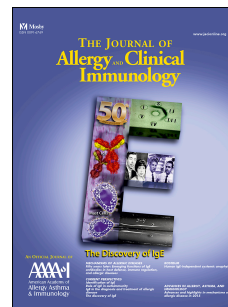


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Outcome of Hematopoietic Cell Transplantation for DNA-Double Strand Breakage Repair Disorders

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139

140

141 **Abstract**

142 Background: Rare DNA breakage-repair disorders predispose to infection and
143 lympho-reticular malignancies. Hematopoietic cell transplantation (HCT) is curative
144 but co-administered chemo- or radio-therapy is damaging due to systemic radio-
145 sensitivity. We collected HCT outcome data for Nijmegen Breakage syndrome
146 (NBS), DNA ligase IV deficiency (LIG4), Cernunnos-XLF deficiency and ataxia-
147 telangiectasia.

148 Methods: Data from 38 centres worldwide, including indication, donor, conditioning
149 regimen, graft-versus-host disease (GvHD) and outcome were analyzed.
150 Conditioning was classified as myeloablative (MAC) if it contained radiotherapy or
151 alkylators and reduced intensity (RIC) if no alkylators and/or fludarabine ≤ 150 mg/m²
152 and cyclophosphamide ≤ 40 mg/kg were used.

153 Results: 55 new, 14 updated and 18 previously published patients were analyzed.
154 Median age at HCT was 48 (range 1.5 – 552) months. 29 were transplanted for
155 infection, 21 malignancy, 13 bone marrow failure, 13 pre-emptively, 5 had multiple
156 indications, and 6 had no information. 22 received MAC, 59 RIC, 4 were infused;-
157 information unavailable for 2. 73/77 patients with LIG4, Cernunnos-XLF deficiency or
158 NBS received conditioning. Survival was 53/77 (69%), worse for MAC than RIC
159 (p=0.006). Most deaths occurred early post-transplant suggesting poor tolerance of
160 conditioning. Survival in ataxia-telangiectasia patients was 25%. 41/83 patients
161 experienced aGvHD (49%): less in RIC compared to MAC, 26/56 (46%) vs 12/21

162 (57%) (p=0.45). Median follow-up was 35 (range 2-168) months. No secondary
163 malignancies were reported during 15 years follow-up. Growth and developmental
164 delay remained post-HCT; immune-mediated complications resolved.

165 Conclusion: RIC-HCT resolves DNA repair disorder-associated immunodeficiency.

166 Long-term follow-up is required for secondary malignancy surveillance. Routine HCT
167 for ataxia-telangiectasia is not recommended.

168 **Key words**: Ataxia-Telangiectasia, Cernunnos-XLF deficiency, DNA repair
169 disorders, DNA Ligase 4 deficiency, Hematopoietic stem cell transplantation,
170 Nijmegen Breakage syndrome,

171

172 **Abbreviations:**

173 AT - Ataxia-Telangiectasia

174 ATG – anti-thymocyte globulin

175 ATM - Ataxia-Telangiectasia mutated

176 Cernunnos-XLF – Cernunnos –XRCC4 like factor

177 CIBMTR - Center for International Blood and Marrow Transplant Research

178 CMC - cytomegalovirus

179 DNA-dsb – DNA double strand breaks

180 DNA-PKcs – DNA protein kinase catalytic subunit

181 EBMT - European Society for Blood and Marrow Transplantation

182 EBV - Epstein-Barr virus

183 GvHD - graft-versus-host disease

184 Gy - Gray

185 HCT - Hematopoietic cell transplantation

186 IEWP - Inborn Errors Working Party

187 LIG4 - DNA ligase 4 deficiency

188 MAC - Myeloablative conditioning

189 NBS – Nijmegen Breakage Syndrome
190 NHEJ - non-homologous end joining
191 NHEJ1 - Non-Homologous End Joining Factor 1
192 PIDTC - North American Primary Immunodeficiency Treatment Consortium
193 PTLD - post-transplant lymphoproliferative disorder
194 RAG1/2 - recombination activating gene 1/2
195 RIC - reduced intensity conditioning
196 SCETIDE - Stem Cell Transplant for primary Immune Deficiencies in Europe
197 SCID - severe combined immunodeficiency
198 XRCC4 - X-ray repair cross-complementing protein 4
199
200

