UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Pilot Myeloid Gene Panels – INTERIM REPORT
(Not Accredited)

Distribution – 232401

Participant –

Date Issued – 30 Oct 2023

Closing Date – 22 Dec 2023

IMPORTANT: A detailed breakdown of nomenclature and educational aspects relating to variant interpretation will be subsequently published in a Myeloid Gene Panels 232401 report addendum to form the finalised trial report. Participants will be notified by email as soon as the final trial report is available. Please accept our apologies for any inconvenience caused.

Trial Comments

This trial was issued to 131 participants; 124 (94.7%) laboratories returned results. Of the 7 participants failing to submit results, two laboratories pre-notified us of this.

Sample Comments

The genomic DNA sample (Myeloid GP 116) was extracted from the peripheral blood of an adult patient with a working diagnosis of myeloproliferative neoplasm (MPN), essential thrombocythaemia (ET), and distributed by UK NEQAS LI. The material was potentially processed >48 hours following collection.

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. We will be next asking for all Laboratory Record information to be re-submitted for trial Myeloid GP 242501.

Sample Myeloid GP 116

Your Results – Variant(s) of strong clinical significance

Gene	Your DNA sequence variant detected	Your protein variant
No variant(s) detected	No variant(s) detected	No variant(s) detected

Your Results – Variant(s) of potential clinical significance

Gene	Your DNA sequence variant detected	Your protein variant
No variant(s) detected	No variant(s) detected	No variant(s) detected

Your Results – Variant(s) of unknown clinical significance

Gene	Your DNA sequence variant detected	Your protein variant
No variant(s) detected	No variant(s) detected	No variant(s) detected

Classification terminology 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].

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	DNA sequence description	Protein level description	
MPL	61/115	22	21	18	NM_005373.3: c.610T>C	p.(Ser204Pro)	11.8 (1.2)
CBL	8/102	4	3	1	NM_005188.4: c.1211G>A	p.(Cys404Tyr)	1.0 (0.2)

[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. Please note for this trial 10 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 (once published) 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 (once published) 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 (version 20.05)[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 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, 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.

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, 10 returning participants failed to provide all the Laboratory Record information requested.

Methodological approach

	Returns
Targeted Gene Panel (DNA seq)	99
Targeted Gene Panel (DNA with RNA fusion transcript seq)	15

