UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Pilot Lymphoid Gene Panels (Not Accredited)

Distribution – Lymphoid GP 242502

Participant –

Date Issued – 24 March 2025

Closing Date – 09 May 2025

Trial comments

This trial was issued to 57 participants, of which 51 (89.5%) returned results. Of the non returns, two participants informed us of their intended non return.

We encourage laboratories to test all samples issued as part of the Lymphoid Gene Panels programme, even if the referral reason is suggestive of a lymphoid neoplasm that would not routinely be tested within the laboratory repertoire. Whilst a referral reason may provide information on the potential lymphoid neoplasm, testing of all EQA sample distributions enables assessment of laboratory Next Generation Sequencing (NGS) panels. There are likely to be samples issued where variants in genes overlap with multiple lymphoid neoplasms, providing insight into the performance of laboratory NGS panels. Furthermore, this programme remains in pilot phase and is still developing and as such, is not currently performance monitored.

This trial report focuses on summarising the variants detected by participants, variant nomenclature provided by participants and educational elements relating to variant biological classification and clinical interpretation.

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

Sample comments

One lyophilised sample (Lymphoid GP 110) was prepared and distributed by UK NEQAS LI. Sample Lymphoid GP 110 was manufactured from the peripheral blood of an adult patient with a working diagnosis of CLL.

Your Laboratory Record status for this trial:	**As submitted for trial Lymphoid GP 232402 with minor amendments requested and applied at Lymphoid GP 242501**

IMPORTANT: To permit meaningful trial data analysis it is essential the information held in your Lymphoid Gene Panels 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 Lymphoid GP 110

Did you detect a reportable DNA sequence change in Sample Lymphoid GP 110: Yes

Your Results – Variant(s) of strong clinical significance

Gene	Your DNA sequence variant detected	Your protein variant
ATM	c.8565_8566delinsAA	p.(Ser2855_Val2856delinsArgIle)

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
CCND3	c.774_775delinsTG	p.(Ser259Ala)

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

Please note, in the interests of clarity we will only summarise variants reported by ≥10 participants.

Gene	n#	Variant classification^			Variant detected (consensus)*		Median VAF (%) (IQR)+
		Strong clinical significance	Potential clinical significance	Unknown clinical significance	DNA sequence description	Protein level description	
ATM	31/35	20	8	3	NM_000051.4: c.8565_8566delinsAA	p.(Ser2855_Val2856delinsArgIle)	48.6 (4.0)
TNFAIP3	23/25	8	13	2	NM_001270508.2:c.359T>A	p.(Leu120*)	23 (2.2)
TNFAIP3	15/25	3	9	3	NM_001270508.2:c.912dup	p.(Glu305Argfs*28)	8.3 (1.6)
TNFAIP3	6/25	1	4	1	NM_001270508.2: c.561_570del	p.(Gln187Hisfs*26)	2.9 (1.1)
IRF4	5/21	0	0	5	NM_002460.4:c.623C>A	p.(Pro208Gln)	48.0 (10.1)

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, participant(s) may also have reported a consensus variant from a gene not stated as included on their panel.
^ 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]. Variant classification by participants utilising alternative systems may have been aligned (where possible) to the equivalent Li *et al* category (if available/applicable).
* 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 (http://varnomen.hgvs.org/)[2] during the compilation of 'All Participants' results table. Protein nomenclature includes parenthesis as it represents a prediction from analysis at the DNA level. Please contact UKNEQAS LI for reference sequence information.
+ Descriptive statistics calculated for any variant with >10 quantification data points. Percentage values quoted have been subjected to rounding up/down to 1 dp. IQR = Interquartile range.

