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Letter to the Editor

Diagnostic Standardization of Leukemia Fusion Gene Detection System using Multiplex Reverse Transcriptase-polymerase Chain Reaction in Korea

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We read the interesting article "Spectra of Chromosomal Aberrations in 325 Leukemia Patients and Implications for the Development of New Molecular Detection Systems" in a recent issue of the Journal of Korean Medical Science by Choi et al. (1). We would like to add some comments on these data for suggesting a diagnostic standardization of detection of leukemia-associated gene rearrangements in Korea.

Choi et al. (1) reported that they were able to detect leukemic fusion genes among 42% of the entire leukemia patient group, a relatively high ratio, by implementing multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) analysis to 325 leukemia patients over 4 yr. The multiplex RT-PCR kits (HemaVision, DNA technology, Aarhus, Denmark) that was used in the study by Choi et al. (1) are useful diagnostic tools that can simultaneously detect 28 different fusion genes and 80 or more breakpoints or splicing variants, enabling concurrent screening and confirmation of various leukemia-related gene rearrangements through "Master PCR" (day 1#) and "Split-out PCR" (day 2#). Based on the experience accumulated by the authors using the same multiplex RT-PCR kits at our institution for recent years, we would like to provide a more detailed comment on the merits and shortcomings of this test kit.

First and foremost, the most important merit of HemaVision kit is that the screening tests by its "Master PCR" can detect unexpected rare chromosomal translocations and/or cryptic gene rearrangements among new patients with various subtypes of leukemia. For example, we were able to identify a very rare *NPM1-MLF1* rearrangement from an acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) patient who accompanied a complex karyotype by utilizing the multiplex RT-PCR testing method (2). Moreover, as the test allows for a clear detection of various alternative splicing and splice variants on gene rearrangements such as *BCR-ABL1*, *PML-RARA*, and *CBFB-MYH11*, we were even able to identify a new type of variant fusion gene that was not reported on rare occasions (3, 4). Also, in case of detection of a rare fusion gene, a "Split-out PCR" of HemaVision kit enables a semiquantitative monitoring and follow up on the minimal residual disease (MRD). From a quality control perspective, HemaVision is better off than the homebrew RT-PCR that has been used in Korean medical institutes in terms of maintaining quality control of the results as one can simultaneously check internal control bands at 911bp size during the test, and also because the consistency and reliability of the test result is high. However, it is impossible to detect all types of gene rearrangements related to leukemia by HemaVision kits (notice that one can only detect 28 types of fusion genes), and there are some practical limitations as to the high cost of the kit itself as well. For instance, HemaVision cannot detect all types of *MLL* partner genes but only about 10 types, while there are about 70 types of *MLL* partner genes including the *CASP8AP2* gene that was recently identified by the authors with respect to *MLL* rearrangements (1, 5). In the future, however, we hope that additional types of *MLL* gene rearrangements could be included in such multiplex RT-PCR kits, based on the *MLL* rearrangement status for Korean acute leukemia patients and on practical data such as domestic incidence of *MLL* partner gene by type, studies that are being conducted by the authors as part of the AML-MDS working party project.

There is no doubt that the research results by Choi et al. is the most representative data related to the detection of leukemia-specific gene rearrangements by multiplex RT-PCR at a single medical center in Korea, but the recommended 3 types of cytogenetic abnormalities in their paper that are to be included in the new multiplex RT-PCR system does not seem to be appropriate. Firstly, t(8;14)(q24;q32) seems to be a cytogenetic abnormality which is better suited for the FISH assay rather than the RT-PCR method since chromosomal translocation occurs by a juxtaposition of the *C-MYC* (8q24) gene towards the *IGH* (14q32) gene, and there is no hybrid gene. Secondly, although t(3;3)(q21;q26) is a recurrent cytogenetic abnormality for AML that

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was newly introduced in the 2008 WHO classification (6), its frequency is known to be relatively low, and it would be more suitable to use EVI1 FISH probe in order to simultaneously detect variants of t(3;3) such as inv(3)(q21q26) and ins(3;3)(q26;q21q26), as well as t(3;21) that is related to *RUNX1-MDS1-EVI1* rearrangement. And the lastly mentioned i(17)(q10) cannot be detected by RT-PCR since it is not a gene rearrangement but a chromosomal abnormality that is related to structural aberration. Additional comments by Choi et al. would be helpful to clarify on these issues.

In conclusion, detecting gene rearrangements for leukemia patients using multiplex RT-PCR kits such as HemaVision seems to be a highly useful molecular diagnostic method for Korean medical institutions in these days. While there are some practical limits as mentioned above, a widespread implementation of this method throughout the majority of medical institutions in Korea will significantly contribute to the standardization of leukemia diagnosis and guarantee high quality test results and efficient quality control. For the issue of what additional gene rearrangements in leukemia patients should be included in the multiplex RT-PCR test panel, a continued research and additional discussion would be necessary rather than adhering to the current provisional conclusions.

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