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A predictive classifier of poor prognosis in transplanted patients with juvenile myelomonocytic leukemia: a study on behalf of the Société Francophone de Greffe de Moelle et de Thérapie Cellulaire.

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Contributions

DM collected data and wrote the manuscript. CA and ACE performed genetic analyses and produced data. SC produced statistical analysis. EL reviewed cytological data. QN collected and reviewed clinical data. AP, FR, GM, DP, CJ, AT, VG, MOC, CP, BB, NB, CP, CC, MF, and AB provided clinical assessments. HC supervised genomic analyses, coordinated the project, and wrote the manuscript. JHD coordinated the project and wrote the manuscript. MS designed and coordinated the project, supervised data collection, and wrote the manuscript. All authors reviewed the manuscript.

Author's disclosures

The authors declare no conflict of interest

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author.

Abstract:

Juvenile myelomonocytic leukemia (JMML) is an aggressive pediatric myeloproliferative neoplasm requiring hematopoietic stem cell transplantation (HSCT) in most cases. We retrospectively analyzed 119 JMML patients who underwent first allogeneic HSCT between 2002 and 2021. The majority (97%) carried a RAS-pathway mutation, and 62% exhibited karyotypic alterations or additional mutations in *SETBP1, ASXL1, JAK3* and/or the RAS pathway. Relapse was the primary cause of death, with a 5-year cumulative incidence of 24.6% (95%CI: 17.1-32.9). Toxic deaths occurred in 12 patients, resulting in treatment-related mortality (TRM) of 9.0% (95%CI: 4.6-15.3). The 5-year overall (OS) and event-free survival were 73.6% (95%CI: 65.7-82.4) and 66.4% (95%CI: 58.2-75.8), respectively. Four independent adverse prognostic factors for OS were identified: age at diagnosis >2 years, time from diagnosis to HSCT ≥6 months, monocyte count at diagnosis >7.2x10^9/L, and the presence of additional genetic alterations. Based on these factors, we proposed a predictive classifier. Patients with three or more predictors (21% of the cohort) had a 5-year OS of 34.2%, whereas those with none (7%) had a 5-year OS of 100%. Our study demonstrates improved transplant outcomes compared to prior published data, which can be attributed to the synergistic impacts of a low TRM and a reduced yet still substantial relapse incidence. By integrating genetic information with clinical and hematological features, we have devised a predictive classifier. This classifier effectively identifies a subgroup of patients who are at a heightened risk of unfavorable post-transplant outcomes who would benefit novel therapeutic agents and post-transplant strategies.

Keywords: Juvenile myelomonocytic leukemia, hematopoietic stem cell transplant, RAS pathway, monocyte, pediatrics.

Introduction

Juvenile myelomonocytic leukemia (JMML) is an aggressive hematologic malignancy of early childhood. It results from the hyperactivation of the RAS signal transduction pathway mainly caused by mutations of *PTPN11*, *KRAS*, *NRAS*, *RRAS, RRAS2, CBL, SH2B3* and *NF1*[1–3]. Except for a small subset of patients who exhibit spontaneous remissions, the disease usually progresses and leads to death within months of diagnosis. Unlike acute leukemia, intensive chemotherapy is insufficient to eradicate the disease and hematopoietic stem cell transplantation (HSCT) is the only curative treatment for most patients[4,5]. However, the disease's rarity has resulted in only a few large cohorts of HSCT being reported over the last twenty years[6,7,8,9,10]. These studies showed that JMML is associated with a poor outcome, with a 5-year overall survival (OS) ranging from 52 to 72% due to high-risk post-transplant relapse combined with high treatment-related mortality (TRM). Predictive variables of poor outcome identified across these trials encompass age at diagnosis and at transplant, abnormal karyotype and HLA disparities. Previously published studies including non-transplanted patients also identified other predictive factors of death and relapse such as low platelet count and high fetal hemoglobin level (HbF)[11,12]. Since then, progress has been made in deciphering the genomic landscape of JMML, contributing to a deeper understanding of the marked heterogeneity that characterizes this disease[13,14]. Indeed, certain initiating mutations, such as *NF1* or *PTPN11* have been shown to correlate with disease aggressiveness. Furthermore, the occurrence of additional genetic mutations, including double RAS mutations, *ASXL1*, *SETBP1* or *JAK3*, while uncommon in JMML, further worsens the overall prognosis of patients[13,14,15,16].

However, it's worth noting that most previously published studies on HSCT in JMML

have provided either incomplete or no genetic information. Consequently, the recent molecular insights associated with aggressive disease have not been challenged within a cohort of transplanted JMML patients. In this study, we report the outcome of 119 children with JMML who underwent HSCT over the past 20 years and were genetically characterized. We evaluated the impact of previously described parameters, as well as the role of the initiating RAS mutation and additional *ASXL1*, *SETBP1* and *JAK3* mutations on prognosis.

METHODS

Patients and data collection

This study investigated 119 consecutive children diagnosed with JMML who received a first allogeneic HSCT between June 2002 and August 2021 in France (Table 1). All patients met the World Health Organization (WHO)'s consensus criteria for JMML[2]. Patients' data were collected retrospectively using the PROMISE database of the European Bone Marrow Transplant group (EBMT) through the *Société Francophone de Greffe de Moelle et de Thérapie Cellulaire* (SFGM-TC). JMML patient samples, bone marrow (BM) and/or peripheral blood (PB), were collected on EDTA at diagnosis. Genomic DNA was extracted from mononucleated cells. Mutational screening using bi-directional Sanger and/or next generation sequencing (NGS) of exons and their flanking intron-exon boundaries was performed on genomic DNA as part of the classic diagnostic workup for JMML, and included *NRAS*, *KRAS*, *PTPN11*, *CBL*, *NF1*, *SH2B3, RRAS*, *RRAS2*, *ASXL1*, *SETBP1* and *JAK3* as previously described[13,17]. Written informed consent for the study was provided by the patients or their guardians in accordance with the Declaration of Helsinki (IRB: 00006477).

Definitions and endpoints

Elevated HbF levels at the time of JMML diagnosis were defined as follows: ≥10% for children aged ≥6 to <12 months, and ≥1% for children aged ≥12 months. HbF levels were considered not interpretable for children under 6 months old.

Relapse was defined as the recurrence of JMML, clinically and morphologically on PB and/or BM analysis, after HSCT. HLA matching and engraftment definitions are provided in the Supplemental Methods. Acute and chronic graft-versus-host disease (GVHD) were diagnosed and graded by each transplantation center according to conventional criteria[18,19]. Treatment of GVHD was based on the protocols used in each center.

TRM was defined as any death occurring from any cause but disease relapse. One patient died of Pneumocystis jirovecii lung infection after JMML relapse while waiting for a second transplant. We considered the death of this patient as related to the HSCT. Event-free survival (EFS) was defined as a composite outcome, including relapse and death, whichever occurred first. In exploratory analyses, we also considered secondary allograft and secondary malignancy as additional events defining a “stringent” EFS.

Statistical analysis

Time-to-event outcomes were measured from the date of transplant to the date of event or date of last follow-up, with a cut-off date of December 30, 2021. TRM and relapse were considered as mutually competing risk events, while death was considered a competing risk for engraftment and GVHD. Engraftment and acute GVHD were arbitrarily censored at 100 days.

The OS and EFS were estimated using Kaplan-Meier estimator. For competing risk analyses of TRM, relapse and GVHD, cumulative incidence functions were estimated[20]. Factors associated with outcomes were analyzed using the Fine and

Gray model for GVHD, proportional hazards models for the cause-specific hazard for relapse and TRM, and Cox proportional hazards models for EFS and OS[21,22]. The proportional hazards assumption was checked by examination of Schoenfeld residuals and Grambsch and Therneau lack-of-fit test[23].

All tests were two-sided, and P-values ≤ 0.05 were considered to indicate a significant association. The analyses were performed using the R statistical software version 4.1.1.

RESULTS

Patients' characteristics at JMML diagnosis and pre-transplant treatment

Table 1 provides a summary of the 119 patients' features. At diagnosis, 64 patients (54%) were less than 2 years. JMML occurred in the setting of a germline predisposing condition in 16 patients (14%): type 1 neurofibromatosis (n=8), CBL syndrome (n=7) and *SH2B3* germline biallelic mutation (n=1). A RAS-pathway mutation was observed in 115 out of 118 patients (97%). The most commonly mutated gene was *PTPN11*, observed in 40% of patients, followed by *KRAS* (22%), *NRAS* (19%), *NF1* (7%), *CBL* (6%) and other less frequent mutations (3%; including *RRAS*, *RRAS2* and *SH2B3*).

The time from diagnosis to HSCT varied among patients, ranging from 1.8 to 44.3 months, but remained consistent throughout the study period and across the genetic subgroups (Table 2, Supplemental Figure 1). Prior to conditioning regimen and transplant, patients received either no treatment (n=13, 11%), low-dose chemotherapy (n=77, 65%, including 6-mercaptopurine, azacytidine (n=8) and low-dose cytarabine), or acute myeloid leukemia-type (AML-type) chemotherapy (n=28,

23.7%) at the physician's discretion (Table 2). The pre-HSCT strategies were uniformly distributed among the *NF1*, *PTPN11*, *KRAS*, and *NRAS* groups, while *CBL* patients exclusively received low-intensity treatment and those without mutation, only intensive chemotherapy (Supplemental Figure 1).

Transplant, engraftment and GVHD occurrence

Transplant characteristics are described in Table 2. All patients underwent myeloablative conditioning regimens, with the majority receiving Busulfan/Fludarabine/Melphalan (Bu/Flu/Mel, n=46) or Busulfan/Cyclophosphamide/Melphalan (Bu/Cy/Mel, n=41) (Table 2, Supplemental Table 1). The Bu/Cy/Mel and Bu/Flu/Mel conditioning regimens were administered at the median year of 2007 (range, 2002-2019) and 2016 (range, 2010-2021), respectively. GVHD prophylaxis according to the donor type is provided in Supplemental Table 2.

Of the 116 patients assessable for engraftment, 100 showed sustained engraftment (Figure 1). The median time to neutrophil recovery was 23 days (range: 12-56), and the median time to a self-sustained platelet count higher than 50 $x10^9$/L was 43 days (range: 14-160). Sixteen patients experienced either primary (n=9) or secondary (n=7) graft failure after a median time of 4 months (range: 2-10). Complete loss of chimerism was concomitant with relapse in 4/16 patients (1 patient with primary and 3 patients with secondary graft failure) (Supplemental Figure 2). Patients who encountered graft failure exhibited a higher prevalence of *CBL* mutations, HLA disparities, cord blood source, and alternative conditioning regimens (other than Bu/Cy/Mel or Bu/Flu/Mel) compared to the rest of the cohort (Supplemental Table 3).

