**Hematopoietic stem cell transplantation for isolated extramedullary relapse of acute lymphoblastic leukemia in children**

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# Hematopoietic stem cell transplantation for isolated extramedullary relapse of acute lymphoblastic leukemia in children

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**Conflict of interest:** The authors have no conflict of interest to disclose.

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**Running title:** Transplantation for acute leukemia extramedullary relapse

41 **Abstract**

42 Relapse of acute lymphoblastic leukemia (ALL) may occur in extramedullary sites, mainly central
43 nervous system (CNS) and testis. Optimal post-remissional treatment for isolated extramedullary
44 relapse (IEMR) is still controversial. We collected data of children treated with hematopoietic stem
45 cell transplantation (HSCT) for ALL IEMR from 1990 to 2015 in Italy. Among 281 patients, 167 had
46 a relapse confined to CNS, 73 to testis, 14 to mediastinum, 27 to other organs. Ninety-seven patients
47 underwent autologous HSCT, 79 received allogeneic HSCT from a matched family donor, 75 from a
48 matched unrelated donor and 30 from an HLA-haploidentical donor. The 10-year overall survival was
49 56% and was not influenced by gender, ALL blast immune-phenotype, age, site of relapse, duration
50 of first remission and type of HSCT. In multivariable analysis, the only prognostic factors were
51 disease status at HSCT and year of transplantation. Patients transplanted in third or subsequent
52 complete remission (CR) had a risk of death 2.3 times greater than those in CR2. Children treated
53 after 2000 had half the risk of death than those treated before that year.
54 Our results suggest that both autologous and allogeneic HSCT may be considered for treatment of
55 pediatric ALL IEMR after the achievement of CR2.

56 **Keywords**

57 Acute Lymphoblastic Leukemia, Extramedullary relapse, Hematopoietic stem cell transplantation

## Introduction

Although current treatment protocols cure up to 85% of children affected by acute lymphoblastic leukemia (ALL), relapse is still the leading cause of treatment failure, affecting approximately 15-20% of patients. Leukemia relapse may occur in extramedullary sites, mainly central nervous system (CNS) and testis, either alone or in combination with bone marrow (BM) relapse [1].

Site of relapse and duration of first remission are the most important prognostic factors in relapsed ALL, early and isolated BM relapse predicting the worst outcome [2, 3]. While the benefit of allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been demonstrated for high-risk relapsed patients, optimal post-remissional treatment for low-risk patients is still controversial [4-8]. Our previous studies [9, 10] demonstrated that autologous HSCT (auto-HSCT) may be a good curative option for children experiencing isolated extramedullary relapse (IEMR). The observation that the immune-surveillance exerted by the allograft against leukemia (graft-versus leukemia, GVL, effect) is more effective in preventing BM relapse than IEMR [11], led us to hypothesize that the agents used in the conditioning regimen (including total body irradiation, TBI) may be sufficient for disease control in patients with IEMR. This approach may reduce the toxicity associated with allo-HSCT and largely related to graft-versus-host disease (GVHD) occurrence.

Therefore, to further address the role of auto- and allo-HSCT in patients experiencing IEMR, we analyzed data of a large cohort of children with first or subsequent ALL IEMR treated with HSCT over a 25-year period in Italy. To the best of our knowledge, this is the largest study that uniformly analyzes the outcome of this subgroup of patients.

## Patients and Methods

This is a retrospective multicenter study involving 20 Italian centers affiliated to the Italian Pediatric Onco-Hematology Association (AIEOP) network.

Data were extracted from the AIEOP-Stem Cell Transplantation (AIEOP-SCT) Registry. We included children (age 1-18 years) with ALL IEMR who underwent HSCT between 1st of January 1990 and 31st of December 2015. Follow up was updated on January 30th, 2018.
Written informed consent was obtained from parents or legal guardians.

IEMR was defined as the presence of lymphoblasts in extramedullary sites with less than 5% blasts in BM. CNS relapse was defined as the presence of >5 cell/μL in the cerebrospinal fluid (CSF) and detection of lymphoblasts by CSF cytomorphology, or alternatively, by clinical or radiological signs. Relapse involving testis or other organs was confirmed by biopsy.

"Very early" relapse was defined when disease recurred less than 18 months from primary diagnosis, "early" when disease recurred later than 18 months from diagnosis and less than 6 months from treatment discontinuation, and "late" when disease recurred more than 6 months from treatment discontinuation [3].

At diagnosis and relapse, patients were treated according to the national protocols available at that time, mainly based on Berlin-Frankfurt-Münster (BFM) Study Group backbone.

HSCT was performed in patients with second or subsequent complete remission (CR), or, in a limited number of cases, with active disease. If an HLA-matched family donor (MFD) was available, allo-HSCT was performed. If not, auto-HSCT, HSCT from a matched unrelated donor (MUD) or haploidentical (haplo-HSCT) were considered. This decision was taken by the single center.

**Statistical analysis**

Overall Survival (OS) was defined as the time from transplantation to either last follow-up or death due to any cause, whereas disease-free survival (DFS) as the time elapsing from transplantation to either last follow-up or disease recurrence or death due to any cause, whichever occurred first. Relapse-free survival (RFS) was defined as the time from transplantation to documented relapse of ALL. Cumulative incidence (CI) of treatment-related mortality (TRM) was defined as the time from

transplantation to death from causes other than disease recurrence/progression, considering relapse as the competing event.

