UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

FLT3 Mutation Status Programme

Distribution - 222303

Participant ID -

Date Issued - 07 March 2023

Closing Date - 07 April 2023

Trial Comments

This trial was issued to 182 participants, of which 176 (96.7%) returned results. Of the non returns, two participants requested an extension to results submission.

Sample Comments

Two lyophilised samples were manufactured and distributed by UK NEQAS LI (sample references FLT3 166 and FLT3 167) for FLT3 ITD analysis and scoring. FLT3 166 was manufactured to be negative for a FLT3 ITD. FLT3 167 was manufactured to be positive for a 30bp FLT3 ITD. In addition, an educational sample (FLT3 Edu H) was issued for tyrosine kinase domain (TKD) analysis. This sample was whole genome amplified DNA, derived from patient material and was positive for the NM_004119.3(FLT3):c.2503G>T p.(Asp835Tyr) variant.

Results and Performance

Your Results

FLT3 Mutation Status	Your Results	Consensus Result
Sample FLT3 166	No Mutation Detected	No Mutation Detected
Sample FLT3 167	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample FLT3 166	1	175
Sample FLT3 167	175	1

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	161
cDNA	15

PCR Type

	Returns
Single PCR	137
Multiplex PCR	28
Sequencing	7
Real-Time PCR	4

Protocol Type

	Returns
In-house Assay	137
Leukostrat FLT3 Mutation Assay	24
Molecular Diagnostic.be	5
Invivoscribe FLT3 (Labelled or Unlabelled)	4
Myeloid Solution by Sophia Genetics	2
Oncomine Myeloid Research Assay	2
Illumina TruSight Myeloid Sequencing Panel	1
Ion Torrent Oncomine Myeloid Panel	1

Analysis Type

	Returns
Capillary Electrophoresis	148
Agarose Gel Electrophoresis	16
Melt Curve Analysis	3
NGS (ThermoFisher Ion Torrent)	3
Next Generation Sequencing (Miseq)	2
Illumina MiniSeq	1
Illumina NextSeq 2000	1
NGS (Illumina)	1
NGS (Other)	1

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Journal Reference for Assay

	Returns
Murphy KM et al (2003) J Mol Diagn 5(2):96-102	41
Thiede C et al (2002) Blood 99(12):4326-4335	33
Yamamoto Y et al (2001) Blood 97(8):2434-2439	18
Kiyoi H et al (1999) Blood 93(9):3074-3080	11
Noguera NI et al (2005) Leukemia 19(18):1479-1482	11
Kiyoi H et al (1997) Leukemia 11(9):1447-1452	10
Dohner, H., et al. (2017) Blood 129(849):424-447.	9
Gale RE et al (2008) Blood 111(5):2776-2784	9
Kottaridis PD et al (2001) Blood 98(6):1752-1759	9
Huang Q et al Br J Haematol (2008) 142 (3):489-492	7
In-House Assay (no published reference available)	7
Nakao M et al (1996) Leukemia 10(2):1911-1918	7
Abu-Duhier FM et al (2000) Br J Haematol 111(1):190-195	6
MolecularDiagnostics.be assay	6
Kottaridis PD et al (2002) Blood 100(7):2393-2398	4
Schnittger S et al (2011) Haematologica 96(12):1799-1807	4
Frohling S et al (2002) Blood 100(13):4372-4380	3
Tan AY et al (2008) J Haematol Oncol 1:10	3
Buban T et al (2011) Clin Chem and Laboratory Medicine 50(2):301-310	2
Gilliland DG and Griffin JD (2002) Blood 100(5):1532-1542	2

Trial Comments

Participant ID:

- 175 out of 176 (99.4%) participants that returned results correctly reported the absence of a *FLT3* internal tandem duplication (ITD) in sample FLT3 166.
- The participant reporting an out of consensus false positive result utilised an in-house assay with capillary electrophoretic analysis.
- For sample FLT3 167, 175 out of 176 (99.4%) participants that returned results correctly identified the presence of a *FLT3* ITD in the sample.
- The participant reporting out of consensus false negative result utilised an in-house assay with capillary electrophoretic analysis.

