Haploidentical transplantation in primary refractory/relapsed secondary versus de novo AML: from the ALWP/EBMT

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Abstract:
We compared the outcomes of haploidentical stem cell transplantation (HaploHSCT) with post-transplant cyclophosphamide (PTCy) in 719 patients (pts) with primary refractory (PR) / first relapse (Rel) secondary acute myeloid leukemia (sAML) (n=129) versus those of de novo AML (n=590), transplanted between 2010 and 2022. A higher percentage of pts with sAML versus de novo AML had PR disease (73.6% vs. 58.6%) (p=0.002). In 81.4% of sAML pts, the antecedent hematological disorder was myelodysplastic syndrome. Engraftment was 83.5% vs. 88.4% in sAML and de novo AML, respectively (p=0.13). In multivariate analysis HaploHSCT outcomes did not differ significantly between the groups; non-relapse mortality (NRM) hazard ratio (HR) =1.38 (95% CI 0.96-1.98, p=0.083), relapse incidence (RI) HR= 0.68 (95% CI 0.4.7.-1.00, p=0.051). The HRs for leukemia-free survival (LFS), overall survival (OS), and GVHD-free, relapse-free survival (GRFS) were 0.99 (95% CI 0.76-1.28, p=0.94), 0.99 (95% CI 0.77-1.29, p=0.97) and 0.99 (95% CI 0.77-1.27, p=0.94), respectively. We conclude that outcomes of HaploHSCT with PTCy are not different for PR/Rel sAML in comparison to PR/Rel de novo AML, a finding of major clinical importance.

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Key points:

Outcomes of haploidentical transplantation with PTCy are similar for primary refractory/relapsed secondary vs de novo AML

- HaploHSCT with PTCy can rescue high-risk patients with PR/ Rel sAML

Abstract

We compared the outcomes of haploidentical stem cell transplantation (HaploHSCT) with post-transplant cyclophosphamide (PTCy) in 719 patients (pts) with primary refractory (PR) / first relapse (Rel) secondary acute myeloid leukemia (sAML) (n=129) versus those of *de novo* AML (n=590), transplanted between 2010 and 2022. A higher percentage of pts with sAML versus *de novo* AML had PR disease (73.6% *vs.* 58.6%) (p=0.002). In 81.4% of sAML pts, the antecedent hematological disorder was myelodysplastic syndrome. Engraftment was 83.5% vs. 88.4% in sAML and *de novo* AML, respectively (p=0.13). In multivariate analysis HaploHSCT outcomes did not differ significantly between the groups; non-relapse mortality (NRM) hazard ratio (HR) =1.38 (95% CI 0.96-1.98, p=0.083), relapse incidence (RI) HR= 0.68 (95% CI 0.4.7.-1.00, p=0.051). The HRs for leukemia-free survival (LFS), overall survival (OS), and GVHD-free, relapse-free survival (GRFS) were 0.99 (95% CI 0.76-1.28, p=0.94), 0.99 (95% CI 0.77-1.29, p=0.97) and 0.99 (95% CI 0.77-1.27, p=0.94), respectively. We conclude that outcomes of HaploHSCT with PTCy are not different for PR/Rel sAML in comparison to PR/Rel *de novo* AML, a finding of major clinical importance.

Introduction

Secondary acute myeloid leukemia (sAML) is a subset of acute myeloid leukemia 82

(AML) with notoriously adverse outcomes evolving from an antecedent hematological 83

disorder, mainly myelodysplastic syndromes (MDS) and myeloproliferative 84

neoplasms (MPNs) or as a complication of prior cytotoxic chemotherapy or radiation 85

therapy (1-5). Patients with sAML have inferior outcomes with lower remission rates 86

and overall survival (OS) compared to *de novo* AML, mainly due to a higher 87

frequency of adverse molecular mutations including secondary type mutations and 88

high-risk cytogenetic abnormalities (6-8), in addition to typically being older and 89

having an antecedent hematological disease (9-12). Allogeneic hematopoietic stem 90

cell transplantation (alloHSCT) represents a potentially curative therapy in this 91

setting, rescuing up to 40% of the patients (13-17) as was already reported in 2010 92

by the Center for International Blood and Marrow Transplant Research (CIBMTR) 93

that described 868 patients with therapy-related AML or MDS including with 94

advanced disease that were transplanted between 1990 and 2004 mainly from 95

matched sibling donors (MSD) or matched unrelated donors (MUD) and 96

myeloablative conditioning (MAC) with a 5-year disease-free survival (DFS) and OS 97

of 21% and 22%, respectively (13). On behalf of the Acute Leukemia Working Party 98

(ALWP) of the European Society for Blood and Marrow Transplantation (EBMT), we 99

evaluated transplantation outcomes in approximately 5000 patients with sAML 100

transplanted between 2000 and 2016, mainly from MSD and MUD, where we 101

observed 2-year OS, leukemia-free survival (LFS) and graft-versus-host disease 102

(GVHD)-free, relapse-free survival (GRFS) of 44.5%, 38.8%, and 27.2%, respectively 103

(18). Notably, transplantation outcomes in sAML are significantly inferior to those 104

achieved in *de novo* AML with a lower OS, LFS, and GRFS due to higher non- 105

relapse mortality (NRM) and relapse incidence (RI) (19). Transplantation outcomes 106

are improving, including those for sAML as we have recently demonstrated in a study 107

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of sAML patients comparing 1337 that were transplanted in 2000 to 2010 with 2887 108

transplanted in 2011 to 2020. We demonstrated a significant reduction in the 2-year 109

