Clinical and molecular features of acute promyelocytic leukemia with variant retinoid acid receptor fusions

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Material and Methods

Patients

From January 2003 to December 2016, a total of 1,381 patients with suspected APL were enrolled in this study. Patients were considered eligible for inclusion only if the following criteria were satisfied: morphologic and immunophenotypic features were consistent with the diagnosis of APL. This study was approved by the ethics committee in accordance with the Declaration of Helsinki protocol. Outcome data were updated as of December 2016.

Molecular identification of alternative *RARA* or *RARG* fusions.

R-banding karyotypic analysis was carried out on bone marrow or peripheral blood cells at diagnosis. Clonal karyotypic abnormalities were described according to the International System for Human Cytogenetic Nomenclature (**ISCN, 2013**). For detection of rearrangements of *PML-RARA* or *RARA* rearrangements, dual color FISH was performed on methanol/acetic acid-fixed cells using Vysis LSI *PML/RARA* and the Vysis LSI *RARA* dual color break apart (Abbott Molecular Inc., Des Plaines, IL, USA). Reverse transcription-polymerase chain reaction (RT-PCR) was performed to characterize the partner genes involved in selected APL patients.

RNA-sequencing (RNA-Seq) and whole-genome sequencing (WGS) were performed to identify molecular aberrations in suspected APL patients lacking classic t(15;17)(q24;q12)/*PML-RARA*. Total RNA and geneomic DNA were extracted using following the standard protocol and the integrity was assessed with an Agilent

Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). For RNA-seq analysis, mRNA samples were prepared by using the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA). Libraries generated were sequenced on the Illumina HiSeq 2500 system (Illumina, San Diego, CA). Paired-end reads from RNA-seq were aligned to the following database files using the Burrows Wheeler Aligner (BWA) aligner (0.5.10): (i) the human grch37-lite reference sequence; (ii) Refseq; (iii) a sequence file representing all possible combinations.

Targeted sequencing

Targeted sequencing of the entire coding sequences of 382 known or putative mutational gene targets in hematologic malignancies was performed on 18 APL patients with alternative *RARA or RARG* fusions with sufficient genomic DNA available. Briefly, the genomic DNA was sheared and the sample libraries prepared using the TruSeq DNA Sample Preparation Kit (Illumina). Sequencing was performed using a custom amplicon-based targeted enrichment assay (Haloplex, Agilent, Boeblingen, Germany) and an Illumina MiSeq instrument (Illumina).

Statistical analysis

Patients' characteristics were analyzed by χ^2 or Fisher's exact tests for univariate analysis. Values for $p<0.05$ were deemed significant. All calculations were performed using the SPSS software package (version 13.0, SPSS, Chicago, IL).