Pilot Myeloid Gene Panels (Not Accredited)

Distribution – 242502 (Technical round)

Participant –

Date Issued – 31 Mar 2025

Closing Date – 16 May 2025

IMORTANT NOTICE: Please accept our apologies for the delay in publication of the Myeloid Gene Panels 242501 trial report educational addendum (sample Myeloid GP 118). We are collaborating with the HGVS Variant Nomenclature Committee (HVNC) to establish the most appropriate application of ISCN (International System for Human Cytogenomic Nomenclature) and HGVS (Human Genome Variation Society) Nomenclature in relation to *KMT2A* Partial Tandem Duplication (PTD).

Trial Comments

This trial was issued to 152 participants; 143 (94.1%) laboratories returned results. Of the nine participants failing to submit results, two laboratories pre-notified us of their non return.

This trial report focuses on summarising the variants detected by participants (including methodological aspects) in the EQA sample provided. It does not contain a detailed breakdown of variant nomenclature or educational elements relating to variant biological classification and clinical interpretation. Such educational aspects will next feature in the Myeloid Gene Panels 252601 trial distribution.

Sample Comments

The genomic DNA trial sample (Myeloid GP 119) was extracted from the peripheral blood of an adult patient with a working diagnosis of chronic myelomonocytic leukaemia (CMML) (no further clinical details available) and distributed by UK NEQAS LI.

Your Myeloid Gene Panels 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 Myeloid GP 119

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] Clinical Significance:			Variant detected (consensus) [c]		Median VAF % (IQR) [d]
		Strong	Potential	Unknown	DNA sequence description	Protein level description	
NRAS	137/140	128	9	0	NM_002524.5:c.38G>A	p.(Gly13Asp)	45.0 (3.0)
SETBP1	135/138	125	9	1	NM_015559.3:c.2608G>A	p.(Gly870Ser)	47.2 (2.0)
TET2	134/138	72	58	4	NM_001127208.3:c.709del[e]	p.(Cys237Valfs*13)[e]	47.6 (2.6)
U2AF1	134/139	99	30	5	NM_006758.3:c.470A>C	p.(Gln157Pro)	47.2 (2.4)
ETV6	129/134	76	47	6	NM_001987.5:c.1106G>A	p.(Arg369Gln)	49.6 (3.0)
EZH2	125/140	42	52	30	NM_004456.5:c.117+1G>A	p.(?)	48.6 (3.0)
ASXL1	121/143	108	12	0	NM_015338.6:c.1934dup	p.(Gly646Trpfs*12)	39.9 (9.0)
KRAS	108/140	57	45	6	NM_004985.5:c.176C>G	p.(Ala59Gly)	4.0 (1.1)
RAD21	64/77	20	32	12	NM_006265.3:c.274+1G>T	p.(?)	31.7 (7.0)
NOTCH2	6/17	0	0	6	NM_024408.4:c.6572C>A	p.(Ala2191Asp)	47.5 (16.2)

[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 (or other variant in the gene). Please note for this trial 4 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, participant(s) may also have reported 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]. Laboratories submitting multiple classifications for the same variant are excluded from this section of the table; therefore, the total number of classifications may not equal the total participants detecting the variant.
[c] Nomenclature provided in the table is based on the MANE Select/Plus Clinical (v1.4)[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) nomenclature (version 21.3)[3-5] 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 parentheses as it 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 (where it was stated), 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] Equivalent to c.772del p.(Cys258Valfs*13) with reference to the ENST00000513237.5 alternative transcript (Ensembl)

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 4 returning participants failed to provide the minimum Laboratory Record information requested.

Methodological approach

	Returns
Targeted Gene Panel (DNA seq)	127
Targeted Gene Panel (DNA with RNA fusion transcript seq)	11
Other	2

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

	Returns
Illumina MiSeq	41
Illumina NextSeq 550	22
Illumina NextSeq 1000/2000	18
Illumina Novaseq 6000	15
Thermo Fisher Scientific Ion Torrent Genexus system	11
Thermo Fisher Scientific (Life Tech) Ion S5	10
Illumina MiniSeq	10
Thermo Fisher Scientific (Life Tech) Ion S5 Plus	7
Thermo Fisher Scientific (Life Tech) Ion S5 Prime	3
Illumina iSeq100	3
Illumina Novaseq X	3
Illumina Novaseq X Plus	2
MGI DNBSQ G400	2