OS, DFS, and RFS were calculated at 10 years using the Kaplan-Meier method; difference in survival between groups was estimated through the log-rank test.

Cumulative incidence of TRM was evaluated at 100 days, 6 months, 1 year and 10 years after transplantation. Incidence curves were compared using the Gray's test. In multivariable Cox regression analysis, all factors with a p-value <0.2 in univariable analysis were included. The risk of death was expressed as the hazard ratio (HR) with 95% confidence interval. Differences in the distribution of various parameters were compared using Chi-square or Fisher exact test as appropriate. A p-value <0.05 was considered statistically significant.

Analysis was performed with SAS software (SASPC, version 9.3, SAS Institute, Cary, NC).

## Results

### Patient characteristics

Two hundred and ninety-two children with IEMR of ALL underwent HSCT from 1990 to 2015 in Italy. Patients included in the study were 281; 11 children were excluded because of insufficient data. Patients' characteristics are detailed in Table 1, while conditioning regimens are listed in Table 2. Mean follow-up from transplantation was 6.9 years (median 4.4 years, range 0.03-25.8 years).

### Outcome

Eighty-three out of 281 patients (29.5%) experienced a second relapse or disease progression at a median time of 176 days (range 15-2345) from HSCT: 49 patients had an isolated BM recurrence, 16 an IEMR and 7 children experienced a combined relapse. The site of recurrence was unknown in 11 patients. One hundred and eighteen patients (42.0%) died at a median time of 219 days (range 12-6623) from HSCT: 63 from relapse, 46 from treatment-related complications (14/46 were in relapse), 4 from a second tumor, 5 from an unknown event. Grade II-IV acute GVHD (aGVHD) occurred in 79 of 184 patients (42.9%) who received an allograft, while chronic GVHD (cGVHD) was diagnosed in 32 out of 151 patients (21.2%) alive at day +100 after allo-HSCT.

### Overall survival

The OS for the entire cohort was 56%±3% at 10 years; it was not influenced by gender, ALL blast immune-phenotype (B-cell precursor [Bcp]-ALL *vs* T-ALL), age (≤10 years *vs* >10 years), site of relapse, source of stem cells, use of TBI during the conditioning regimen and length of first CR (10-year OS for very early, early and late IEMR was 52%±6%, 53%±5%, and 61%±6% respectively, p=0.39). No statistically significant difference was also observed if different type of HSCT were compared: OS for auto-HSCT, MFD, MUD and haplo-HSCT was 57%±5%, 56%±6%, 62%±6% and 46%±10%, respectively, p=0.09 (Figure 1).

In univariable analysis, the prognostic factors associated with OS were: remission status at transplantation and the year in which patients were treated. Patients transplanted in CR2 had a better OS at 10 years (64%±4%), in comparison to both those transplanted in subsequent CR (CR>2) who showed an OS of 44%±7% and patients transplanted with active disease who had an OS of 11%±7% ($p<0.0001$) (Figure 2). For patients given HSCT before 2000, the 10-year OS was 45%±5%, while that of children transplanted after 2000 was 63%±4% ($p=0.0009$).

Disease-free survival

The 10-year DFS for the whole cohort was 54%±3%. Like OS, DFS did not differ in relation to gender, ALL blast immune-phenotype, age, duration of first CR, type of HSCT or stem cell source. As for site of relapse, DFS was slightly better for patients with isolated testicular relapse (65%±6%) compared to CNS relapse (49%±4%), CNS relapse together with other sites (55%±15%), mediastinal relapse (40%±14%) and other sites involvement (65%±13%), but this difference was not statistically significant ($p=0.22$).

Factors influencing DFS were: presence of TBI in the conditioning regimen, remission status at HSCT and year of transplantation. TBI-containing regimens were associated with a better DFS at 10 years compared to non-TBI containing regimens (58%±4% *vs* 37%±8%, $p=0.008$). Remission status at HSCT strongly correlated with DFS: patients transplanted in CR2 had a better 10-year DFS (63%±4%) in comparison to those transplanted in CR>2 (39%±7%) or not in remission (11%±7%) ($p<0.0001$). DFS for patients transplanted either before or after 2000 was 45%±5% and 61%±4%, respectively ($p=0.0008$).

Transplant-Related Mortality

TRM for the entire cohort was 10%±2% at 100 days, 11%±2% both at 6 months and 1 year and 16%±2% at 10 years. TRM for auto-HSCT was 4%±2%, 6%±2%, 6%±2%, and 11%±3%, while TRM for allo-HSCT (MUD, MFD and haplo-HSCT) was 13%±2%, 14%±3%, 14%±3%, and 18%±3% at

100 days, 6 months, 1 year and 10 years, respectively. Comparison resulted not statistically significant (p=0.08).

No statistical significant difference was observed if TRM of patients transplanted before 2000 was compared to that of patients transplanted after 2000 (p=0.33). In detail, TRM of patients transplanted before 2000 was 15%±3%, 16%±4%, 17%±3%, and 17%±4% at 100 days, 6 months, 1 year and 10 years, respectively. TRM of patients transplanted after 2000 was 6%±2%, 8%±2%, 8%±2%, and 15%±3% at 100 days, 6 months, 1 year and 10 years, respectively.