Acute GVHD grade 2-4 was observed in 63 patients (100-day cumulative incidence 53.8%, 95% CI: 44.4-62.3) and acute GVHD grade 3-4 in 38 patients (100-day

cumulative incidence 31.9%, 95% CI: 23.7-40.4) (Supplemental Figure 3). Univariate analyses identified the cytomegalovirus (CMV) status (donor positive/recipient negative) and the absence of elevated HbF as risk factors for developing grade 2-4 acute GVHD, and the *NRAS* mutation as a risk factor for grade 3-4 acute GVHD (Supplemental Table 4). Chronic GVHD was observed in 42 patients (with a 36-month cumulative incidence of 36.0%, 95%CI: 27.2-44.9) (Supplemental Figure 3). Twenty-four had extensive disease and 17 had limited disease (unknown for 1). Only the presence of a mismatched relative HLA status of the donor was significantly associated with the onset of chronic GVHD while having *NF1, KRAS*, and no/other mutation appeared to be protective factors (Supplemental Table 4). The occurrence of acute GVHD grade 3-4 did not significantly impact the 5-year EFS, which was 69.0% (95% CI, 56.8-84.0) with acute GVHD, compared to 65.4% without (95% CI, 54.5-78.6) (p=0.72). In contrast, the occurrence of chronic GVHD led to a reduction in the incidence of relapse or death, although not statistically significant, with a 5-year EFS of 78.8% (95% CI, 64.7-96.1) with chronic GVHD vs 68.3% without (95% CI, 59.5-78.4) (p=0.09) (Supplemental Figure 3).

Patient's outcome

The median follow-up after transplant was 59.5 months (IQR, 21.7 to 118.6). The 5-year OS was 73.6% (95%CI: 65.7 to 82.4), and the 10-year OS 72.2% (95%CI: 64.1 to 81.4) (Figure 2).

Twenty-eight patients relapsed after a median time of 4.6 months (range: 0.5-43.6) from HSCT, resulting in a 5-year cumulative incidence of relapse (CIR) of 24.6% (95%CI: 17.1-32.8). Twelve of them (43%) received a second allogenic transplant, including 6 with the same donor. Six patients remained disease-free over a prolonged post-transplant follow up (median 7 years, range 5-17) while 6 patients relapsed once again (Figure 1). Five died of disease, none from TRM. Time to

relapse, time to second transplant, type of donor and conditioning regimen did not differ between the 2 groups. Of note, 4/6 who did not relapse developed grade 2-4 acute GVHD while it occurred in 1/6 patients in the relapse group. Among the 16 patients who relapsed but did not receive a 2nd transplant, 3 are still alive at 3, 8 and 9 years after HSCT. Patient #192, carrying a *PTPN11* mutation, received 7 cycles of azacitidine. Patient #44, without any identified mutation, received weekly etoposide injections for 3 months, followed by rapamycine until the 6th month post-HSCT. Finally, patient #55, with a *CBL* mutation, achieved CR within a few months of 6-mercaptopurine course.

Twelve patients died from transplant toxicity with a median time of 2.9 months (range: 23 days-67.2 months) resulting in an estimated TRM of 9.0% (95%CI: 4.6-15.3). Toxic causes of deaths included severe GVHD +/- associated with disseminated viral or bacterial infections (n=5), infections (n=4), acute hepatitis of unknown origin (n=1), sinusoidal obstruction syndrome (SOS) (n=1), and thrombotic microangiopathy (n=1). SOS was observed in 32 patients (26.9%).

Of the 16 patients who did not engraft, 9 underwent a second transplant within a year, in the absence of relapse (n=5) or after JMML relapse (n=4) (Figure 1; Supplemental Figure 2). Among the 7 patients who did not receive a subsequent transplant, 3 died of disease recurrence. The remaining 4 patients, comprising one *CBL* patient and 3 *KRAS* patients maintained sustained JMML remission with autologous reconstitution. None had undergone splenectomy before HSCT. The *KRAS* patients have been followed up for 5, 8, and 10 years.

Two patients developed secondary malignancies. One patient with a KRAS mutation, who experienced graft failure, developed T-cell precursor acute lymphoblastic leukemia (ALL) 7 years post-HSCT while still in autologous remission. Remarkably, the same *KRAS p.Gly13Cys* mutation detected in the patient's JMML cells was also

identified in the ALL blast cells. Another patient, who carried a *PTPN11* mutation, developed AML with minimal differentiation 4 years after transplant. The *PTPN11 p.Ala72Val* mutation initially detected in JMML was also found in the AML blasts.

Overall, 39 patients experienced an event, resulting in a 5-year EFS at 66.4% (95%CI: 58.2-75.8), and a 10-year EFS at 65.0% (95%CI: 56.5-74.7) (Figure 2). Also considering second transplants for graft failure without relapse and secondary malignancies as events, the 5-year "stringent EFS" was estimated at 63.6% (95%CI: 55.3-73.3) (Supplemental Figure 4).

Prognostic factors for OS, EFS, relapse and TRM

Table 3 presents the univariate analysis of the patient characteristics that influence the outcomes. Age at diagnosis or age at transplant above 2 years, as well as a monocyte count at diagnosis greater than the third quartile (>7.2 $x10^9$/L), were associated with a lower rate of OS, EFS, and stringent EFS (Table 3, Figure 3, Supplemental Table 5). The negative effect of monocyte count on survival was linked to a higher incidence of relapse (Figure 3). Monocytes >7.2 $x10^9$/L were associated with higher WBC, neutrophil, and lymphocyte counts, as well as a higher BM blasts percentage but were not correlated with platelet count, HbF levels, cytogenetic features, or molecular features (Supplemental Table 6). Although patients with *NF1* mutations tended to exhibit worse outcomes, no significant association was found between RAS initiating variants and OS, EFS, or CIR (Figure 3). Considered individually, abnormal karyotype, pathogenic variants of *SETBP1*, *ASXL1*, *JAK3* or additional *RAS* mutation had no impact on outcome. However, when analyzed collectively, the presence of any of them had a negative impact on OS (Table 3). Conditioning regimens other than Bu/Cy/Mel or Bu/Flu/Mel were associated with

lower EFS. Finally, time to HSCT longer than 6 months impaired OS related to a higher TRM.

The multivariable model confirmed the prognostic impact of monocyte count on OS, EFS and relapse. Age at diagnosis remained significant for OS and EFS. Additionally, time to HSCT and any additional alteration were found significant for OS (Table 4).

Finally, we derived a prognostic classifier based on the 4 predictors of death in the multivariate analysis (age at diagnosis, time to transplant, monocyte count and any additional alteration). This classifier defined prognostic groups of patients with 5-year OS ranging from 34.2% for patients with at least three predictors (n=23, 20.7%) to 100% for patients with none of the four predictors (n=8, 7%) (Figure 4).

Discussion

We present here a comprehensive analysis of the outcome of a large cohort of transplanted JMML patients. All patients met the JMML diagnostic criteria, recently revised in the WHO classification, including genetic characterization, which enables us to formally establish the JMML diagnosis[24,25].

With a 5-year EFS of 66% and OS of 74%, our results compare favorably with previous published studies[5,7,8,9,10,26,27,28,29,30](Supplemental Table 7). The improvement in OS among JMML patients over time has been remarkable, with survival rates increasing from 31% in the 1990s to 72% in the most recent reports.[31]. While the results have plateaued in recent years, it's important to consider that the composition of the transplanted cohorts has changed over time, gradually leading to the exclusion of patients with the most favorable prognosis. Indeed, it has been demonstrated that patients with *CBL* mutations experience a naturally favorable

evolution and may no longer necessitate HSCT, unless they demonstrate an aggressive clinical course or a high mutational burden[32]. Additionally, a smaller subset of *NRAS* patients with non-high-risk features can also avoid HSCT. Despite encompassing a time span of over 20 years, the incidence of *CBL* mutations in our transplanted series (6%) is lower than the typical expectation for JMML at diagnosis. This difference probably reflects the implementation of the watch-and-wait recommendations for these patients. As the proportion of transplanted patients with more severe conditions increased over time, it is reasonable to infer that the observed improvement in OS and EFS in our study are significant.

In our cohort, the survival improvement can be attributed to a reduction in TRM, coupled with a decrease in relapse incidence, which nevertheless still accounts for approximately one-quarter of patient deaths and remains the leading cause of mortality. Indeed, the relapse incidence in our cohort was estimated at 25%, which is lower compared to most reported series where it often surpassed 30% (Supplemental Table 7). Previous studies have demonstrated the critical role of both acute and chronic GVHD in preventing disease recurrence[8,9,28,33]. Consistent with these findings, we noted a significant association between chronic GVHD and improved CIR, which can be attributed to the potent graft-versus leukemia (GVL) effect. The high incidence of GVHD in our cohort may be related to the frequent utilization of unrelated donors and could also reflect physicians' endeavors to enhance alloreactivity in patients known to be sensitive to GVL through the accelerated tapering of immunosuppressors, although this data was not captured. The impact of GVHD on disease control following a 2nd transplant is suggested in our cohort. However, its translation into overall survival could be hindered by the TRM associated with extensive GVHD, as reported in a recent series[34]. The TRM in our cohort was evaluated at 9%, which is below the levels observed in earlier studies,

where reported TRM exceeded 10% (Supplemental Table 7). This improvement may be attributed to the overall advancement in supportive care in transplantation over time, the limited use of TBI in our cohort and the low frequency of HLA mismatched donors. Our analysis identified that an extended duration between diagnosis and transplant has a negative impact on TRM and OS. This variable encompasses several parameters that could contribute to an increased TRM, including iterative transfusions, compromised nutritional status, and infections. The specific factors influencing the decision of the transplant date have not been identified within our cohort. These factors could encompass organizational constraints, including graft availability, or disease-related considerations, such as the aggressive nature of JMML necessitating multiple courses of chemotherapy. However, within our patient series, the median time from diagnosis to HSCT remained consistent, both for patients requiring AML-type chemotherapy and those who had no or low-dose chemotherapy. This finding suggests that transplantation was sometimes planned with a considerable long delay, even for patients with non-aggressive diseases. Given the impact of this delay on TRM, our results suggest the prompt scheduling of the transplant once the diagnosis of JMML is confirmed. The association between *SETBP1* and TRM is more difficult to understand and may be biased by the limited size of this group. The Japanese group adopted the Bu/Flu/Mel regimen in JMML with promising results, aiming to mitigate the toxicity associated with the triple alkylation of the Bu/Cy/Mel regimen[10,35]. Although there is no evidence favoring one of these two conditioning regimens over the other in the literature, it is crucial to recognize the pivotal role of their intensity. Notably, in the only randomized study focusing on JMML conditioning, attempts to reduce the regimen's intensity were unsuccessful, as Bu/Flu resulted in a significantly higher relapse rate when compared to Bu/Cy/Mel[28]. In our study, both Bu/Flu/Mel and Bu/Cy/Mel conditionings led to similar EFS and OS, while other types of conditioning impaired EFS.