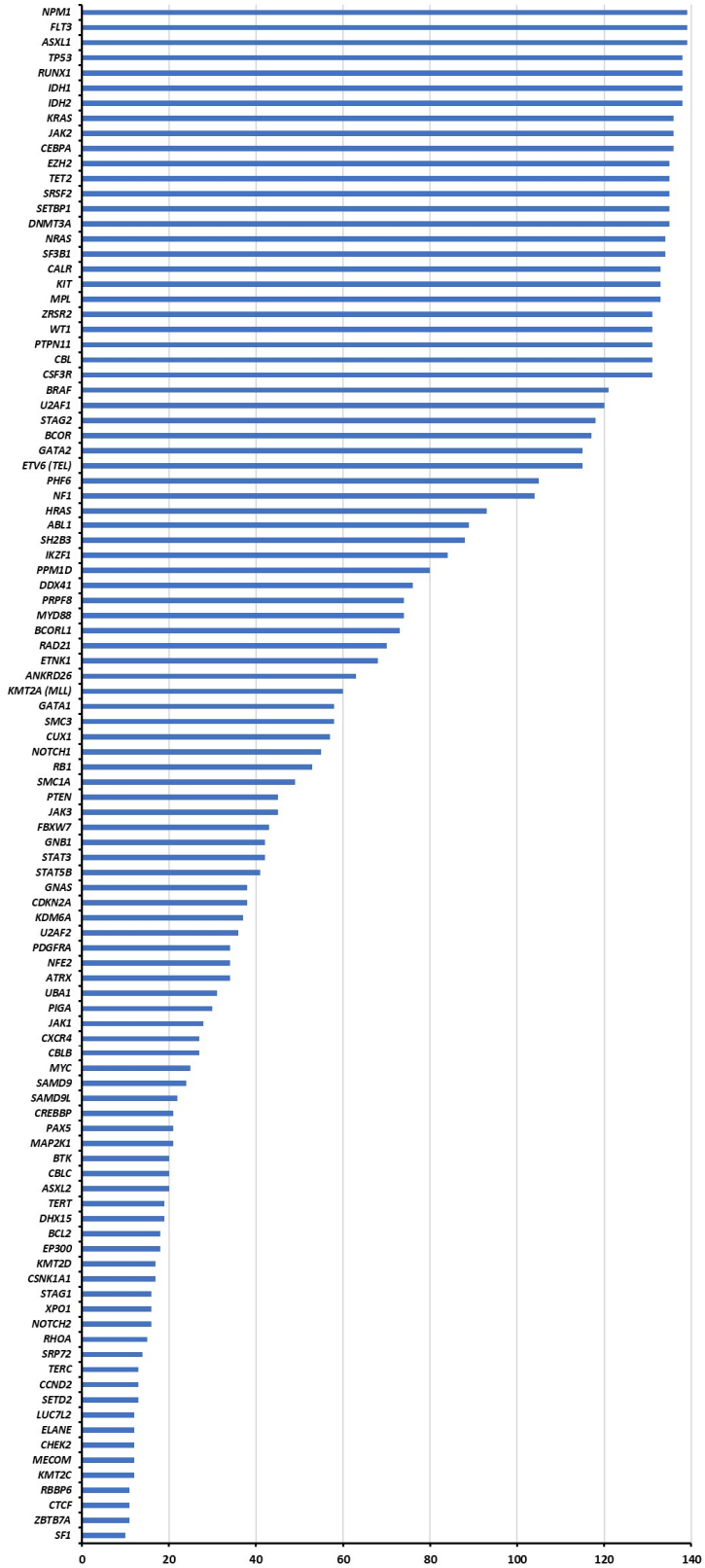
NGS panel description (to analyse the sample in this trial)

	Returns
In house (capture based)	18
Oncomine Myeloid Research Assay	16
Sophia Genetics Myeloid Solution (MYS)	16
Agilent SureSelect Custom XT HS2 Panel	12
Oncomine Myeloid Assay GX v2	11
Qiagen QIASeq Custom Panel	10
Twist Custom Panel	10
In house (amplicon based)	8
AmpliSeq for Illumina Myeloid Panel	7
Archer VARIANTPlex Myeloid panel	6
Illumina TruSight Oncology 500 (TSO500) Panel	2
Agilent SureSelect Custom QXT Panel	2
Sophia Genetics Myeloid Plus Solution (MYS+)	2
Health in Code Haematology OncoKitDx	2
Sophia Genetics CMYS Custom Panel	2

As stated by ≥2 participants.

Genes routinely analysed by participants (in this clinical context).

Information provided by 139 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	110
GRCh38	30

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

	Returns
0.5%	2
1%	6
2%	21
2.5%	8
3%	12
3.5%	2
4%	3
4.5%	1
5%	83
10%	2

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

	Returns
0.5%	1
1%	14
2%	22
2.5%	8
3%	14
3.5%	2
4%	4
4.5%	1
5%	74

Annotation database resources

	Returns
ClinVar (NCBI)	132
COSMIC (Catalogue Of Somatic Mutations In Cancer)	127
TP53 Database (National Cancer Institute) previously hosted by WHO IARC	89
OncoKB (Memorial Sloan Kettering Cancer Center)	60
Seshat (TP53) Database	42
OMIM (NCBI)	42
cBioPortal (Memorial Sloan Kettering Cancer Center *et al*.)	39
Cancer Hotspots (Memorial Sloan Kettering Cancer Center)	34
My Cancer Genome (Vanderbilt-Ingram Cancer Center)	31
Clinical Knowledgebase (CKB) Jackson Laboratory	27
HGMD (The Human Gene Mutation Database)	24
The Cancer Genome Atlas (TCGA)	23
CIViC (Clinical Interpretation of Variants in Cancer)	20
UniProt (EMBL-EBI, SIB, PIR)	17
UMD (Universal Mutation Database) TP53 Database	14
PeCan Pathogenicity Information Exchange (PIE) (St Jude)	7
Molecular Tumor Board Portal (MTB) (Karolinska Institutet)	5

As stated by ≥2 participants.

