UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 232403

Participant ID - 43347

Date Issued - 19 March 2024

Closing Date - 19 April 2024

Trial Comments

This trial was issued to 167 participants, of which 161 (96.4%) returned results. Of the non returns, two participant pre-notified us of their intended non return and two participants requested an extension to results submission.

Sample Comments

Two vials of cell line based lyophilised samples were manufactured and issued by UK NEQAS LI (sample references NPM1 173 and NPM1 174). Sample NPM1 173 was manufactured to be positive for a NPM1 Type A duplication, with sample NPM1 174 formulated to be negative. In addition, an educational sample (NPM1 Edu G) was issued for exon 11 variant analysis. This sample was whole genome amplified DNA, derived from patient material and was positive for a NPM1 Type H (Suzuki et al., Blood 2005) c.863_864insCTTG p.(Trp288Cysfs*12) insertion.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 173	Mutation Detected	Mutation Detected
Sample NPM1 174	No Mutation Detected	No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 173	158	2
Sample NPM1 174	2	158

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	Satisfactory	3	0

N/A = Not Applicable

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Template

	Returns
DNA	120
cDNA	41

PCR Type

	Returns
Single PCR	84
Real-Time PCR	36
Multiplex PCR	24
Sequencing	10
Melting Curve Analysis	6
Single PCR with Clamping	1

Protocol Type

	Returns
In-house Assay	125
Qiagen NPM1 Mutascreen Kit	14
Qiagen NPM1 mut A, B & D MutaQuant Kits	8
Ion Torrent Oncomine Myeloid Panel	5
Oncomine Myeloid Research Assay	3
Cepheid Xpert NPM1 Mutation Assay	2
Illumina TruSight Myeloid Sequencing Panel	2
Myeloid Solution by Sophia Genetics	1
Qiagen NPM1 mut A MutaQuant Kits	1

Analysis Type

	Returns
Capillary Electrophoresis	83
Real-Time PCR Fluorescent Detection	36
NGS (ThermoFisher Ion Torrent)	8
Sanger Sequencing	8
NGS (Illumina)	7
High Resolution Melt	6
Agarose Gel Electrophoresis	3
Digital PCR (Biorad)	3
Next Generation Sequencing (Miseq)	3
Illumina NextSeq 2000	1
Illumina NextSeq 500	1
Illumina NextSeq 550	1
Pyrosequencing	1

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Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6):1103-1108	28
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	21
Falini B. et al (2005) N Engl J Med, 352(3):254-266	17
Gale R. et al (2008) Blood, 111(5):2776-2784	13
Falini B. et al (2007) Blood, 109(3):874-885	11
In-house method (no published reference available)	11
Döhner K. et al (2005) Blood, 106(12):3740-3746	8
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	8
Schnittger S. et al (2005) Blood, 106(12):3733-3739	8
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	7
Thiede C. et al (2006) Blood, 107(10):4011-4020	6
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	6
Boissel N. et al (2005) Blood, 106(10):3618-3620	5
Tan AY. et al (2008) J Haemtol Oncol, 1, 10	4
Belgian Molecular Diagnostic Group	3
Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241	3
Falini B. et al (2006) Blood 108(6):1999-2005	2
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	2
Verhaak RG. et al (2005) Blood, 106(12):3747-3754	2

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Trial Comments Participant ID: 43347

Sample NPM1 173

- In line with sample formulation, 158 of 160 (98.8%) participants returning results identified an *NPM1* variant in samples NPM1 173.
- Of the two participants reporting a false negative for NPM1 173, both utilised an in-house assay, one with capillary electrophoretic analysis and with Sanger sequencing.
- One hundred and eleven participants returned information relating to the type of *NPM1* variant detected. In line with sample formulation, 86 (77.5%) identified a change consistent with the Type A[1] duplication of a TCTG tetranucleotide in exon 11 of the *NPM1* gene (approved HGVS nomenclature NM_002520.7(NPM1):c.860_863dup, systematic exon numbering of the *NPM1* transcript applied). Of these, two participants reported an alternative description of c.863_864insTCTG. HGVS recommendations state that variants should be described as a duplication when a copy of one or more nucleotides are inserted directly 3' of the original nucleotides, when compared to the reference sequence[2]. Furthermore, listing the duplicated nucleotide sequence is not endorsed as this creates a longer description with redundant information.
- A further 19 laboratories (17.1%) reported a 4 bp duplication / insertion but did not specify further details. One participant (0.9%) reported an insertion but did not specify the size of the insertion and two (1.8%) provided protein nomenclature only. One laboratory (0.9%) incorrectly detected *NPM1* type A and D variants and one (0.9%) reportedly detected a *NPM1* type D variant. One participant reported a c.964_965insTCTG variant using the NM_002520.7 reference sequence. This is an invalid cDNA position within the NM_002520.7 MANE reference sequence.

