The detection and quantification of different sequence-variable NPM1 mutations using RNase H-dependent PCR

Gisela Gaina[1*], Dan Soare[1*], Ioana Lambrescu[1,2], Victor Ionescu[1], Alexandra Popa[1], Oana Mosoia[1], Mihai Pavalean[2,3], Laura Cristina Ceafalan[1,2] and Valeriu Cismasiu[1]

1. Cell Biology, Neurosciences and Experimental Myology Laboratory, Victor Babes Institute of Pathology, 050096 Bucharest, Romania
2. Department of Cellular and Molecular Biology and Histology, Carol Davila University of Medicine and Pharmacy, 050474 Bucharest, Romania
3. Urology Department, Central Military Emergency University Hospital, 010825 Bucharest, Romania

*Equal contribution

Background

The most common genetic abnormalities in adult acute myeloid leukemia are due to Nucleophosmin gene (NPM1) mutations.The *NPM1* gene mutation is also included in this category where more than 24 variants of the gene are known of which 6 are more common.

The aim of our study was to establish a qPCR assay and a set of primers for detection and quantification of *NPM1* sequence variations regardless of its sequence by using RNase H-dependent PCR (rhPCR).

Materials and Methods

Synthetic fragments of *NPM1* carrying mutations (AML mutation type A, B, D, I, K) were used. Several sets of rh-primers that show point mismatches in relation to some mutations have been designated and tested. The rh-primers carried a removable PCR blocker at the 3′ end. Only after the removal of this blocker by the RNaseH2 enzyme, the primers are expandable by DNA polymerase. qPCR was used to determine the extent to which the presence of these sequence mismatches could affect the H2-endonuclease activity of ribose digestion and PCR activation. qPCR was performed on QuantStudio 7 Flex RealTime PCR system.

Results

After establishing the optimal combination for the primer sets as well as the reaction conditions, the sensitivity limit was tested in samples with the number of target copies that varied decreasingly: 1000000, 100000, 10000, 1000, 100, 10. It was observed that regardless of the nature of the mutation, the same set of primers can detect 10 copies of mutant *NPM1*.

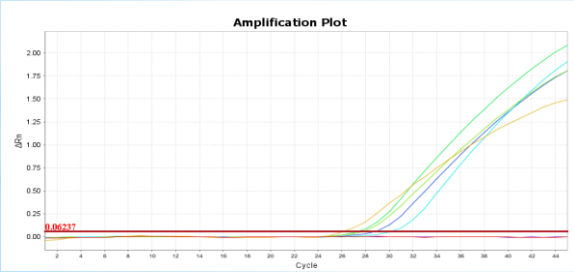

Fig 1 The amplification plots of gBlocks (A…K) with best match qPCR primer sets, along with their respective standard curves

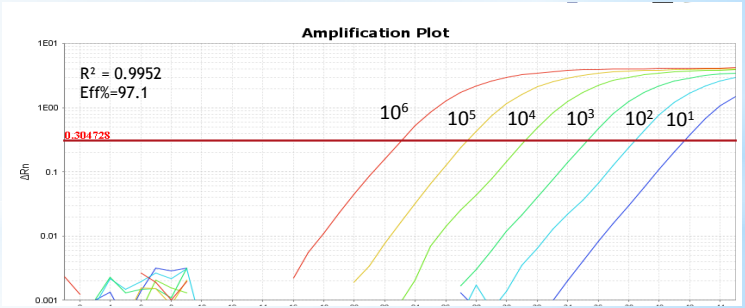

Fig 2 Amplification curve of qPCR showing 10 fold serial dilution of the samples (10^6 to 10 copies per reaction)

Conclusions

The main advantage of this assay is that different sequence-variable *NPM1* mutations can be detected and quantified with high specificity through a single rhPCR reaction. It is no longer necessary to design, synthesize and optimize primers and probes for each individual mutation. Our study demonstrates the potential use of rhPCR for monitoring patients with AML for minimum residual disease and recurrence risk.

Acknowledgement: National Program 1N/2019/PN19.29.01.03.