Subgroup analysis and multivariable analysis

As length of first CR is one of the most important prognostic factors in relapsed ALL, we performed separate analyses for patients with very early, early and late IEMR. Regarding patients experiencing very early relapse (n=87), DFS and OS at 10 years showed a trend in favor of allogeneic HSCT (MFD, MUD and haplo combined) *versus* autologous HSCT (58%±6% *vs* 44%±12% and 59%±6% *vs* 44%±12%, p=0.28 and 0.29 respectively) (Figure 3A). In early relapsed patients (n=97), DFS and OS at 10 years were comparable irrespectively whether patients were treated with either allo- or auto-HSCT (50±7% *vs* 55%±9%, p=0.88 and 52%±7% *vs* 54%±9%, p=0.87) (Figure 3B). In late relapses (n=87), DFS and OS at 10 years were slightly better with auto-HSCT than with allo-HSCT: 65%±8% *vs* 48%±9% and 68%±7% *vs* 52%±9%, respectively (Figure 3C). However, the difference was not statistically significant (p=0.13 and p=0.12).

Remission status at transplantation is well known to influence outcome; thus, we conducted a separate analysis for patients in CR2 at time of HSCT (n=204). RFS and OS for this cohort were 74%±3% and 64%±3%, respectively; outcome of patients given either autologous or allogeneic HSCT was similar. Ten-year RFS of patients transplanted in CR2 after year 2000 was better as compared to that of patients transplanted before 2000 (79%±4% *vs* 64%±6%, respectively, p=0.009).

Since TBI is regarded as the standard regimen conditioning in ALL, we analyzed separately the group of patients who received TBI: 10 year-DFS did not differ regarding the type of transplant (auto *vs* allo: 61%±5% *vs* 58%±4%, p=0.67).

A separate analysis on patients transplanted in more recent years (from 2000 to 2015) was also performed. Results confirmed what we observed analyzing the whole cohort of patients: 10-year OS and DFS were not influenced by site of relapse, presence of TBI, time of relapse and type of HSCT. Ten-year OS for auto, MFD, MUD and haplo-HSCT were 71%±7%, 63%±9%, 66%±6% and 46%±13% (p=0.18). Remission status at transplantation was, again, the only variable influencing outcome: OS was 71%±4% for patients in CR2, 46%±9% for those in CR>2 (p<0.0001); DFS was 69%±4% and 45%±9%(p<0.0001), respectively.

For patients treated with allo-HSCT, occurrence of aGVHD was associated with a better DFS (74%±6% *vs* 48%±7%, p=0.0008) and a better OS (63%±5% *vs* 46%±7%, p=0.028). Considering only patients given an allograft in CR, occurrence of aGVHD conferred a better RFS: 76%±5% *vs* 58%±6%, p=0.009. Bone marrow RFS was 87%±4% for patients who did experience aGVHD versus 74%±6% for those who did not (p=0.02); conversely, extra-medullary RFS was not affected by aGVHD occurrence (90%±4% vs 89%±5%, p=0.79). Presence of cGVHD did not influence patients' outcome (data not shown).

Multivariable analysis was conducted after adjustment for remission status: patients with active disease at transplantation were excluded due to the high incidence of treatment failure in this group. As shown in Table 3, in multivariable analysis the only factors influencing OS in patients with IEMR treated with HSCT were number of relapses and year of transplantation.

## Discussion

Although the vast majority of children affected by ALL are cured with current protocols, relapses still occur and pose remarkable challenges to pediatric hematologists. Allo-HSCT is currently used to treat patients in CR2 with high-risk features (very early/early and isolated BM relapse, recurrence of T-lineage ALL [1,12]), and it is now considered the standard of care also for low-risk patients who present minimal residual disease (MRD) positivity at the end of induction therapy [13,14]. Treatment of extramedullary relapse is less well established. The absence of BM involvement is traditionally considered a favorable prognostic feature [15], and patients with isolated CNS (ICNS) relapse are treated with intensive systemic and intrathecal chemotherapy (CT), followed by either cranio-spinal or cranial radiotherapy (RT) [5, 7, 16, 17]. EFS with this approach ranges from 45% [3, 7, 18, 19] to 70% [16]. Despite the high cure rate obtained in two Children Oncology Group trials [16, 20], with global 5-year EFS approaching 70%, for particular subgroups of IEMR prognosis is still dismal. Patients experiencing very early and early IEMR or ICNS relapses have a survival probability of only 20-30% in most studies [3, 7, 18, 19]. HSCT has been used for treatment of IEMR, but published data are conflicting and limited to small numbers of patients [6, 21-23]. Our previous work [9] showed that EFS of children with early IEMR treated with auto-HSCT was clearly superior to that of patients who received CT/RT (56% *vs* 12%). Moreover, in another report, we demonstrated that auto-HSCT offers a better chance of cure patients in CR2 than in subsequent CR [10]. More recent papers reported comparable outcome in patients with ICNS relapse in CR2 treated with allo-HSCT or CT/RT [2, 5, 7].

In this study, we present the largest cohort of patients with morphologically defined IEMR of ALL and the largest number of HSCT ever performed for this indication, with a long follow-up (up to 26 years from HSCT). In our cohort, 10-year OS and DFS were around 60% with eiher autologous, MFD and MUD-HSCT. Even if a control group of patients treated with CT/RT was not included in this study, our results are comparable with the literature, as reported OS with CT/RT is 45-70% [3, 5, 7, 16-18,

20]. Moreover, if only patients transplanted in CR2 were considered, as in other published series, the 10-year OS of 64% is in line with the most favorable reports [16, 20].