Five patients with autologous reconstitution survived. In *CBL* patients, as in *NRAS* patients, this outcome is expected as many of them show a spontaneously favorable evolution without HSCT. On the contrary, patients with *KRAS* mutations typically have an aggressive disease course and require HSCT to be cured. Nevertheless, case reports have described prolonged remissions in some *KRAS* patients treated with azacitidine or without any treatment at all [36,37,38]. It has also been reported that patients with Ras-associated autoimmune leukoproliferative disorder (RALD), carrying the same somatic *KRAS* mutations than in JMML, can present indolent condition difficult to distinguish from JMML[39]. In our cohort, the three *KRAS* patients who survived with autologous reconstitution had no clinical autoimmune manifestations or immunophenotypic abnormalities. While the conditioning regimen and the transient presence of allogenic stem cells could have contributed to disease control, our findings are consistent with observations in RALD and indolent KRAS JMML cases, suggesting that KRAS-mutated patients might encompass a spectrum of conditions ranging from mild to highly aggressive diseases. Exploring this matter further remains essential to refine treatment strategies and accurately differentiate patients who truly require transplantation from those who could potentially avoid it [40].

Age at diagnosis over 1, 2 or 4 years and time between diagnosis and HSCT over 6 months, which have been consistently described by independent study groups to influence the outcome of transplanted JMML patients, were also identified in our cohort to alter survival in multivariate analysis (Supplemental Table 7). Conversely, other classical prognostic factors such as platelet counts <33 $x10^9$/L, elevated HbF for age, elevated BM blast percentage and abnormal karyotype were not found to influence outcome. Some of these prognostic markers have been demonstrated to be closely related to the genetic background of the disease, *PTPN11* or *NF1*-JMML tending to harbor significantly lower platelet count, higher HbF or more frequent

karyotypic alteration than *CBL* or *NRAS*-JMML[41]. As previously discussed, when considering the comparability of studies conducted over time, the prognostic impact of some markers may have been lost in this selected aggressive group of JMML patients. Additionally, it is plausible that the relatively limited number of patients included in our study may have resulted in insufficient statistical power to detect their influence on survival. The role of pre-transplant chemotherapy in survival for patients with JMML remains controversial. In line with previous studies, we did not find any difference in EFS, or relapse incidence based on the chemotherapy regimen received before transplant[6,8.] This finding reaffirms that patients with a clinical condition compatible with low-intensity treatment do not require intensive chemotherapy. Due to the infrequent utilization of azacitidine in our cohort, we were unable to compare the outcomes of this subgroup with those of patients who received 6-mercaptopurine.

Among our 8 patients who received azacitidine, 3 relapsed after transplant and 4 died consequently to relapse or TRM (n=1). The EWOG group recently published the prospective AZA-JMML-001 trial, investigating the impact of pre-transplant azacitidine in 18 patients. After three cycles, 61% of patients exhibited a partial response, and 14 achieved CR after HSCT during a 2-year follow-up[42]. The recent broader utilization of azacitidine will allow to determine on a larger scale whether this approach indeed yields a positive impact on post-transplant outcomes. In JMML, secondary genetic alterations including karyotype anomalies and additional mutations of the RAS pathway or *SETBP1*, *ASXL1* and *JAK3* have been demonstrated to be associated with an unfavorable prognosis. This has been highlighted by studies conducted by our group and others in unbiased cohorts of JMML patients, including transplanted and non-transplanted ones[13,14]. In this study, we confirm the relevance of these secondary alterations in a selected population of

transplanted patients and found that the number of genetic alterations, rather than the type of the alteration, was the main determinant factor for OS. However, secondary alterations did not impact the incidence of relapse. Unlike secondary alterations, the initiating RAS-pathway alterations were not associated with the outcome in our study, possibly due to insufficient statistical power. Indeed, *CBL* patients exhibited an excellent prognosis while *NF1* patients had the worst survival due to a high incidence of relapse, in line with results from previous studies[13,14]. In recent years, DNA methylation profiling has emerged as a novel prognostic marker in JMML, as demonstrated in three distinct patient cohorts[43,44,41]. This methylation profile has shown associations with disease biology and clinical outcomes. Methylation has not been investigated in the current cohort, but it would be intriguing to explore its relevance as a prognostic marker in a cohort of transplanted JMML patients, predominantly comprising high-risk features patients.

In addition, our study shows that monocyte count higher than 7.2 $x10^9$/L, was an independent factor of adverse outcomes and relapse. To our knowledge, this is the first instance where monocyte count is identified as a prognostic factor for JMML, although this variable has not been often evaluated in the past series. Monocytosis in peripheral blood is the hallmark of JMML and is a mandatory criterion to ascertain the diagnosis. Elevated monocyte count has been linked to disease aggressiveness and worse survival in adult myelodysplastic syndromes, myeloproliferative neoplasms, and chronic myelomonocytic leukemia (CMML)[45,46,47,48]. In CMML, the closest adult counterpart of JMML, different subsets of monocytes, as well as their levels, may play a role in the outcome of patients, with a specific inflammatory fraction being associated with a poor prognosis[49,50,51]. The distribution of the different monocyte fractions in JMML has not yet been thoroughly explored, and therefore, we could not correlate our findings with a comprehensive immunophenotypic and

functional analysis. It would be valuable to investigate this in the future to determine if the adverse outcomes are linked to a specific subset.

Overall, this study underscores promising survival outcome for transplanted JMML patients that can primarily be attributed to the remarkably low TRM. However, it is important to note the persistent high relapse rate, which reflects the limited availability of novel and effective anti-tumoral agents. We confirmed, on a genetically tested cohort, the significant impact of additional alterations on prognosis and found that elevated monocyte count is independently correlated with poor outcome. The prognostic classifier we developed identifies transplanted patients who are most susceptible to relapse and who could benefit from post-transplant interventions. This personalized approach holds promise for improving the outcomes and long-term survival of high-risk JMML patients after transplantation.

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Table 1: Patients characteristics at diagnosis.

	No. of patients assessed	Value
M/F ratio		2.2
Female no. (%)		37 (31.1)
Male no. (%)		82 (68.9)
Median age at diagnosis, (IQR), years	119	1.6 [0.7-3.2]
Hepatomegaly, no. (%)	117	89 (76.1)
Splenomegaly, no. (%)	117	109 (93.2)
Peripheral blood cell count, median (IQR) x10^9/L		
White blood cells	118	25.5 [16.4-46.8]
Platelets	116	59 [33-110]
Platelets <33 x10^9/L, no. (%)	116	30 (25.9)
Monocytes	119	5.1 [2.7-7.2]
Hemoglobin, g/dl	117	8.9 [8-10.3]
Presence of myeloid precursors, n (%)	116	106 (91.4)
Presence of blasts cells, n (%)	115	80 (69.6)
BM blasts percentage, median (range)	118	4 [0-37]
Elevated HbF*, no. (%)	88	73 (82.9)
Karyotypic alterations, no. (%)	113	45 (39.8)
Del7/7q		26 (23.0)
Other aneuploidies		19 (16.8)
Normal		68 (60.2)
RAS-pathway mutations, no. (%)	118	
PTPN11		47 (39.8)
KRAS		26 (22.0)
NRAS		23 (19.5)
CBL		7 (5.9)
NF1		8 (6.8)
Other[$]		4 (3.4)
No mutation		3 (2.5)
Additional mutations, no. (%)	114	39 (34.2)
JAK3		10 (8.8)
SETBP1		9 (7.9)
ASXL1		11 (9.6)
Double RAS pathway mutation		25 (21.9)
Additional alterations, no. (%)**	111	
0		42 (37.8)
≥ 1		69 (62.2)
≥ 2		20 (18.0)

[$]Other mutations include *RRAS*, *RRAS2* and *SH2B3*

IQR, interquartile range; AML, acute myeloid leukemia, HbF, fetal hemoglobin, BM, Bone marrow;
* for patients ≥6 months; ** 0, 1 or 2 alterations among the following genes: *ASXL1, JAK3, SETBP1,* or double RAS mutation or karyotype anomaly.

Table 2: Pre-transplant treatment and transplant characteristics.

	No. of patients assessed	Value
Pre-HSCT treatment, no. (%)	118	
Low-dose chemotherapy		77 (65.3)
AML-like chemotherapy		28 (23.7)
No chemotherapy		13 (11.0)
Splenectomy, n (%)	119	5 (4.2)
Median age at HSCT, years (IQR)	119	2.5 [1.5-3.9]
Median interval from diagnosis to HSCT, months (IQR)		5.8 [4.2-8.6]
Donor, no. (%)	119	
Matched sibling		24 (20.2)
Haploidentical		4 (3.4)
Matched unrelated		46 (38.7)
Mismatched unrelated		45 (37.8)
Source of cells, no. (%)	119	
Cord blood		39 (32.7)
Bone marrow		70 (58.8)
Peripheral blood		10 (8.4)
Donor/recipient gender, no. (%)	114	
Female/Female		15 (13.2)
Female/Male		22 (19.3)
Male/Female		28 (24.6)
Male/Male		49 (43.0)
Donor/recipient CMV status, no. (%)	115	
Negative/Negative		57 (49.6)
Negative/Positive		19 (16.5)
Positive/Negative		19 (16.5)
Positive/Positive		20 (17.4)
Conditioning, no. (%)	119	
Bu/Cy/Mel		41 (34.4)
Bu/Flu/Mel		46 (38.7)
Other		32 (26.9)
Total body irradiation, no. (%)		3 (2.5)
Anti-thymoglobulin, no. (%)		57 (47.9)
GVHD prophylaxis	117	
CsA + MTX		41 (35.0)
CsA		33 (28.2)
CsA + Corticosteroids		27 (23.1)
CsA + MMF		10 (8.5)
CsA + other combinations		6 (5.1)

IQR, interquartile range; HSCT, hematopoietic stem cell transplantation; AML, acute myeloid leukemia; Bu/Cy/Mel, busulfan/cyclophosphamide/melphalan; Bu/Flu/Mel, busulfan/fludarabine/melphalan; GVHD, graft-versus-host disease; CsA, Cyclosporin A; MM, mycophenolate mofetil; MTX, methotrexate.

