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Allogeneic Hematopoietic Stem Cell Transplantation with Myeloablative Conditioning is Associated with Favorable Outcomes in Mixed Phenotype Acute Leukemia

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Abstract

Mixed phenotype acute leukemia (MPAL) represents a poorly characterized group of acute leukemias that lack an accepted therapeutic approach and are typically associated with poor outcomes. We present our experience of genomic profiling, pre-transplant therapy and transplant outcomes for 36 well characterized pediatric and adult patients with MPAL defined according to the 2016 WHO leukemia update. A predominance of ALL-associated mutations and cytogenetic

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abnormalities was noted. Remission rates after induction appeared comparable among adults (20/23) and children (11/13) and among those who received ALL (10/11) or AML-type (21/25) induction. Adults were transplanted in first remission while children were transplanted in the setting of relapse or MLL rearrangement. The median follow-up among the 25 patients who underwent transplantation was 39.6 months and median OS was not reached. Relapse after transplant was associated with MLL rearrangement (p=0.022), reduced intensity (p<0.001), and higher WBC at diagnosis (p=0.034). These data highlight differing therapeutic approaches between adult and pediatric MPAL and demonstrate favorable survival of adult MPAL patients consolidated with allogeneic hematopoietic cell transplantation.

Keywords

Mixed phenotype acute leukemia; allogeneic hematopoietic cell transplantation; somatic mutations

INTRODUCTION

Mixed phenotype acute leukemia (MPAL) is uncommon, accounting for 2–5% of all newly diagnosed acute leukemia [1]. These neoplasms are thought to arise from an immature undifferentiated progenitor that expresses both myeloid and lymphoid antigens. The lack of distinguishing morphologic or genomic features means diagnosis is based solely on blast immunophenotype [2]. Little is known about the biology of MPAL and as a result there is single standard induction chemotherapy approach with ALL and AML-type regimens used interchangeably.

Historically, outcomes in MPAL were thought to be inferior to AML and ALL [3, 4]; however, studies drawing these conclusions may be biased by including patients with high risk myeloid malignancies which frequently have aberrant lymphoid antigen expression such as therapy-related neoplasms and AML with MDS related changes. These cases, as well as those with karyotypes that define therapy-related neoplasms, as well as cases with *TP53* mutation which are typically seen in therapy-related neoplasms [5] should not be categorized as MPAL according to the WHO guidelines [6]. More recent publications show favorable outcomes when patients are consolidated with allogeneic hematopoietic stem cell transplantation (HCT) [7, 8]. There remains a paucity of MPAL transplant data and variables predicting transplant outcome have not been confirmed.

An improved understanding of leukemia genetics has resulted in genomic classification systems for ALL and AML [9, 10]. Genomics offer an understanding of disease biology and identify therapeutic targets and biomarkers that predict relapse after conventional chemotherapy and HCT. MPAL with MLL or Philadelphia rearrangement are the only two genetically defined subgroups, but make up a minority of MPAL cases. B and T myeloid subtypes are defined on the basis of immunophenotype alone but may be better categorized into ALL or AML subgroups on the basis of expression profiling [4] or somatic molecular mutations which are characteristic of lineage-committed leukemia. Here we describe the outcomes with MPAL strictly defined according to the 2016 update to the WHO classification of myeloid neoplasms and acute leukemia [11]. We observe that somatic

mutations and cytogenetic abnormalities in MPAL are characterized by mutations seen frequently in ALL rather than AML. We report favorable outcomes in MPAL consolidated with HCT and identify variables associated with remission induction and relapse after HCT.

MATERIALS AND METHODS

Patients

This was a retrospective analysis of 36 MPAL patients treated at our center between 2005 and 2015. MPAL was defined according to the WHO 2016 update on myeloid neoplasms and acute leukemia [11]. Pediatric patients were defined as those diagnosed below the age of 18 years.

