Patient	**Brandon DAVIS**	**Lab No**	19465433	**Requester**	Dr EMILY KIM
URN	PMEX678749	**Ext Ref**	852914637	**Referral Lab**	Alpha Pathology
DOB	10-Jun-1967	**Collected**	17-Feb-2023		
Sex	M	**Received**	17-Feb-2023		
		Specimen	Bone marrow aspirate		

Clinical Indication Suspected leukaemia

Correlative Morphology Blasts 48.5% on aspirate. Flow cytometry: acute monoblastic and monocytic leukaemia (refer to Alpha Pathology report, Lab ID 852914637)

HAEMATOLOGICAL MALIGNANCY GENE PANEL REPORT

Test Description Somatic variant analysis of 57 genes with clinical significance in haematological malignancy plus analysis of potential germline variants in the DDX41 gene. Refer to Panel Summary for gene list.

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

Clinical Interpretation Variants in ASXL1 and FLT3 (ITD) were detected in this bone marrow aspirate sample consistent with a diagnosis of acute myeloid leukaemia (AML). This molecular profile is associated with inferior outcomes according to ELN 2022 criteria.

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

FLT3-ITD Analysis **FLT3-ITD DETECTED BY SEPARATE ASSAY (see Reportable Variants table for details)**

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 AML
Somatic	**ASXL1**	**c.2842del p.(Glu948Argfs*3)**	**13**	**PROGNOSTIC**
Somatic	**FLT3**	**FLT3-ITD (allelic ratio 0.76)**	**N/A**	**PROGNOSTIC / DRUG TARGET**

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). **Somatic variant analysis –** 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. When performed, FLT3-ITDs are tested for by fragment length analysis using capillary electrophoresis. The FLT3 allelic ratio is calculated by peak height ITD/peak height WT. The detection limit for FLT3-ITDs is approximately 1%. **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. **Germline variant analysis** – All rare germline variants in DDX41 are classified according to ACMG guidelines for the interpretation of sequence variants[2] with class 3 (uncertain significance), class 4 (likely pathogenic) and class 5 (pathogenic) variants reported only. Please note however that germline confirmation is required for all potential clinically significant DDX41 variants.

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 CEBPA (detection limit ~ 10%) and 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. **For somatic variant analysis**, synonymous variants are not routinely reported. **For germline variant analysis**, variant zygosity is assumed to be either heterozygous or homozygous in the germline based on allele frequency for the purpose of clinical interpretation however, the possibilities of hemizygosity or somatic acquisition are not excluded. In haematological specimens, the possibility of a false negative germline result due to loss of the mutant allele through a somatic reversion event cannot be excluded. 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 (%)
ABL1	NM_005157.4	4-10	100	FLT3*	NM_004119.2	14-15,17,20	100	PHF6	NM_001015877.1	7-10	95
ARAF	NM_001654.4	7,10,15	100	FYN	NM_002037.5	7	100	PIGA	NM_002641.3	All coding	100
ASXL1	NM_015338.5	10-12	100	GATA1	NM_002049.3	2-6	100	PLCG1	NM_002660.2	11	100
BCL2	NM_000633.2	All coding	100	GATA2	NM_032638.4	All coding	100	PLCG2	NM_002661.3	16,19-20,24	100
BIRC3	NM_001165.4	6-9	100	ID3	NM_002167.4	All coding	100	RHOA	NM_001664.2	2	100
BRAF	NM_004333.4	15	100	IDH1	NM_005896.2	4,7	100	RUNX1	NM_001754.4	All coding	100
BTK	NM_000061.2	11,15-16	100	IDH2	NM_002168.2	4,7	100	SETBP1	NM_015559.2	4	100
CALR	NM_004343.3	9	100	IRF8	NM_002163.2	3	100	SF3B1	NM_012433.2	14-16	100
CARD11	NM_032415.4	4-9,15,20	100	JAK2	NM_004972.3	12-14,16	100	SH2B3	NM_005475.2	All coding	98.6
CBL	NM_005188.3	8-9	100	JAK3	NM_000215.3	11,13,15	94.9	SRSF2	NM_003016.4	1	100
CD274	NM_014143.3	All coding,3'UTR	100	KIT	NM_000222.2	8,10-11,17	100	STAT3	NM_139276.2	6,13,15,18-21	100
CD79B	NM_000626.2	5,6	100	KRAS	NM_033360.2	2-4	100	STAT5B	NM_012448.3	16	100
CEBPA	NM_004364.3	All coding	100	MAP2K1	NM_002755.3	2-3	100	STAT6	NM_001178078.1	10,13,16	100
CSF3R	NM_156039.3	14,17	100	MPL	NM_005373.2	1-11	100	TCF3	NM_001136139.2	17	100
CXCR4	NM_003467.2	2^	100	MYD88	NM_002468.4	4-5	100	TET2	NM_001127208.2	All coding	100
DDX41	NM_016222.2	All coding	100	NOTCH1	NM_017617.3	26-28,34,3'UTR^	100	TP53	NM_000546.5	All coding	100
DNMT3A	NM_022552.4	All coding	100	NPM1	NM_002520.6	11	100	U2AF1	NM_006758.2	2,6	100
ETNK1	NM_018638.4	3	100	NRAS	NM_002524.4	2-4	97.4	XPO1	NM_003400.3	15-16	100
EZH2	NM_004456.4	All coding	100	PDCD1LG2	NM_025239.3	All coding,3'UTR	100	ZRSR2	NM_005089.3	All coding	100

