Mixed phenotype acute leukemia: outcomes with allogeneic stem cell transplantation. A retrospective study from the Acute Leukemia Working Party of the EBMT

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ABSTRACT

Mixed phenotype acute leukemias are infrequent and considered high risk. The optimal treatment approach and the role of allogeneic hematopoietic stem cell transplantation are not entirely clear. In this study, we investigated 519 patients with mixed phenotype acute leukemia in first complete remission who underwent allogeneic hematopoietic stem cell transplantation between 2000 and 2014, and who were reported to the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT). Median age was 38.1 years (range 18-75). Cytogenetics classified 49.3% as poor risk. At three years, relapse incidence was 31.4% (26.9-35.9), non-relapse mortality was 22.1% (18.4-26.1), the leukemia-free survival was 46.5% (41.7-51.4), and the overall survival was 56.3% (51.5-61.2). At six months, 32.5% had developed acute graft-*versus*-host disease, while at three years, 37.5% had developed chronic graft-*versus*-host disease (32.6-42.3). In a multivariate analysis, age and year of transplant had a strong impact on outcome. Myeloablative conditioning using total body irradiation correlated with a better leukemia-free survival. Our study suggests that mixed phenotype acute leukemia is potentially sensitive to graft-*versus*-leukemia and thus can benefit from allogeneic hematopoietic stem cell transplantation with a potential for cure.

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Introduction

Major progress was made in the last decades in the diagnosis and treatment of acute and chronic leukemias.[1] Mixed phenotype acute leukemias (MPAL), also designated as acute biphenotypic leukemias or hybrid acute leukemias are rare (1-4% of all acute leukemias) and were described many years ago.[2-6] MPAL (bearing markers of myeloid and lymphoid lineage) are considered enigmatic because of their cell of origin which may be a multipotent stem cell. It is now known that MPAL, like other acute leukemias, are heterogeneous.[7] In 1995, the European Group for the Immunologic Characterization of Leukemias (EGIL) established criteria for acute biphenotypic leukemias in which points are assigned to specific markers of B-lymphoid, T-lymphoid and myeloid origin.[8] In 2008, the World Health Organization (WHO) revised the criteria for lineage assignment and introduced the term "mixed phenotype acute leukemia",[9] but excluded those which could be classified under other cytogenetic or clinical categories. The 2016 WHO update of the classification of acute leukemias maintained the definition of MPAL, but introduced clarifications and added the subcategory of Ph1$^+$ MPAL.[10] The optimal treatment approach to MPAL is not entirely clear. In previous case series, ranging in patient numbers between 13 and 117, allogeneic hematopoietic stem cell transplantation (alloSCT) was performed in 7-61%.[6,11] However, many cases were not

classified according to WHO and most studies did not report transplant outcomes. In reviews by 2 experts, chemotherapy according to acute lymphoblastic leukemia (ALL), followed by alloHSCT is the preferred approach,[7,12] but definitive data are still lacking. Generally, MPAL is considered to be high risk with a poor prognosis, although younger patients may have a better outcome. In the pre-transplant era, or in countries with limited resources, a longer-term survival of 15-35% was described.[6,11] In a smaller series from the Center for International Blood and Marrow Transplant Research (CIMBTR) a 3-year leukemia-free survival (LFS) of 56±10% and an overall survival (OS) of 67±10% was described.[13] The aim of the current study is to establish the outcomes of alloHSCT in a large (n=519 patients), recent (2000-2014), adult cohort from the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT).

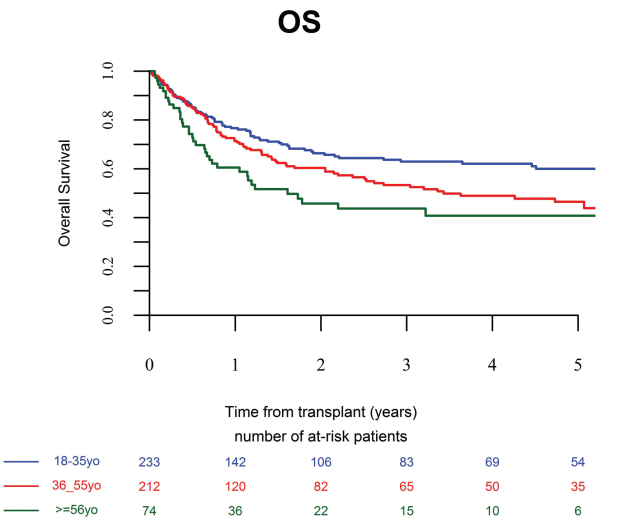

Figure 1. Overall survival (OS) of patients with mixed phenotype acute leukemias (MPAL) who underwent allogeneic hematopoietic stem cell transplantation (alloSCT) according to age groups: 18-35 years (yo), 36-55 yo, and ≥ 56 yo.