Pathology

A single pathologist reviewed all cases. Patients with AML-defining cytogenetic abnormalities, myelodysplastic syndrome transformed to AML, AML with MDS related changes and, therapy-related neoplasms were excluded. Bone marrow cytogenetics was assessed using G-band karyotyping. Molecular sequencing (N=16) was performed using two separate next generation sequencing (NGS) assays. Bone marrow from 8 cases was sequenced using a NGS platform that sequences DNA from 405 genes and RNA from 265 genes of known oncogenic drivers in hematologic malignancies including AML and ALL, sarcomas and pediatric cancer [12]. Additionally, 8 MPAL were sequenced using an institutional NGS platform, which targets 28 genes recurrently mutated in myeloid neoplasms [13]. Minimal residual disease (MRD) at the time of HSCT was assessed using various methods including 10-colour flow cytometry (n=9), quantitative PCR for BCR ABL (n=5), FISH for patient specific leukemia-defining cytogenetic alteration (n=3), presence of IHG or TCR rearrangement (n=3).

Clinical parameters

Induction chemotherapy was grouped as ‘ALL-type’ if it incorporated L-asparaginase, corticosteroids or ‘AML-type’ if it included cytarabine and an anthracycline without L-asparaginase, vincristine or steroids. Transplant conditioning intensities were defined according to consensus guidelines[14]. Complete remission was defined as presence of less than 5% blasts on bone marrow aspirate and relapse was defined as presence of ≥ 5% blasts on bone marrow assessment [15].

Statistical analysis

Fisher’s exact test was used to examine associations between MPAL subtypes and other clinic variables, as well as between remission status after induction. Associations between these clinical variables and overall survival (OS) after transplant were examined using Kaplan-Meier method and the log rank test. Cumulative incidence of relapse (CIR) was estimated, treating death due to other causes and second transplant as competing risks. Gray’s test was used to examine associations between clinical factors and relapse. A test with p-value < 0.05 was considered statistically significant. All statistical analyses were performed in software packages SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 3.1 (The R Foundation for Statistical Computing).

RESULTS

Patients

Clinical variables stratified by WHO MPAL subtype are presented in Table 1. B and T Myeloid cases made up 67% of all patients. The median age was 27 years (range: 1–69) and 13 (36%) were children. Patients with Ph+ MPAL were older while those with MLL MPAL were younger. Five patients had histologically confirmed extramedullary disease at diagnosis (gingival, lymph node, breast, muscle and pericardium) and seven had CNS disease. The median follow-up of all survivors (n=27, 75%) was 42.1 months (95% CI: 19.4–68.4) and median OS after diagnosis was not reached (95% CI: 32 months-NR). OS was not different between adult and pediatric patients (p=0.195) or between MPAL subtypes (p=0.080) (Figure 1A and 1B).

Cytogenetics and molecular typing

Diagnostic cytogenetics was available for 35 patients. Translocations were the most common structural abnormality seen in 20 (56%) patients. Four patients had a normal karyotype, 14 (40%) had a complex karyotype defined as 3 or more structural abnormalities, 6 (17%) had monosomies and ten (29%) had polysomies of which +21 was seen in 7 (20%) and 6 of these had B/myeloid antigen expression. There were no AML, MDS or therapy-related myeloid neoplasm defining cytogenetic abnormalities [11]. Chromosomal changes seen in ALL were common and included high-hyperdiploidy (>50 chromosomes) (n=2) with polysomy of chromosomes typically duplicated in hyperdiploid ALL (chromosomes 4, 7, 11 and 21) and hypodiploidy (<44 chromosomes) in one case. The ALL-associated translocation t(12;21)(p13;q22) was identified in 2 cases. Aside from MLL and BCR-ABL rearrangement, 4/36 patients (11%) had translocations involving 14(q31-q32) and in 3 of these patients this was the only abnormality. All 4 cases with t(v;14)(v;q31-32) had T/myeloid antigen expression. The region on 14(q31-32) encodes several genes associated with B cell Non Hodgkin Lymphoma (*IGH, CL11B*) and T cell leukemia (*TCL1A, TCL6*) and is very frequently rearranged in ALL [16, 17] but not in AML. Rearrangements of 14(q31-32) have been reported in MPAL [18]. One patient had t(10;11)(p15;q21) (*PICALM-MLL10*) rearrangement which has been described in MPAL [18, 19]. *MLL, located on* 11(q23) was always rearranged with 4(q21). Rearrangement of *MLL* is seen in both ALL and AML; however, t(4;11)(q21;q23) is rarely seen in AML [20] but is the most common MLL rearrangement in ALL and the second most common translocation overall in ALL [21]. Ph+ MPAL was seen only in adults and in 4/5 it was associated with a complex karyotype.

