UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

FLT3 Mutation Status Programme

Distribution - 252601

Participant ID - 43347

Date Issued - 15 July 2025

Closing Date - 22 August 2025

Trial Comments

This trial was issued to 184 participants, with all returning results.

Sample Comments

Two lyophilised samples were manufactured and distributed by UK NEQAS LI (sample references FLT3 180 and FLT3 181) for FLT3 ITD analysis and scoring. Sample FLT3 180 was manufactured to be positive for a FLT3 ITD (30bp) and FLT3 181 was formulated to be positive for two ITDs (30bp and 126bp). In addition, an educational sample was provided with this trial distribution. Sample FLT3 Edu P 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:c.2508_2510del p.(Ile836del) variant.

Results and Performance

Your Results

FLT3 Mutation Status	Your Results	Consensus Result
Sample FLT3 180	Mutation Detected	Mutation Detected
Sample FLT3 181	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample FLT3 180	183	1
Sample FLT3 181	180	4

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	Satisfactory	1	0

N/A = Not Applicable

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Template

	Returns
DNA	173
cDNA	11

PCR Type

	Returns
Single PCR	138
Multiplex PCR	30
Sequencing	12
Real-Time PCR	4

Protocol Type

	Returns
In-house Assay	142
Leukostrat FLT3 Mutation Assay	27
Molecular Diagnostic.be	6
Ion Torrent Oncomine Myeloid Panel	3
Myeloid Solution by Sophia Genetics	3
Archer VariantPlex Core Myeloid Kit	1
Invivoscribe FLT3 (Labelled or Unlabelled)	1
Oncomine Myeloid Research Assay	1

Analysis Type

	Returns
Capillary Electrophoresis	157
Agarose Gel Electrophoresis	11
Melt Curve Analysis	3
NGS (Illumina)	3
NGS (ThermoFisher Ion Torrent)	3
Illumina MiniSeq	2
Illumina NextSeq 2000	1
Illumina NextSeq 500	1
Illumina NextSeq 550	1
Next Generation Sequencing (Miseq)	1
Sanger Sequencing	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	37
Yamamoto Y et al (2001) Blood 97(8):2434-2439	15
In-House Assay (no published reference available)	14
Nakao M et al (1996) Leukemia 10(2):1911-1918	12
Noguera NI et al (2005) Leukemia 19(18):1479-1482	12
Kiyoi H et al (1999) Blood 93(9):3074-3080	11
Gale RE et al (2008) Blood 111(5):2776-2784	10
Kottaridis PD et al (2001) Blood 98(6):1752-1759	10
Huang Q et al Br J Haematol (2008) 142 (3):489-492	9
Kiyoi H et al (1997) Leukemia 11(9):1447-1452	9
Dohner, H., et al. (2017) Blood 129(849):424-447.	8
Abu-Duhier FM et al (2000) Br J Haematol 111(1):190-195	7
MolecularDiagnostics.be assay	4
Bacher U et al (2008) Blood 111(1):2527-2537	3
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
Frohling S et al (2002) Blood 100(13):4372-4380	2
Gilliland DG and Griffin JD (2002) Blood 100(5):1532-1542	2
Kiyoi H et al (1998) Leukemia 12(9):1333-1337	2
Schnittger S et al (2011) Haematologica 96(12):1799-1807	2
Tan AY et al (2008) J Haematol Oncol 1:10	2

- One hundred and eighty-three out of 184 (99.5%) participants that returned results correctly reported the presence of a *FLT3* internal tandem duplication (ITD) in sample FLT3 180.
- The participant reporting an out of consensus false negative result, also reported an out of consensus result for FLT3 180. This participant utilised an in-house, real-time PCR assay with melt curve analysis.
- For sample FLT3 181, 180/184 participants (97.8%) returning results correctly identified the presence of at least one *FLT3* ITD in the sample.
- Of the four participants reporting an out of consensus false negative result, one participant also reported an out of consensus result for FLT3 181 and was discussed above. The remaining three participants all utilised a single PCR, in-house assay with capillary electrophoresis.

ITD Analysis

- One hundred and fifty-seven participants provided the size of the ITD(s) detected in sample FLT3 180. In line with sample formulation, 144 (91.7%) participants identified a single ITD. Nine (5.7%) participants reported the detection of two ITDs, three (1.9%) participants reported the detection of three ITDs and one (0.6%) identified four ITDs.
- The median size of the ITD in sample FLT3 180 was 30 bp, reported by 139 out of 157 (88.5%) participants reporting detection of at least one ITD.
- The ITD sizes reported by participants ranged from 9-578 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, 13/157 laboratories (8.3%) reported ITDs that were not a multiple of three in sample FLT3 180.
- One hundred and fifty-three participants provided the size of the ITD(s) detected in sample FLT3 181. In line with sample manufacture, 124 (81.0%) participants identified two ITDs. Eighteen (11.8%) reported the detection of a single *FLT3* ITD, six participants (3.9%) identified three ITDs and five participants (3.3%) identified four ITDs.
- The median size of the first ITD in sample FLT3 181 was 30 bp, reported by 130 out of 153 (85.0%) participants reporting detection of at least one ITD. The median size of the second ITD in sample FLT3 181 was 126 bp, reported by 105/153 (68.6%) participants reporting detection of at least one ITD. This is in line with sample formulation expectations.
- The ITD sizes reported by participants for FLT3 181 ranged from 6-579 bp.
- For participants detecting at least one ITD and reporting ITD size, 31/153 laboratories (20.3%) reported ITDs that were not a multiple of three in sample FLT3 181.

