- Of note, one participant reported detection of the 4 bp (TCTG) tandem duplication despite the *NPM1* gene not featuring on their stated panel. **We ask that participants please check their Laboratory Record (held in the Participant Hub area of the UK NEQAS LI website) carefully during trial results submission to ensure it is complete, and accurately reflects current methodology in relation to this programme.**

76 laboratories identified a missense change at the c.2645 position in the *DNMT3A* gene. The c.2645G>A, p.(Arg882His) variant was identified by 75 (98.7%) laboratories and classified by participants as a variant of strong (n = 68) or potential (n = 8) clinical significance.

- The median VAF (DNA based assays, all panels and platforms) reported for the *DNMT3A* c.2645G>A variant was 44.8 % with an interquartile range of 2.8%.
- Variants (mutations) in *DNMT3A* are frequently identified in patients with AML; position p.Arg882 represents a recurrent 'hotspot'[11-14]. *DNMT3A* is responsible for *de novo* methylation of the genome during mammalian development. Aberrant *DNMT3A* variants are thought to play an important role in the aetiology of leukemogenesis[13].
- Please also refer to the AML and MDS GP 192001 trial report for further comments regarding this variant and available evidence regarding clinical significance. If you are a new participant and do not have access to previous trial reports please contact us for assistance.
- One participant reported an out of consensus *DNMT3A* result at the DNA level (c.2645C>T) and another at the protein level (p.Arg88His); both are suspected to be typographical errors.
- Three laboratories failed to report a *DNMT3A* variant at position c.2645. For one laboratory the *DNMT3A* gene did not feature on their panel. Another participant did not submit the relevant information in their Laboratory Record regarding panel scope to ascertain if their result was a true false negative. The remaining participant (Illumina NextSeq, Qiagen GeneRead DNAseq targeted human myeloid neoplasms panel user) stated the inclusion of *DNMT3A* (NM_022552, all exons) within their assay scope and did not declare any coverage issues.

66 laboratories reported detection of at least one *FLT3* internal tandem duplication (ITD), all classifying the finding as of strong clinical significance.

- Over 70% (n = 47) of participants identifying an ITD(s) reported the detection of two ITDs.
- The *FLT3* ITDs were sized by laboratories as 36 bp (96.2%, 51/53 participants detecting this ITD with size information available) and 54 bp (89.1%, 49/55 participants detecting this ITD with size information available). For the 9 participants reporting an out of consensus ITD size (ranges 30-37 bp and 53-108 bp), 4 laboratories reported an ITD size predicted to produce an out of frame change at the protein level. *FLT3* ITDs are typically 'in-frame' with a size that is a multiple of 3 bp. One participant described the larger ITD as a 108 bp insertion (method information not stated).
- Where available, the nomenclature provided by participants was variable at the DNA level (despite universal reference to the NM_004119/ENST00000241453 transcript). Just over half (n = 37) of laboratories described the *FLT3* ITD(s) appropriately as a duplication event(s). Approximately 20% (n = 14) of participants inappropriately referred to the change(s) as an insertion(s). Six laboratories used mixed terminology (ins and dup). While the remaining 9 participant reported identification of an ITD(s) with no further nomenclature details provided.
- The nomenclature provided at the protein level was in far better agreement regarding positioning of the internal tandem duplication event(s). 89.1% (41/46) of participants described the 36 bp ITD

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as p.(Asn587_Glu598dup) and 90.1% (40/44) described the 54 bp ITD as p.(Asp593_Leu610dup).

- As previously discussed, the HGVS recommends the term duplication (dup) should be used when an additional copy of sequence is directly 3'-flanking the original copy ('tandem duplication'). When there is no evidence that the extra copy of a sequence detected is in tandem (directly 3'-flanking the original copy), the change should be described as an insertion (ins). For either description (dup or ins) the most 3' position possible of the reference sequence should be arbitrarily assigned to have been changed (3' rule) [9,10].
- Mutation of the *FLT3* gene occurs in approximately 30% of all AML cases, with an internal tandem duplication in the juxtamembrane domain representing the most common type (approximately 25% of all AML cases). *FLT3* ITD is a well-established driver variant. Its prognostic impact in patients with AML can be context dependent; adverse outcomes are particularly associated with *FLT3* ITD present at high allelic ratio. Due to the incorporation of *FLT3* ITD into many of the available AML classification systems, guidelines and reviews[7,8,15,16], evidence underpinning clinical significance will not be discussed further here.
- Some participants noted supplementation of their NGS based assay with fragment (size) analysis to ensure robust identification of *FLT3* ITD(s).
- Of note, Illumina platform users employing the Qiagen Gene Read DNAseq targeted human myeloid neoplasms panel (n = 3) or a Qiagen QIASeq custom panel (n = 1) reported additional *FLT3* missense variants of unknown clinical significance; c.1795T>A, p.(Tyr599Asn) (VAF ~6%) and c.1833G>T, p.(Glu611Asp) (VAF 14-41%, depending on read depth). At the time of writing both variants are not listed on any annotation resource accessed via Alamut Visual (version 2.11), reference sequence NM_004119.3 (*FLT3*) (GRCh37)[17].