NRM and a significant improvement in the 2-year GRFS but the 2-year LFS and OS 110

were similar (20) with somewhat better results with MAC versus reduced intensity 111

conditioning (RIC) (13,21). One of the major advances in the field of transplantation 112

is the development of the non-T depleted haploidentical stem cell transplantation 113

(HaploHSCT) with post-transplant cyclophosphamide (PTCy) which has been 114

increasingly used for AML and proven to be highly effective in preventing GVHD and 115

reducing NRM, thus improving transplantation results including for sAML with a 2- 116

year LFS of 49% and OS of 57% in patients transplanted in complete response (CR) 117

(22-25). We have recently analyzed outcomes of HaploHSCT with PTCy in 231 118

patients with sAML in comparison to 1480 patients with *de novo* AML both in first CR 119

(CR1) and observed no significant difference in any transplantation outcome 120

parameter between the sAML versus *de novo* AML groups (26), which is in contrast 121

to our previous results with human leukocyte antigen (HLA) matched alloHSCT (19) 122

However, results of alloHSCT may differ in patients with primary refractory (PR)/ first 123

relapse (Rel) sAML, a group which is very hard to treat and with substantially inferior 124

transplantation outcomes than leukemic patients in remission (27-28). Failure to 125

respond to the induction course and relapse are major unfavorable prognostic factors 126

(4, 5). PR or Rel AML is associated with a dismal prognosis (4-5, 27-28). From a 127

theoretical point of view, it is conceivable that HaploHSCT will improve results in 128

patients with PR/Rel leukemic patients as some reports indicate a stronger graft- 129

versus-leukemia (GVL) effect with haploidentical grafts due to the broad HLA 130

disparity (29-30). We therefore assessed the outcomes of HaploHSCT in patients 131

with PR/Rel sAML comparing them with those of HaploHSCT in *de novo* AML, taking 132

advantage of the ALWP/EBMT registry. 133

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Patients and methods

Study design and data collection

This was a retrospective, multicenter analysis using the dataset of the ALWP of the

EBMT. The EBMT is a voluntary working group of more than 600 transplant centers

that are required to report all consecutive stem cell transplantations and follow-ups

once a year. Since the 1st of January 2003, all transplantation centers have been

required to obtain written informed consent before data registration with the EBMT,

as per the Declaration of Helsinki of 1975. Data accuracy is assured by the individual

transplant centers and by quality control measures such as regular internal and

external audits. In addition, the study protocol was approved by each site and

complied with country-specific regulatory requirements. The results of disease

assessments at HSCT were also submitted and form the basis of this report.

Eligibility criteria for this analysis included adult patients ≥18 years of age with

primary refractory (PR) / first relapse (Rel) de *novo* AML or PR/ first Rel sAML who

underwent a first HSCT from a non-T-cell depleted haploidentical donor with PTCy as

part of GVHD prophylaxis between 2010 and 2022. Active AML was defined by the

failure to achieve CR (bone marrow blasts >5%) despite induction chemotherapy

(27). A haploidentical donor was defined as ≥2 HLA mismatches between donor and

recipient. The exclusion criteria were HSCT from other donor types (sibling,

unrelated, or cord blood donor); previous history of HSCT, and T cell-depleted HSCT.

Data collected included recipient and donor characteristics including the number of

HLA mismatches, age, gender, cytomegalovirus (CMV) serostatus, Karnofsky

performance status (KPS) score, and hematopoietic cell transplantation-specific

comorbidity index (HCT-CI), disease characteristics including cytogenetics (ELN

2017) and disease status at transplantation, antecedent of malignant disorder, year

of transplant, type of conditioning regimen including total body irradiation (TBI), stem

cell source, and GVHD prophylaxis regimen including number of immunosuppressive   163

(IS) compounds. The conditioning regimen was defined as MAC when containing TBI   164

with a dose >6 Gray or a total dose of busulfan (Bu) >8 mg/kg or >6.4 mg/kg when   165

administered orally or intravenously, respectively. All other regimens were defined as   166

RIC (31). Grading of acute (a) GVHD was performed using established criteria (32).   167

Chronic (c) GVHD was classified as limited or extensive according to published   168

criteria (33). For this study, all necessary data were collected according to the EBMT   169

guidelines, using the EBMT minimum essential data forms. The list of institutions   170

contributing data to this study is provided in the Supplemental Appendix.   171

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Statistical analysis   173

The median, interquartile range (IQR), and range were used for quantitative   174

variables, and frequency and percentage for categorical variables. The study   175

endpoints were OS, LFS, RI, NRM, engraftment, aGVHD, cGVHD, and GRFS. All   176

endpoints were measured from the time of transplantation. Engraftment was defined   177

as achieving an absolute neutrophil count (ANC) of 0.5×10^9/L for three consecutive   178

days. OS was defined as time to death from any cause. LFS was defined as survival   179

with no evidence of relapse or progression. NRM was defined as death from any   180

cause without previous relapse or progression. We used modified GRFS criteria.   181

GRFS events were defined as the first event among grade III-IV aGVHD, extensive   182

cGVHD, relapse, or death from any other cause (34). Patient, disease, and   183

transplant-related characteristics for the two cohorts (*de novo* and sAML) were   184

compared using the Mann–Whitney *U* test for numerical data, and the chi-squared or   185

Fisher's exact test for categorical data. Median follow-up was calculated by the   186

reverse Kaplan–Meier method. The probabilities of OS, LFS, and GRFS were   187

calculated using the Kaplan–Meier estimate. The RI and NRM were calculated using   188

cumulative incidence (CI) functions in a competing risk setting, with death in   189

