Molecular and phenotypic diversity of *CBL*-mutated juvenile myelomonocytic leukemia

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ABSTRACT

Mutations in the *CBL* gene were first identified in adults with various myeloid malignancies. Some patients with juvenile myelomonocytic leukemia (JMML) were also noted to harbor mutations in *CBL*, but were found to have generally less aggressive disease courses compared to patients with other forms of Ras pathway-mutant JMML. Importantly, and in contrast to most reports in adults, the majority of *CBL* mutations in JMML patients are germline with acquired uniparental disomy occurring in affected marrow cells. Here, we systematically studied a large cohort of 33 JMML patients with *CBL* mutations and found that this disease is highly diverse in presentation and overall outcome. Moreover, we discovered somatically acquired *CBL* mutations in 15% of pediatric patients who presented with more aggressive disease. Neither clinical features nor methylation profiling were able to distinguish patients with somatic *CBL* mutations from those with germline *CBL* mutations, highlighting the need for germline testing. Overall, we demonstrate that disease courses are quite heterogeneous even among patients with germline *CBL* mutations. Prospective clinical trials are warranted to find ideal treatment strategies for this diverse cohort of patients.

Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive disease of young children that presents with features of both myelodysplasia and myeloproliferation. To date, hematopoietic stem cell transplantation (HSCT) is the only curative therapy and results in long-term survival of about 50-60% of patients.[1,2] The mutational landscape of JMML has been well described in several large studies.[3-6] Nearly all patients have initiating mutations in genes that activate the Ras pathway, most commonly, *PTPN11*, *NRAS*, *KRAS*, *NF1* or *CBL*. Secondary mutations are found in approximately 30% of patients at diagnosis and are associated with inferior survival.

CBL mutations were first described in JMML patients in 2009 by our group and others[7-10] and account for approximately 15% of all JMML cases.[3,11,12] Germline tissue including cord blood and buccal samples were heterozygous for mutations, while leukemia cells were homozygous, indicating that loss of heterozygosity was required for leukemogenesis. The majority of these children presented with syndromic features including facial dysmorphia, growth retardation, cryptorchidism and autoimmune phenomena, specifically vasculitis – a condition that is now called "CBL-syndrome". Patients with CBL-syndrome are at risk of developing JMML. Surprisingly, several of these patients with confirmed JMML experienced spontaneous resolution of their leukemia. Based on this observation and in stark contrast to other forms of JMML, CBL-syndrome JMML has been thought to be associated with relatively good prognosis. Treatment has evolved accordingly for these patients, who undergo a period of close observation of blood counts and assessment of splenomegaly, with HSCT utilized only after more conventional therapies have failed.[13]

In overlapping adult myelodysplastic syndromes/myeloproliferative neoplasms, somatically acquired *CBL* mutations occur in about 10-15% of cases and are associated with a poor prognosis. Several studies in chronic myelomonocytic leukemia (CMML), a disease of adults with many similarities to JMML, have shown that *CBL* mutations are associated with inferior survival.[14-16]

In our experience, we have observed that the spectrum of clinical courses of patients with *CBL*-mutated JMML is wide, ranging from spontaneous resolution to aggressive disease with transformation to acute myeloid leukemia. This observation prompted us to perform a systematic study of clinical presentations and molecular features to identify potential factors that can identify patients who may be observed and those who need immediate therapy.

Methods

Patients

We collected samples and clinical data from 33 patients diagnosed with JMML according to international diagnostic criteria[17] who were found to have a clonal *CBL* mutation. The patients were diagnosed between 2006 and 2019 in 27 different centers across the USA and Canada. Treatment decisions were at the discretion of the treating physicians and were independent of this study. Written consent and specimens were obtained from all patients at the time of routine clinical assessments. The study design was reviewed and approved by the institutional review board of University of California San Francisco, in accordance with the Declaration of Helsinki.

Material and studies

We received bone marrow and/or peripheral blood from all patients, collected at the time of initial diagnosis or shortly thereafter. Mononuclear cells were isolated using a Ficoll method and DNA was extracted using the Gentra Puregene kit (Qiagen, Germany). For identification of genomic mutations, DNA was extracted from bone marrow or peripheral blood mononuclear cells. DNA from peripheral blood was used for DNA methylation analysis. The germline status of *CBL* mutations was determined using material from either a buccal swab, cord blood or after sorting CD3+ T cells from peripheral blood. A somatic mutation was defined as a mutation that was identified in leukemia tissue (peripheral blood or bone marrow), but had a minimum variant allele fraction (VAF) of <5% in any germline tissue (epithelial cells from buccal swab, cord blood, or T cells).

