A multicenter phase II study of intrabone single-unit cord blood transplantation without antithymocyte globulin

Running title: Intrabone single-unit CBT without ATG

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Abstract

To overcome the delayed or failed engraftment after unrelated cord blood transplantation (CBT), we conducted a multicenter phase II study of intrabone single-unit CBT without antithymocyte globulin (ATG) for adult patients with hematological malignancies (UMIN-CTR, UMIN000020997). Sixty-four patients received an intrabone injection of unwashed (n = 61) or washed (n = 3) cord blood after local anesthesia. All injection-related adverse events were mild and resolved spontaneously. Sixty-two patients were evaluable for the efficacy of intrabone CBT of serological HLA-A, -B, and -DR ≥4/6 matched cord blood with a median number of 2.57×10^7/kg cryopreserved total nucleated cells. The probability of survival with neutrophil engraftment on day 28 was 77.4% (95% confidence interval, 67.0–85.8%), which exceeded the threshold value. The cumulative incidences of neutrophils ≥0.5×10^9/L on day 60 was 80.6% (68.2–88.6%), with a median time to recovery of 21 days after transplantation. The cumulative incidences of platelets ≥20 ×

10^9/L and platelets ≥50 × 10^9/L on day 100 were 75.8% (62.6–84.9%) and 72.6% (59.4–82.1%), respectively, with median time to platelets ≥20 × 10^9/L and platelets ≥50 × 10^9/L of 38 and 45 days after transplantation, respectively. The cumulative incidences of grade II–IV and III–IV acute graft-versus-host disease were 29.0% and 6.5%, respectively. All responded to steroid therapy, and secondary treatments were not required. The present study suggests the efficacy of intrabone single-unit CBT without ATG in terms of early engraftment and controllable acute graft-versus-host disease.

Keywords: cord blood transplantation, intrabone, engraftment, graft-versus-host disease, antithymocyte globulin

Introduction

Although hematopoietic cell transplantation (HCT) from an HLA-haploidentical related donor has been increasingly selected as a treatment for patients with hematological malignancies who lack HLA-matched related or unrelated donors, cord blood transplantation (CBT) is still an attractive option, particularly when immediate transplantation is needed [1, 2]. Overall survival or leukemia-free survival after CBT is comparable to those after bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT) from HLA-matched unrelated donors [3–4]. However, delayed or failed engraftment is a serious concern in CBT. The incidence of graft failure has been reported to be approximately 20% after CBT, compared to approximately 5–10% and 5% after unrelated BMT and PBSCT, respectively [3–5]. Various attempts have been made to minimize this disadvantage of CBT. As the dose of cryopreserved total nucleated cells (TNC) and CD34+ cells are major determinants of engraftment, double cord blood

94 units have been widely used as transplant sources [6–8]. However, prospective randomized
95 studies have demonstrated similar engraftment rates between double-unit CBT and
96 single-unit CBT with an adequate cell dose [9, 10]. Expansion of cord blood cells ex vivo
97 before transplantation has been developed to increase the number of transplanted cells [11,
98 12]. A recent phase I/II study of CBT with a single cord blood unit, which was expanded
99 ex-vivo in the presence of nicotinamide, showed promising results [13], although this
100 specialized technique cannot be universally adopted.

101 Intrabone CBT was established by Frassoni F et al., to reduce loss of cord blood cells
102 which might be trapped in other organs after intravenous injection, and to enhance the
103 homing of stem cells to the bone marrow [14]. This strategy requires neither an additional
104 cord blood unit nor specialized technique or cell-processing facility. Their study and
105 subsequent four prospective studies reported good tolerability and potential to enhance
106 engraftment of intrabone CBT [15–18]. In fact, most studies demonstrated neutrophil

engraftment rates of more than 80% in adult patients after intrabone single-unit CBT [14, 16–18]. It is note that, in these studies, the engraftment rate was defined as a cumulative incidence of neutrophil recovery $\geq 0.5 \times 10^9$/L on day 60–100. This is in contrast to BMT and PBSCT, in which presence or absence of engraftment is usually assessed on day 21–28 and if no elevation of neutrophil count is observed by day 28, the patients are considered for a second infusion of stem cells from the original donor or retransplantation from a different donor [19–21]. As we mentioned above, the intrabone CBT is attractive in terms of the enhancement of engraftment, however there is little direct evidence to support the hypothesis that the intrabone CBT improves engraftment rate at a very early time, such as 28 days after transplantation.

Therefore, a multicenter phase II study of intrabone single-unit CBT without ATG for adult patients with hematological malignancies was conducted to assess the probability of survival with neutrophil engraftment on day 28 after transplantation.

Materials and Methods

Patients

From June 2016 to September 2018, 66 patients were enrolled in this study at 10 institutes in Japan: Nagoya University, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Anjo Kosei Hospital, Japanese Red Cross Narita Hospital, Niigata University Medical and Dental Hospital, Tohoku University, Okayama University, Hyogo College of Medicine, Hokkaido University, and Shizuoka Cancer Center. Eligible patients had hematological malignancy and needed CBT. Patients were required to be aged ≥16, to have an Eastern Cooperative Oncology Group performance status of 0, 1 or 2, to have adequate liver, kidney, lung, and heart functions, and to have available serological HLA-A, -B, and -DR ≥4/6 matched cord blood with a cryopreserved TNC at least 2×10^7/kg. The study was approved by the ethics committees at each institute, and written informed

consent was obtained from each patient in accordance with the Declaration of Helsinki. The study has been registered at UMIN-CTR as UMIN000020997.

