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NATA
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Patient	**Adam SMITH**	**Lab No**	18465975		
URN	PMEX996543	**Ext Ref**	3469711335	**Requester**	Dr JOHN SMITH
DOB	01-Jan-1960	**Collected**	31-Dec-2022	**Referral Lab**	Dorevitch Pathology
Sex	M	**Received**	04-Jan-2023		
		Specimen	Bone marrow aspirate		

Clinical Indication Polycythaemia

Correlative Morphology Not provided

Specimen Details N/A

MYELOPROLIFERATIVE NEOPLASM GENE PANEL REPORT

Test Description Somatic variant analysis of 22 genes with clinical significance in myeloproliferative neoplasms. Refer to Panel Summary for gene list.

Result Summary **ASSUMED SOMATIC VARIANTS DETECTED. See Reportable Variants table below for details.**

Clinical Interpretation Variants in DNMT3A and SH2B3 were detected in this bone marrow aspirate sample consistent with a diagnosis of a myeloproliferative neoplasm (MPN) or idiopathic erythrocytosis. No typical MPN driver variants (JAK2/CALR/MPL) were detected in this sample.

Please correlate with morphological, immunophenotypic and cytogenetic features.

Refer to final page of report for further information regarding the clinical utility of genomic testing in this context.

Test Results

Reportable Variants Please note, variant origin (somatic or germline) cannot be determined by this assay. Variant origin is assumed here based on ancillary information (e.g. population databases, literature, variant read frequency) for the purpose of clinical interpretation however testing of a germline sample may be recommended in some circumstances.

ASSUMED ORIGIN	GENE	VARIANT	VRF (%)	CLINICAL SIGNIFICANCE IN MPN
Somatic	**DNMT3A**	**c.2645G>A p.(Arg882His)**	**7**	**CLONAL MARKER**
Somatic	**SH2B3**	**c.1345G>A p.(Glu449Lys)**	**3**	**DIAGNOSTIC**

VRF – variant read frequency

Test Methodology

DNA is analysed by targeted gene sequencing of coding regions and flanking splice sites (within 2 bp) of the genes listed below. Libraries are prepared using a custom QIAGEN QIAseq single primer extension-based panel (Peter MacCallum Cancer Centre AllHaem v1) and sequenced on an Illumina NextSeq500 with 150 bp paired end reads. A customised CLC bioinformatics pipeline including QIAGEN CLC enterprise solutions is used to generate aligned reads and call variants (single nucleotide variants and short insertions or deletions) against the hg19 human reference genome. Variants are analysed using PathOS software (Peter Mac) and described according to HGVS nomenclature version 19.01 (http://varnomen.hgvs.org/) with minor differences in accordance with Peter MacCallum Cancer Centre Molecular Pathology departmental policy. The following population variation and cancer or genetic disease databases are commonly used in addition to literature review to assist with variant interpretation: the Genome Aggregation Database (gnomAD; gnomad.broadinstitute.org), the Catalogue of Somatic Mutations in Cancer (COSMIC; cancer.sanger.ac.uk), ClinVar (ncbi.nlm.nih.gov/clinvar) and the IARC TP53 Database (p53.iarc.fr). Variant origin (i.e. somatic or germline) is assumed based on ancillary information (e.g. population databases, literature, variant read frequency) for the purpose of clinical interpretation. All assumed somatic variants are reported (and generally considered clinically significant). Variants of uncertain origin are also reported, as are likely benign germline polymorphisms if sufficiently rare and otherwise undescribed. Testing of a non-haematological specimen may be recommended to evaluate variant origin. Recurrent population variants are not reported. **Somatic variant categorisation (modified from AMP/ASCO/CAP guidelines[1]) –** Variants are curated and categorised according to the clinical context of the patient and categorised as **DIAGNOSTIC** (the variant either defines a diagnostic category or is sufficiently specific for the clinical context to contribute to diagnostic subcategorisation), **PROGNOSTIC** (the variant has been associated in large trials/series with inferior or superior outcomes in either the context of a specific therapy or independent of therapy. Note this does not take into account interaction between prognostic variants present in the individual patient. Relevant pairwise interactions are presented in the clinical summary), **DRUG TARGET** (the variant or variant class is specifically targeted by a therapeutic agent, this category only includes therapeutic agents that are clinically advanced and generally available through either reimbursement or clinical trials [i.e. not early stage investigational agents]), **DRUG RESISTANCE** (the variant is specifically associated with resistance to a targeted agent [i.e. does not include non-specific resistance to non-targeted therapies]), **MRD MARKER** (the variant is an established biomarker for which assessment at MRD sensitivity after therapy is accepted practice). If the variant is not categorised into any of the