201 **Clinical implications**

202 Hematopoietic cell transplant cures DNA breakage-repair disorders. Cernunnos-XLF
203 deficiency, LIG4 and Nijmegen breakage syndrome patients receiving alkylator or
204 radiotherapy pre-conditioning have worse survival than those receiving reduced
205 intensity conditioning.

206 **Capsule summary**

207 Hematopoietic cell transplant cures DNA breakage-repair disorders. Cernunnos-XLF
208 deficiency, LIG4 and Nijmegen breakage syndrome patients receiving alkylator or
209 radiotherapy pre-conditioning have worse survival than those receiving reduced
210 intensity conditioning. AT patients have very poor outcome.

211

212

213

214 Introduction

215 Maintenance of genomic stability requires repair of DNA, damaged through
216 endogenous processes such as meiotic and mitotic replication errors, and
217 exogenous processes including exposure to oxidising radicals, DNA-damaging
218 chemicals, ultra-violet and ionizing radiation. Several repair pathways regulate the
219 cell cycle, and recognize and repair DNA damage. One of the most serious events to
220 threaten genomic stability, DNA-double strand breaks (DNA-dsb), if unchecked, lead
221 to loss of genomic material, mutagenesis and oncogenesis or cell death¹. Two
222 pathways are employed to repair such damage: homologous recombination, which
223 functions primarily in dividing cells and S phase, and requires a homologous
224 template to maintain replication accuracy, and non-template dependent non-
225 homologous end joining (NHEJ), which is particularly employed during phases of the
226 cell cycle when a homologous template is not present. The latter is an especially
227 error-prone process with some loss of DNA information at the site of the DNA-dsb².

228

229 Development of normal adaptive immunity requires generation of a wide range of T-
230 and B-lymphocyte receptors to recognise unique antigen/MHC combinations and
231 provide effective defence against a broad repertoire of pathogens. Many genetically
232 diverse receptors are generated in the thymus and bone marrow, by breaking,
233 stochastically rearranging and re-joining DNA sequences coding for antigen
234 receptors, a process known as VDJ recombination. Additional diversity is created in
235 B-lymphocytes during immunoglobulin class switch recombination, and somatic
236 hypermutation. The DNA repair mechanisms required to maintain somatic genomic
237 stability are also utilized during lymphocyte VDJ recombination to repair intermediate
238 DNA hairpins and physiological DNA-dsb created following activation of

239 recombination activating gene 1 and 2 (*RAG1/2*)³. Seven ubiquitously-expressed
240 proteins are associated with NHEJ – Ku70/80 and DNA-PKcs, which stabilize the
241 DNA break, the DNA endo/exonuclease Artemis, important for processing RAG-
242 induced hairpin intermediate joins and the DNA ligase 4, Cernunnos-XLF and
243 XRCC4 complex, which together are responsible for the ligation step. Additionally,
244 ataxia-telangiectasia-mutated and nibrin proteins are involved in the initial cell cycle
245 arrest and recruitment of NHEJ proteins to the breakage site⁴ (Supplementary Figure
246 1).

247 Defects in the lymphoid-specific *RAG1/2* proteins lead to T-lymphocyte negative, B-
248 lymphocyte negative, natural killer lymphocyte positive (T-B-NK+) severe combined
249 immunodeficiency (SCID) (5). Defects in Artemis, DNA-PKcs, DNA Ligase 4 and
250 Cernunnos-XLF proteins also lead to T-B-NK+ SCID, and combined
251 immunodeficiencies, often associated with other developmental anomalies,
252 particularly microcephaly in patients with DNA Ligase 4 and Cernunnos-XLF
253 deficiency, as a result of the ubiquitous expression of these proteins⁶⁻¹⁴.