Interestingly, in our study, the use of HSCT seems to abrogate the impact of some “classical” prognostic factors, like site of relapse, duration of first remission and ALL blast immune-phenotype. Similar results were found in the whole cohort, as well as in the group of patients treated in more recent years (from 2000 to 2015). The only factors influencing outcome resulted to be year of HSCT and remission status at transplantation. Taking into account the prognostic impact of year of transplantation, this may reflect improvement in patient selection: the 10-year RFS for patients transplanted before 2000 was better than that of patients transplanted after 2000; on the contrary, 10-year TRM pre and post-2000 did not differ. There is the possibility that MRD assessment during therapy guided decisions on CT administration, time and type of HSCT in single centers. As far as the prognostic significance of remission status before HSCT is concerned, these data confirm what we reported previously [10], namely that outcome is significantly better for children transplanted in CR2 than in subsequent remission. This observation emphasizes the importance of identifying those patients at higher risk of further relapse, who, thus, may benefit of HSCT soon after the achievement of CR2. Very early and early IEMR or ICNS relapses treated with CT/RT have been previously shown to have a survival probability around 20-30% [3, 7, 18, 19]. The use of HSCT in our study improved the OS to 53% for early relapses and 52% for very early relapses. This result is even more relevant considering that patients with third or subsequent CR and even with active disease at time of HSCT were included in this analysis. Based on these data, we suggest considering HSCT for patients with very early/early IEMR once that the CR is achieved.

Furthermore, no difference in outcome was observed regarding the type of HSCT. This finding is in line with our previous study, where we reported that auto-HSCT had the same chance to cure children with ICNS relapse than MFD-HSCT [9]. Present data strengthen this observation, including MUD-HSCT and (although with a low number of cases) haplo-HSCT. The favorable results obtained with either auto and allo-HSCT may be due to the large use of TBI in the conditioning regimen.

Moreover, based on published reports [11, 24, 25], we speculate that the GVL effect of allo-HSCT may be less relevant in extramedullary site, as migration/homing of donor T cells may be impaired at extramedullary sites. In this regard, it was reported that donor cells are absent in extra-medullary sites of patients who relapsed after HSCT [26,27]. Furthermore, donor lymphocytes infusion and recent chimeric antigen receptor T cells have been reported to be less effective in extramedullary disease control [28-30]. In line with these observations and with a previous report [11], in our allo-HSCT cohort, occurrence of aGVHD decreased the incidence of subsequent BM relapses but no of subsequent IEMR. Therefore, we can hypothesize that, in the group of patients with IEMR of ALL, those with higher risk of subsequent BM relapse (i.e., children with positive BM MRD) may benefit more from allo-HSCT, while patients with pure IEMR relapse (i.e., negative BM MRD) could be offered auto-HSCT.

This study shows that both auto- and allo-HSCT are effective treatments for IEMR of ALL, to be considered as soon as CR2 is achieved. Patients with late IEMR, currently treated with CT/RT, may benefit from auto-HSCT also in terms of shorter treatment duration, resulting into better quality of life for patients and their families. Current Italian strategy to treat children with very early and early IEMR recommend allo-HSCT [13]. This study shows that auto-HSCT may be a good alternative, significantly reducing the time patients wait before transplantation and the risk of both GVHD and infection-related mortality/morbidity associated with allo-HSCT. The role of auto-HSCT for patients with late IEMR or very early/early IEM with negative BM MRD remains to be assessed in future trials.

The retrospective nature of this study and the absence of data regarding BM MRD before transplantation represent significant limitations of our study; however, the large number of patients with IEMR and the long follow-up strengthen our results. The role of auto- and allo-HSCT in the treatment of IEMR of pediatric ALL should be further explored in prospective studies including MRD assessment for stratifying patients.

## Conflict of interest

The authors have no conflict of interest to declare.

## Acknowledgments

We are grateful to Dr Veronica Tintori (Bone Marrow Transplant Unit, Azienda Ospedaliero Universitaria Meyer, Firenze, Italy) and to Dr Gabriella Casazza (U.O. Oncoematologia Pediatrica, Azienda Ospedaliero Universitaria di Pisa, Italy) for providing patients' data.

