Clinical and molecular features of *CBL*-mutated juvenile myelomonocytic leukemia

Juvenile myelomonocytic leukemia (JMML) is characterized by excessive myelomonocytic cell proliferation and granulocyte–macrophage colony-stimulating factor hypersensitivity. Approximately 15% of children with JMML harbor homozygous *CBL* mutations.[1,2] Niemeyer *et al.*[1] identified germline *CBL* syndrome with developmental, tumorigenic, and functional consequences caused by hyperactive RAS/RAF/MEK/ERK signaling. Patients with *CBL*-mutated JMML typically have a low-methylation profile and a less aggressive disease course compared to JMML patients with other RAS pathway mutations.[3] In most cases, the disease resolves spontaneously,[4] whereas vasculitides and other autoimmune disorders might develop in some patients.[1] However, prior cohorts in the literature have been restricted to small numbers of patients, specifically those with somatic *CBL* mutations.[2] Thus, this study aimed to retrospectively analyze a cohort along with a review of the literature.

This study retrospectively analyzed 25 children with *CBL*-mutated JMML in Japan between September 1988 and November 2021 (Figure 1). JMML was diagnosed based on previously published internationally accepted diagnostic criteria.[5] Our previous reports included 19 of 25 patients.[6,7] Written informed consent was obtained from the guardians of all patients. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine.

Bone marrow or peripheral blood samples were collected at the initial diagnosis. Ficoll–hypaque density gradient centrifugation was utilized to isolate mononuclear cells, which were cryopreserved until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Chatsworth, CA). Whole-exome sequencing was used for the mutational analysis, as previously described.[7] Canonical RAS pathway gene mutations, i.e., *PTPN11*, *NRAS*, *KRAS*, and *CBL*, were confirmed using Sanger sequencing.

