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CORRESPONDENCE

Assessing the impact of the 2017 European LeukemiaNet recommendations on *FLT3* allelic ratio calculation and reporting in acute myeloid leukaemia

FLT3 internal tandem duplications (*FLT3*-ITDs) are the most common genetic aberration identified in acute myeloid leukaemia (AML) and have historically been associated with higher relapse rate and lower overall survival in AML.[1]

European LeukemiaNet recommendations (ELN) for the diagnosis and prognosis of AML outline three risk stratification groups as a method of standardising the reporting of genetic abnormalities relating to the clinical disease characteristics and prognostics.[2] The risk stratification groups outline the differing clinical outcomes depending on the presence/absence of genetic markers. With regards to *FLT3*, the risk stratification is based on *FLT3*-ITD allelic burden (allelic ratio) in addition to *NPM1* variant status. Patients without *FLT3*-ITDs or with low *FLT3*-ITD allelic ratio (AR) (<0.5) and with mutated *NPM1* are categorised as favourable risk.[2] Patients with high *FLT3*-ITD AR (>0.5) and mutated *NPM1* and patients with low *FLT3*-ITD AR or without *FLT3*-ITD (in the absence of other adverse-risk genetic lesions) and wild-type *NPM1* are considered intermediate risk. Finally, patients with high *FLT3*-ITD AR and wild-type *NPM1* are considered adverse risk.[2]

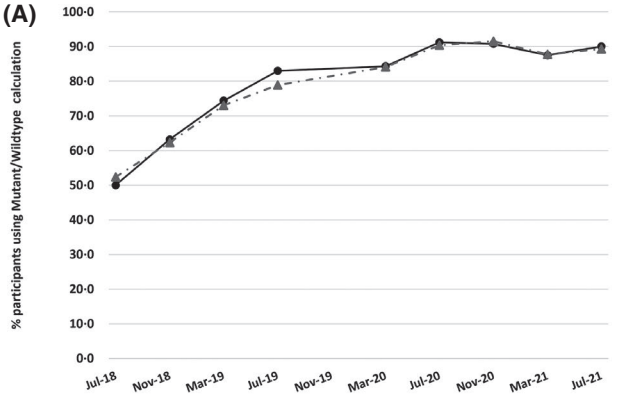
The United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping (UK NEQAS LI), has provided an International Organisation for Standardisation (ISO) 17043 accredited External Quality Assessment (EQA)/proficiency testing (PT) programme for laboratories performing *FLT3*-ITD testing since 2014. The aim of the programme is to promote harmonisation and standardisation of medical laboratory testing, facilitating improvement in testing by highlighting issues with methods returning out of consensus results. Since the publication of the ELN recommendations in 2017, UK NEQAS LI have gathered participant data on the calculation and reporting of *FLT3* AR in a diagnostic setting. We outline herein findings from the UK NEQAS LI *FLT3* EQA programme, over the course of a three-year period (July 2018–July 2021) and the impact of the ELN recommendations on *FLT3*-ITD AR calculation and reporting.

The ELN recommendations outline the calculation approach required for determination of *FLT3*-ITD AR, defined as: area under the curve (AUC) *FLT3*-ITD/AUC *FLT3*-wild type.[2] UK NEQAS LI has seen an increase in the overall use of the ELN-recommended approach to calculating *FLT3*-ITD AR (*FLT3*-ITD/*FLT3*-wild type) from 50.0% (11 out of 22 laboratories) in July 2018 to 90.0% (117 out of 130 laboratories) in July 2021 (Figure 1A). When considering European laboratories alone, there has been an increase in standardisation from 52.4% to 89.3%.

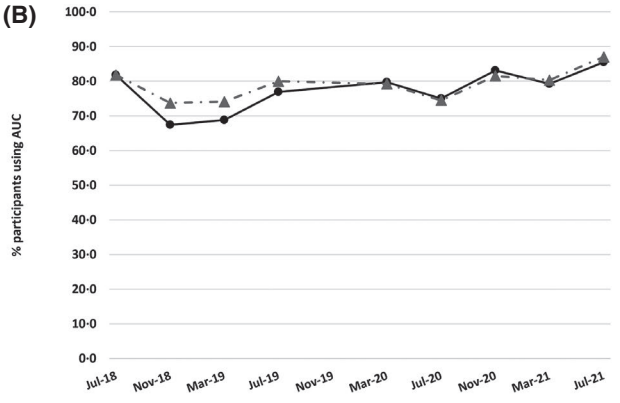
Whilst use of the ELN-recommended approach to calculating *FLT3*-ITD AR has significantly increased between July 2018 and July 2021, standardisation of the data used to quantify *FLT3*-ITD AR when considering use of the mutant/wild type only shows a small increase. In 2018, 81.8% (nine out of 11) laboratories initially reported the use of AUC data in the calculation of *FLT3*-ITD AR, compared to 85.5% (100 out of 117) in 2021 (Figure 1B). When considering European laboratories, 81.8% reported the use of AUC in *FLT3*-ITD/*FLT3*-wild type calculation in 2018, with 87.0% in 2021. Whilst use of AUC data was the most utilised approach to *FLT3*-ITD calculation across the analysis period, peak height was the second most used data approach. Comparative analysis performed across trial samples showed that there were no significant differences in the reported *FLT3*-ITD ARs when using AUC or peak height data (data not shown).