Methods

Data source and methods

This retrospective multicenter study was approved by the ALWP of the EBMT and included adults (≥18 years) diagnosed with *de novo* MPAL and receiving HSCT from a matched related or unrelated donor. Patients with MPAL transplanted between 2000 and 2014 were included in the study. Demographics, MPAL disease characteristics, transplantation and post-transplantation data were extracted from the EBMT database (Med-A forms). The list of centers contributing patients to this study is available in the *Online Supplementary Appendix*. The EBMT is a voluntary working group of more than 600 transplant centers that are required to report all consecutive HSCT procedures and their follow up once a year. Audits are routinely performed to determine the accuracy of the data. Patients have been required to provide informed consent authorizing the use of their personal information for research purposes since 1990. To verify which classification was used, the 11 centers transplanting more than 7 patients with MPAL were contacted. Ten centers responded: 8 used WHO, 1 predominantly WHO, and 1 both EGIL and WHO criteria. HLA matching was performed by standard criteria.

Definitions

Cytogenetic abnormalities were classified as favorable, intermediate or high risk, as previously described.[14] The conditioning regimen was defined as reduced intensity conditioning (RIC) when fludarabine was associated with low-dose total body irradiation (TBI) (6 Gy) or a dose of oral busulfan <8 mg/kg or a dose of intravenous (IV) busulfan <6.4 mg/kg or other immunosuppressive or chemotherapeutic drugs, such as melphalan or cyclophosphamide. Myeloablative conditioning (MAC) was defined as a preparative regimen that contained TBI or busulfan at higher doses.[15] Neutrophil engraftment was defined as an absolute neutrophil count (ANC) over $0.5x10^9$/L for three consecutive days. Platelet engraftment was defined as an absolute platelet count over $20x10^9$/L for three consecutive days.

Statistical analysis

The clinical outcomes studied were overall survival (OS), leukemia-free survival (LFS), relapse incidence (RI), non-relapse mortality (NRM), chronic graft-*versus*-host disease (cGvHD). OS was defined as the time from day 0 of allo-SCT to death or last follow up for survivors. LFS was defined as time from day 0 of allo-SCT to time without evidence of relapse or disease progression censored at the date of death or last follow up. Relapse was defined as any event related to re-occurrence of the disease. NRM was defined as death from any cause without previous relapse or progression. Probabilities of OS and LFS were calculated using the log rank test and Kaplan-Meier graphical representation. Further end points were engraftment, incidence and severity of acute and chronic GvHD (grading of acute GvHD was performed as previously published[16]). Cumulative incidence functions (CIF)[17] were used to estimate RI and NRM in a competing risks setting. In order to study cGvHD, we considered death and relapse as competing events. Survival probabilities are presented as percentages and 95% confidence intervals (95%CI). Univariate analyses were performed using the log rank test for OS and LFS and the Gray test for CIF.

Multivariate analyses were performed using the Cox proportional hazard model. To allow for potential confounding factors between treatments that could influence outcome, propensity score matching was also performed, using the exact matching.[17] Matching on the propensity score was then used to reduce or eliminate confounding effects and estimate treatment effects by matching 3 AML and 4 ALL patients with each MPAL patient. The following factors were included in the propensity score model: patient age [18-35 years (y), 36-55 y and above 56 y], year of transplant, time from diagnosis to transplant (per quantile), conditioning (RIC, MAC TBI, MAC chemotherapy), source of SC [bone marrow (BM)/peripheral blood (PB)], patient sex, female donor to male recipient, type of donor [unrelated donor (UD)/matched sibling donor (MSD)]. The purpose of the propensity score-matching strategy was to reduce confounding effects of these variables, and strengthen causal inferences.[18] Details about the patients used for matching are given in the *Online Supplementary Appendix.*

All tests were two-sided and $P<0.05$ was considered statistically significant. Analyses were performed using the R statistical software v.3.2.3 (available online at: *http://www.R-project.org*), and propensity score analysis was performed using the 'MatchIt' program (last accessed May 18th, 2015; *http://cran.project.org/web/packages/MatchIt/MatchIt.pdf*). Patients with missing values were excluded from propensity score analyses.

