UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

FLT3 Mutation Status Programme

Distribution - 232402

Participant ID -

Date Issued - 21 November 2023

Closing Date - 05 January 2024

Trial Comments

This trial was issued to 183 participants, of which 178 (97.3%) returned results. Of the non returns, one prenotified us of their intention to not return results and a further participant requested an extension to results submission.

Sample Comments

Two lyophilised samples were manufactured and distributed by UK NEQAS LI (sample references FLT3 170 and FLT3 171) for FLT3 ITD analysis and scoring. Both FLT3 170 and FLT3 171 were manufactured to be positive for a FLT3 ITD. In addition, an educational sample was provided with this trial distribution. Sample FLT3 Edu K 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.2505T>G p.(Asp835Glu) variant.

Results and Performance

Your Results

FLT3 Mutation Status	Your Results	Consensus Result
Sample FLT3 170	Mutation Detected	Mutation Detected
Sample FLT3 171	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample FLT3 170	176	2
Sample FLT3 171	176	2

Your Performance

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

N/A = Not Applicable

FLT3 Mutation Status Programme

Template

	Returns
DNA	164
cDNA	13

PCR Type

	Returns
Single PCR	138
Multiplex PCR	29
Sequencing	7
Real-Time PCR	4

Protocol Type

	Returns
In-house Assay	137
Leukostrat FLT3 Mutation Assay	27
Invivoscribe FLT3 (Labelled or Unlabelled)	4
Ion Torrent Oncomine Myeloid Panel	3
Molecular Diagnostic.be	3
Myeloid Solution by Sophia Genetics	2
Archer VariantPlex Core Myeloid Kit	1
Illumina TruSight Myeloid Sequencing Panel	1

Analysis Type

	Returns
Capillary Electrophoresis	149
Agarose Gel Electrophoresis	14
NGS (Illumina)	3
NGS (ThermoFisher Ion Torrent)	3
Illumina MiniSeq	2
Melt Curve Analysis	2
Acrylamide Gel Electrophoresis (PAGE)	1
Illumina NextSeq 2000	1
Illumina NextSeq 550	1
Next Generation Sequencing (Miseq)	1
Sanger Sequencing	1

FLT3 Mutation Status Programme

Journal Reference for Assay

	Returns
Murphy KM et al (2003) J Mol Diagn 5(2):96-102	42
Thiede C et al (2002) Blood 99(12):4326-4335	35
Yamamoto Y et al (2001) Blood 97(8):2434-2439	16
Kottaridis PD et al (2001) Blood 98(6):1752-1759	12
Kiyoi H et al (1999) Blood 93(9):3074-3080	11
Noguera NI et al (2005) Leukemia 19(18):1479-1482	11
Gale RE et al (2008) Blood 111(5):2776-2784	10
In-House Assay (no published reference available)	9
Nakao M et al (1996) Leukemia 10(2):1911-1918	8
Abu-Duhier FM et al (2000) Br J Haematol 111(1):190-195	7
Dohner, H., et al. (2017) Blood 129(849):424-447.	7
Huang Q et al Br J Haematol (2008) 142 (3):489-492	6
Kiyoi H et al (1997) Leukemia 11(9):1447-1452	6
MolecularDiagnostics.be assay	5
Buban T et al (2011) Clin Chem and Laboratory Medicine 50(2):301-310	3
Frohling S et al (2002) Blood 100(13):4372-4380	3
Schnittger S et al (2011) Haematologica 96(12):1799-1807	3
Tan AY et al (2008) J Haematol Oncol 1:10	3
Gilliland DG and Griffin JD (2002) Blood 100(5):1532-1542	2
Schnittger S et al (2002) Blood 100(1):59-66	2

- 176 out of 178 (98.9%) participants that returned results correctly reported the presence of a *FLT3* internal tandem duplication (ITD) in sample FLT3 170.
- For sample FLT3 171, 176/178 participants (98.9%) returning results correctly identified the presence of a *FLT3* ITD in the sample.
- The same two participants reported an out of consensus false negative results for FLT3 170 and 171 and utilised an in-house assay, one with capillary electrophoresis and one with agarose gel electrophoresis.

