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130 patients who underwent HSCT for CD40L deficiency in 36 transplant centers worldwide

HSCT Outcome
- Year of HSCT ≥2000
- Age at HSCT <10 years
- **No organ damage** before HSCT

HSCT Outcome
- Year of HSCT <2000
- Age at HSCT ≥10 years
- **Organ damage** before HSCT

OS DFS
- Use of myeloablative regimens (MAC/MAC low tox)
- HSCT ≤2 years from diagnosis

OS DFS
- Use of RIC
- HSCT >2 years from diagnosis

EFS
- Matched sibling donors
- Use of MAC
- Use of BM-derived stem cells

EFS
- Unrelated or mismatched donors
- Use of RIC/MAC low tox
- Use of PB or CB-derived stem cells

BM, bone marrow   HSCT, hematopoietic stem cell transplantation   OS, overall survival
CB, cord blood   MAC, myeloablative conditioning   PB, peripheral blood
DFS, disease-free survival   MAC low tox, myeloablative conditioning   RIC, reduced intensity conditioning
EFS, event-free survival   with less toxicity

Hematopoietic stem cell transplantation for CD40 ligand deficiency: results from an EBMT/ESID-IEWP-SCETIDE-PIDTC Study

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147

148 **Abstract**

149 **Background:** CD40 ligand (CD40L) deficiency, an X-linked primary immunodeficiency, causes

150 recurrent sinopulmonary, *Pneumocystis* and *Cryptosporidium* infections. Long-term survival with

151 supportive therapy is poor. Currently, the only curative treatment is hematopoietic stem cell

152 transplantation (HSCT).

153 **Objective:** We performed an international collaborative study to improve patients' management,

154 aiming to individualize risk factors and determine optimal HSCT characteristics.

155 **Methods:** We retrospectively collected data on 130 patients who underwent HSCT for CD40L

156 deficiency between 1993-2015. We analyzed outcome and variables relevance with respect to

157 survival and cure.

158 **Results:** Overall survival (OS), event-free survival (EFS) and disease-free survival (DFS) were

159 78.2%, 58.1% and 72.3% 5 years post-HSCT. Results were better in transplants performed ≥2000

160 and in children <10 years old at HSCT. Pre-existing organ damage negatively influenced outcome.

161 Sclerosing cholangitis was the most important risk factor. After 2000, superior OS was achieved

162 with matched donors. Use of myeloablative regimens and HSCT ≤2 years from diagnosis associated

163 with higher OS and DFS. EFS was best with matched sibling donors, myeloablative conditioning

164 (MAC) and bone marrow-derived stem cells. Most rejections occurred after reduced intensity or

165 non-myeloablative conditioning, which associated with poor donor cell engraftment. Mortality

166 occurred mainly early after HSCT, predominantly from infections. Among survivors who ceased

167 immunoglobulin replacement, T-lymphocyte chimerism was ≥50% donor in 85.2%.

168 **Conclusion:** HSCT is curative in CD40L deficiency, with improved outcome if performed before

169 organ damage development. MAC is associated with better OS, EFS and DFS. Prospective studies

170 are required to compare risks of HSCT with those of life-long supportive therapy.

171

6

Key messages

- HSCT can be curative in CD40L deficiency, with best outcome if performed before 10 years of age and without organ damage, especially sclerosing cholangitis.

- Superior OS was achieved with matched donors. HSCT early after diagnosis and use of myeloablative regimens resulted in higher OS and DFS. EFS resulted improved with matched sibling donors, myeloablative conditioning and bone marrow as stem cell source.

- Reduced intensity and non-myeloablative conditioning were associated with poor donor cell engraftment.

Capsule Summary

This manuscript reports the results of a worldwide survey of HSCT outcome in a large cohort of patients with CD40L deficiency. Key findings about survival and cure rate will be relevant to improve patients' management.

Key words

CD40 ligand, hematopoietic stem cell transplantation, X-linked hyper-IgM syndrome, primary immunodeficiency.

Abbreviations

AIHA	AutoImmune Hemolytic Anemia
ATG	Anti-Thymocyte Globulin
BM	Bone Marrow
Bu	Busulfan
CD40L	CD40 ligand
CSM	Class-switched memory
DFS	Disease-Free Survival
DLI	Donor Lymphocyte Infusion
EBMT	European Society for Blood and Marrow Transplantation
EFS	Event-Free Survival
ESID	European Society for Immunodeficiencies
FTT	Failure To Thrive
FU	Follow up
G-CSF	Granulocyte colony-stimulating factor
GVHD	Graft-versus-Host Disease
HSCT	Hematopoietic Stem Cell Transplantation
IEWP	Inborn Errors Working Party
MAC	Myeloablative Conditioning
MMFD	Mismatched Family Donor
MMUD	Mismatched Unrelated Donor
MSD	Matched Sibling Donor
MUD	Matched Unrelated Donor
NMA	Non-myeloablative
OS	Overall Survival

215	PBSC	Peripheral Blood Stem Cell
216	PID	Primary Immune Deficiency
217	PIDTC	Primary Immune Deficiency Treatment Consortium
218	PJP	*Pneumocystis jiroveci* pneumonia
219	PML	Progressive multifocal leukoencephalopathy
220	RIC	Reduced Intensity Conditioning
221	SCETIDE	Stem Cell Transplant for primary Immune Deficiencies in Europe
222	SE	Standard Error
223	TMP-SMX	Trimethoprim-Sulfamethoxazole
224	UCB	Umbilical Cord Blood
225	VOD	Veno-Occlusive Disease
226	vs.	versus

227

Introduction

CD40 ligand (CD40L) deficiency [X-linked hyper-IgM syndrome type 1[1,2] (XHIM, OMIM#308230)] is a rare X-linked primary immunodeficiency (PID) caused by mutations in *CD40LG*, on chromosome Xq26.3-Xq27.1, encoding the transmembrane CD40L glycoprotein[3–8] (CD154, OMIM#300386). Mutations in *CD40LG* result in altered co-stimulatory T-lymphocyte function[9] which impairs B-lymphocyte isotype switching, antibody production, and dendritic cell signaling. Myeloid cell function and development are also impaired[10,11]. This leads to increased susceptibility to bacterial and intracellular pathogens.

Patients usually present in early childhood with recurrent upper and lower respiratory tract infections, and *Pneumocystis jiroveci* interstitial pneumonia (PJP)[12,13]. Acute or chronic diarrhea is frequently associated with *Cryptosporidium spp* infection that may lead to severe biliary tract disease, especially sclerosing cholangitis and cirrhosis, and rarely cholangiocarcinoma, hepatocellular carcinoma, and adenocarcinoma[14].

An increased frequency of central nervous system infections [enteroviral meningoencephalitis[15], JC virus progressive multifocal leukoencephalopathy (PML)[16]], often resulting in neurodegeneration[12,17], has been reported.

Historically, long-term survival with conservative therapy has been poor, with 20-50% of patients surviving to the third decade[12,18,19]. Hepatic disease and severe infections represent the major causes of death[12], and many patients develop chronic comorbidities[18]. More recent data show a median survival time from diagnosis of 25 years in 109 patients with XHIM[20].

Currently, the only curative treatment is hematopoietic stem cell transplantation (HSCT). Numerous published case reports[21–36] and single centre experiences[37–42] report encouraging results, especially with an HLA-matched sibling donor (MSD). However, there is a risk of complications and overall survival (OS) is not optimal[18]. In the European retrospective analysis of 38 CD40L patients receiving HSCT[43], OS was 68%, with 32% of patients dying from infection-related complications, particularly severe cryptosporidiosis. Transplantation was curative in 58% of patients, 72% of those without hepatic disease. Pre-existing lung disease was the most important adverse risk factor.

The choice of performing early HSCT using myeloablative conditioning (MAC) or a later transplant with a reduced intensity conditioning (RIC) or treating patients with full supportive treatment only is still debated. Guidelines for the management of these patients were proposed by the European Society for Blood and Marrow Transplantation (EBMT)/European Society for Immunodeficiencies (ESID) Inborn Errors Working Party (IEWP) in 2011[44]. Recommendations about HSCT based on donor type and disease-related complication status, favored HSCT at diagnosis when a MSD was available and medical support until development of early complications for matched (MUD) or

262   mismatched (MMUD) unrelated donors, and progressive organ damage for mismatched related

263   donors (MMFD). A recently published study[45] reported improved survival in 29 Japanese patients

264   undergoing HSCT (OS 86.2%), with better event-free (EFS) and disease-free (DFS) survival in

265   children younger than 5 years of age at time of transplantation. A multi-centre study comparing

266   outcomes with or without HSCT showed an 85% OS in 67 patients in the transplant group[20].

267   We report the results of a retrospective international collaborative study on patients who underwent

268   HSCT for CD40L deficiency between 1993-2015, reported in the Stem CEll Transplant for primary

269   Immune Deficiencies in Europe (SCETIDE) and EBMT registries, and from North American

270   Primary Immune Deficiency Treatment Consortium (PIDTC) centers. We analyzed outcome and

271   relevance of different variables with respect to survival and cure rate after HSCT, aiming to

272   individualize specific risk factors for patients and determine the optimal timing and type of HSCT.

273

274 **Patients, materials, and methods**

275 *Data collection*

276 Transplant centers known to have performed HSCT in CD40L deficient patients were identified

277 from SCETIDE and EBMT registries (for European, Saudi Arabian and Australian centers) and

278 through the network of PIDTC centers in the United States.

279 Retrospective data collection on the outcome of HSCT was performed by a comprehensive

280 questionnaire for 130 patients with CD40L deficiency, transplanted in 36 centers in 18 countries,

281 over 4 continents (see Table E1 in the Online Repository), between 1993 and 2015, with a follow

282 up (FU) between 0.2 and 17.6 years (median: 4.1 years). Data from 35 patients have been

283 previously published [1,20,21,33,34,36,39,42,43,46–49].

284 Patients in whom the diagnosis of CD40L deficiency was based on molecular genetic analysis

285 and/or evidence of absent protein were included in the study. Five patients (3.8%) had no available

286 molecular diagnosis or protein expression data, but were included based on their clinical history and

287 presentation. Of these, 3 were transplanted before 2000 and died. At that time, molecular diagnosis

288 was not always performed, and it was not possible to pursue diagnosis after death.

289 Centers were responsible for acquiring informed consent from patients and families for data

290 collection and for quality of data entry.

291

292 *Patient characteristics*

293 Patient clinical features pre-HSCT are summarized in Table I by year of HSCT, showing significant

294 differences between the two historical cohorts. In particular, patients transplanted before 2000 were

295 transplanted at an older age and at a greater interval after diagnosis, and they were clinically more

296 compromised (> organ damage, especially liver disease, before transplant).

297 Median age at diagnosis was 11 months (range: 0-131), and was not significantly influenced by the

298 historical period. Forty-seven patients were diagnosed in the first 6 months of life, 11 at birth due to

299 positive family history. CD40L protein expression on activated CD4+ T-lymphocytes was available

300 for 87 patients (66.9%), absent in the majority (81.6%), most frequently quantified using flow

301 cytometry. Diagnosis was confirmed by CD40L gene analysis in 108 patients (83.1%), which

302 showed mainly deletions and missense mutations (see Table E2 in the Online Repository). CD40L

303 expression before HSCT did not significantly differ in patients with these types of mutations.

304 Additional details on the cohort clinical characteristics are reported in the Online Repository

305 material.

306

307 *Transplantation*

308 Patients' performance status at time of transplant was determined by Lansky or Karnofsky score,

309 according to age. Most patients (70.2%) transplanted after 2000 had a score ≥90 at first HSCT.

310 These data were unavailable for most transplants performed before 2000. Characteristics of first

311 HSCT, second HSCT, boosts and donor lymphocyte infusions (DLI) are summarized in Tables II

312 and E3 in the Online Repository. Conditioning regimens were grouped according to their intensity

313 and toxicity features into the following 4 types: MAC, myeloablative with low toxicity (MAC low

314 tox), RIC[50,51] and non-myeloablative (NMA) conditioning (see Table E4 and Figure E1 in the

315 Online Repository). MAC was the most commonly used conditioning for first transplants in the

316 historical group (92%), while after 2000, the use of RIC and MAC low tox regimens has increased

317 (24% and 20% respectively; p=0.0034). NMA was used in 2 first and 2 second transplants. Due to

318 the low numerosity of this group, this was not included in statistical analyses. Since no data about

319 Busulfan (Bu) pharmacokinetics (AUC) were available, Bu-containing regimens were divided

320 between MAC and RIC groups based on the total dose of Bu administered in case of combination

321 with fludarabine (14.3-25.0 mg/kg in MAC, 4.0-13.6 mg/kg in RIC, see Figure E1 in the Online

322 Repository). In the other cases, classification as MAC was based on other features (*e.g.*

323 combination with Cyclophosphamide), not solely on Bu dose.

324 Donor type was defined as: MSD, MUD (10/10, 12/12 or 8/8 HLA match), MMUD (with ≥1

325 mismatch); MMFD (with ≥1 mismatch), usually a haploidentical parent. Data about methods used

326 for HLA match testing were available for only 51.3% of the procedures, with molecular techniques

327 used in the majority of cases (75.3%). Data from donors with unavailable or inaccurate information

328 about degree of matching (number of loci studied <8 for non-sibling donors) were excluded from

329 statistical analysis.

330 MSD were the preferred donor types before 2000. The proportion of unrelated donors has since

331 increased for both matched and mismatched (39% and 31% respectively), mainly represented by

332 adult volunteers (Table II).

333 Stem cell source was bone marrow (BM), peripheral blood stem cells (PBSC) and umbilical cord

334 blood (UCB). Until 1999, BM was the only stem cell source used for first HSCT. Use of PBSC and

335 UCB became subsequently more common (31% and 10% HSCT respectively, p=0.0006 - Table II).

336 T-lymphocyte depletion of the graft was performed in 28 procedures, mainly through positive

337 selection of CD34+ cells (n=19). This technique was used in all cases of PBSC transplants from

338 MMFD (n=4), 8 MMUD and 7 MUD transplants. In 6 recent unrelated donor PBSC transplants

339 performed in a single centre since 2012, TCR alpha-beta depletion was used. *Ex vivo* graft

340 manipulation details are reported (see Table E5 in the Online Repository). In vivo T-lymphocyte

341 depletion was performed mainly by the use of anti-thymocyte globulin (ATG, 51.3%) and

13

342 alemtuzumab (20%), especially in the unrelated donor setting (see Table E4 in the Online

343 Repository and data not shown).

344 Graft-versus-host disease (GVHD) prophylaxis was used in most procedures (92%). No additional

345 GVHD prophylaxis was administered in 8/19 transplants with CD34+ cell selection and in 1 boost.

346 GVHD prophylaxis regimen was based on cyclosporine administration in 88.4% of cases, alone

347 (25.4%) or in combination with other drugs, mainly methotrexate (29.7%), mycophenolate mofetil

348 (19.6%) or corticosteroids (9.5%). Acute GVHD was graded according to EBMT guidelines,

349 defined as severe when ≥grade 3. Chronic GVHD was classified as extensive or limited, based on

350 the clinical severity and extent of target organ involvement.

351 Donor chimerism was defined as complete if ≥95% cells were of donor origin, partial if between

352 5% and 95%, and absent if donor cells represented ≤5% of total cells. Partial chimerism analysis on

353 purified cell subpopulations (granulocytes, CD3+ T-lymphocytes and CD19+ B-lymphocytes) was

354 analyzed in a subgroup of patients, subdivided into predominantly donor (50-94%) and

355 predominantly recipient (6-49%). Fluorescence in situ hybridization or molecular testing based on

356 short tandem repeats analysis, were used to monitor donor cell chimerism.

357 Additional details are reported in the Online Repository material.

358

359 *Statistical analysis*

360 The description of continuous variables was done using median and range or interquartile range,

361 while the comparison between groups was based on the Wilcoxon Rank Sum test. Categorical

362 variables were analyzed through frequency distributions and compared using the Chi-Square or the

363 Fisher's exact test, as appropriate.

364 OS, EFS and DFS calculations were performed both in the whole cohort of patients, and in the

365 subgroups of patients transplanted before ("*historical cohort*") or since 2000. Comparisons of these

366 two groups are shown in Figures 1, 2 and E2 in the Online Repository. Results from the analyses

367 focused on most recently transplanted patients, more representative of current clinical practice, are

368 reported in Table III. EFS was calculated as the time from HSCT to the first of the following

369 events: graft failure/absent engraftment, need for second HSCT, boost or DLI, grade 4 acute GVHD

370 or extensive chronic GVHD, requirement for immunoglobulin supplementation for >2 years after

371 HSCT or death. Events for the calculation of DFS were the ongoing requirement of

372 immunoglobulin supplementation 2 years after the last procedure and death, while the only event

373 considered for OS was death from any cause. Observations of patients were censored at the date of

374 last contact when no events were observed. The Kaplan-Meier method was used to estimate the

375 probabilities of OS, EFS and DFS, with standard errors (SE) calculated according to Greenwood.

376 Curves were compared using the log-rank test and pairwise comparisons were adjusted for
377 multiplicity according to Sidek, while the Cox proportional hazard model was used for
378 multivariable analyses. All the tests were performed two-sided with a 0.05 level of significance.
379 The analyses were performed in SAS 9.3 software (SAS Institute Inc., Cary, USA) and R 3.2.2
380 software (R Foundation for Statistical Computing, Vienna, Austria).
381

Results

Overall Survival

Data from 154 procedures were collected: 130 first, 13 second and 1 third HSCT, 6 cell boosts (infusions of cells from the same donor without conditioning) and 4 DLI. Most were performed since 2000. Median age at first transplant was 4.0 years (range: 0.5-38.3 years). Patients from the historical cohort were transplanted at an older age (median: 8.5 years) compared to those treated after 2000 (median: 3.4 years, p=0.0012). Median time interval between diagnosis and HSCT was 2.0 years, slightly higher for HSCT before 2000 (3.9 years, p=0.0012) (Table I).

