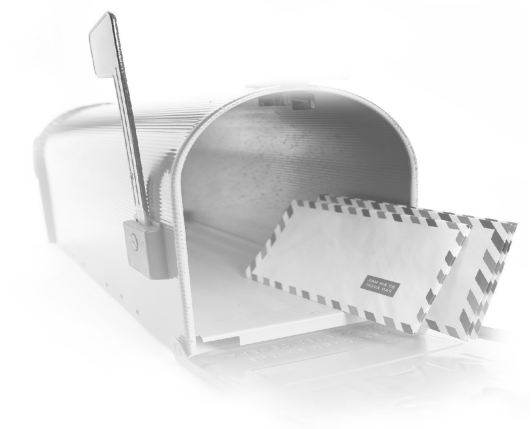

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Letters to the Editor

Comparative evaluation of the developed targeted RNA sequencing system and a commercialized test panel

TO THE EDITOR: Genetic mutations that occur in blood cancer can be broadly classified into nucleotide sequence mutations, including duplications or deletions; fusion gene mutations; and abnormal gene overexpression. Among these genetic mutations, gene fusion not only plays an important role in the development of blood cancers such as leukemia, it is also important as an essential marker for diagnosis, risk assessment, optimal treatment selection, and residual lesion detection. Most clinical hematology laboratories use conventional cytogenetic testing, fluorescent in situ hybridization (FISH), and multiplex reverse transcriptase-PCR to detect gene fusion mutations [1, 2]. However, the above methods currently used have great limitations in detecting various gene fusions that occur in blood cancer. To overcome

