SUPPLEMENTARY APPENDIX

Measurable residual disease monitoring provides insufficient lead-time to prevent morphological relapse in the majority of patients with core-binding factor acute myeloid leukemia

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Supplemental Methods

Treatment and MRD monitoring protocol

The standard treatment protocol for eligible patients with CBF-AML at our institution during the study period was induction chemotherapy with daunorubicin 60 mg/m^2 IV daily for 3 days and continuous infusion of cytarabine 200 mg/m^2 IV daily (if age <60 years old) or 100 mg/m^2 IV daily (if age ≥60 years old) for 7 days. Patients who achieved complete remission received 3 cycles of consolidation chemotherapy with high dose cytarabine 3 g/m^2 IV q12h (if age <60 years old) or 1.5 g/m^2 IV q12h (if age ≥60 years old) on days 1, 3, and 5. Daunorubicin 45 mg/m^2 IV was also administered on days 1 and 2 during the first cycle of consolidation chemotherapy. Allogeneic BMT was not routinely recommended unless there was evidence of molecular progression during follow-up or if adverse prognostic factors were present such as t(8;21) with C-KIT mutation.

Patient samples underwent MRD monitoring using RT-qPCR of RUNX1-RUNX1T1 or CBFB-MYH11 fusion transcripts in an accredited (ISO15189) laboratory at the University Health Network. Total RNA was extracted from BM using a Qiagen column based extraction or PB using a Promega Maxwell automated bead based extraction. 1µg of RNA was reverse transcribed into cDNA using the TaqMan Reverse Transcription Reagents Kit (ThermoFisher, Waltham, MA) in a 25µl final reaction and 5µl cDNA was used for all downstream RT-qPCR and 2ul of cDNA for all downstream nested PCR. All assays were developed and validated by the genomics laboratory and primers/probes were purchased from Integrated DNA Technologies, Inc. (Coralville, Iowa, United States). For quantitation, the standard curve method was used for both RUNX-RUNX1T1 and CBFB-MYH11 RT-qPCR assays. Each patient's own diagnostic level was used as baseline for calculating log reduction. Detection limit for these assays was 1 in 10,000 throughout the study period.