

Pilot *BCR-ABL1* Kinase Domain Mutation Status Programme

Participant No:

Trial No: KDM 151602

Date Issued: 24th February 2016

Closing Date: 25th March 2016

Two vials of lyophilised cell line material (samples KDM 118 and KDM 119) were distributed to 65 participants for *BCR-ABL1* kinase domain mutation (KDM) analysis.

In order to best mimic clinical material, samples were formulated from a mixture of cell lines. Sample KDM 118 contained a background cell line lacking the *BCR-ABL1* p210 (major) transcript, a calculated proportion of a *BCR-ABL1* p210 positive cell line expressing a 'wild-type' (non-point mutated) *BCR-ABL1* fusion transcript and a calculated proportion of a *BCR-ABL1* p210 positive cell line harbouring a specific kinase domain point mutation.

Sample KDM 119 contained a background cell line lacking the *BCR-ABL1* p210 (major) transcript and a calculated proportion of a *BCR-ABL1* p210 positive cell line expressing a 'wild-type' (non-point mutated) *BCR-ABL1* fusion transcript only.

For this trial, 58 (89.2%) participants returned results. Of the 7 participants not to submit data, 1 laboratory pre-notified us of their non return.

We would like to acknowledge Dr Paul La Rosée (University of Jena), who kindly donated the material for this programme. We are also grateful to Novartis who have previously supported this pilot EQA scheme through an educational grant and Prof Johan den Dunnen (Leiden University and Human Genome Variation Society) for guidance regarding nomenclature.

Your Results

Consensus result nomenclature in accordance with Human Genome Variation Society (HGVS) guidelines (*ABL1*:NM_005157.5).

Table 1: Sample KDM 118

	DNA sequence change	Amino acid change
Your result		
Consensus result	c.944C>T	p.Thr315Ile

Please refer to table 3 for all results returned for sample KDM 118.

Table 2: Sample KDM 119

	DNA sequence change	Amino acid change
Your result		
Consensus result	No mutation detected	No mutation detected

Please refer to table 4 for all results returned for sample KDM 119.

Detailed Results Breakdown

Please note results returned by participants, at both the cDNA and protein level, may have been harmonised to the equivalent Human Genome Variation Society (HGVS) approved nomenclature during the compilation of tables 3 and 4 (*ABL1*:NM_005157.5, <http://www.hgvs.org/mutnomen/>). Percentage values quoted have been subjected to rounding up/down to 1 dp.

Table 3: Results returned for sample KDM 118

	Number of participants
DNA sequence change	
c.944C>T	55 (94.8%)
c.943C>T	1 (1.7%)
No mutation detected	2 (3.4%)
Amino acid change	
p.Thr315Ile	56 (96.6%)
No mutation detected	2 (3.4%)

Table 4: Results returned for sample KDM 119

	Number of participants
DNA sequence change	
No mutation detected	58 (100.0%)
Protein amino acid change	
No mutation detected	58 (100.0%)

Method Breakdown

Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

Table 5: Input (template) material

	Number of participants
cDNA	58
DNA	0

Table 6: PCR method

	Number of participants
Nested reverse transcriptase PCR	30
Semi-nested reverse transcriptase PCR	18
Single reverse transcriptase PCR	9
Other	1

Table 7: Target(s) of PCR amplification strategy

	Number of participants
<i>BCR-ABL1</i> fusion (targeting <i>ABL1</i> on the translocated chromosome 9)	50
<i>ABL1</i> (targeting <i>ABL1</i> on the non-translocated chromosome 9)	1
Both	5

Table 8: Analysis method

	Number of participants
Sanger sequencing	55
Ion Torrent PGM (NGS)	2
Allele specific PCR	1
Pacific Biosciences PacBIO RSII (NGS)	1

Table 9: Sequencing approach

	Number of participants
Both forward and reverse (bidirectional)	56
Forward (unidirectional)	2
Reverse (unidirectional)	0

Table 10: Region analysed

	Number of participants
~200-500aa	45
~220-420aa	1
200-400aa	1
207-470aa	1
210-420aa	1
220-400aa	1
220-497aa	1
224-480aa	1
228-490aa	1
230-480aa	1
230-498aa	1
232-486aa	1
235-480aa	1
237-414aa	1

aa = Amino acid position assumed *ABL1*:NM_005157 (NCBI GenBank) derived translation, however reference sequence in use may vary amongst participants.