Next-generation sequencing of juvenile myelomonocytic leukemia-associated mutations

Genomic DNA samples were sequenced using a custom amplicon-based sequencing approach (Paragon Genomics, Hayward, CA, USA) targeting 26 genes that are known to be recurrently mutated in JMML[18] (*Online Supplementary Table S1* and *Online Supplementary Methods*). A VAF of 0.05 (=5%) at diagnosis was required for reporting.

Targeted MethylSeq library preparation, sequencing and hierarchical clustering

Genomic DNA (300 ng) was bisulfite converted and 100 ng of converted single-strand DNA were used as the input for a custom 3000 CpG loci targeted MethylSeq assay (Tecan). Single-index library pools were sequenced on the Illumina NovaSeq with paired-end 150 base-pair (bp) mode averaging 20×10^6 reads per library preparation. MethylSeq hierarchical clustering and classification were performed according to the international consensus definition[12] as described in the *Online Supplementary Methods*.

Statistical analyses

Spontaneous resolution of disease was defined as normalization of monocyte counts in the peripheral blood and reduction of spleen to normal size without any JMML-directed therapy. Persistent disease was defined as not achieving complete remission according to the International Working Group definition[19] with or without therapy excluding HSCT. Overall survival was defined as time from initial diagnosis to death from any cause and was estimated using the Kaplan-Meier method. The median follow-up time was estimated using the reverse Kaplan-Meier method. Differences in clinical features between patients with germline *CBL* mutations and those with somatic-only *CBL* mutations were tested for significance using the Fisher exact test for categorical variables and the Mann-Whitney U test for continuous variables. The level of statistical significance for all tests was a *P*-value less than 0.05. All calculations were performed using Microsoft Excel (version 16.16.3), GraphPad Prism software (version 8.0) and R (version 3.4.1).

Results

Patients' characteristics

The cohort consisted of 33 patients with a median age at diagnosis of 1.1 years (range, 1 month - 25 years). The median follow-up in this study was 3.5 years (range, 0.2-12.1 years). The patients' characteristics at diagnosis are shown in Table 1. Additional clinical information for each patient can be found in *Online Supplementary Table S2*.

Mutational spectrum of *CBL*-mutated juvenile myelomonocytic leukemia and occurrence of secondary mutations

Clonal *CBL* mutations were confirmed for every patient in the cohort and were exclusively located in the linker and ring finger domains, as previously reported[7,16,20] (Figure 1 and *Online Supplementary Table S3*). Twenty-eight patients had germline alterations of *CBL,* including six patients with deletions affecting these domains. All but two of these patients with germline *CBL* mutations showed a higher VAF of the same mutation in their tumor as a result of heterozygosity (all VAF are shown in *Online Supplementary Table S3*). Of the two patients without heterozygosity, one had a deletion and the other a splice site mutation, compatible with previous reports in other patients.[21] Interestingly, we identified five patients (15%) in our cohort with somatic-only *CBL* mutations. The somatic-only mutations were located at the same hotspot positions as those in the germline population and were homozygous in four patients and heterozygous in one patient. We then conducted further experiments in these five patients with somatic-only *CBL* mutations. To confirm that these mutations were in fact somatic in nature, buccal swabs were tested in four patients and a cord blood sample in the remaining patient. No exonic or intronic alterations of *CBL* or any other Ras pathway genes were found in the germline samples of these five patients by targeted deep sequencing. In one of the patients with a somatic-only *CBL* mutation, we discovered a heterozygous *RUNX1* p.R166Q germline mutation, which has been reported in familial platelet disorder with propensity to myeloid malignancy syndrome.

Overall, missense *CBL* mutations at residue Y371 were the most common alteration in both groups. One patient with a germline *CBL* mutation was found to have a secondary somatic *CBL* mutation with a lower VAF, leading to loss of heterozygosity. No other somatic secondary mutations were found in either cohort using our targeted 26-gene JMML panel at diagnosis.

Comparison of patients with germline or somatic-only *CBL* mutations

Table 2 describes the clinical presentation of patients with germline *CBL* mutations compared to those with somatic-only *CBL* mutations. There were no significant differences between the two cohorts in the patients' characteristics at diagnosis (Table 2). Of note, patients in both cohorts presented at a median age of 1 year. Clinical signs of dysmorphia could not be used to distinguish between the groups,

as 13 of 20 patients with germline *CBL* mutations had no overtly syndromic features (65%, data not available for 8 patients). None of the patients with somatic *CBL* mutations presented with obvious signs of dysmorphia. In eight of 15 (53%) patients with germline *CBL* mutations for whom information was available, the mutation was documented to be inherited from a parent with equal rates of maternal and paternal inheritance.