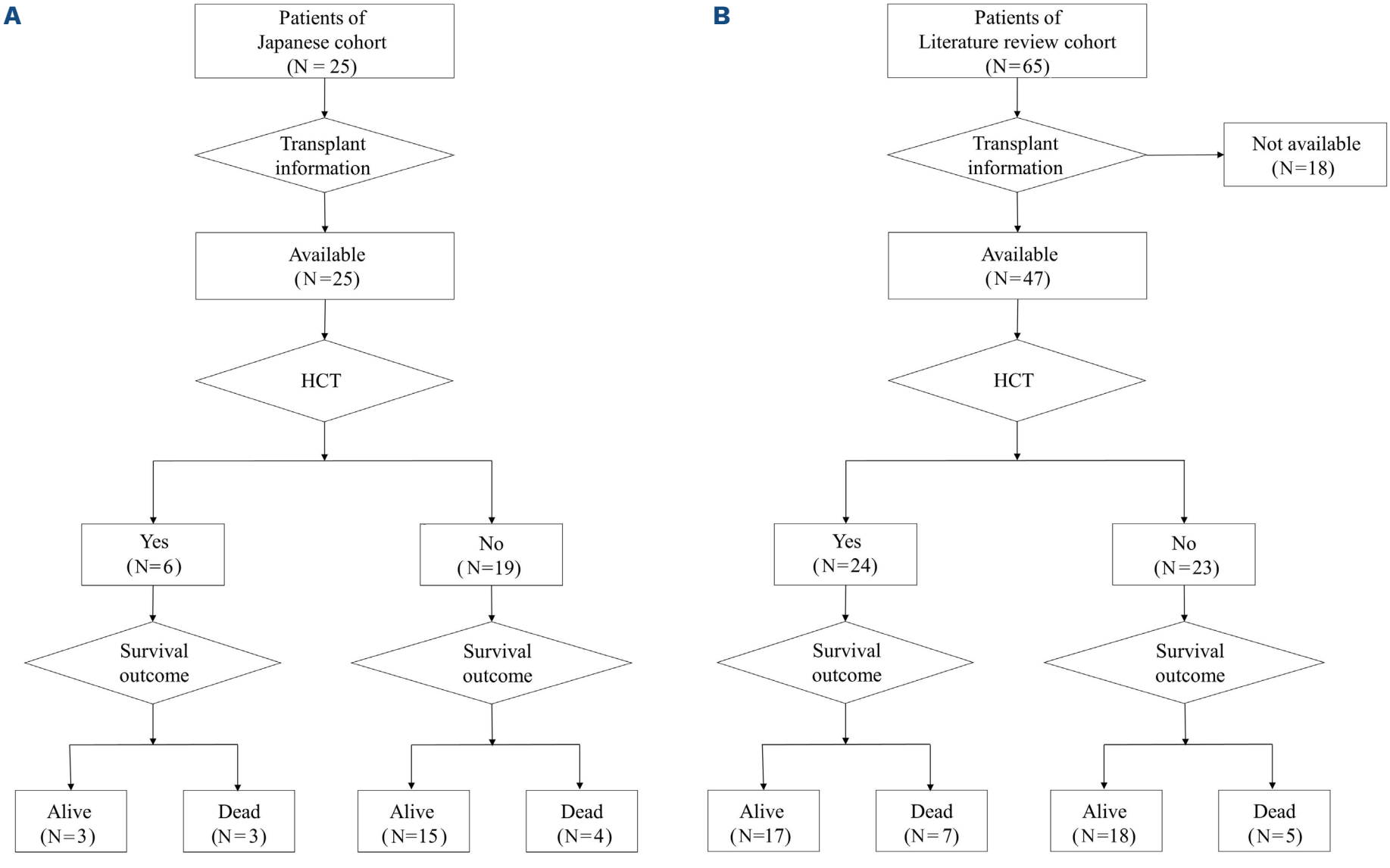

Figure 1. Summary of the Japanese and literature review cohorts. (A) Japanese cohort and (B) literature review cohort. HCT: hematopoietic cell transplantation.

Methylation analysis was performed using digital restriction enzyme analysis of methylation, as previously described.[8] Of the 25 patients, 16 were analyzed with a 450k methylation array, as previously described.[3,7]

The Kaplan–Meier method was used to estimate overall survival (OS) and transplantation-free survival (TFS). Survival differences were evaluated using the log-rank test. OS was defined as the duration from the date of diagnosis to death, and TFS as the duration from the date of diagnosis to transplantation or all-cause death.

All statistical analyses were performed using EZR software (version 1.36; Saitama Medical Center, Jichi Medical University, Saitama, Japan).[9] *P* values were two-tailed in all analyses, and *P* values <0.05 were considered statistically significant.

Literature review

A systematic literature search was conducted using a combination of controlled vocabulary and keywords. PubMed (https://pubmed.ncbi.nlm.nih.gov) was searched for published articles from the date of its inception to February 2022. Searched terminologies included "JMML" and "CBL." In total, 61 articles written in English were found. Of these, 26 abstracts, nine reviews, and seven articles reported from Japan that were identical or potentially identical to the study cases were excluded. The abstracts and text of the remaining 19 articles were carefully evaluated, and ten articles reporting 65 patients with *CBL*-mutated JMML from outside Japan were selected.[1,2,10-17]

Patient characteristics at diagnosis are presented in Figure 2; *Online Supplementary Table S1* and *Online Supplementary Table S2*. Our cohort consisted of 25 patients, with a median age at diagnosis of 1 year (range, 1 month-14 years). The median follow-up was 3.4 years (range, 0.5 months-23.6 years). Splenomegaly with ≥3 cm below the costal margin was determined in 19 patients. *CBL* mutations were homozygous (n=21) or heterozygous (n=4), with 18 missense mutations, four splice site mutations, and three deletions. Three patients with heterozygous gene deletions were identified as having no point mutation in the second allele by whole-exome sequencing. Moreover, 23 patients harbored germline *CBL* mutations, whereas two patients had somatic mutations only. One patient had a concomitant somatic *PTPN11* mutation (UPN160). Methylation analysis classified all cases into the low-methylation (LM) group. Except for one patient who had trisomy 8 (UPN198), no patients had chromosomal abnormalities. In this cohort, three patients had moyamoya disease (*Online Supplementary Figure S1*), of whom one has neovascular glaucoma, whose clinical course was described in detail in a previous publication.[6] Of the 25 patients in this cohort, six received allogeneic hematopoietic stem cell transplantation (HCT); four patients were diagnosed with JMML and had transplantation in the period before the *CBL* mutation was identified as the causative gene for JMML. The remaining two patients underwent HCT because of disease progression.