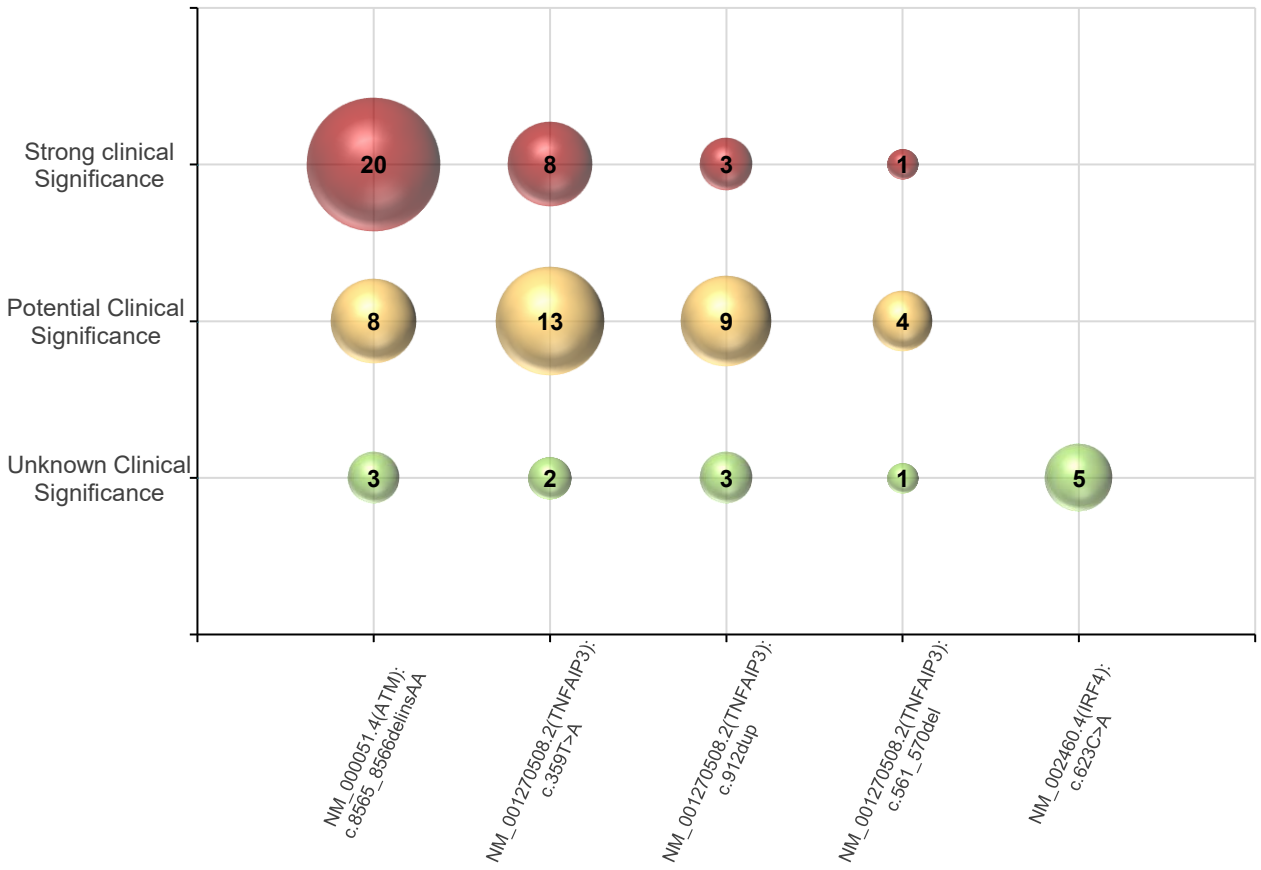

Figure 1: Bubble plot depicting the variant classification for the most frequently identified variants in sample Lymphoid GP 110. The size of the bubble relates to the proportion of participants providing a specific classification[1], with the total number of participants also provided.

Your performance

Performance	Performance Status for this sample	Performance Status Classification Over 12 Month 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 performance monitoring 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.

Methodological approach

	Returns
Targeted Gene Panel (DNA seq)	46
Targeted Gene Panel (DNA with RNA fusion transcript seq)	2

NGS platform used

	Returns
Illumina NextSeq	17
Illumina MiSeq	16
Illumina NovaSeq	6
ThermoFisher Scientific (Life Tech) Ion S5 XL	5
ThermoFisher Scientific Ion Torrent Genexus system	3
Illumina NovaSeq X	1
Illumina MiniSeq	1
Illumina NextSeq 2000	1
Element Biosciences Aviti system	1
MGI Tech DNBSEQ-G400RS	1

NGS panel description

	Returns
Qiagen QIASeq Custom Panel	9
AmpliSeq for Illumina Custom Panel	6
ThermoFisher Scientific Ion AmpliSeq Custom Panel	4
Roche Sequencing KAPA HyperCap/HyperChoice Custom Panel	3
SOPHiA Genetics DDM CLL Panel	3
Agilent SureSelect Custom QXT Panel	3
In-house (capture based)	3
IDT xGen Custom Panel	2
AmpliSeq for Illumina Myeloid Panel	2
Agilent SureSelect XT HS2 Custom Panel	2
Twist Bioscience Custom Panel	2
Agilent HaloPlex HS Custom Panel	1
VariantPlex Core Myeloid	1
In-house Illumina Custom Enrichment Panel	1
Nonacus Cell 3 Custom Pan-Haem	1
ThermoFisher Scientific Lymphoma Core DNA Panel	1
Fluidigm (Standard BioTools) Custom Panel	1
Agilent Custom Myeloid Panel	1
In-house (amplicon based)	1
Univ8 Genomics Euroclonality NDC Panel	1

