

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

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1 **Title:**

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

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38

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

40

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

58 **Introduction**

59 Although current treatment protocols cure up to 85% of children affected by acute lymphoblastic
60 leukemia (ALL), relapse is still the leading cause of treatment failure, affecting approximately 15-
61 20% of patients. Leukemia relapse may occur in extramedullary sites, mainly central nervous system
62 (CNS) and testis, either alone or in combination with bone marrow (BM) relapse [1].

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

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

78

79 **Patients and Methods**

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

82 Data were extracted from the AIEOP-Stem Cell Transplantation (AIEOP-SCT) Registry. We included
83 children (age 1-18 years) with ALL IEMR who underwent HSCT between 1st of January 1990 and
84 31st of December 2015. Follow up was updated on January 30th, 2018.

85 Written informed consent was obtained from parents or legal guardians.

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

90 “Very early” relapse was defined when disease recurred less than 18 months from primary diagnosis,
91 “early” when disease recurred later than 18 months from diagnosis and less than 6 months from
92 treatment discontinuation, and “late” when disease recurred more than 6 months from treatment
93 discontinuation [3].

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

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

100 **Statistical analysis**

101 Overall Survival (OS) was defined as the time from transplantation to either last follow-up or death
102 due to any cause, whereas disease-free survival (DFS) as the time elapsing from transplantation to
103 either last follow-up or disease recurrence or death due to any cause, whichever occurred first.

104 Relapse-free survival (RFS) was defined as the time from transplantation to documented relapse of
105 ALL. Cumulative incidence (CI) of treatment-related mortality (TRM) was defined as the time from

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

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

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

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

117

118 **Results**

119 Patient characteristics

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

124 Outcome

125 Eighty-three out of 281 patients (29.5%) experienced a second relapse or disease progression at a
126 median time of 176 days (range 15-2345) from HSCT: 49 patients had an isolated BM recurrence,
127 16 an IEMR and 7 children experienced a combined relapse. The site of recurrence was unknown
128 in 11 patients. One hundred and eighteen patients (42.0%) died at a median time of 219 days (range
129 12-6623) from HSCT: 63 from relapse, 46 from treatment-related complications (14/46 were in
130 relapse), 4 from a second tumor, 5 from an unknown event. Grade II-IV acute GVHD (aGVHD)
131 occurred in 79 of 184 patients (42.9%) who received an allograft, while chronic GVHD (cGVHD) was
132 diagnosed in 32 out of 151 patients (21.2%) alive at day +100 after allo-HSCT.

133 Overall survival

134 The OS for the entire cohort was 56%±3% at 10 years; it was not influenced by gender, ALL blast
135 immune-phenotype (B-cell precursor [Bcp]-ALL vs T-ALL), age (≤10 years vs >10 years), site of
136 relapse, source of stem cells, use of TBI during the conditioning regimen and length of first CR (10-
137 year OS for very early, early and late IEMR was 52%±6%, 53%±5%, and 61%±6% respectively,
138 p=0.39). No statistically significant difference was also observed if different type of HSCT were
139 compared: OS for auto-HSCT, MFD, MUD and haplo-HSCT was 57%±5%, 56%±6%, 62%±6% and
140 46%±10%, respectively, p=0.09 (Figure 1).

141 In univariable analysis, the prognostic factors associated with OS were: remission status at
142 transplantation and the year in which patients were treated. Patients transplanted in CR2 had a
143 better OS at 10 years ($64\pm 4\%$), in comparison to both those transplanted in subsequent CR ($CR > 2$)
144 who showed an OS of $44\pm 7\%$ and patients transplanted with active disease who had an OS of
145 $11\pm 7\%$ ($p < 0.0001$) (Figure 2). For patients given HSCT before 2000, the 10-year OS was
146 $45\pm 5\%$, while that of children transplanted after 2000 was $63\pm 4\%$ ($p = 0.0009$).