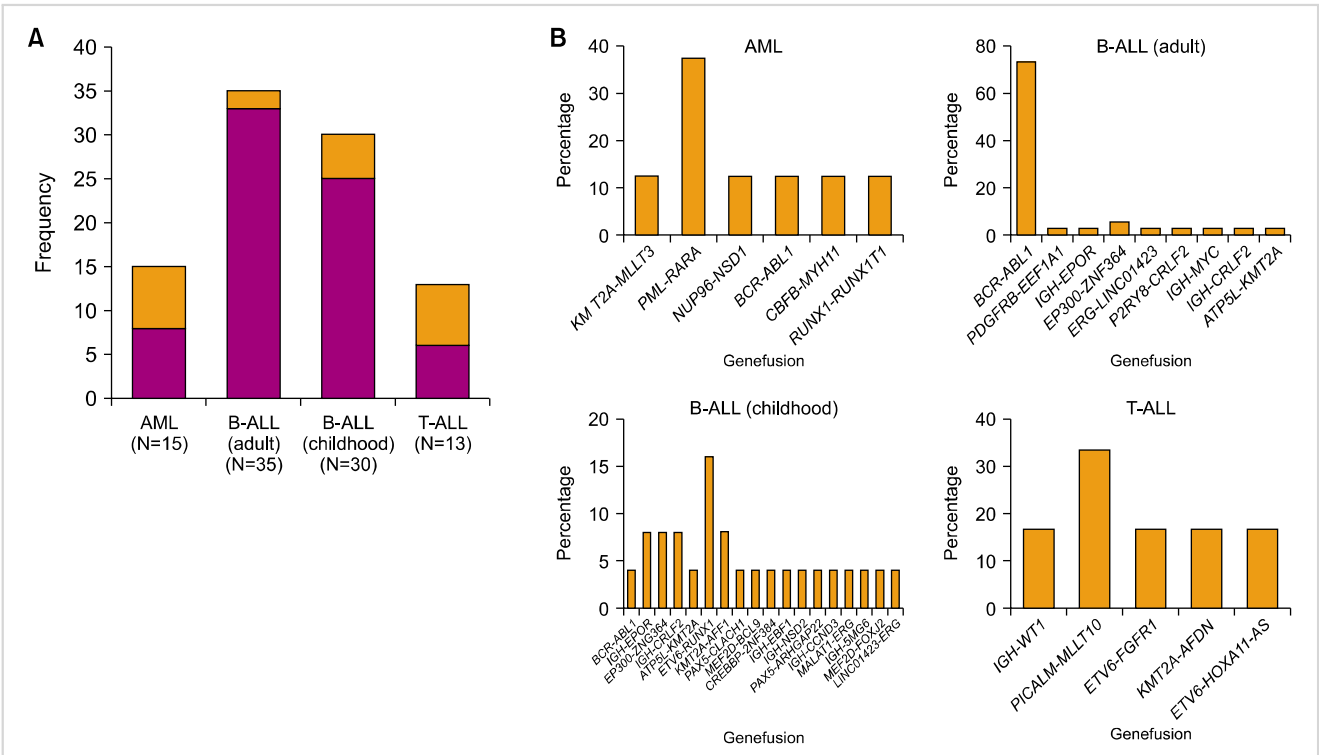

Fig. 1. Frequency of gene fusions detected in various types of leukemia using our targeted RNA-seq system. **(A)** Black bars indicate the frequency of gene fusions detected in patients with each leukemia type. Gene fusion was detected in 77% (72 patients) of all the 93 leukemia patients. Genetic fusion mutations were observed in 94% (33/35) of adult B-ALL patients and 83% (25/30) of pediatric B-ALL patients. **(B)** Gene fusion pattern and frequency for each type of leukemia. Among the gene fusions found in adult B-ALL (N=35), the most common fusion gene was *BCR-ABL1* (24/33, 73%), and the most common fusion gene mutation in childhood B-ALL was *ETV6-RUNX1* (4/26, 15%).
Abbreviation: RNA-seq, RNA sequencing.

these shortcomings, RNA sequencing (RNA-seq) methods are being introduced in clinical laboratories. However, the RNA-seq method is not widely used due to the complexity of the data processing and interpretation. The authors developed a targeted RNA-seq panel (KBB-RNAseq NGS-Leukemia-PHB; KBlueBio Inc., Hwasun, Korea) and bioinformatics pipeline that can be used for targeted RNA-seq to detect gene fusions, sequence variants, and altered gene expression, and is more convenient than traditional RNA-seq methods for use in clinical hematology laboratories [3]. The targeted RNA-seq panel and analysis pipeline, developed using information technology with an automated reporting system and commercialized by the authors, was subjected to a comparative evaluation with a commercialized targeted RNA-seq panel (Archer™ FusionPlex™ Heme Panel version 2, Archer DX, Boulder, CA, USA) using leukemia patient samples.

The targeted RNA-seq system for detecting gene fusions in blood cancers developed by the authors comprised 84 genes, including the self-discovered prohibitin as a companion diagnostic marker for hematological malignancies, and was optimized by MiseqDx (Illumina, San Diego, CA, USA) after library preparation using the capture hybridization method. The test performance of this system was evaluated using bone marrow specimens that were collected when diagnosing 93 patients with leukemia [15 with acute myeloid leukemia (AML), 35 with adult B-acute lymphoid leukemia (B-ALL), 30 with childhood B-ALL, and 13 with

Table 1. Comparative evaluation with a commercially available targeted RNA-seq panel.

