Pilot Acute Myeloid Leukaemia and Myelodysplastic Syndrome Gene Panels (Not Accredited)

Distribution – 212202

Participant –

Date Issued – 30 Mar 2022

Closing Date – 20 May 2022

Trial Comments

This trial was issued to 106 participants; 95 (89.6%) laboratories returned results. Of the 11 participants failing to submit results, seven laboratories pre-notified us of this.

Please note that for future 2022/23 distributions the Acute Myeloid Leukaemia and Myelodysplastic Syndrome Gene Panels (Pilot – Not Accredited) programme will expand to encompass a broader range of myeloid malignancies, which will necessitate a name change to Myeloid Gene Panels (Pilot – Not Accredited). To facilitate a quicker turnaround time for trial report publication, one Myeloid Gene Panels (Pilot – Not Accredited) distribution will focus on summarising the variants detected by participants (including methodological aspects) and the other will additionally provide educational elements related to variant biological classification and clinical interpretation.

Sample Comments

The lyophilised trial sample (AML GP 113) was formulated from the peripheral blood of a patient with a working diagnosis of acute myeloid leukaemia (AML) (no further details known) and distributed by UK NEQAS LI.

Your Laboratory Record status for this trial:	

IMPORTANT: To permit meaningful trial data analysis it is essential the information held in your Laboratory Record is complete and accurately reflects your current practice in relation to this programme. Please provide all the information as requested and/or check it carefully to ensure methodological details are up to date when requested to do so.

Sample AML GP 113

Your Results – Variant(s) of strong clinical significance

Gene	Your DNA sequence variant detected	Your protein variant

Your Results – Variant(s) of potential clinical significance

Gene	Your DNA sequence variant detected	Your protein variant

Your Results – Variant(s) of unknown clinical significance

Gene	Your DNA sequence variant detected	Your protein variant

Please note, due to formatting limitations some rows may appear blank within the tables(s) above. All submitted variant(s) of unknown clinical significance may not be reflected in the above table for individual participants due to formatting and space constraints.

All Participant Results

In the interests of clarity, we have only summarised variants reported by ≥5 participants in the table below.

Gene	n [a]	Variant classification [b]			Variant detected (consensus) [c]		Median VAF % (IQR) [d]
		Strong clinical significance	Potential clinical significance	Unknown clinical significance [not provided]	DNA sequence description	Protein level description	
RUNX1	94/95	86	7	0 [1]	c.497G>A [e]	p.(Arg166Gln) [e]	46.1 (2.1)
FLT3	93/95	92	0	0 [1]	c.2503G>C	p.(Asp835His)	17.2 (1.1)
ASXL1	92/95	76	12	3 [1]	c.1782C>A	p.(Cys594*)	47.0 (2.2)
U2AF1	90/93	56	33	0 [1]	c.470A>G [f]	p.(Gln157Arg) [f]	46.9 (2.1)
PTPN11	79/81	52	24	2 [1]	c.1508G>C [g]	p.(Gly503Ala) [g]	12.0 (1.8)
KRAS	22/87	14	8	0	c.35G>C	p.(Gly12Ala)	1.7 (0.6)
CREBBP	8/9	0	7	1	c.5220dup [h]	p.(Lys1741*) [h]	46.0 (3.3)

[a] Total number of participants reporting this variant/number of participants stating the inclusion of the relevant gene on their panel or known to feature the gene on their panel due to identification of the consensus variant. Please note for this trial three returning participants failed to provide full Laboratory Record information. Not all laboratories provided sufficient gene/region of interest information for their panel to permit identification of all false negative results in the data set. Additionally, please refer to the report comments section regarding any participant(s) reporting a consensus variant from a gene not stated as included on their panel.
[b] Based on Li *et al.* (2017) Joint consensus recommendations from the Association for Molecular Pathology, American Society of Clinical Oncology and College of American Pathologists[1].
[c] Nomenclature provided in the table is based on the MANE Select (v1.0)[2] reference transcript and genome build GRCh38, unless specified. Please refer to the comments section for further information about reference sequences. Results returned by participants, at both the DNA and protein level, may have been harmonised to the equivalent Human Genome Variation Society (HGVS) approved nomenclature[3-4] during the compilation of the 'All Participant results' table. Information regarding a variant(s) reported in any gene listed in the table, which could not be identified as equivalent to a consensus variant has been excluded. Protein nomenclature includes parenthesis as it typically represents a prediction from analysis at the DNA level.
[d] Descriptive statistics calculated for any variant with >2 quantification data points. Median VAF calculated for DNA based assays, all panels and platforms. Percentage values quoted have been subjected to rounding up/down to 1 d.p., IQR = interquartile range. Quantitative data points may have been excluded from the statistics if the associated nomenclature provided was considered equivocal.

[e] Alternative nomenclature: NM_001001890.2(*RUNX1*):c.416G>A p.(Arg139Gln)
[f] Alternative nomenclature: NM_001025204.2(*U2AF1*):c.251A>G p.(Gln84Arg)
[g] Alternative nomenclature: NM_001330437.2(*PTPN11*):c.1520G>C p.(Gly507Ala)
[h] Alternative nomenclature: NM_001079846.1(*CREBBP*):c.5106dup p.(Lys1703*)

Your Performance

Performance	Performance Status for this Sample	Performance Status Classification Over 3 Sample Period	
		Satisfactory	Critical
n/a	n/a	n/a	n/a

Please note: This programme is not currently performance monitored. We will work towards a scoring system as the programme develops.