147 Disease-free survival

148 The 10-year DFS for the whole cohort was $54\pm 3\%$. Like OS, DFS did not differ in relation to gender,
149 ALL blast immune-phenotype, age, duration of first CR, type of HSCT or stem cell source. As for site
150 of relapse, DFS was slightly better for patients with isolated testicular relapse ($65\pm 6\%$) compared
151 to CNS relapse ($49\pm 4\%$), CNS relapse together with other sites ($55\pm 15\%$), mediastinal relapse
152 ($40\pm 14\%$) and other sites involvement ($65\pm 13\%$), but this difference was not statistically
153 significant ($p = 0.22$).

154 Factors influencing DFS were: presence of TBI in the conditioning regimen, remission status at
155 HSCT and year of transplantation. TBI-containing regimens were associated with a better DFS at 10
156 years compared to non-TBI containing regimens ($58\pm 4\%$ vs $37\pm 8\%$, $p = 0.008$). Remission status
157 at HSCT strongly correlated with DFS: patients transplanted in CR2 had a better 10-year DFS
158 ($63\pm 4\%$) in comparison to those transplanted in $CR > 2$ ($39\pm 7\%$) or not in remission ($11\pm 7\%$)
159 ($p < 0.0001$). DFS for patients transplanted either before or after 2000 was $45\pm 5\%$ and $61\pm 4\%$,
160 respectively ($p = 0.0008$).

161 Transplant-Related Mortality

162 TRM for the entire cohort was $10\pm 2\%$ at 100 days, $11\pm 2\%$ both at 6 months and 1 year and
163 $16\pm 2\%$ at 10 years. TRM for auto-HSCT was $4\pm 2\%$, $6\pm 2\%$, $6\pm 2\%$, and $11\pm 3\%$, while TRM
164 for allo-HSCT (MUD, MFD and haplo-HSCT) was $13\pm 2\%$, $14\pm 3\%$, $14\pm 3\%$, and $18\pm 3\%$ at

165 100 days, 6 months, 1 year and 10 years, respectively. Comparison resulted not statistically
166 significant ($p=0.08$).

167 No statistical significant difference was observed if TRM of patients transplanted before 2000 was
168 compared to that of patients transplanted after 2000 ($p=0.33$). In detail, TRM of patients transplanted
169 before 2000 was $15\%\pm 3\%$, $16\%\pm 4\%$, $17\%\pm 3\%$, and $17\%\pm 4\%$ at 100 days, 6 months, 1 year and 10
170 years, respectively. TRM of patients transplanted after 2000 was $6\%\pm 2\%$, $8\%\pm 2\%$, $8\%\pm 2\%$, and
171 $15\%\pm 3\%$ at 100 days, 6 months, 1 year and 10 years, respectively.

172 Subgroup analysis and multivariable analysis

173 As length of first CR is one of the most important prognostic factors in relapsed ALL, we performed
174 separate analyses for patients with very early, early and late IEMR. Regarding patients experiencing
175 very early relapse ($n=87$), DFS and OS at 10 years showed a trend in favor of allogeneic HSCT
176 (MFD, MUD and haplo combined) *versus* autologous HSCT ($58\%\pm 6\%$ vs $44\%\pm 12\%$ and $59\%\pm 6\%$
177 vs $44\%\pm 12\%$, $p=0.28$ and 0.29 respectively) (Figure 3A). In early relapsed patients ($n=97$), DFS and
178 OS at 10 years were comparable irrespectively whether patients were treated with either allo- or
179 auto-HSCT ($50\pm 7\%$ vs $55\%\pm 9\%$, $p=0.88$ and $52\%\pm 7\%$ vs $54\%\pm 9\%$, $p=0.87$) (Figure 3B). In late
180 relapses ($n=87$), DFS and OS at 10 years were slightly better with auto-HSCT than with allo-HSCT:
181 $65\%\pm 8\%$ vs $48\%\pm 9\%$ and $68\%\pm 7\%$ vs $52\%\pm 9\%$, respectively (Figure 3C). However, the difference
182 was not statistically significant ($p=0.13$ and $p=0.12$).

183 Remission status at transplantation is well known to influence outcome; thus, we conducted a
184 separate analysis for patients in CR2 at time of HSCT ($n=204$). RFS and OS for this cohort were
185 $74\%\pm 3\%$ and $64\%\pm 3\%$, respectively; outcome of patients given either autologous or allogeneic
186 HSCT was similar. Ten-year RFS of patients transplanted in CR2 after year 2000 was better as
187 compared to that of patients transplanted before 2000 ($79\%\pm 4\%$ vs $64\%\pm 6\%$, respectively, $p=0.009$).