Table 11: Reference sequence used :

	Number of participants
NM_005157.5 *	20
NM_005157.4 *	12
NM_005157 * (version not provided)	8
ENST00000318560 * (Ensembl Transcript)	4
NM_005157.3 *	3
M14752 ^	2
ENSG00000097007 (Ensembl Gene)	3
X16416 ^	1
ENST00000372348.5 (Ensembl Transcript)	1
Not provided	5

* *ABL1* mRNA isoform a, ^ historic source sequences for NM_005157.

Table 12: Assay journal references (as reported by participants)

	Number of participants
Branford S. et al (2002). Blood 99:9, 3472-3475	16
Hochhaus A. et al (2002). Leukemia, 16:11, 2190-2196	11
Soverini S. et al (2004). Clinical Chemistry, 50:7, 1205-1213	7
Branford S. and Hughes T. (2006). Myeloid Leukemia Methods and Protocols eISBN 1-59745-017-0, 93-106	7
Ernst T. et al (2008). Haematologica, 93:2, 186-192	5
Alikian M. et al (2012). American Journal of Hematology 87:3, 298-304	3
Polakova K. et al (2008). Leuk Res 32:8, 1236-1243	3
Gruber F. et al (2005). Leukemia, 19:12, 2159-2165	1
Branford S. et al (2006). Leukemia, 20, 1925-1930	1
Wang L. et al (2006). Haematologica, 91:2, 235-239	1
MODHEM (Network for Molecular Diagnostics of Hematologic Malignancies)	1
In house method (no reference)	11
Other	4

Quantification

Figures in the tables below have been compiled from quantification data submitted by participants where a defined calculation approach was stated. Descriptive statistics are included for those returns stating a PCR strategy (cDNA template input material) which targets the *BCR-ABL1* fusion (*ABL1* on the translocated chromosome 9) prior to sequencing analysis.

Table 13: Sample KDM 118 quantification data

c.944C>T, p.Thr315Ile					
Calculation approach	n	Mutant quantification			
		Mean	Mode	Median	Range
Mut/(Mut+WT) x 100	9	24.67	25.00	25.00	18-40
Mut/WT x 100	5	30.00	N/A	30.00	20-40
Mut/CG x 100	1	N/A	N/A	N/A	N/A
TOTAL	15				

N/A = Not Applicable, Mut = Mutation, WT= 'Wild-type', CG = Control Gene (*ABL1*) for an RT-PCR based assay.

Since all participants correctly reported sample KDM 119 as having no mutation detectable, there is no quantification data for this sample.

Trial Summary

Sample KDM 118

- Of the 58 laboratories that returned results, 56 (96.6%) detected a kinase domain mutation (KDM).
- Fifty five (94.8%) laboratories reported the sequence change as c.944C>T at the cDNA level. The other laboratory reported a likely equivalent result of c.943C>T. They utilised nested reverse transcriptase PCR and Sanger sequencing and used the NM_005157.4 reference sequence.
- All 56 participants who detected a kinase domain mutation (KDM) described their result as p.Thr315Ile at the protein level.

Sample KDM 119

- All 58 participants returning a result for this sample correctly reported no mutation detectable.

Final Remarks

Sanger sequencing remains the analysis method of choice for the majority of participants (93.2% of returning laboratories). A minority of centres are utilising alternative techniques; including Next Generation Sequencing (NGS) platforms which offer increased sensitivity (Table 8).

With the aim of facilitating the appropriate comparison of quantification data we asked participants to state the amplification target(s) of their PCR approach (Table 7). Please note that for *BCR-ABL1* positive Chronic Myeloid Leukaemia cases, clinically relevant kinase domain point mutations (potentially conferring tyrosine kinase inhibitor (TKI) treatment resistance) are present on the disease associated *BCR-ABL1* fusion allele. Therefore, *ABL1* on the translocated chromosome 9 should be the amplification target for assays involving subsequent sequencing.