Genes recurrently mutated in AML were infrequently altered relative to mutations seen frequently in ALL. There were no mutations in *NPM1, IDH2, TP53* and no biallelic *CEBPA* mutations, which define non-overlapping genomic AML subtypes [9]. Alterations associated with myeloproliferative disorders: *JAK2* and *MPL* mutations were not seen in any patient. There was an overwhelming predominance of ALL-associated mutations among the 8 MPAL who had sequencing using the AML and ALL specific NGS panel (Table 2). Alterations occurred in specific functional pathways including transcription factors critical for lymphoid maturation (*IKZF1 n=1/8, NOTCH n=4/8, ETV6 n=1/16*); kinases involved in *JAK-STAT* signaling (*FLT3-ITD* n=3/16, *IL7R* n=1/8, *JAK1* n=1/16, *JAK3* n=2/16), RAS-

pathway mutations (*PTPN11* n=2/8, *NF1* n=1/8, *RAS* n= 1/16), tumor suppressor genes (*CDKN2A* n=2/8, *PHF6* n=3/16) and epigenetic regulators (*MLL2* mutation n=3/8, *TET2* n=4/16, *RUNX1* n=3/16, *DNMT3A* n=2/16, and *IDH1* n=1/16). There were no *TP53* mutations (0/16) which are strongly associated with therapy-related myeloid neoplasms and no *CRLF2* rearrangements (n=0/8) or alterations in *PAX5* (n=0/8), which appear to be ALL-specific mutations.

Induction and consolidation chemotherapy

ALL-type induction regimens were used in 11 patients (31%) and AML-type in 25 patients (69%) (Table 1). There were no deaths during induction and 31/36 (86%) attained complete remission (CR). There was no association between MPAL subtype or antigen expression (MPO, CD3, CD19) and use of ALL or AML-type induction regimens. All Ph+ MPAL patients received multi-agent induction chemotherapy in combination with a tyrosine kinase inhibitor (imatinib n=2, dasatinib n=3). AML-type regimens were used more frequently in all MPAL subtypes and among adult (16/23, 70%) and pediatric (9/13, 70%) cases. The most frequently used induction regimen in adults was cytarabine plus high dose mitoxantrone [22] (CR in 10/10) and DCTER [23] in children (CR in 7/8). CR rate was significantly lower in patients with T/Myeloid MPAL (7/11, 64%) than B/Myeloid (13/13, 100%), Ph+ MPAL (5/5, 100% and MLL MPAL (5/5, 100%) (p=0.022) (Supplementary table). CR rate after first induction was similar after ALL-type (10/11, 91%) and AML-type regimens (21/25, 84%) (p=0.999) and among adults (20/23, 87%) and children (11/13, 85%) (p=0.999). CR rates were not different following induction with regimens containing a steroid (18/20), L-asparaginase (9/9), anthracycline (30/35), anti-metabolite (20/25), vinca-alkaloid (11/11), alkylating agent (2/2) or topoisomerase inhibitor (7/10).

The majority of adults (n=14/23) and children (n=9/13) received consolidation therapy. Adults were typically treated according to the administered induction regimen, while children were switched from AML-type induction to ALL-type consolidation. Eight children were consolidated with an ALL-type regimen (NY-2)[24] despite 6/8 achieving remission with AML-type induction (DCTER). Seven of 8 adults who achieved remission with an AML-type induction were consolidated with an AML-type consolidation (high dose cytarabine) and 5/7 were consolidated using an ALL-type regimen after initially achieving remission following ALL-type induction. In adults, only 1/14 who received consolidation did not proceed to HCT due to death from relapsed disease. Twelve patients required salvage therapy for relapse. AML-type was used in 9: HIDAC (n=2), ALL-2 (n=5), 7+3 idarubicin (n=1) and 5+2 idarubicin (n=1). ALL-type reinduction was used once (COG AALL-1131) and two children received TVTC (n=2) [25]. CR2 was achieved in 7/9 following AML-type salvage, 1/2 following TVTC and in 1/1 after COG AALL-1131.