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

191 A separate analysis on patients transplanted in more recent years (from 2000 to 2015) was also
192 performed. Results confirmed what we observed analyzing the whole cohort of patients: 10-year OS
193 and DFS were not influenced by site of relapse, presence of TBI, time of relapse and type of HSCT.
194 Ten-year OS for auto, MFD, MUD and haplo-HSCT were 71%±7%, 63%±9%, 66%±6% and
195 46%±13% (p=0.18). Remission status at transplantation was, again, the only variable influencing
196 outcome: OS was 71%±4% for patients in CR2, 46%±9% for those in CR>2 (p<0.0001); DFS was
197 69%±4% and 45%±9%(p<0.0001), respectively.

198 For patients treated with allo-HSCT, occurrence of aGVHD was associated with a better DFS
199 (74%±6% vs 48%±7%, p=0.0008) and a better OS (63%±5% vs 46%±7%, p=0.028). Considering
200 only patients given an allograft in CR, occurrence of aGVHD conferred a better RFS: 76%±5% vs
201 58%±6%, p=0.009. Bone marrow RFS was 87%±4% for patients who did experience aGVHD versus
202 74%±6% for those who did not (p=0.02); conversely, extra-medullary RFS was not affected by
203 aGVHD occurrence (90%±4% vs 89%±5%, p=0.79). Presence of cGVHD did not influence patients'
204 outcome (data not shown).

205 Multivariable analysis was conducted after adjustment for remission status: patients with active
206 disease at transplantation were excluded due to the high incidence of treatment failure in this group.

207 As shown in Table 3, in multivariable analysis the only factors influencing OS in patients with IEMR
208 treated with HSCT were number of relapses and year of transplantation.

209

210 **Discussion**

211 Although the vast majority of children affected by ALL are cured with current protocols, relapses still
212 occur and pose remarkable challenges to pediatric hematologists. Allo-HSCT is currently used to
213 treat patients in CR2 with high-risk features (very early/early and isolated BM relapse, recurrence of
214 T-lineage ALL [1,12]), and it is now considered the standard of care also for low-risk patients who
215 present minimal residual disease (MRD) positivity at the end of induction therapy [13,14]. Treatment
216 of extramedullary relapse is less well established. The absence of BM involvement is traditionally
217 considered a favorable prognostic feature [15], and patients with isolated CNS (ICNS) relapse are
218 treated with intensive systemic and intrathecal chemotherapy (CT), followed by either cranio-spinal
219 or cranial radiotherapy (RT) [5, 7, 16, 17]. EFS with this approach ranges from 45% [3, 7, 18, 19] to
220 70% [16]. Despite the high cure rate obtained in two Children Oncology Group trials [16, 20], with
221 global 5-year EFS approaching 70%, for particular subgroups of IEMR prognosis is still dismal.
222 Patients experiencing very early and early IEMR or ICNS relapses have a survival probability of only
223 20-30% in most studies [3, 7, 18, 19]. HSCT has been used for treatment of IEMR, but published
224 data are conflicting and limited to small numbers of patients [6, 21-23]. Our previous work [9] showed
225 that EFS of children with early IEMR treated with auto-HSCT was clearly superior to that of patients
226 who received CT/RT (56% vs 12%). Moreover, in another report, we demonstrated that auto-HSCT
227 offers a better chance of cure patients in CR2 than in subsequent CR [10]. More recent papers
228 reported comparable outcome in patients with ICNS relapse in CR2 treated with allo-HSCT or CT/RT
229 [2, 5, 7].

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

235 20]. Moreover, if only patients transplanted in CR2 were considered, as in other published series,
236 the 10-year OS of 64% is in line with the most favorable reports [16, 20].