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remission being treated as a competing event for relapse. Death was considered a competing event for engraftment. To estimate the CI of acute or cGVHD, relapse and death were considered as competing events. Univariate analyses were performed using the log-rank test for LFS and OS while Gray's test was used for CI. Multivariate analyses (MVA) were performed using the Cox proportional-hazards regression model (35). All variables differing significantly between the two groups, and potential risk factors were included in the model. To take into account the heterogeneity in the effect of a characteristic or a treatment across centers, we introduce a random effect (or frailty) into the Cox multivariate models (36). We looked at all potential interactions between the core variable and other significant variables. Results were expressed as the hazard ratio (HR) with a 95% confidence interval (95% CI). All *p* values were two-sided with a type 1 error rate fixed at 0.05. Statistical analyses were performed with SPSS 25.0 (SPSS Inc., Chicago, IL, USA) and R 4.0.2 (R Core Team Fifty (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) (37). The scientific boards of the ALWP of the EBMT approved this study

Results

Patient, transplant, and disease characteristics

A total of 719 patients met the inclusion criteria, 129 with sAML and 590 with *de novo* AML. Table 1 shows the baseline demographic and clinical characteristics. Median follow-up was 45.59 (IQR, 39.08-57.85) and 43.48 (IQR, 37.53-47.99) months for patients with sAML and *de novo* AML (p=0.2), respectively. Patients with *de novo* AML were younger, with a median age of 55.4 (range 18-77.8.) versus 61.3 (range 21-78.8) years, (p<0.0001). The median year of transplantation was 2018 (range 2010-2022) *vs.* 2017 (range 2010-2022) (p=0.62), respectively, and 65.1% and 57.6% of the patients with sAML and *de novo* AML, were male (p=0.11), respectively.

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In sAML patients, the most frequent (81.4%) antecedent hematological disorder was myelodysplastic syndrome (MDS), followed by another hematological disorder in 10.9% and solid tumor in 8.3% of the patients, respectively. A higher percentage of pts with sAML vs *de novo* AML had PR disease (73.6% *vs* 58.6%) (p=0.002). The distribution of cytogenetic risk was similar between the two groups and categorized as intermediate (59.4% vs 58.6%), adverse (37.4% vs 34.4%), and favorable (5.9% vs 4%) for patients with sAML and *de novo* AML, respectively (p=0.75). The KPS score was <90 in 50.8% and 44.1 %, of the patients with sAML and *de novo* AML, respectively (p=0.17). The HCT-CI was higher in the sAML group in comparison with the *de novo* AML group, with HCT-CI $\geq$3 in 40.3% vs 21.9%, respectively (p<0.0001). Both patient and donor CMV seropositivity was similar between the two groups with 79.8% and 75.4%; p=0.29, and 53.1% and 59.3%; p=0.2 in sAML and *de novo* AML, respectively. Female donor-to-male patient combination was used in 20.9% of transplants in both sAML and *de novo* AML. Fewer sAML patients received MAC compared to *de novo* AML patients, 39.8% vs 47.8%, respectively, but this was not statistically significant (p=0.10). Graft source was mainly peripheral blood (PB) stem cells in both sAML (69.8%) and *de novo* (66.8%) groups (p=0.51). The most frequent conditioning regimen for both groups was thiotepa/busulfan/fludarabine at 38.8% and 42%, followed by fludarabine/low dose TBI in 17.1% and 12.9% and busulfan/fludarabine in 14.7% and 16%, of patients with sAML, and *de novo* AML, respectively (Supplemental Table S1). For GVHD prophylaxis, PTCY was combined with cyclosporine A (CSA) and mycophenolate mofetil (MMF) in 41.4% and 52.9% of the sAML and *de novo* AML patients, respectively, while in 41.1% and 33.6%, respectively, it was combined with MMF and tacrolimus (Tacro) (Supplemental Table S2*).*

Transplantation outcome

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Engraftment and GVHD incidence did not differ between the sAML vs *de* 244

novo AML groups as depicted in Table 2. Neutrophil recovery 245

(ANC > 0.5 × 10^9/L) was achieved in 83.5% and 88.4% of the patients with 246

sAML and *de novo*, respectively (p=0.13). On univariate analysis, on day 247

+180, the incidence of aGVHD grades II-IV and III-IV was 20% (13.5%- 248

27.4%) vs 26.9% (23.3%-30.6%) (p=0.12) and 8.9% (4.7%-14.7%) vs 10.4% 249

(8%-13.1%), respectively (p=0.61). Two-year incidence of total and extensive 250

cGVHD was 25.3% (17.7-33.5) vs 20.7% (17.3-24.3) (p=0.27) and 12.5% 251

(7.2-19.4) vs 10.3% (7.9-13.1), respectively (p=0.46) (Table 3). The outcomes 252

of LFS, OS, and GRFS did not differ between the sAML and *de novo* AML 253

groups. Two-year NRM and RI were 38.7% (30-47.3) vs 23.8% (20.3-27.4) 254

(p=0.001) and 28.8% (20.9-37.1) vs 46.3 % (42-50.4) (p=0.001) in *de novo* vs 255

sAML, respectively (Table 3). These differences were not confirmed on MVA. 256

Multivariate analysis 257

In the MVA (Table 4), we did not find any statistical difference in transplantation 258

outcomes between the sAML and *de novo* AML groups. The HRs were 1.38 (0.96- 259

1.98, p=0.083) for NRM, 0.68 (0.47-1, p=0.051) for RI, 0.99 (0.76-1.28, p=0.94) for 260