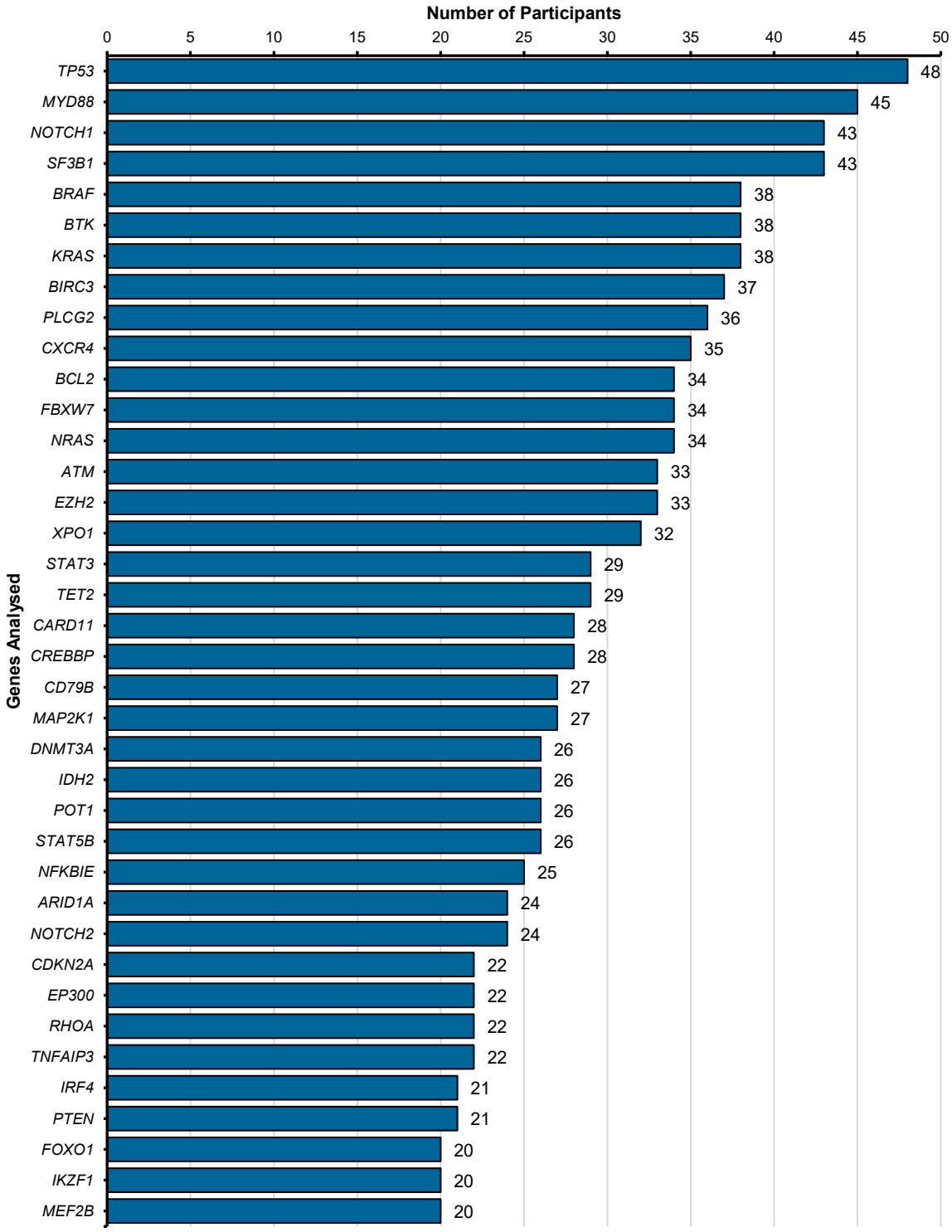

Figure 2: Histogram depicting the genes present on participant NGS panels. Only genes routinely tested by ≥20 participants are recorded in the histogram. Data is derived from participant submissions only. Total numbers of participants may differ from the total numbers outlined in the 'All Participant Results' table (page 4) because where panel content information was not provided, inclusion of a gene was inferred when a variant in that gene was reported.