Transplantation

Twenty-five patients underwent HCT of which 21 (84%) were adults (Table 3). The median follow-up of transplant survivors (n=20) was 39.6 months (95%CI: 14.7–81.1) after transplant and median OS was not reached (Figure 1C). Twenty-one of 23 (91%) adults underwent HCT of which 19 proceeded directly to transplant in first remission. Two adults (both with T/Myeloid MPAL) did not undergo transplant, the first due to comorbidity and

the second due to death from refractory disease. The median time to transplant among adult MPAL was 2 months (2–34 months). Only 4/13 (31%) pediatric patients underwent transplant and only 2 proceeded to HCT directly following remission induction (MPAL with MLL and B/Myeloid MPAL), and the other 2 (MPAL with MLL and B/Myeloid MPAL) underwent HCT in second remission. Two of three pediatric patients with MLL rearrangement underwent HCT and the third died due to disease progression before transplant. Six (5 with B/Myeloid and 1 with T/Myeloid) of 9 pediatric patients who did not undergo transplant were alive and in remission at a median of 16 months (7–118) follow-up, while 3 (MLL MPAL, MPAL NOS and T/myeloid) died due to refractory disease.

Overall 7 patients relapsed after transplant, 5 died including two from treatment-related mortality and 3 from disease relapse. Variables associated with transplant outcome are described in Table 3. Factors associated with shorter OS included reduced intensity conditioning (p=0.010), non TBI containing conditioning (p=0.048), HCT not in CR1 (p=0.006) while relapse was associated with MLL rearrangement (p=0.022) (Figure 1D), reduced intensity conditioning (p<0.001), receiving ALL type induction (p=0.001) and higher diagnostic WBC (above the median 13.5x10^9/L) (p=0.034). Expression of MPO, CD19 and CD3 did not affect transplant outcomes, nor did pre transplant MRD status.

DISCUSSION

AML and ALL have distinct and non-overlapping profiles of molecular and cytogenetic alterations. Our data indicated a strong bias in favor of ALL-associated abnormalities in MPAL. Yan et al [19] reported on somatic mutations in 31 MPAL patients identifying ALL-type mutations including: *IKZF1* (4/31), *NOTCH* (1/31), *CDKN2A* (4/12), *EZH2* (3/31), *ASLX1* (3/31) while no patient had mutations in common AML associated genes: *NPM1, FLT3, DNMT3A, IDH1 or IDH2.* Eckstein et al [26] identified mutations in 21/23 MPAL cases using whole exome sequencing and found that *DNMT3A* was the most frequently mutated gene (6/23). Investigators grouped MPAL alleles into three functional groups including cell signaling pathways (*RAS, NF1, JAK*); tumor suppressors (*TP53, WT1*) and transcription factor (*NOTCH1, RUNX1* and *GATA2*). Notably, mutations in *NPM1* and *PAX5*, which are the most common mutations in AML [27] and ALL [28], respectively, were not seen in any patient in either of the described cohorts or in the cases presented in this report suggesting that these mutations may confer lineage specificity, an observation made by other recent investigators [29]. MPAL with *TP53* mutations may represent inappropriate classification of therapy-related myeloid neoplasms as MPAL given that mutations in this gene are strongly associated with prior cytotoxic exposure and rarely seen in de novo leukemia [5, 30]. Mutations in genes coding for epigenetic regulators (*IDH1 and 2, DNMT3A, TET2, MLL and ASXL1)* that are common in clonal hematopoiesis states, and do not appear to be associated with lineage specificity given they are described in AML, ALL and MPAL[29]. Here, mutation in *DNMT3A* and *TET2* were only identified in adult patients. In our cohort we noted a strong predominance of ALL-associated alterations characteristic of genomic ALL subtypes: ‘Philadelphia-like ALL’ (*IKZF1*, *RAS* and components of the *JAK-STAT* pathway [31]), hyperdiploid ALL (n=2); ALL with *ETV6-RUNX1* fusion (n=2), *MLL* rearranged ALL, of which t(4;11) translocations are most frequent (n=5), ALL with t(v;14q) rearrangement (n=4), *PHF-6 (n=3) and NOTCH* (n=3) mutations which are typical of T-

ALL[32]. Other ALL associated mutations identified in this report included: *IL7R, MLL2, CDKN2A, NF1 and PTPN11*. Gene expression profiling of MPAL was able to categorize most MPAL into either AML or ALL based on gene expression patterns seen in lineage committed leukemia [4]. As we move to a genomic characterization of AML [9] and ALL [33], it is likely that many MPAL cases may be better classified by identification of AML, ALL and therapy-related neoplasm defining mutations and cytogenetic changes rather than by immunophenotype alone.