237 Interestingly, in our study, the use of HSCT seems to abrogate the impact of some “classical”
238 prognostic factors, like site of relapse, duration of first remission and ALL blast immune-phenotype.
239 Similar results were found in the whole cohort, as well as in the group of patients treated in more
240 recent years (from 2000 to 2015). The only factors influencing outcome resulted to be year of HSCT
241 and remission status at transplantation. Taking into account the prognostic impact of year of
242 transplantation, this may reflect improvement in patient selection: the 10-year RFS for patients
243 transplanted before 2000 was better than that of patients transplanted after 2000; on the contrary,
244 10-year TRM pre and post-2000 did not differ. There is the possibility that MRD assessment during
245 therapy guided decisions on CT administration, time and type of HSCT in single centers. As far as
246 the prognostic significance of remission status before HSCT is concerned, these data confirm what
247 we reported previously [10], namely that outcome is significantly better for children transplanted in
248 CR2 than in subsequent remission. This observation emphasizes the importance of identifying those
249 patients at higher risk of further relapse, who, thus, may benefit of HSCT soon after the achievement
250 of CR2. Very early and early IEMR or ICNS relapses treated with CT/RT have been previously shown
251 to have a survival probability around 20-30% [3, 7, 18, 19]. The use of HSCT in our study improved
252 the OS to 53% for early relapses and 52% for very early relapses. This result is even more relevant
253 considering that patients with third or subsequent CR and even with active disease at time of HSCT
254 were included in this analysis. Based on these data, we suggest considering HSCT for patients with
255 very early/early IEMR once that the CR is achieved.

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

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

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

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

285 **Conflict of interest**

286 The authors have no conflict of interest to declare.

287

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292 **References**

- 293 1. Bhojwani D and Pui C.H. Relapsed childhood acute lymphoblastic leukemia. *Lancet Oncol*
294 2013, 14: e205-17
- 295 2. Malempati S, Gaynon P.S, Sather H, La MK, and Stork LC. Outcome After Relapse Among
296 Children with Standard-Risk Acute Lymphoblastic Leukemia: Children's Oncology Group Study
297 CCG-1952. *J Clin Oncol* 2007, 25: 5800-5807
- 298 3. Tallen G, Ratei R, Mann G, Kaspers G, Niggli F, Karachunsky A et al. Long-Term Outcome in
299 Children with Relapsed Acute Lymphoblastic Leukemia After Time-Point and Site-of-Relapse
300 Stratification and Intensified Short-Course Multidrug Chemotherapy: Results of Trial ALL-REZ
301 BFM 90. *J Clin Oncol* 2010, 28: 2339-2347
- 302 4. Jacobs JE and Hastings C. Isolated Extramedullary Relapse in Childhood Acute Lymphocytic
303 Leukemia. *Curr Hematol Malig Rep* 2010, 5: 185–191
- 304 5. Eapen M, Zhang MJ, Devidas M, Raetz E, Barredo JC, Ritchey AK et al. Outcomes after HLA-
305 matched sibling transplantation or chemotherapy in children with acute lymphoblastic leukemia
306 in a second remission after an isolated central nervous system relapse: a collaborative study
307 of the Children's Oncology Group and the Center for International Blood and Marrow Transplant
308 Research. *Leukemia* 2008, 22: 281–286
- 309 6. Harker-Murray PD, Thomas AJ, Wagner JE, Weisdorf D, Luo X, DeFor TE et al. Allogeneic
310 Hematopoietic Cell Transplantation in Children with Relapsed Acute Lymphoblastic Leukemia
311 Isolated to the Central Nervous System. *Biology of Blood and Marrow Transplantation* 2008,
312 14: 685-692
- 313 7. Domenech C, Mercier M, Plouvier E, Puraveau M, Bordigoni P, Michel G et al. First isolated
314 extramedullary relapse in children with B-cell precursor acute lymphoblastic leukaemia: Results
315 of the Coopral-97 study. *European Journal of Cancer* 2008, 4: 2461-2469