Methods

Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques. At the time of reporting, three returning participant failed to provide all the Laboratory Record information requested.

Methodological approach

	Returns
Targeted Gene Panel (DNA seq)	77
Targeted Gene Panel (DNA with RNA fusion transcript seq)	14
Transcriptome (RNA seq)	1
Targeted Gene Panel – no further details known	1

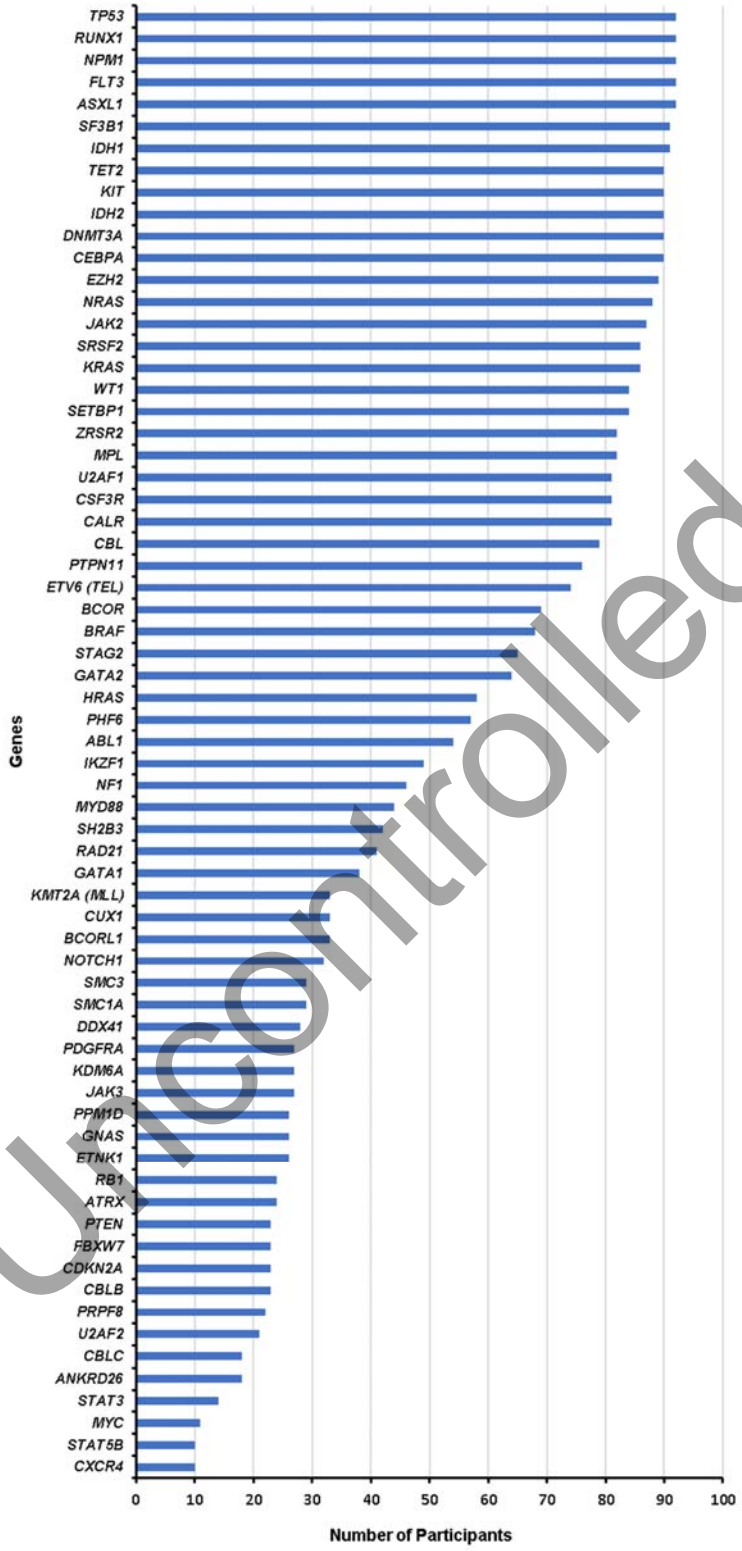
NGS platform(s) used (to analyse the sample in this trial)

	Returns
Illumina MiSeq	37
Illumina NextSeq	20
Thermo Fisher Scientific (Life Tech) Ion S5	18
Illumina Novaseq	9
Illumina MiniSeq	6
Thermo Fisher Scientific (Life Tech) Ion S5 XL	5
Thermo Fisher Scientific Ion Torrent Genexus System	1

NGS panel description (to analyse the sample in this trial)

	Returns
(IonTorrent) Oncomine Myeloid Research Assay	18
Illumina TruSight Myeloid Sequencing Panel	12
Sophia Genetics Myeloid Solution (MYS)	12
In house (capture based)	9
Qiagen QIASeq Custom Panel	9
In house (amplicon based)	8
Archer VariantPlex Myeloid panel	7
Twist Custom Panel	4
Sophia Genetics Extended Myeloid Solution	2
AmpliSeq for Illumina Myeloid Panel	2
Agilent SureSelect Custom QXT Panel	2
Illumina TruSight Oncology 500 (TSO500) Panel	2
Haematology OncoKitDx (Imegen)	2
Other	4

Genes routinely analysed by participants (in this clinical context). Information provided by 92 laboratories; data is presented as submitted by participants (and not subject to comprehensive cross checking with reference to variant(s) detected results from individual laboratories). Only genes routinely analysed by at least 10 participants are represented in the chart.