We identified a high CR rate following ALL (91%) and AML-type (84%) induction regimens with no benefit for regimens containing a particular chemotherapeutic agent. This CR rate was higher than previously published estimates that vary between 22–70% [34, 35]. The difference may be due to strict exclusion of therapy-related neoplasms and secondary AML which often have aberrant lymphoid antigen expression, poor prognosis and can be misclassified as MPAL [36]. Patients who underwent transplantation after receiving ALL-type induction had a higher incidence of post-transplant relapse (5/8 *versus* 2/17 with AML-type); however, the number of patients in this analysis is small. The difference may reflect a selection bias for higher risk patients undergoing treatment with ALL-type regimens. The optimal induction therapy for MPAL is unknown with both ALL [37, 38] and AML-type regimens reported [4]. It is unclear from retrospective studies on what basis investigators selected between AML and ALL-type induction and this was also the case in this report. We noted high CR rate with cytarabine and high dose mitoxantrone (n=10/10) administered according to the induction phase of the ALL-2 protocol [39], which is the preferred induction approach for adult MPAL at our center. Ph+/MPAL are historically associated with poor outcomes likely due to omission of tyrosine kinase inhibitors (TKI) in published cohorts [38]. Here all Ph+/MPAL achieved CR and proceeded to transplant. None received post-transplant TKI maintenance with 1 relapse and 1 treatment-related mortality noted. Patients with MLL rearrangement had equally high CR rates with relapse being the major barrier to long-term survival (Figure 1). Investigation into inhibiting the aberrantly recruited *DOT1L* methyltransferase in *MLL* leukemia is being pursued as a maintenance therapy and results are awaited [40]. MPAL who underwent HCT had favorable OS (Figure 1), supporting recent publications [1, 3, 37]. Adults were referred for allograft in CR1 while pediatric patients were referred in the event of MLL rearrangement or relapse. A recent CIBMTR analysis [3] of 95 carefully defined MPAL showed no difference in survival after transplant with B/Myeloid or T/Myeloid MPAL and similar OS to matched ALL and AML controls, suggesting that MPAL itself may not confer a high transplant specific disease risk as previously thought [41]. The CIBMTR investigators did not find a worse survival for MPAL with *MLL* or Philadelphia chromosome rearrangement. In the present cohort, *MLL* rearrangement was associated with a poor prognosis, with only 1/5 alive and disease free at last follow-up (Figure 1). Variables associated with favorable survival after HCT included ablative and TBI-containing conditioning and transplant in CR1. Conditioning intensity for MPAL was previously associated with favorable transplant outcome [41]. MRD status was not associated with transplant outcome. Although MRD was assessed using different methods, with variable sensitivities, we found no relapses among patients who were transplanted with detected MRD, contrary to recent findings in MPAL [42]. Six patients received cord blood

allografts with no mortality or relapse identified highlighting the potent anti-leukemia affect of cord blood in acute leukemia [43].

It is important to note that these results are derived from a univariate analysis of a small sample size derived from a single center. The number of patients in this analysis precluded a multivariate model to account for potential confounding patient or treatment characteristics. For these reasons, the results presented here should be interpreted with caution and require validation in larger series of patients that would allow for multivariate modeling.

As we move to a genomic classification of acute leukemia where treatment can be personalized based on the spectrum of somatic alterations rather than immunophenotype we are likely to find that some mixed-phenotype leukemias may be more strictly classified into ALL or AML categories by identification of lineage specific somatic mutations and gene expression. Survival analysis after careful classification of adult MPAL and exclusion of high-risk AML subtypes that may have aberrant lymphoid antigen expression suggests that MPAL cases have favorable outcome with either ALL or AML-type induction followed by ablative HCT. HCT remains the standard of care for Ph+ MPAL in the absence of data with a non-transplant approach. It is unclear that HCT benefits MLL rearranged leukemia typically seen in younger MPAL patients. Children with MPAL can be managed expectantly with chemotherapy unless high-risk cytogenetics such as MLL rearrangement or Ph-like genomic alterations are identified [44].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- MPAL had a predominance of ALL-type mutations and cytogenetic abnormalities.
- Remission induction rates were high with both ALL and AML-type induction regimens
- Survival was high after allogeneic transplant with ablative conditioning
- MPAL with MLL rearrangement has an unfavorable prognosis