- 316 8. van den Berg H, de Groot-Kruseman HA, Damen-Korbijn CM, de Bont ESJM, Schouten-van
317 Meeteren AYN and Hoogerbrugge PM. Outcome After First Relapse in Children with Acute
318 Lymphoblastic Leukemia: A Report Based on the Dutch Childhood Oncology Group (DCOG)
319 Relapse ALL 98 Protocol. *Pediatr Blood Cancer* 2011; 57: 210–216
- 320 9. Messina C, Valsecchi MG, Aricò A, Locatelli F, Rossetti F, Rondelli R et al. Autologous bone
321 marrow transplantation for treatment of isolated central nervous system relapse of childhood
322 acute lymphoblastic leukemia. *Bone Marrow Transplantation*, 1998, 21: 9–14
- 323 10. Messina C, Cesaro S, Rondelli R, Rossetti F, Locatelli F, Pession A et al. Autologous bone
324 marrow transplantation for childhood acute lymphoblastic leukaemia in Italy. AIEOP/FONOP-
325 TMO Group. Italian Association of Paediatric Haemato-Oncology. *Bone Marrow*
326 *Transplantation* 1998, 21(10): 1015-21.
- 327 11. Lee JH, Choi SJ, Lee JH, Seol M, Lee YS, Ryu SG et al. Anti-leukemic effect of graft-versus-
328 host disease on bone marrow and extramedullary relapses in acute leukemia. *Haematologica*
329 2005, 90(10): 1380-8.
- 330 12. Borgmann A, von Stackelberg A, Hartmann R, Ebell W, Klingebiel T, Peters C et al. Unrelated
331 donor stem cell transplantation compared with chemotherapy for children with acute
332 lymphoblastic leukemia in a second remission: a matched-pair analysis. *Blood*. 2003; 101:
333 3835-3839
- 334 13. Locatelli F, Schrappe M, Bernardo ME and Rutella S. How I treat relapsed childhood acute
335 lymphoblastic leukemia. *Blood* 2012, 120(14): 2807-2816
- 336 14. Balduzzi A, Galimberti S, Valsecchi MG, Bonanomi S, Conter V, Barth A et al. Autologous
337 Purified Peripheral Blood Stem Cell Transplantation Compare to Chemotherapy in Childhood
338 Acute Lymphoblastic Leukemia After Low-Risk Relapse. *Pediatr Blood Cancer* 2011; 57: 654–
339 659

- 340 15. Nguyen K, Devidas M, Cheng SC, La M, Raetz EA, Carroll WL et al. Factors Influencing
341 Survival after Relapse from Acute Lymphoblastic Leukemia: A Children's Oncology Group
342 Study. *Leukemia* 2008; 22(12): 2142–2150
- 343 16. Ritchey AK, Pollock BH, Lauer SJ, Andejeski Y and Buchanan GR. Improved Survival of
344 Children with Isolated CNS Relapse of Acute Lymphoblastic Leukemia: A Pediatric Oncology
345 Group Study. *J Clin Oncol* 1999, 17: 3745-3752.
- 346 17. Einsiedel HG, von Stackelberg A, Hartmann R, Fengler R, Schrappe M, Janka-Schaub G et al.
347 Long-Term Outcome in Children With Relapsed ALL by Risk-Stratified Salvage Therapy:
348 Results of Trial Acute Lymphoblastic Leukemia-Relapse Study of the Berlin-Frankfurt-Mu"nster
349 Group 87. *J Clin Oncol* 2005, 23: 7942-7950
- 350 18. Roy A, Cargill A, Love S, Moorman AV, Stoneham S, Lim A et al. Outcome after first relapse in
351 childhood acute lymphoblastic leukaemia – lessons from the United Kingdom R2 trial. *Br J*
352 *Haematol* 2005, 130: 67–75
- 353 19. Saarinen-Pihkala UM, Heilmann C, Winiarski J, Glomstein A, Abrahamsson J, Arvidson J et al.
354 Pathways Through Relapses and Deaths of Children with Acute Lymphoblastic Leukemia: Role
355 of Allogeneic Stem Cell Transplantation in Nordic Data. *J Clin Oncol* 2006, 24: 5750-5762
- 356 20. Barredo JC, Devidas M, Lauer SJ, Billett A, Marymont MA, Pullen J et al. Isolated CNS Relapse
357 of Acute Lymphoblastic Leukemia Treated with Intensive Systemic Chemotherapy and Delayed
358 CNS Radiation: A Pediatric Oncology Group Study. *J Clin Oncol* 2006, 24: 3142-3149.
- 359 21. Bordigoni P, Esperou H, Souillet G, Pico J, Michel G, Lacour B et al. Total body irradiation-
360 high-dose cytosine arabinoside and melphalan followed by allogeneic bone marrow
361 transplantation from HLA-identical siblings in the treatment of children with acute lymphoblastic
362 leukaemia after relapse while receiving chemotherapy: a Société Française de Greffe de Moelle
363 study. *Br J Haematol*. 1998,102(3): 656-65