254 Hematopoietic cell transplantation (HCT) is curative for T-B-NK+ SCID, but best
255 results with donor myeloid chimerism and long-term immune reconstitution are
256 obtained if preparative chemotherapy is administered prior to transplantation¹⁵.

257 However, in Artemis-deficient radiosensitive SCID patients, although overall survival
258 is equivalent to patients with *RAG*-deficient SCID, significant long-term sequelae
259 result from the administration of alkylating agents, which are required to gain donor
260 stem cell engraftment with sustained, long-term thymopoiesis. The use of alkylating
261 chemotherapy does not result in increased short-term toxicities or increased
262 transplant-related mortality, but long-term effects on growth and development are
263 observed, due to the effect of chemotherapy on other somatic cells that harbor the

264 genetic defect¹⁵. Similar significant effects of chemotherapy are seen in patients with
265 Fanconi anaemia (OMIM 227650) and dyskeratosis congenita (OMIM 127550) –
266 both DNA fragility syndromes^{16,17}. Given the systemic nature of the DNA-dsb defect
267 in other DNA-dsb repair disorders and the finding that the radiosensitivity is generally
268 more severe than in Artemis-deficiency, it is possible that pre-administration of DNA-
269 damaging chemotherapy prior to transplantation will lead to significant systemic
270 morbidity and possible increased mortality.

271 Due to the primary immunodeficiency phenotype and the frequent occurrence of
272 malignancy, a number of patients with DNA-dsb repair disorders have undergone
273 HCT^{10-13,18-28}. To assess outcome of HCT for DNA-dsb repair disorders, we surveyed
274 patients transplanted for DNA ligase 4 deficiency (LIG4), Cernunnos-XLF deficiency
275 (XLF or NHEJ1), Nijmegen Breakage Syndrome (NBS) and Ataxia-Telangiectasia
276 (AT), using base line data from Stem Cell Transplant for primary Immune
277 Deficiencies in Europe (SCETIDE), Inborn Errors Working Party (IEWP) of the
278 European Society for Blood and Marrow Transplantation (EBMT) registry, the Center
279 for International Blood and Marrow Transplant Research (CIBMTR) and the North
280 American Primary Immune Deficiency Treatment Consortium (PIDTC) and
281 supplemented with additional information from individual centers where available.
282 Patients with mutations in *RAG1/2* and *DCLRE1C* (encoding Artemis) were excluded
283 from the study, as HCT outcomes for these conditions have recently been reported¹⁵.

284

285 Methods

286 Data collection

287 Data on patients with defined mutations in *LIG4* (OMIM 606593), *NBN* (OMIM
288 602667), *NHEJ1* (OMIM 611290) and *ATM* (OMIM 607585), who had undergone
289 HCT were gathered from the IEWP of EBMT, SCETIDE, CIBMTR and the North
290 American PIDTC. Further patients were identified from previously published data and
291 case reports. Centers with identified patients completed a proforma to gather data on
292 genetic diagnosis, patient demographic, reason for HCT, type and source of HCT,
293 conditioning regimen employed, rates and severity of graft-versus-host disease
294 (GvHD) and survival post-HCT.

295 Inclusion criteria were any patient having a confirmed genetic diagnosis and having
296 undergone HCT.

297 The reason to offer HCT was defined as any category or combination of:

- 298 • infection, (defined as any listed severe infection or recurrent infections)
- 299 • malignancy
- 300 • bone marrow failure, (defined as leukopenia, anemia or thrombocytopenia
301 without the presence of infection or malignancy)
- 302 • autoimmunity
- 303 • pre-emptive.