ITD Analysis

- 145 participants provided the size of the ITD(s) detected in sample FLT3 170. In line with sample formulation, one hundred and thirty-three (91.7%) participants identified a single ITD. Nine (6.2%) participants reported the detection of two ITDs, two (1.4%) participants reported the detection of three ITDs and one (0.7%) identified four ITDs.
- The median size of the ITD in sample FLT3 170 was 30 bp, reported by 122 out of 145 (84.1%) participants reporting detection of at least one ITD.
- The ITD sizes reported by participants ranged from 10-569 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, 18/145 laboratories (12.4%) reported ITDs that were not a multiple of three in sample FLT3 170.
- 145 participants provided the size of the ITD(s) detected in sample FLT3 171. In line with sample manufacture, 136 (93.8%) participants identified a single ITD. Seven (4.8%) reported the detection of two *FLT3* ITDs, one (0.7%) participant reported the detection of three ITDs and one (0.7%) identified four ITDs.
- The median size of the ITD in sample FLT3 171 was 30 bp, reported by 119 out of 145 (82.1%) participants reporting detection of at least one ITD.
- The ITD sizes reported by participants for FLT3 171 ranged from 10-571 bp.
- For participants detecting at least one ITD and reporting ITD size, 18/145 laboratories (12.4%) reported ITDs that were not a multiple of three in sample FLT3 171.

FLT3 Mutation Status Programme

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.

- 135 participants provided allelic ratio information relating to the method utilised for allelic ratio calculation.
- 116 out of 135 (85.9%) participants calculated allelic ratio data using the Mutant/Wild-type approach, as outlined in the 2017 ELN recommendations[4].
- Fourteen (10.4%) participants calculated allelic ratio information using the Mutant/(Mutant+Wild-type) approach. Three participants reported the use of variant allele frequency, one reported use of wildtype/mutant and one reported use of absolute counting.
- Of the 116 participants calculating allelic ratio information using the mutant/wildtype approach, 97 (83.6%) calculated allelic ratios using the area under the curve (AUC), with 14 (12.1%) utilising peak height. One (0.9%) participant reported the use both AUC and peak height and one (0.9%) participant utilised an NGS panel calculation approach. Despite stating use of the mutant/wildtype approach, one participant stated use of NGS variant allele frequency. A further two participants provided no information relating to the dataset utilised to calculate allelic ratio information using the mutant/wildtype approach.

The median allelic ratio reported for FLT3 170 (AUC Mutant/AUC Wild-type allelic ratio calculation) was 0.56, with an interquartile range (IQR) of 0.13. Reported allelic ratios for sample FLT3 170 ranged from 0.3-312.

For FLT3 171, 92 participants submitted allelic ratio data utilising the AUC Mutant/AUC Wild-type calculation. The median allelic ratio for FLT3 171 was 0.29 with an IQR of 0.07. Reported allelic ratios for sample FLT3 171 ranged from 0.15-285.

FLT3 Mutation Status Programme
Sample FLT3 Edu K Tyrosine Kinase Domain (TKD) Testing Results

In total, 110 participants returned results from *FLT3* TKD testing for Edu K. Sample FLT3 Edu K was issued as whole genome amplified material (WGA) derived from a patient with a NM_004119.3(*FLT3*):c.2505T>G p.(Asp835Glu) 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 K variant detected?		Variant Detected

All Participant Results

Sample	Variant Detected	No Variant Detected
FLT3 TKD Edu K	106	4

Your Variant Results

	Your DNA Sequence Variant Description	Your Protein Variant Description
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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 K.