LFS, 0.99 (0.77-1.29, p=0.97) for OS and 0.99 (0.77-1.27, p=0.94) for GRFS (Table 261

4). Similarly, the risks of aGVHD II-IV HR=0.69 (0.43-1.11, p=0.13), aGVHD III-IV 262

HR=0.93 (0.47-1.85, p=0.84), cGVHD all grades HR=1.39 (0.87-2.22, p=0.17) and 263

extensive cGVHD HR=1.13 (0.6-2.15, p=0. 7) did not differ between the two groups 264

(Table 4). Significant prognostic factors were adverse cytogenetics risk associated 265

with higher risk of RI and lower LFS, OS, and GRFS; older age associated with 266

higher NRM and inferior OS, KPS >90 was a prognostic factor for lower NRM and RI 267

and higher LFS, OS, and GRFS. A peripheral blood graft was associated with a 268

higher risk of grade II-IV and III-IV aGVHD, NRM, and a lower GRFS, and patient 269

CMV seropositivity was associated with a lower OS (Table 4). No difference was 270

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observed in any transplantation outcome between patients with PR vs those with Rel 271

(Table 4). 272

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Cause of death 275

A total of 484 patients died during the study period comprising 91 with sAML and 393 276

with *de novo* AML (Table 5). The original disease was the main cause of death 277

accounting for 40.9% and 59.3% of the deaths, respectively. The second cause of 278

death was infection at 26.1% and 19.6%, followed by GVHD with 9.1% and 9.4% of 279

deaths, respectively (Table 5). Multi-organ failure accounted for 5.7% and 1.8%, and 280

central nervous system toxicity for 4.5% and 0% of deaths, respectively. Second 281

malignancies accounted for 2.3% and 0.8%, and graft failure/rejection for 3.4% and 282

0.8% of the deaths, respectively. Other causes of death were infrequent and included 283

veno-occlusive disease of the liver, cardiac toxicity, hemorrhage, and interstitial 284

pneumonitis, each accounting for less than 1.5% of total deaths with no difference 285

between the patient groups (Table 5). 286

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Discussion 288

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In this study, we have demonstrated similar transplantation outcomes for patients 290

with PR/Rel sAML in comparison to those with *de novo* AML following non-T 291

depleted HaploHSCT with PTCy. Notably, about a quarter of this very high-risk group 292

of sAML patients, with 73.6% being primary refractory, were relapse-free and GVHD- 293

free at 2 years. These results are similar to those published by Brissot *et al.* who 294

compared 199 HaploHSCT to MUD and MMUD in AML patients with active disease 295

(PR/Rel) with a 2-year OS of 29.3%, LFS of 28%, and GRFS of 16.2% (27). Similarly, 296

in a previous study, we assessed transplantation outcomes in 852 AML patients with 297

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active disease by comparing two MAC regimens, observing an OS of 31.2%-33.4% and LFS of 25%-28.4% at 2 years (38). Comparable data on AML patients with active disease have been previously published from the Memorial Sloan Kettering Cancer Center and others, in the non-HaploHSCT setting (39-41). It is with no surprise that the outcome of HaploHSCT in PR/Ref sAML is worse than that achieved in sAML patients in remission. In a previous study, we analyzed transplantation outcomes in 154 sAML (45% in CR, 55% with active disease) patients undergoing non-T-depleted HaploHSCT between 2006 to 2016, and observed a 2-year LFS, OS, and GRFS of 37.1%, 43.3%, and 42.1%, respectively (42). Active disease at the time of transplantation was associated with inferior outcomes, with a 2-year OS of 35.3% compared to 53.2% in patients in CR ($p = 0.02$). Active disease at the time of transplantation was also an unfavorable prognostic factor for LFS with (30.1% vs 45.7%, $p = 0.01$) and GRFS (21.5% vs 38.4%, $p = 0.03$) in those in CR, respectively (42). In a subsequent study that included 246 HaploHSCT (50% with active disease and 50% in CR), 2-year LFS, OS, and GRFS were 32%, 41%, and 23%, respectively (43). Again, there was a correlation between disease status at transplantation and outcome. In the MVA, patients transplanted in CR had significantly better OS, LFS, and GRFS than those transplanted with active disease with HRs of 1.99, p<0.001; 2.17, p<0.001, and 1.97, p<0.001, respectively. Being with refractory or relapsed leukemia at the time of transplantation may also explain the somewhat lower neutrophil recovery of 83.5% - 88.4% we observed, somewhat similar to previous reports in this setting (28,41).

However, none of these studies have focused on comparing outcomes in sAML versus *de novo* AML. Patients with sAML treated with conventional therapy are known to have inferior outcomes with lower remission rates and OS compared to patients with *de novo* AML (1, 2, 9-11). One of the initial questions was therefore whether the same would also be true for patients undergoing transplantation especially as besides the high-risk disease biology (which may lead to higher post-

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transplant RI), sAML patients are typically older, with comorbidities, leading to   326

reduced tolerability to chemotherapy with increased toxicity and side effects (44-45),   327

factors that may result in a higher NRM both of which will translate into inferior   328

outcomes of alloHSCT in sAML (21, 46). Addressing this question, Schmaelter *et al.*   329

compared transplantation outcomes in 11439 patients with *de novo* AML and 1325   330

with sAML (8600 of whom were in CR1) transplanted mostly from sibling and   331

unrelated donors. They observed a higher RI and NRM in sAML versus *de novo* AML   332

patients, which translated to significantly inferior LFS, OS, and GRFS in the sAML   333

patients with HRs of 1.33, 1.32, and 1.2, respectively (19). We subsequently   334

compared outcomes of HaploHSCT with PTCy in 231 sAML patients versus 1480   335

patients with *de novo* AML, both in CR1, and observed no significant difference in   336

any transplantation outcome parameter between the two groups (26), results that are   337

in contrast to the results of Schmaelter *et al.* in a similar cohort of AML patients   338

undergoing alloHSCT from HLA matched rather than haploidentical donors (19).   339