above categories it is assigned **CLONAL MARKER** indicating its utility in defining the presence of a clonal haematopoietic process in the specimen. These categorisations are general in nature and may not be applicable to the specific clinicopathological context of the patient.

Test Limitations

The detection limit of this assay for specimens sequenced to the target read depth of 500x is a variant allele frequency (VAF) of approximately 2% with the exception of ASXL1 c.1934dup;p.Gly646Trpfs*12 (detection limit ~ 5%). This assay is primarily qualitative however, the variant read frequency (VRF) is provided to assist with variant interpretation and is assumed to approximate VAF in most instances (noting that the VAF of some insertions/deletions may be underrepresented due to assay-based allele bias). The measurement of uncertainty provided as a percentage relative standard uncertainty (i.e. CV%) for variants with VAFs of 5%, 10%-20%, 30%-40% and 50% are on average, 10.2%, 10.4%, 3.5% and 4.4%, respectively. Copy number variations, loss of heterozygosity, structural rearrangements or aneuploidies are not reported. Insertions or deletions (particularly those > 25 bp in length), including FLT3-ITDs, are not reliably detected by this assay. Genes are analysed using the reference transcripts listed below; coding exons found in alternative transcripts are not assessed by this assay. This assay does not distinguish between somatic and germline variants. In addition, the clonal origin of somatic variants (i.e. disease compartment or cell lineage) cannot be determined. Synonymous variants are not routinely reported. Please note Peter Mac assumes sample identification, family relationships, and clinical diagnoses are as stated on the request. Our clinical recommendations may be based on evidence from third-party data sources and should be interpreted in the context of all other clinical and laboratory information for this patient.

Panel Summary

Gene coverage in this sample is as follows

Gene	Transcript	Targeted exons	Coverage at >500x (%)	Gene	Transcript	Targeted exons	Coverage at >500x (%)	Gene	Transcript	Targeted exons	Coverage at >500x (%)
ASXL1	NM_015338.5	10-12	100	JAK2	NM_004972.3	12-14,16	100	SH2B3	NM_005475.2	All coding	100
CALR	NM_004343.3	9	100	KIT	NM_000222.2	8,10-11,17	100	SRSF2	NM_003016.4	1	100
CBL	NM_005188.3	8-9	100	KRAS	NM_033360.2	2-4	100	TET2	NM_001127208.2	All coding	100
CSF3R	NM_156039.3	14,17	100	MPL	NM_005373.2	1-11	100	TP53	NM_000546.5	All coding	100
ETNK1	NM_018638.4	3	100	NRAS	NM_002524.4	2-4	100	U2AF1	NM_006758.2	2,6	100
EZH2	NM_004456.4	All coding	100	RUNX1	NM_001754.4	All coding	100	ZRSR2	NM_005089.3	All coding	100
IDH1	NM_005896.2	4,7	100	SETBP1	NM_015559.2	4	100				
IDH2	NM_002168.2	4,7	100	SF3B1	NM_012433.2	14-16	100				

Please note variants may not be optimally detected in genes with less than 100% coverage. The gene coverage above is considered acceptable given the available information about the clinical context, however please contact the laboratory for further advice should specific genes covered at less than 100% require full coverage. A list of regions with suboptimal coverage is available upon request.