Table 3: Univariable predictive analyses of the outcomes based on Cox models

Outcomes		OS	EFS	Relapse	TRM
	No. of patients	60-month survival, 95% CI	60-month event-free survival, 95% CI	60-month cumulative incidence, 95% CI	60-month cumulative incidence, 95% CI
Overall	119	73.6%, 65.7-82.4	66.4%, 58.2-75.8	24.6%, 17.1-32.8	9.0%, 4.6-15.3
No. of events		30 deaths	39 events	28 relapses	11 toxic deaths
Age at diagnosis ≥ 2 years	55	**3.08 (1.41-6.74)**	**2.34 (1.22-4.51)**	1.99 (0.93-4.24)	3.68 (0.97-13.9)
Platelet < 33 x10^9/L	28	0.88 (0.36-2.18)	1.01 (0.48-2.15)	0.93 (0.38-2.33)	1.21 (0.32-4.58)
Monocyte > Q3 (7.2 x10^9/L)	30	**2.21 (1.06-4.60)**	**2.38 (1.24-4.54)**	**2.90 (1.37-6.14)**	1.36 (0.36-5.15)
Elevated HbF*	73/88	0.84 (0.35-1.97)	1.30 (0.63-2.70)	0.76 (0.32-1.80)	1.23 (0.27-5.70)
Abnormal karyotype	45	1.77 (0.84-3.75)	1.45 (0.76-2.77)	1.82 (0.84-3.96)	1.67 (0.51-5.47)
RAS-pathway mutations					
PTPN11	46	1.00	1.00	1.00	1.00
CBL	7	NA	0.38 (0.05-2.85)	0.43 (0.04-4.13)	NA
KRAS	26	0.35 (0.10-1.21)	0.44 (0.16-1.20)	0.29 (0.06-1.44)	0.50 (0.10-2.49)
NF1	8	2.79 (0.99-7.88)	1.98 (0.73-5.38)	2.10 (0.50-8.83)	NA
NRAS	23	1.38 (0.57-3.33)	1.00 (0.43-2.32)	0.70 (0.17-2.91)	1.05 (0.26-4.18)
No/other	9	0.35 (0.05-2.69)	0.92 (0.27-3.13)	0.71 (0.20-2.56)	NA
Additional mutations					
JAK3	10	1.55 (0.54-4.47)	2.00 (0.84-4.81)	0.69 (0.26-1.87)	1.20 (0.15-9.41)
SETBP1	9	2.58 (0.98-6.77)	2.38 (0.99-5.70)	0.74 (0.22-2.53)	**4.68 (1.24-17.69)**
ASXL1	11	1.65 (0.57-4.74)	1.09 (0.39-3.08)	0.70 (0.20-2.43)	0.96 (0.12-7.50)
Dble RAS pathway mutation	25	0.96 (0.39-2.36)	0.94 (0.43-2.06)	0.86 (0.29-2.53)	2.10 (0.62-7.19)
Additional alteration**					
>=1 Alteration	67	**2.55 (1.04-6.27)**	2.10 (0.99-4.44)	0.82 (0.35-1.95)	NA
>=2 Alterations	20	1.51 (0.64-3.53)	1.68 (0.82-3.47)	0.45 (0.18-1.12)	1.94 (0.51-7.36)
Pre-HSCT treatment					
Low-dose chemotherapy	77	1.00	1.00	1.00	1.00
No chemotherapy	13	0.50 (0.12-2.12)	0.30 (0.07-1.25)	0.28 (0.04-2.12)	0.67 (0.08-5.35)
AML-type chemotherapy	28	0.69 (0.28-1.71)	0.88 (0.45-1.75)	1.41 (0.63-3.13)	0.70 (0.15-3.32)
Pre-HSCT BM ≥5%	42	1.05 (0.50-2.22)	0.84 (0.45-1.55)	0.98 (0.45-2.11)	1.04 (0.31-3.56)

Time to HSCT ≥ 6 months	52	**2.65 (1.26-5.58)**	1.46 (0.82-2.61)	1.52 (0.72-3.18)	**3.78 (1.00-14.2)**
Donor					
Geno-Identical sibling	24	1.00	1.00	1.00	1.00
Matched unrelated	46	0.54 (0.22-1.33)	0.60 (0.27-1.32)	0.41 (0.17-1.02)	1.33 (0.14-12.9)
Mismatched relative	4	NA	0.50 (0.06-3.89)	0.55 (0.07-4.26)	NA
Mismatch unrelated	45	0.62 (0.26-1.49)	1.00 (0.48-2.09)	0.43 (0.17-1.10)	3.43 (0.42-28.0)
HLA disparities ≥ 2	40	0.77 (0.35-1.68)	1.11 (0.61-2.03)	0.64 (0.27-1.50)	3.09 (0.90-10.6)
Stem cells					
Bone marrow	70	1.00	1.00	1.00	1.00
Cord blood	39	0.81 (0.37-1.80)	1.07 (0.58-1.97)	0.54 (0.22-1.35)	2.87 (0.84-9.83)
Peripheral blood	10	0.72 (0.17-3.10)	0.48 (0.11-2.01)	0.70 (0.16-3.01)	NA
Conditioning					
Bu/Cy/Mel	41	1.00	1.00	1.00	1.00
Bu/Flu/Mel	46	1.35 (0.58-3.13)	0.87 (0.42-1.83)	0.34 (0.11-1.04)	4.26 (0.90-20.29)
Other	32	1.20 (0.47-3.05)	**2.04 (1.03-4.05)**	1.77 (0.79-3.95)	0.84 (0.08-9.26)

NA refers to the models that do not converge due to a too low number of events in one stratum. Significant results are in bold letters.
* For patients ≥6 months, ** At least 1 or 2 alterations among the following genes: *ASXL1, JAK3, SETBP1,* or double RAS mutation or karyotype anomaly.
HSCT, hematopoietic stem cell transplantation; CB, cord blood; BM, bone-marrow; PB, peripheral blood; Bu/Cy/Mel, busulfan/cyclophosphamide/melphalan; Bu/Flu/Mel, busulfan/fludarabine/melphalan.

Table 4: Multivariate analyses for OS, EFS and relapse

Outcome/model	HR (95%CI)	*P*
Overall survival		
Age ≥ 2 years at diagnosis	3.14 (1.31-7.51)	0.010
Time to HSCT ≥ 6 months	6.48 (2.84-14.76)	<0.001
Monocyte ≥ 7.2 x10^9/L	3.59 (1.65-7.84)	0.001
≥1 additional alteration	2.88 (1.05-7.86)	0.039
Event-free survival		
Age ≥ 2 years at diagnosis	2.61 (1.35-5.05)	0.0044
Monocyte ≥ 7.2 x10^9/L	2.70 (1.40-5.18)	0.0029
Relapse		
Monocyte ≥ 7.2 x10^9/L	4.07 (1.82-9.09)	0.00061

HSCT, hematopoietic stem cell transplantation.

Figure legends

Figure 1: Flow chart of the transplanted JMML cohort. HSCT, hematopoietic stem cell transplant; TRM, treatment-related mortality; CR, complete remission; DOD, dead of disease.

Figure 2: Estimated Outcomes of the 119 transplanted patients. Overall survival (A) and Event-free Survival (B) were analyzed using Kaplan-Meier methodology. Cumulative incidence function for Relapse and Treatment-related Mortality (C). OS, overall survival; EFS, event-free survival; CI, cumulative incidence; TRM, Treatment-related mortality.

Figure 3: Effect of monocyte count and initiating RAS-mutation on outcomes. **Effect of monocyte count:** Overall survival (A) and Cumulative incidence function of Relapse (B). **Effect of initiating RAS-mutation:** Overall survival (C) and Event-free Survival (D). OS and EFS were analysed using Kaplan-Meier methodology. OS, overall survival; EFS, event-free survival; CI, cumulative incidence.

Figure 4: Prognostic classifier for overall survival. This classifier defined 4 prognostic groups of patients according to the 4 predictor factors from the multivariate analysis. OS, overall survival; HSCT, hematopoietic stem cell transplant.

Figure 1

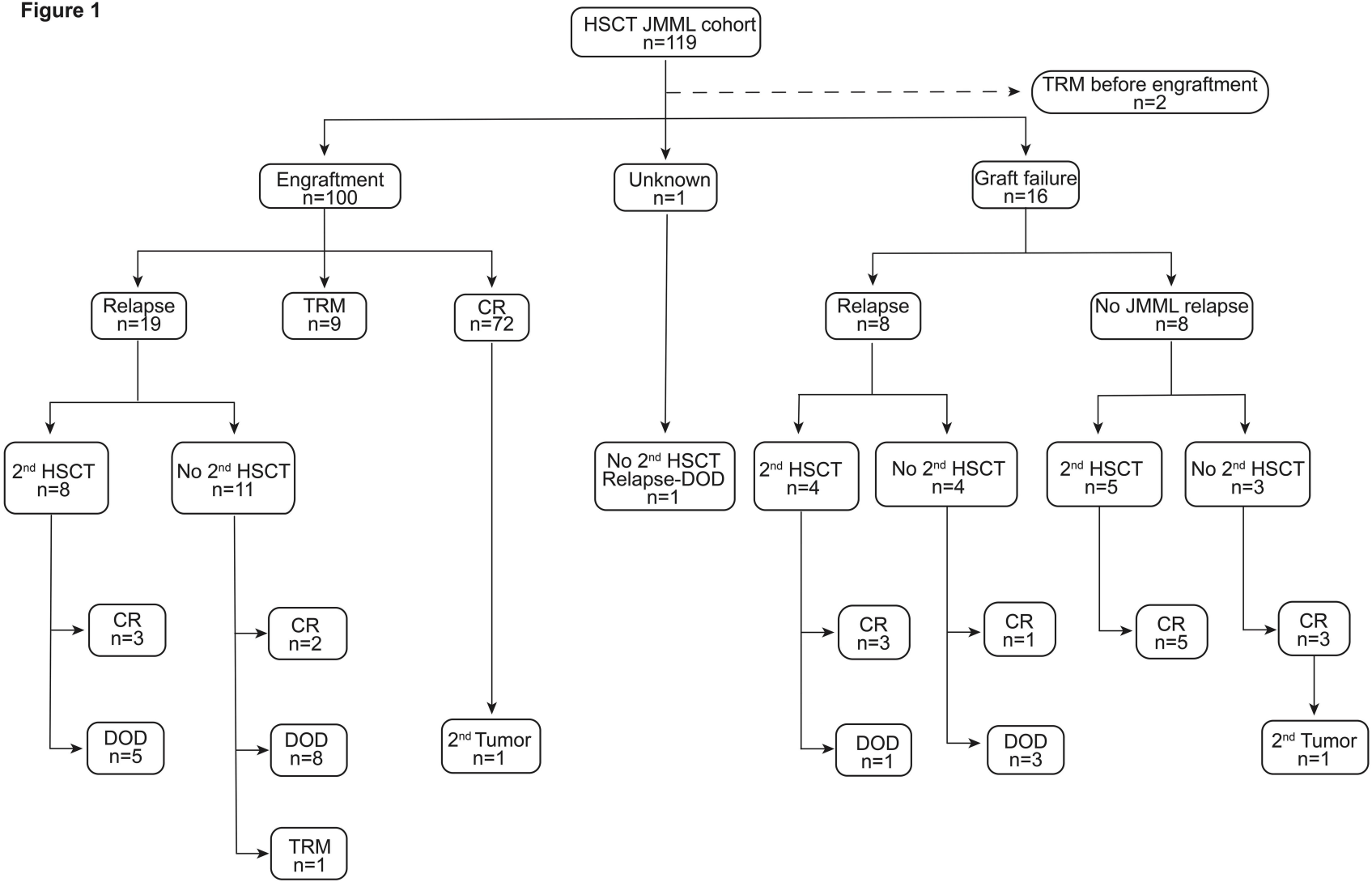

HSCT JMML cohort n=119
TRM before engraftment n=2
Engraftment n=100
Unknown n=1
Graft failure n=16
Relapse n=19
TRM n=9
CR n=72
Relapse n=8
No JMML relapse n=8
2nd HSCT n=8
No 2nd HSCT n=11
No 2nd HSCT Relapse-DOD n=1
2nd HSCT n=4
No 2nd HSCT n=4
2nd HSCT n=5
No 2nd HSCT n=3
CR n=3
CR n=2
DOD n=5
DOD n=8
2nd Tumor n=1
TRM n=1
CR n=3
DOD n=1
CR n=1
DOD n=3
CR n=5
CR n=3
2nd Tumor n=1