Overall survival (OS) after first HSCT improved[43], reaching 81% and 78.2% at 2 and 5 years respectively. In particular, as observed in other PID, outcome improved after 2000, likely due to improvement in transplant-related procedures and patients' management (5 year-OS before 2000, 58.3%; since 2000, 82.2%; p=0.0030).

Patients transplanted younger than 5 years of age reached nearly 90% OS at 2 and 5 years after HSCT. Those older than 10 years at treatment had a 37.8% OS at 5 years (p<0.0001). This "*age-effect*" was also observed in transplants since 2000, although a slight improvement in OS was noted in older patients (OS 43.8% at 5 years, Table III and Figure 1A). Age at diagnosis (< vs. >12 months) did not influence OS. Waiting time between diagnosis and HSCT had an impact on outcome, with significantly better survival for those transplanted within 2 years from diagnosis (Figure 1F).

Pre-existing organ damage (mainly chronic lung disease and/or liver dysfunction) before HSCT negatively influenced outcome (OS 61.5% at 2 years, 55.6% at 5 years; without organ damage, OS 92.9% at 2 and 5 years, p<0.0001). Liver disease, especially sclerosing cholangitis, was the most important adverse risk factor (OS 51.2% and 46.9% at 5 years respectively, p<0.0001), followed by protracted diarrhea (OS 55.5% at 5 years, p=0.0002) and cryptosporidial gastrointestinal infection (OS 59.6% at 5 years, p=0.0004). These clinical features were confirmed to negatively influence outcome also in most recent transplants, even if less profoundly (Figure 1B-E and Table III). Presence of chronic lung disease, previously a significant risk factor[43], did not significantly influence survival in recent transplants. Type of CD40L gene mutation, previous clinical history of respiratory tract infections, including PJP, requirement of ventilation before transplant, neutropenia, oral ulcers, failure to thrive (FTT) and absent *Cryptosporidium* prophylaxis before HSCT had no significant influence on OS.

Use of myeloablative conditioning regimens resulted in better survival as compared to RIC after year 2000 (p=0.0073), with significant differences emerging at pairwise comparison between MAC

16

415   low tox or MAC and RIC (p=0.0197 and p=0.0258, respectively – Table E6). Of note, OS in

416   patients receiving MAC improved in recent years (Table III, Figure E3A).

417   Finally, a significant difference in OS emerged between different donor types (whole cohort,

418   p=0.0198; >2000 p=0.0373), with better survival achieved with matched donors (both sibling and

419   unrelated). However, at pairwise comparison, the difference in OS between MUD and MMUD was

420   attenuated in most recent years (p=0.0545), reflecting an improved outcome also in the mismatched

421   unrelated donor setting. Moreover, among adult volunteer donors, there seemed to be a negative

422   trend in OS with increasing number of mismatches (Table III, Table E7 and Figure E3B).

423

424   *Event-free Survival*

425   EFS after first HSCT was 62.6% and 58.1% at 2 and 5 years respectively, with only a slight

426   improvement after year 2000 (Table III). It was very low (25.2%) in patients transplanted at ≥10

427   years of age, but an improvement was observed in recent years in this subgroup (Figure 2A). Age at

428   diagnosis significantly influenced EFS, which appeared better in those diagnosed early (<1 year of

429   age), while the time interval from diagnosis to HSCT was not relevant (Table III and data not

430   shown).

431   Pre-existing organ damage significantly impacted EFS, in particular the presence of sclerosing

432   cholangitis, both in historical and in recent transplants, in spite of an improvement in the latter

433   (Figure 2B-C and Table III). Other clinical features before HSCT and genotype did not strongly

434   influence EFS.

435   MAC was associated with higher EFS (81.6% at 5 years in patients transplanted since 2000,

436   p<0.0001 – Table III and Figure 2F), as compared to MAC low tox and RIC (Table E6), possibly

437   explained by better engraftment of donor cells with this regimen or use in less compromised

438   patients. Stem cell source resulted in significant differences, with best EFS associated with BM

439   (73% at 5 years FU in patients transplanted since 2000, Table III and Figure 2E).

440   In recent years, no significant differences in EFS emerged between donor types in univariate

441   analysis (Table III, Figure 2D).

442   However, multivariable EFS analysis, performed on patients transplanted after 2000 with complete

443   data (n=96), showed donor type and conditioning regimen as the most significant influences. In

444   particular, patients receiving HSCT from mismatched or MUD donors showed respectively a 4.2-

445   and 3.3-fold increase in the hazard of event compared to those from MSD (p=0.0189, p=0.0607).

446   RIC use was associated with a 3.2-fold increased hazard ratio, as compared to MAC (p=0.0323).

447   Presence of pre-existing organ damage before HSCT was associated with a 2.7-fold increased

448 hazard (p=0.1036). Pre-transplant sclerosing cholangitis and age at HSCT had no relevant role on

449 EFS (see Table E8 in the Online Repository material).

450

451 Results of DFS analysis are described in the Online Repository material (see Figure E2 in the

452 Online Repository).

453

454 *Causes of death*

455 Twenty-six deaths were reported, most of them transplant-related (n=22, 84.6%). Most occurred

456 within 6 months of HSCT (n=20, 76.9%), mainly caused by infections (see Figure E4 in the Online

457 Repository). Liver failure was the cause of death of 2 patients with pre-existing sclerosing

458 cholangitis, who experienced severe liver GVHD, *Cryptosporidium* infection and VOD after

459 transplant. Graft rejection was reported as primary cause of death in 3 patients.

460 Four non-transplant-related deaths were due to progression of original disease. In 2 cases,

461 neurological complications occurred, with progressive neurodegeneration in one patient and

462 worsening PML in another patient with history of JC virus encephalitis before transplant. In the

463 other 2 cases, infection (n=1) and deteriorating liver function (n=1) were complicated by previous

464 graft rejection (Table IV).

465

466 *Rejection*

467 Eighteen patients (14.8% of 122 patients with available data) experienced graft rejection after first

468 transplant (Table IV). Most occurred within 6 months of HSCT (72.2%), mainly after unrelated

469 donor transplant (83.3%, 10 MUD, 5 MMUD of which 3 adult volunteers and 2 UCB). Stem cell

470 source was BM, PBSC or UBC in 8, 8 and 2 patients respectively. Positive selection of CD34+ cells

471 was performed in 3 procedures. RIC was the most common conditioning regimen (n=8), followed

472 by MAC (n=5), MAC low tox (n=3) and NMA (n=2). Most patients experienced infections in the

473 first 6 months of FU after first transplant, mainly of viral origin. No signs of acute GVHD were

474 observed in 72.2% patients in this subgroup.

475 Most patients who rejected their first HSCT received further therapeutic interventions (10 second

476 HSCT, 1 third HSCT and 1 cell boost) after a median of 11.7 months from the first transplant. Most

477 were alive at last FU (81.8%), and in 66.7% immunoglobulin supplementation could be

478 discontinued. Seven patients did not receive additional cell therapy procedures. Three of these

479 patients continued supportive care with immunoglobulin supplementation and are alive, while the

480 remaining 4 died. Deaths occurred at a median of 25 months after HSCT, mainly due to disease

481 progression (infections, deteriorating liver function). Donor type, stem cell source and the

482 occurrence of viral infections early after HSCT or acute GVHD did not significantly influence the

483 risk of rejection.

484

485 Information on additional procedures can be found in the Online Repository material (see Table E3

486 in the Online Repository).

487

488 *Engraftment and cure rate*

489 Transplantation resulted in complete or partial donor chimerism in most patients, stable over time to

490 last FU (Figure 3A). Data about lineage-specific donor chimerism were available only for a

491 subgroup of patients. Median lineage-specific chimerism remained stably ≥88% up to last FU (>1

492 year after last procedure) in both granulocytes and T-lymphocytes, while in B-lymphocytes a slight

493 reduction in donor chimerism was observed over time (median donor chimerism: 75%) (Figure 3B).

494 At last available FU (>1 year) after last procedure (see Figure E5 in the Online Repository), donor

495 cell engraftment in granulocytes (CD15+ cells) and in T-lymphocytes (CD3+ cells) was complete

496 or predominantly donor in 78.1% and 82.9% patients with available data, respectively, while in B-

497 lymphocytes, a higher percentage of predominantly recipient chimerism was observed (35.7%

498 patients).

499 Decreasing lineage-specific chimerism was observed over time in 27.8% transplants (with FU ≥1

500 year, among those with available data). However, in another 25% transplants, increasing donor cell

501 chimerism in T- and B-lymphocyte subpopulations was observed (Figure 4A). In this subgroup, 3

502 patients received DLI infusion with favorable effect on donor cell chimerism.

503 Among survivors who ceased immunoglobulin replacement ≥2 years after last procedure and for

504 whom data were available, T-lymphocyte chimerism was complete or predominantly donor in

505 85.2%. B-cell chimerism was full donor in 7, and predominantly recipient (range: 18-43% donor

506 chimerism) in 5 of them (Figure 4B).

507 A higher percentage of complete donor chimerism (63.2%) was observed in transplants in which

508 patients did not experience viral infections after HSCT (Figure 4C). Moreover, viral infections after

509 HSCT may have influenced T-lymphocyte chimerism kinetics: in the majority of transplants in

510 which decreasing T-lymphocyte chimerism was observed (91.7%), viral infections occurred in early

511 FU, likely favoring the expansion of autologous lymphocytes to replenish the niche (Figure 4D and

512 data not shown).

513

514 Immune reconstitution and data regarding complications (see Table E9 in the Online Repository)

515 can be found in the Online Repository material.

Discussion

This is the largest HSCT series for CD40L deficiency collected worldwide to date. It includes data from 130 patients transplanted over more than 20 years. Interestingly, the comparison of the 2 historical cohorts of patients, treated before and after 2000, clearly shows how patients' features have changed over time, mainly thanks to improvement in diagnostic tools and clinical management. Most recent patients have been transplanted at a younger age, with shorter time interval after diagnosis, and with lower organ damage burden. All this factors have contributed to the general HSCT outcome improvement observed in the past years.

These differences, though interesting, represented a difficulty in data analysis that was hampered by the presence of potential confounding between variables. For this reason, for main outcome measures, we analyzed historical periods separately. In particular, we decided to perform multivariate analysis only on most recent transplant cohort since it could not be performed including the "*period effect*" due to statistical model limitations. Moreover, while the heterogeneity induced by the period is relevant, we think that the evaluation of the more recent patients' cohort is more interesting since it reflects more closely the current clinical practice.

Other limitations of the study are represented by the sample heterogeneity typical of retrospective observational studies, including many different centers and spanning over long time frames, and by unavoidable intrinsic correlations between variables, such as the choice of conditioning regimen and patient's clinical status. Furthermore, in spite the total number of patients included in the study is the highest ever collected for this disease, analyses on patients' subgroups were limited by small sample size, especially when evaluating different conditioning regimens, donor types and lineage-specific donor cell chimerism. This makes it difficult to draw strong conclusions, especially at longest follow up, but our study provides a number of novel and interesting findings that should be further explored in the future.

In spite of these difficulties, a number of important new observations emerge from this report. Firstly, overall survival post transplantation is now 80%, although there remain significant differences between those transplanted <10 years of age, and those transplanted when older, even in more recent years. Linked with this was a superior survival in those transplanted within 2 years of the diagnosis of CD40L deficiency and in those without organ damage, specifically liver disease. Importantly, in recent years, transplants from MSD and MUD had reached similar good results in terms of OS, but not EFS, which remained lower with unrelated or mismatched donors. Most patients who received MAC showed complete engraftment at last FU, whereas RIC was associated with absent engraftment. New conditioning regimens, specifically low toxicity MAC, had superior OS and DFS, but not EFS, as compared to RIC. This could likely be explained by the tendency to

550 reach lower level of myeloid chimerism over time in patients who received these conditioning

551 regimens, which may reflect decreased stem cell engraftment.

552 DFS was more likely with the use of myeloablation. Patients who ceased immunoglobulins were

553 stable over time, even if additional procedures (repeat HSCT, boost infusions) were required to

554 attain this in some cases. Among those with FU ≥2 years, median CD40L expression on activated

555 CD4+ T cells was 49% in those who stopped immunoglobulin supplementation and 14.5% in those

556 who still needed it. T-lymphocyte chimerism was complete or predominantly donor in most cured

557 patients, but unfortunately, a minimum T cell donor percentage reliably associated with

558 immunoglobulin independence could not be retrieved based on available data.

559 Deaths were mainly related to transplant-associated complications including graft rejection,

560 although a few were due to progression of pre-existing neurological disease. Rejection rate was

561 15%, usually occurring early after transplant, although re-transplantation was usually successful.

562 Among those who rejected their first transplant, only 11.1% received HSCT from MSD, in line with

563 the finding of lower EFS in transplants from other type of donors.

564 A higher percentage of complete donor chimerism (63.2%) was observed in transplants in which

565 patients did not experience viral infection after HSCT. Moreover, viral infection after HSCT may

566 have influenced T-lymphocyte chimerism kinetics: in the majority of transplants in which

567 decreasing T-lymphocyte chimerism was observed (91.7%), viral infections occurred in early FU,

568 likely favoring the expansion of autologous T-lymphocytes to replenish the niche.

569 Although we did not compare our results with non-transplanted patients, previous reports have

570 demonstrated similar survival as ours, although improved quality of life in those undergoing

571 HSCT[20]. However, from our data, clear trends emerge. HSCT is curative, but best results continue

572 to be seen in younger patients, with least organ damage and infection-free. Furthermore, MAC is

573 associated with a better immunological outcome than RIC regimens, again favoring earlier HSCT.

574 There is a need for prospective studies directly comparing risks of HSCT with those of life-long

575 immunoglobulin and prophylaxis. Additionally, advances in gene therapy, and particularly gene

576 editing may be attractive as a potential therapeutic alternative for those for whom HSCT is too risky

577 because of associated clinical features and poor donor options, particularly given that infusion of

578 gene-corrected T-lymphocytes may be curative[52].

579

580 **Conflicts of interest:** none.

581

582

21

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743

744 **Table list**

745

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758 **Figure 1. Characteristics influencing overall survival (OS) in patients receiving first**

759 **hematopoietic stem cell transplantation (HSCT) before/after 2000. (A)** Age at HSCT. Survival

760 curves of patients <5 and 5-10 years old at HSCT, transplanted before 2000, are superimposed. **(B)**

761 Organ damage before HSCT. **(C)** *Cryptosporidium* infection before HSCT. **(D)** All liver alterations.

762 **(E)** Sclerosing cholangitis. **(F)** Waiting time to HSCT from diagnosis. Under each graph, the

763 number of patients at risk at each follow up time point after HSCT is reported for all patient groups.

764 OS curves of the different patients' groups are represented by solid or dashed lines. For each of

765 them, a specific label is reported nearby the corresponding curve. yrs, years.

766

767 **Figure 2. Characteristics influencing event-free survival (EFS) in patients receiving first**

768 **HSCT before/after 2000. (A)** Age at HSCT. **(B)** Organ damage before HSCT. **(C)** Sclerosing

769 cholangitis before HSCT. **(D)** Donor type. **(E)** Source of stem cells. **(F)** Conditioning regimen.

770 Under each graph, the number of patients at risk at each follow up time point after HSCT is

771 reported for all patient groups. EFS curves of the different patients' groups are represented by solid

772 or dashed lines. For each of them, a specific label is reported nearby the corresponding curve. yrs,

773 years; BM, bone marrow; CB, cord blood; PB, peripheral blood; MAC, myeloablative conditioning;

774 MAC low tox, myeloablative conditioning with low toxicity; RIC, reduced intensity conditioning.

775

776 **Figure 3. Donor cell engraftment after first HSCT and after last procedure. (A)** Overall donor

777 cell engraftment over time, represented by percentage (%) of subjects with complete, partial or

778 absent engraftment on unsorted cells at different time points after first HSCT (*left panel*) and after

779 last procedure (*right panel*). *3 patients with full chimerism received donor lymphocyte infusions

780 (DLI); **% of those with available data. **(B)** Median lineage-specific donor cell engraftment over

781 time, at different time points after first HSCT (*left panels*) and after last procedure (*right panels*).

782 Data on unsorted cells, sorted myeloid cells (CD15), T lymphocytes (CD3) and B lymphocytes

783 (CD19) are reported. For each median value, interquartile range is plotted and the number of

784 subjects for whom data where available at each FU is reported in brackets. FU, follow up; mo.,

785 months.

786

787 **Figure 4. Engraftment kinetics and T-cell chimerism. (A)** Donor cell engraftment kinetics,

788 represented by the percentage (%) of transplant procedures in which increasing, declining or stable

789 donor cell engraftment was observed over time, ≥1 year after last procedure. °,1 or °°°,3 patient(s)

28

790   received DLI. Data on unsorted cells, sorted myeloid cells (CD15+), B lymphocytes (CD19+) and T

791   lymphocytes (CD3+) are reported. **(B)** T-cell and B-cell chimerism at last follow up (FU) in

792   survivors, OFF immunoglobulin replacement (IG) at 2 or more years (yr) after last procedure (*).

793   **(C)** T-cell chimerism at last FU, according to the occurrence of viral infections after HSCT

794   (YES/NO). **(D)** Donor T-cell chimerism kinetics over time (increasing/declining/stable), according

795   to the occurrence of viral infections after HSCT (YES/NO). °°°,3 patients received DLI. **% of

796   transplants (or subjects) with available data.