304 Conditioning was categorized as either myeloablative conditioning or reduced
305 intensity conditioning. Myeloablative conditioning (MAC) was defined as any regimen
306 using high dose alkylating agents, typically melphalan or busulphan, thiotepa, or total
307 body irradiation at any dose. Although a low dose 200-400cGy regimen can normally
308 be considered non-myeloablative, we reasoned that radiation-sensitive cells were

309 best not exposed to ionising radiation. If the regimen did not use alkylating agents
310 and/or had doses of fludarabine ≤ 150 mg/m² and cyclophosphamide ≤ 40 mg/kg it
311 was defined as reduced intensity conditioning (RIC)²⁹. A modified Fanconi-regimen
312 was based on fludarabine 120-150 mg/m² (30 mg/m²/day in 4-5 divided doses),
313 cyclophosphamide 20-40 mg/kg (in 4 divided doses) with or without anti-thymocyte
314 globulin (ATG) or alemtuzumab serotherapy^{30,31} or fludarabine 180/m² (in 6 divided
315 doses), busulphan 1.6mg/kg (in 2 divided doses) and cyclophosphamide 40mg/kg (in
316 2 divided doses)³². The use of targeted agents such as antibodies, for example
317 alemtuzumab, did not affect the classification of the conditioning.

318 The primary outcome that was measured was survival. Secondary outcome
319 measures sought were presence, severity and outcome of GvHD, other transplant-
320 related complications and survival.

321 Analysis

322 Significance of results was determined by use of Fisher's exact test, utilising 2x2
323 contingency tables. A two-tailed p value of ≤ 0.05 was considered significant.

324 Kaplan-Meier curves were created based on last known status at time of proforma
325 received, cases where survival was not listed have been excluded from the survival
326 analysis. All statistics were calculated using GraphPad Prism 6 (GraphPad Software,
327 Inc., La Jolla, California).

328

329 **Results**

330 Data were collected from 38 centers worldwide, culminating in 55 newly identified
331 patients, and 14 previously published patients with updated new information, giving
332 new information on 69 patients. Available data from 18 previously published cases¹⁰⁻
333 ^{14,18-28} were included where possible, totalling 87 cases. The median age of patients
334 at HCT was 48 months (range 1.5 - 552 months), 47 were male (54%).

335 Mutations in *LIG4* were most commonly represented, (36 patients, 32 unpublished or
336 with new information) (Table S1), 26 with *NBN* mutations (17 unpublished or with
337 new information) (Table S2), 17 with *NHEJ1* mutations (12 unpublished or with new
338 information) (Table S3), and 8 with *ATM* mutations (all with new information, 2
339 previously published, updated in this report) (Table S4). All patients received
340 allogeneic hematopoietic stem cells, except two published cases who died
341 immediately prior to HCT, whilst receiving MAC, but whose data were included in the
342 study.

343 Information was provided on the primary reason for HCT in 83 patients (figure 1).
344 Significant or repeated infections were the most commonly cited reason (29 patients,
345 35% - 12 with *LIG4* mutations, 11 with *NHEJ1* mutations), 13 patients were
346 transplanted for bone marrow failure (15%) and 21 patients (24%) for malignancy (17
347 with *NBN* mutations). Thirteen patients were transplanted pre-emptively on the basis
348 of a SCID-like diagnosis (15%), 10 with *LIG4* mutations. Five patients had a mixture
349 of the above indications, and in 6 patients, the reason for HCT was not available.

350 Twenty two patients received MAC, and 59 RIC, of which 30 were based on a
351 modified Fanconi anaemia conditioning regimen. Four patients received a stem cell

352 infusion without prior conditioning, data were unavailable for 2 patients. Two
353 received radiotherapy (5Gy, 2Gy) as part of the conditioning regimen.