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| Number of pts (%) | AUTO-HSCT (n=97) | MFD-HSCT (n=79) | MUD-HSCT (n=75) | Haplo-HSCT (n=30) | total (n=281) | p value |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | 0.83 |
| Male | 67 (69.1%) | 58 (73.4%) | 55 (73.3%) | 23 (76.7%) | 203 (72.3%) | |
| Female | 30 (30.9%) | 21 (26.6%) | 20 (26.7%) | 7 (23.3%) | 78 (27.7%) | |
| **Median age at relapse, years (range)** | 4.9 (0.3-15.2) | 5.6 (1.0-17.8) | 5.3 (0.4-18.0) | 5.8 (1.5-11.5) | | 0.55 |
| **Blast immune-phenotype #** | | | | | | 0.003* |
| Bcp | 82 (84.5%) | 59 (74.7%) | 55 (73.3%) | 15 (50.0%) | 211 (75.1%) | |
| T | 7 (7.2%) | 10 (12.6%) | 15 (20.0%) | 9 (30.0%) | 41 (14.6%) | |
| Other | 2 (2.1%) | 1 (1.3%) | 0 | 0 | 3 (1.1%) | |
| Not known | 6 (6.2%) | 9 (11.4%) | 5 (6.7%) | 6 (20.0%) | 26 (9.2%) | |
| **Site of relapse #** | | | | | | 0.23 |
| CNS | 57 (58.8%) | 51 (64.5%) | 44 (58.7%) | 15 (50.0%) | 167 (59.4%) | |
| Testis | 34 (35.0%) | 17 (21.5%) | 14 (18.7%) | 8 (26.7%) | 73 (26.0%) | |
| Mediastinum | 1 (1.0%) | 2 (2.6%) | 8 (10.7%) | 3 (10.0%) | 14 (5.0%) | |
| CNS+other | 2 (2.1%) | 3 (3.8%) | 5 (6.6%) | 1 (3.3%) | 11 (3.9%) | |
| *CNS+ cerebral parenchima* | *1* | *0* | *2* | *1* | *4* | |
| *CNS+testis* | *0* | *2* | *1* | *0* | *3* | |
| *CNS+mediastinum* | *0* | *0* | *2* | *0* | *2* | |
| *CNS+eye* | *1* | *1* | *0* | *0* | *2* | |
| Other | 3 (3.1%) | 6 (7.6%) | 4 (5.3%) | 3 (10.0%) | 16 (5.7%) | |
| *Eye* | *0* | *3* | *0* | *1* | 4 | |
| *Lymph-nodes* | *1* | *1* | *0* | *1* | 3 | |
| *Other sites (liver, ovary, kidney, skin..)* | *2* | *2* | *4* | *1* | 9 | |
| **Time to relapse #** | | | | | | 0.004* |
| Very early | 16 (16.5%) | 27 (34.2%) | 33 (44.0%) | 11 (36.7%) | 87 (31.0%) | |
| Early | 33 (34.0%) | 28 (35.4%) | 26 (34.7%) | 10 (33.3%) | 97 (34.5%) | |
| Late | 42 (43.3%) | 21 (26.6%) | 16 (21.3%) | 8 (26.7%) | 87 (31.0%) | |
| not known | 6 (6.2%) | 3 (3.8%) | 0 | 1 (3.3%) | 10 (3.5%) | |
| **Remission status at HSCT** | | | | | | 0.003* |
| CR2 | 78 (80.4%) | 58 (73.4%) | 56 (74.7%) | 12 (40.0%) | 204 (72.6%) | |
| CR>2 | 13 (13.4%) | 16 (20.3%) | 15 (20.0%) | 15 (50.0%) | 59 (21.0%) | |
| Active disease | 6 (6.2%) | 5 (6.3%) | 4 (5.3%) | 3 (10.0%) | 18 (6.4%) | |
| **TBI-based conditioning #** | | | | | | 0.056 |
| Yes | 82 (84.5%) | 71 (89.9%) | 55 (73.3%) | 27 (90.0%) | 235 (83.6%) | |
| No | 14 (14.5%) | 7 (8.9%) | 18 (24.0%) | 3 (10.0%) | 42 (15.0%) | |
| not known | 1 (1.0%) | 1 (1.2%) | 2 (2.7%) | 0 | 4 (1.4%) | |
| **Stem cell source #** | | | | | | <0.0001* |
| BM | 60 (61.9%) | 71 (89.9%) | 52 (69.4%) | 7 (23.3%) | 190 (67.6%) | |
| CB | 0 | 2 (2.5%) | 17 (22.6%) | 1 (3.3%) | 20 (7.1%) | |
| PBSC | 36 (37.1%) | 3 (3.8%) | 6 (8.0%) | 22 (73.4%) | 67 (23.9%) | |
| BM+other | 1 (1.0%) | 3 (3.8%) | 0 | 0 | 1 (0.4%) | |
| **Year of HSCT** | | | | | | <0.0001* |
| 1990-2000 | 57 (58.8%) | 37 (46.8%) | 7 (9.3%) | 6 (20%) | 107 (38.1%) | |
| 2000-2015 | 40 (41.2%) | 42 (53.2%) | 68 (90.7%) | 24 (80%) | 174 (61.9%) | |

**Table 1:** Characteristics of 281 children who underwent HSCT for isolated extramedullary relapse of ALL from 1990 to 2015 in Italy.

* statistically significant ($p<0.05$). # analysis of significance was performed among most representative groups: immune-phenotype (T *vs* Bcp), site of relapse (CNS *vs* testis), time to relapse (very early *vs* early and late), TBI-based conditioning (Yes *vs* No), stem cell source (BM *vs* CB *vs* PBSC).

Abbreviations: auto autologous, Bcp B cell precursor, BM bone marrow, CB cord blood, CNS central nervous system, CR complete remission, haplo haploidentical, HSCT hematopoietic stem cell transplantation, MFD

matched family donor, MUD matched unrelated donor, n number, PB peripheral blood, PBSC peripheral blood stem cells, pts patients, TBI total body irradiation.

| Conditioning Regimen | number of patients (%) |
|---|---|
| **Cyclo+Thiotepa+ TBI** | 52 (18.5%) |
| **Ara-c+TBI** | 44 (15.7%) |
| **Thiotepa+Cyclo+ATG+TBI** | 24 (8.5%) |
| **Etoposide+TBI** | 18 (6.4%) |
| **Vincristine+Cyclo+TBI** | 18 (6.4%) |
| **Etoposide+Cyclo+TBI** | 14 (5.0%) |
| **Thiotepa+Fludara+TBI** | 13 (4.6%) |
| **Cyclo+TBI** | 10 (3.6%) |
| **Thiotepa+L-Pam+TBI** | 10 (3.6%) |
| **others+TBI** | 36 (12.8%) |
| **NON TBI** | 42 (14.9%) |
| **Bus+Thiotepa+Cyclo** | 10 |
| **Bus+Cyclo** | 5 |
| **Bus+Thiotepa+Fludara** | 4 |
| **Other** | 23 |