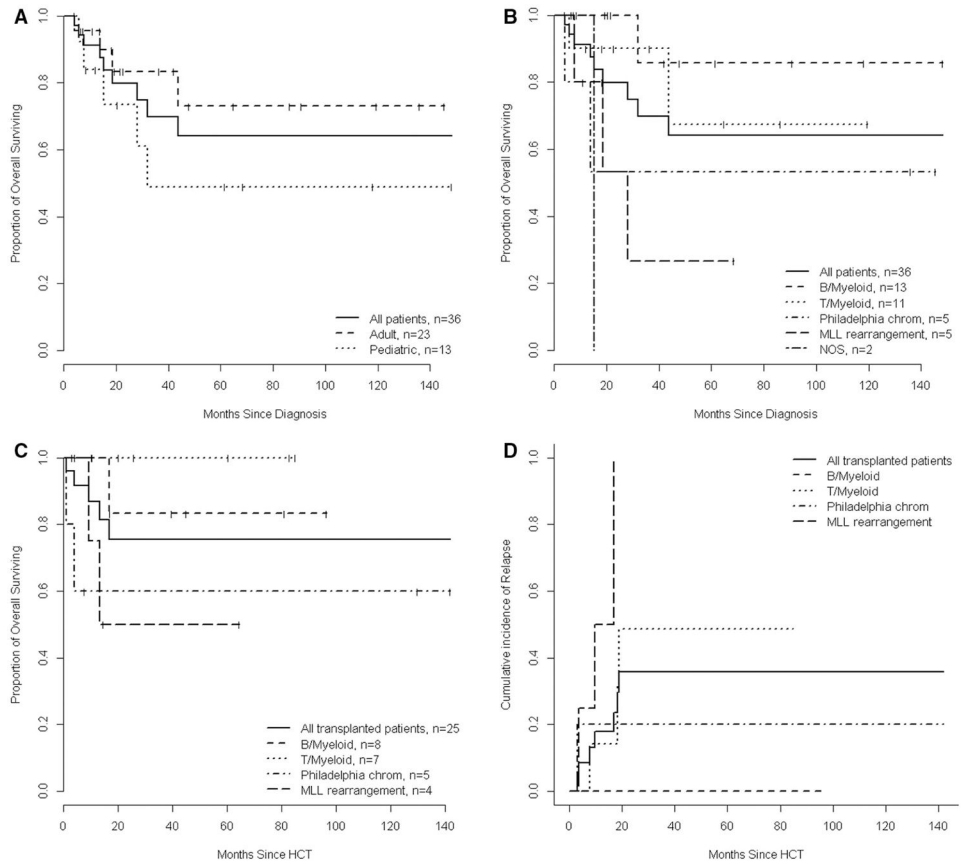

Figure 1.

(A) OS for all adult and pediatric MPAL from time of disease diagnosis. (B) OS for all patients by MPAL subtypes from time of disease diagnosis. (C) OS by MPAL who underwent HCT by MPAL subtype from time of transplant. (D) Estimated cumulative incidence of relapse after HCT by MPAL subtype. In figure C and D a separate curve for the single MPAL NOS patient who underwent HCT is not presented; however, this patient is included in the curve showing HCT outcome of all MPAL.