Genome Assembly

	Returns
GRCh37/hg19	82
GRCh38	11

Minimum variant allele frequency (VAF) for reporting identification of an indel (deletion/duplication/insertion) variant

	Returns
1%	10
2%	7
2.5%	3
3%	7
4%	3
5%	60
8%	1
10%	2

Minimum variant allele frequency (VAF) for reporting identification of a single nucleotide variant (SNV) or substitution variant

	Returns
1%	14
2%	9
2.5%	3
3%	10
4%	2
5%	54
10%	1

Annotation database resources

	Returns
COSMIC (Catalogue Of Somatic Mutations In Cancer)	92
ClinVar (NCBI)	88
WHO IARC TP53 Database hosted by National Cancer Institute (NCI)	53
My Cancer Genome (Vanderbilt-Ingram Cancer Center)	33
OMIM (NCBI)	29
Seshat (TP53) Database	27
OncoKB (Memorial Sloan Kettering Cancer Center *et al*.)	32
HGMD (The Human Gene Mutation Database)	19
The Clinical Knowledgebase (CKB) Jackson Laboratory (Boost)	16
The Cancer Genome Atlas (TCGA)	16
CIViC (Clinical Interpretation of Variants in Cancer)	15
cBioPortal (Memorial Sloan Kettering Cancer Center *et al*.)	14
UMD (TP53) Database	13

As stated by ≥3 participants.

Large population dataset/resources routinely consulted

	Returns
gnomAD (Genome Aggregation Database)	78
dbSNP (Short Genetic Variations, NCBI)	67
1000 Genomes	46
ESP (Exome Sequencing Project, NHLBI GO)	19

As stated by ≥3 participants.

Aggregation tool(s) utilised to access annotation resources

	Returns
Varsome (SAPHETOR)	37
Alamut (SOPHiA GENETICS)	34
Franklin (GENOOX)	8

As stated by ≥3 participants.

Trial Comments

Methodology

- All but one returning participant (with the relevant information provided in their Laboratory Record) described the application of a DNA based targeted gene panel NGS testing approach (n=92). At least 14 laboratories stated the additional inclusion of RNA fusion gene transcript sequencing. Please note, for this programme laboratories are not requested to report large changes affecting genome architecture or copy number variants (>50 kb).
- The single laboratory utilising transcriptome (RNA) sequencing was only able to report one of the consensus variants due to sample AML GP 113 failing local quality control (QC) across multiple regions of interest (ROI). This was an anticipated outcome, given the sample was formulated from patient peripheral blood (diagnostic waste), which was only available to UK NEQAS LI for processing >48 hours after collection.
- The average number of genes currently analysed by laboratories on a given panel is 44 with a range of 11 – 185 genes in total. For the genes most frequently included on participant gene panels (and analysed in this clinical context) please refer to the chart on page 6.
- Comparable to the previous trial (AML and MDS GP 212201), approximately 75% (n=72) of returning participants providing the relevant information employed bridge amplified reversible dye terminator-based platforms from Illumina to analyse sample AML GP 113. The remaining laboratories stated the use of Thermofisher Scientific Ion Torrent technology (n=24).
- The most utilised 'off the shelf' commercially available panel kits included the Oncomine Myeloid Research Panel (n=18), Sophia Genetics Myeloid Solution (n=12), Illumina TruSight Myeloid Sequencing Panel (n=12) and Archer VariantPlex Myeloid Panel (n=7).
- At 11.8% (n=11), the proportion of participants known to be working to the GCh38 human genome assembly continues to steadily increase (AML and MDS GP 212201 = 9.1%, 202102 = 6.7%).

Annotation and interpretation

- COSMIC (n=92), ClinVar (n=88) and gnomAD (n=78) remain the annotation resources most widely utilised by participants. Nevertheless, the list of databases and tools accessed by centres continues to expand, please refer to the tables on page 8 for further information.
- A growth in the use of Franklin (GENOOX) (n=8) was noted. Along with other established aggregation tools including Varsome (SAPHETOR) (n=37) and Alamut (SOPHiA GENETICS) (n=34), such approaches can be extremely useful but should always be employed with caution. Submissions to resource databases may not be subject to a level of curation sufficient for clinical diagnostic application; it is prudent to check the underpinning publication and/or supporting source information. Many resources access the same primary dataset(s); laboratories are encouraged to be mindful of duplicated evidence when classifying variants in terms of biological and/or clinical significance.
- In keeping with the previous trial, 64.5% (n=60) and 58.1% (n=54) of responding laboratories stated application of a 5% minimum variant allele frequency (VAF) threshold for reporting identification of a deletion/duplication/insertion (indel) and single nucleotide variant (SNV) (substitution), respectively. For an indel, 32.3% (n=30) of participants quoted a minimum threshold below 5%. For an SNV the thresholds were set lower with 40.9% (n=38) laboratories applying a minimum VAF below 5%. The single laboratory utilising a transcriptome approach (RNA sequencing, Illumina Novaseq) quoted a 10% VAF threshold for the reporting of both types of variant.

