressing each of the research questions developed for this MSAC assessment. These databases are described in Table 67. Molecular testing for the diagnosis of SMCD, HES and CEL appears in the literature only since 1992, so the search period was restricted to 1992 or, if inception of the database was later, from that date until February 2009.

Table 67 Electronic databases searched for relevant literature

Database	Period covered
CINAHL	1992 – 02/2009
Cochrane Library – including, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Database, the NHS Economic Evaluation Database	1992 – 02/2009
Current Contents	1992 – 02/2009
Embase.com (including Embase and Medline)	1992 – 02/2009
Pre-Medline	1992 – 02/2009
Web of Science – Science Citation Index Expanded	1992 – 02/2009
EconLit	1992 – 02/2009

Search terms for identifying literature within these bibliographic databases are given below (Table 68).

Table 68 Search terms used to identify potential studies

Element of clinical question	Suggested search terms
Population	(systemic mast cell disease [text] OR SMCD [text] OR systemic mastocytosis [text] OR Urticaria pigmentosa [MeSH] OR cutaneous mast cell dis* OR cutaneous mastocytosis [MeSH] OR cutaneous mastocytosis [text] OR Mastocytosis, Systemic [MeSH] OR indolent systemic mastocytosis [text] OR aggressive systemic mastocytosis [text] OR Hypereosinophilic Syndrome [MeSH] OR HES [text] OR hypereosinophil* [text] OR eosinophil* [text] OR CEL [text] OR chronic eosinophilic leukaemia [text])
Intervention/test	(Receptors, Platelet-Derived Growth Factor/genetics [MeSH] OR Proto-Oncogene Proteins c-kit/genetics [MeSH] OR KIT [text] OR D816V [text] OR PDGFR* [text] OR Receptor, Fibroblast Growth Factor, Type 1/genetics [MeSH] OR FGFR1 [text] OR Translocation, Genetic [MeSH] OR Mutation [MeSH] OR Gene Rearrangement [MeSH] OR gene rearrangement [text] OR muta* [text] OR oncogene [MeSH] OR oncogene [text])
Comparator (if applicable)	n/a
Outcomes (if applicable)	n/a
Limits	Human

Additional sources of literature—peer-reviewed or grey literature—were sought from the sources listed in Table 69 and from the HTA agency websites listed in Table 70.

Table 69 Additional sources of literature

Source	Location
Internet	
Australian Clinical Trials Registry	http://www.actr.org.au
NHMRC - National Health and Medical Research Council (Australia)	http://www.health.gov.au/nhmrc/
US Department of Health and Human Services (reports and publications)	http://www.os.dhhs.gov/
New York Academy of Medicine Grey Literature Report	http://www.nyam.org/library/greylit/index.shtml
Trip database	http://www.tripdatabase.com
Current Controlled Trials metaRegister	http://controlled-trials.com/
National Library of Medicine Health Services/Technology Assessment Text	http://text.nlm.nih.gov/
U.K. National Research Register	http://www.update-software.com/National/
Google Scholar	http://scholar.google.com/
Hand searching (journals 2007–09)	
Blood	Electronic access
Leukemia research	Electronic access
Leukemia	Electronic access
Best practice & research clinical haematology	Electronic access
American Journal of Hematology	Electronic access
European Journal of Haematology	Electronic access
Haematologica	Electronic access
Journal of Molecular Diagnostics	Electronic access
British Journal of Haematology	Electronic access
New England Journal of Medicine	Electronic access
Acta haematologica	Electronic access
American Journal of Clinical Pathology	Electronic access
Expert clinicians	
Studies other than those found in regular searches	MSAC Advisory Panel
Speciality websites	
American Society of Hematology	www.hematology.org
MPD Foundation	www.mpdfoundation.org
National Cancer Institute	www.cancer.gov/cancertopics/pdq/treatment/myeloproliferative/patient
The Myeloproliferative Disorders Research Consortium	www.mpd-rc.org/home.php
MPD Online Resource	www.mpdinfo.org/index.html
The Leukaemia Foundation	www.leukaemia.org.au/web/aboutdiseases/related_index.php
The British Society for Haematology	www.b-s-h.org.uk/
The Leukemia and Lymphoma Society	www.leukemia.org/hm_lls
Haematology Society of Australia and New Zealand	www.hsanz.org.au
International Society of Laboratory Hematology	www.islh.org
Royal College of Pathologists of Australasia	www.rcpa.edu.au
International Society of Haematology, Asian-Pacific Division	www.ishapd.org
Australasian Leukaemia and Lymphoma Group	www.petermac.org/allg/
Association of Cancer Online Resources	www.acor.org
MPD Support Group	http://members.aol.com/mpdsupport/
HES Resource	www.hesresource.com/
The Mastocytosis Society	www.tmsforacure.org/patientinfo.shtml