Sample no.	Diagnosis	Multiplex RT-PCR or FISH	Commercial targeted RNA-seq system [4]	Breakpoint	Our targeted RNA-seq system [3]	Breakpoint
1	APL	*PML-RARA*	*PML-RARA*(ex6-ex3)	chr15:74325755 chr17:38504568	*PML-RARA*(ex6-ex3)	chr15:74325755 chr17:38504568
2	B-ALL	*BCR-ABL1*	*BCR-ABL1*(ex1-ex2)	chr22:23524426 chr9:133729451	*BCR-ABL1*(ex1-ex2)	chr22:23524426 chr9:133729451
					BCR-ABL1(ex2-ex1)	chr22:23595986 chr9:133589842
3	AML	*RUNX1-RUNX1T1*	*RUNX1-RUNX1T1*(ex6-ex2)	chr21:36231771 chr8:93029591	*RUNX1-RUNX1T1*(ex6-ex2)	chr21:36231771 chr8:93029591
			RUNX1-RUNX1T1(ex6-ex1)	chr21:36231771 chr8:93074855	*RUNX1-RUNX1T1*(ex6-ex3)	chr21:36231771 chr8:93074937
			RUNX1-RUNX1T1(ex6-ex1)	chr21:36231771 chr8:93074937	*RUNX1-RUNX1T1*(ex6-ex1)	chr21:36231875 chr8:93074774
4	B-ALL	*ETV6-RUNX1*	*ETV6-RUNX1*(ex5-in2)	chr12:12022900 chr21:36265263	*ETV6-RUNX1*(ex5-ex3)	chr21:36265260 chr12:12022903
			ETV6-RUNX1(ex5-ex3)	chr12:12022903 chr21:36265260	*ETV6-RUNX1*(ex5-ex4)	chr21:36259393 chr12:1202290
			ETV6-RUNX1(ex5-ex4)	chr12:12022903 chr21:36259393		
			ETV6-RUNX1(ex5-in2)	chr12:12022903 chr21:36340571		
5	B-ALL	Negative	*P2RY8-CRLF2*(ex1-ex1)	chrX:1655814 chrX:1331530	*P2RY8-CRLF2*(ex1-ex1)	chrX:1655814 chrX:1331529
			EIF4E3-FOXP1(ex7-ex3)	chr3:71739161 chr3:71542706		
6	B-ALL	Negative	Negative		*IGH-CRLF2*	chr14:106329453 chrX:1351126
					IGH-CRLF2	chr14:106322373 chrX:1351121
					IGH-CRLF2	chr14:106330849 chrX:1351119
7	AML	*CBFB-MYH11*	*CBFB-MYH11*(ex5-ex33)	chr16:67116211 chr16:15814908	*CBFB-MYH11*(ex5-ex33)	
			CBFB-MYH11(ex5-ex33)	chr16:67116242 chr16:15814908		
8	T-ALL	Negative	*PICALM-MLLT10*(ex17-ex4)	chr11:85692172 chr10:21875223	*PICALM-MLLT10*(ex17-ex4)	chr11:85692172 chr10:21875223
			PICALM-MLLT10(ex17-ex4)	chr11:85689133 chr10:21875228	*PICALM-MLLT10*(ex7-ex3)	chr11:85687725 chr10:21827841
			PICALM-MLLT10(ex17-ex3)	chr11:85692184 chr10:21827830	*PICALM-MLLT10*(ex19-ex3)	chr11:85670103 chr10:21827841

Abbreviations: FISH, fluorescence in situ hybridization; RNA-seq, RNA sequencing; RT-PCR, reverse transcriptase-PCR.

T-acute lymphoid leukemia (T-ALL)]. Out of all 93 leukemia patients, tier 1 or tier 2 gene fusions were observed in 72 (77%) patients. Fusion gene mutations were detected in 83% (25/30) of the childhood B-ALL and 94% of the adult B-ALL samples (33/35). In patients with AML or T-ALL, fusion gene mutations were found in 53% (8/15) and 46% (6/13), respectively (Fig. 1).