Sample NPM1 174

- One hundred and fifty-eight (98.8%) returning results for NPM1 174 did not detect an *NPM1* variant.
- Of the two participants reporting a false positive one utilised an in-house assay and one the Qiagen NPM1 mut A, B & D MutaQuant kit. Both reported use of Real-Time PCR fluorescent detection as the analysis method.

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NPM1 Educational Sample Edu G

Sample Information

Sample NPM1 Edu G was issued as whole genome amplified material (WGA) derived from a patient with a NM_002520.7(NPM1):c.863_864insCTTG p.(Trp288Cysfs*12). Results for this sample have not been scored.

In total, 93 participants returned results for the educational DNA sample NPM1 Edu G.

Your Result

Sample	Participant	Your Result
NPM1 Edu G variant detected?	43347	Not Tested

All Participant Results

Sample	Variant Detected	No Variant Detected
NPM1 Edu G	92	1

Your Variant Results

	Your DNA sequence variant description	Your protein variant description
NPM1 Edu G	Not Tested	Not Tested

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- Ninety out of 92 participants (97.8%) indicated that they detected a variant in sample NPM1 Edu G.
- The laboratory reporting a false negative result utilised Next Generation Sequencing (ThermoFisher Scientific Ion Torrent) with the Oncomine Myeloid Research Assay.

Sample Edu G was formulated to contain a Type H[3]/I[4]/J[5]/Pm[6]/DD-1[7]/4[8] variant. This variant is a 4 base pair insertion of a CTTG tetranucleotide in exon 11 of the *NPM1* gene (approved HGVS nomenclature[2] NM_002520.7:c.863_864insCTTG p.(Trp288Cysfs*12), systematic exon numbering of the *NPM1* transcript applied).

- Forty-seven participants reported HGVS nomenclature for the *NPM1* variant detected in sample Edu G, of which, 39 (83.0%) participants reported the variant as c.863_864insCTTG, in line with sample formulation.
- Four (7.5%) participants reported a c.860_863dup (Type A[1] variant). Of these, one reported an alternate variant description of c.863_864insTCTG. HGVS recommendations state that variants should be described as a duplication when a copy of one or more nucleotides are inserted directly 3' of the original nucleotides, when compared to the reference sequence[2]. Furthermore, listing the duplicated nucleotide sequence is not endorsed as this creates a longer description with redundant information[2].
- Two participants (4.3%) reported detection of a c.863_864insTGCT variant, one (2.1%) reported a c.863_864insCCTG and one (2.1%) reported a c.863_864insCAGA.
- When asked to define the variant type (e.g. Type A, B etc.) there were a range of subtypes submitted by the participants. However, within the literature, the c.863_864insCTTG variant has been reported using six different subtype classifications (Type H, I, J, DD-1, Pm, 4)[3-8]. Of the 41 participants reporting variant subtype classifications, 32 (78.0%) reported one of the six subtype classifications. The breakdown of which is shown below:

NPM1 variant type described	Number of participants (%)
Type I	10 (24.4)
Type J	7 (17.1)
Type H	6 (14.6)
Type 4	4 (9.8)
Type Pm	3 (7.3)
Type DD-1	2 (4.9)

- Of the remaining participants, six (14.6%) participants reported a Type A, one (2.4%) a B, one (2.4%) a type D, with the remaining participant reporting a Type G *NPM1* variant.
- The educational sample questionnaire asked participants about the use of HGVS and *NPM1* subtypes in the clinical reporting of *NPM1* insertion/duplication variants. Overall, 68 participants provided this information, with 39 (57.4%) participants stating that they report both the HGVS nomenclature and the *NPM1* subtype within their clinical report. Twenty-one (30.9%) indicated that they report the *NPM1* subtype information only. A further eight (11.8%) participants stated that they report the HGVS nomenclature only.
- Within the literature, more than 50 insertion/duplication variant types have been described within exon 11 of *NPM1*[9]. The most frequently identified variants are Type A,

accounting for 75-80% of all *NPM1* variants in AML, followed by Type B, accounting for 10% and Type D, accounting for 5% of *NPM1* variants[9].