Outcome and diversity of clinical courses of *CBL*-mutated juvenile myelomonocytic leukemia

The overall survival rate of the whole cohort after a median follow-up of 3.7 years was 41% (95% confidence interval [95% CI]: 7-75%; median survival: 7.9 years) (Figure 2A). However, 45% of the patients (15 of 33) underwent HSCT at a median time of 0.5 years after initial diagnosis (range, 0.3-2.6 years). Overall survival of patients who underwent HSCT was 69% (95% CI: 36-87%; median survival not reached) compared to 31% (95% CI: 1-74%;

Table 1. Patients' characteristics at diagnosis.

|	Whole cohort (n=33)
Median age at diagnosis, years Range	1.1 0.1-25.3

Gender
    Male
    Female
	
	14
	19

Median WBC at diagnosis, x10⁹/L Range	34.2 6.9-196.0
Median absolute monocyte count at diagnosis, x10⁹/L Range	5.8 0.8-31.1
Median platelet count at diagnosis, x10⁹/L Range	68 10-204
Median hemoglobin at diagnosis, g/dL Range	9.9 6.2-11.8
Elevated hemoglobin F for age	24% (6 of 25 with data available)
Abnormal cytogenetics	3% (1 of 30 with data available)
Monosomy 7	0
Splenomegaly	97% (29 of 30 with data available)
Dysmorphic features present	35% (7 of 20 with data available)

Germline *CBL* mutation inheritance
    Maternal origin
    Paternal origin
	53% (8 of 15 with data available)
	4
	4

WBC: white blood cell count.
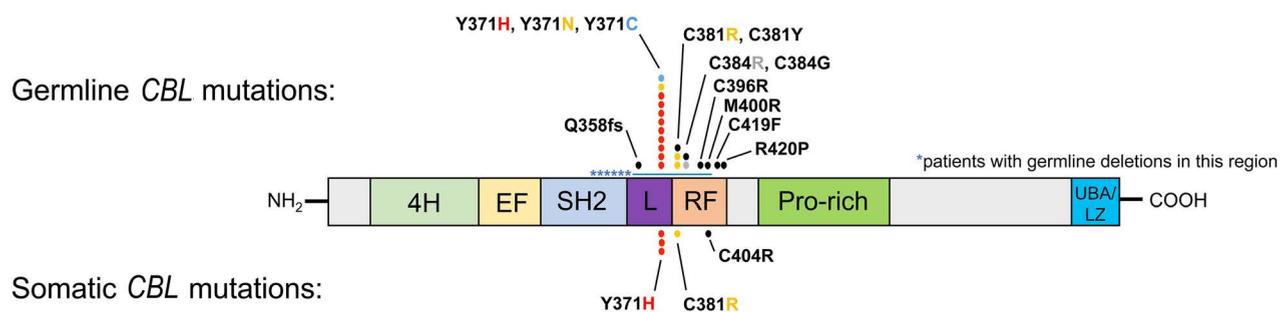
Figure 1. Germline and somatic *CBL* mutations in patients with juvenile myelomonocytic leukemia. Each dot represents one mutation found at the specified codon. The resulting change in amino acid is coded by colors. The stars represent six patients found to have deletions. 4H: four helix bundle; EF: EF-hand like domain; SH2: Src homology 2 domain; L: linker domain; RF: ring finger domain; Pro-rich: proline-rich domain; UBA/LZ: ubiquitin-associated/leucine zipper domain.

median survival: 7.9 years) for patients who did not undergo HSCT (Figure 2B, C). Of note, all three deaths in the non-transplanted cohort were due to other organ failure associated with CBL-syndrome unrelated to JMML. There was no difference in overall survival between the patients with germline *CBL* mutations and those with somatic *CBL* mutations (*Online Supplementary Figure S1*).

We observed that all five patients with somatic *CBL* mutations were refractory to moderately-intense myeloid-based chemotherapy (i.e., 2 g/m²/day cytarabine alone or in combination with 30 mg/m²/day fludarabine).[1] Three of

these five patients received an allogeneic HSCT, and two are alive and disease-free at the time of last follow-up. One relapsed with frank acute myeloid leukemia following allogeneic HSCT for JMML and died of infectious complications (Vignette 1).

The clinical courses of the 28 patients with germline *CBL* mutations were heterogeneous. Three patients experienced spontaneous resolution of JMML without any treatment. Two patients underwent splenectomy as their only treatment but both died several years later of CBL-syndrome-associated organ failure (Vignette 2). Twelve patients received allogeneic HSCT following different pretransplant therapies. Nine of the transplanted patients went into remission after the transplant (2 after graft failure [Vignette 5 in the *Online Supplementary Material* and a separate case previously described by Oshrine.[22]]). Among the remaining three patients who were transplanted, two died of relapsed JMML and one died of transplant-related complications.