354 Survival

355 Of patients with DNA ligase 4, Cernunnos-XLF deficiency and NBS, there were
356 survival data for 77, of whom 73 received conditioning. Overall survival was 53/77
357 (69%) (Figure 2A), of whom 2 died from relapse of malignancy giving a transplant-
358 related survival of 71%. One patient with NBS rejected the graft, and is alive with
359 disease. One rejected and succumbed to malignancy. Survival among those
360 receiving myeloablative conditioning was significantly worse at 41% (7/17) compared
361 with 79% (44/56) for those receiving reduced intensity conditioning, ($p=0.006$)
362 (Figure 2B), describing 2 patients who died of malignancy relapse as survivors.
363 There was no significant difference in transplanted-related mortality between those
364 who received a modified Fanconi or other reduced intensity conditioning regimens
365 ($p=0.13$). The Kaplan Meier curve demonstrates that the majority of deaths occur
366 early in the course of transplant, particularly in those receiving myeloablative
367 conditioning, suggesting poor tolerance of the conditioning regimen.

368 In patients with Ataxia-Telangiectasia, overall survival was 25%. Of the 2 patients
369 who survived, both received a modified Fanconi conditioning regimen and neither
370 experienced GvHD, unlike all patients who received myeloablative conditioning. The
371 6 patients who died experienced GvHD grade 2-3 (67%), despite well-matched
372 donors. Death was due to multi-organ failure, viral activation or post-transplant
373 lymphoproliferative disorder (PTLD).

374 Transplant-related survival in the entire cohort for whom data were available was
375 66% (56/85), with a survival of 75% (45/60) following reduced intensity conditioning

376 and 32% (7/22) following myeloablative conditioning ($p=0.0006$). There was no
377 significant difference in outcome between those who underwent HCT for malignancy
378 (12/22 survivors) or for other indications (37/57 survivors, $p=0.44$). There was no
379 significant difference in survivors for those receiving RIC (11/17) or MAC (1/5)
380 conditioning when malignancy was the reason for HSCT ($p=0.14$). There was also
381 no significant difference in survivors for those receiving RIC (5/25) or MAC (4/9)
382 conditioning when infection was the reason for HSCT ($p=0.09$). There were too few
383 cases who were transplanted for bone marrow failure to make a similar comparison.

384 There were no differences in survival between donor sources whether matched
385 sibling, matched unrelated or mis-matched unrelated donors were used (18/25,
386 20/27, 5/8 respectively).

387 Graft versus host disease

388 Data on presence or absence of acute (a) GvHD was available for 83 patients; in 41
389 of these, aGvHD was present (49%). Of the reported patients with aGvHD, 24 (59%)
390 had mild (grade 1-2), and 15 (37%) had severe (grade 3-4) aGvHD (a grade was
391 unavailable for 2 patients). Rates of aGvHD were lower in the RIC group, 26/56
392 cases (46%) for which data were available, compared to the MAC group, in whom
393 12/21 cases (57%) experienced aGvHD although this was not statistically significant
394 ($p=0.45$). Three of 4 patients who received infused stem cells with no pre-
395 conditioning experienced aGvHD grade 1, 3 and 4 respectively. There was no
396 significant difference in survival between those experiencing grades 0-1 compared
397 with grades 2-4 aGvHD ($p=0.22$).

398 Mortality

399 Overall mortality was 29/85 (34%) - information was unavailable for 2 previously
400 published patients²⁴. Two patients died of multi-organ failure during the conditioning
401 process – both received full myeloablative conditioning. Eleven others died of multi-
402 organ failure post-transplantation, making multi-organ failure the most common
403 cause of death (45%). Eleven deaths were predominantly infectious (38%)

404

405 Other Complications

406 The most common non-aGvHD complication was viremia due to adenovirus,
407 cytomegalovirus (CMV), Epstein-Barr virus (EBV) or a combination, reported in
408 24/79 patients (30%), of whom 6 died. There were 6 cases (8%) of EBV-related
409 PTLD. Six patients (8%) experienced severe mucositis, 14 developed chronic (c)
410 GvHD (18%). Seven patients rejected the graft – 2 after stem cell infusion (1 with
411 serotherapy), 2 after T-lymphocyte depleted transplants (1 myeloablative, 1 reduced
412 intensity conditioning) and 3 after MAC or RIC transplants. Patients receiving RIC
413 were less likely to develop severe mucositis, veno-occlusive disease or PTLD than
414 those who received MAC (7/59 vs 8/22, $p=0.0215$).