Table I – Clinical features of CD40L deficient-patients before first HSCT

Patients' features before HSCT	Total*	All patients (n=130)		HSCT up to 1999 (n=24)		HSCT since 2000 (n=106)		
		Median (range)		Median (range)		Median (range)		p-value
Age at diagnosis (months)	126	11.0	(0-131)	13.0	(3-129)	10.7	(0-131)	0.2466
Age at HSCT (years)	130	4.0	(0.5-38.3)	8.5	(1.0-18.1)	3.4	(0.5-38.3)	**0.0012**
Interval between diagnosis and HSCT (years)	126	2.0	(0-27.4)	3.9	(0.9-16.2)	1.5	(0-27.4)	**0.0012**
	Total*	n	(%)	n	(%)	n	(%)	p-value
CD40L expression	87							0.4525
- absent		71	(82)	11	(92)	60	(80)	
- low		16	(19)	1	(8)	15	(20)	
Age at HSCT (years)	130							**0.0320**
- 0-5		79	(61)	10	(42)	69	(65)	
- 5-10		26	(20)	5	(21)	21	(20)	
- >10		25	(19)	9	(37)	16	(15)	
Organ damage before HSCT	119	45	(38)	15	(71)	30	(31)	**0.0005**
Infections before HSCT								
- all	129	117	(91)	22	(96)	95	(89)	0.6919
- URTI	124	60	(48)	14	(67)	46	(45)	0.0659
- LRTI	125	86	(69)	15	(71)	71	(68)	0.7756
- PJP	108	47	(44)	7	(39)	40	(44)	0.6643
- Cryptosporidium	118	29	(25)	9	(47)	20	(20)	**0.0189**
Need of ventilation	106	38	(36)	6	(38)	32	(36)	0.8812
Chronic lung disease	114	17	(15)	5	(29)	12	(12)	0.1305
Neutropenia	123	57	(46)	11	(52)	46	(45)	0.5422
Oral ulcers	122	26	(21)	6	(29)	20	(20)	0.3869
Failure to thrive (FTT)	125	37	(30)	7	(33)	30	(29)	0.6812
Protracted diarrhoea	126	31	(25)	10	(48)	21	(20)	**0.0073**
Liver disease**	126	33	(26)	11	(50)	22	(21)	**0.0052**
Sclerosing cholangitis	125	28	(22)	9	(43)	19	(18)	**0.0211**
Autoimmunity	111	6	(5)	1	(7)	5	(5)	0.5636
Malignancies	119	3	(3)	2	(10)	1	(1)	0.0800
IG supplementation	125	123	(98)	19	(90)	104	(100)	**0.0271**
Cryptosporidium prophylaxis	100	31	(31)	7	(54)	24	(28)	0.1035
PJP prophylaxis	113	109	(97)	15	(88)	94	(98)	0.1068

CD40L, CD40 ligand; HSCT, hematopoietic stem cell transplantation; Q1, first quartile; Q3, third quartile; URTI, upper respiratory tract infections; LRTI, lower respiratory tract infections; PJP, *Pneumocystis jiroveci* pneumonia; IG, immunoglobulins.

Organ damage was defined as the presence of chronic lung disease and/or liver alterations (sclerosing cholangitis or liver fibrosis or hepatitis). Significant p-values (p <0.05) are in bold.

* Number of patients with available data.

** All liver alterations, including also ascending cholangitis, mild hepatic portal inflammation and minimal alterations.

Table II – Characteristics of first HSCT performed on 130 CD40L deficient-patients

First HSCT characteristics	Total*	All patients (n=130)		HSCT up to 1999 (n=24)		HSCT since 2000 (n=106)		p-value
		n	(%)	n	(%)	n	(%)	
Conditioning regimen	129							**0.0034**
- MAC		79	(61)	22	(92)	57	(54)	
- MAC low tox		21	(16)	0	(0)	21	(20)	
- RIC		27	(21)	2	(8)	25	(24)	
- NMA		2	(2)	0	(0)	2	(2)	
GVHD prophylaxis	129							1.0000
- Yes		123	(95)	23	(96)	100	(95)	
- No		6	(5)	1	(4)	5	(5)	
Donor type	123							0.3092
- MSD		37	(30)	10	(45)	27	(27)	
- MUD		46	(37)	7	(32)	39	(39)	
- ad. vol.		46	(100)	7	(100)	39	(100)	
- UCB		0	(0)	0	(0)	0	(0)	
- MMUD		36	(29)	5	(23)	31	(31)	
- ad. vol.		29	(81)	5	(100)	24	(77)	
- UCB		7	(19)	0	(100)	7	(23)	
- MMFD		4	(3)	0	(0)	4	(4)	
Stem cell source	129							**0.0006**
- BM		86	(67)	24	(100)	62	(59)	
- PBSC		33	(25)	0	(0)	33	(31)	
- UCB		10	(8)	0	(0)	10	(10)	

CD40L, CD40 ligand; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning; MAC low tox, myeloablative conditioning with low toxicity; NMA, non-myeloablative conditioning; RIC, reduced intensity conditioning; GVHD, graft-versus-host-disease; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; MMFD, mismatched family donor; BM, bone marrow; PBSC, peripheral blood stem cells; UCB, umbilical cord blood; DLI, donor lymphocyte infusion; ad. vol., adult volunteer.

Significant p-values (p <0.05) are in bold.

* Number of patients with available data.

Table III – OS, EFS and DFS in CD40L deficient-patients transplanted since year 2000

Characteristics	OS						EFS						DFS					
	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value
Overall	16/106	86.1	3.5	82.2	4.3	-	37/106	64.2	3.6	61.3	5.1	-	20/106	78.7	4.5	77.1	4.7	-
Age at HSCT (yrs)						**0.0005**						**0.0238**						**0.0001**
<5	6/69	91.0	3.5	91.0	3.5		24/69	64.3	6.1	62.1	6.3		8/65	85.4	4.9	85.4	4.9	
5-10	3/21	89.3	7.2	82.4	9.4		4/21	85.2	7.9	78.1	9.3		4/26	85.5	7.9	79.8	9.2	
≥10	7/16	58.3	13.8	43.8	16.1		9/16	33.3	13.3	33.3	13.3		8/15	38.1	14.3	38.1	14.3	
Age at diagnosis (mos)						0.2777						**0.0148**						0.06
<12	7/59	89.6	4.0	86.8	4.8		15/59	72.8	6.1	72.8	6.1		8/60	87.2	4.6	84.4	5.3	
>12	9/45	80.6	6.2	75.8	7.5		22/45	51.0	8.0	44.6	8.2		12/43	64.8	8.5	64.7	8.5	
Time between diagnosis and HSCT (yrs)						**0.0014**						0.1226						**0.0025**
≤2	3/59	94.3	3.2	94.3	3.2		17/59	69.7	6.5	66.8	6.9		4/53	90.5	4.6	90.5	4.6	
>2	13/45	74.8	6.6	67.2	7.9		20/45	55.8	7.7	52.8	7.8		16/50	65.5	7.4	62.5	7.7	
Organ damage before HSCT						**0.0014**						**0.0071**						**<0.0001**
No	5/68	92.2	3.4	92.2	3.4		16/68	74.5	5.6	74.5	5.6		4/60	92.9	3.4	92.9	3.4	
Yes	10/30	72.4	8.4	62.7	9.8		15/30	49.5	9.6	45.7	9.6		12/28	58.3	9.7	53.9	10.0	
Chronic lung disease						0.2545						0.1433						0.1026
No	10/85	89.0	3.5	86.9	4.0		24/85	71.0	5.2	69.0	5.4		11/79	85.1	4.5	82.7	5.0	
Yes	3/12	73.3	13.2	73.3	13.2		6/12	45.8	15.0	45.8	15.0		4/12	64.8	14.3	64.8	14.3	
Cryptosporidium infection (gastrointestinal)						**0.001**						0.0603						**<0.0001**
No	7/79	90.7	3.4	90.7	3.4		23/79	69.9	5.5	67.9	5.7		7/74	89.7	4.0	89.7	4.0	
Yes	7/20	68.8	10.7	60.2	12.3		9/20	50.0	12.1	50.0	12.1		8/18	55.7	13.2	44.6	14.5	
Protracted diarrhea						**0.0023**						0.5314						**0.0371**
No	8/84	90.2	3.3	90.2	3.3		28/84	65.8	5.6	61.9	5.9		10/76	84.4	4.7	84.4	4.7	
Yes	8/21	70.2	10.2	56.3	12.2		9/21	56.1	11.0	56.1	11.0		8/22	65.5	10.7	60.1	11.1	
Sclerosing cholangitis						**0.0003**						**0.0126**						**<0.0001**
No	8/85	90.0	3.4	90.0	3.4		26/85	67.7	5.5	65.7	5.6		8/79	88.3	4.0	88.3	4.0	
Yes	8/19	67.5	11.0	52.1	12.9		11/19	43.0	12.0	36.8	11.8		10/18	46.0	12.4	38.3	12.5	
Liver disease**						**0.002**						0.0666						**0.0009**
No	8/82	89.7	3.5	89.7	3.5		26/82	66.7	5.6	64.6	5.8		10/80	85.3	4.4	85.3	4.4	
Yes	8/22	71.8	9.9	57.6	12.1		11/22	49.7	11.4	44.2	11.4		10/22	53.8	11.6	47.1	12.0	
Pneumonias						0.6865						0.7624						0.6436
No	6/33	84.2	6.5	76.5	9.4		13/33	65.4	8.5	56.7	9.3		7/32	71.4	9.5	71.4	9.5	
Yes	10/71	86.7	4.2	84.4	4.6		23/71	64.6	6.7	64.6	6.7		11/65	82.9	5.0	80.2	5.5	
PJP						0.6862						0.9663						0.9081
No	6/50	87.2	4.9	87.2	4.9		16/50	68.0	6.9	64.9	7.2		8/51	82.0	6.0	82.0	6.0	
Yes	6/40	87.2	5.4	83.1	6.5		13/40	63.6	8.3	63.6	8.3		6/35	83.6	6.9	78.3	8.2	
URTI						0.4377						0.1809						0.1457
No	7/57	88.3	4.5	84.6	5.7		16/57	66.6	7.1	66.6	7.1		7/55	86.1	5.5	82.0	6.6	
Yes	9/46	82.4	5.7	78.5	6.6		20/46	60.0	7.3	54.8	7.6		11/40	70.0	7.7	70.0	7.7	

Characteristics	OS						EFS						DFS					
	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value
Need of ventilation before HSCT						0.5732						0.8708						0.6827
No	7/58	89.2	4.2	86.2	5.0		19/58	65.9	6.7	63.3	6.9		10/55	80.8	5.9	77.4	6.6	
Yes	5/32	82.7	7.2	82.7	7.2		10/32	67.3	8.6	67.3	8.6		4/29	84.1	7.4	84.1	7.4	
Neutropenia						0.3152						0.3861						0.8773
No	10/56	82.6	5.3	79.3	6.0		17/56	67.3	6.7	67.3	6.7		10/55	80.8	6.0	77.1	6.7	
Yes	5/46	88.8	4.7	88.4	4.7		18/46	62.1	7.6	55.9	8.0		7/39	79.2	7.2	79.2	7.2	
Oral ulcers						0.3384						0.8886						0.8351
No	9/81	89.7	3.5	87.6	4.0		26/81	68.1	5.5	64.2	5.8		13/81	82.4	4.8	80.2	5.1	
Yes	4/20	83.8	8.6	73.3	12.4		7/20	61.5	11.5	61.5	11.5		2/14	80.2	12.8	80.2	12.8	
FTT						0.868						0.74						0.4987
No	11/74	87.4	3.9	81.7	5.5		25/74	63.3	5.9	63.3	5.9		11/69	84.1	4.7	81.6	5.2	
Yes	5/30	81.8	7.4	81.8	7.4		12/30	63.4	9.5	51.9	10.7		6/27	70.6	10.6	70.6	10.6	
No Cryptosporidium prophylaxis before HSCT						0.8896						0.9309						0.9141
No	6/63	84.8	4.7	84.8	4.7		21/63	65.7	6.4	63.1	6.6		10/62	80.9	5.6	80.9	5.6	
Yes	3/24	87.5	6.8	87.5	6.8		8/24	61.9	10.9	61.9	10.9		3/21	85.7	7.6	85.7	7.6	
Conditioning regimen						**0.0073**						**<0.0001**						**0.0031**
MAC	5/57	92.7	3.5	90.0	4.3		10/57	81.6	5.3	81.6	5.3		6/58	91.0	3.9	88.3	4.6	
RIC	8/25	71.8	9.1	62.8	11.5		16/25	41.9	10.2	32.6	9.8		9/23	55.0	11.6	55.0	11.6	
MAC low tox	1/21	93.3	6.4	93.3	6.4		8/21	42.8	15.8	42.8	15.8		1/17	83.3	15.2	83.3	15.2	
NMA^	1/2	50.0	35.4	°	°		2/2	0	§	°	°		2/3	33.3	27.2	33.3	27.2	
Donor type						**0.0373**						0.0605						0.2619
MSD	3/27	88.8	6.1	88.8	6.1		5/27	85.0	6.9	80.8	7.8		4/27	88.8	6.1	84.6	7.1	
MUD	2/39	94.0	4.1	94.0	4.1		13/39	61.6	9.0	56.9	9.5		5/38	94.2	4.0	77.6	9.3	
MMUD ad. vol.	7/24	72.7	9.8	58.1	15.2		10/24	52.1	11.9	52.1	11.9		7/24	72.6	9.8	63.6	12.0	
MMFD+mmUCB	3/11	81.8	11.6	70.1	14.7		6/11	45.5	15.0	45.5	15.0		2/11	90.9	8.7	77.9	14.1	
Stem cell source						0.0936						**0.0035**						0.1123
BM	6/62	91.7	3.6	88.3	4.8		15/62	75.5	5.8	73.0	6.1		8/60	84.1	5.3	84.1	5.3	
PBSC	7/33	78.4	8.0	72.8	9.2		17/33	43.6	10.1	37.4	10.4		10/36	65.2	10.0	58.7	10.9	
UCB	3/10	70.0	14.5	70.0	14.5		5/10	50.0	15.8	50.0	15.8		2/8	75.0	15.3	75.0	15.3	

Organ damage was defined as the presence of chronic lung disease and/or liver alterations (sclerosing cholangitis or liver fibrosis or hepatitis). EFS and OS were calculated from first HSCT, while DFS from the last procedure (i.e. second HSCT, boost or DLI), thus the analyses were performed considering the covariates at the proper procedure. * Number of patients with available data. ** All liver alterations, including also ascending cholangitis, mild hepatic portal inflammation and minimal alterations. ^ NMA group is reported for descriptive purposes only, but it has not been included in the statistical analyses (Log Rank test) due to its low numerosity. § SE not estimable at this time point. °No subjects at risk at this time point. Significant p-values (p <0.05) are in bold.

Ev, events; pts, patients; SE, standard error; OS, overall survival; EFS, event-free survival; DFS, disease-free survival; yrs, years; mos, months; FU, follow up; HSCT, hematopoietic stem cell transplantation; URTI, upper respiratory tract infections; PJP, *Pneumocystis jiroveci* pneumonia; FTT, failure to thrive; MAC, myeloablative conditioning; NMA, non-myeloablative; RIC, reduced intensity conditioning; MAC low tox, myeloablative conditioning with low toxicity; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; MMFD, mismatched family donor; ad. vol., adult volunteer; mm, mismatched; UCB, umbilical cord blood; BM, bone marrow; PBSC, peripheral blood stem cells.

Table IV – Transplant features, therapeutic intervention and outcome in 18 patients who experienced graft rejection after 1st HSCT for CD40L deficiency

	Pt. no.	Year of 1st HSCT	1st HSCT stem cell source	1st HSCT Donor type	1st HSCT conditioning regimen	Timing of rejection/ declining chimerism	Therapeutic intervention (months after 1st HSCT)	Infections in the early FU*	Acute GVHD (grade)	Outcome (at last FU)
Graft rejection	**8**	2012	BM	MUD	**RIC** (Flu/Mel/ATG)	6 mo. FU	**2nd HSCT** (28.4)	ADV, EBV Bacterial sepsis	Yes (grade I)	Alive *(on IVIG)*
	9	2012	PBSC (TCR αβ depl.)	MUD	**MAC low tox** (Treo/Flu/ATG)	6 mo. FU	**2nd HSCT** (8)	ARVI	Yes (grade II)	Alive **(OFF Ig)**
	15	2007	BM	MSD	**RIC** (Flu/Mel/Alemtuzumab)	> 12 mo. FU (6y)§	None	ADV, Crypto.	No	Alive *(on IVIG)*
	33	2009	PBSC	MUD	**NMA** (Flu/ATG)	12 mo. FU§	**2nd HSCT** (15.4) ^	HHV6, Crypto.	No	Alive *(on IVIG)*
	37	1996	BM (Positive selection of CD34+ cells)	MUD	**MAC** (Bu/Cy/aLFA1-2)	6 mo. FU	None	No	No	Alive *(on IVIG)*
	41	2001	PBSC (Positive selection of CD34+ cells)	MMFD (haplo)	**MAC** (BU/Cy/ATG)	6 mo. FU	None	Whipworm	No	Deceased
	49a	2001	BM (Positive selection of CD34+ cells)	MUD	**MAC** (BU/Cy/ATG)	6 mo. FU	**2nd HSCT** (12.5)	HHV6, ADV CVL infection	No	Alive **(OFF Ig)**
	74	2014	BM	MUD	**MAC low tox** (Treo/Flu/Alemtuzumab)	19 mo. FU §	**2nd HSCT** (21.4)	CMV, Parainfl. URTI	No	Alive *(on IVIG)*
	77	2004	PBSC	MMUD	**MAC low tox** (Treo/Flu/ATG)	6 mo. FU	**2nd HSCT** (10.9) **3rd HSCT** (31.1)	CMV reactiv. Clostridium diff.	No	Alive **(OFF Ig)**
	83	2001	BM	MMUD	**RIC** (Flu/Mel/ATG)	12 mo. FU	None	EBV, Crypto. BK virus	Yes (grade I)	Deceased
	85	2003	BM	MSD	**RIC** (Flu/Mel/Alemtuzumab)	6 mo. FU	**2nd HSCT** (21.1)	No	No	Alive **(OFF Ig)**
	86	2006	PBSC	MUD°	**NMA** (Flu/Cy/Alemtuzumab + anti-CD45)	6 mo. FU	None	Mycobacteria (gut)	No	Deceased
	89	2011	PBSC	MUD	**RIC** (Flu/Mel/Alemtuzumab)	> 12 mo. FU (3y)	None	ADV	No	Alive *(on SCIG)*
	98	2007	UCB	MMUD	**MAC** (Bu/Cy/ATG)	<1 mo. FU	**2nd HSCT** (1.3)	CMV	No	Alive **(OFF Ig)**
	102	1997	BM (T-cell depleted)	MUD	**MAC** (BU-Cy-ATG + in vivo LFA1 CD2)	<1 mo. FU	**Cell boost** (1.1)	Aspergillus, Gram - sepsis	No	Deceased
	107	2011	PBSC	MUD°	**RIC** (Flu/Mel/Alemtuzumab)	< 3 mo. FU	**2nd HSCT** (3.3)	NA	NA	Alive **(OFF Ig)**
	124	2014	PBSC (CD45RA-depleted)	MMUD	**RIC** (Bu/Flu/TT/ATG)	< 3 mo. FU	None	ADV, Rhinovirus Crypto.	No	Deceased
	125	2003	UCB	MMUD	**RIC** (Bu/Flu/ATG)	< 2 mo. FU	**2nd HSCT** (2)	NA	NA	Deceased

* first 6 months after 1st HSCT; ° no. of HLA loci studied not specified; § chimerism declining since 6 months of FU.