Results

Patients' and disease characteristics

Patients', disease and transplant characteristics are summarized in Table 1. A total of 519 patients (all in first complete remission) underwent allo-SCT in the study period. These patients were contributed by 189 centers, 30 of which transplanted 5 or more patients with MPAL during the study period. Sixty-three percent of the patients were male. Median age at transplant was 38.1 years. Complete cytogenetics were available in 203 patients. Among these, 50.7% had intermediate risk and 49.3% poor risk cytogenetics, respectively. Among the poor risk patients, 50% were defined by positivity for bcr/abl or Ph$^+$. Four hundred patients received MAC (260 including TBI, 140 without TBI), 119 received RIC. The majority of the patients were transplanted with stem cells harvested from PB (73.0%), while 26.4% received BM. Hematopoietic recovery from transplant was observed in 492 patients (97.0%). In 6 patients (1.2%), the engraftment was transient. Nine patients (1.8%) never engrafted. Donor lymphocyte infusions (DLI) post transplant were documented in 55 patients (25 prophylactic or pre-emptive, 29 for clinical relapse). Among the 29 patients who received DLI for relapse, 10 also underwent a second transplant. The 1- and 2-year OS of these patients was 44 and 35%, respectively.

Table 1. Patients', disease and transplant characteristics.

Characteristic	N (%)
Total patient number	519 (100%)
Median age, years (range)	38.1 (18-75)
18-35	232 (44.9)
36-55	212 (40.9)
≥ 56	74 (14.3)
Sex male / female	329 (63.4) / 190 (36.6)
Time of transplant	
2000- 2004	69 (13.3)
2005- 2010	238 (45.9)
2011- 2014	212 (40.9)
Cytogenetics	
Available	203 (39.1)
Good risk	0 (0)
Intermediate risk	103 (50.7)
Poor risk (including 50 bcr-abl or Ph$^+$ and 11 11q23$^+$ patients)	100 (49.3)
Unavailable	316 (60.9)
Conditioning regimen	
MA with TBI	260 (50.1)
MA, no TBI	140 (27.0)
RIC	119 (22.9)
Median time from CR-transplant (days)	99 (± 36)
Graft type	
Bone marrow	137 (26.4)
Peripheral blood stem cells	379 (73.0)
Both	3 (0.6)
In vivo T-cell depletion	
ATG	153 (33.0)
Alemtuzumab	56 (12.1)
No *in vivo* T-cell depletion	254 (54.9)
CMV status	
Patient positive	285 (63.2)
Patient negative	166 (36.8)
Donor positive	240 (53.7)
Donor negative	207 (46.3)
Follow up of survivors, median (range), months	32.1 (0.9-181.2)

MA: myeloablative conditioning; TBI: total body irradiation; RIC: reduced intensity conditioning; ATG: anti-T-cell globulin. Definition of cytogenetics: Good risk: t(8;21) or inv16. Poor risk: complex or del5 or del7 or mono5 or mono7 or 11q23 or 3q26 or inv3 or t(6;9) or t(11;19) or Ph+ or t(4;11). Intermediate risk: all other.

Prognostic factors for outcome

A univariate analysis of outcomes at three years is shown in Table 2. Age at transplant had a strong impact on LFS, RI and NRM. More recent transplants had less NRM and a trend for better OS. No impact was observed according to donor type, patient sex, or cytomegalovirus (CMV) status of donor or recipient. Female donors were associated with more cGvHD. The combination of a female donor for a male recipient was particularly strongly associated with cGvHD. MAC, especially with TBI, correlated with better LFS and less RI. The source of stem cells did not impact on LFS or OS, though PB had a trend for higher RI and more cGvHD, but less NRM. In a multivariate analysis (Table 3), the best outcome (NRM, LFS, and OS) was seen in younger patients and in patients transplanted more recently. Matched unrelated donors compared with matched sibling donors and female donors for male recipients had a significant increase in cGvHD, but similar RI, LFS and OS. Adverse cytogenetics (both Ph$^+$ and other poor cytogenetics) were associated with decreased OS. MAC with TBI compared with myeloablation with chemotherapy alone was associated with better LFS and a lower RI. *In vivo* T-cell depletion (TCD) was associated with less cGvHD, but did not impact on survival. Similarly, the use of PB *versus* BM correlated with more

