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Blood 142 (2023) 6025–6026

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Establishment of an NPM1 Mutation Copy Number Estimator for Xpert ® NPM1 Mutation Test

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Objectives: The nucleophosmin (*NPM1*)is the most mutated gene (~30%) in Acute Myeloid Leukemia (AML) [1]. Three NPM1 mutations (type A, B, and D) represent ~84% in NPM1-mutated AML cases while other uncommon subtypes occupy ~16% [2]. Xpert® NPM1 mutation, an automated cartridge-based test for measuring NPM1 mutation transcript levels (type A, B and D), is standardized to quantify the amount of mutated NPM1 relative to ABL1 control gene based on delta Ct in peripheral blood [3]. Since mutated NPM1 level is crucial for risk assessment, medication selection, and ongoing therapeutic monitoring in AML [4,5], it is important to obtain the NPM1 mutation copy number (CN). The aim of this work is to develop NPM1 mutation CN estimator and to compare %NPM1 mutation/ABL1 reporting between delta Ct-based and CN-based methods.
Methods: Five levels of NPM1 mutations (A, B, D) and ABL1 IVT-RNA panels as well as two lots of Xpert® NPM1 mutation tests were used to generate standard curves for CN and %CN reporting. The cell lysates from cell lines carrying either NPM1 mutation A, B, or D and AML clinical samples containing NPM1 mutations were examined to evaluate the CN and %CN between two lots of the Xpert® NPM1 mutation tests and to compare the delta Ct-based and CN-based methods for reporting %NPM1 mutation/ABL.
Results: Linearity was demonstrated in Ct vs CN input for NPM1 mutation and ABL1 with R^2 above 0.96 for Lot1 and Lot2. Less than 3-fold difference was exhibited for CN and %CN across two lots of Xpert® NPM1 mutation test. Less than 3-fold difference was observed in %NPM1 mutation/ABL1 reporting between delta Ct-based and CN-based approaches.
Conclusion: An NPM1 mutation copy number estimator for Xpert® NPM1 mutation test was established, which will provide diagnostic and prognostic values for NPM1-mutated AML patients.

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Disclosures Yuan: *Cepheid:* Current Employment.

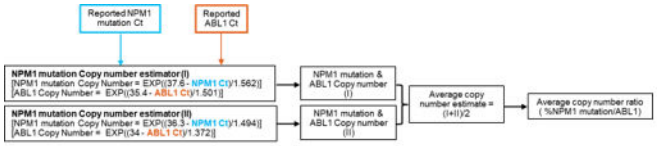

Figure 1: Two sets of NPM1 mutation copy number estimator for Xpert® NPM1 mutation test, which will provide diagnostic and prognostic values for NPM1-mutated AML patients. Enter reported NPM1 mutation Ct and ABL1 Ct into the formulas (I) and (II) to calculate the copy number. Average copy number of NPM1mutation and ABL1 from both formulas will be utilized in obtaining averaged %NPM1 mutation/ABL1.

Figure 1

https://doi.org/10.1182/blood-2023-172557