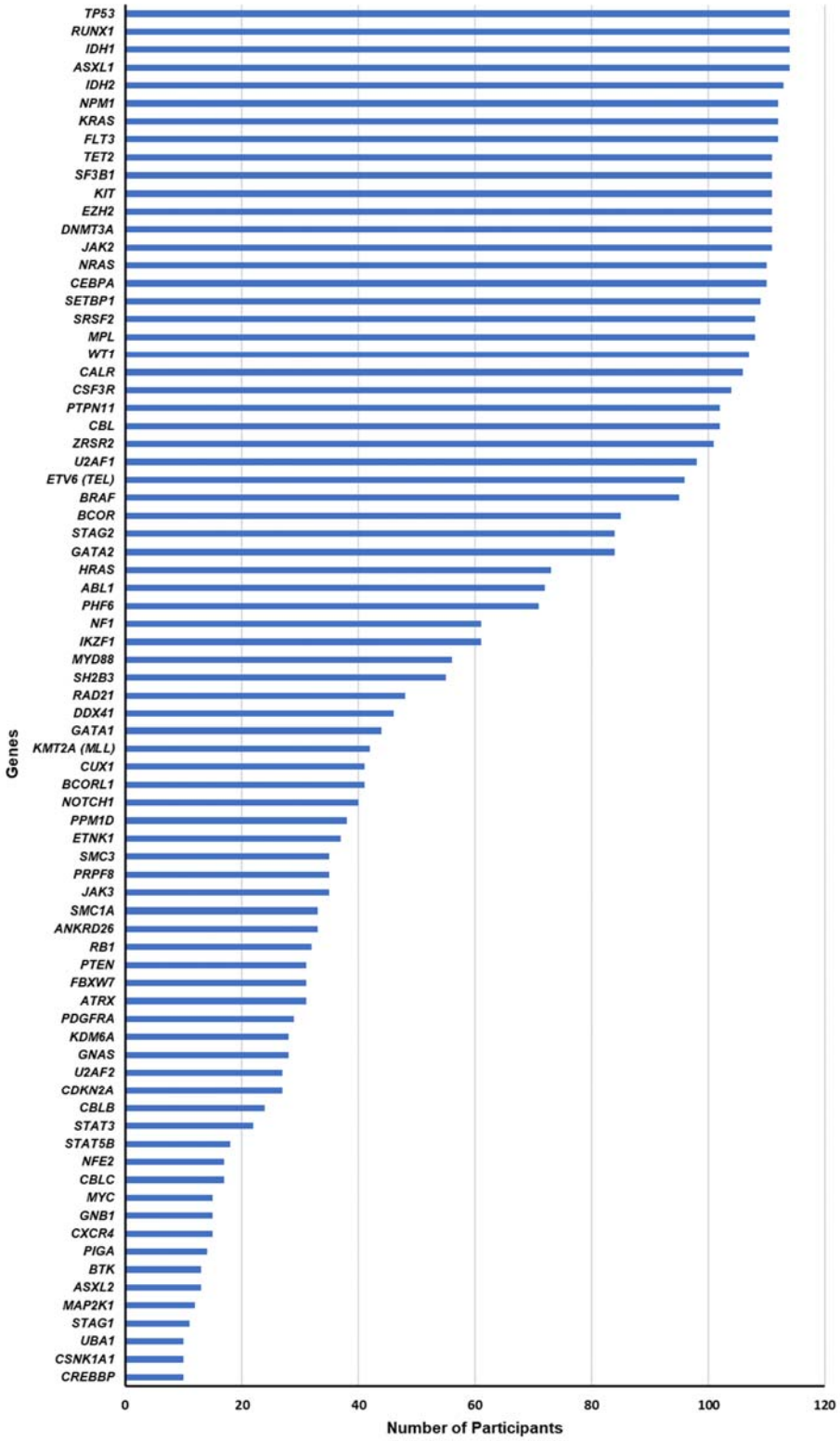
NGS platform(s) used (to analyse the sample in this trial)

	Returns
Illumina MiSeq	41
Illumina NextSeq	31
Thermo Fisher Scientific Ion Torrent (Life Tech) Ion S5	14
Illumina Novaseq	12
Illumina MiniSeq	10
Thermo Fisher Scientific Ion Torrent Genexus system	5
Thermo Fisher Scientific Ion Torrent (Life Tech) Ion S5 XL	2
Illumina iSeq 100	2
Element Bioscience AVITI	1

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

	Returns
(IonTorrent) Oncomine Myeloid Research Assay	18
Sophia Genetics Myeloid Solution (MYS)	17
In house (capture based)	13
Other	11
Qiagen QIASeq Custom Panel	10
Illumina TruSight Myeloid Sequencing Panel	10
Archer VariantPlex Myeloid panel	6
Twist Custom Panel	6
In house (amplicon based)	5
Sophia Genetics Extended Myeloid Solution	4
AmpliSeq for Illumina Myeloid Panel	4
Agilent SureSelect Custom QXT Panel	4
Oncomine™ Myeloid DNA Assay GX v2- all genes included in the kit	3
Illumina TruSight Oncology 500 (TSO500) Panel	2
Imegen Haematology OncoKitDx	2

Genes routinely analysed by participants (in this clinical context). Information provided by 114 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	98
GRCh38	16

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

	Returns
<1%	1
1%	12
>1-2%	14
>2-3%	12
>3-4%	4
>4-5%	70
10%	1

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

	Returns
1%	18
>1-2%	14
>2-3%	14
>3-4%	4
>4-5%	64

Annotation database resources

	Returns
COSMIC (Catalogue Of Somatic Mutations In Cancer)	111
ClinVar (NCBI)	105
The TP53 Database (National Cancer Institute) [previously WHO IARC TP53 Database]	65
OncoKB (Memorial Sloan Kettering Cancer Center *et al*.)	43
OMIM (NCBI)	36
My Cancer Genome (Vanderbilt-Ingram Cancer Center)	32
Seshat (TP53) Database	32
CIViC (Clinical Interpretation of Variants in Cancer)	25
The Clinical Knowledgebase (CKB) Jackson Laboratory (Boost)	23
HGMD (The Human Gene Mutation Database)	23
cBioPortal (Memorial Sloan Kettering Cancer Center *et al*.)	22
The Cancer Genome Atlas (TCGA)	20
UMD (TP53) Database	16

As stated by ≥3 participants.