ITD Analysis

- 146 participants provided the size of the ITD(s) detected in sample FLT3 167. In line with sample formulation, one hundred and thirty-four (91.8%) participants identified a single ITD. Ten (6.8%) participants reported the detection of two ITDs and two (1.4%) identified four ITDs.
- The median size of the ITD in sample FLT3 167 was 30 bp, reported by 117 out of 146 (80.1%) participants reporting detection of at least one ITD.
- The ITD sizes reported by participants ranged from 6-570 bp. *FLT3* ITDs normally range in size from approximately 15-153bp[1], with ITDs >400bp also reported[2]. These variants 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, 18/146 laboratories (12.3%) reported ITDs that were not a multiple of three in sample FLT3 167.

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Allelic Ratio Quantification

The recent publication of the 2022 updated ELN recommendations for the diagnosis and management of AML in Adults[3] has revised several aspects of AML disease risk classification. **The updated guidelines indicate that the *FLT3*-ITD allelic ratio is no longer considered in the risk stratification, with all *FLT3*-ITD positive AML cases categorised in the intermediate-risk group, irrespective of the presence of *NPM1* co-mutation.** This change relates to the methodological issues surrounding standardisation of the approaches to calculating the *FLT3*-ITD allelic ratio, the modifying impacts of midostaurin-based therapy on *FLT3*-ITD without *NPM1* mutation and the increasing role of measurable residual disease (MRD) in treatment decisions.

For now, UK NEQAS LI will continue to provide the option to submit allelic ratio information for our trial samples. This is to monitor the uptake of the new ELN recommendations. All allelic ratio information continues to be summarised in trial reports.

- 138 participants provided allelic ratio information relating to the method utilised for allelic ratio calculation.
- 129 out of 138 (93.5%) participants calculated allelic ratio data using the Mutant/Wild-type approach, as outlined in the 2017 ELN recommendations[4].
- Five (3.6%) participants calculated allelic ratio information using the Mutant/(Mutant+Wild-type) approach. Two (1.4%) participants reported the use of variant fraction, one (0.7%) participant utilised absolute counting and one (0.7%) reported the use of Wildtype/Mutant.
- Of the 129 participants calculating allelic ratio information using the mutant/wildtype approach, 106 (82.2%) calculated allelic ratios using the area under the curve (AUC), with 18 (14.0%) utilising peak height. One (0.8%) participant reported the use both AUC and peak height, one (0.8%) participant used NGS variant allele frequency and one participant (0.8%) utilised an NGS panel calculation approach. Two further participants provided no information on the method used to calculate allelic ratio information.

The median allelic ratio reported for FLT3 167 (AUC Mutant/AUC Wild-type allelic ratio calculation) was 3.75, with an interquartile range (IQR) of 1.3. Reported allelic ratios for sample FLT3 167 ranged from 0.08-391.

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Sample FLT3 Edu H Tyrosine Kinase Domain (TKD) Testing Results

In total, 110 participants returned results from *FLT3* TKD testing for Edu H. Sample FLT3 Edu H was issued as whole genome amplified material (WGA) derived from a patient with a NM_004119.3(*FLT3*):c.2503G>T p.(Asp835Tyr) variant in the tyrosine kinase domain of *FLT3*. Results for this sample have not been scored.

Your Result

Sample	Participant	Your Result
FLT3 TKD Edu H variant detected?		Not Tested

All Participant Results

Sample	Variant Detected	No Variant Detected
FLT3 TKD Edu H	105	5

Your Variant Results

	Your DNA Sequence Variant Description	Your Protein Variant Description
	Not Tested	Not Tested

PCR Type

The breakdown of participant returns regarding methodological information may not be equal to the total number of participant result submissions for *FLT3* TKD testing for sample FLT3 Edu H.

	Returns
Single PCR	56
Restriction Fragment Length Polymorphism	27
Multiplex PCR	18
Next Generation Sequencing	9
Melt Curve Analysis	2
Real-Time PCR	1

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Protocol Type

	Returns
In-house designed Assay	82
LeukoStrat™ FLT3 Mutation Assay	14
Ion Torrent™ Oncomine™ Myeloid Panel	3
AmpliSeq for Illumina Myeloid Panel	3
Illumina TruSight Myeloid Panel	2
Invivoscribe FLT3 Mutation Assay	2
Ion Torrent™ Oncomine™ Myeloid Panel v2	1
SOPHiA™ Myeloid Solution Panel	1
Qiagen QiaSeq Custom Panel	1
Other	1