Annotation database resources

	Returns
COSMIC (Catalogue Of Somatic Mutations In Cancer)	46
ClinVar (NCBI)	46
The TP53 Database hosted by NCI (previously IARC TP53 database)	34
The Genome Aggregation Database (gnomAD)	30
dbSNP (Short Genetic Variations, NCBI)	25
OncoKB (Memorial Sloan Kettering Cancer Center)	20
The Clinical Knowledgebase (CKB) Jackson Laboratory	19
Seshat TP53 database	18
cBioPortal (Memorial Sloan Kettering Cancer Center *et al*.)	16
My Cancer Genome (Vanderbilt-Ingram Cancer Center)	14
OMIM (NCBI)	14
HGMD (The Human Gene Mutation Database)	9
The Cancer Genome Atlas (TCGA)	8
Franklin by Genoox	8
Alamut (SOPHiA GENETICS)	2

As stated by ≥2 participants.

Large-scale sequencing project dataset(s) routinely consulted during variant interpretation

	Returns
The Genome Aggregation Database (gnomAD)	43
dbSNP (Short Genetic Variations, NCBI)	18
1000 Genomes	15
NHLBI-GO Exome Sequencing Project (ESP)	9

Published guideline(s) and/or recommendation(s) referenced to inform classification of somatic variant clinical significance/pathogenicity (in a Haemato-Oncology context)

	Returns
Li, M.M. *et al.* Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. *J Mol Diagn.* 2017; **19**(1):4-23.	34
Horak, P. *et al*. Standards for classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC). *Genet Med*. 2022; **24**(5):986-998.	16
Froyen, G. *et al.* Standardization of Somatic Variant Classifications in Solid and Haematological Tumours by a Two-Level Approach of Biological and Clinical Classes: An Initiative of the Belgian ComPerMed Expert Panel. *Cancers (Basel)*. 2019; **11**(12): 2030.	10
Koeppel, F. *et al*. Standardisation of pathogenicity classification for somatic alterations in solid tumours and haematological malignancies. *Eur J Cancer*. 2021; **159**:1-15.	7
Sukari, M.A. *et al.* A classification system for clinical relevance of somatic variants identified in molecular profiling of cancer. *Genet Med.* 2016; **18**(2):128-136.	4

As stated by ≥2 participants.

Genome Assembly

	Returns
GRCh37/hg19	35
GRCh38	13

Minimum variant allele frequency (VAF) for reporting the identification of a single nucleotide variant

	Returns
5%	26
4%	5
3%	6
1-2%	11

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

	Returns
7%	1
5%	28
4%	5
3%	5
1-2%	9

Trial Comments

Methodology

- The vast majority of participants employed bridge amplified reversible dye terminator-based platforms from Illumina (n=40 data returns, 78.4%). The semiconductor-based platforms from Thermo Fisher Scientific were the next most common methodology (n=7, 13.7%).
- Three participants utilised a myeloid based panel in this trial distribution.
- Of the 48 laboratories providing information regarding genome assembly, 35 participants referenced GRCh37/hg19, with 13 participants referenced the GRCh38/hg38 genome-based assembly. At the time of reporting, GRCh38.p14 (equivalent to the UCSC hg38) is the latest human genome release (26th August 2024) from NCBI Genome Data Viewer (https://www.ncbi.nlm.nih.gov/genome/gdv/).
- The minimum Variant Allele Frequency (VAF) quoted for reporting single nucleotide variants ranged from 1-5%, with a median of 5%. For indel (insertion/duplication and deletion) variants minimum VAF quoted for reporting ranged from 1-7%, with a median of 5%
- Forty-eight out of 51 participants returning results provided information relating to the number of genes on the NGS panel. A total of 263 different genes were present on participant NGS panels. The median number of genes tested on a given panel by laboratories for sample Lymphoid GP 110 was 40 (range 4-137).

Sample Lymphoid GP 110

Thirty-nine (76.5%) out of 51 participants returning results for this trial indicated the detection of at least one DNA sequence variant in sample Lymphoid GP 110. A summary of the most frequently reported variants (five variants across three genes) has been summarised in the 'All Participant results' table on page 3.

Of the 12 participants that did not detect a variant in sample Lymphoid GP 110, nine (75.0%) did not include *ATM*, *TNFAIP3* or *IRF4* on their NGS panel, in the context of lymphoid neoplasms. One participant did not provide information relating to the NGS panel utilised within the laboratory. A further participant reported the inclusion of *IRF4* and *TNFAIP3* on their NGS assay panel, however, these genes were not included in the bioinformatic analysis for sample Lymphoid GP 110 given the clinical scenario provided. One participant reported the inclusion of *ATM* (full coding region), *TNFAIP3* (full coding region) and *IRF4* (gene coverage region information not provided) on their NGS assay panel. The laboratory did not report any coverage or internal quality control (QC) issues within these genes.