Of the 11 patients who were not transplanted and did not experience spontaneous resolution, nine have persistent disease without having been transplanted to date. The median follow-up of these patients is 3.5 years and their treatments included low-dose chemotherapy (Vignette 3), moderately-intense acute myeloid leukemia-directed chemotherapy, azacitidine, and/or targeted agents. Several patients received no therapy, while others were refractory to multiple lines of therapy. One patient with multiple congenital abnormalities died from complications of multi-organ failure in infancy. One patient was lost to follow up. An overview of all disease courses is shown in Figure 3.

Methylation-based clustering of patients' samples

In order to investigate whether DNA methylation profiles are capable of distinguishing patients who will experience spontaneous resolution from those with more aggressive disease, we assessed the methylation of approximately 3,000 CpG loci using peripheral blood of each patient at diagnosis. Patients were designated as having low, intermediate or high methylation based on minimum distance to the centroid of the international consensus definition
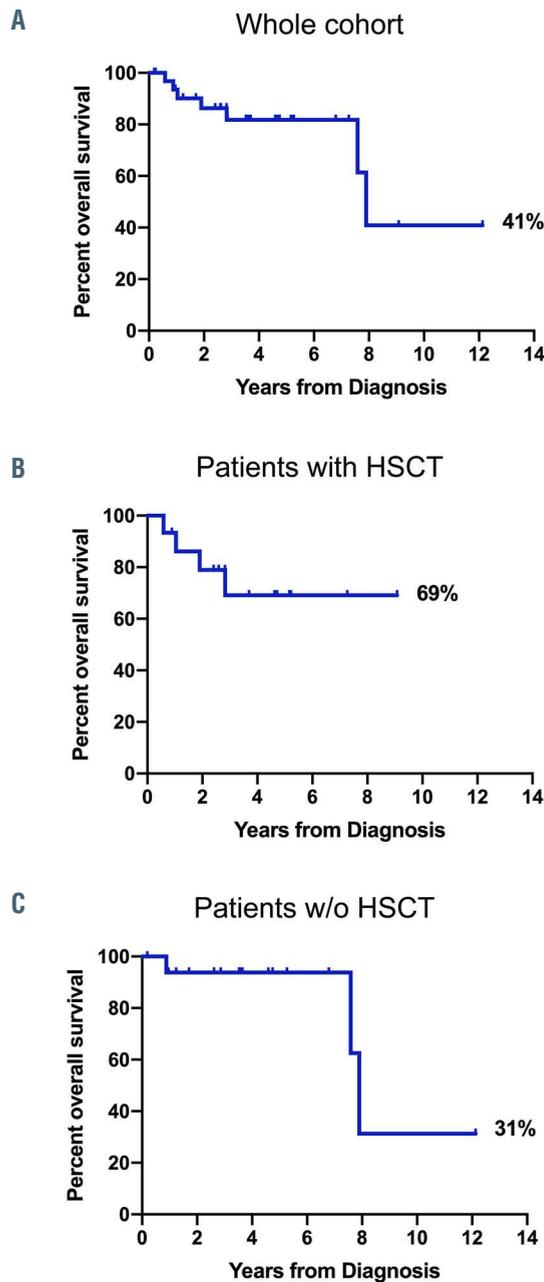

Figure 2. Kaplan-Meier survival curves for *CBL*-juvenile myelomonocytic leukemia. (A) Overall survival of the whole cohort (n=33). (B) Overall survival of patients who underwent hematopoietic stem cell transplantation (n=15). (C) Overall survival of patients who did not undergo hematopoietic stem cell transplantation (n=17). One patient who was lost to follow-up was not considered in analyses for (B) or (C). HSCT: hematopoietic stem cell transplantation; w/o: without.

Table 2. Comparison of clinical characteristics of patients with germline or somatic-only *CBL* mutations.