Large population dataset/resources routinely consulted

	Returns
gnomAD (Genome Aggregation Database)	125
dbSNP (Short Genetic Variations, NCBI)	82
1000 Genomes	52
ESP (Exome Sequencing Project, NHLBI GO)	28

As stated by ≥2 participants.

In silico tools utilised to predict impact on splicing

	Returns
Splice AI	65
SpliceSiteFinder	36
GeneSplicer	30
MaxEntScan	25
NNSPLICE	22

Aggregation tool(s) utilised to access annotation resources

	Returns
Franklin (GENOOX)	69
Varsome (SAPHETOR)	66
Alamut (SOPHiA GENETICS)	45
QCI Interpret (Qiagen)	5
Mobidetails (MoBiDiC, Montpellier University Hospital)	4

As stated by ≥2 participants.

Trial Comments

Methodology

- The majority of returning participants (with the relevant information provided in their Laboratory Record) described the application of a DNA based targeted gene panel next generation sequencing (NGS) testing approach (n=139). At least 12 laboratories stated the additional inclusion of 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 average number of genes currently analysed by laboratories on a given panel is 55 (range 8-361). For the genes most frequently included on participant gene panels (and analysed in this clinical context) please refer to the chart on page 7.
- Overall, 77.6.% (n=114) returning participants providing the relevant information employed bridge amplified reversible dye terminator-based platforms from Illumina to analyse sample Myeloid GP 119. The remaining laboratories stated the use of ThermoFisher Scientific (Ion Torrent) (n=31) or MGI DNBSEQ (n=2) technology.
- The most utilised ‘off the shelf’ commercially available panel kits included the ThermoFisher Scientific Oncomine Myeloid Research Panel (n=16), Sophia Genetics Myeloid Solution (n=16), ThermoFisher Scientific Oncomine Myeloid Assay GX v2 (n=11) and Archer VARIANTPlex Myeloid Panel (n=6).
- For a deletion/duplication event, 39.3% (n=55) of participants quoted a minimum threshold for reporting of <5% VAF for this trial.

- For a SNV the thresholds for reporting continue to be set lower with 47.1% (n=66) laboratories applying a minimum VAF <5% for this trial.

Annotation and interpretation

- The proportion of participants known to be working to the GRCh38 human genome assembly was 21.4% (n=30).
- ClinVar (n=132), COSMIC (n=127) and gnomAD (n=125) remain the resources most widely utilised by participants. The list of databases and tools accessed by centres continues to expand, please refer to the tables on pages 9-10 for further information.
- The *in silico* tools utilised most frequently to predict impact on splicing included Splice AI (n= 65) and are summarised in the table on page 10.
- An increased uptake of aggregation tools (incorporating AI powered interpretation), particularly Franklin (GENOOX/Qiagen) (n=69), was noted. Such tools can be extremely useful. However, 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.
- The Association for Clinical Genomic Science (ACGS) guidelines for the classification of oncogenicity of somatic variants in cancer: recommendations by the UK somatic variant interpretation group (SVIG-UK) have very recently been published online ahead of print[6].

Sample Myeloid GP 119

All returning participants (n=143) reported at least one sequence variant in sample Myeloid GP 119 (please refer to the summary 'All Participant Results' table on page 4 for further details).

One laboratory employing the OGT SureSeq Myeloid Plus assay on the Illumina MiniSeq platform returned an out of consensus *NRAS* variant, NM_002524.5:c.182A>T p.(Gln61Leu), and classified it as of strong clinical significance. No consensus variants were returned. Aspects of their methodology appeared equivocal. However, they are expected to have featured the relevant genes on their panel. It is feasible that this centre performed a sample transposition error and we strongly encourage the laboratory to investigate this.

A total of 137 returning participants reported detection of a variant equivalent to NM_002524.5:c.38G>A p.(Gly13Asp) in exon 2 of the *NRAS* gene. The majority of laboratories (93.4%) classified this missense variant as of strong clinical significance (n=128). The remaining participants designated it as of potential clinical significance (n=9).

- The median variant allele frequency (VAF) (all panels and platforms) reported for the NM_002524.5:c.38G>A *NRAS* variant was 45.0% with an interquartile range of 3.0% (n=136).
- One laboratory erroneously returned the *NRAS* consensus variant via the *HRAS* gene data entry fields. However, the NM_002524.4 *NRAS* reference sequence was quoted. The data was incorporated during the trial analysis process.
- Nomenclature was in good agreement at the DNA and protein levels; all laboratories quoted a MANE Select transcript NM_002524/ENST00000369535 based reference sequence (various

versions). However, 16.9% of centres (n=23) failed to include parentheses to indicate the predicted status of the protein nomenclature (genomic DNA was analysed). Just 4.4% of laboratories (n=6) employed the less preferable single letter amino acid notation.