^ This patient also received 2 liver transplantations, 1 before 1st HSCT, 1 after 2nd HSCT. He also experienced cGVHD after 2nd HSCT.

ADV, Adenovirus; Crypto., Criptosporidium spp.; DLI, donor lymphocyte infusion; depl., depleted; NA, not available; Parainfl., Parainfluenza virus; reactiv., reactivation; HSCT, hematopoietic stem cell transplantation; FU, follow up; BM, bone marrow; PBSC, peripheral blood stem cells; UCB, umbilical cord blood; MMFD, mismatched family donor; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; MAC, myeloablative conditioning; NMA, non-myeloablative; RIC, reduced intensity conditioning; no., number; CMV, cytomegalovirus; EBV, Epstein Barr virus; HHV6, human herpes virus 6; RSV, Respiratory Syncytial virus; URTI, upper respiratory tract infection; ARVI, acute respiratory viral infection; CVL, central venous line; IVIG, intravenous immunoglobulins; Ig, immunoglobulins; SCIG, subcutaneous immunoglobulins; NA, not available.

Figure 1 – Overall Survival

(A) Age at HSCT
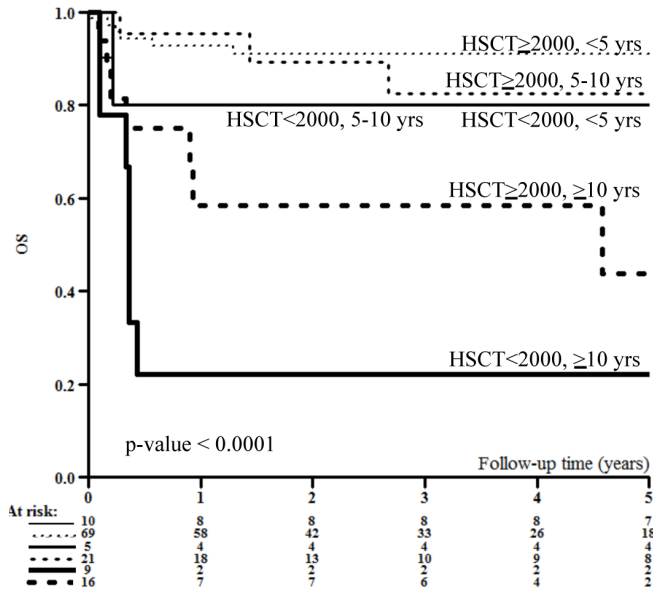
(B) Organ damage before HSCT
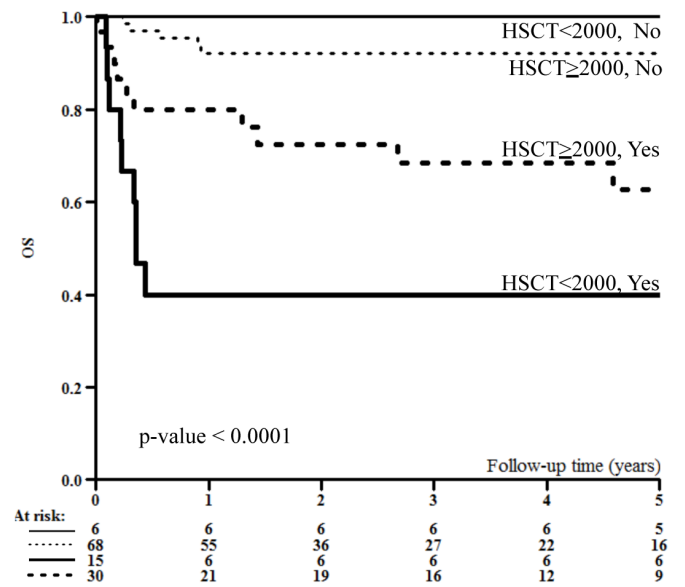
(C) *Cryptosporidium* infection
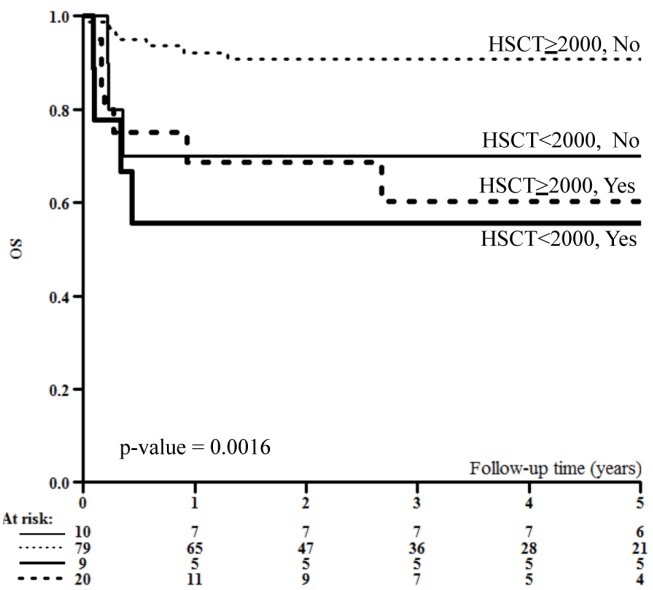
(D) Liver disease (all)
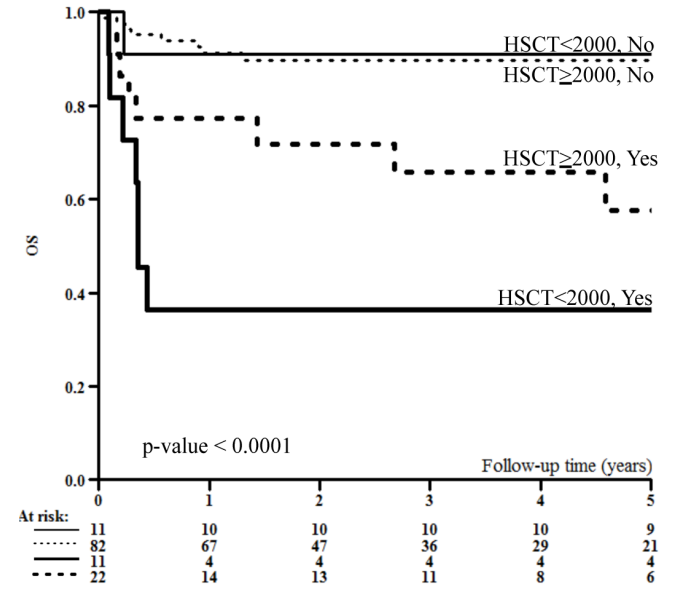
(E) Sclerosing cholangitis
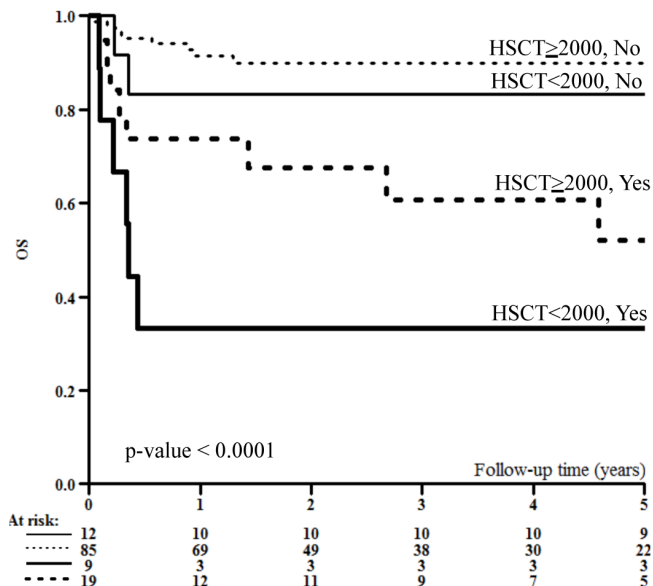
(F) Waiting time to HSCT
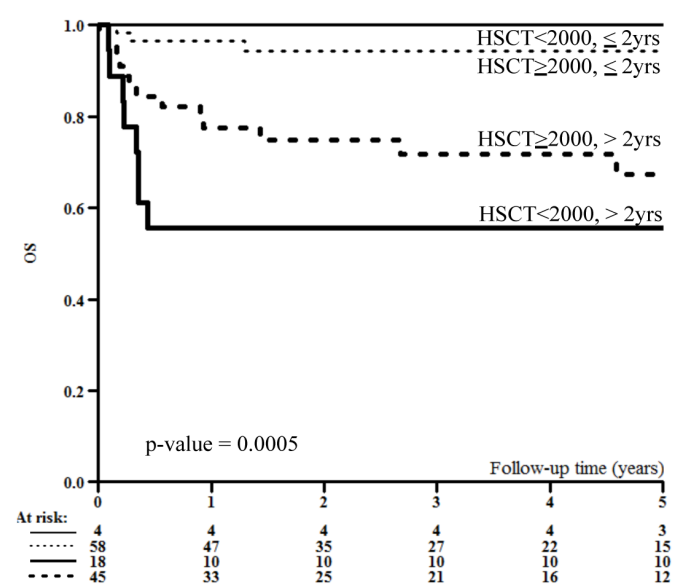
Figure 2 – Event-free Survival