Figure 2

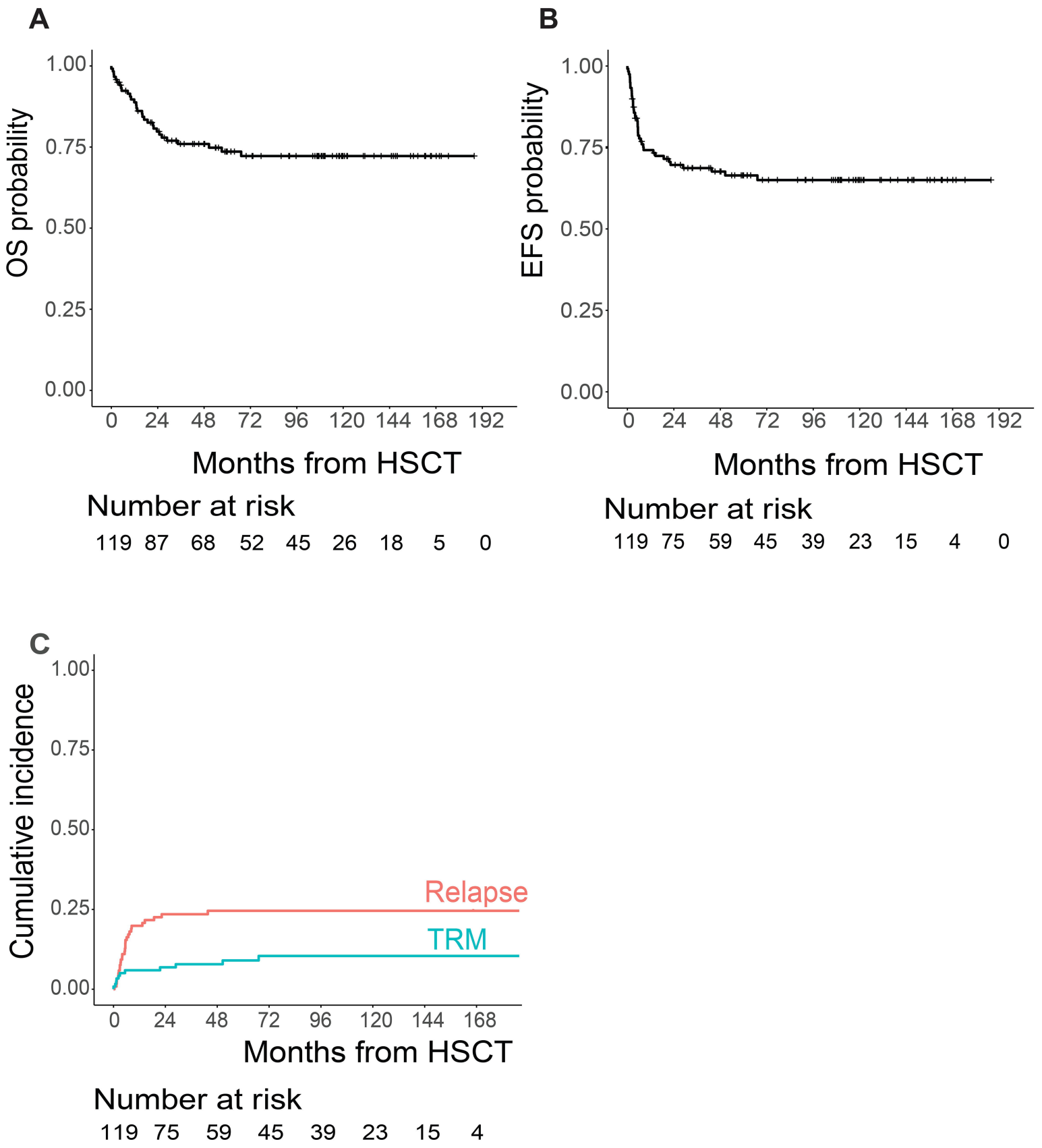

A
OS probability
1.00
0.75
0.50
0.25
0.00
0 24 48 72 96 120 144 168 192
Months from HSCT
Number at risk
119 87 68 52 45 26 18 5 0
B
EFS probability
1.00
0.75
0.50
0.25
0.00
0 24 48 72 96 120 144 168 192
Months from HSCT
Number at risk
119 75 59 45 39 23 15 4 0
C
Cumulative incidence
1.00
0.75
0.50
0.25
0.00
Relapse
TRM
0 24 48 72 96 120 144 168
Months from HSCT
Number at risk
119 75 59 45 39 23 15 4

Figure 3

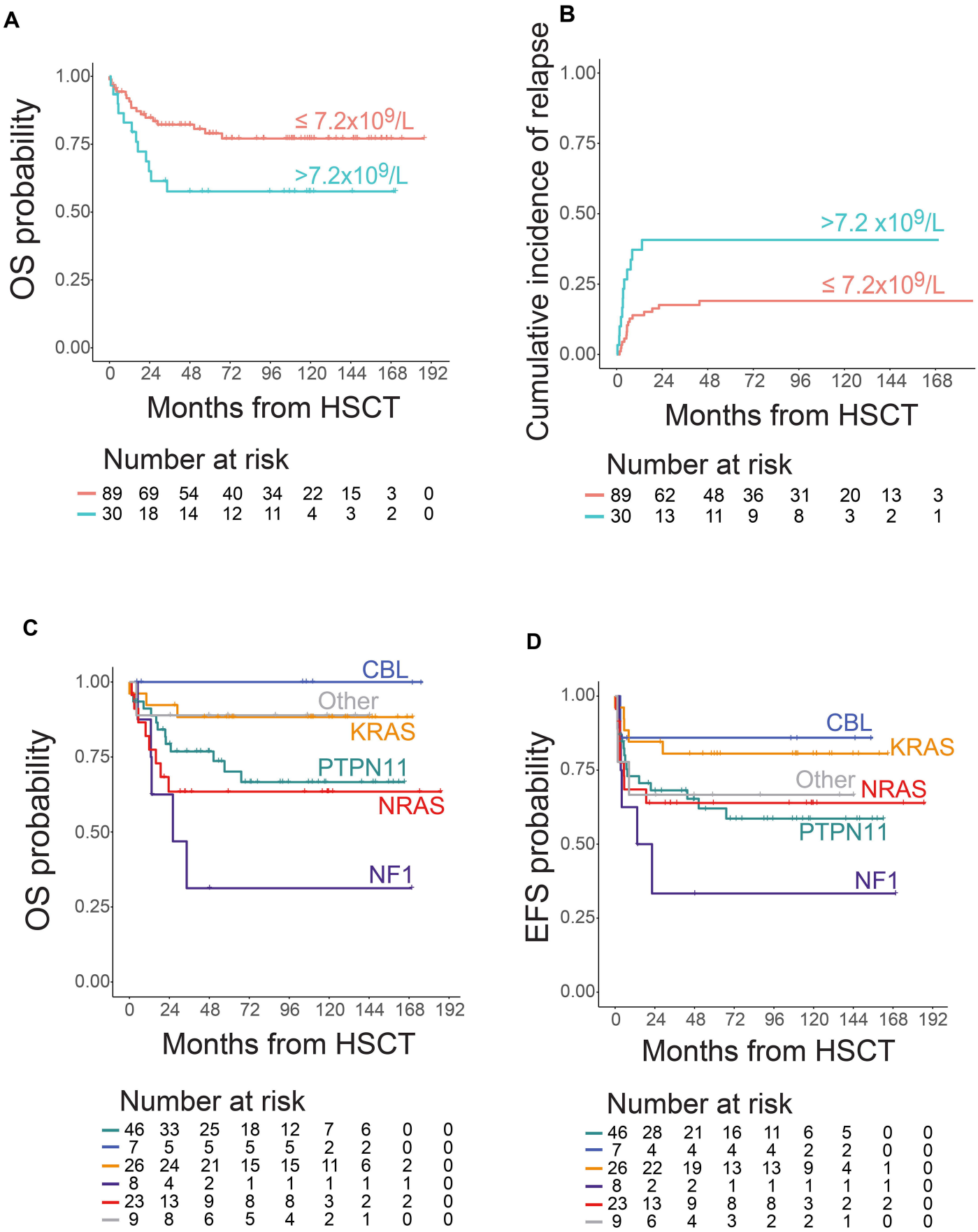
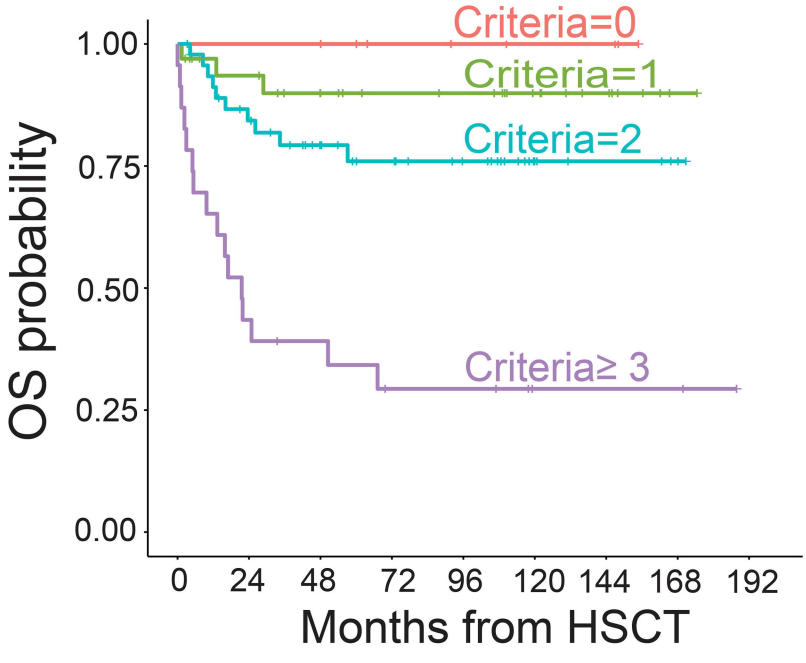

A
OS probability
1.00
0.75
0.50
0.25
0.00
≤ 7.2x10⁹/L
>7.2x10⁹/L
0 24 48 72 96 120 144 168 192
Months from HSCT
Number at risk
89 69 54 40 34 22 15 3 0
30 18 14 12 11 4 3 2 0
B
Cumulative incidence of relapse
1.00
0.75
0.50
0.25
0.00
>7.2 x10⁹/L
≤ 7.2x10⁹/L
0 24 48 72 96 120 144 168
Months from HSCT
Number at risk
89 62 48 36 31 20 13 3
30 13 11 9 8 3 2 1
C
OS probability
1.00
0.75
0.50
0.25
0.00
CBL
Other
KRAS
PTPN11
NRAS
NF1
0 24 48 72 96 120 144 168 192
Months from HSCT
Number at risk
46 33 25 18 12 7 6 0 0
7 5 5 5 5 2 2 0 0
26 24 21 15 15 11 6 2 0
8 4 2 1 1 1 1 1 0
23 13 9 8 8 3 2 2 0
9 8 6 5 4 2 1 0 0
D
EFS probability
1.00
0.75
0.50
0.25
0.00
CBL
KRAS
Other
NRAS
PTPN11
NF1
0 24 48 72 96 120 144 168 192
Months from HSCT
Number at risk
46 28 21 16 11 6 5 0 0
7 4 4 4 4 2 2 0 0
26 22 19 13 13 9 4 1 0
8 2 2 1 1 1 1 1 0
23 13 9 8 8 3 2 2 0
9 6 4 3 2 2 1 0 0