- Given the prevalence and rarity of a large proportion of exon 11 *NPM1* duplication/insertion variants and the assignment of different NPM1 subtypes for a variant sharing the same HGVS nomenclature, the system for classifying *NPM1* variant subtypes appears to lack the harmonisation that reporting of HGVS nomenclature offers in a clinical setting. This is particularly evident when the same genetic variant is assigned different classifications within the literature, as described for the variant in NPM1 Edu G.
- The identification of *NPM1* duplication/insertions as a stable MRD marker in AML increases the importance of ensuring accurate, standardised information is available to enable monitoring of the correct *NPM1* variant type. As such, clinical reporting of HGVS nomenclature only may offer several advantages over the historical *NPM1* molecular subtype classification, promoting consistency among laboratories, enabling accurate monitoring of a variant and increasing standardisation of clinical reporting.
- It is worth noting that there is no evidence to suggest that different *NPM1* variants within exon 11 have different prognosis. The 2022 European LeukaemiaNet guidelines for diagnosis and management of AML in adults[10] state that the presence of mutated *NPM1* in patients has favourable prognosis when there is absence of *FLT3*-ITD variants and intermediate prognosis when *NPM1* variants are present in addition to *FLT3*-ITD variants.

We would like to take this opportunity to thank participants who returned data for NPM1 Educational Sample G.

The persistent presence of the *NPM1* variant(s) in patients with *NPM1*+ AML has shown that this is a stable marker to determine molecular assessment of measurable residual disease (MRD) at specific clinical time points[11]. **For participants interested in EQA for MRD assessment using *NPM1* (and other AML markers), UK NEQAS LI have recently developed a new pilot programme, 'Acute Myeloid Leukaemia Measurable Residual Disease by Molecular Methods'[12]. If participants require further information about this programme, please contact admin@uknegasli.co.uk.**

References

1. Falini, B. *et al.* Cytoplasmic Nucleophosmin in Acute Myelogenous Leukemia with a Normal Karyotype. *N. Engl. J. Med.* **352,** 254–266 (2005).

2. Human Genome Variation Society (HGVS), https://varnomen.hgvs.org/ (v20.05).

3. Suzuki, T. *et al*. Clinical characteristics and prognostic implications of *NPM1* mutations in acute myeloid leukemia. *Blood*. **106**(8), 2854-2861 (2005).

4. Alpermann, T. *et al*. Molecular subtypes of NPM1 mutations have different clinical profiles, specific patterns of accompanying molecular mutations and varying outcomes in intermediate risk acute myeloid leukemia. *Haematologica*. **101**(2): e55-58 (2016).

5. Verhaak, R. G. W. *et al*. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. *Blood*. **106**(12), 3747-3754 (2005).

6. Schnittger, S. *et al*. Nucleophosmin gene mutations are predictors of favourable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood*. **106**(12), 3733-3739 (2005).

7. Thiede, C. *et al*. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood*. **107**(10), 4011-4020 (2006).

8. Döhner, K. *et al*. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood*. **106**(12), 3740-3746 (2005).

9. Hindley, A *et al*. Significance of *NPM1* gene mutations in AML. *Int. J. Mol. Sci*. **22**(18), 10040 (2021).

10. Döhner, H. *et al*. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*. **140**(12), 1345-1377 (2022).

11. Schuurhuis, G.J. *et al.* Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*. **131**(12), 1275-1291 (2018).

12. Scott, S. *et al*. Assessment of acute myeloid leukemia molecular measurable residual disease testing in an interlaboratory study. *Blood Adv*. **7**(14), 3686-3694 (2023).

NPM1 Mutation Status Programme

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 and post closure 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/