- 364 22. Afify Z, Hunt L, Green A, Guttridge M, Cornish J and Oakhill A. Factors affecting the outcome
365 of stem cell transplantation from unrelated donors for childhood acute lymphoblastic leukemia
366 in third remission. *Bone Marrow Transplantation* 2005, 35: 1041–1047
- 367 23. Yoshihara T, Morimoto A, Kuroda H, Imamura T, Ishida H, Tsunamoto K et al. Allogeneic stem
368 cell transplantation in children with acute lymphoblastic leukemia after isolated central nervous
369 system relapse: our experiences and review of the literature. *Bone Marrow Transplantation*
370 2006, 37: 25–31
- 371 24. Shem-Tov N, Saraceni F, Danylesko I et al. Isolated Extramedullary Relapse of
372 Acute Leukemia after Allogeneic Stem Cell Transplantation: Different Kinetics and Better
373 Prognosis than Systemic Relapse. *Biol Blood Marrow Transplant.* 2017, 23(7): 1087-1094
- 374 25. Lee K-H, Lee J-H, Kim S et al. High frequency of extramedullary relapse of acute leukemia after
375 allogeneic bone marrow transplantation. *Bone Marrow Transplantation* 2000, 26:147–152
- 376 26. Huck K, Laws H-J, Meisel R et al. Three cases of renal relapse after allogeneic hematopoietic
377 stem cell transplantation for childhood acute lymphoblastic leukemia. *Haematologica* 2006,
378 91:(2) e20-e25
- 379 27. Kantarcioglu B, Bekoz HF, Ogret YD et al. Isolated extramedullary cutaneous relapse despite
380 concomitant severe graft-vs.-host disease and tissue chimerism analysis in a patient with acute
381 lymphoblastic leukemia after allogeneic hematopoietic stem cell transplantation: A case report.
382 *Molecular and Clinical Oncology* 2016, 5: 745-749
- 383 28. Singhal S, Powles R, Kulkarni S et al. Long-term follow-up of relapsed acute leukemia treated
384 with immunotherapy after allogeneic transplantation: the inseparability of graft-versus-host
385 disease and graft-versus-leukemia, and the problem of extramedullary relapse. *Leuk*
386 *Lymphoma.* 1999 Feb;32(5-6):505-512.

- 387 29. Neumann M, Blau IW, Burmeister T et al. Intrathecal application of donor lymphocytes in
388 leukemic meningeosis after allogeneic stem cell transplantation. *Ann Hematol* 2011, 90(8): 911-
389 916
- 390 30. Park JH, Rivière I, Gonen M et al. Long-Term Follow-up of CD19 CAR Therapy in Acute
391 Lymphoblastic Leukemia. *N Engl J Med* 2018, 378(5): 449-459.

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%)	
<i>CNS+ cerebral parenchima</i>	1	0	2	1	4	
<i>CNS+testis</i>	0	2	1	0	3	
<i>CNS+mediastinum</i>	0	0	2	0	2	
<i>CNS+eye</i>	1	1	0	0	2	
Other	3 (3.1%)	6 (7.6%)	4 (5.3%)	3 (10.0%)	16 (5.7%)	
<i>Eye</i>	0	3	0	1	4	
<i>Lymph-nodes</i>	1	1	0	1	3	
<i>Other sites (liver, ovary, kidney, skin..)</i>	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