(A) Age at HSCT
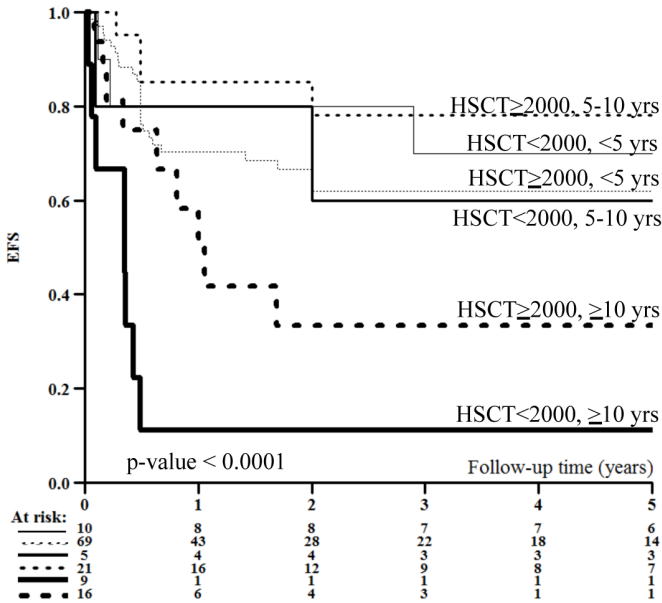
(B) Organ damage before HSCT
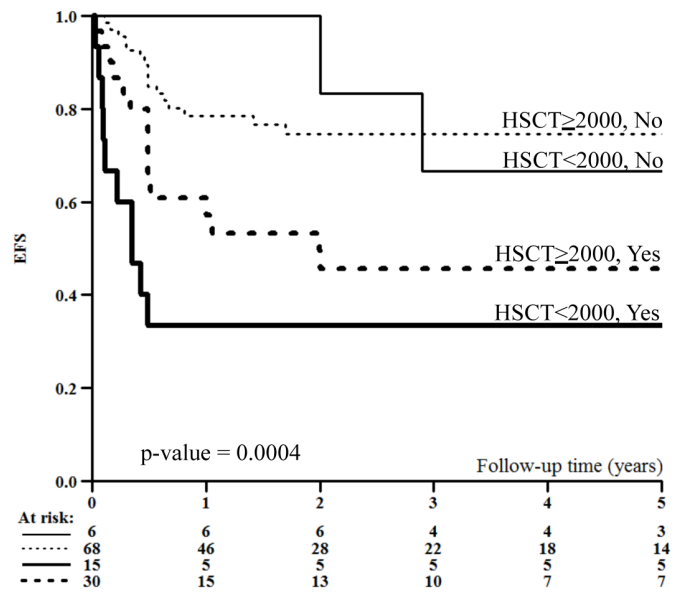
(C) Sclerosing cholangitis
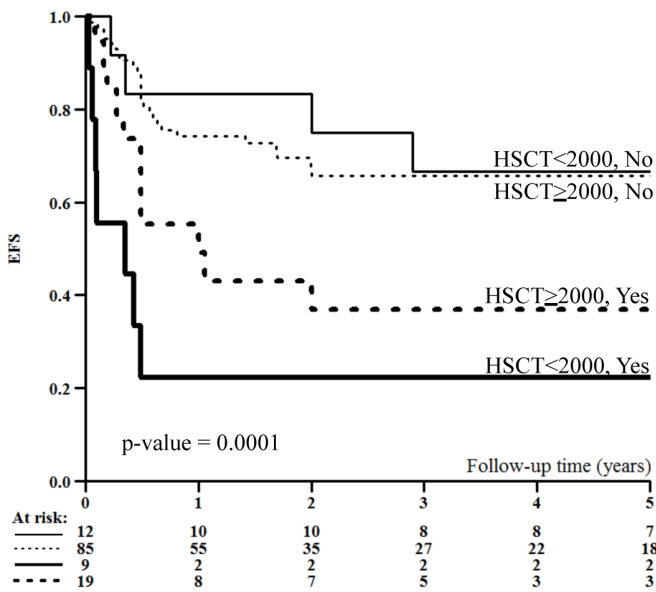
(D) Donor type
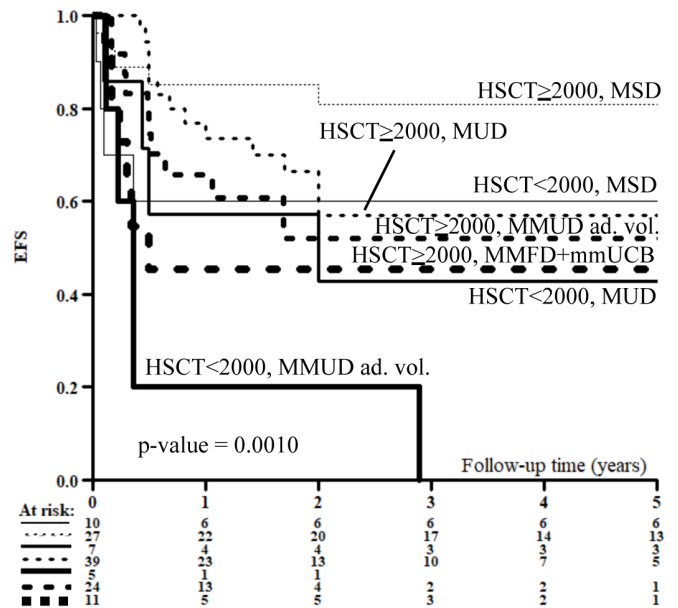
(E) Stem cell source
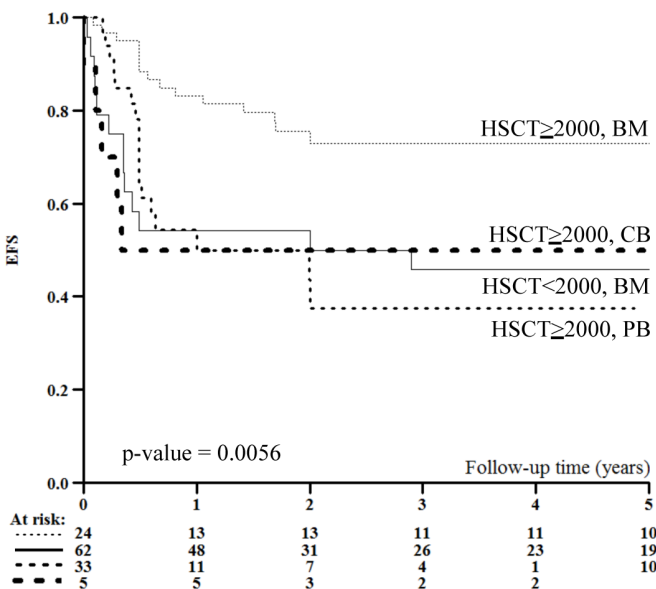
(F) Conditioning regimen
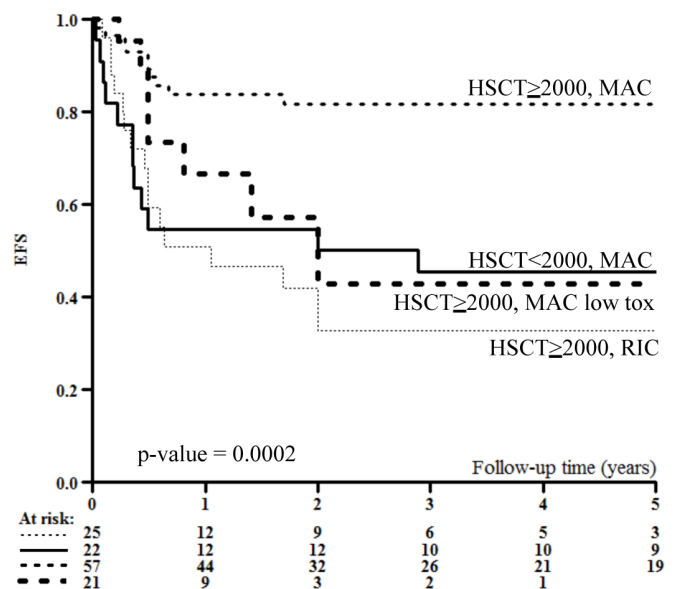
Figure 3 – Donor cell engraftment
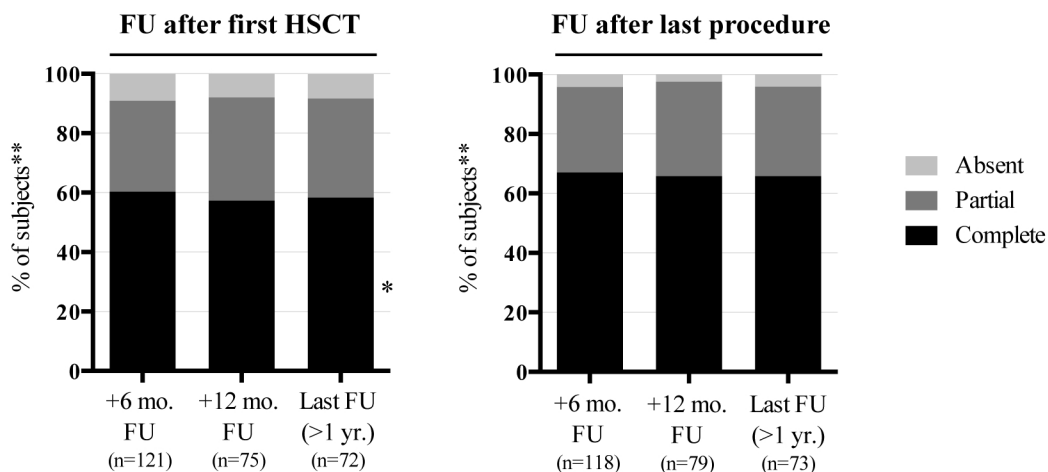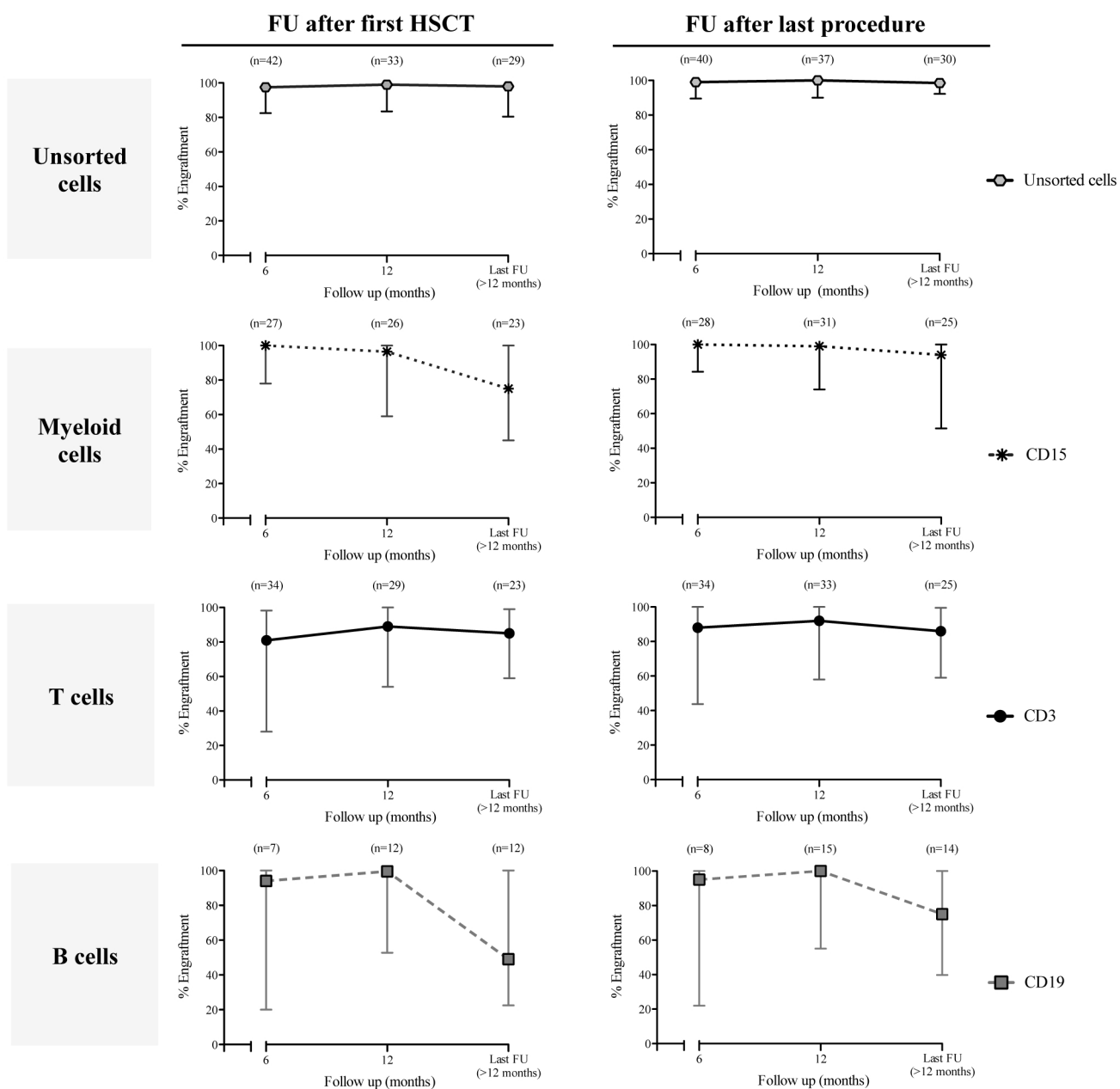
Figure 4 – Engraftment kinetics and T-cell chimerism
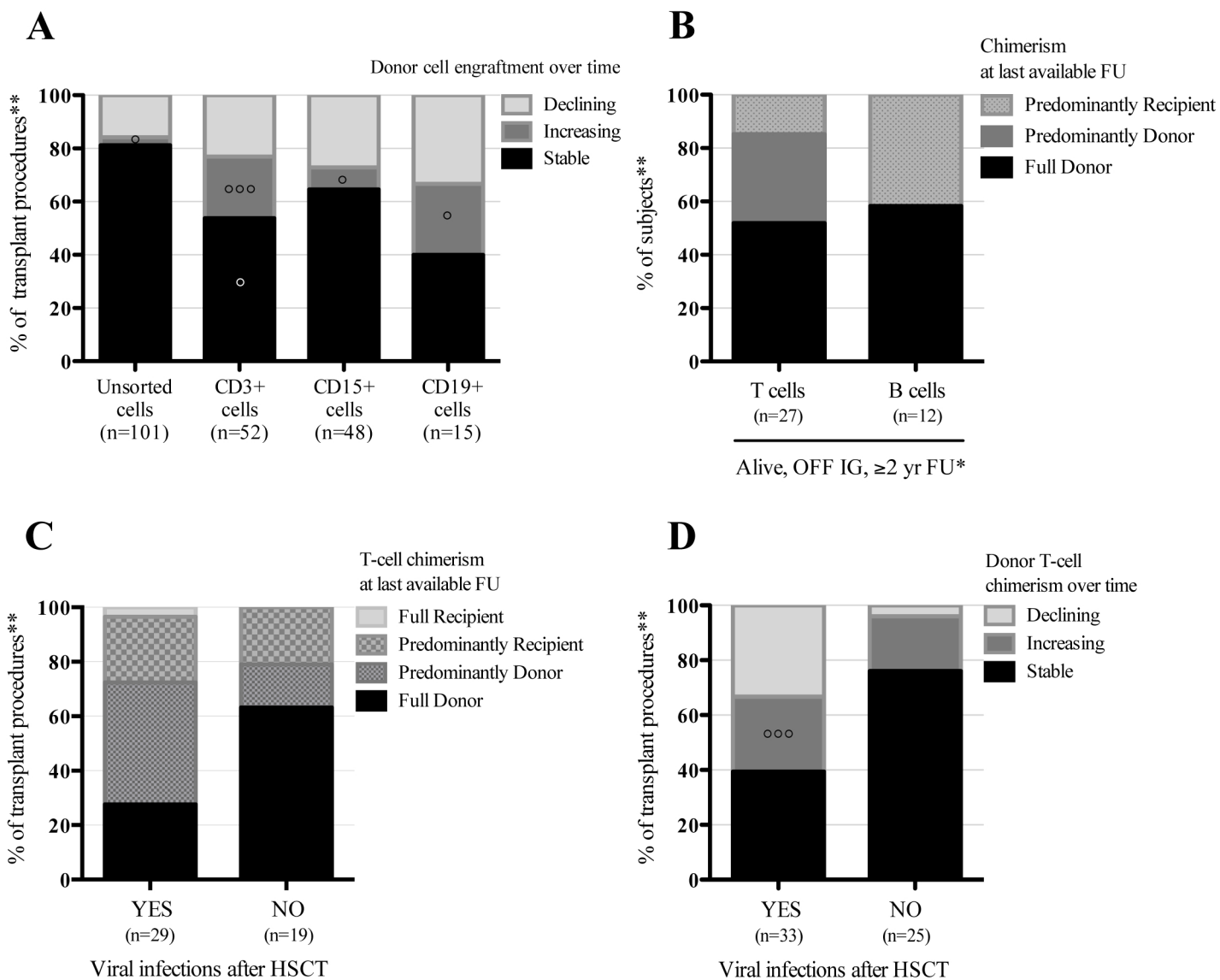
1 **Online Repository (OR) material**

2

3 **Patients, materials, and methods**

4 *Patient characteristics*

5 Clinical history was characterized by recurrent infections in most patients (Table I), mainly

6 involving the respiratory tract, requiring ventilation in 36%. Forty-seven patients experienced PJP.

7 Chronic lung disease developed in 15%. *Cryptosporidium* infection was more frequent in patients

8 transplanted before 2000 (47% patients, p=0.0189), as well as protracted diarrhea (48% patients,

9 p=0.0073). Sclerosing cholangitis was more prevalent in this group (43% patients) as compared to

10 the more recent transplant group (18%, p=0.0211). Liver disease affected 26% of all patients, 50%

11 of those transplanted before year 2000. Four patients underwent orthotopic liver transplantation

12 before HSCT. Neutropenia was detected in 46% patients, treated with G-CSF in 26%. Oral ulcers

13 and failure to thrive (FTT) were reported in 21% and 30% of patients, respectively. Central nervous

14 system involvement was described in 10 patients: 4 had meningo-encephalitis, and developmental

15 delay was described in 6 patients. In summary, organ damage before HSCT was present in 38%

16 patients, significantly higher in the historic transplant cohort (71%, p=0.0005), when HSCT

17 candidates were more compromised than those transplanted after 2000.

18 The type of CD40L gene mutation (deletion or missense) did not significantly influence infection

19 rate or organ damage burden before transplant. Only a tendency to less pre-HSCT liver disease

20 (9.4%) emerged in patients with missense mutations, as compared to those with deletions (27.8%),

21 but this was not statistically significant (p=0.0686).

22 Most patients received immunoglobulin supplementation and PJP prophylaxis before HSCT, with a

23 higher prevalence after 2000 for immunoglobulin supplementation (p=0.0271). *Cryptosporidium*

24 prophylaxis was less common (31% patients).

25

26 *Transplantation*

27 Median infused cell dose was 5.08×10^8 nucleated cells/kg (range: 0.03 – 337.55), with 6.90×10^6

28 CD34+cells/kg (range: 0.10 – 43.72) and 29.85×10^6 CD3+ cells/kg (range: 0.001 - 1000).

29 Neutrophil and platelet engraftment were defined as first day of 3 consecutive days >500/μl and

30 >50.000/μl, respectively. Median engraftment occurred 17 days after transplant for neutrophils and

31 22 days for platelets.

32

33 **Results**

34 *Conditioning*

35 The most common conditioning regimen in first transplants was MAC (61%), more prevalent

36 before 2000 (92%, Table II), mainly based on the combination of busulfan (Bu) and

37 cyclophosphamide (Cy) [no longer recommended due to the risk of veno-occlusive disease (VOD)],

38 followed by Bu at myeloablative dose and fludarabine (Flu) (Table E4). RIC usage increased in

39 subsequent years, mainly based on Flu/Melphalan (Mel) or Flu/Bu at reduced intensity dose. The

40 use of MAC low toxicity has been introduced since 2004, with the administration of treosulfan

41 (Treo) and Flu ± Thiotepa (TT).

42 The choice of the conditioning regimen in first transplants was strongly influenced by clinical

43 condition. Notably, RIC was used in older patients compared to myeloablative regimens [median

44 age at HSCT (years), before 2000: RIC 12.8, MAC 7.2; after 2000: RIC 6.0, MAC 2.4, MAC low

45 tox 3.6]. Moreover, 54.6% of patients receiving RIC had organ damage before HSCT. Most patients

46 who received MAC showed complete engraftment at 6-month, 12-month and last follow up after 1st

47 HSCT (70.7%, 68.2% and 66.7% respectively). Among patients with absent engraftment, most

48 received RIC for first HSCT (50.0%, 50.0% and 80.0% at 6-month, 12-month and last follow up).

49 Of note, in patients receiving MAC low toxicity or RIC regimens, a tendency to reach a lower level

50 of myeloid (CD15) engraftment could be observed over time, especially at FU ≥ 1 year after first or

51 last HSCT (Figure E6). In the 2 patients who received NMA for first transplant, engraftment of

52 donor cells was poor, leading to graft rejection in both cases (Table IV).

53

54 *Additional procedures (second/third HSCT/boosts/DLI)*

55 Twenty-two patients (16.9%) received one or more additional procedures after the first HSCT,

56 generally due to poor engraftment.

57 Thirteen patients (10%) underwent a second HSCT, mainly due to first HSCT failure/rejection

58 (76.9%, Table E3), at a median of 11 months after the first procedure. In one case, a 2nd transplant

59 was performed due to a refractory AIHA. All these patients received their first transplant after 2000.

60 Stem cell source was BM (n=6), PBSC (n=5) and UCB (n=2), mainly from unrelated donors

61 (12/13). MAC low toxicity and RIC were the most used conditioning regimens for the first HSCT

62 in these patients (n=5 and n=4 respectively, Table E3).

63 For the second procedure, in 5 cases, the cell source or donor type was changed, with an increased

64 use of PBSC (n=8) and MMFD (n=2). The intensity of conditioning was augmented in 6 cases.

65 Most patients were alive and off immunoglobulin supplementation (53.9%) at last FU. However, 2

66 required a third procedure (respectively, a stem cell boost and a third HSCT) to achieve this result

67 (Table E3).

68 Six patients transplanted for the first time between 1997 and 2004 received a stem cell boost

69 thereafter, mainly due to slipping donor chimerism, especially in T cells, and declining CD40L

70 expression (Table E3). In most cases, these patients first received T-cell depleted unrelated BM

71 HSCT preceded by MAC.

72 Cell boosts, consisting of BM-derived stem cell infusions from the same donor, were performed at a

73 median of 20.7 months after the last procedure, with no conditioning regimen. In one case (pt.49),

74 alemtuzumab was administered between day -22 and -18. In 50% cases, boosts stabilized donor cell

75 engraftment with favorable effects on immune reconstitution, resulting in survival free from

76 immunoglobulin supplementation.

77 In most recent years (since 2009), DLI were used in cases of low/absent engraftment of donor cells

78 (especially T lymphocytes) in order to re-establish full donor chimerism, or in cases of absent or

79 delayed immune recovery in the early FU phase (Table E3). In our cohort, 4 patients received this

80 treatment after a first PBSC HSCT (T cell depleted and TCR αβ depleted in 2 cases, respectively),

81 from 3 MUD and 1 MMFD (haplo). RIC and MAC low toxicity conditioning regimens were

82 administered to 2 patients each. All of them experienced viral infections in the first 6 months after

83 HSCT. Each patient received 2 or 3 DLI infusions, within the first year of FU. This approach was

84 well tolerated by patients and was successful in 75% enabling cessation of immunoglobulin

85 supplementation.

86

87 *Immune reconstitution after HSCT*

88 Median total lymphocyte, T cell (both CD4+ and CD8+ subsets) and B cell count normalized[E1] by

89 the first 12 months of FU. Most B cells were naïve (CD19+/CD27-/IgM+), but at last FU, class-

90 switched memory B cells resulted normal for age[E2] in 6 out of the 12 patients for whom data were

91 available. Serum IgA level was still low/absent in most patients (67.1%) at 6 month-FU, but

92 increased over time, reaching normal level for age in 57.8% patients and level compatible with

93 partial IgA deficiency in 21.1% at last FU. Serum IgM level was normal in most patients (69%),

94 and high in only 3 of them, at last FU. Data on specific vaccination response was available for a

95 subgroup of patients (n=32), showing a normal antibody response to tetanus toxoid, *type B*

96 *Haemophilus influenzae* and conjugated pneumococcal vaccines in most of them (75.7%, 66.7%

97 and 55.6% respectively). In some, evidence of antibody production after measles-mumps-rubella

98 vaccine was observed too. One patient had demonstrated good ability to mount adequate antibody

99 response to VZV infection. Among those with FU ≥2 years, median CD40L expression on activated

3

100 CD4+ T cells was 49% in those who ceased immunoglobulin supplementation and 14.5% in those

101 who still needed it.

102

103 *Complications after HSCT*

104 Infections represented the most common complication after transplant, occurring in 74.2% patients,

105 mostly of viral etiology (51.9% patients), although no association with acute GVHD was observed.

106 Bacterial and fungal infections were reported in 25.6% and 11.6% patients respectively (Table E9).

107 *Cryptosporidium* infection was reported in 10.9% patients, significantly less after 2000 (7.6%,

108 p=0.0240).