For clarity, variant classifications in this dataset have been aligned to Li *et al.,* (2017) joint consensus recommendations from the Association for Molecular Pathology, American Society of Clinical Oncology and College of American Pathologists[1] (where possible). This classification system utilises a tier system from I-IV, ranging from variants of strong, potential, or unknown clinical significance and includes benign/likely benign variants. **Please note for the purposes of this EQA programme, we only require the reporting of variants of strong, potential, or unknown clinical significance. Variants considered benign or likely benign do not need to be reported.**

Thirty-one returning participants reported detection of the NM_000051.4(*ATM*):c.8565_8566delinsAA p.(Ser2855_Val2856delinsArgIle) missense variant in exon 58 of the gene. Of the 31 participants reporting the variant, 20 (64.5%) classified the variant as of strong clinical significance, eight classified the variant as potential clinical significance (25.8%) and three (9.7%) participants classified the variant as having unknown clinical significance.

- The median variant allele frequency (VAF) reported for the variant was 48.6% with an interquartile range of 4.0% and a median read depth of 1,504x coverage.
- The variant has been reported in dbSNP (rs587781353)[3], however, this variant is absent from the COSMIC[4] database.
- Furthermore, the variant is present in ClinVar[5] (VCV000140897.62), reported in a germline capacity in association with *ATM*-cancer predisposition syndromes, (familial) breast cancer and ataxia telangiectasia.
- In addition to the 31 laboratories reporting the c.8565_8566delinsAA variant, two participants reported two individual missense changes, c.8565T>A and c.8566G>A (p.(Ser2855Arg) and p.(Val2856Ile)). HGVS recommendations state that changes involving two or more consecutive nucleotides should be described as deletion/insertion variants and not as separate variants[2].
- For the predicted protein change associated with the *ATM* variant; there was variable use of the HGVS nomenclature, as outlined in the table below.

Protein nomenclature *ATM* variant	n	Comments	
p.(Ser2855_Val2856delinsArgIle)	17	Compliant with HGVS recommendations. Parentheses reflect the analysis of DNA and the predicted status of the protein level description.	
p.(Ser2855_Val2856elinsArgIle)	1	Largely compliant with HGVS recommendations. Small typographical error in describing the 'delins' variant.	
p.Ser2855_Val2856delinsArgIle	4	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected^.	
p.S2855_V2856delinsRI	1	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. Three letter amino acid code is preferred when describing protein changes^.	
p.[Ser2855Arg;Val2856Ile]	3	Changes involving two or more consecutive amino acids should be described as 'delins' variants and not individually as separate variants.	
p.2855_2856delinsArgIle	1	Protein coordinates are always prefixed with the reference amino acid at that position. 'Ser' should be the prefix to position 2855 and 'Val' the prefix to position 2856.	
p.(SerVal2855ArgIle)	1		
p.(Ser2855_Val2856)	1	The protein coordinates are prefixed with the correct reference amino acids for the given positions. However, the description fails to effectively communicate the predicted changes of the protein product ('delins' is missing, along with the amino acid sequence inserted).	
p.Ser2855_Val2856delins	1	The protein coordinates are prefixed with the correct reference amino acids for the given positions. The description fails to effectively communicate the predicted changes of the protein product, with the amino acid sequence inserted not specified.	
p.(Ser2855delinsArgIle)	1	Positional error. The 'delins' variant affects Ser2855 and Val2856 (Ser2855_Val2856).	

^ Please note that if RNA or cDNA was the source material for sequencing parentheses are not required.

Colour coding reflects the level of compliance with current HGVS recommendations (v21.1.3): **green** = fully compliant **amber** = generally compliant with some omission(s)/minor issues and **red** = nomenclature error(s)/ fails to comply with the recommendations/ positional errors.

Please refer to the Human Genome Variation Society (HGVS) recommendations for detailed guidance regarding variant nomenclature http://varnomen.hgvs.org/

Of the remaining two participants that did not report the *ATM* variant in sample Lymphoid GP 110, one participant utilised a Qiagen QIASeq Custom Panel and sequenced exons 17, 40 and 63 of the *ATM* gene. The NM_000051.4(*ATM*):c.8565_8566delinsAA variant reported in sample Lymphoid GP 110 is located in exon 58, thus is outside the region of interest (ROI) of their assay.

The final participant has previously been discussed as one of the laboratories that did not report a genetic variant in sample Lymphoid GP 110. This participant utilised a Qiagen QIASeq Custom Panel (for the Illumina NextSeq). The participant stated that the custom panel targeted 54 genes in the clinical context of lymphoid neoplasms, including *ATM*. The participant indicated that all coding regions of *ATM* were assayed (reference sequence: NM_000051.4). The participant did not declare any coverage issues for *ATM* in sample Lymphoid GP 110.