	Returns
Single PCR	54
Restriction Fragment Length Polymorphism	27
Multiplex PCR	17
Next Generation Sequencing	10
Melt Curve Analysis	2
Real-Time PCR	1
Other (Not Specified)	3

FLT3 Mutation Status Programme

Protocol Type

	Returns
In-house designed	75
LeukoStrat™ FLT3 Mutation Assay	18
ThermoFisher Scientific Oncomine Myeloid Research Assay	4
Illumina TruSight Myeloid Sequencing Panel	2
ThermoFisher Scientific Oncomine Myeloid GX v2 Assay	2
Invivoscribe FLT3 Mutation Assay	2
Illumina AmpliSeq™ Myeloid Panel	1
SOPHiA™ Myeloid Solution Panel	1
Archer VariantPlex Core Myeloid	1
Other (Not Specified)	3

Analysis Type

	Returns
Capillary Electrophoresis	56
Next Generation Sequencing – Illumina	15
Sanger Sequencing	12
Agarose Gel Electrophoresis	11
Restriction Enzyme Polymorphism	9
Next Generation Sequencing – ThermoFisher Scientific Ion Torrent	7
High Resolution Melt Analysis	2
Acrylamide Gel Electrophoresis	1
Other (Not Specified)	1

FLT3 Mutation Status Programme

Journal Reference for Assay

	Returns
Murphy, J. M. *et al. J Mol Diagn*. 2003; 5(2): 96-102	27
Yamamoto, Y. *et al. Blood*. 2001; 97(8): 2434-2439	13
Thiede, C. *et al. Blood*. 2002; 99(12): 4326-4335	13
Noguera, N.I. *et al*. Leukemia. 2005; 19(8): 1479-1482	7
Kottaridis, P.D. *et al*. *Blood*. 2001; 98(6): 1752-1759	4
Kiyoi, H. *et al. Leukemia*. 1997; 11(9): 1447-1452	3
Nakao, M. *et al*. *Leukemia*. 1996; 10(12): 1911-1918	2
Döhner, H. *et al*. *Blood*. 2017; 129(4): 424-447	2
In-house	2

As stated by ≥2 participants

FLT3 Mutation Status Programme

FLT3 Edu K TKD Testing Comments

- 106 out of 110 (96.4%) participants detected a *FLT3* TKD variant in sample FLT3 Edu K.
- The four participants reporting a false negative result for FLT3 Edu K utilised an in-house assay, with two utilising capillary electrophoretic analysis, one using agarose gel electrophoretic analysis and one Sanger sequencing.
- In total, 42 participants returned informative data 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.2505T>G p.(Asp835Glu) variant.
- Thirty-seven out of 42 (88.1%) participants reported a c.2505T>G *FLT3* TKD variant. Three participants (7.1%) reported alternate nucleotide substitutions: one reported a c.2503G>T variant, one a c.2506A>C variant and one reported a c.2508C>G variant. Of the remaining participants, one (2.4%) participant reported a c.1772_1801dup and one (2.4%) reported a c.2508_2510del variant.
- For the protein level HGVS nomenclature, informative data relating to the amino acid substitution was returned by 43 participants; 37 out of 43 (86.0%) participants reported the substitution of aspartic acid with glutamic acid at position 835 of the protein.
- In line with HGVS recommendations, 22 out of 37 (59.5%) participants reported the protein nomenclature as p.(Asp835Glu). Nine (24.3%) reported p.Asp835Glu. Four participants (10.8%) participant reported the protein nomenclature as p.D835E. One participant (2.7%) reported the protein nomenclature as p.(D835E) and one (2.7%) as (p.Asp835Glu.
- 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/).
- Six participants reported out of consensus HGVS nomenclature at the protein level. One participant (2.3%) reported a p.(Asp835Tyr) substitution, one (2.3%) a p.(I836L) substitution, one (2.3%) a p.Ile836Met variant, one (2.3%) a p.(Ile836del) variant, and one (2.3%) a p.(Asp600_Leu601insHisValAspPheArgGluTyrGluTyrAsp) variant. A further participant (2.3%) reported a p.(Asp385Glu) variant.

- In total, 49 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 31 participants (63.3% of returns), followed by the Mut/WT calculation reported by 12 participants (24.5% of returns) and the Mut/WT x 100 calculation, reported by two participants (4.1% of returns). One participant reported the use of absolute counting. Three participants did not specify the quantification calculation information.
- The median variant load reported (Mut/(Mut+WT) x 100 quantification calculation) was 27.0%, with an interquartile range (IQR) of 4.7%. Variant loads utilising this calculation method ranged from 19.5-50.0%.

FLT3 Mutation Status Programme

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).

FLT3 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/