These to some degree unexpected results may be due to a reduction in transplant-   340

related mortality (TRM) which is known to be high in sAML transplants (13,16-   341

17,19,21,46) as the HaploHSCT PTCy platform was previously demonstrated to lead   342

to a remarkable reduction in TRM and GVHD incidence (22-24,47). As for the 2-year   343

incidence of extensive cGVHD of 20-25%, we observed, which may be somewhat   344

higher than previously reported in the HaploHSCT PTCy setting (22-24), it may be   345

due to early withdrawal of immune suppression utilized to prevent relapse in this very   346

high-risk patient population, however being a registry-based study we do not have   347

this information. Of major importance, particularly for transplantation in patients with   348

active leukemia, is the fact that the haploidentical procedure may be associated with   349

an enhanced anti-leukemic effect. A stronger GVL effect was recently demonstrated   350

in a mouse-leukemic model where mismatched cytotoxic T lymphocytes possessed   351

higher cytotoxic activity against the leukemia than their matched counterparts (48).   352

Several clinical studies from China showed faster clearance of post-transplant   353

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measurable residual disease (MRD), reduced post-transplant disease progression, and relapse, and better results in high-risk leukemia patients with positive MRD pre-transplantation, with haploidentical compared to sibling transplantation (29-30,49). Furthermore, PTCy may provide a direct immune-mediated, specific anti-leukemic effect, distinct from GVHD, that is probably mediated by the release of cytokines or other molecules to which leukemic cells may be more sensitive than normal cells (50). Notably, using modern immune profiling and machine learning techniques, unique immune signatures and T-cell subset reconstitutions were recently demonstrated with PTCy, which may allow a potent GVL effect while reducing GVHD (51). Indeed, PTCy was shown to impair the proliferation and cytokine production of alloreactive T-cells but did not completely eradicate them and thus reduce the progression of severe forms of GVHD while maintaining the GVL effect (52). However, from a clinical point of view, the possible stronger GVL effect associated with HaploHSCT may not be translated to a reduced relapse rate due to the HLA-loss phenomenon which is one of the major mechanisms of relapse after HaploHSCT (53-54).

Altogether, the reduced toxicity and potentially stronger anti-leukemic effect associated with HaploHSCT may explain the lack of difference we observed with the Haplo SCTs in patients with PR/Rel sAML versus those with PR/Rel *de novo* AML. The other factors observed to be associated with HaploHSCT outcomes included cytogenetic risk, age, KPS, CMV seropositivity, and peripheral blood grafts and are in agreement with previous publications of allogeneic transplantations including HaploHSCTs in sAML (16, 18, 19, and 26). This study, being a retrospective and registry-based transplantation study, has several limitations including the risk of selection bias and the possibility of unavailable data that could not have been considered, such as frontline therapies as well as the number of bone marrow and PB blasts, mutation profile, molecular, and MRD data.

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In conclusion, in this real-life registry-based retrospective analysis of HaploHSCT for 381
PR/Rel sAML in comparison to HaploHSCT in PR/Rel *de novo* AML, we observed 382
similar transplantation outcomes with HaploHSCT, with about a quarter of the very 383
high-risk group of sAML patients, 73.6% being primary refractory, reaching relapse- 384
free and GVHD-free status at 2 years. Hopefully, with the recent advances in our 385
understanding of the biology of sAML as well as the approval of novel agents 386
including vyxeos (CPX-351) and venetoclax (55-56), it may be possible to further 387
improve PR/Rel sAML outcomes. 388

Supplementary Appendix: Contributing Centers 389

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Marseille, France; Universita Cattolica S. Cuore, Rome, Italy; Azienda Ospedaliero 394
Universitaria di Udine, Udine, Italy; University Hospital Erlangen, Erlangen, Germany; 395
CHU Bordeaux, Hopital Haut-Leveque, Pessac, France; University Hospital 396
Maastricht, Maastricht, Netherlands; CHU Grenoble Alpes - Université Grenoble 397
Alpes, Grenoble, France; Medicana International Hospital Istanbul, Istanbul, Turkey; 398
S.S.C.V.D Trapianto di Cellule Staminali, Torino, Italy; Charles University Hospital, 399
Pilsen, Czech Republic; Hospital Gregorio Marañón, Madrid, Spain; Goethe- 400
Universitaet, Frankfurt Main, Germany; IRCCS, Casa Sollievo della Sofferenza, San 401
Giovanni, Italy; University Hospital Eppendorf, Hamburg, Germany; RM Gorbacheva 402
Research Institute, Pavlov University, Petersburg, Russian Federation; University 403
Hospital | Basel, Basel, Switzerland; Hannover Medical School, Hannover, Germany; 404
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Author's contribution

AN wrote the manuscript, designed the study, and interpreted the data. ML and MM designed the study, performed the statistical analyses, interpreted the data, and edited the manuscript.

JT, AMR, DK, JV, DB, SS, RF, JW, EF, GVG, and FC reviewed the manuscript and provided clinical data. All authors approved the final version of the manuscript.