* Please note FLT3-ITDs are not detected with this assay. A separate assay may have been performed, result included in Test Results if sample tested. ^Partial coverage of region

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)** **Ms Vanessa Singh (Medical Scientist)**
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.
2. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**(5): 405-23.

ABN 42 100 504 883

NATA & RCPA ACCREDITED LABORATORY Number 2465

CLINICAL UTILITY OF MOLECULAR TESTING IN ACUTE MYELOID LEUKAEMIA

DIAGNOSTIC UTILITY

- In the WHO revised 4th edition classification, acute myeloid leukaemia (AML) with recurrent genetic abnormalities includes AML with mutated *NPM1*, AML with biallelic mutation of *CEBPA*, and AML with mutated *RUNX1* (provisional)[1].
- The presence of a mutation in *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR* or *STAG2* has been shown to be highly specific (>95%) for a diagnosis of secondary AML, even without a known antecedent MDS diagnosis[3].
- *KIT* mutations are rarely observed in non-core binding factor AML[2] and therefore if detected, specific testing for t(8;21) and inv(16) should be considered.
- *JAK2* Val617Phe mutations are infrequent in *de novo* AML (approximately 1%) and therefore a preceding myeloproliferative neoplasm should be considered if detected[2].
- AML with plasmacytoid dendritic cell expansion (pDC-AML) is a recently described entity representing a subset of AML with pDC expansion and high frequency of *RUNX1* mutations (70%)[4].
- The molecular profile of blastic plasmacytoid dendritic cell neoplasm (BPDCN) is not specific and resembles that of other myeloid neoplasms such as MDS and CMML, however *RUNX1* mutations are rarely observed[5,6].
- Some mutations have potential germline predisposition: *CEBPA*, *DDX41*, *RUNX1*, *ANKRD26*, *ETV6*, *GATA2* and *TP53*. Testing a remission and/or germline sample in the appropriate clinical context should be considered.

PROGNOSTIC UTILITY

- The ELN 2022 risk stratification incorporates baseline cytogenetic and molecular factors (Table)[7]. Major changes include *CEBPA* in-frame mutations in the bZIP domain, secondary AML-like gene mutations, and removal of the allelic ratio threshold for *FLT3*-ITD.
- Other examples of prognostication models include the knowledge bank approach and the AML Classification and Risk Stratification Calculator[8,9].
- MRD assessment is an independent prognostic indicator post therapy for AML, and may be a more potent predictor of outcome compared to the baseline clinical and molecular profile[10,11].
- *TP53* mutations and complex karyotype provide independent and additive prognostic information, with the combination having the worst outcome[2].