Hesyndrome	www.hesyndrome.com
American Partnership for Eosinophilic Disorders	www.apfed.org

Table 70 Health Technology Assessment Agency websites

AUSTRALIA	
Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP-S)	http://www.surgeons.org/open/asernip-s.htm
Centre for Clinical Effectiveness, Monash University	http://www.mihsr.monash.org/cce
Centre for Health Economics, Monash University	http://www.buseco.monash.edu.au/che/
AUSTRIA	
Institute of Technology Assessment / HTA unit	http://www.oeaw.ac.at/ita/e1-3.htm
CANADA	
Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (AETMIS)	http://www.aetmis.gouv.qc.ca/site/home.php/
The Canadian Agency for Drugs And Technologies in Health (CADTH)	http://www.cadth.ca/index.php/en/
Centre for Health Economics and Policy Analysis (CHEPA), McMaster University	http://www.chepa.org
Centre for Health Services and Policy Research (CHSPR), University of British Columbia	http://www.chspr.ubc.ca
Health Utilities Index (HUI)	http://www.fhs.mcmaster.ca/hug/index.htm
Institute for Clinical and Evaluative Studies (ICES)	http://www.ices.on.ca
Institute of Health Economics	http://www.ihe.ca
Saskatchewan Health Quality Council (Canada)	http://www.hqc.sk.ca
DENMARK	
Danish Centre for Evaluation and Health Technology Assessment (DACEHTA)	www.sst.dk/Planlaegning_og_behandling/Medicinsk_teknologivurdering.aspx?lang=en
Danish Institute for Health Services Research (DSI)	http://www.dsi.dk/engelsk.html
FINLAND	
Finnish Office for Health Technology Assessment (FINOHTA)	http://www.stakes.fi/EN/index.htm
FRANCE	
L'Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES)	http://www.anaes.fr/
GERMANY	
German Institute for Medical Documentation and Information (DIMDI) / HTA	http://www.dimdi.de/static/en
THE NETHERLANDS	
Health Council of the Netherlands Gezondheidsraad	http://www.gr.nl/index.php
Institute for Medical Technology Assessment (Netherlands)	http://www.imta.nl/
NEW ZEALAND	
New Zealand Health Technology Assessment (NZHTA)	http://nzhta.chmeds.ac.nz/
NORWAY	
Norwegian Knowledge Centre for Health Services	http://www.nokc.no/About+us
SPAIN	
Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III"l/Health Technology Assessment Agency (AETS)	http://www.isciii.es/htdocs/en/investigacion/Agencia_quees.jsp
Andalusian Agency for Health Technology Assessment (Spain)	http://www.juntadeandalucia.es/salud/orgdep/AETSA/default.asp?V=EN
Catalan Agency for Health Technology Assessment (CAHTA)	http://www.aatrm.net/html/en/Du8/index.html
SWEDEN	
Center for Medical Health Technology Assessment	http://www.cmt.liu.se/english/publications
Swedish Council on Technology Assessment in Health Care (SBU)	http://www.sbu.se/www/index.asp
SWITZERLAND	

