UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

# NPM1 Mutation Status Programme

Distribution - 222303

Participant ID -

Date Issued - 06 March 2023

Closing Date - 07 April 2023

## Trial Comments

This trial was issued to 166 participants, of which 159 (95.8%) returned results. Of the non returns, three participants pre-notified us of their intended non return.

## Sample Comments

Two vials of cell line based lyophilised samples were manufactured and issued by UK NEQAS LI (sample references NPM1 167 and NPM1 168). Both trial samples were formulated to be positive for a NPM1 Type A duplication. In addition, an educational sample (NPM1 Edu F) 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 G c.863_864insTTTG p.(Trp288Cysfs*12) insertion.

## Results and Performance

### Your Results

| NPM1 Mutation Status | Your Results | Consensus Result |
|---|---|---|
| Sample NPM1 167 | Mutation Detected | Mutation Detected |
| Sample NPM1 168 | Mutation Detected | Mutation Detected |

### All Participant Results

| | Mutation Detected (Returns) | No Mutation Detected (Returns) |
|---|---|---|
| Sample NPM1 167 | 155 | 4 |
| Sample NPM1 168 | 159 | 0 |

### Your Performance

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

N/A = Not Applicable

# NPM1 Mutation Status Programme

## Template

| | Returns |
|---|---|
| DNA | 122 |
| cDNA | 37 |

## PCR Type

| | Returns |
|---|---|
| Single PCR | 88 |
| Real-Time PCR | 29 |
| Multiplex PCR | 23 |
| Sequencing | 12 |
| Melting Curve Analysis | 6 |

## Protocol Type

| | Returns |
|---|---|
| In-house Assay | 126 |
| Qiagen NPM1 Mutascreen Kit | 15 |
| Qiagen NPM1 mut A, B & D MutaQuant Kits | 6 |
| Ion Torrent Oncomine Myeloid Panel | 4 |
| Oncomine Myeloid Research Assay | 3 |
| Illumina TruSight Myeloid Sequencing Panel | 2 |
| Agilent Custom Haloplex HS panel | 1 |
| Imegen NPM1 Kit | 1 |
| Myeloid Solution by Sophia Genetics | 1 |

## Analysis Type

| | Returns |
|---|---|
| Capillary Electrophoresis | 85 |
| Real-Time PCR Fluorescent Detection | 29 |
| Sanger Sequencing | 11 |
| NGS (ThermoFisher Ion Torrent) | 8 |
| High Resolution Melt | 6 |
| Next Generation Sequencing (Miseq) | 6 |
| Agarose Gel Electrophoresis | 5 |
| NGS (Illumina) | 3 |
| Digital PCR (Biorad) | 2 |
| Illumina NextSeq 2000 | 1 |
| Illumina NextSeq 500 | 1 |
| Illumina NextSeq 550 | 1 |
| Pyrosequencing | 1 |

# NPM1 Mutation Status Programme

## Journal Reference for Assay

| | Returns |
|---|---|
| Gorello P. et al (2006) Leukemia, 20(6):1103-1108 | 26 |
| Falini B. et al (2005) N Engl J Med, 352(3):254-266 | 20 |
| Noguera N. et al (2005) Leukemia, 19(8):1479-1482 | 15 |
| Gale R. et al (2008) Blood, 111(5):2776-2784 | 10 |
| Thiede C. et al (2006) Blood, 107(10):4011-4020 | 10 |
| Schnittger S. et al (2005) Blood, 106(12):3733-3739 | 9 |
| Döhner K. et al (2005) Blood, 106(12):3740-3746 | 8 |
| Falini B. et al (2007) Blood, 109(3):874-885 | 7 |
| In-house method (no published reference available) | 7 |
| Boissel N. et al (2005) Blood, 106(10):3618-3620 | 6 |
| Huang Q. et al (2008) Br J Haematol, 142:(3)489-492 | 6 |
| Belgian Molecular Diagnostic Group | 5 |
| Lin LI. et al (2006) Leukemia, 20(10):1899-1903 | 5 |
| Tan AY. et al (2008) J Haemtol Oncol, 1, 10 | 5 |
| Falini B. et al (2006) Blood 108(6):1999-2005 | 4 |
| Scholl S. et al (2007) Leuk Res, 31(9):1205-1211 | 4 |
| Thiede C. et al (2006) Leukemia, 20(10):1897-1899 | 4 |
| Chou WC. et al (2007) Leukemia, 21(5):998-1004 | 2 |
| Pitiot AS. et al (2007) Leukemia 21(7):1564-1566 | 2 |
| Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241 | 2 |

## NPM1 Mutation Status Programme

**Trial Comments** **Participant ID:**

### Sample NPM1 167

- In line with sample formulation, 155 of 159 (97.5%) participants returning results identified an *NPM1* variant in samples NPM1 167.
- Of the four participants reporting a false negative for NPM1 167, three utilised an in-house assay, one with capillary electrophoretic analysis, one utilised Sanger sequencing and one utilised Next Generation Sequencing (NGS) (Illumina). The remaining participant utilised the Oncomine Myeloid Research Assay on the ThermoFisher Scientific Ion Torrent platform.
- One hundred and eight participants returned information relating to the type of *NPM1* variant detected. In line with sample formulation, 87 (80.6%) 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, three 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 (17.6%) 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 one provided protein nomenclature only.