Table 1

Clinical characteristic of MPAL at diagnosis

	Total	B/Myeloid	T/Myeloid	Ph+MPAL	MLL MPAL	MPAL NOS
N	36	13	11	5	5	2
Men	21 (58%)	7 (54%)	6 (55%)	4 (80%)	2 (40%)	2 (100%)
Median age, years (range)	27 (1–69)	17 (1–65)	37 (14–69)	45 (18–60)	15 (2–29)	15 (3–27)
Age <18	13 (36%)	7 (54%)	2 (18%)	0 (0%)	3 (60%)	1 (50%)
Clinical features						
Splenomegaly	9 (25%)	4 (33%)	1 (9%)	2 (50%)	1 (20%)	1 (50%)
Adenopathy	11 (32%)	2 (17%)	6 (55%)	1 (25%)	0 (0%)	2 (100%)
Sarcoma	5 (15%)	0 (0%)	3 (27%)	0 (0%)	2 (40%)	0 (0%)
CNS involvement	7 (19%)	4 (31%0	1 (9%)	0 (0%0	1 (20%)	1 (50%)
CBC, median (range)						
WBC (x10^9/L)	13.5 (0.5–200)	9.9 (0.5–71)	37.2 (8.1–143)	60.2 (34.7–130.5)	169.3 (1.9–200)	13.5
Hb (g/dL)	10 (5.5–14.1)	9.4 (5.5–13.1)	10.7 (6.6–13.3)	10.8 (10–14.1)	7 (6.5–7.3)	12.7 (12.7–12.7)
Plt (x10^9/L)	114 (9–341)	109.5 (28–341)	104.5 (14–333)	139 (123–327)	11 (9–26)	235 (235–235)
Cytogenetics						
Normal	4 (11%)	2 (15%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)
Complex	14 (40%)	3 (23%)	4 (36%)	4 (80%)	2 (40%)	1 (50%)
-7/7q	5 (14%)	1 (8%)	2 (18%)	0 (0%)	1 (20%)	0 (0%)
t 14(q31-32)	4 (11%)	0 (0%)	4 (36%)	0 (0%)	0 (0%)	0 (0%)
+21	6 (17%)	5 (38%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)
Molecular						
Rearranged TCR	10/15 (67%)	3 (75%)	4 (67%)	1 (33%)	1 (100%)	1 (100%)
Rearranged BCR	3/8 (38%)	1 (50%)	0/2	2 (100%)	0/1	0/0
Induction type						
AML-type[#]	25 (69%)	9	7	4	3	1
ALL-type[^]	11 (31%)	4	4	1	2	1

[#]AML-type induction: 7+3 idarubicin (n=4), 7+3 daunorubicin (n=1), ALL-2 (n=10) [39], COG AAML 0531 (n=1) [45] and DCTER (n=9) [23].

[^]ALL-type induction: Institutional Pediatric Inspired Adult ALL protocol (unpublished) (n=2), CALGB 10102 (n=2) [46], CCG 1991 (n=1) [47], COG AALL-0434 (n=1) [48], Hyper-CVAD (n=2) [49], BFM (n=2) [50], COG-AALL 0232 (n=1) [48].

Table 2

Genomic variants in 8 patients who had extended DNA and RNA sequencing for ALL and AML type somatic mutations.

Age	MPAL	Cytogenetics	Molecular mutations
1	B/myeloid	46,XY,?inv(11)(q22q23),der(16)t(1;16)(q21;q12)	PASK R451*
3	MPAL, NOS rare type	46,XY,t(1;5;9)(p32;q33;p22)	**IL7R** L243_T244insGES GPCL, **NOTCH1** P2415fs*5, **IKZF1** K91fs*3, **PHF6** Y105fs*38
4	B/myeloid	46,XY,del(6)(q?23q?25),add(12)(p13)[12] 46,idem,add(4)(p?14)[4]/46,XY[4] FISH positive for t(12;21)(p13;q22)	**ETV6-RUNX1** fusion
15	MLL/MPAL	53,X,+X,-Y,+1,der(1)inv(1)p12q12)del(1)(p12); t(4;11)(q21;q23),+der(1)t(4;11),+8,+10,+13,+21, +22	**PTPN11** p.E76K, **CDKN2A** p.16INK4a **CDKN2A** p.H63Y, **CDKN2A** p.14ARF **CDKN2A** p.A97V, MLL-AFF1 fusion, CD36 N53fs*24, ETV6 p.E392*
25	MLL/MPAL	46,XX,t(4;11)(q21;q23)	MLL-AFF1 fusion
27	MPAL, NOS rare type	47,XY,+8,del(12)(p12)[8]47,idem,del(9)(q?34)[4] 46,XY[8]	RUNX1 R320*, JAK3 p.A573V, JAK3 p.M511I, JAK3 p.V674A, **NOTCH1** truncation intron 2, **NUP214 SET-NUP214** fusion, **PHF6** R128*, **PTPN11** p.G503V, SF3B1 p.E862K, SUZ 12 S53fs*32, TYK2 p.V15A
29	B/myeloid	45,XX,der(13;14)(q10;q10)[8]51,idem,+10,+11,+ 17,+18,+21x2	FLT3 p.Y589S, **CDKN2A** p16INK4a loss, **CDKN2A** p14ARF loss exon 2–3, **EP300 C14orf119 - EP300** fusion, MLL2 R1702*
37	T/myeloid	45,XY,add(1)(p?22),der(3)t(1;3)(p22;q21),-9, add(10)(p11.2),del(11)(q23),del(12)(p11.2), add(14)(q32),i(17)(q10),der(18)t(9;18)(q13;q23)	**NF1** deletion exon 31–35, MLL PICALM-MLL T10 fusion^, **NOTCH2** p.I1549M, **MLL3** p.M711T, FANCE p.R141* #