Competing interests

The authors declare that they have no relevant conflict of interest and no competing financial interests.

Ethics approval and consent to participate

The scientific boards of the ALWP of the EBMT approved this study.

Consent for publication

Not applicable.

Availability of data materials

AN, ML, FC, and MM had full access to all study data (available upon data-specific request).

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Table 1: Patient and transplant characteristics

	de novo AML (n=590)	secAML (n=129)	P	Overall (n=719)
Follow-up (months), median [IQR]	43.48 [37.53-47.99]	45.59 [39.08-57.85]	0.20	43.48 [39.47-47.69]
Patient age (years), median (min-max) [IQR]	55.4 (18-77.8) [42.6-63]	61.3 (21-78.8) [55-67.3]	< 0.0001	57.2 (18-78.8) [44.2-63.8]
Patient sex	339 (57.6%)	84 (65.1%)	0.11	423 (58.9%)
Male	250 (42.4%)	45 (34.9%)		295 (41.1%)
Female	1	0		1
Year transplant, median (min-max)	2017 (2010-2022)	2018 (2010-2022)	0.62	2017 (2010-2022)
Cytogenetic risk group				
Favorable	28 (5.9%)	4 (4%)	0.75	32 (5.6%)
Intermediate	282 (59.4%)	58 (58.6%)		340 (59.2%)
Adverse	165 (34.7%)	37 (37.4%)		202 (35.2%)
Missing	115	30		145
Status at transplant				
Primary refractory	346 (58.6%)	95 (73.6%)	0.002	441 (61.3%)
First Relapse	244 (41.4%)	34 (26.4%)		278 (38.7%)
Donor age (years)				
Median (min-max) [IQR]	38.3 (13-73.9) [29.4-48]	36.9 (16.6-64) [29.1-43.9]	0.21	38.2 (13-73.9) [29.3-47.1]
Missing	20	3		23
Donor sex				
Donor male	365 (62.1%)	81 (62.8%)	0.88	446 (62.2%)
Donor female	223 (37.9%)	48 (37.2%)		271 (37.8%)
Missing	2	0		2
Female to male combination				
No F->M	466 (79.1%)	102 (79.1%)	0.99	568 (79.1%)
F->M	123 (20.9%)	27 (20.9%)		150 (20.9%)
Missing	1	0		1

Conditioning intensity				
MAC	281 (47.8%)	51 (39.8%)	0.10	332 (46.4%)
RIC	307 (52.2%)	77 (60.2%)		384 (53.6%)
Missing	2	1		3
Cell source				
BM	196 (33.2%)	39 (30.2%)	0.51	235 (32.7%)
PB	394 (66.8%)	90 (69.8%)		484 (67.3%)
Karnofsky score				
<90	249 (44.1%)	64 (50.8%)	0.17	313 (45.3%)
>=90	316 (55.9%)	62 (49.2%)		378 (54.7%)
Missing	25	3		28
HCT-CI				
HCT-CI 0-2	422 (78.1%)	74 (59.7%)	< 0.0001	496 (74.7%)
HCT-CI>=3	118 (21.9%)	50 (40.3%)		168 (25.3%)
Missing	50	5		55
Patient CMV				
Pat. CMV neg	143 (24.6%)	26 (20.2%)	0.29	169 (23.8%)
Pat. CMV pos	439 (75.4%)	103 (79.8%)		542 (76.2%)
Missing	8	0		8
Donor CMV				
Don. CMV neg.	233 (40.7%)	60 (46.9%)	0.2	293 (41.8%)
Don. CMV pos	340 (59.3%)	68 (53.1%)		408 (58.2%)
Missing	17	1		18
TBI				
CT	484 (82.2%)	105 (81.4%)	0.83	589 (82%)
TBI	105 (17.8%)	24 (18.6%)		129 (18%)
Missing	1	0		1

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Abbreviations: secAML-secondary acute myeloid leukemia; IQR-interquartile range; min-minimum; max-maximum; F-female; M-male; Pat.-patient, CMV- cytomegalovirus; neg-negative; pos-positive; Don. -donor; BM-bone marrow; PB-peripheral blood; MAC – myeloablative conditioning; RIC – reduced intensity conditioning; TBI – total body irradiation; CT-chemotherapy; BM – bone marrow; HCT-CI - hematopoietic cell transplantation specific comorbidity index

Table 2: Transplantation outcomes: Engraftment and GVHD

	de novo AML (n=590)	secAML (n=129)	P	Overall (n=719)
Engraftment				
Graft failure	66 (11.6%)	21 (16.5%)	0.13	87 (12.5%)
Engrafted	502 (88.4%)	106 (83.5%)		608 (87.5%)
Missing	22	2		24
Cumulative incidence of PMN>500	88.2%[85.2-90.6]	83.3%[75.5-88.9]	0.042	
Acute GVHD				
Grade I	121 (21.3%)	22 (17.5%)	ND	143 (20.6%)
Grade II	89 (15.7%)	13 (10.3%)		102 (14.7%)
Grade III	39 (6.9%)	5 (4%)		44 (6.3%)
Grade IV	23 (4%)	7 (5.6%)		30 (4.3%)
Present, grade unknown	3 (0.5%)	1 (0.8%)		4 (0.6%)
No aGvHD present (Grade 0)	293 (51.6%)	78 (61.9%)		371 (53.5%)
Missing	22	3		25

Abbreviations: secAML-secondary acute myeloid leukemia; PMN- polymorphonuclear neutrophils; GVHD- graft-versus-host disease; a-acute; ND-not done; unless otherwise stated, results expressed as frequency (%). The numbers are the raw percentages for each grade GVHD.

