UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

# NPM1 Mutation Status Programme

Distribution - 232402

Participant ID - 43347

Date Issued - 22 November 2023

Closing Date - 05 January 2024

## Trial Comments

This trial was issued to 166 participants, of which 162 (97.6%) returned results. Of the non returns, one participant 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 171 and NPM1 172). Sample NPM1 171 was manufactured to be positive for a NPM1 Type A duplication, with sample NPM1 172 formulated to be negative.

## Results and Performance

### Your Results

| NPM1 Mutation Status | Your Results | Consensus Result |
|---|---|---|
| Sample NPM1 171 | Mutation Detected | Mutation Detected |
| Sample NPM1 172 | No Mutation Detected | No Mutation Detected |

### All Participant Results

| | Mutation Detected (Returns) | No Mutation Detected (Returns) |
|---|---|---|
| Sample NPM1 171 | 161 | 1 |
| Sample NPM1 172 | 1 | 161 |

### 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 | 125 |
| cDNA | 37 |

## PCR Type

| | Returns |
|---|---|
| Single PCR | 90 |
| Real-Time PCR | 31 |
| Multiplex PCR | 26 |
| Sequencing | 8 |
| Melting Curve Analysis | 6 |
| Single PCR with Clamping | 1 |

## Protocol Type

| | Returns |
|---|---|
| In-house Assay | 128 |
| Qiagen NPM1 Mutascreen Kit | 13 |
| Qiagen NPM1 mut A, B & D MutaQuant Kits | 7 |
| Ion Torrent Oncomine Myeloid Panel | 5 |
| Cepheid Xpert NPM1 Mutation Assay | 2 |
| Illumina TruSight Myeloid Sequencing Panel | 2 |
| Qiagen NPM1 mut A MutaQuant Kits | 2 |
| Agilent Custom Haloplex HS panel | 1 |
| Myeloid Solution by Sophia Genetics | 1 |
| Oncomine Myeloid Research Assay | 1 |

## Analysis Type

| | Returns |
|---|---|
| Capillary Electrophoresis | 86 |
| Real-Time PCR Fluorescent Detection | 34 |
| Sanger Sequencing | 9 |
| NGS (Illumina) | 7 |
| High Resolution Melt | 6 |
| NGS (ThermoFisher Ion Torrent) | 6 |
| Agarose Gel Electrophoresis | 4 |
| Digital PCR (Biorad) | 3 |
| Next Generation Sequencing (Miseq) | 3 |
| Illumina NextSeq 500 | 2 |
| Illumina NextSeq 2000 | 1 |
| Pyrosequencing | 1 |

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## Journal Reference for Assay

| | Returns |
|---|---|
| Gorello P. et al (2006) Leukemia, 20(6):1103-1108 | 26 |
| Noguera N. et al (2005) Leukemia, 19(8):1479-1482 | 20 |
| Falini B. et al (2005) N Engl J Med, 352(3):254-266 | 19 |
| Gale R. et al (2008) Blood, 111(5):2776-2784 | 12 |
| In-house method (no published reference available) | 11 |
| Falini B. et al (2007) Blood, 109(3):874-885 | 10 |
| Schnittger S. et al (2005) Blood, 106(12):3733-3739 | 8 |
| Döhner K. et al (2005) Blood, 106(12):3740-3746 | 7 |
| Huang Q. et al (2008) Br J Haematol, 142:(3)489-492 | 7 |
| Lin LI. et al (2006) Leukemia, 20(10):1899-1903 | 7 |
| Thiede C. et al (2006) Blood, 107(10):4011-4020 | 7 |
| Tan AY. et al (2008) J Haemtol Oncol, 1, 10 | 5 |
| Belgian Molecular Diagnostic Group | 4 |
| Boissel N. et al (2005) Blood, 106(10):3618-3620 | 4 |
| Thiede C. et al (2006) Leukemia, 20(10):1897-1899 | 4 |
| 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

### Sample NPM1 171

- In line with sample formulation, 161 of 162 (99.4%) participants returning results identified an *NPM1* variant in sample NPM1 171.
- The participant reporting a false negative result for NPM1 171 utilised an in-house assay with capillary electrophoretic analysis.
- One hundred and fifteen participants returned information relating to the type of *NPM1* variant detected. In line with sample formulation, 93 (80.9%) 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, 33 participants reported HGVS nomenclature for the *NPM1* variant. Twenty-five (75.8%) reported c.860_863dup, with seven (21.2%) reporting c.860_863dupTCTG and one (3.0%) 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 18 laboratories (15.8%) reported a 4 bp insertion but did not specify further details. One participant (0.9%) reported a 4 bp insertion/duplication variant and one participant (0.9%) reported an insertion but did not specify the size. Additionally, one laboratory (0.9%) reported the detection of Type A and Type D *NPM1* variants in FLT3 171 and one participant (0.9%) reported the presence of a Type D *NPM1* variant.

### Sample NPM1 172

- One hundred and sixty-one out of 162 (99.4%) participants returning results did not detect an *NPM1* variant in sample NPM1 172.
- The participant reporting an out of consensus false positive result utilised an in-house assay with Real-Time fluorescent detection.

## NPM1 Mutation Status Programme

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[3]. **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'[4]. 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. Schuurhuis, G.J. *et al.* Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*. 2018; **131**(12), 1275-1291.

4. Scott, S. *et al*. Assessment of acute myeloid leukemia molecular measurable residual disease testing in an interlaboratory study. *Blood Adv*. 2023; **7**(14): 3686–3694 (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
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
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) No activities in relation to this EQA exercise were subcontracted.

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/