Transplantation

Conditioning regimens and supportive care were assigned according to each institutional protocol at the clinical sites. Cyclosporine (CsA) or tacrolimus (Tac) with short-term methotrexate (MTX) or mycophenolate mofetil (MMF) were used for prophylaxis of GVHD. ATG was not used as GVHD prophylaxis.

Procedure of intrabone injection

A unit of cord blood was thawed in a 37°C water bath, collected into a syringe, and aliquoted into two or four syringes. In some cases, to remove dimethysulphoxide, cord blood was washed with a saline solution plus dextran and human albumin, resuspended in

10ml of the solution, and aliquoted into two syringes [22]. Decision on cord blood washing was institution dependent. After local anesthesia, standard bone marrow aspiration needles were inserted into the iliac bone. Intravenous anesthesia was not used. A small volume of bone marrow was aspirated to confirm that the needle was inserted into the bone marrow cavity. Subsequently, an aliquot of cord blood was gently injected. The procedure was repeated for the remaining aliquots across the iliac crest. The adverse events associated with intrabone injection were assessed by the National Cancer Institute-Common Terminology Criteria for Adverse Events version 4.0.

Donor chimerism

Quantitative analysis of donor chimerism was performed using polymerase chain reaction of short tandem repeat on days 28 and 56 after transplantation. Fluorescence in situ hybridization for sex chromosomes was permitted for sex-mismatched transplantation.

159

160 **Definitions**

161 Engraftment was defined as the first of three consecutive days of neutrophil count ≥0.5 ×

162 10^9/L with donor chimerism ≥95%. The time to reticulocyte and platelet recoveries were

163 defined as the first of three consecutive days of reticulocyte ≥1%, platelet ≥20 × 10^9/L and

164 platelet ≥50 × 10^9/L without transfusion support. Engraftment failure was defined if

165 neutrophil count did not achieve ≥0.5 × 10^9/L by day 60. Engraftment failure was also

166 defined if both of the following criteria were met before day 60; (1) the bone marrow was

167 severe hypoplastic after day 28, (2) donor chimerism of the T cell fraction was ≤50% and

168 further decreased compared to the previous time point. Acute and chronic GVHD were

169 diagnosed and graded by clinicians at each institution according to the consensus criteria

170 [23, 24]. Acute myelogenous leukemia in the first or second remission, acute lymphoblastic

171 leukemia in the first remission, chronic myelogenous leukemia in the first chronic phase,

and myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk disease; all others were defined as high-risk disease. Conditioning regimen was classified as myeloablative if at least one of the following criteria was met: total body irradiation (TBI) >8 Gy, melphalan (Mel) >140mg/m^2, intravenous busulfan (iv BU) ≥7.2mg/kg was used. Other conditioning regimens were classified as nonmyeloablative [25].

Statistical analysis

The primary endpoint of this study was the probability of survival with neutrophil engraftment on day 28 after transplantation. Fifty-eight patients were required to provide at least 80% power to differentiate the primary endpoint of 78% with the width of the 95% confidence interval (CI) at 15%. Assuming a 10% drop-out rate, 65 patients were required for this study. The probabilities of hematological recoveries, GVHD, relapse, and

non-relapse mortality (NRM) were estimated based on cumulative incidence curves, considering the following competing events: death without engraftment and second transplantation for hematological recoveries, death or relapse without GVHD and second transplantation for GVHD, death without relapse for relapse, and relapse for NRM. Comparisons between the groups were performed using the Grey test. Overall survival after transplantation was estimated according to the Kaplan-Meier method. All statistical analyses were performed using EZR software version 1.41 [26]. *P*-values of less than 0.05 were regarded as statistically significant.

Results

Patient characteristics

Sixty-six patients were enrolled in this study. Two patients did not undergo intrabone CBT due to deterioration of sepsis (n = 1) and cerebral infarction (n = 1) after enrollment. Of 64

198 patients who received intrabone CBT, two were assessed only for the safety of intrabone

199 injection, because the study monitoring conference concluded that an HCV-positive patient

200 who had received fewer than 2×10^7/kg of cryopreserved TNC (n = 1) and a patient with

201 EB virus lymphoproliferative disorder (non-malignant disease) (n = 1) should be excluded

202 from evaluable patients for efficacy. The characteristics of 62 patients who were evaluable

203 for the efficacy of intrabone single-unit CBT are summarized in Table 1. The median age

204 was 48 years (range, 17–69 years). Thirty-eight patients received myeloablative

205 conditioning regimens including TBI-based regimen (n = 23) and BU-based regimen (n =

206 15), and 24 patients received non-myeloablative conditioning regimens including

207 fludarabine (Flu) + Mel-based regimen (n = 14), Flu + cyclophosphamide (CY)-based

208 regimen (n = 6), and Flu + BU-based regimen (n = 4). No patient received ATG as a GVHD

209 prophylaxis. Although 23 patients (37%) had anti-HLA antibody, no patient had

210 donor-specific anti-HLA antibody, which is reported to be associated with an increased risk

of engraftment failure [27]. The median number of cryopreserved TNC and CD34+ cells were 2.57×10^7/kg (range, 2.02–4.00×10^7/kg) and 0.99×10^5/kg (range, 0.44–2.30×10^5/kg), respectively.