Figure 4



Number at risk

Criteria=0	8	8	8	5	4	3	3	0	0
Criteria=1	33	27	21	18	17	12	7	1	0
Criteria=2	47	36	26	21	16	6	4	2	0
Criteria≥ 3	23	10	8	5	5	2	2	2	0

Criteria
Age ≥ 2 years at diagnosis
Time to HSCT ≥ 6 months
Monocyte > $7.2x10^9$/L
≥1 additional alteration

Supplemental Methods

Both bone marrow and peripheral stem cell donors underwent 4-digit molecular typing for HLA A, B, C, DR, and DQ subtypes, while cord blood (CB) had class 1 HLA typing (A and B subtypes) conducted via the serologic method, and class 2 (DR) typing performed using 4-digit molecular methods. Compatibility was defined as a 10 out of 10 HLA match for both matched sibling donors (MSD) and matched unrelated donors (MUD), and a 6 out of 6 matches for CB. Less than either 10 or 6 was classified as mismatched. Neutrophil engraftment was defined as absolute neutrophil count $\geq 0.5 \times 10^9$/L for 3 consecutive days and platelet engraftment as platelet count $\geq 50 \times 10^9$/L for 7 consecutive days, without transfusion. Graft failure was defined as either the absence of hematopoietic reconstitution of donor origin on day 60 (primary graft failure) or a loss of donor cells after transient donor engraftment (secondary graft failure)[7]. Full donor chimerism was defined as >95% of donor cells and mixed donor chimerism between 5 and 95%.

Supplemental Table 1: Conditioning regimen

Conditioning regimen	n
Busulfan-Fludarabine-Melphalan (Bu/Flu/Mel)	46
Busulfan-Cyclophosphamide-Melphalan (Bu/Cy/Mel)	41
Busulfan-Cyclophosphamide	17
Busulfan-Fludarabine-Thiothepa	9
TBI-Cyclophosphamide-Fludarabine	2
Busulfan-Cyclophosphamide-Clofarabine-Cytarabine	1
Busulfan-Fludarabine-Cyclophosphamide	1
Busulfan-Cyclophosphamide-Melphalan-Etoposide	1
TBI-Etoposide	1

TBI, total body irradiation. Busulfan was given orally (n=14, before October 2005) or intravenously (n=102, since November 2005)

Supplemental Table 2: Graft-versus-host disease prophylaxis according to donor.

	Identical sibling, n=24	Matched unrelated, n=46	Mismatched unrelated, n=45	Haploidentical, n=4
CSA	19	4	10	0
CSA/corticosteroids	0	8	19	0
CSA/MTX	4	31	6	1
CSA/MMF	0	2	7	1
CSA/MTX/sirolimus	0	0	1	0
CSA/MTX/tacrolimus	0	1	1	0
CSA/MMF/Cyclophosphamide	0	0	0	2
Missing	1	0	1	0

Supplemental Table 3: Patients characteristics of according to engraftment status

	Engraftment, n=100	No/lost engraftment, n=16
M/F ratio	2.3	1.6
Pre-HSCT treatment, no. (%)	n=99	
No or low-dose chemotherapy	73	14 (87.5)
AML-type chemotherapy	26	2 (12.5)
Splenectomy	2	3 (18.7)
RAS-pathway mutations, no. (%)	n=99	
PTPN11	42	4 (25.0)
KRAS	21	5 (31.2)
NRAS	19	3 (18.7)
CBL	4	3 (18.7)
NF1	6	1 (6.2)
Other	4	0
No mutation	3	0
Donor, no. (%)		
Match siblings	21	2 (12.5)
Haploidentical	3	1 (6.2)
Match unrelated	42	4 (25.0)
Mismatched unrelated	34	9 (56.2)
Source of cells, no. (%)		
Cord blood	29	8 (50.0)
Bone marrow	62	7 (43.7)
Peripheral blood	9	1 (6.2)
Conditioning, no. (%)		
Bu/Cy/Mel	37	2 (12.5)
Bu/Flu/Mel	42	3 (18.7)
Other	21	11 (68.7)

HSCT, hematopoietic stem cell transplant; AML, acute myeloid leukemia; HbF, fetal hemoglobin; Bu/Cy/Mel, busulfan/cyclophosphamide/melphalan; Bu/Flu/Mel, busulfan/fludarabine/melphalan;

Supplementary Table 4: Univariable predictive analyses of GVHD based on Fine & Gray models

Outcomes	Acute GVHD 2–4	Acute GVHD 3–4	Chronic GVHD
	100-day CI, 95%CI	100-day CI, 95%CI	36-month CI, 95%CI
Overall	53.8%, 44.4-62.3	31.9%, 23.7-40.4	36.0%, 27.2-44.9
Patient characteristics at diagnosis			
Age ≥ 2 years at diagnosis	1.14 (0.70-1.84)	1.02 (0.64-1.71)	0.86 (0.47-1.59)
Platelets ≥ 33 x10^9/L	0.82 (0.47-1.43)	0.57 (0.24-1.35)	0.93 (0.44-1.96)
Monocyte >7.2 x10^9/L	0.88 (0.49-1.56)	0.88 (0.47-1.63)	0.86 (0.41-1.80)
Myeloid precursors in PB	1.45 (0.50-4.16)	0.72 (0.25-2.03)	0.95 (0.38-2.39)
BM blasts ≥5%	1.36 (0.84-2.21)	1.39 (0.74-2.62)	1.13 (0.61-2.07)
Pre-HSCT BM ≥5%	1.45 (0.88-2.39)	1.16 (0.68-2.00)	1.19 (0.64-2.23)
Elevated HbF*	**0.52 (0.30-0.90)**	0.75 (0.37-1.51)	0.73 (0.35-1.52)
Abnormal karyotype	1.25 (0.76-2.05)	0.85 (0.44-1.63)	0.87 (0.47-1.62)
Monosomy 7	1.08 (0.62-1.89)	0.61 (0.25-1.46)	1.10 (0.54-2.24)
RAS-pathway mutations			
PTPN11	1.00	1.00	1.00
CBL	0.15 (0.02-1.24)	0.79 (0.13-4.75)	0.14 (0.02-1.13)
KRAS	0.65 (0.25-1.70)	0.75 (0.19-2.92)	**0.22 (0.07-0.64)**
NF1	0.82 (0.25-2.70)	1.10 (0.22-5.47)	**0.11 (0.01-0.91)**
NRAS	1.10 (0.43-2.82)	**3.44 (1.01-11.78)**	0.40 (0.14-1.13)
No/other	0.62 (0.25-1.52)	1.77 (0.54-5.88)	**0.38 (0.15-0.96)**
Additional alteration**	1.30 (0.78-2.16)	0.80 (0.42-1.51)	0.99 (0.52-1.87)
Pre-HSCT treatment			
No	1.00	1.00	1.00
Low-dose chemotherapy	0.90 (0.38-2.09)	0..71 (0.27-1.90)	1.37 (0.40-4.70)
AML-type chemotherapy	0.95 (0.38-2.35)	0.84 (0.29-2.44)	2.25 (0.62-8.13)
Transplant characteristics			
Female recipient sex	1.24 (0.75-2.05)	1.50 (0.80-2.84)	1.52 (0.82-2.82)
Female donor to male recipient	1.18 (0.63-2.20)	1.11 (0.51-2.44)	0.89 (0.45-1.75)
Age ≥ 2 years at HSCT	1.03 (0.63-1.68)	1.36 (0.70-2.65)	1.05 (0.56-1.97)
Time to HSCT ≥ 6 months	1.25 (0.76-2.04)	1.25 (0.66-2.35)	1.02 (0.55-1.87)
Donor			
Identical sibling	1.00	1.00	1.00
Matched unrelated	1.14 (0.56-2.29)	1.05 (0.42-2.66)	1.56 (0.60-4.07)
Mismatched relative	0.80 (0.22-2.95)	1.53 (0.40-5.91)	**3.24 (1.02-10.3)**
Mismatch unrelated	0.99 (0.48-2.02)	1.02 (0.40-2.56)	1.26 (0.47-3.39)
HLA disparities ≥ 2	0.69 (0.41-1.16)	0.81 (0.42-1.56)	0.87 (0.45-1.66)
Cord Blood source	0.63 (0.37-1.08)	0.65 (0.32-1.30)	0.62 (0.30-1.26)
Donor/recipient CMV status			
Negative/Negative	1.00	1.00	1.00
Negative/Positive	0.70 (0.31-1.58)	0.69 (0.26-1.84)	0.64 (0.24-1.69)
Positive/Negative	**1.64 (1.00-2.71)**	1.00 (0.43-2.18)	0.78 (0.32-1.90)
Positive/Positive	0.65 (0.29-1.45)	0.81 (0.33-2.00)	0.78 (0.31-1.98)
Conditioning			
Other	1.00	1.00	1.00
Bu/Cy/Mel	1.24 (0.65-2.36)	0.79 (0.33-1.90)	0.96 (0.46-2.03)
Bu/Flu/Mel	1.40 (0.76-2.58)	1.32 (0.62-2.78)	0.79 (0.37-1.70)
Anti-thymoglobulin	0.69 (0.42-1.12)	0.52 (0.27-1.02)	0.78 (0.42-1.45)

NA refers to the models that do not converge due to a low number of events in one stratum. Significant results are in bold letters.

* For patients ≥6 months,

** At least 1 alteration among the following genes: *ASXL1, JAK3, SETBP1,* or double RAS mutation or karyotype anomaly.
GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; BM, bone marrow; HbF, fetal hemoglobin; AML, acute myeloid leukemia; Bu/Cy/Mel, busulfan/cyclophosphamide/melphalan; Bu/Flu/Mel, busulfan/fludarabine/melphalan.