415

416 Follow up

417 Given the retrospective and multi-institutional nature of the study, detailed
418 information regarding long term (> 5years) follow up was scarce. Median length of
419 follow up was 35 months (range 2-168 months). No secondary malignancies were
420 reported during the follow up period, which although short overall, does include
421 patients with almost 15 years follow up. Pre-existing growth retardation and

422 developmental issues appear to remain post- HCT: more detailed examination would
423 be required to determine whether HCT ameliorates these features. A predisposition
424 to infection or hematological cytopenia pre-existing before HCT appears to have
425 been abolished.

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428 Discussion

429 Many patients with DNA-dsb repair defects exhibit immunodeficiency, ranging from
430 mild to severe combined immunodeficiency. They are at increased risk of developing
431 lymphoid malignancy. Allogeneic HCT is curative for many immunodeficiencies³³.
432 Establishment of effective DNA repair mechanisms in lymphoid progenitors leading
433 to restoration of functional adaptive immunity may prevent the future development of
434 lymphoid malignancy in this cohort of patients. Lymphoid malignancy is difficult to
435 treat effectively when established because of the aggressive nature of the tumours
436 and poor tolerance of patients to cytotoxic radio- and chemotherapy³⁴. It is therefore
437 a reasonable strategy to consider HCT in these patients. However, as most patients
438 have some residual immunity, and even in the SCID phenotype, natural killer cells
439 are present, rejection and poor stem cell engraftment are likely without some
440 preparative cytoreductive pre-conditioning. The systemic nature of the genetic
441 defect, however, increases the risk of substantial morbidity or mortality from
442 chemotherapy or ionising radiation administered prior to transplantation. Only a few
443 small case series of patients with DNA-dsb repair defects undergoing HCT have
444 been published. To date there has been no formal large case series from which to
445 gauge experience.

446 We now report a multi-institutional retrospective survey on outcome of HCT for 55
447 previously unpublished patients and update information for 18 previously reported
448 patients with DNA-dsb repair defects. We have demonstrated that HCT can correct
449 the hematopoietic defect and underlying immunodeficiency. Furthermore we have
450 demonstrated that survival is significantly superior when reduced intensity
451 conditioning is used. It is likely that chemotherapy agents, especially alkylating
452 agents, induce systemic double strand breaks, which are not readily repaired

453 because of the underlying genetic defect. These systemic double strand breaks may
454 contribute to the early mortality seen following myeloablative therapy. This
455 intolerance, clinically manifest as severe toxicity, sometimes followed by higher
456 grade GvHD, suggests that when considering HCT, a reduced intensity conditioning
457 regimen should be used in patients with known ionising radiation sensitivity and/or
458 proven diagnosis of a DNA-dsb repair defect, and that radiotherapy should be
459 omitted. Given the equivalence of outcome results when comparing modified
460 Fanconi anemia-based regimens with other reduced intensity regimens, the former
461 may be preferred. Longer term-follow up is required to determine impact of HCT on
462 future prevention of lymphoid malignancy.