The *ATM* gene, located on the long arm of chromosome 11 (11q22-23), encodes a serine/threonine kinase, ataxia telangiectasia-mutated protein involved in DNA repair and cell cycle control. One of the most common chromosome aberrations identified in the CLL is deletion of 11q, present in 10-20% patients presenting at first diagnosis, encompassing the *ATM* gene[6].

In addition, *ATM* is one of the most frequently mutated genes found in CLL, with variants identified in 10-15% patients prior to first treatment. *ATM* variants associated with CLL frequently affect the PI3-Kinase (PI3K) domain[7], with the NM_000051.4(*ATM*):c.8565_8566delinsAA p.(Ser2855_Val2856delinsArgIle) variant identified in sample Lymphoid GP 110 located in the PI3K domain. The PI3K domain is a catalytic domain that, when ATM is active, phosphorylates serine/threonine residues in downstream protein targets involved in DNA damage repair, apoptosis and cell cycle checkpoint control[8].

Previous analysis assessing the impact of *ATM* variants have been evaluated when considering the co-occurrence of variants with del(11q). Analysis from the UK LRF CLL4 trial showed that CLL patients with co-occurrence of del(11q) and *ATM* showed a reduction in median overall survival (OS) compared to patients without *ATM* variants (42 vs. 91 months) and also lower median progression-free survival (PFS) (10 vs. 46 months)[9].

In contrast, multivariate analysis evaluating the impact of genetic variants in several genes frequently mutated in CLL (*TP53*, *ATM*, *BIRC3*, *MYD88*, *FBXW7*, *POT1*, *SF3B1* and *NOTCH1*) alongside other independent prognostic factors, including treatment, del(11q), del(17p) and IGHV variant status showed that *ATM* mutation does not significantly shorten progression-free survival (PFS) or overall survival (OS) and whilst *ATM* variants co-occur with del(11q), they do not add additional prognostic value to the impact of del(11q) on PFS and OS[10].

A recent multi-centre study assessed the impact and prognostic significance of somatic *ATM* mutations in 3631 untreated CLL patients on the time to first treatment (TTFT). *ATM* mutations were identified in 246 (6.8%) patients, with 112/246 (45.5%) frequently co-occurring with del(11q) aberrations and 56/246 (22.8%) co-occurring with *SF3B1* gene variants. Isolated *ATM* mutations were rarely reported, occurring in 1.3% of Binet A cases and in 0.7% IGHV-mutated CLL[11].

Univariate analysis suggested that the presence of *ATM* variants in patients with Binet stage A disease was associated with a shorter TTFT when compared to patients who tested negative for *ATM* variants (wildtype-*ATM*)[11]. However, the prognostic impact of recurrent gene variants in CLL can be influenced by IGHV variant status and the co-occurrence of other gene variants. Multivariate analysis only identified del(11q) as a predictor of overall survival. The investigators note that caution must be applied to this finding due to the variability in treatment strategies amongst patients across the multi-centre analysis[11].

It is worth noting that *ATM* gene variants are not listed as one of the most relevant prognostic and predictive markers in CLL in the 5th edition of WHO Classification of Haematolymphoid tumours[6].

For this trial laboratories identified three consensus variants in the *TNFAIP3* gene. Overall, 92.0% of returning participants reported at least one of the *TNFAIP3* variants (n=23 / 25).

Of the two participants that did not report any *TNFAIP3* variants (or any other gene variants) in sample Lymphoid GP 110, one utilised a Qiagen QIASeq Custom Panel (for the Illumina NextSeq). The participant stated that the custom panel targeted 54 genes in the clinical context of lymphoid neoplasms, including *TNFAIP3*. The participant indicated that all coding regions of *TNFAIP3* were assayed (reference sequence: NM_001270508.2). The participant did not declare any coverage issues for *TNFAIP3* in sample Lymphoid GP 110. The remaining participant reported the inclusion of *TNFAIP3* on their NGS assay panel; however, this gene was not included in the bioinformatic analysis for sample Lymphoid GP 110 given the clinical scenario provided. This participant has previously been discussed as one of the laboratories who did not report a genetic variant in sample Lymphoid GP 110.

A total of 23 returning participants identified the NM_001270508.2(*TNFAIP3*):c. c.359T>A p.(Leu120*) variant in exon 3 of the gene. Of the 23 participants reporting the variant, 13 participants classified the variant as having potential clinical significance (56.5%), eight (34.8%) classified the variant as strong clinical significance and two classified the variant as potential clinical significance (8.7%).