Figure 3 shows an overview of all disease courses. The 5-

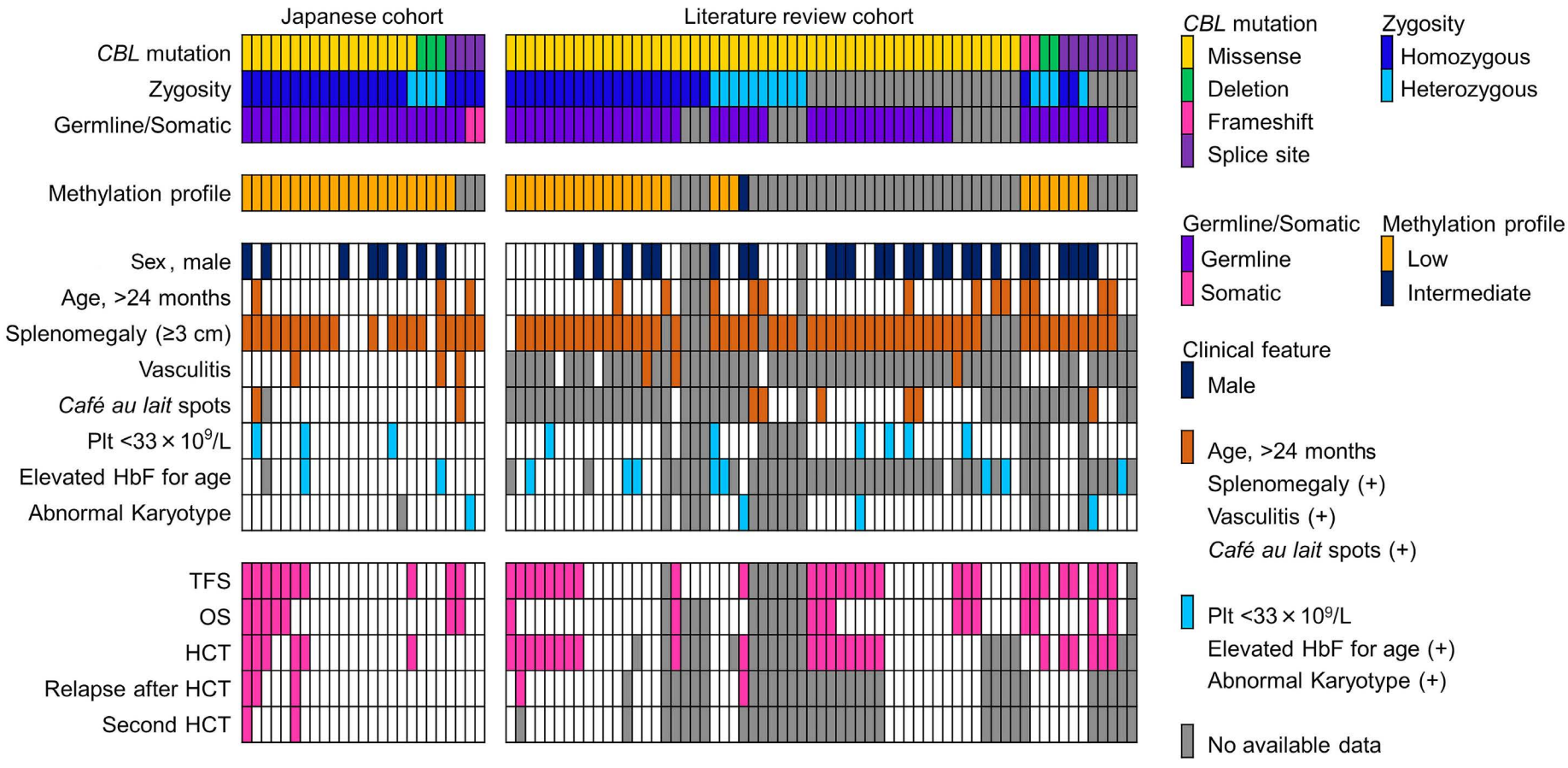

Figure 2. Clinical and genetic profiles of the Japanese and literature review cohorts. Each column indicates 1 patient. Methylation profiling data were available for 50 of 87 patients (22 in the Japanese cohort and 28 in the literature review cohort). Plt: platelet count; HbF: fetal hemoglobin; TFS: transplantation-free survival; OS: overall survival; HCT: hematopoietic cell transplantation.

year OS and TFS rates were 70.3% (95% confidence interval [CI]: 47.4-84.6) and 52.7% (95% CI: 27.8-72.6), respectively. Spontaneous JMML resolution was experienced by 14 patients, without treatment (n=11) or with oral administration of 6-mercaptopurine (n=3). Four patients died of leukemia before transplantation, and six patients underwent allogeneic HCT after receiving different pretransplant treatments. Among patients who underwent HCT, two went into remission, three died of JMML relapse, and one died of transplant-related complications.