Large population dataset/resources routinely consulted

	Returns
gnomAD (Genome Aggregation Database)	99
dbSNP (Short Genetic Variations, NCBI)	84
1000 Genomes	53
ESP (Exome Sequencing Project, NHLBI GO)	32

As stated by ≥3 participants.

Aggregation tool(s) utilised to access annotation resources

	Returns
Varsome (SAPHETOR)	46
Alamut (SOPHiA GENETICS)	42
Franklin (GENOOX)	22

As stated by ≥3 participants.

Trial Comments

Methodology

- All returning participants (with the relevant information provided in their Laboratory Record) described the application of a DNA based targeted gene panel NGS testing approach (n=114). At least 15 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 average number of genes currently analysed by laboratories on a given panel is 45 with a range of 8 – 186 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.
- Overall, 81.4% (n=96) of returning participants (providing the relevant information) employed bridge amplified reversible dye terminator-based platforms from Illumina to analyse sample Myeloid GP 116. The remaining laboratories stated the use of Thermofisher Scientific Ion Torrent (n=21) or Element Bioscience technology (n=1).
- The most utilised 'off the shelf' commercially available panel kits included the Ion Torrent Oncomine Myeloid Research Panel (n=18), Sophia Genetics Myeloid Solution (n=17), Illumina TruSight Myeloid Sequencing Panel (n=10) and Archer VariantPlex Myeloid Panel (n=6).
- The proportion of returning participants (providing the relevant information) working to the GRCh38 human genome assembly was 14.0% (n=16), all Illumina platforms users.

Annotation and interpretation

- COSMIC (n=111), ClinVar (n=105) and gnomAD (n=99) remain the annotation resources most widely employed by participants. Please refer to the tables on page 8 for further information.
- Growth in the use of Franklin (GENOOX) (n=22) continues. Along with other established aggregation tools including Varsome (SAPHETOR) (n=46) and Alamut (SOPHiA GENETICS) (n=42), 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.
- Regarding assay performance, 60.5% (n=69) and 55.3% (n=63) 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, 38.6% (n=44) of participants quoted a minimum threshold below 5%. For an SNV the thresholds were set lower with 44.7% (n=51) laboratories applying a minimum VAF below 5%.

Sample Myeloid GP 116

Of the 124 participants returning results for this trial, 67 (54.0%) submitted at least one reportable sequence variant in sample Myeloid GP 116 (please refer to the summary table on page 3 for details).

A detailed breakdown of nomenclature and educational aspects relating to variant interpretation will be subsequently published in a Myeloid Gene Panels 232401 report addendum to form the finalised trial report. Participants will be notified by email as soon as the final trial report is available. Please accept our apologies for any inconvenience caused.

Please note the recent release of v21.0.2 of the HGVS Nomenclature (dated 15 Mar 2024)[5]. 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 and utilise transcript reference sequences designated by the MANE collaboration[2].

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.

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.

Each financial year one Myeloid Gene Panels (Pilot – Not Accredited) trial 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. 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.

References

1. Li, MM *et al.* Standards and guidelines for the interpretation and reporting of sequence variants in cancer. *J Mol Diagn.* 19(1):4-23 (2017).

2. Morales, J *et al.* A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* 604:310–315 (2022).

3. Human Genome Variation Society (HGVS), https://varnomen.hgvs.org/ (v20.05) – effective during the live trial period and accessed for analysis of trial Myeloid GP 232401.

4. Dunnen, JT *et al.* HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat*. 37(6):564-9 (2016).

5. Human Genome Variation Society (HGVS), https://hgvs-nomenclature.org/stable/ (v21.0.2) – accessed March 2024.

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:
UK NEQAS for Leucocyte Immunophenotyping
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e-mail: amanda.newbould@ukneqasli.co.uk

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/

4.8.4) The UK NEQAS LI Policy for the Use of Reports by Individuals and Organisations states that all EQA reports are subject to copyright, and, as such, permission must be sought from UK NEQAS LI for the use of any data and/or reports in any media prior to use. See associated policy on the UK NEQAS LI website: http://www.ukneqasli.co.uk/eqa-pt-programmes/new-participant-information/