Several reports have highlighted the existence of multiple *FLT3*-ITDs presenting in patients with AML; the prevalence of these have ranged from 21.5% to 35.0%.[3–6] In July 2020, UK NEQAS LI issued a survey to laboratories regarding *FLT3*-ITD AR-reporting in patients with multiple *FLT3*-ITDs.

In total, 52 laboratories returned survey results. The demographic of the data returns indicated that 47 (90.4%) were within Europe. When considering the reporting of *FLT3*-ITD AR in patients presenting with multiple *FLT3*-ITDs, 28 (53.8%) participants report a combined AR to clinicians (the sum of all *FLT3*-ITD AUC divided by the *FLT3*-wild type AUC). Sixteen (30.8%) report each *FLT3*-ITD AR separately and eight (15.4%) participants reported both separate and combined *FLT3*-ITD AR to clinicians. The clinical significance of multiple *FLT3*-ITDs in AML patients is unclear in the *FLT3* inhibitor era. In one study 139 AML patients treated with the combination of *FLT3* inhibitor and intensive chemotherapy and having multiple *FLT3*-ITDs had no difference in overall survival (OS) when compared to patients with single *FLT3*-ITD mutations.[6] However, in a small



	Jul-18	Nov-18	Mar-19	Jul-19	Mar-20	Jul-20	Nov-20	Mar-21	Jul-21
% All (n=)	50·0 (11)	63·2 (43)	74·4 (32)	83·0 (39)	84·3 (59)	91·2 (52)	90·8 (59)	87·5 (77)	90·0 (117)
% Europe (n=)	52·4 (11)	62·3 (38)	73·0 (27)	78·9 (30)	84·1 (53)	90·4 (47)	91·5 (54)	87·7 (71)	89·3 (100)



	Jul-18	Nov-18	Mar-19	Jul-19	Mar-20	Jul-20	Nov-20	Mar-21	Jul-21
% All (n=)	81·8 (9)	67·4 (29)	68·8 (22)	76·9 (30)	79·7 (47)	75·0 (39)	83·1 (49)	79·2 (61)	85·5 (100)
% Europe (n=)	81·8 (9)	73·7 (28)	74·1 (20)	80·0 (24)	79·2 (42)	74·5 (35)	81·5 (44)	80·3 (57)	87·0 (87)

FIGURE 1 Assessment of laboratories performing *FLT3* internal tandem duplications (ITD) allelic ratio (AR) calculations in line with European LeukemiaNet (ELN) recommendations. (A) Laboratories utilising the correct approach in the calculation (*FLT3*-ITD/wild type). (B) Laboratories utilising the correct approach and data [area under the curve (AUC)] in the calculation

subset of patients, the benefit of treatment was more noticeable, particularly in younger individuals, with high *FLT3*-ITD AR, single *FLT3*-ITD and *NPM1* co-mutation. Prior to *FLT3* inhibitor treatment, some studies suggested AML patients with 2+ *FLT3*-ITDs have a reduced overall survival (OS[7] while others demonstrated no impact on OS.[3] Whilst different outcomes have been reported in patients presenting with multiple *FLT3*-ITDs, the method by which the studies calculated the AR also varied. Although these studies were published prior to publication of ELN recommendations, large cohort studies contribute to the synthesis of evidence required to inform the development of clinical recommendations. As such, this highlights the importance of clarification of the *FLT3*-ITD AR calculation method within the ELN recommendations in the hope of improving standardisation, allowing comparative analysis of patient data.

Overall, the findings indicate that there has been successful uptake and adoption of the guidelines for calculating the *FLT3*-ITD AR across international and European laboratories, but better in Europe, particularly when considering the calculation method. Recent clinical practice recommendations on haematopoietic stem cell transplant for patients with *FLT3*-ITD-positive AML have suggested that there is a global need to standardise the methodology for determining *FLT3*-ITD AR.[8] The recommendations suggest the need for universal calibration of all laboratories, in a similar manner to global efforts resulting in the introduction of the International Scale for *BCR–ABL1* quantification.[9,10] Whilst it is important to ensure continued adoption of the current ELN recommendations amongst laboratories, in light of the suggestions of universal *FLT3*-ITD AR calibration, there is a need for review of the *FLT3*-ITD AR calculation methods in future iterations of ELN recommendations, to guide laboratories on how to calculate and report multiple *FLT3*-ITD scenarios and to account for the transitions from traditional capillary electrophoresis methods towards identification of *FLT3*-ITD using modern Next-Generation Sequencing technologies.

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