**Table 2**: Conditioning regimens.

Abbreviations: Ara-C Cytarabine, ATG anti-thymocyte globulin, Bus Busulphan, Cyclo Cyclophosphamide, Fludara Fludarabine, L-Pam Melphalan, TBI total body irradiation

| Characteristics | Categories | Pts n | Events | 10-yr OS % (SE%) | Univariable p-value | Multivariable p-value | Hazard Ratio (95% CI) |
|---|---|---|---|---|---|---|---|
| **Age** | ≤ 10 yrs | 215 | 82 | 59 (4) | 0.50 | - | |
| | > 10 yrs | 48 | 16 | 66 (7) | | | |
| **Gender** | Female | 73 | 25 | 60 (6) | 0.58 | - | |
| | Male | 190 | 73 | 60 (4) | | | |
| **Blast Immune-phenotype** | Bcp | 203 | 76 | 61 (4) | 0.42 | - | |
| | T | 33 | 9 | 68 (9) | | | |
| **Relapse site** | Testis | 72 | 24 | 67 (6) | 0.23 | 0.56 | |
| | CNS | 154 | 60 | 58 (4) | | | |
| **TBI in conditioning regimen** | No | 36 | 17 | 43 (10) | 0.21 | 0.86 | |
| | Yes | 224 | 80 | 60 (4) | | | |
| **Year of HSCT** | Before 2000 | 94 | 49 | 51 (5) | 0.0064 | **0.0035** | **0.5 (0.3-0.8)** |
| | After 2000 | 169 | 49 | 65 (4) | | | |
| **HSCT type** | Autologous | 91 | 36 | 62 (5) | 0.63 | - | |
| | Allogeneic | 172 | 62 | 60 (4) | | | |
| **Status at HSCT** | CR2 | 204 | 65 | 65 (4) | <0.0001 | **0.0005** | **2.3 (1.4-30.7)** |
| | CR>2 | 59 | 33 | 44 (7) | | | |

**Table 3**: Multivariable analysis of factors influencing outcome in children with isolated extramedullary relapse of ALL.

Abbreviations: Bcp B cell precursor, CI Confidence Interval, CNS central nervous system, CR complete remission, HSCT hematopoietic stem cell transplantation, n number, OS overall survival, Pts patients, TBI total body irradiation, yrs years.

**Figure legends**

**Figure 1**: Overall survival of patients transplanted for extramedullary relapse of ALL according to the type of HSCT employed.

Abbreviations: ALL acute lymphoblastic leukemia, auto autologous hematopoietic stem cell transplantation, haplo HLA-haploidentical donor, HSCT hematopoietic stem cell transplantation, MFD matched family donor, MUD matched unrelated donor.

**Figure 2:** Overall survival of patients transplanted for extramedullary relapse of ALL: stratification per remission status at HSCT

Abbreviations: ALL acute lymphoblastic leukemia, CR complete remission, HSCT hematopoietic stem cell transplantation.

**Figure 3**: Overall survival of patients with very early (A), early (B) and late (C) isolated extramedullary ALL relapse: auto-HSCT *versus* allo-HSCT (MFD, MUD and haplo-HSCT combined).

Abbreviations: ALL acute lymphoblastic leukemia, allo-HSCT allogeneic hematopoietic stem cell transplantation, auto-HSCT autologous hematopoietic stem cell transplantation, haplo HLA-haploidentical donor, MFD matched family donor, MUD matched unrelated donor.