109 Acute GVHD was reported in 45.2% patients after first HSCT, mostly of grade I/II (76.4%

110 patients), involving skin only (40.4%) or with gut (21.1%). Liver GVHD associated with pre-

111 existing sclerosing cholangitis (61.5%). Severe acute GVHD (grade III/IV) was reported in 13

112 patients. Incidence of chronic GVHD was lower (3.9%), occurring in only 5 patients transplanted

113 after 2000, extensive in 4.

114 VOD was reported in 13.2% patients, and other liver/biliary complications in 10.1%. A significant

115 improvement was observed after year 2000 (p=0.0178, p=0.0157 respectively – Table E9).

116 Pulmonary complications were uncommon (7% patients), and ventilator dependency during HSCT

117 was reported in 3.6% cases only. Neurological complications were rare (3.1%, n=4), but were fatal

118 for 2 patients.

119

120 *Disease-free Survival*

121 Disease-free survival (DFS) aimed to estimate disease cure, in terms of survival without

122 requirement for continuous immunoglobulin replacement ≥2 years after the last procedure. Overall,

123 DFS was 73.4% and 72.3% at 2 and 5 years respectively, stable over time. Notably, DFS improved

124 significantly since 2000 (78.7% and 77.1% at 2 and 5 years, vs 47.6% in patients receiving HSCT

125 before 1999, p = 0.0011 – Table III and data not shown).

126 Among survivors that ceased immunoglobulin replacement ≥2 years after the last treatment, 10

127 received an additional procedure after the first HSCT (2nd HSCT n=6, 3rd HSCT n=1, boost n=3,

128 DLI n=2). Age at HSCT ≥10 years and presence of organ damage, especially liver disease,

129 sclerosing cholangitis and *Cryptosporidium* infection, were the most relevant variables to

130 negatively influence DFS in patients transplanted after 2000 (Table III, Figure E2A-D). Patients'

131 genotype did not have any impact on DFS.

132 Conditioning regimen was more significant in influencing DFS as compared to OS, with better DFS

133 when myeloablative regimens were used, instead of RIC (Table III, Figure E2E, Table E6). No role

134   for donor type or stem cell source emerged in DFS. A waiting time ≤2 years between diagnosis and

135   HSCT positively influenced DFS (Table III, Figure E2F).

136

137   **OR References**

138   E1.   Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, et
139   al. Immunophenotyping of blood lymphocytes in childhood. Reference values for
140   lymphocyte subpopulations. J Pediatr. 1997;130:388–93.

141   E2.   van Gent R, van Tilburg CM, Nibbelke EE, Otto SA, Gaiser JF, Janssens-Korpela PL, et al.
142   Refined characterization and reference values of the pediatric T- and B-cell compartments.
143   Clin Immunol. 2009;133:95–107.

144

145 **OR Table list**

146

147 **Table E1.** Participating centers

148

149 **Table E2.** CD40L gene mutations in the cohort of transplanted patients (n=130)

150

151 **Table E3.** Characteristics of second transplants (A), boosts (B) and donor lymphocyte infusions (C)

152

153 **Table E4.** Conditioning regimens

154

155 **Table E5.** Ex vivo Graft Manipulation

156

157 **Table E6.** Pairwise comparison between different conditioning regimens and HSCT outcome

158

159 **Table E7.** OS, EFS and DFS in CD40L deficient-patients who received first HSCT from unrelated

160 adult volunteers, according to the degree of match

161

162 **Table E8.** Results of the Cox regression model on EFS

163

164 **Table E9.** Complications post-first HSCT

165

166

167   **OR Figure legends**

168

169   **Figure E1. Busulfan (Bu) total dose in RIC versus MAC Bu/fludarabine (Flu) recipients.**

170   Median Bu total dose (with range) administered in RIC versus MAC Bu/Flu recipients is shown to

171   support the breakpoint chosen between the 2 groups based upon the Bu total dose (mg/kg) reported

172   by the different centers, because no data about Bu pharmacokinetics (AUC) were available. This

173   cut-off was used for the classification between RIC and MAC categories only of conditioning

174   regimens containing Bu/Flu. Other conditioning regimens were included in the MAC category

175   based on other features (*e.g.* administration of Cyclophosphamide), not solely on Bu dose. Bu,

176   busulfan; Flu, fludarabine; RIC, reduced intensity conditioning; MAC, myeloablative conditioning.

177

178   **Figure E2. Variables influencing disease-free survival (DFS) in patients receiving first**

179   **hematopoietic stem cell transplantation (HSCT) before/after 2000. (A)** Age at HSCT. **(B)**

180   Organ damage before HSCT. **(C)** Sclerosing cholangitis. **(D)** *Cryptosporidium* infection before

181   HSCT. **(E)** Conditioning regimen. **(F)** Waiting time to HSCT from diagnosis. Under each graph, the

182   number of patients at risk at each follow up time point after HSCT is reported for all patient groups.

183   DFS curves of the different patients' groups are represented by solid or dashed lines. For each of

184   them, a specific label is reported nearby the corresponding curve. yrs, years; MAC, myeloablative

185   conditioning; MAC low tox, myeloablative conditioning with low toxicity; RIC, reduced intensity

186   conditioning.

187

188   **Figure E3. Influence of conditioning regimen (A) or donor type (B) on overall survival (OS) in**

189   **patients receiving first hematopoietic stem cell transplantation (HSCT) before/after 2000.**

190   Under each graph, the number of patients at risk at each follow up time point after HSCT is

191   reported for all patient groups. OS curves of the different patients' groups are represented by solid

192   or dashed lines. For each of them, a specific label is reported nearby the corresponding curve.

193   MAC, myeloablative conditioning; MAC low tox, myeloablative conditioning with low toxicity;

194   RIC, reduced intensity conditioning; MSD, matched sibling donor; MUD, matched unrelated donor;

195   MMFD, mismatched family donor; mmUCB, mismatched umbilical cord blood; MMUD,

196   mismatched unrelated donor; ad. vol., adult volunteer.

197

198   **Figure E4. Causes of post-transplant deaths.** Each cause of death is represented by a different

199   color. The height of each colored bar in the graph is proportional to the number of patients who died

200   for that specific cause. *One patient died for Aspergillum infection early after 2nd HSCT performed

8

201 for refractory autoimmune hemolytic anemia. °One patient did not reconstitute immunity after
202 HSCT with subsequent inability to control viral infections and steroid resistant-graft-versus-host
203 disease (GVHD), for which received anti-thymocyte globulin on day+34 and +36. §During
204 transplant infusion. MOF, multiple organ failure; PML, Progressive Multifocal
205 Leukoencephalopathy.

206

207 **Figure E5. Lineage-specific chimerism at different time points after last procedure.** Lineage-
208 specific donor cell engraftment over time, represented by percentage (%) of subjects with different
209 degree of donor cell chimerism in myeloid cells (CD15+ cells), T lymphocytes (CD3+ cells) and B
210 lymphocytes (CD19+ cells) at different time points after last procedure. **% of subjects with
211 available data. mo., months; yr., year; FU, follow up.

212

213 **Figure E6. Myeloid chimerism over time in patients receiving different conditioning regimens**.
214 Myeloid cell chimerism, represented by percentage (%) of subjects with full donor, predominantly
215 donor, predominantly recipient or full recipient chimerism, at different time points after first HSCT
216 **(A)** and after last HSCT **(B)**. **% of those with available data within the same conditioning group
217 at the specified time point. HSCT, hematopoietic stem cell transplantation; mo., months; FU, follow
218 up.

Table E1 - Participating centers

Center	No. of patients	Country
Newcastle	17	UK
London GOSH	15	UK
Paris Necker (children)	12*	France
Brescia	6	Italy
Moscow	6	Russia
Prague	5	Czech Republic
Riyadh	5	Saudi Arabia
Lyon	4	France
Melbourne	4	Australia
Wroclaw (DCTK)	4	Poland
Copenaghen	3	Denmark
Dallas	3	USA
Gothenburg	3	Sweden
Leiden	3	The Netherlands
Nancy	3	France
Paris Necker (adults)	3	France
Philadelphia	3	USA
San Francisco	3	USA
Sydney	3	Australia
Ulm	3	Germany
Utrecht	3	The Netherlands
Zagreb	3	Croatia
Munich	2	Germany
Wroclaw	2	Poland
Ankara	1	Turkey
Barcelona V. Hebron	1	Spain
Budapest	1	Hungary
Columbia	1	USA
Cracow	1	Poland
Freiburg	1	Germany
Gent	1	Belgium
Leuven	1	Belgium
Marseille	1	France
Minneapolis	1	USA
Ohio	1	USA
Stockholm	1	Sweden

No., number; UK, United Kingdom; GOSH, Great Ormond Street Hospital; USA, United States of America.
*1 additional 2nd transplant performed on a Lyon patient.

Table E2 – CD40L gene mutations in the cohort of transplanted patients (n=130)

CD40L gene mutation	All patients (n=130)		HSCT up to 1999 (n=24)		HSCT since 2000 (n=106)	
	n	(%)	n	(%)	n	(%)
Present	108	83.1	18	75	90	84.9
Deletion	36	33.3	7	29.2	29	27.4
Missense	32	29.6	3	12.5	29	27.4
Intronic	12	11.1	2	8.3	10	9.4
Nonsense	4	3.7	0	0	4	3.8
Insertion	3	2.8	0	0	3	2.8
Other	5	4.6	1	4.2	4	3.8
Not specified	16	14.8	5	20.8	11	10.4
No mutation found	7	5.4	0	0	7	6.6
Unknown	15	11.5	6	25	9	8.5

Table E3-A - Second transplants (n=13)

Pt. no.	First HSCT				Second HSCT				Reason for 2nd HSCT	Months between 1st and 2nd HSCT	Outcome (at last FU)
	Year	Stem cell source	Donor type	Conditioning regimen	Year	Stem cell source	Donor type	Conditioning regimen			
8	2012	BM	MUD	**RIC** (Flu/Mel/ATG)	2014	BM	MUD	**RIC** (Bu/Flu/ATG)	1st Graft failure/rejection	28.4	Alive *(on IVIG)*
9	2012	PBSC (TCR αβ depletion)	MUD	**MAC low tox** (Treo/Flu/ATG)	2013	PBSC (TCR αβ depletion)	MUD	**NMA** (TLI/Flu/Cy)	1st Graft failure/rejection	8.0	Alive (**OFF Ig**)
10	2009	PBSC	MUD	**MAC low tox** (Treo/Flu/TT/ Alemtuzumab)	2012	PBSC	MUD	**MAC** (Bu/Flu/ATG)	Mixed chimerism	33.9	Alive (**OFF Ig**)
25	2011	BM	MUD	**MAC** (Bu/Flu/ATG)	2012	PBSC (Positive selection of CD34+ cells)	MMFD	**RIC** (Cy-Mel-TT-ATG-Rtx)	Mixed chimerism	8.2	Alive *(on IVIG)*
32	2010	BM	MUD	**MAC low tox** (Treo/Flu/TT/ Alemtuzumab)	2011	BM	MUD	**MAC** (BU/Cy/ATG)	Refractory AIHA	9.9	Deceased
33	2009	PBSC	MUD	**NMA** (Flu/ATG)	2010	PBSC	MUD	**MAC low tox** (Treo/Flu/ATG)	1st Graft failure/rejection	15.4	Alive *(on IVIG)*
49[a]	2001	BM (Positive selection of CD34+ cells)	MUD	**MAC** (BU/Cy/ATG)	2002	BM	MUD	**RIC** (Flu/Mel/Alemtuzumab)	1st Graft failure/rejection	12.5	Alive (**OFF Ig**)
74	2014	BM	MUD	**MAC low tox** (Treo/Flu/ Alemtuzumab)	2015	PBSC (Positive selection of CD34+ cells)	MUD	**MAC** (Bu/Flu/Cy)	1st Graft failure/rejection	21.4	Alive *(on IVIG)*
77[b]	2004	PBSC	MMUD	**MAC low tox** (Treo/Flu/ATG)	2005	PBSC	MMUD	**MAC low tox** (Treo/Cy/ATG)	1st Graft failure/rejection	10.9	Alive (**OFF Ig**)
85	2003	BM	MSD	**RIC** (Flu/Mel/Alemtuzumab)	2005	BM	MSD	**MAC** (Bu/Cy)	1st Graft failure/rejection	21.1	Alive (**OFF Ig**)
98	2007	UCB	MMUD	**MAC** (Bu/Cy/ATG)	2007	PBSC (Positive selection of CD34+ cells)	MMFD	**RIC** (Flu/TT/ATG)	1st Graft failure/rejection	1.3	Alive (**OFF Ig**)
107	2011	PBSC	MUD[c]	**RIC** (Flu/Mel/Alemtuzumab)	2012	NK	NK	**MAC** (Bu/Cy/Flu/ATG)	1st Graft failure/rejection	3.3	Alive (**OFF Ig**)
125	2003	UCB	MMUD	**RIC** (Bu/Flu/ATG)	2003	PBSC	NK	**NMA** (Flu/ATG)	1st Graft failure/rejection	2	Deceased

[a] After 2nd HSCT, pt.49 received also a stem cell boost due to lack of donor T cells (see table E3-B). [b] After 2nd HSCT, pt.77 received also a third HSCT 31.1 months after the first HSCT and 20.2 months after the second HSCT [donor: MMUD, stem cell source: PBSC; conditioning regimen: MAC (Bu-Cy-ATG)].[c] Number of HLA loci studied not specified.

HSCT, hematopoietic stem cell transplantation; no., number; FU, follow up; BM, bone marrow; PBSC, peripheral blood stem cells; UCB, umbilical cord blood; MMUD, mismatched unrelated donor; MMFD, mismatched family donor; MUD, matched unrelated donor; MSD, matched sibling donor; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; NMA, non-myeloablative conditioning; MAC low tox, myeloablative conditioning with low toxicity; AIHA, autoimmune hemolytic anemia; IVIG, intravenous immunoglobulins; Ig, immunoglobulins. NK, not known.

Table E3-B - Boosts (n=6)

Pt. no.	Year of 1st HSCT	HSCT stem cell source	Donor	Conditioning regimen	Infections in the early FU*	Boost cell source and timing (months after last HSCT)	Reason for Top Up	Effect	Outcome (at last follow up)
48	1999	T cell-depleted BM (positive selection of CD34+ cells)	MMUD	**MAC** (Bu/Cy/ATG)	CVL infection	T cell-depleted BM (35.2)	Declining CD40L expression and slipping chimerism	Stable mixed chimerism and CD40L expression (even if low)	Alive (**OFF Ig**)
49	2001 (1st) 2002 (2nd)	BM (1st HSCT T cell-depleted, 2nd HSCT whole marrow)	MUD	1st- **MAC** (Bu/Cy/ATG) 2nd- **RIC** (Flu/Mel/Alemtuzumab)	ADV, CVL infection	BM° (3.8)	Presence of donor chimerism in all cell lines, except for T cells	Successful donor T cell engraftment and CD40L expression	Alive (**OFF Ig**)
51	2004	BM	MUD	**MAC** (Bu/Cy/ATG)	CVL infection	Cryopreserved BM (20.7)	Slipping T-cell chimerism	Restarted IVIG – Stable engraftment and CD40L, but unable to make immunoglobulins	Alive *(on IVIG)*
60	1999	T cell-depleted BM (positive selection of CD34+ cells)	MUD	**MAC** (Bu/Cy/ATG)	ADV, Rotavirus, Astrovirus, UTI, CVL infection	BM (33.8)	Slipping chimerism in T and B cells and subsequently declining CD40L expression (failing graft)	Good immune reconstitution	Alive (**OFF Ig**)
92	2000	BM	MMUD	**RIC** (Flu/Mel/Alemtuzumab)	No	BM (20.6)	NK	NK	Alive *(on IVIG)*
102	1997	T cell-depleted BM	MUD	**MAC** (BU-Cy-ATG + in vivo LFA1 CD2)	Aspergillus, Gram - sepsis	BM (1.1)	1st graft failure/rejection	Rejection	Deceased

* first 6 months after 1st HSCT.

° This boost was preceded by administration of Alemtuzumab between day-22 and day-18. No conditioning regimen was administered to other patients before cell boosts.

HSCT, hematopoietic stem cell transplantation; FU, follow up; BM, bone marrow; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; CVL, central venous line; CMV, cytomegalovirus; ADV, adenovirus; RSV, Respiratory Syncytial virus; UTI, urinary tract infection; IVIG, intravenous immunoglobulins; Ig, immunoglobulins. NA, not available; NK, not known.

Table E3-C - Donor lymphocyte infusions (DLI) (n=4)

Pt. no.	Year of 1st HSCT	Stem cell source	Donor	Conditioning regimen	Infections in the early FU*	DLI infusions	Aim of infusion	Outcome *(at last follow up)*
20	2009	PBSC (Positive selection of CD34+ cells)	MMFD (haplo)	**MAC low tox** (Treo/Cy/Flu/ATG)	CMV reactivation	2, low dose (d+85, d+108)	- re-establishment of full donor chimerism - delayed immune recovery - clearance of CMV reactivation	Alive (**OFF Ig**)
88	2009	PBSC	MUD°	**RIC** (Flu/Mel/Alemtuzumab)	ADV	3 (12 mo. FU)	- absent engraftment of donor T cells at FU +6 months after HSCT	Alive (**OFF Ig**)
91	2011	PBSC	MUD	**RIC** (Flu/Mel/Alemtuzumab)	ADV, RSV	NA (6 mo. FU)	- predominantly recipient chimerism at FU +6 months after HSCT	Alive *(on IVIG)*
127	2014	PBSC (TCR αβ depletion)	MUD	**MAC low tox** (Flu/Mel/Treo/ATG)	Enterocolitis, HSV, viral RTI	2 (d+153, d+195)	- absent immune recovery (almost absent T cells in PB)	Alive (**OFF Ig**)

* first 6 months after 1st HSCT; ° nb of HLA loci studied not specified. Patient 20 was already reported in Jasinska A, *et al.* Pediatr Transplant 2013.
HSCT, hematopoietic stem cell transplantation; FU, follow up; PBSC, peripheral blood stem cells; MMFD, mismatched family donor; MUD, matched unrelated donor; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; CMV, cytomegalovirus; ADV, adenovirus; RSV, Respiratory Syncytial virus; RTI, respiratory tract infection; IVIG, intravenous immunoglobulins; Ig, immunoglobulins. NA, not available.