	Germline *CBL* (n=28)	Somatic *CBL* (n=5)	*P*-value
Median age at diagnosis, years	1.1	1.0	0.79
Range	0.1-25.3	0.6-3.5	
Gender			
Male	12	2	0.85
Female	16	3	
Median WBC at diagnosis, x10⁹/L	31.7	43.0	0.58
Range	6.9-196.0	24.1-88.0	
Median absolute monocyte count at diagnosis, x10⁹/L	5.5	6.7	0.96
Range	0.8-31.1	4.4-12.0	
Median platelet count at diagnosis, x10⁹/L	62	102	0.56
Range	10-204	42-167	
Median hemoglobin at diagnosis, dg/L	9.7	10.4	0.34
Range	6.2-11.8	8.9-11.7	
Elevated hemoglobin F for age	18%	20%	0.91
Abnormal cytogenetics	4%	0%	N/A
Splenomegaly	96%	100%	N/A

WBC: white blood cell count; N/A: not applicable.
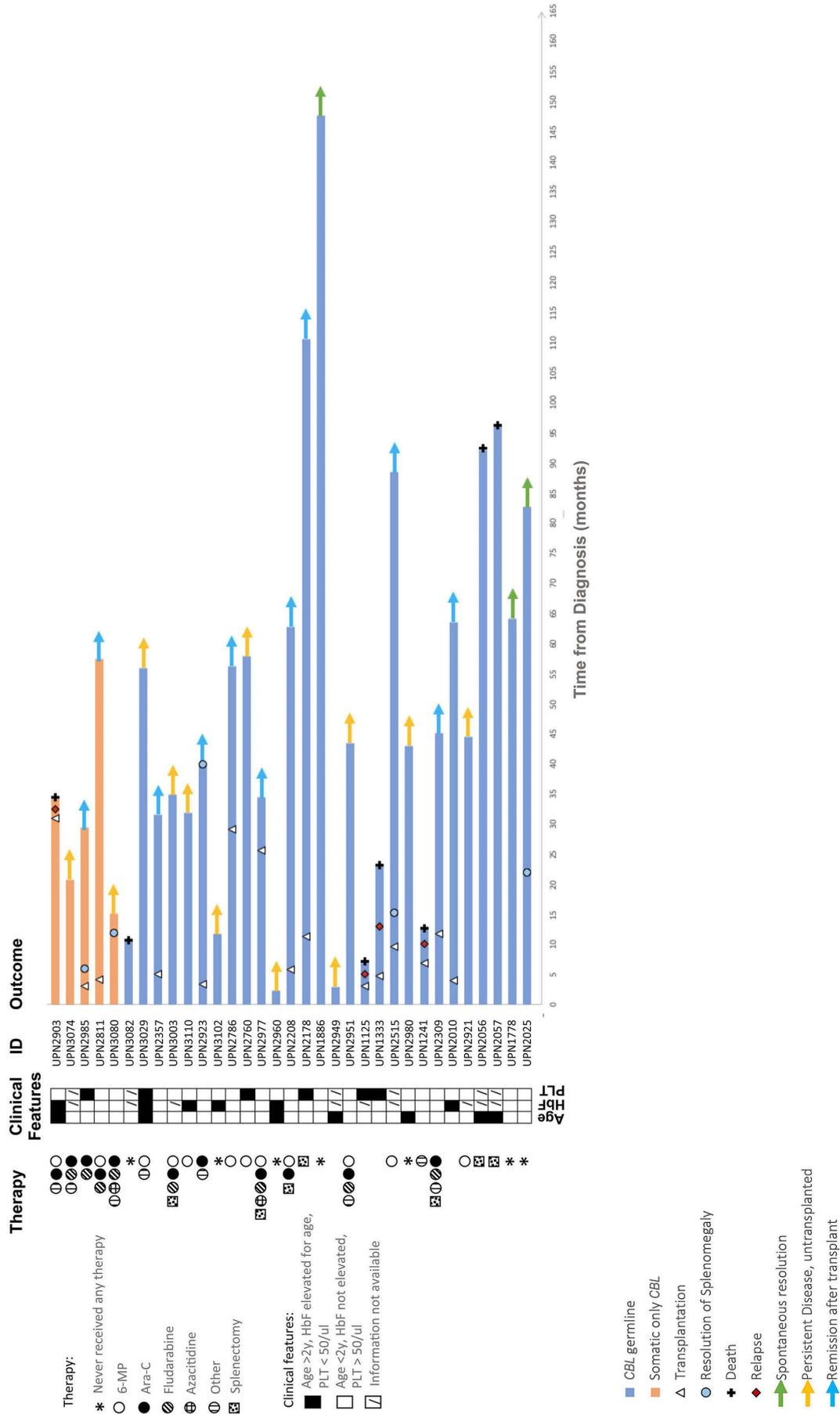
Figure 3. Swimmer plot showing the clinical course of each patient over time. Each bar represents one patient color-coded based on the presence of germline *CBL* (blue) or somatic-only *CBL* (orange). Dates of hematopoietic stem cell transplantation (HSCT), relapse, death or resolution of splenomegaly are depicted by symbols. The current ongoing status of the patient is depicted as a color-coded arrow. Therapeutic agents received by the patient (before HSCT if applicable) are shown on the left side with pattern-coded