A commercial targeted RNA-seq analysis system developed by constructing a cDNA library for specific genes using anchored multiplex PCR [3] was compared to the detection system (KBB-RNAseq NGS-Leukemia-PHB) developed by the authors [4]. For this comparative evaluation, four cases of B-ALL, two cases of AML, and one case each of acute promyelocytic leukemia and T-ALL were used. The results of the two analysis systems were consistent for seven of the eight comparatively evaluated patients, however, B-ALL accompanied by an *IGH-CRLF2* gene fusion was detected only in the panel developed by the authors (Table 1). Philadelphia chromosome (Ph)-ALL is found in 20–25% of B-ALL cases, and it is known that approximately 61% of the fusion gene mutations related to the *CRLF2* gene are causative genes. Ph-like B-ALL has a very poor patient prognosis and has been reclassified as a new subtype of ALL [5, 6]. Therefore, the diagnosis of Ph-like B-ALL through the genetic analysis of ALL patients, especially B-ALL patients, is very important for treating leukemia with precision medicine. Our targeted RNA-seq analysis system was found to be more effective at detecting Ph-like B-ALL than the cDNA library-based targeted RNA-seq using anchored multiplex PCR, which is currently commercially available and used in some clinical testing sites [4].

Information technology (IT)-based data analytics software (NGeneAnalySys, NGeneBio, Seoul, Korea) was used to analyze the data produced by the next-generation sequencing (NGS) equipment using the RNA-seq panel (KBB-RNAseq NGS-Leukemia-PHB) developed by the authors (Supplementary Fig. 1). After selecting the data generated by the NGS equipment, the data are uploaded to the analysis server, the panel type is selected, and an automatic analysis is performed. The status of the analysis can be checked in real-time, as can the list of mutations found by the automatic analysis and the correct classification of the results according to the importance of the mutations. Variation filtering using annotation information is performed, a clinical report is first prepared, and a final report is issued after confirming the patient, test information, and the type and importance of the detected mutation. Information (VAF, HGVS, sequence, COSMIC, population, and IGV information check), a mutation analysis according to guidelines such as association for molecular pathology, a quality control report, and a customizable clinical report are provided.

In conclusion, our targeted RNA-seq system accurately and efficiently detected various fusion gene mutations that occur in acute leukemia. Importantly, our targeted RNA-seq panel detected the fusion gene mutations that cause Ph-like B-ALL more accurately than a commercially-available targeted system.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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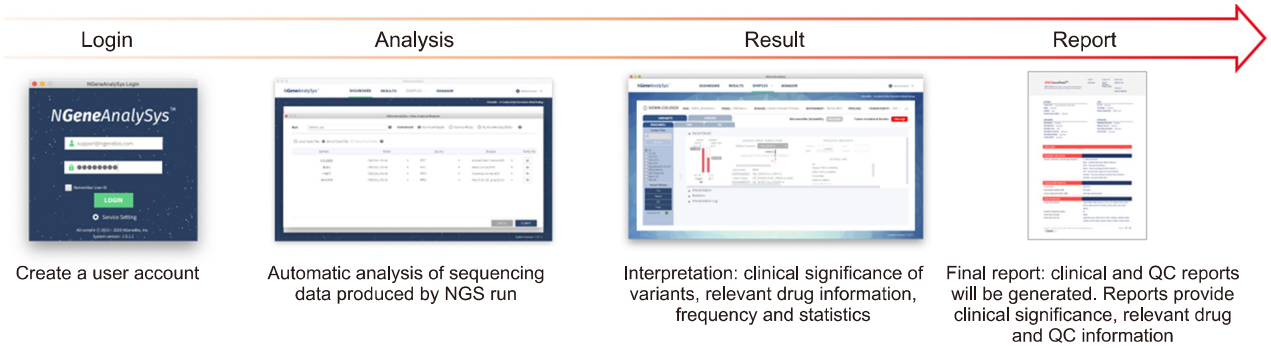

Supplementary Fig. 1. Outline of the IT-based automatic analysis and reporting system. After selecting the data generated by the NGS equipment, the data areuploaded to the analysis server, the panel type is selected, and an automatic analysis is performed. Variation filtering using annotation information is performed, a preliminary clinical report is written, and a final report is issued after confirming the patient, test information, and the type and importance of the detected mutation.
Abbreviations: IT, information technology; NGS, next-generation sequencing.