Table E4 – Conditioning regimens

Conditioning regimen		n 1st tx. (2nd /3rd tx.)	% 1st tx. (2nd /3rd tx.)
MAC		**79 (5/1)**	**61.2 (38.5/100)**
- Bu/Cy	//	26 (1)	41.9 (15.4/100)
	+ ATG	22 (1/1)	
	+ in vivo LFA-1+CD2	2	
	+ LFA-1/2	1	
	+ Alemtuzumab	2	
	+ ATG + in vivo LFA-1+CD2	1	
- Bu/Flu	//	1	14.7 (7.7)
	+ ATG	14 (1)	
	+ Alemtuzumab	4	
- Bu/Flu/Mel	+ ATG	1	0.8
- Bu/Flu/Cy	//	1 (1)	2.3 (15.4)
	+ATG	2 (1)	
- Bu/Cy/TBI	+ATG	1	0.8
- TBI*/Cy	+ Alemtuzumab	1	0.8
MAC low tox		**21 (2)**	**16.3 (15.4)**
- Treo/Cy	//	1	1.6 (7.7)
	+ ATG	0 (1)	
	+ Alemtuzumab	1	
- Treo/Flu	+ ATG	3 (1)	6.2 (7.7)
	+ Alemtuzumab	5	
- Treo/Flu/TT	+ATG	1	3.9
	+ Alemtuzumab	4	
- Treo/Flu/Cy	+ATG	1	0.8
- Treo/Flu/Mel	+ ATG	3	2.3
- Treo/Flu/Mel/Rtx	+ ATG	2	1.6
RIC		**27 (4)**	**20.9 (30.8)**
- Flu/Mel	+ ATG	9	13.2 (7.7)
	+ Alemtuzumab	8 (1)	
- Bu/Flu	+ ATG	4 (1)	4.7 (7.7)
	+ Alemtuzumab	2	
- Cy/Mel/TT/Rtx	+ ATG	0 (1)	0 (7.7)
- Flu/Cy/TBI°	+ ATG	1	0.8
- Flu/TT	+ ATG	0 (1)	0 (7.7)
- Flu/Mel/TT	+ ATG	1	0.8
	+ Alemtuzumab	1	0.8
- Bu/Flu/TT	+ ATG	1	0.8
NMA		**2 (2)**	**1.6 (15.4)**
- TLI/Flu/Cy	//	0 (1)	0 (7.7)
- Flu	+ ATG	1 (1)	0.8 (7.7)
- Flu/Cy	+ Alemtuzumab + anti-CD45	1	0.8

Data about conditioning regimen are missing for one patient. Second HSCT n=13, third HSCT n=1. Tx., transplant; MAC, myeloablative conditioning; MAC low tox, myeloablative conditioning with low toxicity; Bu, busulfan; Cy, cyclophosphamide; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Treo, treosulfan; TT, thiotepa; Rtx, rituximab; TLI, total lymphoid irradiation; ATG, antithymocyte globulin; LFA1, lymphocyte function-associated antigen 1; RIC, reduced intensity conditioning; NMA, non-myeloablative conditioning. No Bu pharmacokinetics (AUC) data were available. Bu-containing regimens were divided between MAC and RIC groups based on the total dose of Bu administered in case of combination with fludarabine (14.3-25.0 mg/kg in MAC, 4.0-13.6 mg/kg in RIC, see Figure E1 in the Online Repository). In the other cases, classification as MAC was based on other features (*e.g.* combination with Cyclophosphamide), not solely on Bu dose. *900 cGy; °200 cGy. Total TT dose in RIC was ≤ 10 mg/kg.

Table E5 – Ex vivo Graft Manipulation (total procedures = 150, DLI excluded)

Graft Manipulation		BM (n=96) n	PBSC (n=42) n	UCB (n=10) n	Total (n=148) n
- No manipulation		67 - 1st tx. 60 - 2nd tx. 4 - 3rd tx. 0 - Boost 2	26 - 1st tx. 22 - 2nd tx. 3 - 3rd tx. 1 - Boost 0	8 (all 1st tx.)	101 - 1st tx. 91 - 2nd tx. 7 - 3rd tx. 1 - Boost 2
- T-cell depletion	- Positive selection of CD34+ cells	11 - 1st tx. 10 - 2nd tx. 0 - Boost 1	8 - 1st tx. 5 - 2nd tx. 3 - Boost 0	0	19 - 1st tx. 15 - 2nd tx. 3 - Boost 1
	- TCR αβ-depletion	0	6 - 1st tx. 5 - 2nd tx. 1	0	6
	- in vitro C1G (+RBC depletion)	1	0	0	1
	- other (C1M in vitro)	1	0	0	1
	- other (CD2+CD7+CD19+complement)	1	0	0	1
- RBC depletion	- only	4 - 1st tx. 3 - Boost 1	0	0	4 - 1st tx. 3 - Boost 1
	- + plasma reduction	2	0	0	2
	- + MNC enrichment (buffy coat)	1	0	0	1
- Plasma reduction		4	0	0	4
- MNC enrichment	- Fycoll	0	0	1	1
	- Buffy coat	1	0	0	1
- Other		3 - 1st tx. 2 - 2nd tx. 0 - Boost 1	2 - 1st tx. 1 - 2nd tx. 1 - Boost 0	0	5 - 1st tx. 3 - 2nd tx. 1 - Boost 1
- Unknown		1 (1 boost)	0	1 (1st tx)	2

Data about cell source are missing for n=2 procedures (one first and one second transplant). Where it is not specified, data refer to first transplants.
DLI, donor lymphocyte infusion; BM, bone marrow, PBSC, peripheral blood stem cell; UCB, umbilical cord blood; tx., transplant; TCR, T-cell receptor; C1G, Campath 1G; C1M, Campath 1M; RBC, red blood cells; MNC, mononuclear cells.

Table E6 – Pairwise comparison between different conditioning regimens and HSCT outcome

	Comparison		OS	EFS	DFS
				p-value	
			OS	EFS	DFS
All periods	MAC	MAC low tox	0.9638	0.1071	1.000
	MAC	RIC	0.3705	**0.0024**	0.1973
	MAC low tox	RIC	**0.0374**	0.1643	**0.0302**
HSCT>2000	MAC	MAC low tox	0.9322	**0.0088**	0.7332
	MAC	RIC	**0.0258**	**<0.0001**	**0.0089**
	MAC low tox	RIC	**0.0197**	0.13	**0.0109**

Reported p-values are adjusted for multiple comparisons. Significant p-values (p <0.05) are in bold.
HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning; MAC low tox, myeloablative conditioning with low toxicity; RIC, reduced intensity conditioning; OS, overall survival; EFS, event-free survival; DFS, disease-free survival.

Table E7 – OS, EFS and DFS in CD40L deficient-patients who received first HSCT from unrelated adult volunteers, according to the degree of match

Variables	OS					EFS					DFS				
	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value **	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value **	no. ev/ no. pts*	2 yrs FU (%)	SE (%)	5 yrs FU (%)	SE (%)	p-value **
Donor match *(All periods)*						**0.0003**						0.2615						**0.0142**
no mm	2/41	94.4	3.9	94.4	3.9		15/41	61.8	8.6	53.5	9.2		6/39	74.5	9.3	74.5	9.3	
1 mm	8/21	67.4	10.3	44.4	19.4		9/21	60.6	11.0	30.3	22.1		8/21	53.3	14.5	53.3	14.5	
>1 mm	2/3	33.3	27.2	33.3	27.2		3/3	0	§	°			2/3	33.3	27.2	33.3	27.2	
Donor match *(HSCT>2000)*						**0.0209**						0.7527						0.2383
no mm	2/38	93.8	4.2	93.8	4.2		13/38	61.0	9.1	56.3	9.5		5/37	77.5	9.3	77.5	9.3	
1 mm	4/16	81.2	9.8	40.6#	29.1		4/16	73.1	11.7	73.1	11.7		4/17	65.9	16.5	65.9	16.5	
>1 mm	2/3	33.3	27.2	33.3#	27.2		3/3	0	§	°			2/3	33.3	27.2	33.3	27.2	

EFS and OS were calculated from first HSCT, while DFS from the last procedure (i.e. second HSCT, boost or DLI), thus the analyses were performed considering the covariates at the proper procedure. * Number of patients with available data. ** p-value calculated not including the >1 mm subgroup, due to its very small size. § SE not estimable at this time point. °No subjects at risk at this time point. # This value should not be considered as reliable because of the too low number of subjects at risk in this subgroup at this FU time point. Significant p-values (p <0.05) are in bold. Ev, events; pts, patients; SE, standard error; OS, overall survival; EFS, event-free survival; DFS, disease-free survival; yrs, years; FU, follow up; HSCT, hematopoietic stem cell transplantation; mm, mismatch.

Table E8 – Results of the Cox regression model on EFS

Characteristic	HR	95% CI	p-value
Donor type			
MUD *vs* MSD	3.26	(0.95-11.2)	0.0607
MMFD+mmUCB and MMUD ad. vol. *vs* MSD	4.22	(1.27-14.05)	**0.0189**
Conditioning regimen:			
MAC low tox *vs* MAC	2.00	(0.76-5.23)	0.1602
RIC *vs* MAC	3.16	(1.10-9.08)	**0.0323**
Organ damage before HSCT: yes *vs* no	2.66	(0.82-8.64)	0.1036
Sclerosing cholangitis before HSCT: yes *vs* no	1.01	(0.24-4.25)	0.9885
Age at HSCT (years)	0.99	(0.91-1.08)	0.7737

Legend: EFS, event-free survival; HR, hazard ratio; CI, confidence interval; *vs*, versus; MUD, matched unrelated donor; MSD, matched sibling donor; MMFD, mismatched family donor; mmUCB, mismatched unrelated umbilical cord blood; MMUD, mismatched unrelated donor; ad. vol., adult volunteer; MAC, myeloablative conditioning; MAC low tox, myeloablative conditioning with low toxicity; RIC, reduced intensity conditioning; HSCT, hematopoietic stem cell transplantation.
Significant p-values (p <0.05) are in bold.

Table E9 - Complications in the first 6 months after first HSCT

Complication	All patients (n=130)			HSCT up to 1999 (n=24)		HSCT since 2000 (n=106)		p-value
	Total*	n	(%)	n	(%)	n	(%)	
Acute GVHD								
- all grades	126	57	(45.2)	12	(52.2)	45	(43.7)	0.6118
- grade I-II		42	(33.3)	6	(26.1)	36	(35.0)	0.0787
- grade III-IV		13	(10.3)	6	(26.1)	7	(6.8)	
- grade not known		2	(1.6)	0	(0)	2	(1.9)	
Chronic GVHD	128	5	(3.9)	0	(0.0)	5	(4.8)	0.5844
-Extensive		4	(3.1)	0	(0.0)	4	(3.8)	1.0000
-Limited		1	(0.8)	0	(0.0)	1	(1.0)	
Infections (all)	128	95	(74.2)	18	(75.0)	77	(74.0)	0.9227
Viral infections								
- all	129	67	(51.9)	9	(37.5)	58	(55.2)	0.1166
- CMV	129	21	(16.3)	3	(12.5)	18	(17.1)	0.7630
- Adenovirus	129	30	(23.3)	6	(25.0)	24	(22.9)	0.8226
- EBV	129	8	(6.2)	0	(0.0)	8	(7.6)	0.3502
- other	129	36	(27.9)	2	(8.3)	34	(32.4)	**0.0178**
Bacterial infections	129	33	(25.6)	5	(21.0)	28	(26.7)	0.7402
Fungal infections	129	15	(11.6)	6	(25.0)	9	(8.7)	**0.0348**
Cryptosporidium infection	129	14	(10.9)	6	(25.0)	8	(7.6)	**0.0240**
VOD	129	17	(13.2)	7	(29.2)	10	(9.5)	**0.0178**
Liver complications (other than VOD)	129	13	(10.1)	6	(25.0)	7	(6.7)	**0.0157**
Hemorrhagic Cystitis	129	5	(3.9)	1	(4.2)	4	(3.8)	1.0000
Autoimmune complications	128	6	(4.7)	1	(4.2)	5	(4.8)	1.0000
Need of ventilation during HSCT hospitalization	111	4	(3.6)	2	(11.8)	2	(2.1)	0.1103

* number of patients with datum available. Significant p-values (p <0.05) are in bold.
CMV, Cytomegalovirus; EBV, Epstein-Barr Virus; GVHD, graft-versus-host-disease; HSCT, hematopoietic stem cell transplant; VOD, veno-occlusive disease.

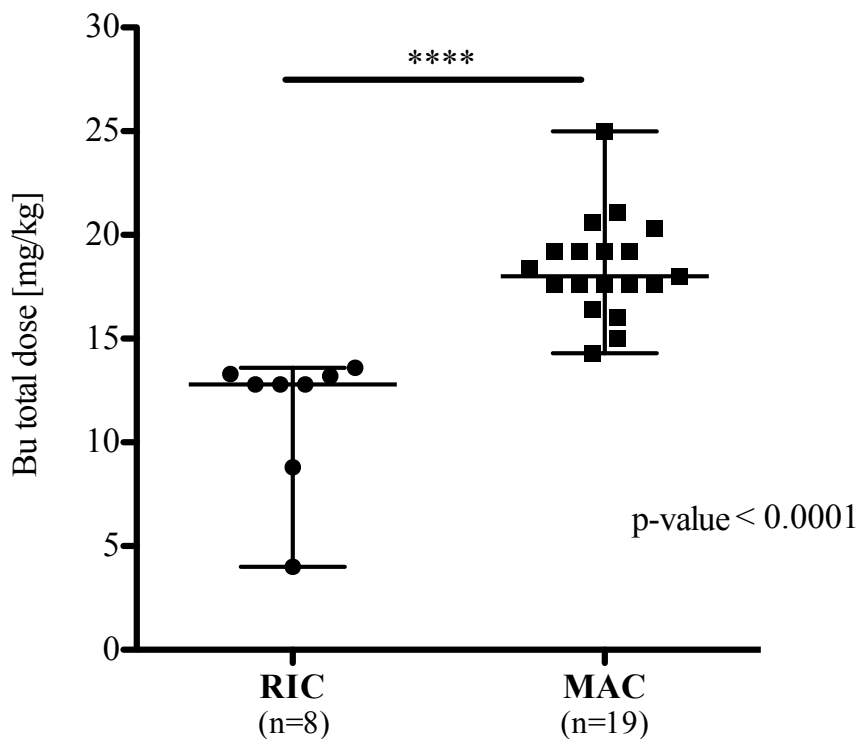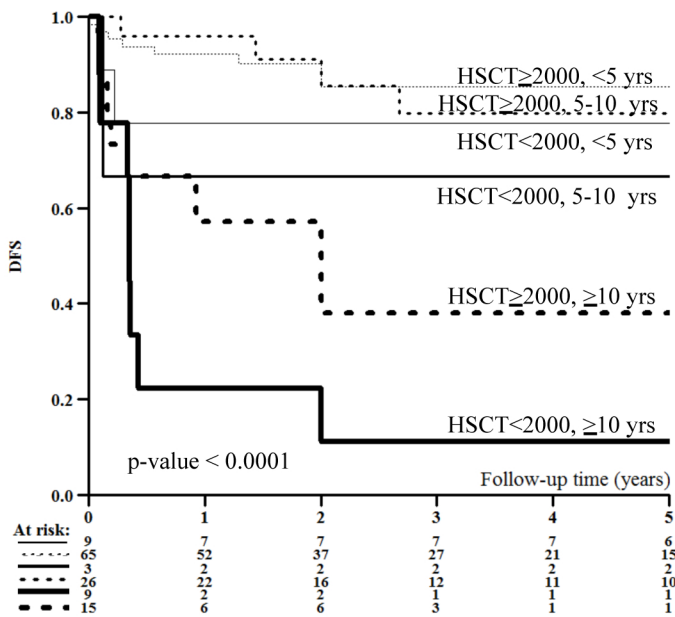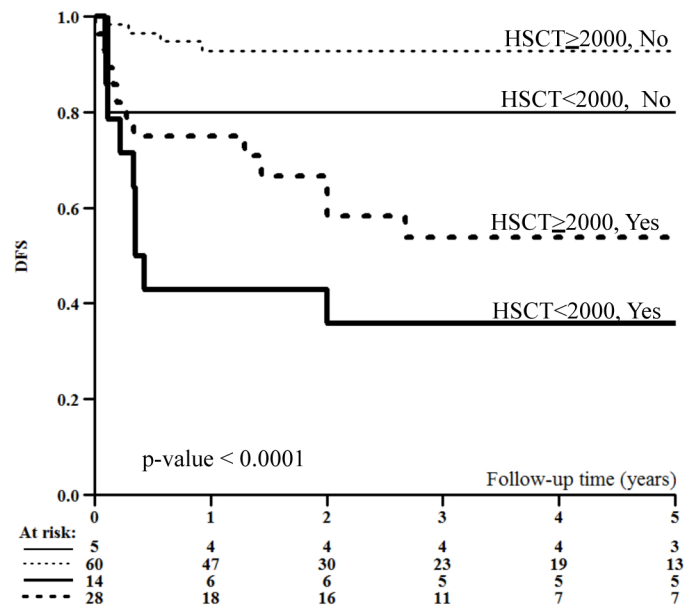# Figure E1 – Busulfan total dose in RIC versus MAC Bu/Flu recipients
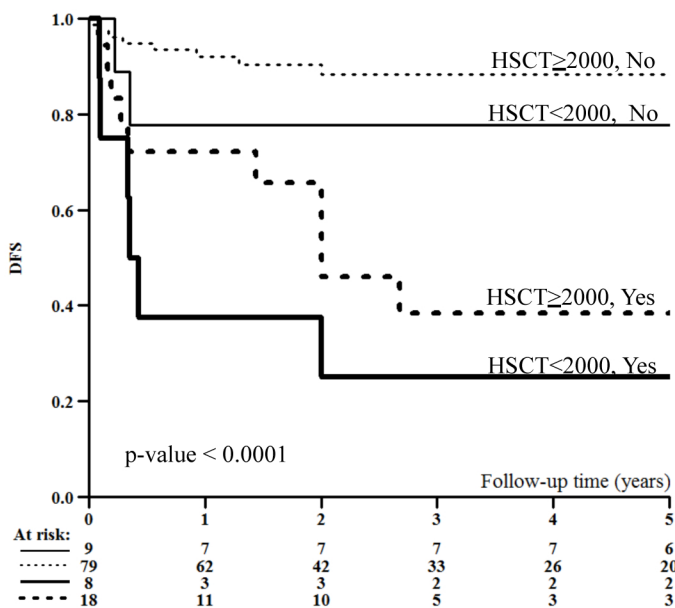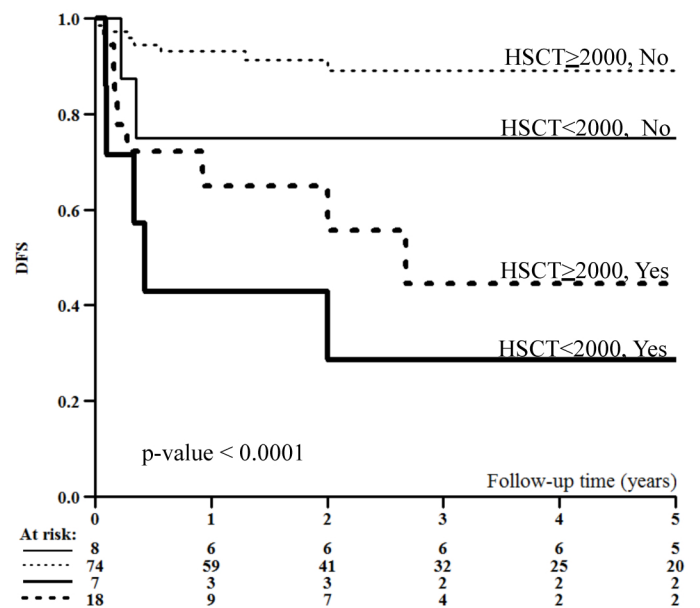
Figure E2 – Disease-free Survival

