Data S1

Bone marrow aspirate assessment. Bone marrow aspirate smears were air-dried, fixed in absolute methanol for 10 min at 22˚C, and stained with Wright's stain for 10 min at room temperature. Stained smears were examined using an Olympus BX51 light microscope (Olympus Corporation).

Histological and staining methods. Bone marrow biopsy specimens were processed as follows: Tissues were fixed in 10% neutral buffered formalin at room temperature for 24-48 h. Fixed tissues were embedded in paraffin and sectioned at a thickness of 4 μm. All staining procedures were performed at room temperature.

Reticulin silver staining. Sections were treated sequentially with potassium permanganate (6-8 min), oxalic acid (1 min), ferric ammonium sulfate (6-8 min), ammoniacal silver solution (15-20 min), formalin (1 min), gold chloride (2 min) and sodium thiosulfate (1 min).

Periodic acid-Schiff staining. Sections were treated sequentially with periodic acid (20 min), Schiff reagent (30-40 min) and sodium metabisulfite (1 min), rinsed under running water and then counterstained with hematoxylin (1-2 min).

Masson's trichrome staining. Sections were treated sequentially with hematoxylin (1-2 min), Masson's composite staining solution (3-5 min), phosphomolybdic acid (1-3 min) and aniline blue (5-6 min), and differentiated with acetic acid (1 min).

Hematoxylin and eosin staining. Sections were baked, deparaffinized in xylene and then stained with hematoxylin (5-8 min). The sections were rinsed, blued (1 min), rinsed again, stained with eosin (1-2 min), rinsed, dehydrated through a graded alcohol series, cleared in xylene and mounted.

Microscopy. Stained sections were examined using a light microscope.

Flow cytometry. The sample type was a bone marrow aspirate. Red blood cells were lysed using a commercial ammonium chloride-based lysing solution. A single-cell suspension was prepared and washed prior to antibody staining. The sample was processed within 24 h of collection.

The immunophenotyping panel was designed for comprehensive lineage assessment and minimal residual disease detection in hematopoietic malignancies. The conjugated antibodies used were as follows: CD38 (FITC; catalog no. 340909, BD Biosciences), CD5 (PE; catalog no. 665749; BD Biosciences), CD45 (PerCP; catalog no. 8931017; Agilent Technologies, Inc.), CD10 (PerCP; catalog no. 8930045; Agilent Technologies, Inc.), CD11b (FITC; catalog no. IM0530; Beckman Coulter, Inc.). Flow cytometry was performed using the BD FACSCanto II (BD Biosciences) with NovoExpress software (version 1.4.1; Agilent Technologies, Inc.). Cells processed without fluorochrome-conjugated antibodies were used as an unstained control to assess autofluorescence.

Next-generation sequencing. Sequencing was performed by Hangzhou Chain Medical Laboratory Co., Ltd. using the QIAamp DNA Mini Kit (catalog no. 51304; Qiagen, Inc.) for bone marrow DNA isolation.

Quality verification. Purity and concentration were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Integrity was evaluated using an Agilent 2100 Bioanalyzer with High Sensitivity DNA Kit (cat. no. 5067-4626; Agilent Technologies, Inc.). The sample requirement was a minimum DNA input of 50 ng with an A260/280 ratio >1.8 and DNA integrity number >7.0.

Library preparation and sequencing. Target enrichment was performed using a custom-designed panel for hematological malignancy genes (for example, covering genes relevant to MDS and AML). The library construction kit was the xGen Pan-Cancer Panel v2 (catalog no. 1056116; Integrated DNA Technologies, Inc.) or equivalent.