### Sample NPM1 168

- All participants (n=159) returning results for sample NPM1 168 identified an *NPM1* variant.
- One hundred and fourteen participants returned information relating to the type of *NPM1* variant detected. In line with sample formulation, 91 (79.8%) 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, three participants reported a 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 (16.7%) 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, one gave protein nomenclature only, one laboratory reported the detection of a Type D NPM1 variant and one (0.9%) reported detection of either a Type B or D variant (but submitted HGVS nomenclature described a type A variant).

## NPM1 Mutation Status Programme

# NPM1 Educational Sample Edu F

### Sample Information

Sample NPM1 Edu F was issued as whole genome amplified material (WGA) derived from a patient with a NM_002520.7(NPM1):c.863_864insTTTG p.(Trp288Cysfs*12). Results for this sample have not been scored.

In total, 91 participants returned results for the educational DNA sample NPM1 Edu F.

### Your Result

| Sample | Participant | Your Result |
|---|---|---|
| NPM1 Edu F variant detected? | | Not Tested |

### All Participant Results

| Sample | Variant Detected | No Variant Detected |
|---|---|---|
| NPM1 Edu F | 90 | 1 |

### Your Variant Results

| | Your DNA sequence variant description | Your protein variant description |
|---|---|---|
| NPM1 Edu F | Not Tested | Not Tested |

## NPM1 Mutation Status Programme

- Ninety out of 91 participants (98.9%) indicated that they detected a variant in sample NPM1 Edu F.
- The laboratory reporting a false negative result utilised the Qiagen NPM1 Mutascreen Kit with capillary electrophoresis.

**Sample Edu F was formulated to contain a Type $G^3/O_M^4$ variant. This variant is a 4 base pair insertion of a TTTG tetranucleotide in exon 11 of the *NPM1* gene (approved HGVS nomenclature[2] NM_002520.7:c.863_864insTTTG p.(Trp288Cysfs*12), systematic exon numbering of the *NPM1* transcript applied).**

- Fifty-one participants reported HGVS nomenclature for the *NPM1* variant detected in sample Edu F, of which, 38 (74.5%) participants reported the variant as c.863_864insTTTG, in line with sample formulation. Of these, one participant reported this as a c.861_862insTGTT insertion.
- Eleven (21.6%) participants reported a c.860_863dup (Type $A^1$ variant). Of these, three participants reported a c.860_863dupTCTG and two reported a c. 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.
- One participant (2%) reported detection of a c.860_863dupTTTG variant. The type of variant described at position c.860_863 would result in the duplication of a TCTG tetranucleotide and not TTTG. A further participant (2%) described the variant as a c.863_864insTGTT.
- When asked to define the variant type (e.g. Type A, B etc.) there were differences between the mutation type defined by the HGVS nomenclature and the mutation type submitted by the participants. Forty-eight participants returned information relating to the variant type. In total 24 (50.0%) participants defined the variant as a Type $G/O_M$ *NPM1* variant, with 11 (22.9%) reporting the insertion as a Type A variant. The remaining participants described various variant types, as outlined in the table below.

| NPM1 variant type described | Number of participants (%) |
|---|---|
| Type L | 4 (8.3) |
| Non type A, B or D | 3 (6.3) |
| Type D | 1 (2.1) |
| Type B/D | 1 (2.1) |
| Type DD-25 | 1 (2.1) |
| Non type A, B, D or DD1 | 1 (2.1) |
| Atypical | 1 (2.1) |
| Rare | 1 (2.1) |

## NPM1 Mutation Status Programme

- Of the 38 participants reporting HGVS nomenclature indicative of a Type G/$O_M$ *NPM1* variant, 20 (52.6%) participants defined this as a Type G/$O_M$ variant.
- Participants describing the NPM1 variant type as Type L, Type B/D and Type DD-25 reported HGVS nomenclature indicative of a Type G variant (c.863_864insTTTG).
- Within the literature, more than 50 insertion/duplication variant types have been described within exon 11 of *NPM1*[5]. 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[6]. The observed differences in HGVS classification and individual participant classifications could be a result of the Type G/$O_M$ *NPM1* variant being present in a small proportion of *NPM1* insertion/duplication events reported in acute myeloid leukaemia.
- Given the rarity of a large proportion of the remaining variants, and the differences observed herein between variant type classification and HGVS nomenclature, it suggests that the system for classification of *NPM1* variant type is outdated and less reliable than reporting of the HGVS nomenclature within a clinical setting. Clinical reporting of HGVS nomenclature only rather than variant type in *NPM1* exon 11 duplication/insertion events would improve standardisation.
- 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[7] 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 F.**

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[8]. **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'[9]. If participants require further information about this programme, please contact admin@uknegasli.co.uk.**

## NPM1 Mutation Status Programme

### 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. 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).

5. Rau, R. and Brown, P. Nucleophosmin (*NPM1*) mutations in adult and childhood acute myeloid leukemia: towards a definition of a new leukemia entity. *Hematol Oncol*. **27**(4), 171-181 (2009).

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

7. 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).

8. 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).

9. Scott, S. *et al*. Assessment of acute myeloid leukemia molecular measurable residual disease testing in an interlaboratory study. *Blood Adv*. (2023). doi: https://doi.org/10.1182/bloodadvances.2022009379

# 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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United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
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/