(A) Age at HSCT

HSCT≥2000, <5 yrs
HSCT≥2000, 5-10 yrs.
HSCT<2000, <5 yrs
HSCT<2000, 5-10 yrs
HSCT≥2000, ≥10 yrs
HSCT<2000, ≥10 yrs
p-value < 0.0001
Follow-up time (years)

At risk:
0	1	2	3	4	5
9	7	7	7	7	6
65	52	37	27	21	15
3	2	2	2	2	2
26	22	16	12	11	10
9	2	2	1	1	1
15	6	6	3	1	1

(B) Organ damage before HSCT

HSCT≥2000, No
HSCT<2000, No
HSCT≥2000, Yes
HSCT<2000, Yes
p-value < 0.0001
Follow-up time (years)

At risk:
0	1	2	3	4	5
5	4	4	4	4	3
60	47	30	23	19	13
14	6	6	5	5	5
28	18	16	11	7	7

(C) Sclerosing cholangitis

HSCT≥2000, No
HSCT<2000, No
HSCT≥2000, Yes
HSCT<2000, Yes
p-value < 0.0001
Follow-up time (years)

At risk:
0	1	2	3	4	5
9	7	7	7	7	6
79	62	42	33	26	20
8	3	3	2	2	2
18	11	10	5	3	3

(D) *Cryptosporidium* infection

HSCT≥2000, No
HSCT<2000, No
HSCT≥2000, Yes
HSCT<2000, Yes
p-value < 0.0001
Follow-up time (years)

At risk:
0	1	2	3	4	5
8	6	6	6	6	5
74	59	41	32	25	20
7	3	3	2	2	2
18	9	7	4	2	2

(E) Conditioning regimen

HSCT≥2000, MAC
HSCT≥2000, MAC low tox
HSCT≥2000, RIC
HSCT<2000, MAC
p-value = 0.0004
Follow-up time (years)

At risk:
0	1	2	3	4	5
23	15	12	7	5	3
18	10	10	9	9	8
58	49	36	28	23	20
17	10	6	4	2	

(F) Waiting time to HSCT

HSCT<2000, ≤ 2yrs
HSCT≥2000, ≤ 2yrs
HSCT≥2000, > 2yrs
HSCT<2000, > 2yrs
p-value < 0.0001
Follow-up time (years)

At risk:
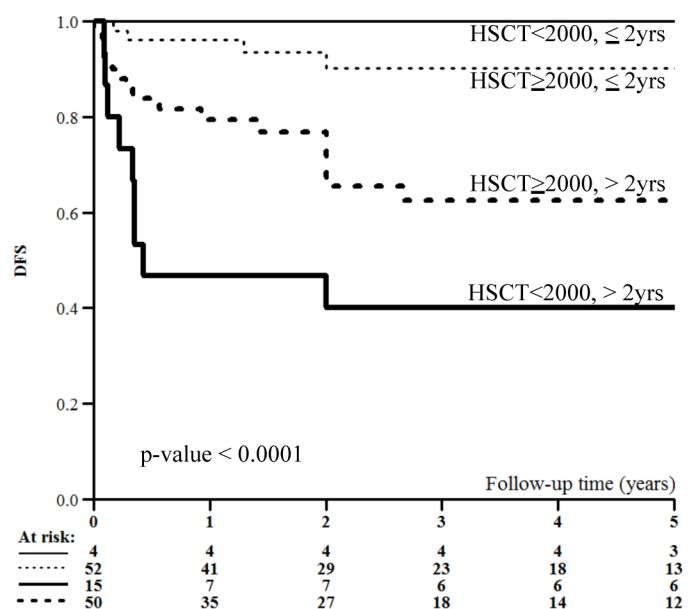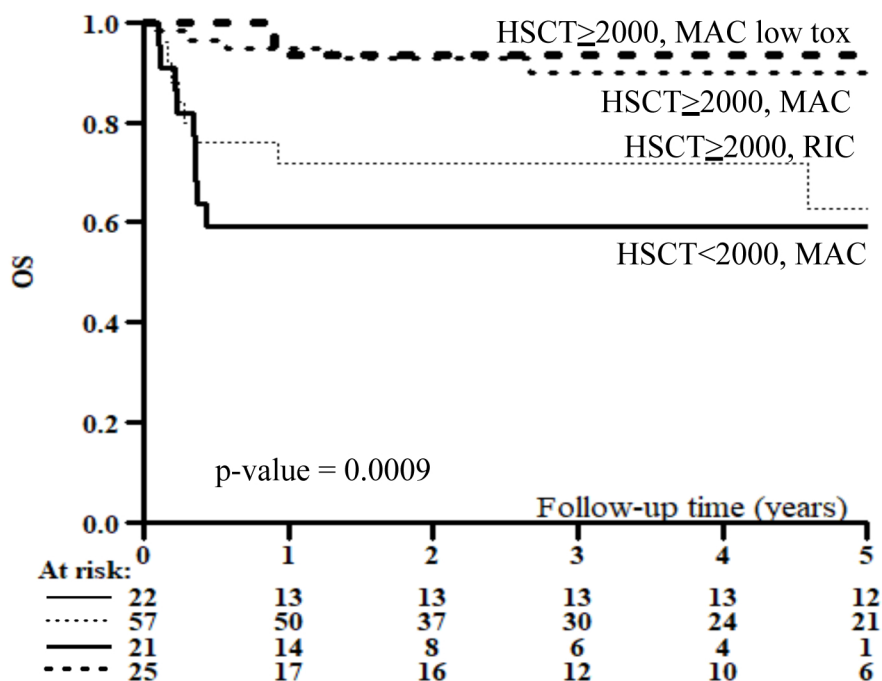| 0	1	2	3	4	5
4	4	4	4	4	3
52	41	29	23	18	13
15	7	7	6	6	6
50	35	27	18	14	12

Figure E3 – OS according to conditioning regimen and donor type

(A) Conditioning regimen

(B) Donor type
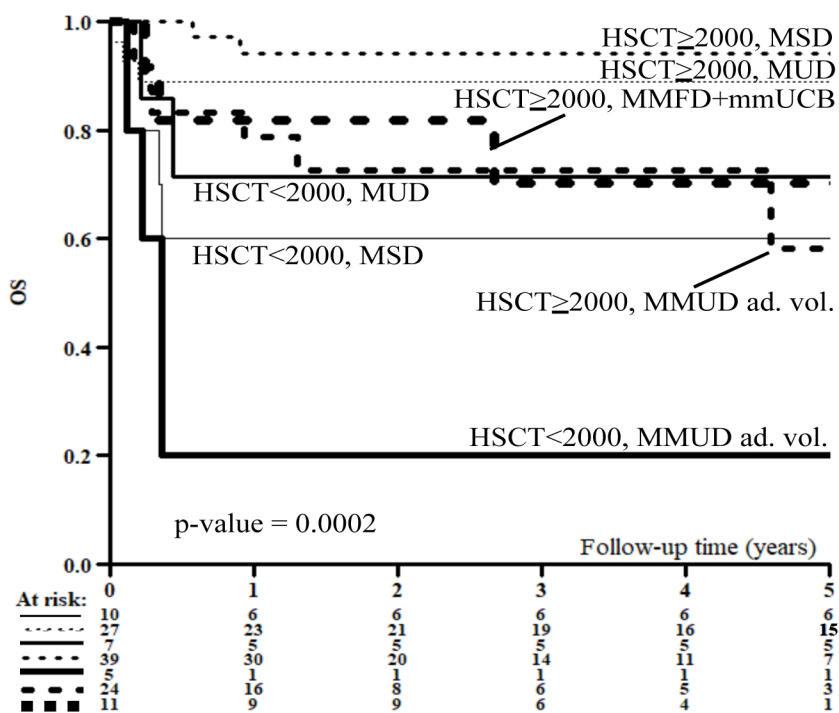
Figure E4 – Causes of death

n = 26

Legend:
- Infection (early)*
- Infection (late)
- Infection with GVHD (early)
- Infection with GVHD (late)°
- Graft rejection
- Liver failure
- MOF
- PML
- Progressive neurodegeneration
- Deteriorating liver function
- GVHD
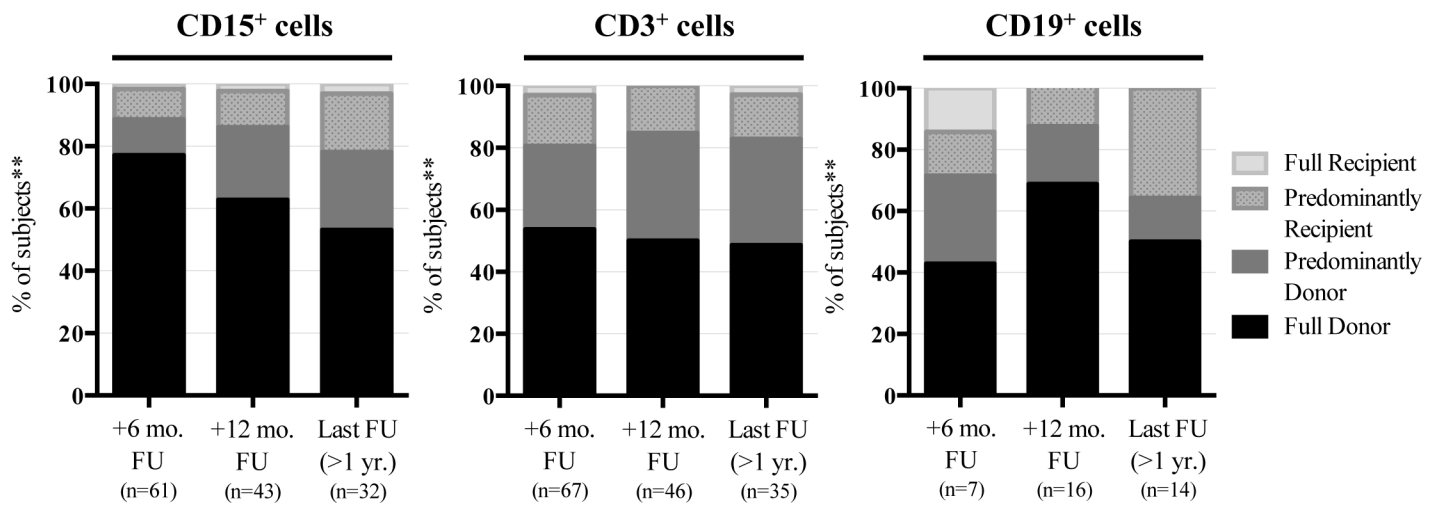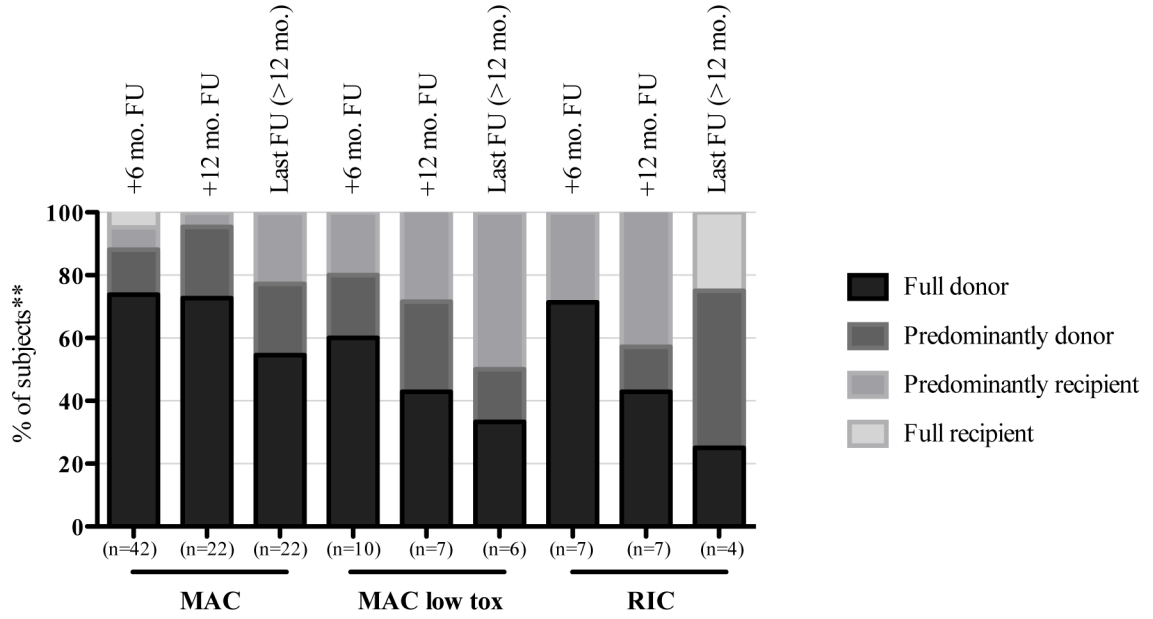- Cardiorespiratory arrest §
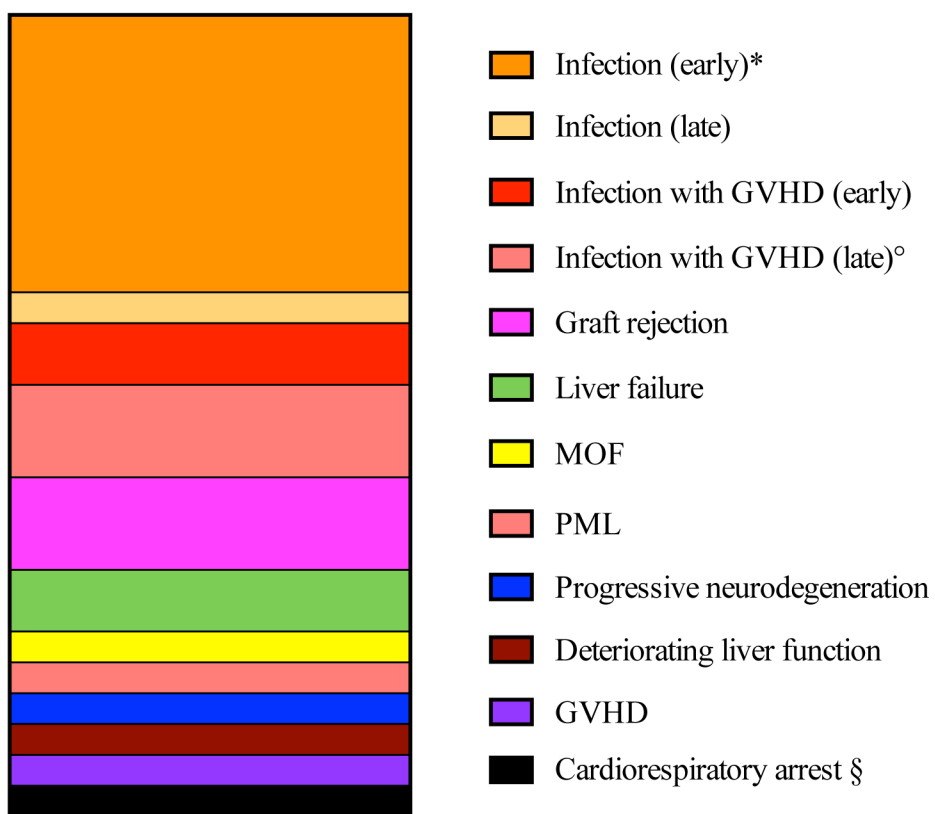
Figure E5 – Lineage-specific chimerism

Figure E6 – Myeloid chimerism and conditioning