The *TET2* gene features on almost all participant panels (n = 74). However, only a few laboratories (n = 5, 6.8%) report the c.86C>G, p.(Pro29Arg) missense variant, classifying it as of potential (n = 1) or unknown clinical significance (n = 4).

- The median reported VAF (47.3%) (DNA based assays, all panels and platforms) is in keeping with potential germline status.
- At the time of writing the NM_001127208.2(*TET2*):c.86C>G, p.(Pro29Arg) variant is listed on the COSMIC database (COSV54402572 with legacy reference COSM5020248)[18]. However, the entry is limited to 9 listed reports with only 1 of AML origin (no literature reference provided).
- The variant is listed on ClinVar (RCV000122131.1) by a single submitter (germline), clinical significance not provided[19]. The cited publication (Bodian *et al.*, 2014) describes whole genome sequencing of an ancestrally diverse cohort of 681 healthy individuals with the *TET2* c.86C>G, p.(Pro29Arg) variant detected at 6.7%[20].
- The current dbSNP entry (rs12498609, chr4:105234028 (GRCh38.p12)[21] features a global population MAF of 2.9% (sub-population range 2.2% - 22%) and gnomAD (4-106155185-C-G (GRCh37))[22] states an overall MAF of 6.1% (17,073/282,210) with 954 homozygotes noted for this *TET2* variant.
- Taken together, the information available supports the case for the *TET2* c.86C>G, p.(Pro29Arg) variant as a germline polymorphism of likely neutral clinical significance.
- Two laboratories reported identification of variants of strong clinical significance in the *TET2* gene. One laboratory (using Illumina Novaseq, Transcriptome (RNA-seq)) reported the missense change c.3638T>C, p.(Val1213Ala) with a VAF of 25.7% (low read depth noted at the variant position = 35). A further participant (using Illumina MiSeq, Agilent SureSelect custom QXT protocol DNA-seq) detected the c.626delA, p.(Asn209Metfs*41) variant with a VAF of 7% (read depth at the variant position = 604). Both laboratories stated a NM_001127208 or Ensembl equivalent transcript reference but no further annotation information was provided to support the

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classification. At the time of writing both variants are not listed on any annotation resource accessed via Alamut Visual (version 2.11), reference sequence NM_001127208.2 (*TET2*) (GRCh37)[17].

In conclusion, participant results generated a good consensus regarding the well characterised variants detected in sample AML GP 109. The interpretive classification of the extensively observed *NPM1* 4bp (TCTG) duplication and *FLT3* ITD(s) were in overall agreement for this trial. As previously discussed, (see AML and MDS GP 192001 trial report) the classification of the recurrent *DMNT3A* missense variant revealed a little less consistency amongst laboratories.

The approach to missense variant nomenclature was predominantly in line with HGVS recommendations. However, many laboratories inappropriately described the *NPM1* 4 bp (TCTG) duplication and *FLT3* ITD(s) as insertion events. Nevertheless, there was a clear consensus regarding the size of the identified *FLT3* ITD(s) when this information was provided by participants.

Despite similar median values, the VAFs reported for the *NPM1* 4 bp (TCTG) duplication demonstrated a wider interquartile range (10%, n = 76) than that for the *DNMT3A* missense substitution (2.8%, n = 73). Unfortunately, due to the nature of the quantitative information returned for the *FLT3* ITD(s) (a mixture of VAF % and allelic ratio, with some confusion regarding multiple ITDs) we were unable to calculate similar statistics. We acknowledge the limitations of the dataset.

Despite the *TET2* gene featuring widely on assay panels the restricted reporting of the *TET2* c.86C>G, p.(Pro29Arg) missense variant for this trial suggests an increased awareness amongst many participants of the occurrence of germline missense likely neutral variants in this polymorphic gene.

Thank you to laboratories for taking the time to answer the additional survey questions regarding this programme (>95% response rate). We are pleased that the implementation of the Laboratory Record system and educational elements of the trial report content have been so well received by participants. The feedback provided will help shape the future direction of this pilot programme.

The information provided herein is for participant information only. Clinical decision making with regards to variant interpretation, pathogenicity, actionability and predicted disease outcomes should not be based solely on comments provided by UK NEQAS LI in this EQA trial report.

Please do contact us if you have any suggestions regarding how this developmental (pilot) programme could be improved for future trial distributions.

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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:
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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/

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