Sample AML GP 113

All participants returning results for this trial (n=95) reported at least one sequence variant in sample AML GP 113 (please refer to the summary table on page 3 for details). As previously discussed, a single laboratory utilising transcriptome (RNA) sequencing was only able to report one of the consensus variants (*RUNX1* c.497G>A p.(Arg166Gln)) due to QC issues.

Of note, one participant failed to identify any of the consensus variants for sample AML GP 113 but did report detection of the NM_004972.3(*JAK2*):c.1849G>T p.Val617Phe (V617F) canonical driver and NM_004456.5(*EZH2*):c.965A>G, p.(Asn322Ser) missense variant. This laboratory is suspected to have undertaken a sample switch.

All but one returning participant (n=94) reported detection of a variant equating to NM_001754.5:c.497G>A p.(Arg166Gln) in exon 5 of the *RUNX1* gene. Laboratories classified the variant as of strong (n=86) or potentially strong (n=7) clinical significance. The remaining participant provided no clinical significance classification.

- The median VAF (DNA based assays, all panels and platforms) reported for this missense variant was 46.1% with an interquartile range of 2.1 (n=93).
- The majority of participants (n=90) provided nomenclature using a NM_001754 based Matched Annotation from NCBI and EMBL-EBI (MANE) Select[2] transcript for *RUNX1* encoding protein isoform AML1c (480 amino acids). However, three centres returned an alternative description (c.416G>A p.(Arg139Gln)) based on the longer *RUNX1* mRNA transcript NM_001001890, which actually encodes the shorter protein isoform AML1b (453 amino acids) which features a distinct N-terminus. Please note, the Human Genome Variation Society (HGVS) advocate use of the transcript reference sequence indicated by the MANE project[2-3].
- One participant provided erroneous DNA based nomenclature (c.497G>C) using Ensembl reference sequence ENST00000300305.3 (encoding a 480 amino acid protein) but was in consensus at the protein level.
- The single laboratory utilising transcriptome (RNA) sequencing was able to successfully identify the c.497G>A p.(Arg166Gln) missense variant in sample AML GP 113.
- A single Illumina MiniSeq user employing an in-house amplicon based panel encompassing the relevant region of the *RUNX1* gene failed to identify the variant but is suspected to have undertaken a sample switch.
- *RUNX1* molecular testing is recommended standard of care for AML diagnostic evaluation[5-7]. The World Health Organisation (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues (4th edition)[5] expanded the AML with recurrent genetic abnormalities section to include the provisional category, 'AML with mutated *RUNX1*'. However, the pending WHO update (5th edition)[8] will no longer recognise a distinct entity due to the lack of specificity. *RUNX1* somatic cases remained in the European LeukemiaNet (ELN) classification under the 'AML with myelodysplasia-related gene mutations' category and feature in the adverse-risk group[6].
- The *RUNX1* c.497G>A p.(Arg166Gln) variant is listed on COSMIC[9] in a haematopoietic neoplasm context (COSV55867644, n=22), which includes myeloid leukemogenesis underpinning references[10-11].
- This missense variant is absent from the general population datasets accessed via gnomAD[12].
- ClinVar[13] (VCV000417961.7) describes the variant in a germline context and concludes it to be 'pathogenic' in relation to familial platelet disorder with associated myeloid malignancy. The entry

has been reviewed (3 stars) using *RUNX1* ClinGen myeloid malignancy expert panel specifications[14].

- The VAF reported for the *RUNX1* c.497G>A p.(Arg166Gln) variant is in keeping with potential germline status. The importance of confirming germline status for the patient and their family has been discussed in previous trials reports (including AML and MDS GP 212201) and is covered elsewhere in the literature[6,15-16]. Patients with an autosomal dominant familial platelet disorder with propensity to myeloid malignancies associated with *RUNX1* typically demonstrate a broad age of onset but experience life-long thrombocytopenia[17].

In total 92 laboratories detected the c.1782C>A p.(Cys594*) variant in exon 13 of the *ASXL1* gene. The variant was classified by participants as of strong (n=76), potential (n=12) or unknown (n=3) clinical significance. A single participant provided no clinical significance classification.