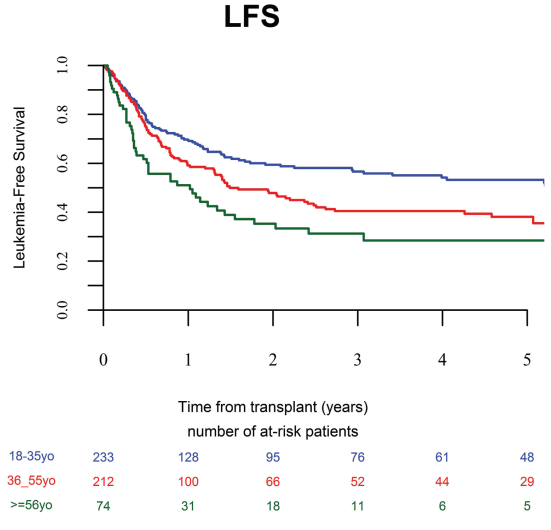

Figure 2. Leukemia-free survival (LFS) of patients with mixed phenotype acute leukemias (MPAL) who underwent allogeneic hematopoietic stem cell transplantation (allo-SCT) according to age groups: 18-35 years (yo), 36-55 yo, and ≥56 yo.

Table 2. Univariate analysis of outcomes at three years after allogeneic hematopoietic stem cell transplantation for mixed phenotype acute leukemia.