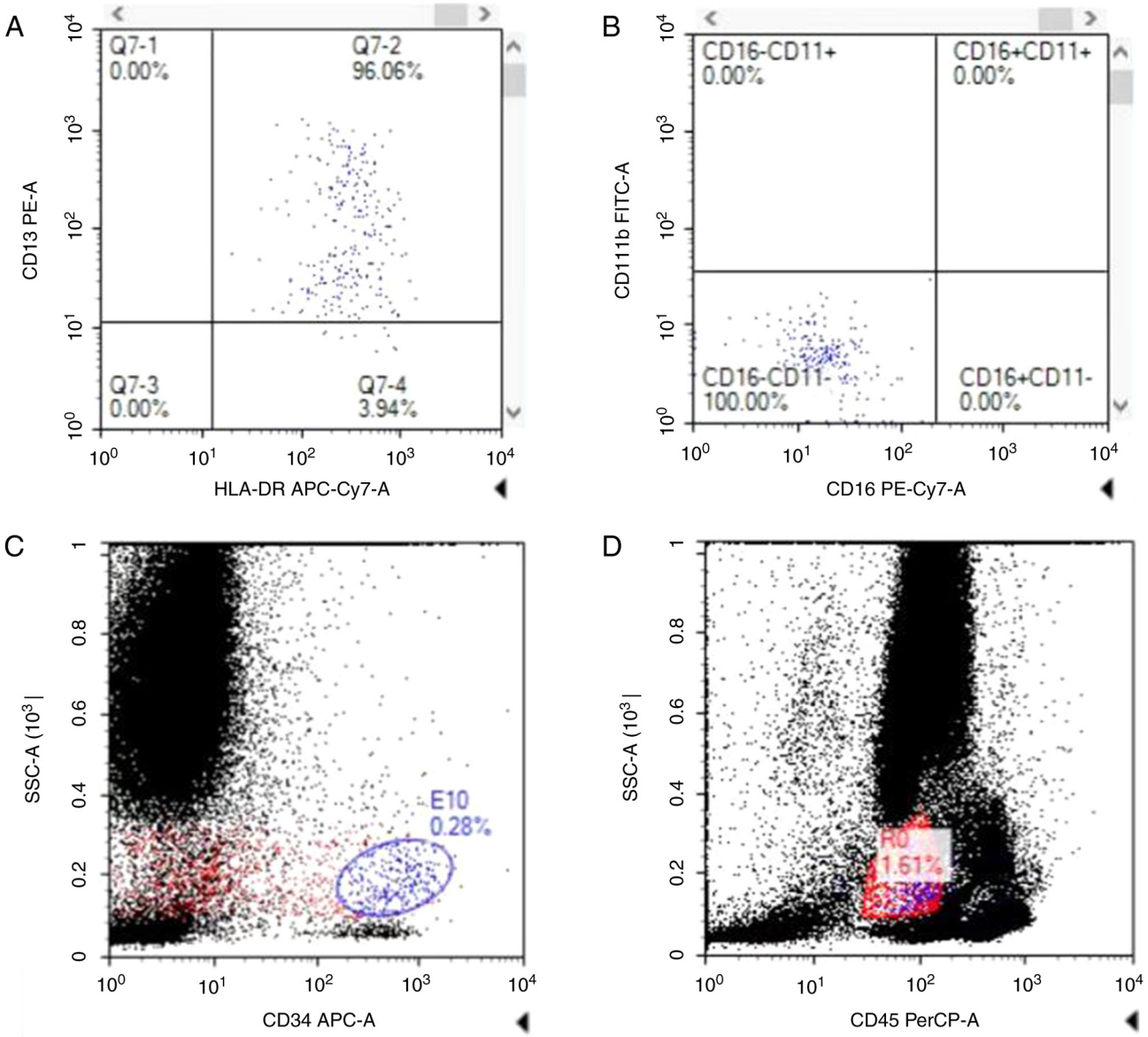
Genomic DNA was fragmented to a target size of 250-300 bp using a Covaris S220 system (Covaris, Inc.). Adapter ligation was performed using xGen UDI-UMI Adapters (catalog no. 10005910; Integrated DNA Technologies, Inc.), followed by 8 cycles of PCR amplification with KAPA HiFi HotStart ReadyMix (catalog no. 7958935001; Roche). Library concentration was quantified by quantitative PCR using the KAPA Library Quantification Kit for Illumina platforms (catalog no. 07960140001; Roche Diagnostics GmbH) on an Applied Biosystems QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, Inc.). The final library was loaded at a concentration of 300 pM.

Sequencing was performed on the Illumina NovaSeq 6000 system (Illumina, Inc.) using the NovaSeq 6000 S4 Reagent Kit (300 cycles; catalog no. 20028316; Illumina, Inc.) with 2x150 bp paired-end reads. A sequencing quality threshold of >93% of bases achieving Q30 was applied, consistent with published standards.

Bioinformatics analysis. Base calling and demultiplexing were performed using the Illumina DRAGEN Bio-IT Platform (Illumina, Inc.). Reads were aligned to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner. Variant calling for single nucleotide variants and small insertions/deletions was conducted following the GATK Best Practices pipeline. Annotation was performed using ANNOVAR (https://annovar.openbioinformatics.org/en/latest/), with reference to public databases including dbSNP (https://www.ncbi.nlm.nih.gov/snp/), ClinVar, (https://www.ncbi.nlm.nih.gov/clinvar/) and COSMIC (https://cancer.sanger.ac.uk/cosmic/login).

Data quality metrics. The following data quality metrics were achieved: Average sequencing depth >500x for targeted regions; >95% of target bases covered at ≥100x; and variant detection sensitivity of ~5% allele frequency at 500x depth.

Figure S1.


A
Q7-1
0.00%
Q7-2
96.06%
Q7-3
0.00%
Q7-4
3.94%
CD13 PE-A
HLA-DR APC-Cy7-A
B
CD16-CD11+
0.00%
CD16+CD11+
0.00%
CD16-CD11-
100.00%
CD16+CD11-
0.00%
CD111b FITC-A
CD16 PE-Cy7-A
C
E10
0.28%
SSC-A (10³)
CD34 APC-A
D
R0
1.61%
SSC-A (10³)
CD45 PerCP-A