- The median VAF (DNA based assays, all panels and platforms) returned for the NM_015338.6(*ASXL1*):c.1782C>A p.(Cys594*) variant was 47.0% with an interquartile range of 2.2% (n=92).
- Nomenclature provided at both the DNA and protein level was in good consensus (* or Ter are both permitted by the HGVS to indicate a translation termination (stop) codon)[3-4]. Most laboratories reported the nonsense variant using the MANE Select transcript NM_015338 (ENST00000375687) encoding protein isoform 1. One laboratory provided in consensus nomenclature but erroneously quoted an *ABL1* derived reference sequence.
- Three of the returning laboratories omitted to provide gene panel information but were able to successfully detect the *ASXL1* c.1782C>A p.(Cys594*) variant.
- Overall, three centres failed to identify this consensus variant. All stated inclusion of the *ASXL1* final exon (NM_015338 exon 13) on their panels. One of the participants employed RNA sequencing and declared the region as failing local QC. A Qiagen QIASeq Custom Panel (Illumina MiSeq) user reported an out of consensus *ASXL1* variant (NM_015338.6:c.2444C>T p.Pro815Leu). The other laboratory is suspected to have undertaken a sample switch.
- Molecular analysis of the *ASXL1* gene is standard of care for AML investigation and prognostication[5-7]. *ASXL1* driver variants are a common early event in myeloid malignancy and associated with adverse outcome[6].
- The pending WHO classification update (5th edition)[8] category of 'AML, myelodysplasia-related' incorporates AML cases featuring *ASXL1* pathogenic variants regardless of any prior history of myelodysplastic neoplasia (MDS). The ELN include *ASXL1* as a defining gene in their 'AML with myelodysplasia-related gene mutations' classification[6].
- The *ASXL1* c.1782C>A p.(Cys594*) variant is listed in COSMIC[9] (COSV60106156). The record is limited to only three myeloid malignancy entries, one of which is noted as AML (confirmed somatic, case with normal karyotype). The variant has not been reviewed on ClinVar[13] to date.
- As discussed previously in the AML and MDS GP 202102 trial report, there are multiple reports (in a myeloid malignancy context) of similar *ASXL1* truncating (suspected loss of function) variants occurring within the exon 13 region (large final exon, commonly referred to as exon 12 in the legacy literature)[9-11]. Nevertheless, the location of the c.1782C>A p.(Cys594*) variant in the final exon may have led to some laboratories classifying this nonsense variant as of only potential (n = 12) or unknown clinical significance (n = 3).

- *ASXL1* variants can be associated with clonal haematopoiesis of indeterminate potential (CHIP) and aging and thus, are not generally considered reliable measurable residual disease (MRD) markers[6-7].

The *FLT3* c.2503G>C p.(Asp835His) tyrosine kinase domain (TKD) variant was identified by 93 laboratories. All but one participant classified the missense change as of strong clinical significance (n=92). The remaining participant provided no clinical significance information.

- The median VAF (DNA based assays, all panels and platforms) returned for the NM_004119.3(*FLT3*):c.2503G>C p.(Asp835His) variant was 17.2% with an interquartile range of 1.1% (n=93).
- Nomenclature was in good general agreement at the DNA and protein levels. However, confusion persists for some participants (n=15) regarding the use and/or position of parenthesis, which should be included when a protein description is predicted from the analysis of gDNA[3-4]. The preferred three letter amino acid code was utilised by 96% of laboratories.
- Three of the returning participants failed to provide gene panel information but were able to successfully detect the *FLT3* c.2503G>C p.(Asp835His) variant.
- Two laboratories did not report this variant. Both stated the inclusion of *FLT3* exon 20 in their panel. One of the participants employed RNA sequencing and declared the region as failing local QC and the other centre is suspected to have undertaken a sample switch.
- The prognostic impact of *FLT3*-TKD variants are less well defined than *FLT3* internal tandem duplications (ITDs)[7,18]. However, in line with the interpretive classification findings from this trial, the c.2503G>C p.(Asp835His) *FLT3*-TKD activating variant is therapeutically actionable[6,18-20].

Overall 90 laboratories identified a *U2AF1* c.470A>G p.(Gln157Arg) missense variant. The variant was classified by participants as of strong (n=56) or potential (n=33) clinical significance. A single participant provided no clinical significance classification.

- The median VAF (DNA based assays, all panels and platforms) returned for the NM_006758.3(*U2AF1*):c.470A>G p.(Gln157Arg) variant was 46.9% with an interquartile range of 2.1% (n=90).
- Many laboratories described the missense variant with reference to the NM_006758 (ENST00000291552) based MANE Select transcript (n=77) with the substitution residing in exon 6. However, there was some variety in the reference sequences employed by participants which impacted the resulting nomenclature in some instances, please refer to the table over the page. One laboratory omitted to provide a *U2AF1* transcript reference sequence.
- Of note, 13 centres reporting this variant entered their results in the *U2AF2* field(s) of the data entry page but provided an accompanying *U2AF1* transcript reference sequence. The *U2AF2* gene encodes the large subunit (65kDa) of the U2 small nuclear RNA auxiliary factor 1 protein rather than the small (35 kDa) subunit encoded by *U2AF1.*

	U2AF1 transcript reference sequence NCBI RefSeq (Ensembl)	Nomenclature		n	Variant location	Comments
		cDNA	Protein			
	NM_006758[.3] **(ENST00000291552)**	c.470A>G	p.(Gln157Arg)	77	Exon 6	**MANE Select transcript** (945 bp) 8 exons total Encodes Splicing factor U2AF small (35 kDa) subunit isoform a (240 aa)
	NM_001025203[.1]	c.470A>G	p.(Gln157Arg)	9	Exon 6	Alternative transcript (971 bp) 8 exons total Encodes Splicing factor U2AF small (35 kDa) subunit isoform b (240 aa)
	NM_001025204[.2]	c.251A>G	p.(Gln84Arg)	1	Exon 7	Longest transcript (1012 bp) 9 exons total Encodes Splicing factor U2AF small (35 kDa) subunit isoform c (167 aa)
	NM_001320651[.2] **(U2AF1L5)***	c.251A>G	p.(Gln84Arg)	2	Exon 7	U2 small nuclear RNA auxiliary factor 1 like 5, transcript variant 4 (1012 bp) 9 exons total Encodes a 167 aa protein Annotated on a scaffold that is thought to be a false duplication, accession is likely redundant with NM_001025204

* https://www.ncbi.nlm.nih.gov/gene/102724594. Two returning laboratories quoted the U2AF1L5 (LOC102724594) transcript references sequence but provided c.470A>G p.(Gln157Arg) nomenclature.
[ ] latest available version for the NCBI accession.