- As previously discussed, a single laboratory (equivocal method information provided) reported identification of an out of consensus *NRAS* variant, NM_002524.5:c.182A>T p.(Gln61Leu) with VAF = 48%.
- Overall, just five participants failed to report a variant in the *NRAS* gene:
    - An AmpliSeq for Illumina Myeloid Panel user provided insufficient methodological information to ascertain if the variant position was encompassed by the region of interest of their assay. No relevant coverage issues were declared by the participant. Of note, other users of this commercial kit successfully identified the consensus *NRAS* variant (n=6).
    - Additionally, one laboratory submitted the variants c.35G>A p.(Gly12Asp) and c.38G>A p.(Gly13Asp) into the *KRAS* data entry fields and cited the NM_033360.4 (*KRAS*) reference sequence. It is not clear if this was purely a clerical error (variant actually detected in the *NRAS* gene) or rather the outcome of a sample transposition event. The only other variants identified by this laboratory were the NM_015338.6:c.1934dup p.(Gly646Trpfs*12) *ASXL1* change at VAF=13% (see discussion page 16) and an out of consensus *FLT3* variant, NM_004119.3:c.2505T>G p.(Asp835Glu), both classified by the centre as of strong clinical significance. We urge the laboratory to investigate this.
    - Three centres did not include the *NRAS* gene on their panel.

Overall, 135 returning participants reported detection of a variant equivalent to NM_015559.3:c.2608G>A p.(Gly870Ser) in exon 4 of the *SETBP1* gene. Most laboratories (92.6%) classified this missense variant as of strong clinical significance (n=125). The remaining participants designated it as of potential (n=9) or unknown (n=1) clinical significance.

- The median VAF (all panels and platforms) reported for the NM_015559.3:c.2608G>A p.(Gly870Ser) *SETBP1* variant was 47.2% with an interquartile range of 2.0% (n=133).
- Most of the centres returning this variant (94.1%) utilised the MANE Select transcript NM_015559\ENST00000649279 (various versions) (isoform a, n=127). However, a minority of laboratories quoted an alternative transcript reference sequence (n=7). This included the legacy Ensembl GRCh37.p13 ENST00000282030 based transcript, which does not appear to be part of the current Ensembl build (GRCh38.p14) *SETBP1* transcript table (n=6) but does have documented equivalence to NM_015559.2. There was no impact on nomenclature.
- Two labs submitted the *SETBP1* consensus variant via the *SETDB1* gene data entry fields, fortuitously this was picked up during trial analysis and the data incorporated (NM_015559.3 *SETBP1* reference sequenced quoted). This added complexity to the trial analysis process.
- Nomenclature was generally handled well by participants. Just two laboratories submitted positional numbering errors for this missense consensus variant, one at the DNA level (c.2068G>A) and the other at the protein level (p.(Gly59Ser)). Again, the absence of applicable parentheses (n=22) and use of the less preferable single letter amino acid notation (n=6) was observed.
    - In total, eight participants failed to report the NM_015559.3:c.2608G>A p.(Gly870Ser) *SETBP1* consensus variant:
        - Three laboratories stated the inclusion of the *SETBP1* gene on their panel:
            - An OGT SureSeq Myeloid Plus (n=1) and an Oncomine Myeloid Assay GX v2 (n=1) user both returned a false negative result, as *SETBP1* exon 4 was encompassed by their stated assay scopes.

- A further participant employing an in-house amplicon-based panel (Thermo Fisher Scientific Ion S5 Prime platform) did not provide sufficient region of interest information for their assay to ascertain if the variant position was included.
- No relevant coverage issues were declared for sample Myeloid GP 119 by any of these three centres.

- Two participants failed to provide their Laboratory Record information and therefore, the scope of their assays were not known.
- Three centres were known not to include the *SETBP1* gene on their panel.

In total, 134 laboratories identified the NM_001127208.3:c.709del p.(Cys237Valfs*13) variant in exon 3 of the *TET2* gene. Participants classified this frameshift variant as of strong (n=72), potential (n=58) or unknown (n=4) clinical significance.

