Materials and Methods

Cell culture

Isolated of CD34+ cells from ALL patient bone marrow using magnetic bead separation system (Miltenyi Biotec) and cultured of CD34+ cells in Stemline II Hematopoietic Stem Cell Expansion Medium (Sigma) with SCF, TPO and G-CSF (R&D Systems). Normal bone marrow derived human iPSC (IISH1i-BM1) were purchased from the WiCell Research Institute (Madison, WI) and we handled according to the supplier instructions.

Generation of iPSCs

ALL_CD34+ cells were generated using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Life Technologies) according to manufacturer's instructions: cells were transduced using 1×10^5 onto 0.1% gelatin-coated dishes containing hPCCM and the recommended MOI. To confirm pluripotency, cells were live stained with a TRA1–60 antibody (Stemgent).

Detection of alkaline phosphatase activity

Alkaline phosphatase (ALP) activity was detected using the ES Cell Characterization Kit (Millipore) according to manufacturer's instructions.

Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde for 10minutes, permeabilized with 0.1% Triton X-100, and blocked for 1h with 3% normal horse serum (Gibco; Thermo Fisher Scientific), and incubated with primary antibodies for Oct3/4, SSEA4 and SOX2 (Table 2). Images were acquired using a fluorescence microscope (Olympus). Scale bars represent 200 μm.

Reverse transcription qPCR

qPCR (Bio-Rad iCycler iQ system) was performed using iQ SYBR Green qPCR Master Mix (Bio-Rad). Primers used for qPCR are listed in Table 2. The cycle threshold values of genes of interest were normalized to those of GAPDH.

Karyotype

The iPSCs were incubated in 0.075M KC1 for 20min at 37C. After fixation with a solution of 3:1 methanol/acetic acid and determined at the 300-band level of resolution.

Fluorescence in situ Hybridization

FISH analyzed for CD34+ cells and iPSCs from ALL_patient performed at department of diagnostic examination, Korea university anam hospital.

Short tandem repeat genotyping

STR analysis of the generated hiPSCs and parental cells was performed using a PowerPlex 16 System (Promega) to detect 16 loci at the Dawoojin Gene Research Institute (Seoul, Korea).

Differentiation

For three germ layers, undifferentiated colonies of hPSCs were cultured in low-attachment surface plates with DMEM-F12 medium containing 20% knockout serum replacement for 7days and seeded on gelatin-coated dishes and cultured for 7days. For hematopoietic cells, incubated with BMP4 for the first 3 days and changed in Stemline II serum-free medium (Sigma) supplemented with vascular

endothelial growth factor, basic fibroblast growth factor, stem cell factor, FMS-like tyrosine kinase 3 and Thrombopoietin (Gibco) for 7days.

Colony-forming unit assay

CFU assay was performed 10,000 differentiated hematopoietic cells or CD34+ cells were seeded in methylcellulose (Stem Cell Technologies). After incubation for 14 days at 37 °C in 5% CO_2, hematopoietic cell clusters were counted on the basis of morphology.

Mycoplasma test

e-Myco™ Mycoplasma PCR Detection Kit (iNtRON) was performed according to manufacturer's instructions to confirm the absence of mycoplasma.