Intrabone injection of cord blood

Sixty-four patients received an intrabone injection of unwashed (n = 61) or washed (n = 3) cord blood after local anesthesia. The number of the injection sites was two in four patients and four in 60 patients. All injection-related adverse events were mild; nine were skin reactions and 25 were pains at the injection site, all of which were resolved spontaneously. No moderate or severer adverse event was observed.

Hematological recovery

Two patients died within 28 days after transplantation due to multiple organ failure (day 21)

224 and sepsis (day 26). The probability of survival with neutrophil engraftment on day 28 was

225 77.4% (95% CI, 67.0–85.8%). Finally, 50 patients achieved engraftment. The cumulative

226 incidence of engraftment on day 60 was 80.6% (95% CI, 68.2–88.6%), and the median time

227 to engraftment was 21 days (range, 15–31 days) (Fig. 1a). Two of 50 patients with

228 engraftment achieved ≥95% of donor chimerism after day 28. One patient received

229 myeloablative conditioning regimen (iv BU 12.8mg/kg + CY 120mg/kg + TBI 3Gy), and

230 the other received nonmyeloablative conditioning regimen (Flu 200mg/m^2 + CY 50mg/kg +

231 TBI 3Gy). The cumulative incidences of reticulocytes ≥1% on day 60, platelets ≥20 × 10^9/L

232 on day 100, and platelets ≥50 × 10^9/L on day 100 were 79.0% (95% CI, 66.4–87.4%),

233 75.8% (62.6–84.9%), and 72.6% (59.4–82.1%), respectively (Fig. 1b-d). Among patients

234 with hematological recovery, the median time to reticulocytes ≥1%, platelets ≥20 × 10^9/L,

235 and platelets ≥50 × 10^9/L were 30 (range, 18–77 days), 38 (18–81 days), and 45 (27–118

236 days) days, respectively. The numbers of cryopreserved TNC (≥2.5 × 10^7 cells/kg vs. < 2.5

237 $\times 10^7$ cells/kg) and CD34+ cells ($\geq 1.0 \times 10^5$ cells/kg vs. $< 1.0 \times 10^5$ cells/kg) did not affect

238 neutrophil (Fig. 2a, b) and platelet recoveries (Fig. 3a, b). When patients were categorized

239 into four groups according to the combination of numbers of cryopreserved TNC and

240 CD34+ cells, no significant differences were observed among these groups (data not

241 shown). The degree of HLA mismatches in host-versus-graft direction (0–1 antigen

242 mismatch vs. 2 antigens mismatch) was not associated with neutrophil recovery (Fig. 2c)

243 and platelet recoveries (Fig. 3c). Neither preconditioning regimens nor GVHD prophylaxis

244 affected neutrophil and platelet recoveries (data not shown).

245

246 **GVHD, relapse, NRM and survival**

247 The cumulative incidences of grade II–IV and III–IV acute GVHD on day100 after

248 intrabone CBT were 29.0% (95% CI, 18.3–40.7%) and 6.5% (2.1–14.5%), respectively

249 (Fig. 4a, b). Twenty-four patients received treatment for acute GVHD using topical (n = 8)

250 or systemic (n = 16) corticosteroid. All of them responded to steroid therapy, and secondary
251 treatments were not required. The cumulative incidence of chronic GVHD at 1 year was
252 9.9% (95% CI, 4.0–19.1%).
253 The cumulative incidences of relapse and NRM at 1 year after intrabone CBT were
254 19.4% (95% CI, 10.6–30.1%) and 21.0% (11.8–31.9%), respectively.
255 Overall survival at 1 year after intrabone CBT for all 62 patients was 66.1% (95% CI,
256 52.9–76.4%) (Fig. 5). Of 10 patients with engraftment failure, nine underwent a second
257 transplantation from another cord blood (n = 7) or an HLA-haploidentical related donor (n
258 = 2), and five were alive at 1 year after intrabone CBT. One patient died of exacerbation of
259 sepsis during pretreatment prior to second transplantation. Of 50 patients who achieved
260 engraftment, 14 died after intrabone CBT. The most frequent cause of death was relapse of
261 hematological malignancies (n = 7). Other causes included non-infectious pulmonary
262 complications (n = 3), sinusoidal obstruction syndrome (n = 2), respiratory syncytial virus

infection (n = 1), and thrombotic microangiopathy (n = 1).