Please contact the laboratory on 03 8559 7284 if you wish to discuss this report further.

Reported by **Dr Ing Soo Tiong (Consultant Haematologist)**

Authorised by **A/Prof Piers Blombery (Consultant Haematologist)**
Reported **16-Mar-2023**

References

1. Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017; **19**(1): 4-23.

CLINICAL UTILITY OF MOLECULAR TESTING IN CLASSICAL MYELOPROLIFERATIVE NEOPLASMS

DIAGNOSTIC UTILITY

- Classical *BCR-ABL1*-negative myeloproliferative neoplasms (MPN) include polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF). The presence of mutations in *JAK2* (>95% PV and 50%-60% of ET/PMF), *CALR* (25%-30% ET/PMF) or *MPL* (~5% ET/PMF) constitutes a major diagnostic criterion in these neoplasms.
- *JAK2* exon 12 mutations are seen in Val617Phe negative PV and are associated with isolated erythrocytosis. They may also be rarely observed in myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)[1].
- Approximately 10% of ET/PMF lack the typical driver mutations in *JAK2*/*CALR*/*MPL* ('triple-negative MPN'); the diagnosis can be supported by the presence of an alternative clonal marker (e.g. *ASXL1*, *EZH2*, *TET2*, *IDH1*, *IDH2*, *SRSF2*) in the appropriate clinicopathological context.
- Co-mutation of *JAK2*/*CALR*/*MPL* is a rare but recognised phenomenon, most frequently occurring in cases with a low *JAK2* allele burden (<5%)[2]. *BCR-ABL1* may also rarely co-occur.
- Acquired uniparental disomy / copy-neutral loss of heterozygosity of 9p24 is observed in approximately 30%-50% of PV and 20% of PMF, often manifesting as a high *JAK2* Val617Phe allele burden. This is rarely seen in ET, therefore a high *JAK2* Val617Phe should prompt consideration of a diagnosis PV, PMF or post-ET/PV MF[3,4].
- *JAK2* Val617Phe mutations can also occur in chronic myelomonocytic leukaemia (CMML) and are typically associated with proliferative features[5]. *MPL, CALR* and *CSF3R* mutations are rarely observed in CMML.
- The diagnosis of MDS/MPN-RS-T is strongly supported by the presence of an *SF3B1* mutation together with a *JAK2* (most common), *CALR* or *MPL* mutation[6].
- Mutations in *ASXL1*, spliceosome genes, and RAS pathway genes are uncommon in chronic phase MPN (ET and PV), therefore a diagnosis of PMF, post-ET MF and post-PV MF should be considered[4,7].
- The presence of mutations in *IDH1*/*IDH2* or *TP53* in addition to *JAK2*/*CALR*/*MPL* should raise suspicion of transformation to blast-phase MPN[4,8].
- Somatic mutations in *SH2B3* are observed in 5%-7% of MPNs and may co-occur with another typical MPN driver mutation (*JAK2*/*CALR*/*MPL*), as well as being also observed in idiopathic erythrocytosis[9].

PROGNOSTIC UTILITY

- 'Triple-negative' PMF has been associated with inferior leukaemia-free and overall survival (noting that there is no standard definition of this entity)[10].
- In PMF (MIPSS70+v2.0), absence of type 1/type-1 like *CALR* and presence of high molecular risk mutations (*ASXL1*, *SRSF2*, *U2AF1* Gln157, *EZH2* and *IDH1*/*IDH2*) are considered adverse risk features[11]. *NRAS/KRAS* mutations have also been associated with inferior survival in both primary and secondary myelofibrosis[12,13].
- Integrated clinical-molecular prognostic scoring systems for MPNs that incorporate high risk molecular features include: MIPSS-PV (*SRSF2*)[14], MIPSS-ET (*SF3B1*, *SRSF2*, *U2AF1* and *TP53*)[14], MYSEC-PM for post-PV/ET MF (*CALR*-unmutated)[15], Myelofibrosis Transplant Scoring System (MTSS) for both primary and post-PV/ET MF (non-*CALR*/*MPL* driver mutation and *ASXL1* mutation)[16], and the MPN personalised risk model[4].