The literature review cohort consisted of 65 patients with a median age at diagnosis of 1 year (range, 1 month-25 years). The patient characteristics are provided in the *Online Supplementary Table S2*. Fifty-one patients had sple-

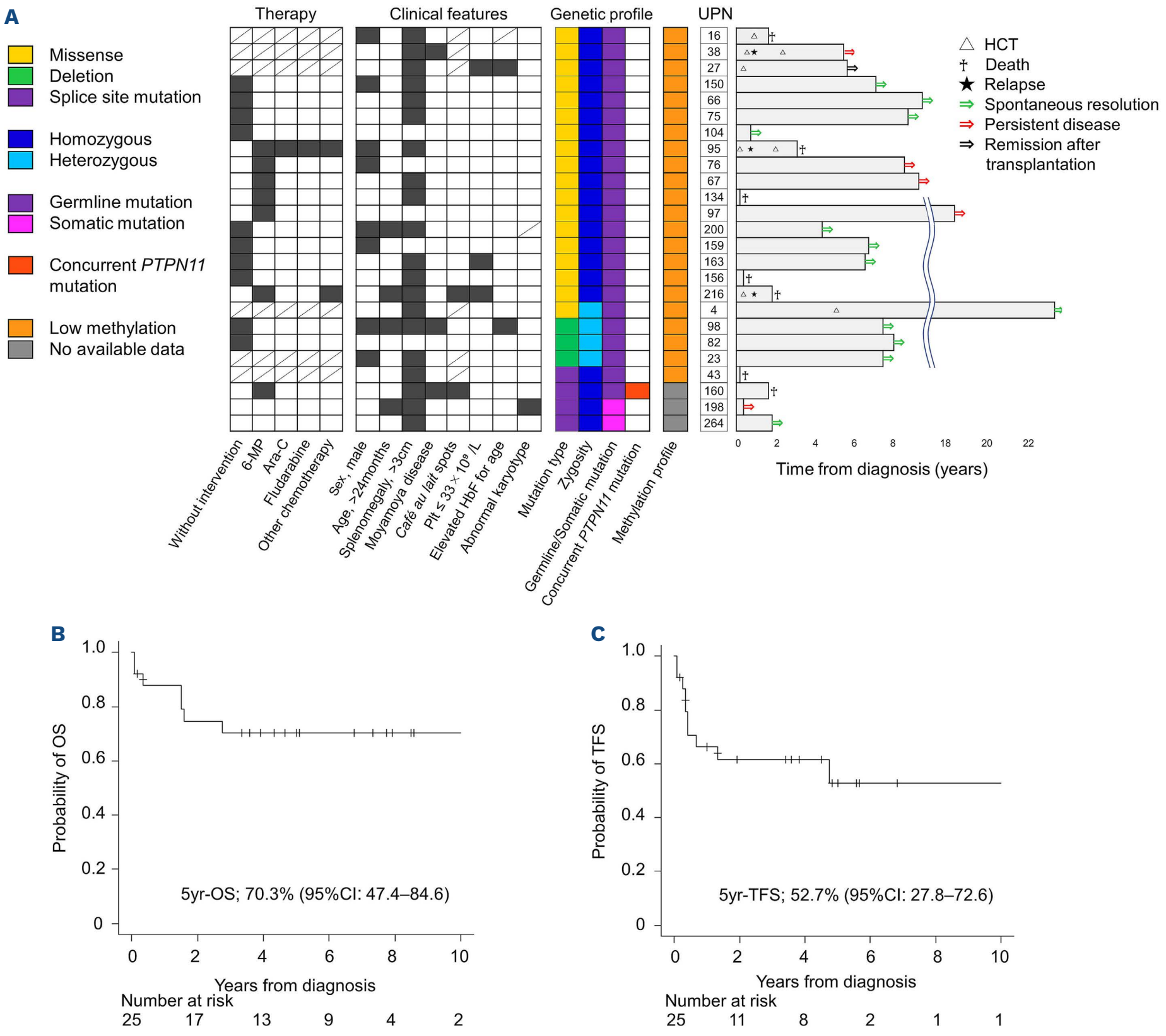

Figure 3. Swimmer plot and survival curves of the Japanese cohort. (A) Swimmer plot showing the clinical course of patients in the Japanese cohort. Each bar shows the clinical course of 1 patient. Symbols indicate the dates of hematopoietic cell transplantation (HCT), death, relapse, or spontaneous resolution. A color-coded arrow indicates the current status of the patient. Therapeutic agents received by the patient are shown on the left side using pattern-coded dots. Filled (if true) or empty (if false) boxes indicate the clinical features (sex, age, splenomegaly, moyamoya disease, *Café au lait* spot, platelet count [Plt], elevated fetal hemoglobin [HbF] for age, and abnormal karyotype). Dashed boxes are used to indicate unavailable data. 6-MP: 6-mercaptopurine; Ara-C: cytosine arabinoside. (B, C) Kaplan–Meier estimates of overall survival (OS) and transplantation-free survival (TFS). Five-year (yr) OS and TFS rate were 70.3% (95% confidence interval [CI]: 47.4-84.6) and 52.7% (95% CI: confidence interval: 27.8-72.6), respectively.