Discussion

We conducted the largest prospective study of intrabone single-unit CBT for adult patients with hematological malignancies. The probability of survival with neutrophil engraftment on day 28 (primary endpoint) was 77.4% (95% CI, 67.0–85.8%), which exceeded the threshold value of 63%. The cumulative incidence of neutrophil engraftment on day 60 was 80.6%. In a recent large retrospective study for single-unit intravenous CBT comparing European and Japanese populations, the cumulative incidences of neutrophil engraftment on day 100 were 81% in the European cohort and 78% in the Japanese cohort. However, those on day 28 were no more than 63% and 69%, respectively (numerical values based on visual measurement of its Supplemental Figure) [28]. These data suggest that intabone CBT is effective in terms of early neutrophil engraftment rather than a high neutrophil

276 engraftment rate. In contrast, this study exhibited earlier platelet recovery (median time to

277 platelets ≥50 × 10^9/L, day 45) and higher platelet recovery rate (cumulative incidence of

278 platelets ≥50 × 10^9/L on day100, 72.6%), compared to the Japanese historical data on

279 intravenous CBT (median time to platelets ≥50 × 10^9/L, day 78; cumulative incidence of

280 platelets ≥50 × 10^9/L on day100, 58%) [16]. These data suggest that intrabone CBT is

281 effective in terms of both the speed and rate of platelet recovery.

282 The doses of TNC and CD34+ cells in transplanted cord blood unit are major factors

283 affecting the speed and/or rate of engraftment [29, 30]. Cord blood units comprising TNC

284 ≥2.5 × 10^7/kg and CD34+ cells ≥1.5 × 10^5/kg are recommended for single-unit CBT

285 according to the National Marrow Donor Program and the Center for International Blood

286 and Marrow Transplant Research [31]. However, a single cord blood unit with TNC <2.5 ×

287 10^7/kg is widely used in Japan, although a significant association between TNC dose <2.5 ×

288 10^7/kg and lower engraftment rate has been confirmed in a retrospective study of

289 single-unit CBT [28]. Some studies have shown a greater impact of the number of CD34+
290 cells for achievement of engraftment compared with the number of TNC [30, 32–34]. For
291 intrabone CBT, small-sized studies demonstrated an association of the TNC or CD34+ cell
292 dosage with neutrophil recovery [15, 18]. In the present study, neither TNC nor CD34+ cell
293 dose affected the speed and rate of engraftment and even the group that had a combination
294 of low TNC and low CD34+ cells showed no difference in neutrophil recovery from other
295 three groups. Thus, a question left unresolved is whether intrabone injection has the
296 potential to overcome the delayed and/or failed engraftment, especially in CBT with low
297 numbers of TNC and CD34+ cells.

298 The HLA disparity has also been reported to be a risk factor for the engraftment failure
299 in single-unit CBT [35–38]. However, the present study demonstrated no difference in both
300 neutrophil and platelet engraftment rate between 0–1 antigen-mismatched and 2
301 antigens-mismatched cord blood units. Thus, the HLA disparity may have no impact on the

302 engraftment in intrabone CBT.

303 In addition to the present study, five prospective studies of intrabone single-unit CBT
304 have been reported to date (Table 2). Relatively young patients underwent intrabone CBT
305 with myeloablative regimens in Europe [14, 18], while relatively elderly patients underwent
306 intrabone CBT with nonmyeloablative regimens in Japan, excluding this report [15–17].
307 The combination of CsA and MMF together with ATG was used as GVHD prophylaxis in
308 Europe, while the combination of CsA or Tac and short-term MTX or MMF without ATG
309 was used in Japan. Interestingly, three reports from Japan demonstrated faster neutrophil
310 engraftment compared with the other reports [15–17], which might be due to the use of
311 nonmyeloablative preconditioning [39]. Two reports from Europe demonstrated lower
312 incidence of acute GVHD compared with the other reports [14, 18], which might be due to
313 the use of ATG as GVHD prophylaxis [40].

314 The use of ATG as GVHD prophylaxis for CBT is controversial [41]. ATG has been

315 widely used since the early phases of CBT development [42, 43]. However, the exposure to
316 ATG after CBT has a detrimental effect on early T-cell immune reconstitution [44, 45]. A
317 recent retrospective study in Japan demonstrated a negative impact of ATG on NRM after
318 intravenous single-unit CBT [46]. A retrospective study in Europe comparing intrabone
319 single-unit CBT and intravenous double-unit CBT demonstrated a significantly lower
320 incidence of acute GVHD in intrabone CBT [47]. In the present study, 29% of patients
321 developed grade II or higher acute GVHD, however, all patients responded to first-line
322 steroid therapy. Taken together, it is critical to determine whether ATG provides benefits to
323 patients undergoing intrabone single-unit CBT in terms of GVHD prophylaxis, immune
324 reconstitution, NRM and survival.

325 In the previous prospective randomized study to compare intrabone infusion of bone
326 marrow with intravenous infusion, the infused cells were traced by bone marrow
327 scintigraphy [48]. The bone marrow cells that had been infused intrabone first passed the

328 lung and then were distributed more widely in the human body. In fact, there were no
329 differences in engraftment, GVHD or survival between the intrabone and intravenous bone
330 marrow transplantation [48,49]. However, there is a critical difference between CBT and
331 bone marrow transplantation. The former is characterized by delayed engraftment, lower
332 engraftment rate, infusion of small-volume of thawed cord blood (< 20~25 mL), etc., and
333 the latter is characterized by surefire engraftment, large-volume of bone marrow
334 (approximately 600~1000mL), etc. Further investigation to compare the behavior of cord
335 blood graft after intrabone and intravenous injection in human body should be performed.
336 The major advantages of intrabone CBT compared to double-unit CBT and ex vivo
337 expanded CBT are an inexpensive method and easy technique, and additional cord blood
338 units, recombinant cytokines, drugs, and cell processing technique and facilities are all not
339 required. The present study as well as a previous study [17] confirmed safety of the
340 injection of unwashed cord blood into the bone marrow cavity. Further efforts to optimize

341 preconditioning regimen, GVHD prophylaxis, and cord blood selection for intrabone

342 single-unit CBT is needed.