Analysis Type

	Returns
Capillary Electrophoresis	57
Next Generation Sequencing – Illumina	15
Sanger Sequencing	12
Agarose Gel Electrophoresis	10
Next Generation Sequencing – ThermoFisher Scientific Ion Torrent	7
High Resolution Melt Analysis	3
Next Generation Sequencing – Other (Not Specified)	1
TapeStation	1
Mass Spectrometry	1
Pyrosequencing	1

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Journal Reference for Assay

	Returns
Murphy, J. M. *et al.* J Mol Diagn 2003; 5(2): 96-102	33
Yamamoto, Y. *et al.* Blood 2001; 97(8): 2434-2439	14
Thiede, C. *et al.* Blood 2002; 99(12): 4326-4335	11
Noguera, N.I. *et al*. Leukemia 2005, 19(8): 1479-1482	3
Abu-Duhier, F.M. *et al*. Br J Haematol 2000, 111(1): 190-195	3
Kottaridis, P.D. *et al.* Blood 2001; 98(6): 1752-1759	3
Kiyoi, H. *et al.* Leukemia 1997; 11(9): 1447-1452	3
Nakao, M. *et al*. Leukemia 1996; 10(12): 1911-1918	2

As stated by ≥2 participants

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FLT3 Edu H TKD Testing Comments

- 105 out of 110 (95.5%) participants detected a *FLT3* TKD variant in sample FLT3 Edu H.
- The five participants reporting a false negative result for FLT3 Edu H utilised an in-house assay, with three utilising capillary electrophoretic analysis and two using Sanger sequencing.
- In total, 52 participants returned information relating to the TKD variant identified (DNA level HGVS nomenclature). This sample was WGA material derived from a patient with a NM_004119.3(*FLT3*):c.2503G>T p.(Asp835Tyr) variant.
- Forty-seven out of 52 (90.4%) participants reported a c.2503G>T *FLT3* TKD variant. Two participants (5.7%) reported a c.2505T>G variant, one (2.0%) reported a c.2503G>C variant and one reported a c.2039C>T variant.
- For the protein level HGVS nomenclature, informative data relating to the amino acid substitution was returned by 51 participants; 46 out of 51 (90.2%) participants reported the substitution of aspartic acid for tyrosine at position 835 of the protein. In line with HGVS recommendations, 32 (69.6%) participants reported the protein nomenclature as p.(Asp835Tyr). Ten (21.7%) reported p.Asp835Tyr. Three (6.5%) participants reported the protein nomenclature as p.D835Y. One (2.2%) participant reported the protein nomenclature as p.IAsp835Tyr.
- Please note, parentheses are required in this context as WGA DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. Furthermore, use of the three-letter amino acid code is preferred when describing changes at the protein level. For further information, please refer to the HGVS website: (https://varnomen.hgvs.org/recommendations/protein/variant/deletion/).
- Five participants reported out of consensus HGVS nomenclature at the protein level. Three of the five participants reported the protein nomenclature as p.(Asp835Glu), one as p.(Asp835His) and one p.(Ala680Val).
- A further three participant stated that amino acid residue 835. Whilst these participants reported the involvement of a specific amino acid residues, they did not go on to specify the type of variant induced due to the limitations of the methodology utilised.

- In total, 52 participants returned quantification data for the *FLT3* TKD variant. The most commonly used quantification method was using the Mut/(Mut+WT) x 100 calculation, reported by 30 participants (57.7% of returns), followed by the Mut/WT calculation reported by 13 participants (25.0% of returns) and the Mut/WT x 100 calculation, reported by five participants (9.6% of returns). One participant reported the use of (Mut/WT) x 100 for quantification, one reported the use of TapeStation. A further participant stated use of absolute counting. One participant did not specify the quantification calculation information and a further participant stated 'other' but provided no further information.
- The median variant load reported (Mut/(Mut+WT) x 100 quantification calculation) was 23.0%, with an interquartile range (IQR) of 4.8%. Variant loads utilising this calculation method ranged from 0.2-40.47%.

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References

1. Stirewalt, D. L. *et al.* 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: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* **140**(12), 1345-1377 (2022).
4. Döhner, H. *et al.* Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* **129**(4), (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:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
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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) 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/