dots. Clinical features (age, hemoglobin F levels and platelet counts at diagnosis are depicted as filled (if true) or empty (if false) boxes. Dashed boxes are used if data were not available. Information on treatment was missing for patients UPN1125, UPN2949 and UPN 2357; patient UPN2949 was lost to follow-up. HbF: hemoglobin F; PLT: platelet count.
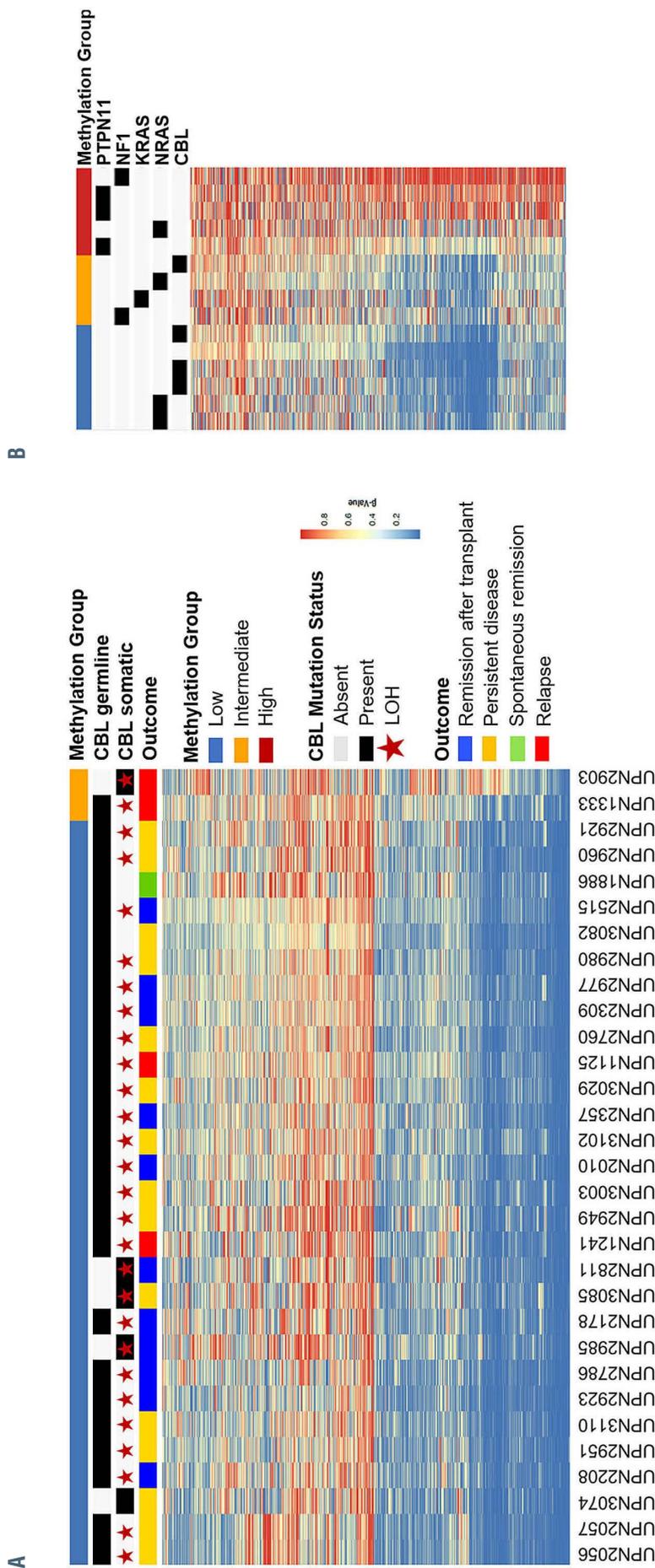
Figure 4. DNA methylation profiles of patients with *CBL*-mutated juvenile myelomonocytic leukemia. (A) *CBL* mutant samples profiled by MethylSeq classified according to the international juvenile myelomonocytic leukemia (JMML) consensus signature. (B) DNA methylation groups low, intermediate, and high were defined using an international cohort of JMML samples described by Schönung *et al.*[12] Each *CBL* mutant sample was classified into one of the three methylation groups based on minimum distance to the nearest centroid. Heatmaps (A and B) show the β values of 1,386 CpG loci used for methylation classification. LOH: Loss of heterozygosity. Two patients in the low methylation group are not depicted in (A) because their methylation data were generated with an Illumina 450k array.