- The median VAF (all panels and platforms) reported for the NM_001127208.3:c.709del p.(Cys237Valfs*13) *TET2* variant was 47.6% with an interquartile range of 2.6% (n=133).
- Most participants cited the MANE Select NM_001127208/ENST00000380013 based transcript (various versions, isoform a) to describe this single nucleotide deletion variant (n=114).
- Additionally, 17 laboratories quoted only a NM_017628/ENST00000305737 based (various versions) alternative transcript encoding a shorter protein with distinct C-terminus (isoform b). However, on this occasion, it had no bearing on nomenclature.
- One centre identified the consensus variant but submitted alternative nomenclature, c.772delT p.(Cys258Valfs*13, citing the Ensembl ENST00000513237 transcript encoding a 2023 amino acid protein for which a RefSeq (NCBI) equivalent could not be identified. A further laboratory returned a variant description with the same positional numbering but quoted an incompatible *TET2* transcript reference sequence (NM_001127208.3). Participants are urged to be diligent when reporting a variant with multiple protein encoding expressed transcripts. Use of reference transcripts curated by the MANE project[2] are strongly advocated to facilitate consistency and reduce the opportunities for misunderstanding during variant communication. Versioned reference sequence identifiers are required when the reference sequence database uses this system to distinguish between unique sequences (e.g. RefSeq and Ensembl). In the context of such reference sequences, the HGVS Nomenclature specification states only those with version numbers are suitable for defining and describing a sequence variant within a given gene[3].
- A further laboratory cited an erroneous reference sequence (NM_017629) which equates to the *AGO4* gene.
- One participant provided an incorrect DNA description (c.708del) but was in consensus at the protein level. Another laboratory returned a protein level description featuring a likely typographic error, p.(Cys7237ValfsTer13) (DNA level nomenclature was as consensus). Both centres cited a MANE Select NM_001127208 derived reference transcript.
- In general, participants demonstrated various approaches to the syntax and symbols employed to describe this single nucleotide deletion, particularly at the protein level. A detailed breakdown of the submitted protein level nomenclature is beyond the scope of this report. However, centres are encouraged to consult the HGVS Nomenclature website[3] for recommendations regarding the description of a single nucleotide deletion and the predicted frameshift event at the protein level:

  https://hgvs-nomenclature.org/stable/recommendations/DNA/deletion/

  https://hgvs-nomenclature.org/stable/recommendations/protein/frameshift/

- Nine participants failed to report the NM_001127208.3:c.709del p.(Cys237Valfs*13) variant in the *TET2* gene.
    - Four laboratories returned a false negative result. The *TET2* gene featured on their panel within the stated region of interest encompassing the consensus variant position (exon 3). No relevant coverage issues were declared for sample Myeloid GP 119.
    - Two participants failed to provide their Laboratory Record information and therefore, the scope of their assays were not known.
    - Three centres were known not to include the *TET2* gene on their panel.

A total of 134 returning participants identified a variant equivalent to NM_006758.3:c.470A>C p.(Gln157Pro) in exon 6 of the *U2AF1* gene. Over 96% of laboratories classified this missense variant as of strong (n=99) or potential (n=30) clinical significance. The remaining participants designated it as of unknown clinical significance (n=5).

- The median variant allele frequency (VAF) (all panels and platforms) reported for the NM_006758.3:c.470A>C p.(Gln157Pro) *U2AF1* variant was 47.2% with an interquartile range of 2.4% (n=134).
- On the whole nomenclature was consistent at the DNA and protein levels; most laboratories quoted a MANE Select transcript NM_006758.3/ENST00000291552.9 based reference sequence (various versions, isoform a, n=130). Four centres cited a NM_001025203 based reference transcript (isoform b) but this did not impact the subsequent nomenclature.
- Of note, 14.2% of laboratories returning the *U2AF1* missense consensus variant submitted it via the *U2AF2* data entry fields with a *U2AF1* reference sequence stated (n=19). Again, this added complexity to the trial analysis process.
- An absence of parentheses to indicate the predicted status of protein nomenclature (genomic DNA was analysed) and use of the less preferable single letter amino acid format was noted in some descriptions, as previously discussed. Additionally, a single participant omitted to include an appropriate p. prefix at the protein level, Gln157Pro.
- Overall, 9 participants failed to identify the *U2AF1* missense consensus variant:
    - Five laboratories stated the inclusion of the *U2AF1* gene on their panel:
        - One Agilent SureSelect Custom XT HS2 Panel user highlighted coverage issues for *U2AF1* exon 6 for sample Myeloid GP 119.
        - An in-house amplicon-based panel user (Thermo Fisher Scientific Ion S5 Prime platform) provided insufficient methodological information to ascertain if the variant position (exon 6) was encompassed by the region of interest of their assay. No relevant coverage issues were declared by the centre.
        - The remaining three laboratories, AmpliSeq for Illumina Myeloid Panel on Illumina MiSeq (n=1), Oncomine Myeloid Assay GX v2 on Thermo Fisher Scientific Ion Torrent Genexus system (n=1) and OGT SureSeq Myeloid Plus on Illumina MiniSeq (n=1), reported a false negative result. No relevant coverage issues were declared by these participants.
    - One laboratory did not provide any Laboratory Record information. Therefore, the scope of their assay was not known.
    - Three centres did not include the *U2AF1* gene on their panel.

A total of 129 returning participants reported detection of a variant equivalent to NM_001987.5:c.1106G>A p.(Arg369Gln) in exon 6 of the *ETV6* gene. Over 95% of laboratories classified this missense variant as of strong clinical (n=76) or potential significance (n=47). The remaining participants designated it as of unknown clinical significance (n=6).