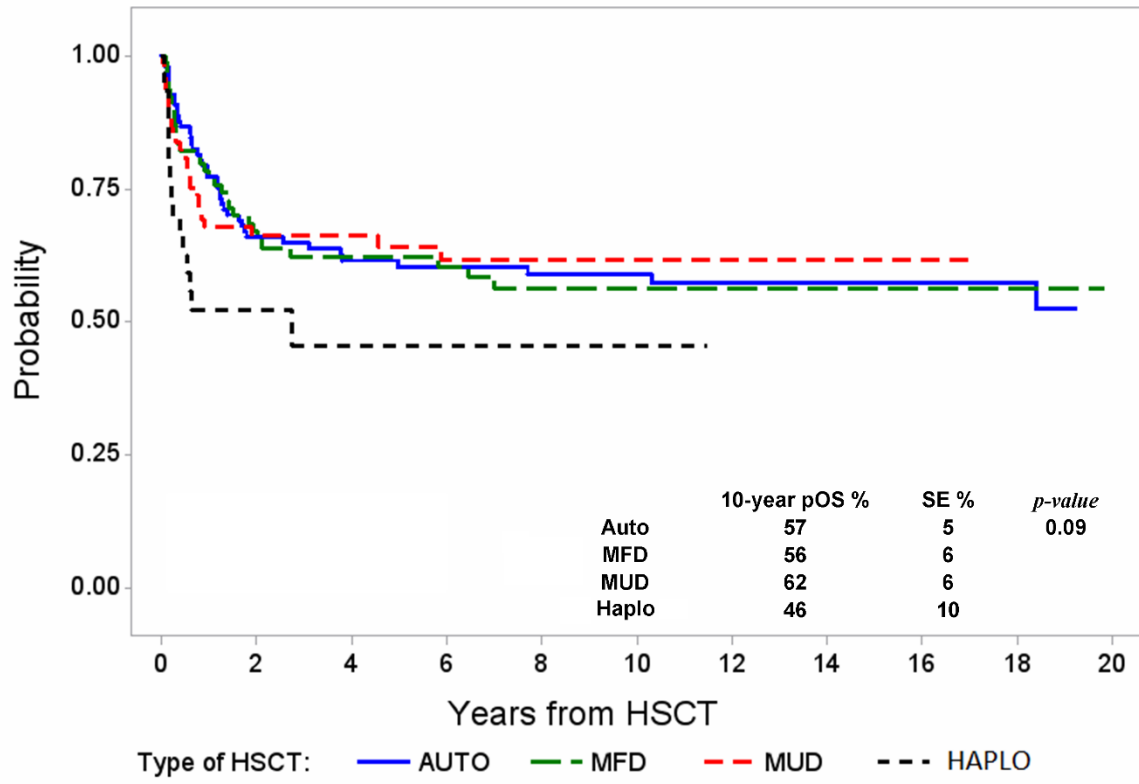


Figure 2

Overall Survival

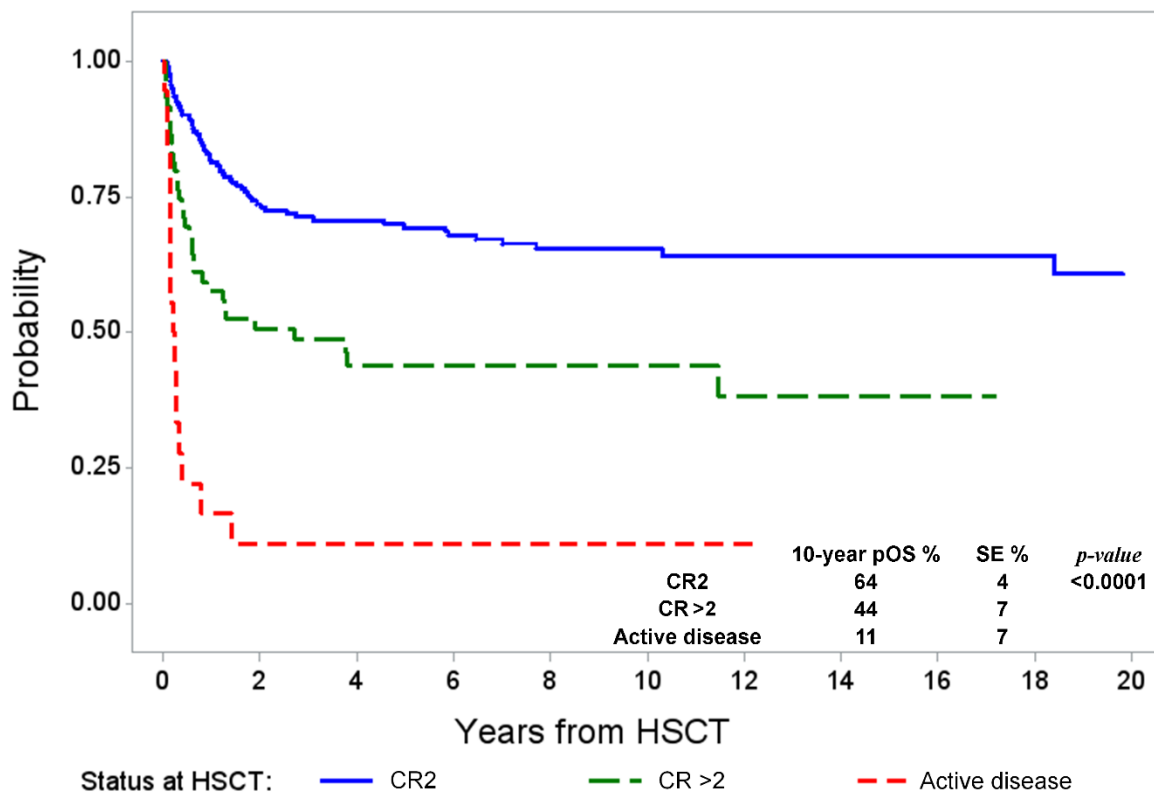