- *U2AF1* is among the genes (which also includes *ASXL1)* associated with secondary AML (s-AML) arising post MDS or MDS/MPN and is listed in the forthcoming WHO classification (5th edition)[8] as defining in the 'AML, myelodysplasia-related (AML-MR)' entity (formally designated 'AML with myelodysplasia-related changes' (AML-MRC))[5]. *U2AF1* also features in the ELN 'AML with myelodysplasia-related gene mutations' classification. It is listed (along with *ASXL1 and RUNX1*) in the ELN adverse risk category[6].
- Approximately 60% of patients with MDS or chronic myelomonocytic leukaemia (CMML) and ~55% of secondary AML cases have biologically significant variants in a gene encoding a component of the spliceosome machinery. *U2AF1* (likely) oncogenic variants are found in around 10% of s-AML patients[21].
- Lindsley *et al.* describe a group of 8 chromatin modifiers, spliceosome and cohesion genes (including *U2AF1* and *ASXL1*) which are found to be mutated with >95% specificity in s-AML compared with *de novo* AML,)[22]. Of note, when detected in apparent *de novo* AML, assumed biologically significant variants in these genes conferred the same poor prognosis as seen for s-AML[6,22].
- The U2 auxiliary factor proteins form the U2AF1/2 heterodimer complex which recognises the 3' splice site. Position p.Gln157 appears to represent a 'hot spot' for missense variants and is located within one of the two zinc finger domains[21,23].
- The *U2AF1* c.470A>G p.(Gln157Arg) variant is listed in COSMIC[9] (COSV52341147) with >65 haematopoietic neoplasm entries, the majority of which are in a myeloid context and includes 15 AML cases.
- The variant is listed on ClinVar[13] as 'likely pathogenic' in association with AML (somatic) by a single submitter (no assertation criteria provided). The submitter provided an underpinning reference[24] (this case is potentially the same patient reported in the Shen *et al.* study)[23].

In total 79 laboratories detected the c.1508G>C p.(Gly503Ala) variant in exon 13 of the *PTPN11* gene. This missense variant was classified by participants as of strong (n=52), potential (n=24) or unknown (n=2) clinical significance. A single participant provided no clinical significance classification.

- The median VAF (DNA based assays, all panels and platforms) returned for the NM_002834.5(*PTPN11*):c.1508G>C p.(Gly503Ala) variant was 12.0% with an interquartile range of 1.8% (n=79).
- Nomenclature was in good agreement at both the DNA and protein levels with all but one laboratory using a MANE Select based transcript NM_002834 (ENST00000351677) encoding tyrosine-protein phosphatase non-receptor type 11 isoform 1. A single centre submitted the variant as c.1520G>C p.(Gly507Ala) with reference to a longer alternative transcript (NM_001330437.2) which encodes isoform 3.
- Seventy six participants stated the inclusion of *PTPN11* on their panel. Three returning laboratories failed to provide gene panel information but were able to successfully detect the *PTPN11* c.1508G>C p.(Gly503Ala) variant.
- Two laboratories known to feature the *PTPN11* gene on their panel failed to identify the missense variant; including an Archer VariantPlex Myeloid panel (Illumina NextSeq) user. The remaining laboratory is suspected to have undertaken a sample switch.
- The c.1508G>C p.(Gly503Ala) missense variant specifically is absent from the general population datasets via gnomAD[12]. Although several participants cited dbSNP[25] (rs397507546), on closer inspection it appears that the record features only two occurrences of a G>A substitution at the same position, which does not result in the same amino acid change. Please take care when citing records representing multiple alternative alleles.
- The COSMIC[9] entry (COSV61004973) lists >25 occurrences in a haematopoietic neoplasm, including nine AML cases.
- The ClinVar[13] (VCV000162464.1) record describes the variant as 'pathogenic' in the context of a RASopathy (single submitter, no citation or assertion criteria provided).
- The c.1508G>C p.(Gly503Ala) variant (somatic) has also been previously reported in a case of juvenile myelomonocytic leukaemia (JMML)[26]. The authors note the occurrence of *PTPN11* gain of function variants exclusively in advanced MDS associated with a high incidence of progression to AML with a short duration of survival (small cohort study).
- The clinical impact of *PTPN11* RAS pathway activating variants in adult AML has been historically less well understood due to their infrequency. However, more recently Alfayez *et al.* published a large cohort study (>1400 patients) and found *PTPN11* oncogenic variants to also be associated with an adverse prognosis in adult AML[27]. The *PTPN11* variants identified were exclusively missense (112 positive cases) with the p.Gly503 residue noted as a common variant site. The Gly>Ala change was detected in treatment naïve (n=3) and refractory/relapsed (n=3) AML cases. A further 4 cases were described as *PTPN11* negative at diagnosis but went on to acquire the p.Gly503Ala variant. Echoing the findings of smaller studies, the authors observed that *PTPN11* oncogenic variants were more common in myelomonocytic/monocytic AML and that they appeared to be mutually exclusive with core binding factor (CBF) leukaemia.
- Fu *et al.* report functional data utilising a *PTPN11* p.(Gly503Ala) retroviral transduction/transplantation mouse model (in the context of a co-occurring *KMT2A* rearrangement), which demonstrated accelerated disease progression[28].
- The ELN has included *PTPN11* in a group of additional genes for which analysis is recommended for patients with suspected AML but findings are not presently defining at diagnosis[6].