Supplementary Table 5: Univariable predictive analyses of the “stringent” EFS when additionally including beside relapses and deaths, secondary malignancies and secondary allografts as events

Outcomes		“Stringent” EFS
	No. of patients	60-month event-free survival, 95% CI
Overall	119	63.6%, 55.3-73.3
No. of events		46 events
Patient characteristics at diagnosis		
Age ≥ 2 years	55	**2.62 (1.42-4.82)**
Platelet ≥ 33 x10^9/L	28	0.93 (0.46-1.88)
Monocyte > Q3 (7.2 x10^9/L)	30	**1.92 (1.05-3.53)**
Myeloid precursors in PB	106	2.24 (0.54-9.26)
BM blasts ≥5%	49	0.98 (0.54-1.76)
Elevated HbF*	73/88	1.11 (0.53-2.31)
Abnormal karyotype		1.28 (0.71-2.32)
Monosomy 7	26	0.67 (0.31-1.44)
RAS-pathway mutations		
PTPN11	46	1.00
CBL	7	1.37 (0.27-6.78)
KRAS	26	0.56 (0.14-2.24)
NF1	8	2.14 (0.51-8.99)
NRAS	23	1.47 (0.40-5.34)
No/other	9	1.24 (0.37-4.18)
Additional mutations		
JAK3	10	1.67 (0.70-3.95)
SETBP1	9	1.93 (0.81-4.56)
ASXL1	11	0.90 (0.32-2.50)
Double RAS pathway mutation	25	1.22 (0.62-2.40)
Additional alteration**		
>=1 Alteration	67	1.59 (0.83-3.03)
>=2 Alterations	20	1.33 (0.66-2.68)
Pre-HSCT treatment		
Low-dose chemotherapy	77	1.00
No chemotherapy	13	0.30 (0.07-1.25)
AML-type chemotherapy	28	0.88 (0.45-1.75)
Transplant characteristics		
Male recipient sex	82	0.88 (0.48-1.64)
Female donor to male recipient	28	1.40 (0.73-2.68)
Age ≥ 2 years at HSCT	71	**2.28 (1.18-4.41)**
Pre-HSCT BM ≥5%	42	0.84 (0.45-1.55)
Time to HSCT ≥ 6 months	52	1.46 (0.82-2.61)
Donor		
Geno-Identical sibling	24	1.00
Matched unrelated	46	0.60 (0.27-1.32)
Mismatched relative	4	0.50 (0.06-3.89)
Mismatch unrelated	45	1.00 (0.48-2.09)
HLA disparities ≥ 2	40	1.11 (0.61-2.03)
Stem cells		
Bone marrow	70	1.00
Cord blood	39	1.07 (0.58-1.97)
Peripheral blood	10	0.48 (0.11-2.01)
Donor/recipient CMV status		
Negative/Negative	57	1.00

Negative/Positive	19	1.43 (0.64-3.19)
Positive/Negative	19	1.32 (0.55-3.16)
Positive/Positive	20	1.92 (0.86-4.29)
Conditioning		
Bu/Cy/Mel	41	1.00
Bu/Flu/Mel	46	0.87 (0.42-1.83)
Other	32	**2.04 (1.03-4.05)**
Anti-thymoglobulin	57	0.93 (0.52-1.66)

* For patients ≥6 months,
** At least 1 alteration among the following genes: *ASXL1, JAK3, SETBP1,* or double RAS mutation or karyotype anomaly.
NA refers to the models that do not converge due to a too low number of events in one stratum. Significant results are in bold letters.
EFS, event free survival; HSCT, hematopoietic stem cell transplantation; CB, cord blood; BM, bone-marrow; PB, peripheral blood; CMV, cytomegalovirus; Bu/Cy/Mel, busulfan/cyclophosphamide/melphalan; Bu/Flu/Mel, busulfan/fludarabine/melphalan.

Supplementary Table 6: Characteristics of patients at diagnosis according to monocyte count in peripheral blood

	AMC >7.2G/L n=30	AMC ≤7.2G/L n=89	*p-value*
M/F (ratio)	22/8 (2.75)	60/29 (2.1)	0.65
Age at diagnosis, median [IQR]	1.7 [0.7-3.1]	1.6 [0.8-3.3]	0.50
Peripheral blood cell count, median [IQR] x10^9/L			
White blood cells	59.2 [40.0-73.0]	21.6 [14-29.2]	<0.0001
Platelets <33 x10^9/L, n (%)	9/29 (31.0)	19/87 (21.8)	0.33
Platelets	53 [28-107]	60 [34-113]	0.82
Hemoglobin, g/dl	8.8 [7.2-10.1]	9.1 [8.1-10.4]	0.36
Neutrophils	17.7 [6.3-31.0]	6.9 [3.5-10.6]	0.0002
Eosinophils	0.7 [0-1.8]	0.27 [0.06-0.64]	0.22
Basophils	0.5 [0.3-0.9]	0.27 [0.06-0.41]	0.14
Lymphocytes	13.3 [9.1-19.4]	6.3 [4.7-10.1]	<0.0001
BM Blasts %, median [IQR]	5.2 [3.6-11.5]	3.5 [2.0-7.0]	0.008
Elevated HbF*, no. (%)	19/25 (76.0)	64/79 (81.0)	0.58
RAS-pathway mutations, no. (%)			
PTPN11	10 (33.3)	37 (41.6)	0.28
KRAS	5 (16.7)	21 (23.6)	
NRAS	6 (20)	17 (19.1)	
CBL	2 (6.7)	5 (5.6)	
NF1	5 (16.7)	3 (3.4)	
Other	0	4 (4.5)	
No mutation	1 (3.3)	2 (2.2)	
Abnormal karyotype, n (%)	12/29 (41.4)	33/84 (39.3)	1.00
Additional mutations, no. (%)			
JAK3	3/29 (10.3)	7/85 (8.2)	0.71
SETBP1	3/29 (10.3)	6/85 (7.1)	0.69
ASXL1	1/29 (3.4)	10/85 (11.7)	0.28
Double RAS pathway mutation	6/29 (20.7)	19/85 (22.3)	1.00
≥ 1 additional alteration, n (%)**	18/29 (62.1)	51/82 (62.2)	1.00
≥ 2 additional alterations, n (%)**	6/29 (20.7)	14/82 (17.0)	0.78

IQR, interquartile range; HbF, fetal hemoglobin; BM, Bone marrow.
* for patients ≥6 months; ** At least 1 or 2 alteration among the following genes: *ASXL1, JAK3, SETBP1,* or double RAS mutation or karyotype anomaly.

Supplementary Table 7: Patient characteristics and outcomes following HSCT in main studies published in JMML

Reference	HSCT period	N° of pts	RAS mut (%)	Time to HSCT (months)	PB/CB/BM (%)	HLA disparity ≥1 (%)	URD (%)	Conditioning (%)	Graft failure (%)	5-y EFS (%)	Risk factors for EFS	5-y OS (%)	Risk factors for OS	5-y TRM (%)	Risk factors for TRM	5-y RI (%)	Risk factors for RI
Smith et al, 2002	1990-1997	46	NA	8,7	NA	37	100	TBI-based (76), Bu-Cy (11), Bu-Cy-Mel (13)	4	24 (2y)	no	42 (2y)	no	18 (2y)	NA	58 (2y)	no
Manabe et al, 2002	1990-1997	27	NA	9	11/4/85	19	41	TBI-based (67), Bu-Cy-based (22), Others (11)	4	54 (4y)	NA	58 (4y)	**Abn karyotype**, age>1y	NA	NA	NA	NA
Locatelli et al, 2005	1993-2002	100	NA	6	14/7/79	NA	52	Bu-Cy-Mel (100)	5	52	age at dg≥4y, **age at HSCT ≥4y, female sex**	64	NA	13	female donor	35	**age at HSCT ≥4y,** female sex, age at dg≥4y, HbF≥40%, blast BM at HSCT≥20%.
Locatelli et al, 2013	1995-2010	110	NA	5,6	0/100/0	84	100	Bu-Cy-Mel (45), TBI-based (17), RIC (7), Others (41)	18	44	**Age at dg>1.4y, monosomy 7, HLA MM≥2**	52	**Age at dg >1.4y, monosomy 7,** AML-type chemotherapy	22	**monosomy 7,** female sex, no chemotherapy, **HSCT<2003**	33	**Age at dg >1.4y**
Stieglitz et al, 2015	2001-2006	44	NA	1.8	NA	NA	50	TBI-Cy (100)	4	39	NA	57	NA	7	NA	43	NA
Yabe et al, 2015	2001-2011	30	67	6	7/17/77	43	73	Bu-Flu-Mel (100)	20	53	HLA MM	72	NA	NA	NA	NA	NA
Dvorak et al, 2018	2013-2015	15	100	1.2 to 1.4	7/7/87	13	67	Bu-Cy-Mel (40), Bu-Flu (60)	7	47 (1.5y)	NA	64 (1.5y)	NA	NA	NA	NA	NA
Lin et al, 2019	2010-2018	47	94	4 to 4.4	91/0/9	60	26	Bu-Cy-based (43), Bu-Cy-VP16-based (53), Others (4)	4	55	Age at dg>2.6y	64	NA	11	NA	35	**HLA MM<2,** Age>2.6y, *PTPN11, KRAS, NRAS*
Yoshida et al, 2020	2000-2011	129	NA	6	8/23/69	48	66	Bu-Flu-Mel (46), Bu-Cy (23), other MAC (6), RIC (10), TBI-based (16)	12	46	Age at HSCT≥2y, Abn karyotype, **interval dg-HSCT>6 months, HSCT<2005, HLA MM≥2, CB**	64	Abn karyotype, **HSCT<2005, HLA MM≥2, TBI**	14	TBI	34	age at HSCT≥2y, **Abn karytype**, **CB,** HLA MM≥2
Eun Sang Yi et al, 2023	2000-2019	68	74	NA	61/22/16	54	65	Bu-Flu or Bu-Mel-based (43), Bu-Cy-based (48), TBI-based (9)	10	53	HbF≥40%, no *NRAS* mutation	62	NA	30	NA	26	**HbF≥40%, Abn karyotype, MSD**

In bold, variables significant in multivariate analysis

HSCT, hematopoietic stem cell transplantation; CB, cord blood; BM, bone-marrow; PB, peripheral blood; URD: unrelated donor; EFS, event free survival; OS, overall survival; TRM, treatment related mortality; RI, relapse incidence; NA, not available; TBI, total body irradiation; Bu, busulfan; Cy, cyclophosphamide; Mel, melphalan; Flu, fludarabine; RIC, reduced intensity conditioning; Dg, diagnostic; HLA MM, HLA mismatch; Abn, abnormal; HbF, fetal hemoglobin; MSD, matched sibling donor

Supplemental Figure legends

Supplemental figure 1: Pretransplant strategy according to the initiating RAS-mutation and effect of pre-HSCT treatment on BM blast percentage. (A) Time from JMML diagnosis to transplant (months, IQR) according to the initiating RAS mutation, n=118. (B) Pre-HSCT chemotherapy intensity according to the initiating RAS mutation, n=118. (C) BM blast (%) at JMML diagnosis (n=118) and prior to HSCT (n=88) according to the pre-HSCT chemotherapy received. Diagnostic BM blast count in patients with AML-type, low-dose, and no chemotherapy (10.8%, 4.5% and 4.2%, respectively). Pre-HSCT blasts count in patients with AML-type, low-dose, and no chemotherapy (4.3%, 5.7% and 2.8%, respectively). AML, acute myeloid leukemia; Dg, diagnostic; HSCT, hematopoietic stem cell transplant; BM, bone marrow.