BIOMARKERS OF RESPONSE TO THERAPY

- Ruxolitinib and other JAK inhibitors may be effective in PMF and post-PV/ET MF regardless of *JAK2* mutation status[17].
- Treatment with interferon alpha in patients with MPN may induce a reduction in mutant allele burden (including complete molecular remission) in a subset of patients[18].

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1. Scott LM. The JAK2 exon 12 mutations: a comprehensive review. *Am J Hematol* 2011; **86**(8): 668-76. **2.** Mora B, et al. Platelet count predicts driver mutations' co-occurrence in low JAK2 mutated essential thrombocythemia and myelofibrosis. *Leukemia* 2021; **35**(5): 1490-3. **3.** Wang L, et al. Acquired uniparental disomy of chromosome 9p in hematologic malignancies. *Exp Hematol* 2016; **44**(8): 644-52. **4.** Grinfeld J, et al. Classification and Personalized Prognosis in Myeloproliferative Neoplasms. *N Engl J Med* 2018; **379**(15): 1416-30. **5.** Pich A, et al. JAK2V617F activating mutation is associated with the myeloproliferative type of chronic myelomonocytic leukaemia. *J Clin Pathol* 2009; **62**(9): 798-801. **6.** Swerdlow SH CE, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, editor. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (revised 4th edition). Lyon: IARC; 2017. **7.** Stein BL, et al. Disruption of the ASXL1 gene is frequent in primary, post-essential thrombocytosis and post-polycythemia vera myelofibrosis, but not essential thrombocytosis or polycythemia vera: analysis of molecular genetics and clinical phenotypes. *Haematologica* 2011; **96**(10): 1462-9. **8.** Tefferi A, et al. IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. *Leukemia* 2010; **24**(7): 1302-9. **9.** Maslah N, et al. The role of LNK/SH2B3 genetic alterations in myeloproliferative neoplasms and other hematological disorders. *Leukemia* 2017; **31**(8): 1661-70. **10.** Rumi E, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. *Blood* 2014; **124**(7): 1062-9. **11.** Tefferi A, et al. MIPSS70+ Version 2.0: Mutation and Karyotype-Enhanced International Prognostic Scoring System for Primary Myelofibrosis. *J Clin Oncol* 2018; **36**(17): 1769-70. **12.** Santos FPS, et al. Prognostic impact of RAS-pathway mutations in patients with myelofibrosis. *Leukemia* 2020; **34**(3): 799-810. **13.** Coltro G, et al. RAS/CBL mutations predict resistance to JAK inhibitors in myelofibrosis and are associated with poor prognostic features. *Blood Adv* 2020; **4**(15): 3677-87. **14.** Tefferi A, et al. Mutation-enhanced international prognostic systems for essential thrombocythaemia and polycythaemia vera. *Br J Haematol* 2020; **189**(2): 291-302. **15.** Passamonti F, et al. A clinical-molecular prognostic model to predict survival in patients with post polycythemia vera and post essential thrombocythemia myelofibrosis. *Leukemia* 2017; **31**(12): 2726-31. **16.** Gagelmann N, et al. Comprehensive clinical-molecular transplant scoring system for myelofibrosis undergoing stem cell transplantation. *Blood* 2019; **133**(20): 2233-42. **17.** Harrison C, et al. JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis. *N Engl J Med* 2012; **366**(9): 787-98. **18.** Yoon SY, Won JH. The clinical role of interferon alpha in Philadelphia-negative myeloproliferative neoplasms. *Blood Res* 2021; **56**(S1): S44-S50.