A further RAS pathway gene was implicated in sample AML GP 113 by a subset of laboratories. The *KRAS* c.35G>C p.(Gly12Ala) variant was identified at a low level (<3% VAF) by 22 laboratories. This activating missense variant in exon 2 was classified by participants as of strong (n=14) or potential (n=8) clinical significance.

- The median VAF (DNA based assays, all panels and platforms) returned for the NM_004985.5(*KRAS*):c.35G>C p.(Gly12Ala) variant was 1.7% with an interquartile range of 0.6% (n=22).
- Fifteen laboratories reported the variant with reference to a MANE Select based transcript (NM_004985) which represents the predominant variant and encodes GTPase KRas isoform b. The remaining participants (n=7) utilised the longer but rare NM_033360 transcript which encodes isoform a. There was no impact on nomenclature as the discrepancy between the two transcripts occurs C-terminal to exon 2. Nevertheless, as previously discussed, we strongly encourage participants to utilise transcript reference sequences designated by the MANE project[2].
- Eighty-six laboratories stated the inclusion of the *KRAS* gene in their panel. Those identifying the low level c.35G>C p.(Gly12Ala) variant used a variety of methodological approaches, including both 'in-house' and commercial assays. Nonetheless, the group featured multiple Sophia Genetics Myeloid Solution (MYS) panel users (9/12 returned the variant). Overall, 11/22 participants submitting the variant had quantified it as below their stated minimum VAF for reporting identification of a SNV (substitution).
- The *KRAS* c.35G>C p.(Gly12Ala) activating variant is typically associated with solid tumours. However, it has also been observed in AML cases and myeloid neoplasia more widely (COSMIC COSV55497479)[9].
- Activating *KRAS* variants are generally considered a late event in AML and their clinical relevance appears less well established in the literature[29-30]. In accordance the ClinVar record is not informative for a myeloid malignancy context (VCV000045122.6)[13].
- Nevertheless, the ELN has included *KRAS* in a group of additional genes (along with *PTPN11* for which analysis is recommended for patients with suspected AML but findings from which are not currently defining at diagnosis[6].

Seven of the eight laboratories stating the *CREBBP* gene as present on their panel identified the c.5220dup p.(Lys1741*) variant. Additionally, one participant omitting to state the gene as included on their panel also detected the variant. Overall, laboratories classified this truncating variant as of potential (n=7) or unknown (n=1) clinical significance.

- The median VAF (DNA based assays, all panels and platforms) returned for the NM_004380.3(*CREBBP*):c.5220dup p.(Lys1741*) variant was 46.0% with an interquartile range 3.3% (n=8).
- Six participants reported the variant using the NM_004380 based MANE Select transcript encoding CREB-binding protein isoform a (longest transcript and protein, duplication residing in exon 31). Two centres provided alternative nomenclature, c.5106dup p.(Lys1703*), utilising a NM_001079846 based reference encoding isoform b (duplication in exon 30). Again, we advocate use of the transcript reference sequence indicated by the MANE project to facilitate the exchange of variant information and avoid confusion. Please note the *CREBBP* gene is frequently referred to as *CBP* in the legacy literature.
- One Sophia Genetics Myeloid Solution (Illumina MiSeq) user featured *CREBBP* on their panel but returned a negative result for the c.5220dup p.(Lys1741*) variant. However, their stated region of interest for the gene did not encompass the applicable exon.

- The *CREBBP* c.5220dup p.(Lys1741*) variant is not cited in ClinVar[13] or COSMIC[9]. Nor is it referenced in the general population data sets[12,25].
- The *CREBBP* gene encodes a lysine acetyltransferase involved in transcriptional regulation and chromatin remodelling**.** Germline loss-of-function *CREBBP* variants result in Rubinstein-Taybi syndrome, a rare autosomal dominant congenital developmental disorder with distinctive dysmorphology[31].
- *CREBBP* variants are known to have a pathogenic role in multiple cancers. Kongkiatkamon *et al.* has recently characterised *CREBBP* variants in a myeloid neoplasia context[32].

Conclusion

Recent advances in the genetic characterisation of AML have markedly improved the understanding of leukemogenesis and AML further informed risk stratification[6]. Oncogenic variants in established genes, including *FLT3*, *RUNX1* and *ASXL1,* provide important prognostic and/or therapeutic information for AML patients. This was reflected in the clinical significance classifications applied by trial participants to the variants identified in these genes for sample AML GP 113 (albeit it with slightly less of a consensus from laboratories regarding the *ASXL1* variant).

