UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

BRAF p.Val600Glu (V600E) Mutation Status for Hairy Cell Leukaemia Programme

Distribution - 232402

Participant ID -

Date Issued - 03 July 2023

Closing Date - 11 August 2023

Trial Comments

This trial was issued to 75 participants, of which 71 (94.7%) submitted results. One participant prenotified us of their intention not to return results, and one requested an extension to the submission deadline.

Sample Comments

Two vials of lyophilised cell line derived material were manufactured and issued by UK NEQAS LI . Both BRAF 158 and BRAF 159 were manufactured to be positive for the BRAF p.Val600Glu (V600E) variant.

Results and Performance

Your Results

BRAF Mutation Status	Your Results	Consensus Result
Sample BRAF 158	Mutation Detected	Mutation Detected
Sample BRAF 159	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample BRAF 158	66	4
Sample BRAF 159	71	0

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 Type

	Returns
DNA	69
cDNA	2

Please note that only PCR, Protocol and Analysis types used by ≥2 participants are included in the tables below.

PCR Type

	Returns
Allele Specific PCR	16
Droplet Digital PCR	16
PCR for Next generation Sequencing	12
Real-Time PCR	12
Single PCR	10
Multiplex PCR	4

Protocol Type

	Returns
In-house Assay	44
BioRad PrimePCR ddPCR kit	13
Biocartis Idylla	3
Custom QIAseq Lymphoid Panel	2
Diatech Pharmacogenetics Easy Kit	2

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	16
Digital PCR	12
NGS (Illumina)	11
Capillary Electrophoresis	6
Digital PCR (Biorad)	6
NGS (ThermoFisher Ion Torrent)	5
Agarose Gel Electrophoresis	4
Biocartis Idylla	3
Sanger Sequencing	3
Acrylamide Gel Electrophoresis (PAGE)	2

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Journal Reference for Assay

	Returns
Tiacci E. et al (2012). Blood, 119:1 - 192-195	10
Arcaini L. et al (2012). Blood, 119:1, 188-191	8
Wong C. et al (2005). J Clin Pathol, 58, 640-644.	5
Huang T. et al (2013) Biomark Res, 16:1, 3	3
Rustad EH. et al. (2015) Blood Cancer J 5: e299	3
Ellison G. et al (2010). J Exp Clin Cancer Res; 115, 21-28	2
Guerrini, F. et al., (2016) Front Pharmacol, 7:363.	2
Liu X. et al. (2020) Front Genet 11: 147	2

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Trial Summary

Sample BRAF 158

- Sample BRAF 158 was manufactured to have a *BRAF* p.Val600Glu (V600E) variant load towards the lower end of those observed in patients with hairy cell leukaemia (HCL); hairy cells have previously been reported at levels as low as 2% in peripheral blood from HCL patients[1].
- In line with sample formulation, 66 participants (93.0%) returning results for this trial detected the *BRAF* p.Val600Glu (V600E) variant in sample BRAF 158.
- Four participants failed to detect *BRAF* p.Val600Glu (V600E), all employing different techniques. One participant utilised the NGS based Oncomine Focus Assay (ThermoFisher Ion Torrent), the other three used in-house assays: single PCR followed by Sanger sequencing; single PCR followed by SNaPshot (Mini sequencing); Next Generation Sequencing (Illumina platform) with cDNA as the input material. We suggest that these participants review the limit of detection of their assay in the context of HCL patients with low *BRAF* p.Val600Glu (V600E) allele burdens[1].
- Furthermore, the NGS user (cDNA as input material) is known to have initially experienced difficulties producing nucleic acid of a suitable quality for downstream processing. We remind participants not to submit results obtained using nucleic acid that does not meet local quality control criteria; instead, repeat samples are generally available for all our programmes and can be requested via email: repeatsamples@ukneqasli.co.uk
- A fifth participant also submitted an out of consensus negative result for this sample, however they informed us that they routinely perform cell selection (prior to DNA extraction and Sanger sequencing), in order to improve test sensitivity. Since it is not possible to apply their enrichment methodology to the lyophilised cell samples provided in this programme, this participant was excluded from scoring for sample BRAF 158.
- Of the three participants stating the use of Sanger sequencing as an analysis method in this trial, only the participant discussed above also stated the use of an enrichment technique for clinical samples, and of the two other Sanger sequencing users, only one was able to detect the lower level *BRAF* p.Val600Glu (V600E) variant in this sample. **We remind participants that Sanger sequencing, in the absence of an enrichment method, is considered an inadequate technique for the detection of *BRAF* p.Val600Glu in HCL.**

Sample BRAF 159

- In line with formulation of sample BRAF 159, all 71 participants (100%) correctly detected the *BRAF* p.Val600Glu (V600E) variant in this sample. BRAF 159 was formulated to have a high allele burden.

General Comments

- The Human Genome Variation Society (HGVS) nomenclature guidelines now include a recommendation to use transcript reference sequences specified by the Matched

Annotation from the NCBI and the EMBL-EBI (MANE) collaboration project[2]. In the preliminary versions of the MANE project, the MANE Select transcript for *BRAF* was designated as NM_001374258.1 / ENST00000644969.2 (producing the *BRAF* c.1919T>A p.Val640Glu description). However, in response to feedback and in recognition of the legacy nomenclature for this gene, the MANE Select transcript has now been revised to NM_004333.6 / ENST00000646891.2, as specified in the recently published MANE v1.0 transcript set[3]. The NM_001374258.1 reference sequence (longest transcript), has been retained as the MANE Plus Clinical transcript, and its use is also acceptable. The use of Locus Reference Genomic (LRG) reference sequences is no longer advocated by the HGVS when a MANE transcript option is available[2].

- The nomenclature used in this programme, *BRAF* c.1799T>A p.Val600Glu, complies with the current MANE Select transcript NM_004333.6. Please note that some annotation resources may not yet be updated to specify the current MANE Select transcript, and therefore caution should be exercised when using software tools that may not be aligned with MANE v1.0.
- We thank participants for their continued engagement in the *BRAF* p.Val600Glu (V600E) Mutation Status for Hairy Cell Leukaemia programme.

References

1. Arcaini, L. *et al.* The BRAF V600E mutation in hairy cell leukemia and other mature B-cell neoplasms. *Blood* **119**: 188–191 (2012).
2. den Dunnen, J.T. *et al*. HGVS recommendations for the description of sequence variants: 2016 update. *Hum.Mutat.* **37**: 564-569 (2016). https://varnomen.hgvs.org/recommendations/general/ (Accessed October 2022).
3. Morales, J. *et al*. A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* **604**: 310-315 (2022).

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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
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) Pre issue testing of samples for this programme is subcontracted, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were subcontracted. Where subcontracting occurs, it is placed with a competent subcontractor and the EQA provider is responsible for this work.

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/