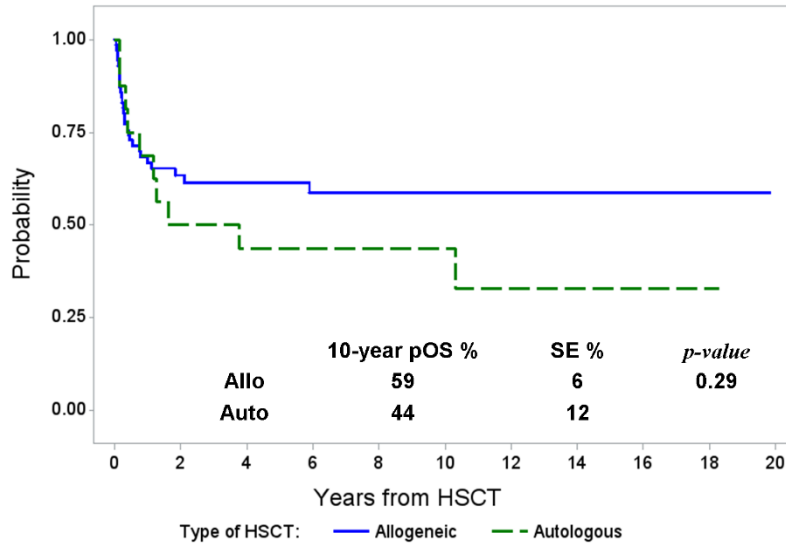
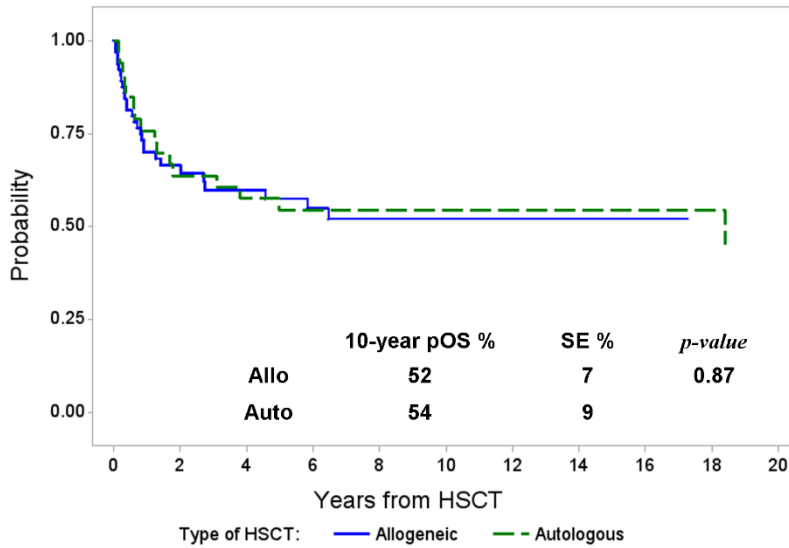


Figure 3

A



B



C