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Allelic Ratio Quantification

The 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.

- 131 participants provided method information relating to the allelic ratio calculation.
- 116 out of 131 (88.5%) participants calculated allelic ratio data using the Mutant/Wild-type approach, as outlined in the 2017 ELN recommendations[4].
- Thirteen (9.9%) participants calculated allelic ratio information using the Mutant/(Mutant+Wild-type) approach. One participant (0.8%) reported the use of a variant fraction approach and one participant stated 'other' but provided no further details.
- Of the 116 participants calculating allelic ratio information using the mutant/wildtype approach, 95 (81.9%) calculated allelic ratios using the area under the curve (AUC), with 17 (14.7%) utilising peak height. One participant utilised a method bespoke to their Next Generation Sequencing assay (0.9%), one (0.9%) participant reported the use of both AUC and peak height and one (0.9%) participant stated use of 'gel band intensity'. A further participant 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 180 (AUC Mutant/AUC Wild-type allelic ratio calculation) was 1.15 (n=91 datasets), with an interquartile range (IQR) of 0.17. Reported allelic ratios for sample FLT3 180 ranged from 0.31-11919.

FLT3 181 was manufactured to contain two FLT3 ITDs, one at 30bp and one at 126bp in size. Overall, 126 participants submitted allelic ratio information for this sample, of which 113 (89.7%) reported a single allelic ratio, combining both FLT3 ITDs. Thirteen out of 126 (10.3%) reported allelic ratios of the individual ITDs identified in FLT3 181.

The median combined allelic ratio reported (single ITD data only) for FLT3 181 (AUC Mutant/AUC Wild-type allelic ratio calculation) was 0.46 (n= 81), with an IQR of 0.16. Reported combined allelic ratios for sample FLT3 181 ranged from 0.06-4745.

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

In total, 130 participants returned results from *FLT3* TKD testing for Edu P. Sample FLT3 Edu P was issued as whole genome amplified material (WGA) derived from a patient with a NM_004119.3:c.2508_2510del p.(Ile836del) 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 P variant detected?	43347	Yes

All Participant Results

Sample	Variant Detected	No Variant Detected
FLT3 TKD Edu P	127	3

Your Variant Results

Your DNA Sequence Variant Description	Your Protein Variant Description
Not determined	Not determined

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 P.

	Returns
Single PCR	61
Restriction Fragment Length Polymorphism	30
Multiplex PCR	24
Next Generation Sequencing	4
Sequencing	2
Melt Curve Analysis	3
Real-Time PCR	1
Allele Specific PCR	1

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Protocol Type

	Returns
In-house designed	91
LeukoStrat™ FLT3 Mutation Assay	19
Qiagen QiaSeq Custom Panel	4
ThermoFisher Scientific Oncomine Myeloid Research Assay	4
Invivoscribe FLT3 mutation assay	3
SOPHiA GENETICS™ Myeloid Solution	2
ThermoFisher Scientific Oncomine Myeloid GX v2 Assay	1
ThermoFisher Scientific Oncomine Precision Assay GX Assay	1
Illumina AmpliSeq™ Myeloid Panel	1
Agilent SureSelect Panel	1
BioRad droplet digital PCR	1
Archer VariantPlex Myeloid	1

Analysis Type

	Returns
Capillary Electrophoresis	76
Next Generation Sequencing – Illumina	20
Restriction Enzyme Polymorphism	18
Next Generation Sequencing – ThermoFisher Scientific Ion Torrent	12
Sanger Sequencing	10
Agarose Gel Electrophoresis	9
High Resolution Melt Analysis	5
Next Generation Sequencing – Other	1
Droplet digital PCR	1
Mass spectrometry (MASSArray)	1
MALDI-TOF	1

FLT3 Mutation Status Programme

Journal Reference for Assay

	Returns
Murphy, J. M. *et al.* J Mol Diagn. 2003; 5(2): 96-102	41
Yamamoto, Y. *et al. Blood*. 2001; 97(8): 2434-2439	17
Thiede, C. *et al. Blood*. 2002; 99(12): 4326-4335	15
Kiyoi, H. *et al*. Leukemia. 1997; 11(9):1447-1452	4
Kottaridis, P.D. *et al*. *Blood*. 2001; 98(6): 1752-1759	3
Noguera, N.I. *et al*. Leukemia. 2005; 19(8): 1479-1482	2
Nakao, M. *et al*. Leukemia. 1996; 10(12): 1911-1918	2

As stated by ≥2 participants.