- The median VAF (all panels and platforms) reported for the NM_001987.5:c.1106G>A p.(Arg369Gln) *ETV6* variant was 49.6% with an interquartile range of 3.0% (n=129).
- Nomenclature was in good agreement at the DNA and protein levels; all laboratories quoted a MANE Select transcript NM_001987/ENST00000396373 based reference sequence (various versions). An absence of parentheses to indicate the predicted status of protein nomenclature (genomic DNA was analysed) and use of the less preferable single letter amino acid format was noted in some descriptions, as previously discussed.
- Overall, 14 participants failed to report the *ETV6* missense consensus variant:
    - Five laboratories stated the inclusion of the *ETV6* gene on their panel:
        - Four laboratories reported a false negative result: Sophia Genetics Myeloid Plus Solution (MYS+) on the Illumina MiSeq (n=1), Agilent SureSelect Custom XT HS2 Panel on the Illumina MiSeq (n=1), Oncomine Myeloid Assay GX v2 on the Thermo Fisher Scientific Ion Torrent Genexus system (n=1) and OGT SureSeq Myeloid Plus on the Illumina MiniSeq platform (n=1). All stated assay regions of interest that encompassed the *ETV6* missense consensus variant position (exon 6). No relevant coverage issues were declared by these participants.
        - A further participant employing an in-house amplicon-based panel (Thermo Fisher Scientific Ion S5 Prime platform) omitted to provide their assay region of interest information and therefore, the scope of their assay was not known.
    - Nine centres did not include the *ETV6* gene on their panel.

Overall, 125 returning participants reported detection of a variant equivalent to NM_004456.5:c.117+1G>A in intron 2 of the *EZH2* gene. Classification of this splice variant was spread across strong (n=42), potential (n=52) or unknown (n=30) clinical significance.

- The median VAF (all panels and platforms) reported for the NM_004456.5:c.117+1G>A *EZH2* variant was 48.6% with an interquartile range of 3.0% (n=124).
- Nomenclature at the DNA level was in full agreement; all laboratories cited a MANE Select transcript NM_004456/ENST00000320356 based reference sequence (various versions).
- Two participants offered a p.X39 description of the predicted event at the protein level However, most laboratories completing the relevant data entry field submitted p.? (n=63) or p.(?) (n=14). We refer participants to the HGVS Nomenclature website[3] for details regarding the communication of variants that likely affect RNA splicing but for which RNA (or cDNA) was not analysed.

  https://hgvs-nomenclature.org/stable/recommendations/uncertain/

  https://hgvs-nomenclature.org/stable/recommendations/uncertain/#protein
- One participant returned the *EZH2* intronic consensus variant in both the strong and potential clinical significance data entry tables and has been excluded from the classification section of the 'All Participant Results' table on page 4 of this report. **We request that participants submit a relevant variant detected with only one concluding classification**. Multiple classifications by a laboratory for the same variant confounds the data analysis process.

- An AmpliSeq for Illumina Myeloid Panel (Illumina MiniSeq platform) user reported an out of consensus *EZH2* exonic variant, NM_004456.5:c.221T>A p.(Val74Glu). The variant was reported by the participant at a low level (VAF= 6.9%) and classified by the laboratory as of potential clinical significance.
- Overall, 17 participants failed to report a variant in the *EZH2* gene:
    - Of these laboratories, 14 stated the inclusion of the *EZH2* gene on their panel:
        - The assays of at least 11 participants (various methods) encompassed *EZH2* exon 2 and therefore presumably the relevant exon/intron boundary to identify the NM_004456.5:c.117+1G>A splice variant. No applicable coverage issues were declared by the participants and thus, they have been designated to have returned a false negative result. We acknowledge some laboratories may restrict analysis to exonic coding sequence only. However, given the known oncogenic contribution of splicing associated variants (including those that disrupt a *cis* evolutionary conserved sequence position at a natural 5' donor site)[7], we advocate that relevant centres review their approach.
        - One centre stating the use of the 'GTC Hematology Profile' assay (Illumina NextSeq 1000/2000 platform) failed to provide sufficient detail regarding assay scope to ascertain if the exon 2/intron 2 junction formed part of the region of interest.
    - Three centres did not include the *EZH2* gene on their panel.

All returning laboratories (n=143) included the *ASXL1* gene within their assay scope. The NM_015338.6:c.1934dup p.(Gly646Trpfs*12) single base duplication in exon 13 was reported by 121 laboratories. Classification of this frameshift truncating variant was predominantly of strong (n=108) clinical significance. However, some centres designated it of potential (n=12) clinical significance.

