UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

FLT3 Mutation Status Programme

Distribution - 202101

Participant ID -

Date Issued - 21 July 2020

Closing Date - 28 August 2020

Trial Comments

FINAL REPORT: This trial was issued to 162 participants, of which 152 (93.8%) returned results. Of the non returns, four laboratories notified us of their intended non return and one laboratory submitted a request for an extension in results submission in light of the ongoing Covid-19 pandemic.

Sample Comments

Two lyophilised samples were manufactured and distributed by UK NEQAS LI (sample references FLT3 150 and FLT3 151) for FLT3 ITD analysis and scoring. Both FLT3 150 and FLT3 151 were manufactured to be positive for a FLT3 ITD, with the samples comprised of cell line material spiked into a pooled buffy coat.

Results and Performance

Your Results

FLT3 Mutation Status	Your Results	Consensus Result
Sample FLT3 150		Mutation Detected
Sample FLT3 151		Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample FLT3 150	151	1
Sample FLT3 151	151	1

Your Performance

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

N/A = Not Applicable

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Template

	Returns
DNA	138
cDNA	15

PCR Type

	Returns
Single PCR	119
Multiplex PCR	25
Sequencing	5
Real-Time PCR	4

Protocol Type

	Returns
In-house Assay	118
Leukostrat FLT3 Mutation Assay	18
Molecular Diagnostic.be	7
Invivoscribe FLT3 (Labelled or Unlabelled)	4
Illumina TruSight Myeloid Sequencing Panel	3
Archer Variantplex FLT3-NPM1 custom assay	1
Ion AmpliSeq Cancer Hotspot Panel v2	1
Myeloid Solution by Sophia Genetics	1

Analysis Type

	Returns
Capillary Electrophoresis	126
Agarose Gel Electrophoresis	16
NGS (Other)	7
Melt Curve Analysis	2
Acrylamide Gel Electrophoresis (PAGE)	1
Microfluidic Electrophoresis	1

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Journal Reference for Assay

	Returns
Murphy KM et al (2003) J Mol Diagn 5(2):96-102	36
Thiede C et al (2002) Blood 99(12):4326-4335	25
Kiyoi H et al (1997) Leukemia 11(9):1447-1452	12
Kottaridis PD et al (2001) Blood 98(6):1752-1759	12
Yamamoto Y et al (2001) Blood 97(8):2434-2439	12
Noguera NI et al (2005) Leukemia 19(18):1479-1482	11
In-House Assay (no published reference available)	9
Gale RE et al (2008) Blood 111(5):2776-2784	8
Abu-Duhier FM et al (2000) Br J Haematol 111(1):190-195	7
Huang Q et al Br J Haematol (2008) 142 (3):489-492	7
Nakao M et al (1996) Leukemia 10(2):1911-1918	7
Kiyoi H et al (1999) Blood 93(9):3074-3080	6
MolecularDiagnostics.be assay	6
Schnittger S et al (2011) Haematologica 96(12):1799-1807	4
Buban T et al (2011) Clin Chem and Laboratory Medicine 50(2):301-310	3
Schnittger S et al (2002) Blood 100(1):59-66	3
Chang TL et al (2003) Haematologica 88(2):21-22	2
Frohling S et al (2002) Blood 100(13):4372-4380	2
Gilliland DG and Griffin JD (2002) Blood 100(5):1532-1542	2
Tan AY et al (2008) J Haematol Oncol 1:10	2

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Trial Comments

- 151 out of 152 (99.3%) participants that returned results correctly identified at least one *FLT3* internal tandem duplication (ITD) in sample FLT3 150.
- The participant that reported a false negative in sample FLT3 150 utilised an in-house assay with capillary electrophoresis.
- For sample FLT3 151, 151 out of 152 (99.3%) participants that returned results correctly identified a *FLT3* internal tandem duplication (ITD) in the sample.
- The participant that reported a false negative in sample FLT3 151 used an in-house Next Generation Sequencing approach.