Swiss Network on Health Technology Assessment (SNHTA)	http://www.snhta.ch/
UNITED KINGDOM	
National Health Service Health Technology Assessment (UK) / National Coordinating Centre for Health Technology Assessment (NCCHTA)	http://www.hta.nhsweb.nhs.uk/
NHS Quality Improvement Scotland	http://www.nhshealthquality.org/
National Institute for Clinical Excellence (NICE)	http://www.nice.org.uk/
The European Information Network on New and Changing Health Technologies	http://www.euroscan.bham.ac.uk/
University of York NHS Centre for Reviews and Dissemination (NHS CRD)	http://www.york.ac.uk/inst/crd/
UNITED STATES	
Agency for Healthcare Research and Quality (AHRQ)	http://www.ahrq.gov/clinic/techix.htm
Harvard School of Public Health – Cost-Utility Analysis Registry	http://www.tufts-nemc.org/cearegistry/
Institute for Clinical Systems Improvement (ICSI)	http://www.icsi.org
Minnesota Department of Health (US)	http://www.health.state.mn.us/
National Information Centre of Health Services Research and Health Care Technology (US)	http://www.nlm.nih.gov/hsrph.html
Oregon Health Resources Commission (US)	http://egov.oregon.gov/DAS/OHPPR/HRC/about_us.shtml
U.S. Blue Cross/ Blue Shield Association Technology Evaluation Center (Tec)	http://www.bcbs.com/consumertec/index.html
Veteran's Affairs Research and Development Technology Assessment Program (US)	http://www.va.gov/resdev

Appendix I Classification of myeloid neoplasms

Table 71 2008 WHO classification scheme for myeloid neoplasms

1. Acute myeloid leukaemia
2. Myelodysplastic syndromes (MDS)
3. Myeloproliferative neoplasms (MPN) 3.1 Chronic myelogenous leukaemia 3.2 Polycythaemia vera 3.3 Essential thrombocythaemia 3.4 Primary myelofibrosis 3.5 Chronic neutrophilic leukaemia 3.6 Chronic eosinophilic leukaemia, not otherwise categorized 3.7 Hypereosinophilic syndrome 3.8 Mast cell disease 3.9 MPNs, unclassifiable
4. MDS/MPN 4.1 Chronic myelomonocytic leukaemia 4.2 Juvenile myelomonocytic leukaemia 4.3 Atypical chronic myeloid leukaemia 4.4 MDS/MPN, unclassifiable
5. Myeloid neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1* 5.1 Myeloid neoplasms associated with *PDGFRA* rearrangement 5.2 Myeloid neoplasms associated with *PDGFRB* rearrangement 5.3 Myeloid neoplasms associated with *FGFR1* rearrangement (8p11 myeloproliferative syndrome)

Adapted from Tefferi & Vardiman (2008)

Appendix J Studies included in the review

Study profiles of included studies in the assessment of the effectiveness of molecular testing for the diagnosis of SMCD, HES and CEL

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
(Bacher et al 2006) Germany	Retrospective case series Level IV diagnostic evidence CX P1 Q2	40 patients with unexplained and persistent eosinophilia Males n=27 Females n=13 Median age = 60 (range 19–89) years Median WCC = 14.7x10^9/L Median eosinophil count = 50%	Not reported	BM histology, cytogenetics and molecular testing	Diagnostic yield	n/a
(Droogendijk et al 2006) University Medical Centers of Rotterdam and Groningen, The Netherlands	Level IV intervention evidence CX P1 Q1	14 patients with SMCD Females n = 5 Age range: 43–73 years	*Inclusion:* Men and non-pregnant women between 18 and 75 years with smoldering, indolent or aggressive SMCD Histologically proven SMCD, elevated serum tryptase levels > 20 µg/L and/or markedly elevated urinary-methylhistamine *Exclusion:* Life expectancy < 3 months, NYHA classification of functional class III or IV, or a Karnofsky performance	Imatinib mesylate therapy 400 mg/day orally	Treatment effectiveness	Not reported