- The median VAF (all panels and platforms) reported for the NM_015338.6:c.1934dup p.(Gly646Trpfs*12) *ASXL1* variant was 39.9% with an interquartile range of 9.0% (n=118).
- One participant returned the *ASXL1* consensus variant in the strong, potential and unknown clinical significance data entry fields and has been excluded from the classification section of the 'All Participant Results' table on page 4 of this report. This may have been related to the previously contentious status of the NM_015338.6:c.1934dup p.(Gly646Trpfs*12) finding (see below). However, we request that participants submit a relevant detected variant with only a single concluding classification. Multiple classifications by a laboratory for the same variant confounds the data analysis process.
- One laboratory erroneously returned the *ASXL1* consensus variant via the *ABL1* gene data entry fields. However, the ENST00000375687.10 *ASXL1* reference sequence was quoted. The data was incorporated during the trial analysis process.
- All participants cited a MANE Select NM_015338\ENST00000375687 based transcript (various versions) to describe the variant. Please note that in some publications, legacy exon numbering is applied and the large *ASXL1* terminal exon (exon 13) may be referred to as exon 12.
- Two laboratories returned protein level descriptions featuring erroneous positional information, p.(Gly641TrpfsTer12) and p.Gly546fs. Additionally, a single participant submitted nomenclature containing a likely typographic mistake, p.ly646TrpfsTer12. DNA level nomenclature was comparable to the consensus variant for all three of these results.

- Participants demonstrated various approaches to the syntax and symbols employed to describe this single nucleotide duplication. A detailed breakdown of the submitted nomenclature is beyond the scope of this report. However, centres are encouraged to follow the guidance available on the HGVS Nomenclature website[3], particularly regarding the prioritisation of a duplication-based description (rather than an insertion, when applicable) and the 3' rule.

  https://hgvs-nomenclature.org/stable/recommendations/DNA/duplication/

  https://hgvs-nomenclature.org/stable/recommendations/protein/frameshift/

- Overall, 22 participants failed to report detection of the NM_015338.6:c.1934dup p.(Gly646Trpfs*12) variant in the *ASXL1* gene:
  - Of these participants, at least 19 laboratories (various methods) are deemed to have returned a false negative result. The *ASXL1* gene was stated on their panel with the region of interest encompassing the consensus variant position. No relevant coverage issues were declared by these laboratories for sample Myeloid GP 119.
  - A further centre omitted to provide sufficient detail regarding assay scope to ascertain if *ASXL1* exon 13 (historically exon 12) formed part of their in-house amplicon-based assay region of interest (Thermo Fisher Scientific Ion S5 Prime platform). No relevant coverage issues were declared.
  - Of concern, one Oncomine Myeloid Assay GX v2 (Thermo Fisher Scientific Ion Torrent Genexus system) user noted (in an applicable comments field of their Laboratory Record) that they do not report the NM_015338.6:c.1934dup p.(Gly646Trpfs*12) variant. However, it may be possible that this remark is limited to NGS analysis and the laboratory employs an alternative technique, such as a targeted real time PCR assay to detect this variant.
  - An additional laboratory featured the *ASXL1* gene on their panel but noted region of interest coverage issues likely impacting exon 13 (equivalent to exon 12 legacy exon numbering).

- **We strongly encourage the relevant laboratories failing to report the NM_015338.6:c.1934dup p.(Gly646Trpfs*12) variant to review their variant calling/filtering settings and reporting policy for this *ASXL1* variant**. Although it was proposed in an early study that this controversial sequence change (also referred to as c.1934_1935insG in the legacy literature) may be an artifact of PCR and/or sequencing[8], subsequent investigation indicates that c.1934dup can represent a genuine somatic acquired variant[9].

- Alberti *et al.* (2018)[10] and Montes-Moreno *et al.* (2018)[11] both discuss considerations for discriminating the presence of the *ASXL1* c.1934dup somatic variant from a technical artifact using NGS in the clinical context of myeloid malignancies.

- **Notably, 62.5% (10/16) of Oncomine Myeloid Research Assay panel (Thermo Fisher Scientific (Life Tech) Ion platform) users failed to report the NM_015338.6:c.1934dup p.(Gly646Trpfs*12) consensus *ASXL1* variant in sample Myeloid GP 119.** The additional guanine (G) nucleotide is located within a mononucleotide tract (8G). Semiconductor-based NGS platforms are known to find homopolymer regions particularly challenging to sequence[12].

A total of 108 returning participants identified the low-level NM_004985.5:c.176C>G p.(Ala59Gly) variant in exon 3 of the *KRAS* gene. Most participants (94.5%) classified this missense variant as of strong (n=57) or potential (n=45) clinical significance. The remaining participants designated it as of unknown clinical significance (n=6).

- The median VAF (all panels and platforms) reported for the NM_004985.5:c.176C>G p.(Ala59Gly) *KRAS* variant was 4.0% with an interquartile range of 1.1% (n=108).