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FLT3 Edu P TKD Testing Comments

- One hundred and twenty seven of 130 (97.7%) participants returning data for FLT3 Edu P detected a *FLT3* TKD variant.
- Of the three participants reporting a false negative result for FLT3 Edu P one utilised droplet digital PCR, one utilised an in-house assay with mass spectrometry (MASSArray) analysis and one used a Qiagen QiaSeq custom panel with ThermoFisher Ion Torrent next generation sequencing.
- In total, 53 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:c.2508_2510del p.(Ile836del) variant.
- In line with expectation, 48 (90.6%) participants reported HGVS consistent with a c.2508_2510del *FLT3* TKD variant. Of these, nine participants described the deleted nucleotide material (c.2508_2510delCAT). HGVS Nomenclature recommendations do not endorse listing the duplicated nucleotide sequence as this creates a longer description with redundant information[5].
- A further participant reported an out of consensus c.2503_2505del variant. This participant utilised an in-house assay with Sanger sequencing.
- Two participants reported a NM_004119.3:c.2503G>T variant, with a further participant reporting a NM_004119.3:c.2505T>G variant. All three participants employed in-house assays, with those detecting the c.2503G>T variant using agarose gel electrophoresis and the c.2505T>G participant using capillary electrophoretic analysis.
- One participant identified a variant but reported HGVS nomenclature suggesting that the genetic variation could not be mapped to a unique location, so multiple possible descriptions were given, separated by a ^ (caret)[5]. The reported DNA HGVS was NM_004119.3(FLT3):c.(2503_2508)delinsN[?]^ NM_004119.3(FLT3):c.2505T>V^ NM_004119.3(FLT3):c.2508C>D.
- For the protein level HGVS nomenclature, informative data relating to the amino acid substitution was returned by 57 participants; 49 (86.0%) reported the deletion of isoleucine at position 836 of the protein.
- Two participants reported the substitution of aspartic acid with glutamic acid at position 835 (p.(Asp835Glu)). A further two participants reported the substitution of aspartic acid with tyrosine at position 835 (p.(Asp835Tyr)).
- Two participants reported the substitution of aspartic acid with an unknown amino acid (p.Asp835? (n=1) / X (n=1)). HGVS recommendations utilise International Union of Pure and Applied Chemistry (IUPAC) symbols for DNA and protein descriptions. The X symbol in protein descriptions represents an unknown amino acid, however, given that X can be utilised to indicate a translation stop codon in a nonsense variant, HGVS suggest use of the three-letter amino acid code, Xaa[5].
- An additional participant reported the deletion of aspartic acid at position 835 of the protein, p.Asp835del.
- One participant, who reported possible HGVS DNA descriptions, also provided protein descriptions: NP_004110.2:p.(Asp835Tyr^His^Asn^Glu^Val^Gly)^ NP_004110.2:p.(Asp835_Ile836)delinsX[?].
- From all HGVS Nomenclature submissions by participants, 13 out of 57 (22.8%) reported information without parentheses. 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. For further information, please refer to the HGVS Nomenclature website: (https://hgvs-nomenclature.org/stable/)[5].

- In total, 58 participants returned quantification data for the *FLT3* TKD variant. The most commonly used quantification method was the Mut/(Mut+WT) x 100 calculation, reported by 38 participants (65.5% of returns), followed by the Mut/WT calculation reported by eight participants (13.8% of returns), with three participants (5.2%) reporting use of the Mut/WT x 100 calculation.
- Calculation using Next Generation Sequencing based methods was reported by two participants (3.4% of returns). A further participant reported the use of area under the curve. Six participants did not specify the quantification calculation information.
- The median variant load reported (Mut/(Mut+WT) x 100 quantification calculation) (n=38) was 27.1%, with an interquartile range (IQR) of 4.2%. Variant loads utilising this calculation method ranged from 16.7-62.0%.

We would like to take this opportunity to thank participants who returned data for FLT3 Educational Sample P.

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).
5. Human Genome Variation Society (HGVS) Nomenclature. Available at: https://hgvs-nomenclature.org/stable/. (v21.1.3) (Accessed: 21 October 2025).

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
e-mail: amanda.newbould@ukneqasli.co.uk

4.8.2 b) The coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (acting Director).

4.8.2 c) Person(s) authorising this report: Mr Stuart Scott (acting Director) of UK NEQAS LI.

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited.

4.8.2 d) Pre issue and post closure testing of samples for this programme is externally provided, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were externally provided.

4.8.2 d) Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

4.8.2 g) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel is informed 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/