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
			score < 50 Treatment with chemotherapy or radiotherapy in previous 6 months Use of warfarins or systemic corticosteroids with a daily dose > 15 mg prednisone or equivalent			
(Johnson et al 2009) MD Anderson Center, USA	Retrospective case series Level IV diagnostic evidence CX P1 Q2	59 patients with a high clinical suspicion of SMCD Males n=25 Females n=34 Median age = 54 (range 23–75) years	High clinical suspicion of SMCD who underwent a BM aspiration and biopsy, and for whom ancillary testing was performed	Serum tryptase levels, BM histology with immunohistochemical staining, flow cytometry analysis for CD2, CD25, CD45 and CD117, and molecular testing for *KIT* D816V and *FIP1L1-PDGFRA*	Diagnostic yield	n/a
(Krokowski et al 2005) Germany *Likely overlap of patients with study by Sotlar et al (2004)*	Diagnostic case-control Level III-3 diagnostic evidence CX P2 Q2	88 patients with either SMCD or MCH *Cases*: SMCD (n=57) Indolent SMCD = 30 Smoldering SMCD = 3 SM-AHNMD = 17 Aggressive = 4 MCL = 3 *Controls:* MCH (n=31)	Not reported	2001 WHO criteria	Diagnostic accuracy—sensitivity, specificity, PPV, NPV	n/a
(Loules et al 2009) Greece	Case series Level IV diagnostic evidence CX	15 patients with eosinophilia (> $1.5x10^9$/L) with unambiguous history of allergic diseases. Male n=7	Not reported	Diagnosis using standard criteria or molecular test results. Standard criteria included results from cytogenetic	Not reported	n/a

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
	P1 Q2	Female n=8 Mean age = 45 (range 22–72) years		analysis, flow cytometry analysing CD3, CD4 and CD8, and molecular testing for *KIT* D816V and *FIP1L1-PDGFRA*		
(Metzgeroth et al 2007) Germany	Prospective case series Level IV diagnostic evidence CX P1 Q2	580 patients with persistent eosinophilia	Not reported	All available laboratory tests including PB morphology and BM histology, and molecular testing for the presence of *FIP1L1- PDGFRA* fusion gene	Diagnostic yield	n/a
(Pardanani et al 2006b) Mayo Clinic, USA	Case series Level IV intervention evidence CX P1 Q2	830 patients with suspected or known HES and/or SMCD	Not reported	All pertinent laboratory and clinical data including BM histology. Diagnosis was according to 2001 WHO criteria	Treatment response	In patients with *CHIC2* deletion/translocation, range = 1–40 months
(Pardanani et al 2003) Mayo Clinic, USA	Prospective case series Level IV intervention evidence CX P1 Q1	5 patients with symptomatic mast cell disease with eosinophilia ASM = 4 ISM = 1	Symptomatic mast cell disease proven by BM histology and deemed to require cytoreductive therapy	2001 WHO criteria	Treatment response to imatinib mesylate	Range: 1–19 months
(Sonneck et al 2007)	Case series Level IV diagnostic evidence CX P1 Q2	6 patients with hypotension following insect stings	Not reported	BM histology, serum tryptase levels, immunohistochemistry and molecular testing	Diagnostic yield	n/a
(Sotlar et al 2004)	Diagnostic case-control	148 patients with SMCD or	Not reported	*Cases:*	Diagnostic accuracy—	n/a