(Figure 4).[1,5,12,23] Thirty-one of the 33 *CBL*-mutated patients were classified as having low methylation irrespective of whether their mutation was germline or somatic in nature, while the other two patients, one with a germline mutation, the other with a somatic mutation, were classified as having intermediate methylation. Notably, both patients who were classified as having intermediate methylation relapsed after HSCT. Unsupervised hierarchical clustering did not reveal any differences in methylation patterns among patients with germline *CBL* mutations who experienced spontaneous remission, survived more than 4 years without HSCT or died without receiving treatment.

To demonstrate the wide spectrum of clinical presentations and the variability in treatment decisions we share the following vignettes.

Vignette 1: UPN2903

A previously healthy 12-month-old female infant was noted to have thrombocytopenia with a normal hemoglobin concentration and white blood cell count on a routine full blood count. The patient was asymptomatic and was observed. At 3 years of age, leukoerythroblastosis was noted on her peripheral blood smear, and her fetal hemoglobin concentration was noted to be elevated for age. Her bone marrow was normocellular for age with trilineage hematopoiesis and no dysplasia. Genetic sequencing revealed a CBL p.Y371H mutation at 70% VAF. The patient was noted to have possible facial dysmorphology. A presumptive diagnosis of germline CBL-associated JMML was made, although no germline testing was performed at that time. The patient was observed for nearly 2 years at which point her white cell count increased to 40x10⁹/L. A repeat bone marrow examination was performed, which revealed transformation to acute myeloid leukemia with 20% blasts and a der(16)t(1;16) on cytogenetics resulting in partial deletion of 16q and partial trisomy for 1q. Repeat tumor testing revealed that the CBL mutation had decreased to 52% VAF, and additional NRAS p.G13D (22%) and KRAS p.T58I (3%) mutations were noted. The patient went on to receive various chemotherapeutic regimens, including daunorubicin, cytarabine and etoposide, mitoxantrone and cytarabine, 6-mercaptopurine, hydroxyurea, trametinib and sorafenib, but was never able to achieve remission. The patient was transplanted with 10% bone marrow blasts using busulfan, cyclophosphamide, and melphalan conditioning, but died of infection and graft-versus-host disease shortly after the transplant. Post-mortem, a cord blood sample was analyzed and confirmed the absence of a germline CBL mutation. However, a heterozygous RUNX1 p.R166Q mutation was detected in the cord blood, indicating that the patient had an autosomal dominant familial platelet disorder with propensity to myeloid malignancy syndrome.

Vignette 2: UPN2056

A 6-month-old patient with several congenital anomalies including hypoplastic kidneys, midgut malrotation, webbed neck, and choanal atresia was suspected of having Kabuki or Noonan syndrome. Over time, he displayed significant developmental delay and short stature. At 3 years of age, this patient developed a myeloproliferative disorder with features suggestive of JMML, including monocytosis, anemia, and juvenile xanthogranulomas, and fewer than 5% blasts in his bone marrow. At this time, tumor sequencing revealed a novel homozygous frameshift mutation in exon 7 of CBL. Germline testing revealed the identical heterozygous deletion. Of note, the patient had an identical twin brother who developed a myeloproliferative disorder at nearly the same time with similar congenital anomalies and was found to carry the same mutation. The patient had a splenectomy at age 4 years, but his monocytosis persisted. The patient had multiple medical comorbidities, including chronic renal disease, seizures and hypothyroidism, and died of complications unrelated to JMML.

Vignette 3: UPN2977

A previously healthy 9-month-old female was evaluated for fatigue and splenomegaly. A full blood count revealed a white blood count of 52x10⁹/L, hemoglobin concentration of 9.0 g/dL, and platelet count of 68x10⁹/L. Bone marrow evaluation demonstrated monocytosis with normal cytogenetics. Genetic testing revealed a germline heterozygous CBL p.Y371H mutation with loss of heterozygosity in the bone marrow. She was initially observed, but subsequently failed to thrive and developed worsening splenomegaly and leukocytosis (peak WBC of 121x10⁹/L). She was started on 6-mercaptopurine with a transient improvement in her white blood cell count. She experienced recurrent splenomegaly, leukocytosis, and thrombocytopenia, so went on to receive two cycles of fludarabine/cytarabine and underwent splenectomy. Despite these interventions, she had persistent leukocytosis. Repeat bone marrow evaluation was consistent with persistent JMML by morphology, but demonstrated new cytogenetic changes with a 3q deletion. Given her refractory JMML and cytogenetic evolution, she received four cycles of azacitidine followed by a haplo-identical peripheral blood stem cell transplant from her mother with busulfan, cyclophosphamide, and melphalan conditioning. She had engraftment failure, so received a stem cell boost and then ultimately received 5/6 unrelated umbilical cord blood transplant with fludarabine, cyclophosphamide, and total body irradiation as conditioning. Her post-transplant course was complicated by recurrent bacteremia and cytomegalovirus viremia. She is now in clinical remission more than 1 year after transplantation and is doing well.