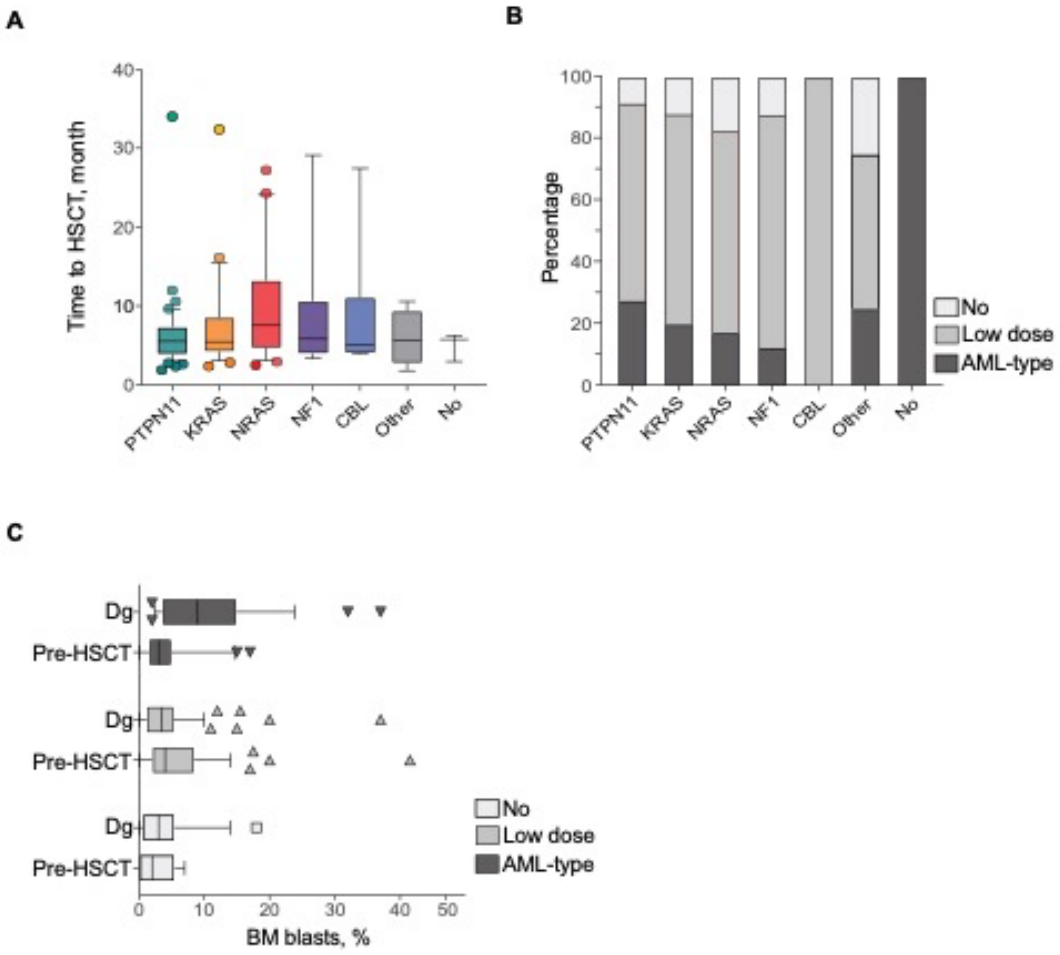
Supplemental figure 2: Outcome of the 16 patients who failed to engraft. HSCT, hematopoietic stem cell transplant; JMML, juvenile myelomonocytic leukemia.

Supplemental figure 2: GvHD and impact on JMML Relapse. Cumulative Incidence for grade 2-4 acute GvHD (A), and for chronic GvHD (B). Relapse-free survival according to the presence or absence of grade 2-4 acute GvHD (C) and Relapse-free survival according to the presence or absence of chronic GvHD (D) were analyzed using Kaplan-Meier methodology. CI, cumulative incidence; HSCT, hematopoietic stem cell transplant; RFS, relapse-free survival

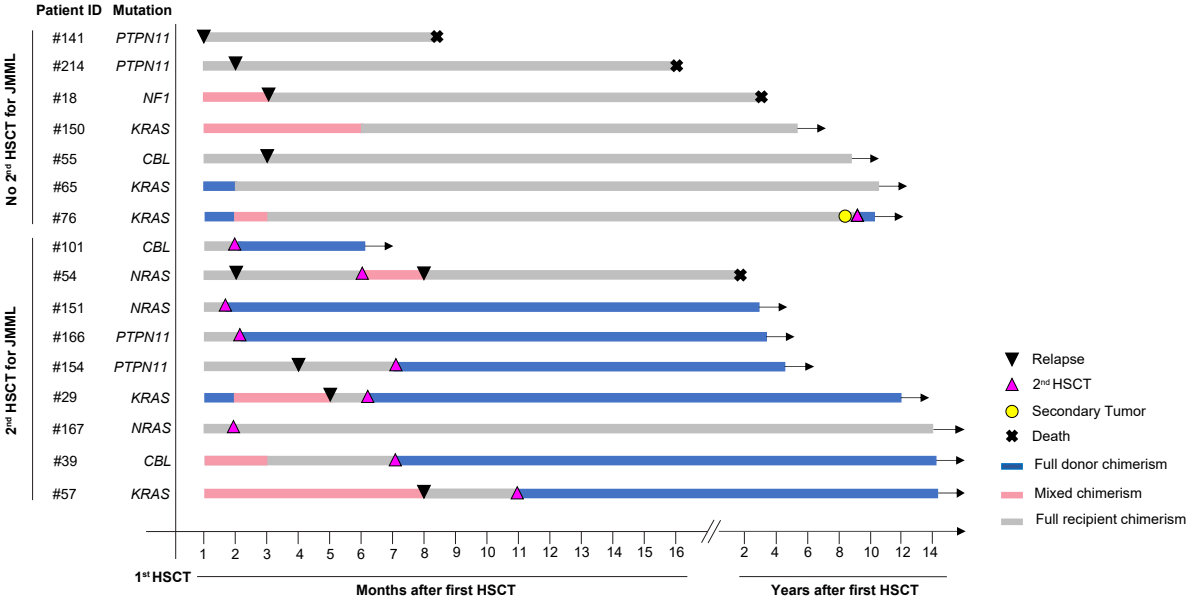
Supplemental figure 4: Stringent EFS probability. Stringent EFS was analyzed using

Kaplan-Meier methodology. Stringent EFS considers relapse, death, secondary allograft, and secondary malignancy as events. EFS, event-free survival; HSCT, hematopoietic stem cell transplant.

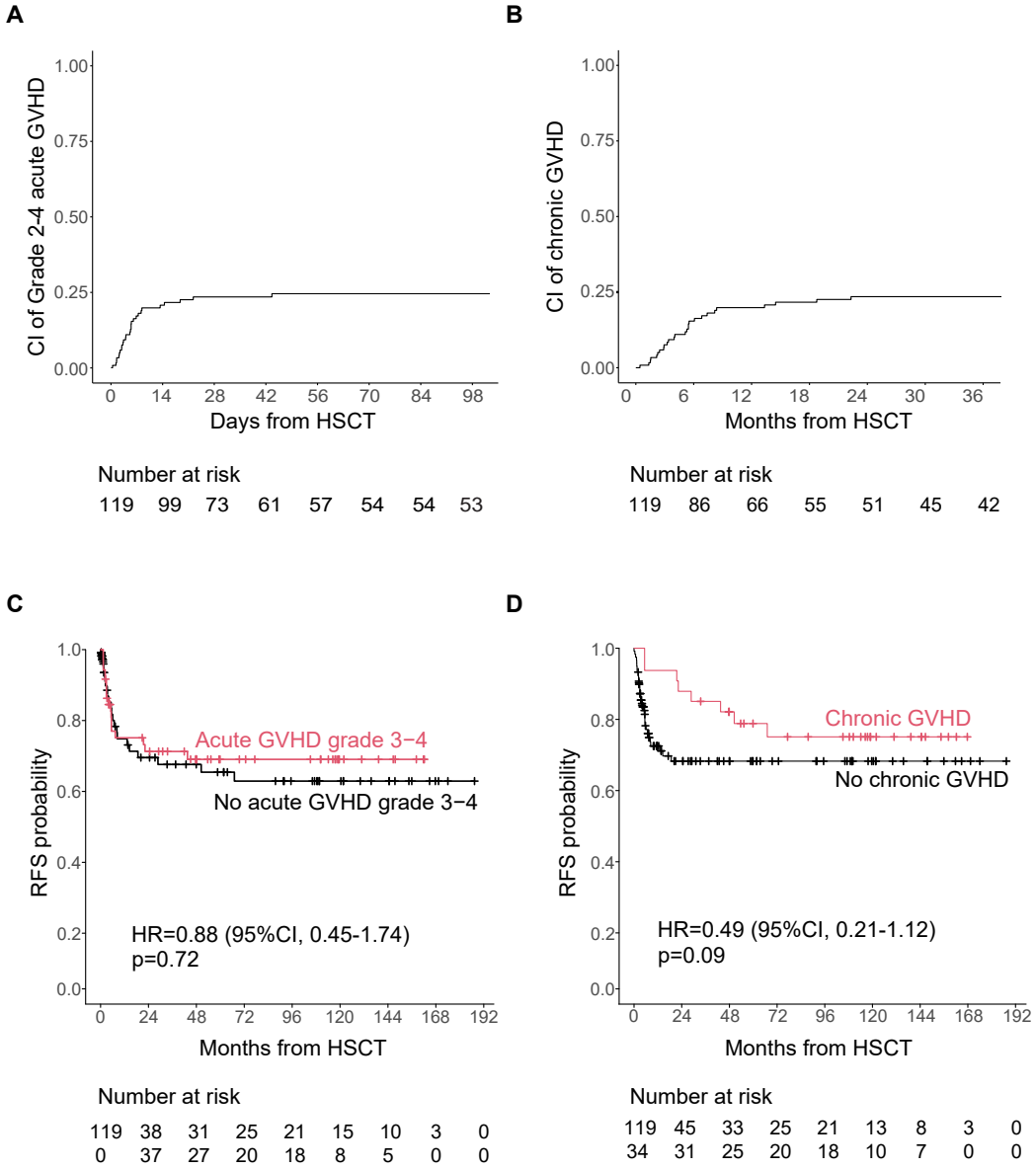
Supplemental Figure 1



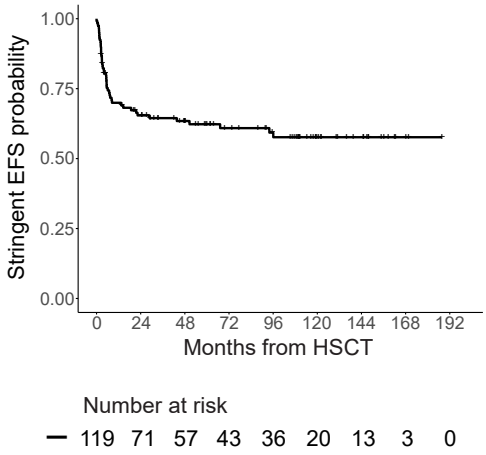
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Number at risk

—	119	71	57	43	36	20	13	3	0