Study and location	Study design and appraisal Level of evidence Comparison Population Quality	Study participants	Inclusion/exclusion criteria	Intervention and comparator / reference standard	Outcomes assessed	Duration of follow-up
Likely overlap of patients with study by Krokowski et al (2005)	Level III-3 diagnostic evidence CX P1 Q2	MCH *Cases:* SMCD (n=73) ISM = 43 SM-AHNMD = 20 ASM = 7 MCL = 3 *Controls:* MCH (n=75) Reactive BM = 54 Myelogenous neoplasms other than SM or without coexisting SM = 16 Myelomastocytic leukaemia = 5		43 patients had PNA-PCR of BM samples followed by melting curve analysis. If negative, then nested PCR of microdissected mast cells and melting curve analysis. The remaining 30 patients had RT-PCR and *HinfI* digestion of isolated mononuclear cells. *Controls*: PNA-PCR of BM samples followed by melting curve analysis of PCR products if mutation present. *Reference standard:* 2001 WHO criteria for the diagnosis of SMCD	sensitivity, specificity, PPV, NPV	
(Tan et al 2006) Australia	Case series Level IV diagnostic evidence CX P1 Q2	26 patients with SMCD or suspicious of SMCD SMCD (n=13) Clinical suspicion of SMCD (n=13)	Not reported	2001 WHO criteria for the diagnosis of SMCD	Diagnostic yield	n/a
(Wang et al 2008) China	Case series Level IV diagnostic evidence CX P1 Q2	24 patients with persistent hypereosinophilia Males n=18 Females n=6 Median age = 43 (range 16–72) years	Not reported	2001 WHO criteria for the classification of HES/CEL plus molecular testing	Diagnostic yield and survival	n/a

ASM = aggressive systemic mastocytosis; BM = bone marrow; HES = hypereosinophilic syndrome; CEL = chronic eosinophilic leukaemia; ISM = indolent systemic mastocytosis; MCL = mast cell leukaemia; n/a = not applicable; PB = peripheral blood; SMCD = systemic mast cell disease; SM–AHNMD = systemic mastocytosis with associated clonal haematologic non-mast cell lineage disease; PNA-PCR = Peptide nucleic acid mediated polymerase chain reaction; MCH = mast cell hyperplasia; PPV = positive predictive value; NPV = negative predictive value; WCC = white cell count; NYHA = New York Heart Association

Appendix K Excluded studies

Incorrect intervention

Abruzzo, L.V., Jaffe, E.S. et al (1992). 'T-cell lymphoblastic lymphoma with eosinophilia associated with subsequent myeloid malignancy', *American Journal of Surgical Pathology,* 16 (3), 236–245.

Akin, C., Jaffe, E.S. et al (2002). 'An immunohistochemical study of the bone marrow lesions of systemic mastocytosis: expression of stem cell factor by lesional mast cells', *American Journal of Clinical Pathology,* 118 (2), 242–247.

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Aslan, V. & Entok, E. (2006). 'Factor V Leiden mutation and deep venous thrombosis in a patient with hypereosinophilic syndrome', *Saudi Medical Journal,* 27 (9), 1418–1420.

Autier, J., Buffet, M. et al (2005). 'Non-pigmented micromastocytomas: an exceptional presentation of cutaneous mastocytosis in an adult', *Dermatology,* 211 (4), 376–378.

Avshalumov, K., Pichardo, R. et al (2008). 'Bullous mastocytosis: report of a patient and a brief review of the literature', *American Journal of Dermatopathology,* 30 (5), 455–457.

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Chim, C.S. & Ma, S.K. (2005). 'Eosinophilic leukemic transformation in polycythemia rubra vera (PRV)', *Leukemia & Lymphoma,* 46 (3), 447–450.

Cogan, E., Schandene, L. et al (1994). 'Brief report: clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome', *New England Journal of Medicine,* 330 (8), 535–538.

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Egesten, A., Hagerstrand, I. et al (1997). 'Hypereosinophilic syndrome in a child mosaic for a congenital triplication of the short arm of chromosome 8', *British Journal of Haematology,* 96 (2), 369–373.

Engelmann, M.G., Kolbe, T. et al (2004). 'Hypereosinophilic syndrome associated with heterozygous factor V gene mutation: an unusual combination resulting in an acute coronary syndrome and recurrent cerebral stroke - a case report', *Angiology,* 55 (2), 221–225.

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