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507 **Figure legends**

508 **Fig. 1.** Hematological recovery after intrabone single-unit CBT without ATG. Cumulative

509 incidences of neutrophil $\geq 0.5 \times 10^9$/L (a), reticulocytes $\geq$ 1% (b), platelet $\geq 20 \times 10^9$/L (c),

510 and platelet $\geq 50 \times 10^9$/L (d) are shown.

511

512 **Fig. 2.** Neutrophil recoveries according to the cell dose and HLA compatibility. Cumulative

513 incidences of neutrophil $\geq 0.5 \times 10^9$/L according to total nucleated cell dose (high: $\geq 2.5 \times$

514 10^7 cells/kg, low: $< 2.5 \times 10^7$ cells/kg) (a), CD34+ cell dose (high: $\geq 1.0 \times 10^5$ cells/kg, low:

515 $< 1.0 \times 10^5$ cells/kg) (b), and the number of HLA-A, -B, and -DR antigen mismatches (c) are

516 shown.

517

518 **Fig. 3.** Platelet recoveries according to the cell dose and HLA compatibility. Cumulative

519 incidences of platelets $\geq 50 \times 10^9$/L according to total nucleated cell dose (high: $\geq 2.5 \times 10^7$

cells/kg, low: <2.5×10^7 cells/kg) (a), CD34+ cell dose (high: ≥1.0×10^5 cells/kg, low: <1.0×10^5 cells/kg) (b), and the number of HLA-A, -B, and -DR antigen mismatches (c) are shown.

Fig. 4. Acute GVHD after intrabone single-unit CBT without ATG. Cumulative incidences of grade II–IV (a) and grade III–IV (b) acute GVHD are shown.

Fig. 5. Overall survival for all patients.

Table 1 Patient characteristics (n = 62)

Age at transplant, median (range), y	48 (17−69)
Sex, n	
Male/female	31/31
Disease, n (%)	
AML	33 (53%)
ALL	12 (19%)
MDS	8 (13%)
CML	3 (5%)
ATLL	2 (3%)
T-LBL	2 (3%)
MPAL	1 (2%)
CMML	1 (2%)
Disease risk, n (%)	
Standard-risk†	35 (56%)
High-risk‡	27 (44%)
Preconditioning, n (%)	
Myeloablative	38 (61%)
Nonmyeloablative	24 (39%)
GVHD prophylaxis, n (%)	
CsA + sMTX	11 (18%)
CsA + MMF	3 (5%)
Tac + sMTX	42 (67%)
Tac + MMF	6 (10%)
HLA compatibility, n (%)	
Host-versus-graft direction	
6/6	5 (8%)
5/6	16 (26%)
4/6	41 (66%)
Graft-versus-host direction	
6/6	5 (8%)
5/6	20 (32%)
4/6	37 (60%)
Cord blood	
TNC, median (range), × 10^7/kg	2.57 (2.02−4.00)
CD34+ cells, median (range), × 10^5/kg	0.99 (0.44−2.30)

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; ATLL, adult T-cell leukemia/lymphoma; T-LBL, T-cell lymphoblastic lymphoma; MPAL, mixed phenotype acute leukemia; CMML, chronic myelomonocytic leukemia; GVHD, graft-versus-host disease; CsA, cyclosporine; sMTX, short-term methotrexate; MMF, mycophenolate mofetil; Tac, tacrolimus; TNC, total nucleated cells

† AML in the first or second remission, ALL in the first remission, CML in the first chronic phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts; ‡ All other diseases than standard-risk disease

Table 2 Summary of the prospective studies of intrabone single-unit cord blood transplantation

Reports	Frassoni et al. Lancet Oncol 2008 [14]	Bonifazi et al. Bone Marrow Transplant 2019 [18]	Murata et al. Cancer Sci 2017 [15]	Kurita et al. Bone Marrow Transplant 2017 [16]	Okada et al. Eur J Haematol 2018 [17]	This report
N	32	23	21	15	40	62
Age at transplant, median (range), y	36 (18−66)	36 (16−57)	57 (38−66)	59 (32−64)	62 (28−70)	48 (17−69)
Disease, n (%)						
Acute leukemia	32 (100)	20 (87)	11 (52)	13 (87)	24 (60)	45 (73)
Other malignancies	0 (0)	3 (13)	10 (48)	2 (13)	16 (40)	17 (27)
Cord blood						
TNC, median (range), × 10^7/kg	2.6 (1.4−4.2)	3.04 (1.91−5.48)	2.7 (2.0−4.9)	2.8 (2.0−5.0)	2.59 (1.96−4.08)	2.57 (2.02−4.00)
CD34+ cells, median (range), × 10^5/kg	1.0 (0.47−2.13)	1.29 (0.12−3.46)	0.92 (0.44−3.14)	no data	0.68 (0.30−2.01)	0.99 (0.44−2.30)
Preconditioning, n (%)						
Myeloablative	30 (94)	23 (100)	0 (0)	5 (33)	0 (0)	38 (61)
Nonmyeloablative	2 (6)	0 (0)	21 (100)	10 (67)	40 (100)	24 (39)
GVHD prophylaxis, n (%)						
CsA + MMF	32 (100)	23 (100)	0 (0)	0 (0)	40 (100)	3 (5)
CsA + sMTX	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	11 (18)
Tac + MMF	0 (0)	0 (0)	0 (0)	12 (80)	0 (0)	6 (10)
Tac + sMTX	0 (0)	0 (0)	21 (100)	2 (13)	0 (0)	42 (67)
Tac alone	0 (0)	0 (0)	0 (0)	1 (7)	0 (0)	0 (0)
Use of ATG as GVHD prophylaxis, n (%)	32 (100)	23 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Cumulative incidence of neutrophil engraftment, %	85	82	76	87	86	81
Median time to neutrophil engraftment, d	23	21.5	17	17	17.5	21
Acute GVHD, %						
Grade II-IV	15	14	44	38	61	29
Grade III-IV	0	5	19	0	15	7