Of note, the recent divergence of the WHO[8] and ELN[6] classification systems regarding the consideration of *RUNX1* variants when classifying AML at diagnosis was highlighted by sample AML GP 113.

At the time of sourcing this EQA material clinical information was scant. Nevertheless, the trial results for sample AML GP 113 appear consistent with a s-AML evolved from prior MDS. The chromatin-spliceosome subgroup of AML (including *ASXL1* and *U2AF1*) resemble a variant pattern more commonly observed in MDS[33]. It is possible that an AML patient with chromatin-spliceosome mutations may have experienced a prodromal MDS period even if they do not necessarily meet the formal criteria for the current WHO entity AML with myelodysplasia-related changes (AML-MRC)[5,21.]

The ELN recently defined a group of additional genes, which includes *PTPN11* and *KRAS*, for which analysis is recommended at presentation[6]. The identification of variants in these genes are not required for establishing a diagnosis of AML, nor are they presently actionable therapeutic targets. Rather variants in such genes are beginning to be employed for the subsequent monitoring of disease via NGS methods.

Standard of care analysis via NGS multi-gene panels at diagnosis has revealed a number of germline haematological malignancy predisposition disorders, now recognised to be much more common than previously understood. Identification of a germline susceptibility risk variant in a patient impacts the clinical management and surveillance strategies for the index case and any relatives who may share the familial variant. Of particular importance, the confirmation of a suspected deleterious germline variant is crucial for the selection of suitable familial donors if the patient is a candidate for hematopoietic stem cell transplantation (HSCT)[15-16].

The consistent use of standardised nomenclature with an appropriate reference sequence is critical for the effective communication of genetic testing results across the literature/databases and within a clinical setting. We strongly urge participants to comply with the latest HGVS recommendations for variant nomenclature[3] and utilise transcript reference sequences designated by the MANE collaboration[2].

Final remarks

As referenced in this trial report, as a reflection of the increased understanding of the AML genetic landscape and subsequent progress in prognostication and treatment, the ELN have very recently published an electronic updated version of their diagnosis and management guidelines for adult AML[6]. At the time of writing, the applicable the National Comprehensive Cancer Network (NCCN) clinical practice guidelines for AML are marked as under review[7].

Further, the WHO tumours of haematopoietic and lymphoid tissues classification "blue book series" monograph[5] has also undergone a revision with the upcoming 5th edition pending full publication[8].

Of note, the independent International Consensus Classification (ICC) group have this month published their approach to the classification of myeloid neoplasms and acute leukaemia[34]. This new classification framework represents a major revision of the previous collaborations unified under the auspices of the WHO. The ICC group includes many authors of the previous WHO monograph editions but is no longer affiliated with the WHO and the International Agency for the Research on Cancer (IARC). In the companion paper Cazzola and Sehn summarise the distinctive features of the proposed system, which focuses on integrating genomic data to designate discrete disease entities that may be selectively treated[35]. We will look to incorporate the work of the ICC group into the educational comments section of future Myeloid GP trial reports, as applicable. We acknowledge the challenge faced by laboratories and clinical colleagues if a cohesive approach to myeloid neoplasm classification cannot be maintained by the field.

Please also be aware of the recent joint recommendations from the Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC) and Variant Interpretation for Cancer Consortium (VICC)[36]. The proposed systematic procedure focuses on determining the oncogenicity of a somatic variant using population frequency, functional, and *in silico* data or somatic frequency. The authors suggest their approach can be used in combination with that of Li *et al.* (2017)[1] to first classify biological 'driver' status and then clinical significance in terms of prognosis and actionability.

It is beyond the scope of this programme to comment conclusively on the clinical significance of the variants reported by participants. We acknowledge the limitations of this EQA exercise.

The information provided herein is for participant information only. Clinical decision making with regards to variant interpretation, pathogenicity (driver status), actionability and predicted disease outcomes should not be based solely on comments provided by UK NEQAS LI in this EQA trial report.

Thank you to all participants who provided their full Laboratory Record information, as requested. The valuable methodological information supplied, including details regarding panel region of interest (ROI) and related reference sequences, facilitates an informative trial report.

The expansion of this programme to encompass the NGS multi-gene testing aspects of the previous Myeloproliferative Neoplasms Gene Panels programme necessitates a name change to Myeloid Gene Panels (Pilot – Not Accredited) for the forth coming 2022/23 trial distributions.

Please do contact us if you have any suggestions regarding how this developmental (pilot) programme could be improved for future trial distributions: admin@ukneqasli.co.uk.

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Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
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4.8.2 b) The coordinators of UK NEQAS LI programmes are Mr Liam Whitby (Director) and Mr Stuart Scott (Centre Manager).

4.8.2 c) Person(s) authorizing this report:
Mr Liam Whitby (Director) or Mr Stuart Scott (Centre Manager) of UK NEQAS LI.

4.8.2 d) Pre issue testing of samples for this programme is subcontracted, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were subcontracted. Where subcontracting occurs, it is placed with a competent subcontractor and the EQA provider is responsible for this work.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance (for BS EN ISO/IEC 17043:2010 accredited programmes only). Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided. Alternatively, if you are unhappy with your performance classification for this trial, please find the appeals procedure at www.ukneqasli.co.uk/contact-us/appeals-and-complaints/

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