In bold are alteration recurrently identified in ALL.

^MLL PICALM-MLL T10 fusions are described to occur in acute leukemia with mixed phenotype and typically in patients with T/myeloid phenotype as in this case [51].

#Mutations in FANCE are associated with Fanconi anemia [52] but to the best of our knowledge they have never been reported in MPAL.

Table 3

Variables associated with transplant outcome

	N	Death N	2-year OS (95% CI)	Relapse N	2-year CIR (95% CI)
Remission status at BMT[1]					
Non-CR1	4	3	25% (1–67%)	2	50% (0–100%)
CR1	21	2	88% (60–97%)	5	34% (8–60%)
Conditioning intensity[1,2]					
Ablative	22	3	83% (56–94%)	4	26 (3–49%)
Reduced	3	2	NA (NA)	3	NA (NA)
TBI containing conditioning[1]					
No	10	4	53% (17–79%)	5	56% (19–93%)
Yes	15	1	92% (54–99%)	2	19% (0–45%)
Induction type[2]					
ALL-type	8	3	43% (6–78%)	5	NA (NA)
AML-type	17	2	87% (58–97%)	2	15% (0–36%)
MPAL Subtype[2 *]					
B/Myeloid	8	1	83% (27–98%)	0	0% (0–0%)
T/Myeloid	7	0	100% (100–100%)	3	49% (3–94%)
Ph+ MPAL	5	2	60% (13–88%)	1	20% (0–60%)
MLL MPAL	4	2	50% (6–85%)	3	NA (NA)
Age (years)					
≥18	21	3	83% (55–94%)	5	32% (7–58%)
<18	4	2	50% (6–85%)	2	50% (0–100%)
Diagnostic WBC[2]					
≤13.5x10^9/L	10	0	100% (100–100%)	0	0% (0–0%)
>13.5x10^9/L	11	5	80% (39–95%)	4	49% (10–89%)
Cytogenetics					
No complex karyotype	16	3	77% (44–92%)	4	29% (3–55%)
Complex karyotype	9	2	76% (33–94%)	3	51% (3–99%)
No monosomy	20	4	74% (44–90%)	4	26% (2–49%)

	N	Death N	2-year OS (95% CI)	Relapse N	2-year CIR (95% CI)
Monosomy	5	1	80% (20–97%)	3	60% (6–100%)
GVHD prophylaxis					
Non-TCD	13	2	82% (44–95%)	5	48% (13–83%)
TCD	12	3	69% (30–89%)	2	22% (0–51%)
Donor					
HLA Mismatched	10	2	73% (28–93%)	1	11% (0–33%)
HLA Matched	15	3	77% (44–92%)	6	50% (19–80%)
Cell source					
Peripheral blood or bone marrow	19	5	69% (40–86%)	7	44% (18–69%)
Cord blood	6	0	100% (100–100%)	0	0% (0–0%)
MRD at time of transplant					
No	13	4	65% (31–86%)	3	26% (0–54%)
Yes	9	0	100% (100–100%)	1	25% (0–74%)

[1] A significant variable in OS;

[2] A significant variable in relapse.

[#] Ablative conditioning was busulfan based (n=4); clofarabine, melphalan and thiotepa (n=2); TBI based (n=9); or other.

[*] One patient with NOS MPAL was not included in the survival analysis.

CIR: Cumulative incidence rate; NA: not available; MRD: minimal residual disease, TBI: total body irradiation.