Table 3: Transplantation outcomes: Univariate analysis

	2 years				
	Relapse	NRM	LFS	OS	GRFS
de novo AML	46.3%[42-50.4]	23.8%[20.3-27.4]	30%[26.1-33.9]	33.5%[29.4-37.6]	23.8%[20.3-27.6]
secAML	28.8%[20.9-37.1]	38.7%[30-47.3]	32.5%[24.3-41]	34.4%[25.9-43.1]	25%[17.6-33.1]
P value	*0.001*	*0.001*	*0.58*	*0.35*	*0.49*

	180 days		2 years	
	Acute GVHD II-IV	Acute GVHD III-IV	Chronic GVHD	Ext. chronic GVHD
de novo AML	26.9%[23.3-30.6]	10.4%[8-13.1]	20.7%[17.3-24.3]	10.3%[7.9-13.1]
secAML	20%[13.5-27.4]	8.9%[4.7-14.7]	25.3%[17.7-33.5]	12.5%[7.2-19.4]
P value	*0.12*	*0.61*	*0.27*	*0.46*

Abbreviations: secAML-secondary acute myeloid leukemia; NRM-non-relapse mortality; LFS-leukemia-free survival; OS-overall survival; GVHD-graft- versus-host disease; GRFS-GVHD-free, relapse-free survival; Ext-extensive

Table 4: Transplantation outcomes: Multivariate analysis

	RELAPSE		NRM		LFS	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Secondary AML	0.68 (0.47-1)	0.051	1.38 (0.96-1.98)	0.083	0.99 (0.76-1.28)	0.94

	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Patient age (per 10y)	0.92 (0.84-1.02)	0.11	1.39 (1.19-1.62)	< 0.0001	1.06 (0.98-1.15)	0.13
Adverse risk group	1.95 (1.49-2.55)	< 0.0001	0.97 (0.67-1.39)	0.85	1.47 (1.18-1.82)	5.00E-04
First relapse vs PR	0.98 (0.75-1.29)	0.89	0.94 (0.67-1.33)	0.72	0.95 (0.77-1.18)	0.65
KPS>=90	0.69 (0.53-0.89)	0.005	0.52 (0.37-0.73)	0.0001	0.62 (0.5-0.76)	< 0.0001
HCT-CI>=3	1.03 (0.75-1.42)	0.83	1.21 (0.85-1.73)	0.29	1.12 (0.88-1.42)	0.36
PBSC vs. BM	1.14 (0.86-1.53)	0.36	1.54 (1.04-2.27)	0.032	1.26 (1-1.6)	0.052
RIC vs. MAC	1.03 (0.78-1.36)	0.82	1.25 (0.88-1.79)	0.22	1.14 (0.91-1.41)	0.25
Female donor to male R	0.94 (0.69-1.27)	0.68	0.96 (0.66-1.4)	0.84	0.96 (0.75-1.21)	0.72
Pat. CMV pos	1.17 (0.83-1.63)	0.37	1.39 (0.91-2.15)	0.13	1.24 (0.95-1.61)	0.12
Don. CMV pos	1.25 (0.94-1.67)	0.13	0.89 (0.63-1.24)	0.48	1.09 (0.87-1.35)	0.45
Year of HCT	0.96 (0.92-1.01)	0.15	0.97 (0.91-1.04)	0.4	0.97 (0.93-1.01)	0.15

Table 4- Continued…

	OS		GRFS		acute GVHD grade II-IV	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Secondary AML	0.99 (0.77-1.29)	0.97	0.99 (0.77-1.27)	0.94	0.69 (0.43-1.11)	0.13
Patient age (per 10y)	1.09 (1.01-1.19)	0.034	1.06 (0.98-1.15)	0.12	1.01 (0.89-1.15)	0.87
Adverse risk group	1.42 (1.14-1.77)	0.002	1.29 (1.05-1.59)	0.015	0.9 (0.62-1.31)	0.6

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	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
First relapse vs PR	0.93 (0.75-1.16)	0.53	0.94 (0.76-1.15)	0.52	0.88 (0.62-1.26)	0.49
KPS>=90	0.62 (0.5-0.76)	< 0.0001	0.62 (0.5-0.75)	< 0.0001	1.27 (0.88-1.81)	0.2
HCT-CI>=3	1.13 (0.89-1.44)	0.32	1.06 (0.85-1.34)	0.59	0.84 (0.55-1.27)	0.4
PBSC vs. BM	1.23 (0.97-1.56)	0.086	1.34 (1.06-1.68)	0.013	1.64 (1.07-2.53)	0.024
RIC vs. MAC	1.17 (0.94-1.46)	0.16	1.03 (0.84-1.27)	0.76	1.14 (0.79-1.65)	0.47
Female donor to male R	1 (0.79-1.28)	0.98	1.03 (0.82-1.3)	0.79	0.85 (0.56-1.28)	0.44
Pat. CMV pos	1.33 (1.01-1.75)	0.042	1.17 (0.91-1.51)	0.23	0.9 (0.58-1.38)	0.62
Don. CMV pos	1.05 (0.84-1.31)	0.65	1.07 (0.87-1.32)	0.5	1.45 (0.99-2.13)	0.056
Year of HCT	0.98 (0.94-1.02)	0.42	0.98 (0.94-1.02)	0.29	0.96 (0.9-1.02)	0.19

Table 4- Continued...