- The median VAF reported for this variant was 23.0% with an interquartile range of 2.2% and a median read depth of 1,169x coverage (n=23).
- The variant is not documented in dbSNP[3] or COSMIC database[4]. However, variants at this nucleotide position (c.359T>G, COSV52800949 and c.359T>C, COSV99397433) have been reported in association with colon adenocarcinoma, caecum adenocarcinoma and MALT lymphoma.
- Nomenclature was in good agreement at both the cDNA and protein level for this frameshift variant. Sixteen (69.6%) participants provided fully compliant HGVS nomenclature at the protein level, as outlined below.

Protein nomenclature *TNFAIP3* variant	n	Comments	
p.(Leu120Ter)	8	Compliant with HGVS recommendations. Parentheses reflect the analysis of DNA and the predicted status of the protein level description^. * or Ter are equally acceptable to indicate a termination/STOP codon.	
p.(Leu120*)	8		
p.Leu120Ter	4	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected^.	
p.L120*	1	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected^. The three-letter amino acid code is preferred when describing protein changes.	
p.L120	1	The description fails to effectively communicate the predicted changes of the protein product. The defined HGVS format states that the protein coordinates (prefixed with the reference amino acid at that position) should be noted, with the alternate new base then described.	
p.(Leu210Ter)	1	Incorrect positional information.	

^ Please note that if RNA or cDNA was the source material for sequencing parentheses are not required.

Colour coding reflects the level of compliance with current HGVS recommendations (v21.1.3): **green** = fully compliant **amber** = generally compliant with some minor issues and **red** = fails to comply with the recommendations.
Please refer to the Human Genome Variation Society (HGVS) recommendations for detailed guidance regarding variant nomenclature http://varnomen.hgvs.org/

The second *TNFAIP3* consensus variant, NM_001270508.2(*TNFAIP3*):c.912dup p.(Glu305Argfs*28) in exon 6 of the gene, was reported by 15 centres. Classification of the variant was divided between potential (n=9, 60.0%), strong (n=3, 20.0%) and unknown (n=3, 20%) clinical significance.

- The median VAF reported for this variant was 8.3% with an interquartile range of 1.6% and a median read depth of 693x coverage (n=15).
- The variant has not been reported in dbSNP[3] or COSMIC[4] however, a frameshift variant affecting c.911_912del is listed in association with diffuse large B-cell lymphoma (COSV105103527).
- Nomenclature was in good agreement at both the cDNA and protein level for this frameshift variant. Twelve (80.0%) participants provided fully compliant HGVS nomenclature at the protein level, as outlined below.

Protein nomenclature *TNFAIP3* variant	n	Comments	
p.(Glu305Argfs*28)	7	Compliant with HGVS recommendations. Parentheses reflect the analysis of DNA and the predicted status of the protein level description^. * or Ter are equally acceptable to indicate a termination/STOP codon. Similarly, the short description of a frameshift variant is compliant.	
(Glu305ArgfsTer28)	4		
p.(Glu305fs)	1		
p.E305Rfs*28	2	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected^. The three letter amino acid code is preferred when describing protein changes.	
p.Glu305ArgfsTer28	1	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected^.	

^ Please note that if RNA or cDNA was the source material for sequencing parentheses are not required.

Colour coding reflects the level of compliance with current HGVS recommendations (v21.1.3): **green** = fully compliant **amber** = generally compliant with some minor issues.
Please refer to the Human Genome Variation Society (HGVS) recommendations for detailed guidance regarding variant nomenclature http://varnomen.hgvs.org/

The third *TNFAIP3* consensus variant, NM_001270508.2(*TNFAIP3*):c.561_570del p.(Gln187Hisfs*26) in exon 4 of the gene, was reported by six centres. Classification of the variant was divided between potential (n=4, 66.7%), strong (n=1, 16.7%) and unknown (n=1, 16.7%) clinical significance.

- The median VAF reported for this variant was 2.9% with an interquartile range of 1.1% and a median read depth of 1,595x coverage (n=6).
- The variant has not been reported in dbSNP[3] or COSMIC[4] and is absent from the gnomAD database.
- Of the six laboratories identifying this low-level variant, four stated the minimum VAF for reporting the identification of an indel (deletion/insertion/duplication) variant was ≤ 2%. The remaining participants who reported the variant states a minimum VAF for reporting of 4 and 5%.
- Of the 19 participants who did not report the detection of the NM_001270508.2(*TNFAIP3*):c.561_570del, 18 (94.7%) stated the minimum VAF for reporting the identification of an indel (deletion/insertion/duplication) variant was ≥3%, above the median VAF for the variant identified in sample Lymphoid GP 110. The remaining participant reported a minimum VAF of 2% and reported no coverage issues across *TNFAIP3*.
- No nomenclature symbol or positional errors were noted for this single base pair substitution at either DNA or protein levels, with only one minor non-compliance in the application of HGVS noted (see tabulated breakdown of protein HGVS provided below).