TNC, total nucleated cells; GVHD, graft-versus-host disease; CsA, cyclosporine; MMF, mycophenolate mofetil; sMTX, short-term methotrexate; Tac, tacrolimus; ATG, antithymocyte globulin

Fig. 1

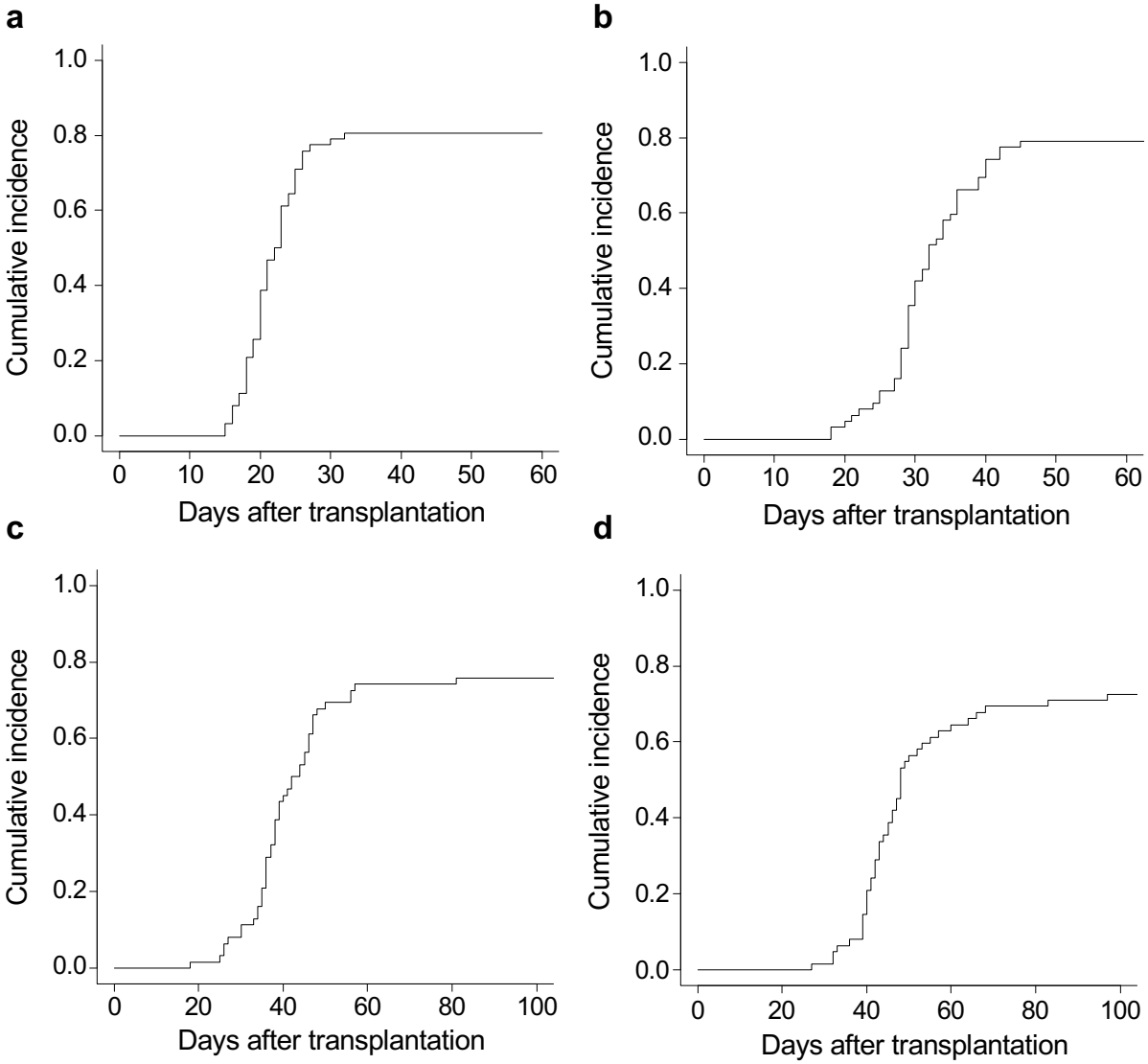

a
b
c
d
Cumulative incidence
Days after transplantation
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
80
100