Table. 2022 European LeukemiaNet (ELN) risk classification

Risk Category[b]	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*[b,c] • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*[b,c] • Mutated *NPM1*[b,d] without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*[e]
Intermediate	• Mutated *NPM1*[b,d] with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD • t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*[b,f] • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1)/*DEK::NUP214* • t(v;11q23.3)/*KMT2A*-rearranged[g] • t(9;22)(q34.1;q11.2)/*BCR::ABL1* • t(8;16)(p11;p13)/*KAT6A::CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)* • t(3q26.2;v)/*MECOM(EVI1)*-rearranged • -5 or del(5q); -7; -17/abn(17p) • Complex karyotype,[h] monosomal karyotype[i] • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*[j] • Mutated *TP53*[k]

BIOMARKERS OF RESPONSE TO THERAPY

- *FLT3*-ITD and *FLT3*-TKD mutations (clinical trials included only TKD mutations at Asp835 and Ile836 codons) are the target of midostaurin[12] (in newly diagnosed AML) and gilteritinib[13] (in relapsed/refractory AML).
- Repeat *FLT3* testing at relapse or disease progression is recommended as ~20% of patients have a change (gain or loss) in *FLT3* mutation status[14].
- *IDH1* (Arg132) and *IDH2* (both Arg140 and Arg172) mutations are the target of IDH1 and IDH2 inhibitors, respectively[15].
- Second-site *IDH1*/*IDH2* mutations have been described in patients with acquired resistance to IDH1/IDH2 inhibitors[16].

REFERENCES

1. Swerdlow S, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (revised 4th edition). Lyon: IARC; 2017. **2.** Papaemmanuil E, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med* 2016; **374**(23): 2209-21. **3.** Lindsley RC, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood* 2015; **125**(9): 1367-76. **4.** Xiao W, et al. Plasmacytoid dendritic cell expansion defines a distinct subset of RUNX1-mutated acute myeloid leukemia. *Blood* 2021; **137**(10): 1377-91. **5.** Stenzinger A, et al. Targeted ultra-deep sequencing reveals recurrent and mutually exclusive mutations of cancer genes in blastic plasmacytoid dendritic cell neoplasm. *Oncotarget* 2014; **5**(15): 6404-13. **6.** Menezes J, et al. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. *Leukemia* 2014; **28**(4): 823-9. **7.** Döhner H, et al. Diagnosis and Management of AML in Adults: 2022 ELN Recommendations from an International Expert Panel. *Blood* 2022. **8.** Gerstung M, et al. Precision oncology for acute myeloid leukemia using a knowledge bank approach. *Nat Genet* 2017; **49**(3): 332-40. **9.** Tazi Y, et al. Unified classification and risk-stratification in Acute Myeloid Leukemia. *Nature Communications* 2022; **13**(1): 4622. **10.** Jourdan E, et al. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. *Blood* 2013; **121**(12): 2213-23. **11.** Ivey A, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. *N Engl J Med* 2016; **374**(5): 422-33. **12.** Stone RM, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. *N Engl J Med* 2017; **377**(5): 454-64. **13.** Perl AE, et al. Gilteritinib or Chemotherapy for Relapsed or Refractory FLT3-Mutated AML. *N Engl J Med* 2019; **381**(18): 1728-40. **14.** Daver N, et al. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia* 2019; **33**(2): 299-312. **15.** Dohner H, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017; **129**(4): 424-47. **16.** Intlekofer AM, et al. Acquired resistance to IDH inhibition through trans or cis dimer-interface mutations. *Nature* 2018; **559**(7712): 125-9.