Discussion

In this study, we comprehensively analyzed the largest cohort of *CBL*-mutated JMML patients described to date. The clinical courses and outcomes of the 33 patients analyzed varied dramatically, and a typical "watch and wait" approach was not appropriate for all patients.

Interestingly, we found that five of the 33 patients had somatically acquired *CBL* mutations. To our knowledge, this is the first time somatic *CBL* mutations have been described in JMML. All mutations, whether germline or somatic in nature, were located at the known hotspot loci in the *CBL* gene. No secondary mutations were found in any other JMML-associated genes in any of these patients at diagnosis. Moreover, patients with germline or somatic *CBL* mutations did not differ in their clinical presentation or laboratory features at diagnosis. Surprisingly, the overall survival rate of patients with germline *CBL* mutations in our cohort was lower than that in previous reports. One question that remains unanswered is whether transplantation for germline *CBL*-mutant patients can effectively decrease or prevent the known non-hematologic complications of CBL-syndrome and potentially increase overall survival. There was a trend towards improved overall survival in patients with germline mutations who underwent HSCT but transplant-related mortality remains a concern.

In a prior study, five of six patients with CBL-syndrome

JMML who were not transplanted experienced spontaneous resolution of their hematologic diseases.[8] In our cohort, we observed spontaneous resolution in only three of the 18 non-transplanted *CBL*-mutated JMML patients. In particular, none of the patients with somatic-only *CBL* mutations experienced spontaneous resolution, which is consistent with other JMML subtypes harboring somatic Ras pathway mutations. In adults with chronic myelomonocytic leukemia, somatic *CBL* mutations are found in about 10-15% of cases and are associated with a higher rate of transformation to acute myeloid leukemia and inferior overall outcomes.[14-16] Similarly, while germline *PTPN11* mutations are associated with Noonan syndrome and a typically self-limited form of JMML,[24] patients with somatic *PTPN11* mutations experience aggressive leukemia and rarely, if ever, have spontaneous remission.[23,25] In our cohort, all five patients with somatic-only *CBL* mutations were refractory to medium-intensity myeloid-based chemotherapy and required HSCT for definitive disease control. We thus maintain that patients with somatic-only *CBL*-mutated JMML should not be observed without therapeutic intervention, but instead should be treated with HSCT. Furthermore, somatic-only testing of JMML samples is insufficient for proper identification of these patients, and dedicated germline testing should also be performed in all patients with *CBL* mutations.

Aberrant DNA methylation profiles have shown to be a promising tool for risk stratification in JMML.[5,11,23] We therefore assessed the methylome of each patient at diagnosis. Four of the patients had been assessed for methylation in previous methylation studies (UPN1333, UPN2056, UPN2178, UPN2309) that utilized an array-based approach. The current study assessed methylation status using a sequencing-based approach and all four patients were found to have the same methylation designation as in the previous studies. All but two of the 33 patients clustered with the low-methylation group and both of the patients with intermediate methylation relapsed after HSCT. However, methylation analysis was not able to distinguish between patients who experienced spontaneous remission and those with persistent disease. At this time, methylation analysis does not appear to be a biomarker capable of predicting outcome in patients with *CBL*-mutated JMML, in contrast to other JMML subtypes.

The optimal treatment for patients who require therapy due to splenomegaly or cytopenias is still unclear. The patients reported in this manuscript were diagnosed between 2006 and 2019. The optimal management of *CBL*-mutated patients with JMML has evolved over time, and no consensus currently exists given the relatively recent identification of this JMML subtype and the heterogeneity of its presentation. Several experimental studies have hinted at the potential use of targeted therapies in *CBL*-mutated disease. Mutant CBL is known to engage LYN/PI3K/AKT and JAK2 proteins, and targeted kinase inhibitors of these signaling pathways have been proposed as potential therapeutic strategies.[26,27] Preclinical studies have specifically investigated the SRC/LYN inhibitor dasatinib and have shown decreased sensitivity of *CBL*-mutant JMML cells to granulocyte-macrophage colony-stimulating factor and increased chemotherapy sensitivity.[28,29] Another preclinical study using patient-derived induced pluripotent stem cells with *CBL* mutations reported potential efficacy of JAK2 and PI3K/mTOR signaling inhibition and decreased granulocyte-macrophage colony-stimulating factor hypersensitivity.[30] To date, there have been no prospective clinical trials using these agents in JMML. Further research and international collaboration are warranted to investigate the potential use of targeted therapies in patients with *CBL*-mutated JMML, as a "watch and wait" approach is likely to be effective in only a minority of patients.

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