nomegaly. *CBL* mutations were homozygous (n=24) or heterozygous (n=14) or with no available data about zygosity (n=28) with 53 missense mutations, two deletions, two frameshift, and eight splice site mutations. Three patients had chromosomal abnormalities, including chromosome 16 deletion (n=1), chromosome 8 derivation (n=1), and absence of detailed karyotype information (n=1). Methylation data were available for 28 cases, of which 27 were classified with an LM profile and one with an intermediate methylation profile. This literature review cohort identified three patients with vasculitides, including Takayasu arteritis and small vessel vasculitis. Information on transplantation was available for 47 of 65 patients. Of the 23 patients without transplantation, 18 (78%) survived and five (22%) died. Information on the cause of death of these five patients was unavailable. Of the 24 transplant recipients, 17 (71%) survived and seven (29%) died (transplant-related mortality, n=1; and no detailed information, n=6).

We conducted a retrospective analysis of 25 cases of *CBL*-mutated JMML diagnosed in Japan and identified an additional 65 cases by literature review. Studies[1,4] have reported that most children with *CBL*-mutated JMML show a self-limiting clinical course with persistent clonal hematopoiesis, and observation is generally recommended, although splenomegaly and thrombocytopenia may require therapeutic intervention. However, of the 25 patients in the present study, four died of leukemia before HCT, and three died after HCT, resulting in a 5-year OS rate of 70.3%. Of the 65 patients with *CBL*-mutated JMML, 12 died (pre-transplant, n=5; post-transplant, n=7), indicating that *CBL*-mutated JMML is a heterogeneous population, and some patients experienced aggressive disease courses. A recent international collaborative study identified methylation classification as a potent prognostic factor in JMML, and the presence of *CBL* mutations is tightly associated with the LM subgroup.[3] Therefore, of the 50 patients (Japanese cohort, n=22; literature review cohort, n=28) with evaluable methylation data, 49 were classified in the LM subgroup and one in the IM group. These data suggest that we need to develop additional biomarkers besides methylation profiling to understand the molecular pathogenesis and heterogeneity of *CBL*-mutated JMML.

Moyamoya disease is a chronic cerebrovascular disease that is characterized by bilateral stenosis or artery occlusion around the progressive circle of Willis. CBL syndrome with germline *CBL* mutation shows a phenotype overlapping with Noonan syndrome, but it is associated with various vasculitides forms, including Takayasu disease, optic atrophy, hypertension, and acquired cardiomyopathy.[1] Moyamoya disease was complicated in three patients with CBL syndrome without clinical JMML manifestations.[18,19] In the present cohort, moyamoya disease was found in three of 25 patients with *CBL*-mutated JMML, including the previously reported case.[6] Prospective screening for moyamoya disease and other vasculitides complications in patients with *CBL*-mutated JMML is recommended using imaging studies, including magnetic resonance angiography.

This study has several limitations. First, the number of patients was insufficient to fully characterize the clinical features of *CBL*-mutated JMML associated with poor prognosis, although this is one of the largest *CBL*-mutated JMML studies so far. Second, there were only two cases with somatic *CBL* mutations and one case with secondary mutations in this cohort, making it difficult to evaluate the association between these genetic conditions and prognosis. Third, a significant proportion of the patients with *CBL*-mutated JMML in the literature review cohort lack basic genetic information such as *CBL* mutation zygosity and DNA methylation classification.

In conclusion, patients with *CBL*-mutated JMML represent a heterogeneous patient population that includes cases requiring therapeutic interventions, such as HCT. Further international collaborative studies are needed to accurately assess the clinical profile of *CBL*-mutated JMML and identify clinical factors associated with a poor prognosis.

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Disclosures

No conflicts of interest to disclose.

Contributions

TY gathered clinical information, designed the research, analyzed data, and wrote the paper. HM, YO and WM designed and performed the research, led the project, and wrote the paper. DS, NM and HK performed laboratory analyses. OY collected clinical samples and information. RT, SK, AN, AH and YT cooperatively designed and performed the research.

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Data-sharing statement

Data used in this study will be provided to qualified researchers on reasonable request.

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