Protein nomenclature *TNFAIP3* variant	n	Comments	
p.(Gln187Hisfs*26)	2	Compliant with HGVS recommendations. Parentheses reflect the analysis of DNA and the predicted status of the protein level description^. * or Ter are equally acceptable to indicate a termination/STOP codon. Similarly, the short description of a frameshift variant is compliant.	
p.(Gln187HisfsTer26)	2		
p.(Gln187fs)	1		
p.Q187Hfs*26	1	Mostly compliant with HGVS recommendations; however, parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected^. The three-letter amino acid code is preferred when describing protein changes.	

^ Please note that if RNA or cDNA was the source material for sequencing parentheses are not required.

Colour coding reflects the level of compliance with current HGVS recommendations (v21.1.3): **green** = fully compliant **amber** = generally compliant with some minor issues.
Please refer to the Human Genome Variation Society (HGVS) recommendations for detailed guidance regarding variant nomenclature http://varnomen.hgvs.org/

Five returning participants reported detection of the NM_002460.4(*IRF4*):c.623C>A p.(Pro208Gln) variant in exon 5 of the gene. Of the five participants reporting this missense variant, all classified the variant as of unknown clinical significance.

- The median variant allele frequency (VAF) reported for the variant was 48.0% with an interquartile range of 10.1% and a median read depth of 2,268x coverage (n=5).
- The variant has been reported in dbSNP (rs757910134)[3] and has been reported 213 times in gnomAD (v4.1.0) across global exome and genome analysis[12]. Of these, 207 were reported in a European (non-Finnish) population, two in an African/African American population, one in an Admixed American population and three entries listed as 'remaining' where the individuals cannot be listed in a given ancestry based on the lack of information available.
- The variant is not listed in the COSMIC database, however, a variant affecting the same amino acid position (c.622C>T, p.(Pro208Ser)) (COSV101110762)[4] is listed one time, in association with adenocarcinoma. Furthermore, the variant is present in ClinVar[5] (VCV001405517.8).
- No nomenclature symbol or positional errors were noted for this single base pair substitution at either DNA or protein levels, with only minor non compliances in application of HGVS noted.
    - One participant provided protein nomenclature information without parentheses, despite utilising targeted gene panel DNA sequencing.
    - One participant utilised the single amino acid code without parentheses for describing the predicted protein, when the three-letter amino acid code is preferred when describing protein changes.

Final Comments

There was a general observation in relation to the reporting of HGVS nomenclature, in particular, protein descriptions. When reporting predicted protein changes, HGVS recommendations indicate that when DNA is utilised as input material, parentheses are required as any protein change is only predicted based on the DNA variant detected. Furthermore, the three-letter amino acid code is preferred when describing protein changes.

When providing the reference sequence utilised during analysis, it is important to ensure that a sequence identifier must only identify one reference sequence[2]. HGVS recommendations state that version numbers are required to distinguish between sequences. Only reference sequences with version numbers are suitable for defining and describing a sequence variant within a given gene. Furthermore, to better standardise variant description and facilitate clinical reporting, the HGVS advocate use of the transcript reference sequence(s) specified by the MANE Select collaboration project[13].

Poorly curated variant nomenclature and use of incomplete or alternative reference sequence information impedes the ability of a laboratory to effectively search the relevant published data sets and literature during the variant classification process and thus, has the potential to impact a patient's diagnosis, prognostication and/or treatment. **We strongly encourage laboratories to verify the nomenclature generated by automated software systems/pipelines, as it may not fully comply with the current HGVS recommendations.**

We would like to thank participants for their continued engagement with the Lymphoid Gene Panels programme, particularly when considering the complexity of the data returns. The creation of the participant laboratory record means that methodology, panel content and coverage will be held on record for future trial distributions. These will be stored within the Participant Hub online. At each trial distribution, participants will be invited to make any necessary changes for each trial if required.

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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 coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (acting Director).

4.8.2 c) Person(s) authorising this report: Mr Stuart Scott (acting Director) of UK NEQAS LI.

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited.

4.8.2 d) 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; no other activities in relation to this EQA exercise were externally provided.

4.8.2 d) 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.

4.8.2 g) 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 is informed 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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