**Figure 1**

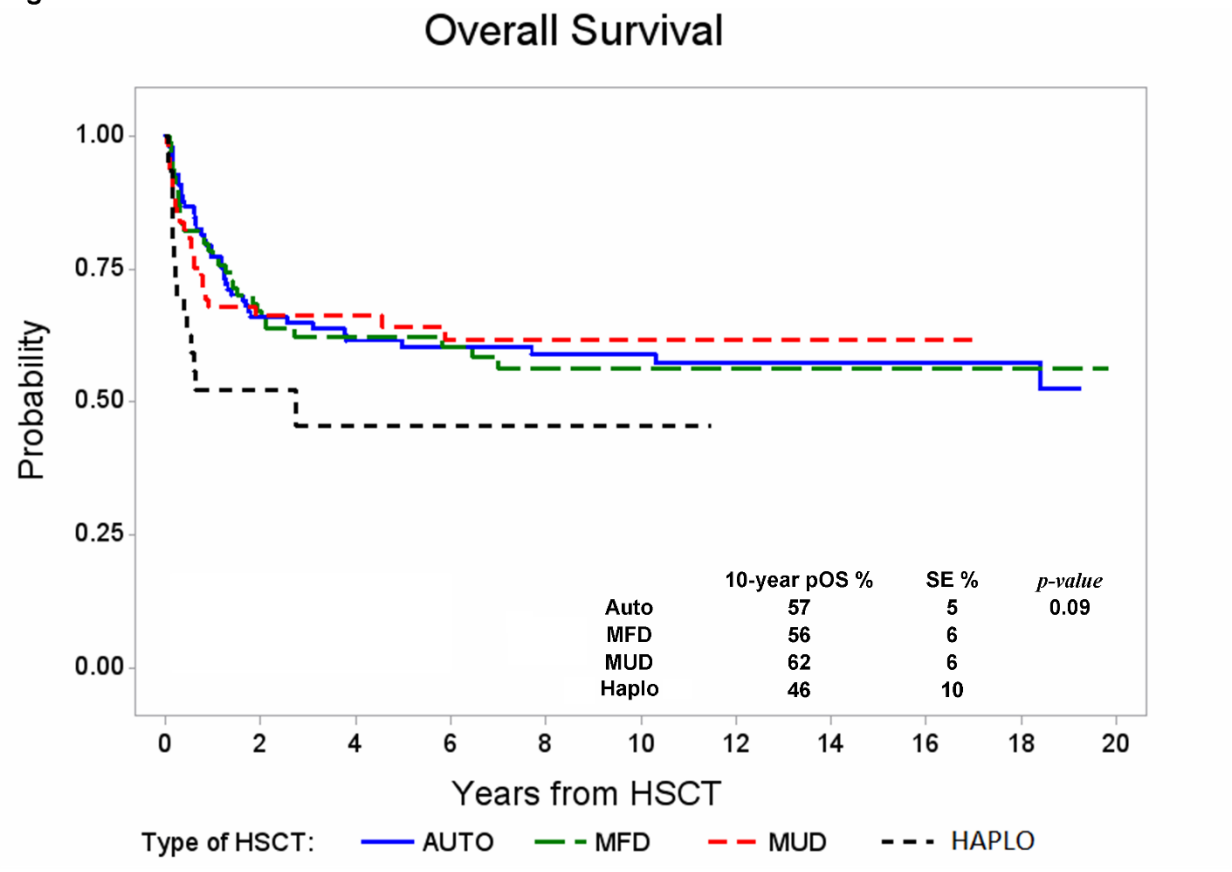
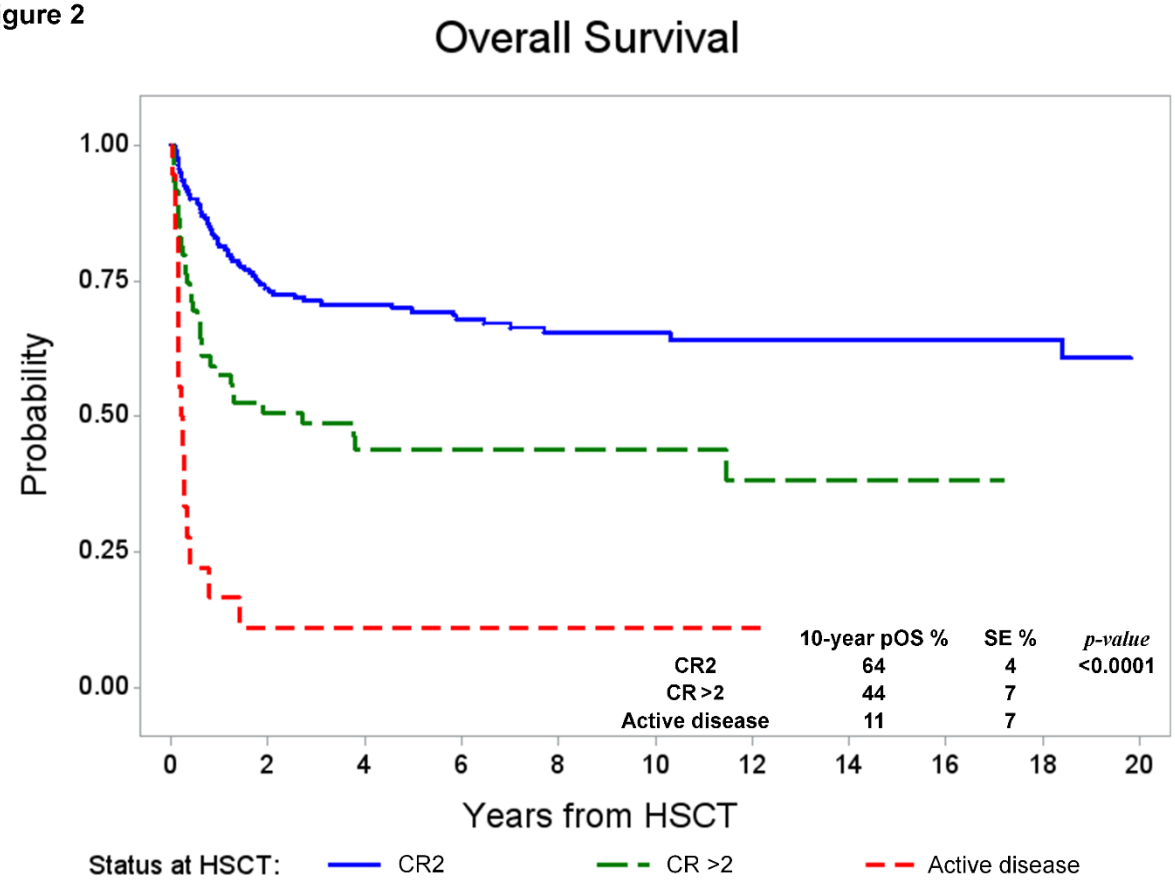
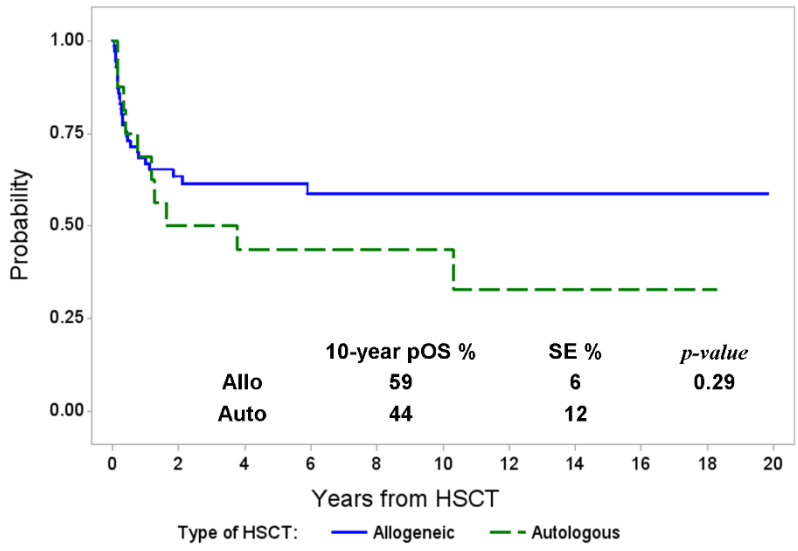