Characteristic	LFS (% ± SD) P	OS (% ± SD) P	RI (% ± SD) P	NRM (% ± SD) P	cGvHD (% ± SD) P
Age (years)					
18-35	56.6 (49-63)	62.9 (56-70)	27.4 (21-34)	16.0 (11-21)	39.5 (32-47)
36-55	40.5 (33-48)	53.3 (46-61)	32.1 (25-39)	27.4 (21- 34)	38.1 (31-46)
≥56	31.3 (20-43)	43.7 (31-56)	41.8 (29-54)	26.9 (17-38)	30.2 (19-42)
	P<0.0001	*P*=0.0017	*P*=0.031	*P*=0.026	*P*=0.567
Year of transplant					
2004-2004	41.8 (30-54)	44.7 (33-57)	24.9 (15-36)	33.2 (22-45)	31.2 (20-43)
2005-2010	46.8 (40-54)	55.6 (49-62)	32.9 (27-39)	20.2 (15-26)	37.7 (31-45)
2011-2014	46.0 (37-55)	61.8 (53-71)	33.7 (25-43)	20.3 (14-27)	40.3 (32-49)
	P=0.361	*P*=0.057	*P*=0.464	*P*=0.039	*P*=0.639
Donor type					
Matched sib donor	45.6 (39-52)	54.5 (48-61)	32.6 (27-39)	21.8 (17-27)	34.6 (28-41)
Unrelated donor	47.7 (41-55)	58.0 (50-66)	29.7 (23-37)	22.4 (17-28)	40.7 (33-48)
	P=0.690	*P*=0.477	*P*=0.569	*P*=0.918	*P*=0.310
Patient sex					
Male	45.3 (39-51)	55.3 (49-61)	32.3 (27-38)	22.4 (18-28)	41.1 (35-47)
Female	48.7 (41-57)	58.0 (50-66)	29.7 (23-37)	21.6 (16-28)	31.2 (24-39)
	P=0.922	*P*=0.897	*P*=0.805	*P*=0.930	*P*=0.116
Donor sex					
Male	45.9 (40-52)	59.1 (53-65)	34.8 (29-41)	19.3 (15-24)	33.3 (27-39)
Female	47.1 (39-55)	51.4 (43-59)	25.9 (19-33)	27.0 (20-34)	43.0 (35-51)
	P=0.978	*P*=0.131	*P*=0.079	*P*=0.065	*P*=0.028
Matching by sex					
No F-> M	45.4 (40-51)	57.5 (52-63)	33.9 (28-39)	20.7 (17-25)	32.5 (27-38)
F-> M	49.8 (39-60)	51.3 (41-62)	22.6 (15-32)	27.6 (19-37)	53.6 (42-64)
	P=0.492	*P*= 0.624	*P*=0.062	*P*=0.229	*P*=0.000
CMV status patient					
Negative	50.2 (42-59)	59.2 (51-68)	29.7 (22-38)	20.0 (14-27)	35.1 (27-43)
Positive	45.4 (39-52) *P*=0.789	55.1 (49-62) *P*=0.420	29.6 (24-36) *P*=0.293	24.9 (20-31) *P*=0.166	40.0 (33-47) *P*=0.235
CMV status donor					
Negative	48.4 (41-56)	58.3 (51-66)	30.1 (23-37)	21.5 (16-28)	40.4 (33-48)
Positive	46.9 (40-54)	56.8 (50-64)	29.7 (23-36)	23.4 (18-30)	37.2 (30-44)
	P=0.379	*P*=0.306	*P*=0.729	*P*=0.499	*P*=0.502
Type of conditioning					
MAC chemotherapy	43.1 (34-53)	55.2 (46-65)	33.2 (24-42)	23.7 (17-2)	30.9 (22-41)
MAC TBI	56.3 (17-29)	60.0 (53-67)	22.6 (17-29)	21.1 (16-27)	42.3 (35-49)
RIC	29.1 (38-58)	49.4 (39-60)	48.3 (38-58)	22.6 (15-31)	33.5 (24-43)
	P<0.001	*P*=0.151	*P*<0.001	*P*=0.764	*P*=0.146
Cytogenetics					
Intermediate	54.8 (44-65)	65.6 (56-76)	28.4 (19-38)	16.8 (10-25)	37.2 (27-48)
Poor	46.1 (36-56)	55.0 (45-65)	34.1 (25-44)	19.8 (12-28)	34.4 (25-44)
Not available	43.3 (37-50)	53.2 (47-60)	31.5 (26-38)	25.2 (20-31)	38.8 (32-45)
	P=0.132	*P*=0.159	*P*=0.574	*P*=0.344	*P*=0.616
Source of stem cells					
Bone marrow	45.7 (37-55)	53.6 (45-63)	25.9 (19-34)	28.3 (21-37)	30.9 (23-40)
Peripheral blood	46.9 (41-53)	57.4 (52-63)	33.5 (28-39)	19.6 (16-24)	40.3 (34-46)
	P=0.993	*P*=0.314	*P*=0.066	*P*=0.055	*P*=0.055

LFS: leukemia-free survival; SD: Standard Deviation; OS: overall survival; RI: relapse incidence; NRM: non-relapse mortality; cGvHD: chronic graft-*versus*-host disease; *P*: *P*-value; sib: sibling; F: female; M: male; CMV: cytomegalovirus; MAC: myeloablative conditioning; TBI: total body irradiation; RIC: reduced intensity conditioning.

Table 3. Multivariate analysis of major outcomes after transplant.