Fifty three (91.4%) participants submitting results provided an *ABL1* reference sequence with 20 laboratories utilising the latest version of the most appropriate transcript (nuclear isoform a, NM_005157.5). No laboratories stated the use of an isoform b (NM_007313) based reference sequence (Table 11). The NCBI GenBank *ABL1* gene webpage (www.ncbi.nlm.nih.gov/gene/25) is a valuable resource for obtaining relevant reference sequences at both a protein and DNA level.

As highlighted in the previous *BCR-ABL1* kinase domain mutation (KDM) trial reports some participants are beginning to return results (both at the DNA and protein levels) which incorporate the HGVS symbols intended to communicate allelic relationships and/or associated uncertainty. For example, the use square brackets or parentheses to envelope a sequence description and the implementation of various separators (forward slash or semi-colon). There is currently only limited best practice guidance from the HGVS¹ and relevant professional bodies^{2,3} regarding the specific reporting requirements for somatic mutations; including the nomenclature symbols and syntax to be employed for the communication of allelic relationships when reporting multiple acquired mutations (for example, polyclonal or compound kinase domain mutations)⁴.

Parentheses are typically used in the context protein level nomenclature to indicate a deduced prediction from the direct analysis of DNA (without supporting experimental evidence). When reporting amino acid change(s) at the protein level identified from the analysis of RNA (or cDNA) the use of parentheses may not be required. Further

clarification from the Human Genome Variation Society (HGVS) has been sought by UKNEQAS LI and is ongoing. Please refer to the related HGVS links below.

- <http://www.hgvs.org/mutnomen/recs-prot.html>
- <http://www.hgvs.org/mutnomen/disc.html> (Discussion, see 'Protein description between brackets' section)

Please also refer to the related general recommendation for the description of multiple sequence variants identified in an individual and the modifications made to the recommendations for mosaicism based nomenclature (following the acceptance of proposal SVD-WG001) provided in the HGVS links below.

- www.hgvs.org/mutnomen/recs.html#2in1
- www.hgvs.org/mutnomen/examplesDNA.html (see 'Miscellaneous' section)
- www.hgvs.org/mutnomen/disc.html#1or3 (Discussion, see 'Two sequence variants in one individual' section)
- <http://www.hgvs.org/mutnomen/FAQ.html#mosaic> (FAQs, see 'Mosaic cases')

The cell lines utilised in this pilot programme have been pre-validated by the The European Treatment Outcome Study (EUTOS) group. However, it is important to note they are cell lines stably transfected with cDNA constructs and therefore do not exactly reflect the genetic context of clinical samples. At this time no alternative suitable material exists for the purposes of *BCR-ABL1* kinase domain mutation testing EQA.

It is important to critically evaluate whether unanticipated variants are biological or technical artefacts. It appears evident that some of the engineered cell lines employed in this programme harbour change(s) in addition to the target *BCR-ABL1* point mutation. It is hoped that continued work by the cell line creators, UK NEQAS LI and our programme participants will assist in better determining their exact mutational profile.

The pilot phase of this programme aims to improve understanding of the available material and survey the methodologies employed by participants in clinical practice. We hope to further develop the programme to enable UKAS ISO:17043 accreditation and the application of a validated scoring system in the future. We would encourage participants to share any comments they may have that might help us to improve subsequent *BCR-ABL1* kinase domain mutation (KDM) trials. Please do contact us (admin@ukneqasli.co.uk) with any related suggestions or comments.

Reference(s)

1. Den Dunnen, J. T. & Antonarakis, S. E. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum. Mutat.* **15**, 7–12 (2000).
2. Smith, K. *et al.* ACGS General Genetic Laboratory Reporting Recommendations (2015).
(www.acgs.uk.com/media/925294/acgs_general_genetic_laboratory_reporting_recommendationdraft.pdf)
3. Richards, S *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine* **17**, 405-424 (2015).
4. Khorashad, J. S. *et al.* BCR-ABL1 compound mutations in tyrosine kinase inhibitor-resistant CML: frequency and clonal relationships. *Blood* **121**, 489–498 (2012).

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: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:

Prof David Barnett, Director or Mr Liam Whitby, Operations 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/