- Broadly nomenclature was good agreement. The choice of transcript reference sequence was split between the MANE Select NM_004985/ENST00000311936 (various versions) (n=64) and MANE Plus Clinical NM_033360/ENST00000256078 (various versions) (n=44) transcript options. However, there was no impact on variant nomenclature.
- Overall, 31 laboratories stated inclusion of the *KRAS* gene on their panel but did not submit the NM_004985.5:c.176C>G p.(Ala59Gly) or equivalent variant:
    - In total 27 laboratories included *KRAS* exon 3 in their assay region of interest but did not report identification of the variant (various methods). However, this low-level variant (median VAF <5%) may have fallen below the analytical sensitivity of some assays.
        - Two of these laboratories, a Nonacus Cell3 Custom Oncology Panel with the Illumina Novaseq 6000 (n=1) and Oncomine Myeloid Research Assay with the Thermo Fisher Scientific Ion S5 (n=1) user, stated incomplete coverage of *KRAS* exon 3 was achieved for sample Myeloid GP 119.
    - Four laboratories provided insufficient methodological information to ascertain if the variant position was encompassed by the region of interest of their assay. No relevant coverage issues were declared by the participants. This included two AmpliSeq for Illumina Myeloid Panel users. Of note, other users of this commercial kit successfully identified the consensus *KRAS* variant (n=5).
- An additional three centres did not report detection of the *KRAS* variant but did not feature the gene on their panel.

Sixty-four returning participants reported detection of a variant equivalent to NM_006265.3:c.274+1G>T in intron 3 of the *RAD21* gene. Classification of this spice variant was spread across strong (n=20), potential (n=32) or unknown (n=12) clinical significance.

- The median VAF (all panels and platforms) reported for the NM_006265.3:c.274+1G>T *RAD21* variant was 31.7% with an interquartile range of 7.0% (n=63).
- Nomenclature at the DNA level was in full agreement; all laboratories cited a MANE Select transcript NM_006265/ENST00000297338 based reference sequence (various versions). Please refer to page 15 for related information regarding protein level nomenclature for variants that likely affect RNA splicing but for which RNA (or cDNA) was not analysed.
- The *RAD21* gene was known to feature on less than 50% of returning laboratory panel scopes. Identification of the NM_006265.3:c.274+1G>T variant will not be discussed in detail. However, please refer to pages 15-16 for general comments regarding splice variants.

Six returning participants reported identification of the NM_024408.4:c.6572C>A p.(Ala2191Asp) variant in exon 34 of the *NOTCH2* gene. All classified the missense variant as of unknown clinical significance.

- The median VAF (all panels and platforms) reported for the NM_024408.4:c.6572C>A p.(Ala2191Asp) *NOTCH2* variant was 47.5% with an interquartile range of 16.2% (n=6). This small dataset featured a gross outlier, which was likely to represent a decimal representation (0.47) of a 47% quantification result. The data entry pages for this programme currently request participants to submit VAF in a percentage [% = variant reads/total reads] format.

Final Remarks

Many thanks to those 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.

Please accept our apologies for the delay issuing this trial report. Continued growth in participation for this pilot programme has produced a large dataset requiring manual analysis and independent checking. We acknowledge that currently maintaining the Laboratory Record and standard trial data entry are time consuming for participants; however, absent, duplicated and/or inconsistent information has hampered the progression of analysis and compounded the delay in reporting this distribution. A frequently occurring challenge to data analysis was caused by the ability of multiple participants to detect a variant(s) in a gene which was not included in their stated assay panel scope. It is the responsibility of participants to ensure that their Laboratory Record for this programme is reviewed at each trial distribution, as appropriate. We will be reviewing data entry and reporting procedures in 2026-2027.

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 Nomenclature specifications[3] and utilise a uniquely identifiable transcript reference sequence(s) designated by the MANE collaboration[2].

Variant classifications have been aligned to Li *et al*., (2017) joint consensus recommendations from the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP)[1]. The classification system utilises a tier-based system (I-IV): variants of strong, potential or unknown clinical significance and benign/likely benign variants. Please note for the purposes of this EQA programme, participants are not required to submit variants considered to be benign/likely benign (neutral).

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, oncogenicity/pathogenicity (driver status), actionability and predicted disease outcomes should not be based solely on comments provided by UK NEQAS LI.

Please do contact us if you have any suggestions regarding how this 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 17043:2023

7.4.3.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600
e-mail: admin@ukneqasli.co.uk

7.4.3.2 b) Person(s) authorising this report: Mr Stuart Scott (Director) of UK NEQAS LI.

7.4.3.2 c) Administration and shipping for this programme is provided by EQA International Limited.

7.4.3.2 c) Pre issue and post closure testing of samples for this programme is externally provided, although the final decision about sample suitability lies with the EQA provider. Aside from the activities mentioned above, no other activities in relation to this EQA exercise were externally provided.

7.4.3.2 c) Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

7.4.3.2 f) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel (NQAAP) is informed when a UK participant is identified as having performance issues. Please note, the activities of the NQAAPs are currently paused, whilst alternative funding mechanisms are sought.

7.4.3.2 h) All EQA samples are prepared in accordance with strict standard operating procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue.

7.4.3.2 j), m), n), o) & r) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the design and implementation of the programme, example annotated reports including and the performance systems applied to assess performance (for BS EN ISO/IEC 17043:2023 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.

7.4.3.2 l) We do not assign values against reference materials or calibrants.

7.4.3.2 q) Details of the programme designs as authorised 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.

7.4.3.2 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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