	acute GVHD grade III-IV		chronic GVHD		extensive chronic GVHD	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Secondary AML	0.93 (0.47-1.85)	0.84	1.39 (0.87-2.22)	0.17	1.13 (0.6-2.15)	0.7
Patient age (per 10y)	0.98 (0.81-1.2)	0.88	1 (0.87-1.15)	1	1.2 (0.97-1.47)	0.095
Adverse risk group	0.52 (0.27-0.99)	0.045	0.71 (0.45-1.12)	0.14	0.96 (0.54-1.72)	0.9
First relapse vs. PR	0.88 (0.52-1.48)	0.62	0.85 (0.57-1.25)	0.4	1.1 (0.65-1.86)	0.72
KPS>=90	0.93 (0.56-1.56)	0.8	0.75 (0.51-1.09)	0.13	0.71 (0.43-1.18)	0.18

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HCT-CI>=3	0.88 (0.48-1.62)	0.68	1.02 (0.66-1.59)	0.92	1.01 (0.55-1.85)	0.98
PBSC vs. BM	2.49 (1.29-4.79)	0.006	1.43 (0.95-2.15)	0.086	1.68 (0.94-2.99)	0.078
RIC vs. MAC	1.13 (0.67-1.93)	0.64	0.84 (0.57-1.25)	0.4	0.64 (0.37-1.09)	0.1
Female donor to male R	0.75 (0.39-1.45)	0.39	1.46 (0.98-2.2)	0.066	1.67 (0.96-2.92)	0.07
Pat. CMV pos	0.98 (0.52-1.84)	0.94	0.71 (0.46-1.1)	0.13	0.63 (0.36-1.11)	0.11
Don. CMV pos	1 (0.57-1.74)	1	1.22 (0.81-1.81)	0.34	0.83 (0.48-1.42)	0.49
Year of HCT	0.98 (0.89-1.07)	0.63	0.99 (0.92-1.06)	0.73	0.98 (0.89-1.09)	0.71

Abbreviations: AML- acute myeloid leukemia; HR-hazard ratio; CI-confidence interval; y-year; pos-positive; neg-negative; R-recipient; PR-primary refractory; MAC – myeloablative conditioning; RIC – reduced intensity conditioning; GVHD- graft versus host disease; a-acute: NRM – non-relapse mortality; LFS – leukemia-free survival; OS - overall survival; GRFS - GVHD-free and relapse-free survival; HCT-hematopoietic cell transplantation; CMV- cytomegalovirus; neg-negative; pos-positive; Don.-donor; Pat.-patient ;BM-bone marrow; PBSC-peripheral blood stem cells; BM – bone marrow; HCT-CI - hematopoietic cell transplantation specific comorbidity index; KPS-Karnofsky performance status

Table 5: Cause of Death

	de novo AML (n=393)	secAML (n=91)	Overall (n=484)
Original disease	227 (59.3%)	36 (40.9%)	263 (55.8%)
Infection	75 (19.6%)	23 (26.1%)	98 (20.8%)
GVHD	36 (9.4%)	8 (9.1%)	44 (9.3%)
Non HSCT-related	14 (3.7%)	3 (3.4%)	17 (3.6%)
Other transp-related	8 (2.1%)	1 (1.1%)	9 (1.9%)
MOF	7 (1.8%)	5 (5.7%)	12 (2.5%)
Hemorrhage	4 (1%)	1 (1.1%)	5 (1.1%)
Failure/Rejection	3 (0.8%)	3 (3.4%)	6 (1.3%)
IP	3 (0.8%)	1 (1.1%)	4 (0.8%)
Other second malignancy	3 (0.8%)	2 (2.3%)	5 (1.1%)
VOD	2 (0.5%)	1 (1.1%)	3 (0.6%)
Cardiac toxicity	1 (0.3%)	0 (0%)	1 (0.2%)
CNS toxicity	0 (0%)	4 (4.5%)	4 (0.8%)
Missing	10	3	13

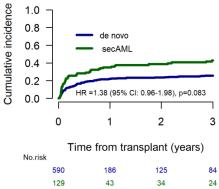Abbreviations: secAML-secondary acute myeloid leukemia; HSCT- hematopoietic stem cell transplantation; VOD-veno occlusive disease of the liver, GVHD-graft-versus-host disease; MOF-multiorgan failure; CNS-central nervous system; transp-transplantation; IP-interstitial pneumonitis
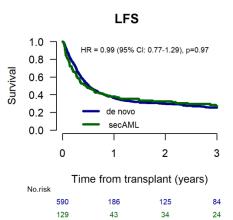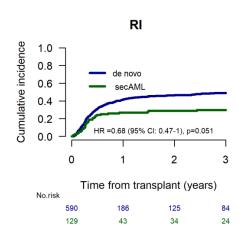
Figure Legend.

Figure 1

Outcomes of haploidentical transplantation with post-transplant cyclophosphamide in first relapse/primary refractory secondary AML *versus* first relapse/primary refractory *de novo* AML: non-relapse mortality (NRM), relapse incidence (RI), leukemia-free survival (LFS), overall survival (OS), acute graft-versus- host disease (GVHD) II-IV, chronic GVHD, and GVHD-free, relapse-free survival (GRFS).
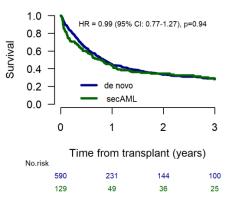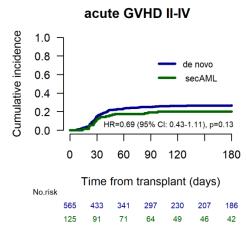
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Figure 1