Fig. 2

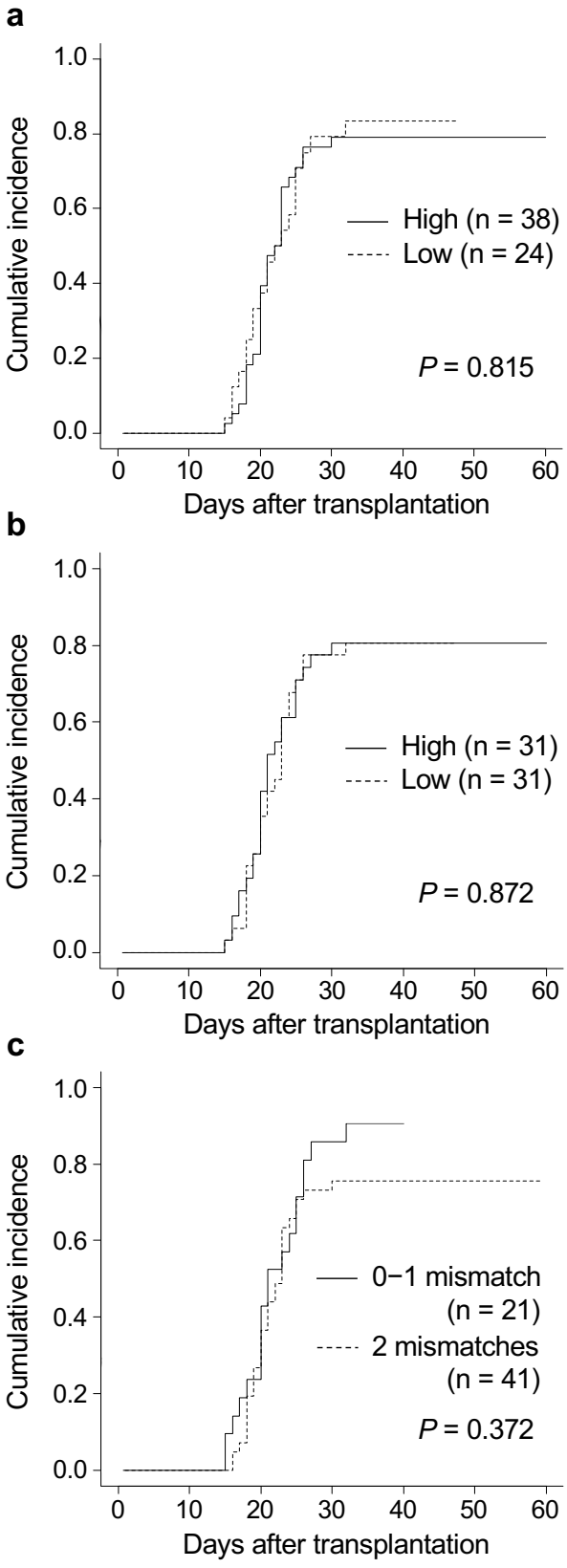
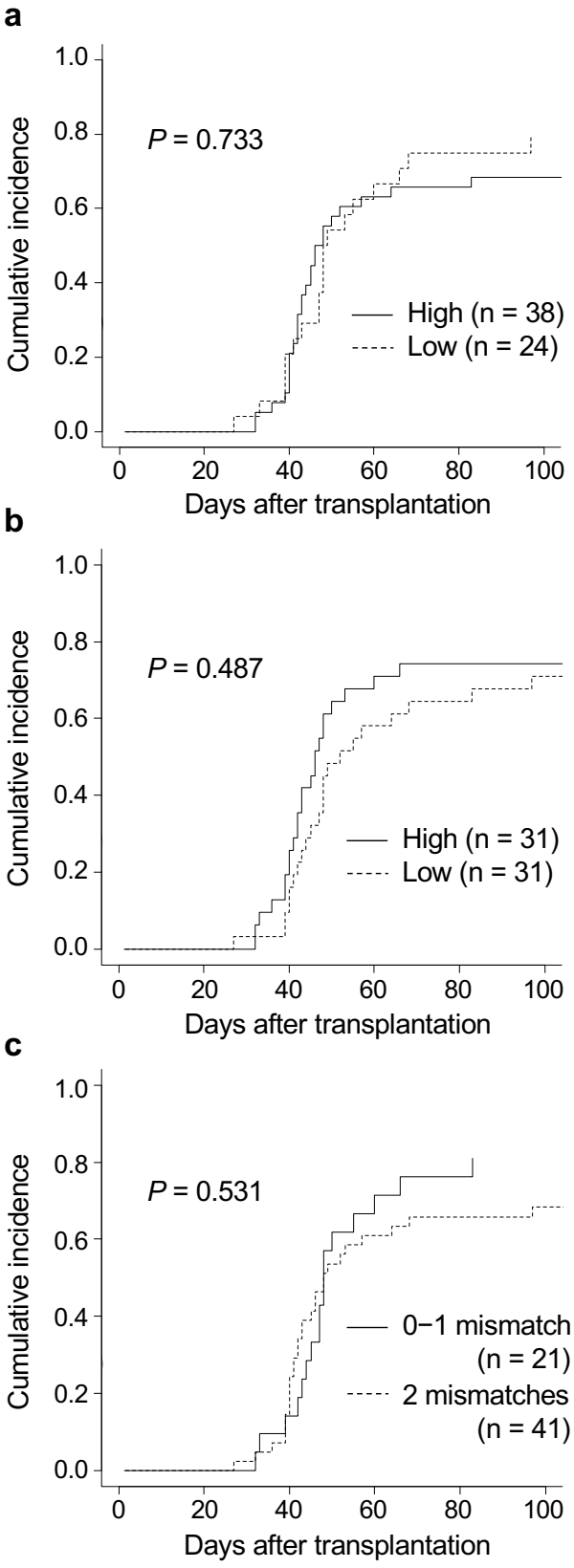