	RI			NRM			LFS			OS			Chronic GvHD		
	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*
Age per decade	0.96	0.83-1.11	0.59	1.43	1.19-1.71	<0.001	1.13	1.01-1.27	0.03	1.19	1.05-1.35	0.006	1.01	0.87-1.18	0.88
Year of transplant	0.98	0.92-1.04	0.42	0.92	0.86 -0.98	0.01	0.95	0.91-0.99	0.02	0.93	0.89-0.98	0.003	0.96	0.91-1.01	0.15
UD *vs.* MSD	0.79	0.52-1.21	0.28	1.08	0.64-1.84	0.77	0.89	0.64-1.24	0.49	0.93	0.64-1.35	0.69	1.82	1.19-2.79	0.006
Female D -> male R	0.80	0.50-1.28	0.35	1.19	0.71-1.97	0.51	0.94	0.67-1.33	0.72	1.11	0.77-1.59	0.59	2.23	1.51-3.30	<0.001
Intermediate cytogenetics (ref)	1			1			1			1			1		
Poor cytogenetics	1.13	0.71-1.79	0.61	1.76	0.98-3.15	0.06	1.39	0.97-1.99	0.07	1.52	1.02-2.26	0.04	1.31	0.86-2.01	0.21
Cytogenetics NA or failed	1.39	0.83-2.30	0.21	1.40	0.73-2.70	0.31	1.40	0.94-2.09	0.10	1.40	0.91-2.22	0.13	1.02	0.62-1.67	0.95
MAC chemo (ref)	1			1			1			1			1		
MAC TBI *vs.* MAC chemo	0.50	0.31-0.79	0.003	0.78	0.46-1.31	0.35	0.61	0.43-0.86	0.005	0.73	0.50-1.06	0.10	1.35	0.85-2.14	0.20
RIC *vs.* MAC chemo	1.34	0.82-2.19	0.24	0.63	0.33-1.18	0.15	1.00	0.68-1.47	1.00	0.86	0.56-1.34	0.51	1.82	1.02-3.25	0.043
In vivo TCD	1.40	0.88-2.22	0.16	0.82	0.46-1.46	0.51	1.12	0.78-1.61	0.54	0.95	0.64-1.42	0.80	0.52	0.33-0.84	0.01
PB *vs.* BM	0.99	0.63-1.55	0.97	0.83	0.51-1.35	0.44	0.92	0.66-1.27	0.60	0.89	0.63-1.26	0.51	1.66	1.08-2.55	0.02

RI: relapse incidence; NRM: non-relapse mortality; LFS: leukemia-free survival; OS: overall survival; GvHD: graft-*versus*-host disease; HR: Hazard Ratio; CI: Confidence Interval; *P*: *P*-value; UD: unrelated donor; MSD: matched-sibling donor; D: donor; R: recipient; ref: reference value; NA: not available; MAC: myeloablative conditioning; chemo: chemotherapy; TBI: total body irradiation; RIC: reduced intensity conditioning; TCD: T-cell depletion; PB: peripheral blood; BM: bone marrow.

cGvHD, but was not associated with RI, NRM, LFS or OS. The outcome in smaller centers (1-5 patients with MPAL in the study period) was not statistically different from larger centers (more than 5 patients with MPAL; *data not shown*). In a univariate analysis, the occurrence of ≥grade 2 aGvHD and cGvHD correlated with higher NRM and lower RI, but made no significant impact on LFS and OS (although a trend for worse LFS was observed with aGvHD; *data not shown*). An additional 51 patients with MPAL were transplanted in second complete remission (CR2). Three-year survival was inferior, mostly due to higher NRM (*data not shown*).

Matched-pair analysis

In a matched-pair analysis, 498 patients with MPAL were matched with 1371 patients with acute lymphoblastic leukemia (ALL) and 498 patients were matched with 1412 patients with acute myelogenous leukemia (AML). Overall, the groups were well-matched although the ALL group had more high-risk cytogenetics. The comparison MPAL *versus* ALL showed no significant differences. When MPAL was compared with AML, MPAL had higher NRM and lower LFS (See *Online Supplementary Tables 1 and 2*).

Discussion

Mixed phenotype acute leukemias are high-risk acute leukemias often with poor cytogenetics. As in other types of acute leukemia, the prognosis can potentially be improved by allo-SCT. We present here, extracted from the EBMT database, the largest study of patients with MPAL who underwent allo-SCT. Our results (56.3% OS at 3 years and 46.5% LFS at 3 years, with an RI of 31%) are highly encouraging and definitely improved over registry data from the Surveillance, Epidemiology and End Results Program (SEER). In the SEER study, a survival of 20-40% at three years was reported for patients aged over 20 years.[6] In Table 4, a synopsis with outcomes of previous smaller studies investigating allo-SCT for MPAL is presented. In single institution studies, 9-59 patients with MPAL were transplanted. In a survey of 100 patients with MPAL treated at European hematology centers and classified according to WHO, only 20 underwent allogeneic or autologous transplantation. In this survey, no data on outcome of transplant are given. However, the overall median survival of adults with MPAL was only 11 months.[23]

The Center for International Blood and Marrow Transplant Research (CIBMTR) recently published a thoroughly investigated study which included 95 patients with a median age of 20 years. The CIBMTR study differs by including cord blood as source of stem cells and patients transplanted in CR2. Only 33% of patients in the CIBMTR study had PB as source of stem cells and only 11% received RIC (compared with 23% in the present study). The present study confirms and extends the CIBMTR study: allo-SCT is an effective treatment for adult patients with MPAL if a matched donor can be found.