ITD Analysis

- 126 participants provided the size of the ITD(s) detected in sample FLT3 150. 119/126 (94.4%) participants identified a single ITD, five (4.0%) participants found two ITDs and two (1.6%) participants reported three ITDs.
- The median size of the ITD in sample FLT3 150 was 30 bp, reported by 97 (77.0%) participants, in line with sample formulation expectations.
- ITD sizes reported by participants for FLT3 150 ranged from 10-481bp. *FLT3* ITDs normally range in size from approximately 15-153bp[1], with ITDs >400bp also reported[2]. These mutations are typically 'in-frame' and comprise duplicated genetic material, with a size that is a multiple of three. For participants detecting at least one ITD and reporting ITD size, 16/126 laboratories (12.7%) reported ITDs that were not a multiple of three.
- For FLT3 151, 125 participants provided information on the size of the ITD(s) detected. 119 participants found a single ITD, three participants identified two ITDs and three participants found three ITDs.
- The median size of the ITD in sample FLT3 151 was 30bp, as reported by 96 (76.8%) participants, in line with sample formulation. ITD sizes reported ranged from 10-482bp. For participants reporting at least one ITD and reporting the size, 19/125 (15.2%) reported an ITD size that was not a multiple of three.
- Across both samples, nine laboratories reported ITD sizes ranging from 327-482bp, which appear out of consensus in comparison with other participant data returns. All nine laboratories used capillary electrophoresis. As such, it is possible that these represent the total size of the PCR product used for analysis.

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Quantification Analysis

- Fifty-two participants returned informative quantification data in line with ELN diagnostic recommendations[4] (allelic ratio: mutant/wildtype) for samples FLT3 150.
- The median allelic ratio for FLT3 150 was 0.9, with an interquartile range (IQR) of 0.3. Reported allelic ratios ranged from 0.49-3.12. In this scenario, based on the median allelic ratio, FLT3 150 would be considered FLT3 high (>0.5)[3].
- For sample FLT3 151, 49 participants provided informative quantification data in line with ELN diagnostic recommendations[3] (allelic ratio: mutant/wildtype).
- The median allelic ratio for FLT3 151 was 1.97, with an interquartile range (IQR) of 0.6. Reported allelic ratios ranged from 0.70-5.94. In this scenario, based on the median allelic ratio, FLT3 151 would be considered FLT3 high (>0.5)[3].

Reporting of allelic ratios for multiple ITDs

- In FLT3 192003, one sample was formulated to contain two ITD and there were observed differences in the methods for calculating *FLT3* allelic ratios.
- In this trial distribution, UK NEQAS LI sought to determine how participants are reporting allelic ratios in AML patients with multiple ITDs.
- In total, 52 participants returned information relating to laboratory reporting of allelic ratios in patients presenting with multiple ITDs.
- 28 out of 52 (53.8%) participants report a combined allelic ratio to clinicians (the sum of all ITD area under the curve/peak height divided by the wild type area under the curve/peak height). 16/52 (30.8%) report each ITD allelic ratio separately and 8 out of 52 (15.4%) participants reported both separate ITD allelic ratios and a combined allelic ratio to clinicians.
- Döhner *et al.,* (2017) guidelines outline three risk stratification groups based on genetics. With regards to *FLT3* (in combination with *NPM1* mutation status), patients with mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD low (allelic ratio <0.5) are categorised as favourable risk[3]. Patients with mutated *NPM1* and *FLT3*-ITD high (allelic ratio >0.5) or patients with wild-type *NPM1* with *FLT3*-ITD low or without *FLT3*-ITD (in the absence of other adverse-risk genetic lesions) are considered intermediate risk[3]. Finally, patients with wild-type *NPM1* and *FLT3*-ITD high are considered adverse risk[3].
- Application of diagnostic guidelines are subjective, depending on the end user's interpretation. However, the ELN diagnostic recommendations outline the calculation approach required for determination of *FLT3* ITD allelic ratio as the area under the curve for *FLT3*-ITD divided by the area under the curve of *FLT3*-wild type. Whilst this does not specifically mention the approach to multiple ITD reporting, it could be assumed that the area under the curve for *FLT3*-ITD encompasses the total sum in cases where multiple *FLT3*-ITD present in patients.
- **When considering the risk stratification guidelines, the findings from FLT3 192003 and the results above currently highlight the potential for patients presenting with multiple ITDs to be stratified into different risk categories based on laboratory approaches to *FLT3*-ITD reporting.**

References

1. Stirewalt, D. L. Size of FLT3 internal tandem duplication has prognostic significance in patients with acute myeloid leukemia. *Blood* **107,** 3724–3726 (2006).
2. Meshinchi, S. & Appelbaum, F. R. Structural and functional alterations of FLT3 in acute myeloid leukemia. *Clin. Cancer Res.* **15,** 4263–4269 (2009).
3. Döhner, H. *et al.* Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* **129,** (2017).

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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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e-mail: nicola.rose@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/