Overall Survival
Probability
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
14
16
18
20
Years from HSCT
10-year pOS %
SE %
p-value
Auto 57 5 0.09
MFD 56 6
MUD 62 6
Haplo 46 10
Type of HSCT:
AUTO
MFD
MUD
HAPLO

**Figure 2**

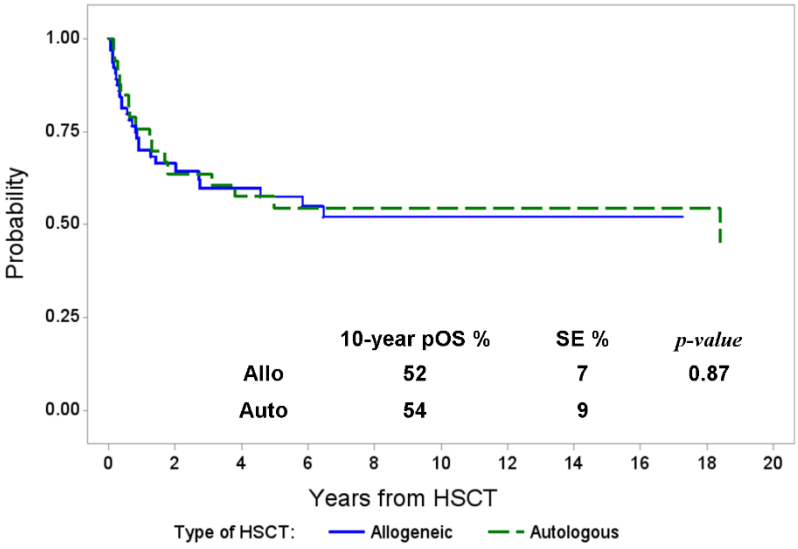

Overall Survival
Probability
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
14
16
18
20
Years from HSCT
10-year pOS %
SE %
p-value
CR2
64
4
<0.0001
CR >2
44
7
Active disease
11
7
Status at HSCT:
CR2
CR >2
Active disease

## Figure 3

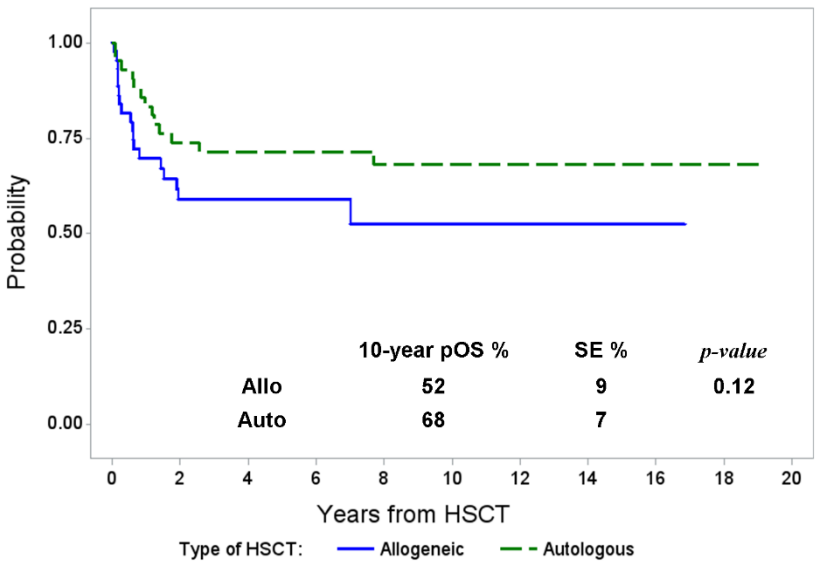
A


1.00
0.75
0.50
0.25
0.00
Probability
10-year pOS % SE % p-value
Allo 59 6 0.29
Auto 44 12
0 2 4 6 8 10 12 14 16 18 20
Years from HSCT
Type of HSCT: Allogeneic Autologous

B


1.00
0.75
0.50
0.25
0.00
Probability
10-year pOS % SE % p-value
Allo 52 7 0.87
Auto 54 9
0 2 4 6 8 10 12 14 16 18 20
Years from HSCT
Type of HSCT: Allogeneic Autologous

C


1.00
0.75
0.50
0.25
0.00
Probability
10-year pOS % SE % p-value
Allo 52 9 0.12
Auto 68 7
0 2 4 6 8 10 12 14 16 18 20
Years from HSCT
Type of HSCT: Allogeneic Autologous