Classically, chronic myelogenous leukemia, AML, ALL, and to a lesser degree, myelodysplastic syndromes, are diseases considered to be sensitive to the graft-*versus*-leukemia (GvL) effect of allo-SCT.[24,25] In all these diseases, GvL effects are associated to a variable degree with GvHD. We propose here, supported by the CIBMTR study, to add MPAL to the list of potentially GvL-sensitive leukemias. A slightly lower rate of aGvHD and cGvHD than in the previous CIBMTR study was observed in the present study which may be due to the common use of *in vivo* TCD. However, treatment intensity also plays a role since MAC (especially with TBI) in our study yields better outcomes than RIC.

In the study presented here, a matched pair-analysis showed transplant outcomes for MPAL are comparable with ALL and slightly worse than AML. This has to be put in perspective given that allo-SCT is a treatment offered to most patients with standard-risk AML, whereas transplant for ALL in younger patients is offered only in high-risk situations. The overall outcome in the multicenter EBMT setting (with a more homogeneous patient population: all patients in CR1, only matched related or unrelated donors) is comparable or slightly better than in the CIBMTR set-

Table 4. Synopsis of previous and current studies of patients with MPAL undergoing alloHSCT.

Region	Years of study	Patient #	Median age (years)	% in CR1	% of aGvHD	LFS at 3 years	OS at 3 years	Reference
Korea	1995- 2008	9	6	n.r.	n.r.	n.r.	≈ 30 %	19
China	2002-	24@	22	n.r.	n.r.	17 %#	24 %#	20
	2011	35@	26	n.r.	n.r.	56 %#	64 %#	
Japan	2001- 2010	18	n.r.	n.r.	75	40 %#	48 %#	21
China	2006- 2013	29	30	72	n.r.	n.r.	77 %	22
CIBMTR	1996- 2011	95	20	82	48	56 %	67 %	13
EBMT	2000- 2014	519	38	100	33	47 %	56 %	This study

MPAL: mixed phenotype acute leukemias; alloHSCT: allogeneic hematopoietic stem cell transplantation; CR1: first complete remission; aGvHD: graft-*versus*-host disease; LFS: leaukemia-free survival; OS: overall survival; CIBMTR: Center for International Blood and Marrow Transplantation Research; EBMT: European Society for Blood and Marrow Transplantation; @Patients treated according to 2 different protocols; #at five years.

ting. In the current, more recent EBMT study, the outcomes improved in recent years. Although not documented (being a registry-based study), we can assume that most patients who were bcr/abl positive were treated with tyrosine kinase inhibitors. A limiting factor of our study is that no central review was performed and that the WHO classification for MPAL was introduced only in 2008. This is compensated by the large patient numbers in a multi-center and multi-national setting.

An important and unreported finding is the favorable effect of TBI as part of conditioning. In the multivariate analysis (see Table 3), MAC with TBI had a lower RI and higher LFS. Similar data were recently published for adult patients with T-cell ALL.[26,27] Based on our present study, we recommend MAC with TBI for fit patients with MPAL as standard regimen. The superiority of MAC over RIC was recently also shown for AML and myelodysplastic syndromes.[28]

Looking into the future, two groups have performed whole exome or genome sequencing in patients with MPAL. The first study examined 23 adult and pediatric cases and found frequent mutations of epigenetic modifiers especially DNMT3A.[29] The second study examined 115 pediatric cases of MPAL and found 35 recurrently mutated genes (including *WT1, FLT3, NRAS, JAK3,* and numerous other genes) and correlated these mutations with subtypes of MPAL.[30] If targeted agents can be introduced into the treatment algorithm of MPAL, the prognosis of this rare, and until recently poor prognosis leukemia, may further improve.

In conclusion, consolidation with alloHSCT in CR1 provides a favorable disease control to adult patients with MPAL with a moderate relapse risk. This resembles the outcome observed in patients with ALL. The observation of a possible beneficial impact of TBI as part of the conditioning regimen deserves further investigation.

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