a
Cumulative incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Days after transplantation
High (n = 38)
Low (n = 24)
P = 0.815
b
Cumulative incidence
Days after transplantation
High (n = 31)
Low (n = 31)
P = 0.872
c
Cumulative incidence
Days after transplantation
0−1 mismatch
(n = 21)
2 mismatches
(n = 41)
P = 0.372

Fig. 3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0.8
0.6
0.4
0.2
0.0
Cumulative incidence
P = 0.733
High (n = 38)
Low (n = 24)
0
20
40
60
80
100
Days after transplantation
b
1.0
0.8
0.6
0.4
0.2
0.0
Cumulative incidence
P = 0.487
High (n = 31)
Low (n = 31)
0
20
40
60
80
100
Days after transplantation
c
1.0
0.8
0.6
0.4
0.2
0.0
Cumulative incidence
P = 0.531
0−1 mismatch
(n = 21)
2 mismatches
(n = 41)
0
20
40
60
80
100
Days after transplantation

Fig. 4

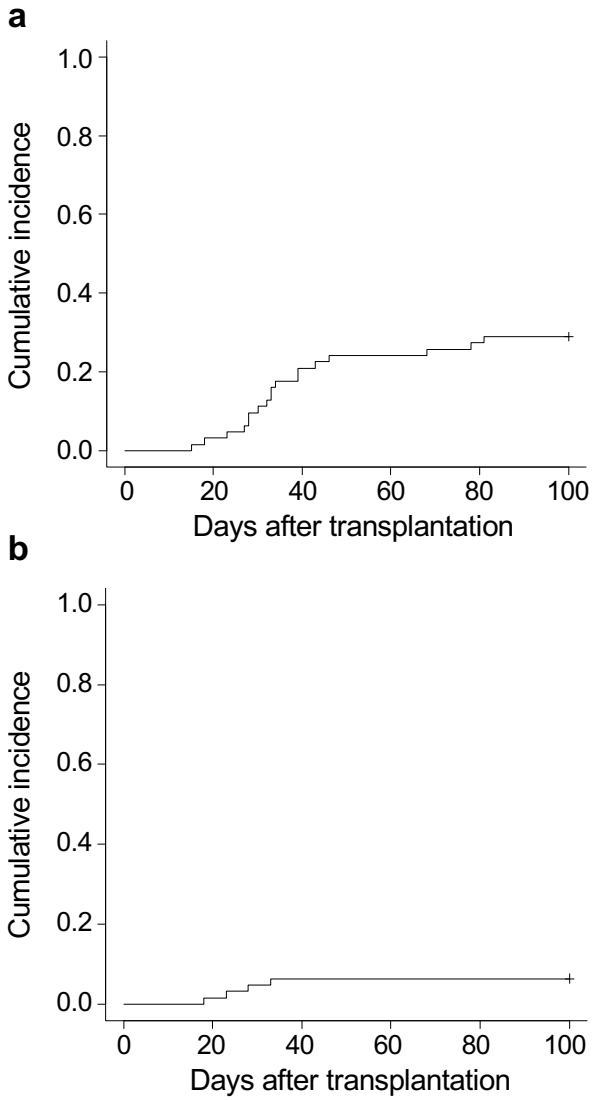
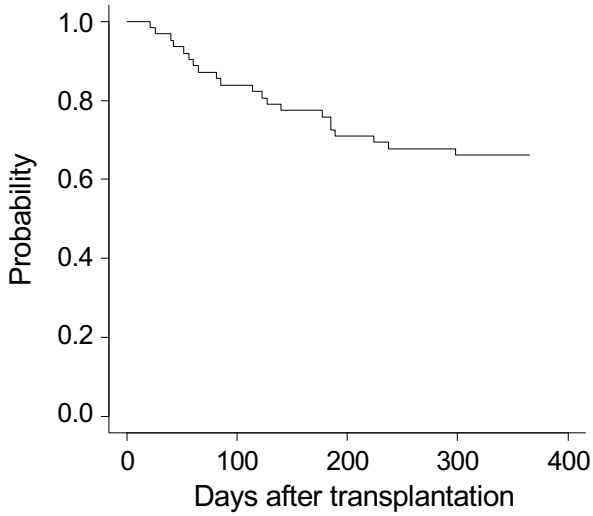

a
Cumulative incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Days after transplantation
b
Cumulative incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Days after transplantation

Fig. 5


1.0
0.8
0.6
0.4
0.2
0.0
Probability
0
100
200
300
400
Days after transplantation