463 The rate of aGvHD overall was 49%, of which 37% was grades 3-4. The rate of
464 cGvHD was 18%. The incidence of severe (grade 3-4) aGvHD and cGvHD is higher
465 than that reported for transplantation of patients with other primary
466 immunodeficiencies³⁵⁻³⁸. It is not clear whether this is due to the greater use of
467 matched unrelated donors, rather than matched sibling donors (although, in the
468 modern era, outcome of HCT using matched siblings or unrelated donors
469 approaches equivalence), particularly in those receiving reduced intensity
470 conditioning. Significant co-morbidities may also have contributed to the increased
471 incidence of GvHD. However, it may be that the underlying molecular defect causing
472 impaired DNA repair and reduced cellular repair capability predisposes to GvHD
473 following cellular damage, as is found in Fanconi anemia or dyskeratosis
474 congenita^{17,39}.

475 Patients showed a range of other early post-HCT complications in addition to GvHD.
476 Most common were viral reactivations, which in the case of EBV led to PTLN in 6

477 patients. Severe mucositis and veno-occlusive disease were commonly
478 encountered.

479 Three patients experienced veno-occlusive disease and two who had undergone
480 transplant for malignancy, experienced relapse of the primary malignancy. Three
481 patients developed autoimmune thyroid disease, and autoimmune cytopenias were
482 also manifest.

483 Within this patient cohort there are few data on long-term follow up. Transplantation,
484 unsurprisingly given the systemic nature of the defect, appears not to improve the
485 effects of the primary disease on growth or neurological development. It may be, as
486 in patients with Artemis-SCID, that use of any alkylating agent leads to long-term
487 sequelae¹⁵. It will be difficult to predict whether growth or development has been
488 improved or deteriorated as a result of chemotherapy, given the scarce data
489 available on the natural history of these diseases, and the variability of phenotype
490 already reported. However, determining the long-term and late beneficial and
491 adverse effects of HCT in DNA-dsb defects will be important to inform about the
492 utility of this treatment approach. A recent report on a cohort of patients with
493 mutations in *NBN* documented poor survival in those developing malignancy²⁴.
494 Given the good survival outcome in this cohort amongst those who received reduced
495 intensity conditioning regimens – a pre-emptive approach to transplant may be
496 considered. Of particular importance, therefore, will be long-term follow up to
497 determine frequency of secondary malignancies – not reported so far in other
498 primary immunodeficiency transplant series, but a well-recognised complication in
499 patients transplanted for Fanconi anemia³⁹.

500 Whilst the outcome of HCT in patients with mutations in *LIG4*, *NBN* and *NHEJ1* is
501 favourable, particularly when reduced intensity conditioning regimens are employed,
502 the data for patients with Ataxia-Telangiectasia undergoing HCT are disappointing.
503 Whether this is specifically due to the use of myeloablative conditioning regimens, or
504 the presence of malignancy, precipitating transplantation as a therapeutic option, is
505 not clear. With current results, it is difficult to recommend HCT as a treatment option
506 for patients with Ataxia-Telangiectasia, except in clinical trials. In contrast, patients
507 with the other conditions described have transplant outcomes similar to other primary
508 immunodeficiencies when choosing a reduced intensity conditioning. Therefore,
509 transplantation could be considered more favourably as a pre-emptive therapeutic
510 approach, particularly if radiotherapy is omitted from the conditioning regimen, and
511 low intensity conditioning regimens are employed. The high rate of post-transplant
512 complications, including GvHD, remains a concern however, and should drive the
513 development of alternative low or non-toxic conditioning approaches that relieve
514 these patients of the deleterious effects of alkylating therapy but enable full T- and B-
515 lymphocyte reconstitution. In the meantime, careful follow up is required to observe
516 further systemic benefits from transplantation, if any, and importantly to monitor for
517 long-term adverse events. In the future, gene therapy may be an acceptable
518 alternative treatment strategy for this group of patients.

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530 Author contributions:

531 ARG conceived and designed the study, interpreted the data and wrote the
532 manuscript, JS and MAS collated and helped interpret the data and write the
533 manuscript, all other authors provided and assisted in analysis of data, and
534 helped write and revise the manuscript. All authors have seen and approved
535 the final version for submission.

536

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722 Figure 1. Indication for hematopoietic cell transplantation

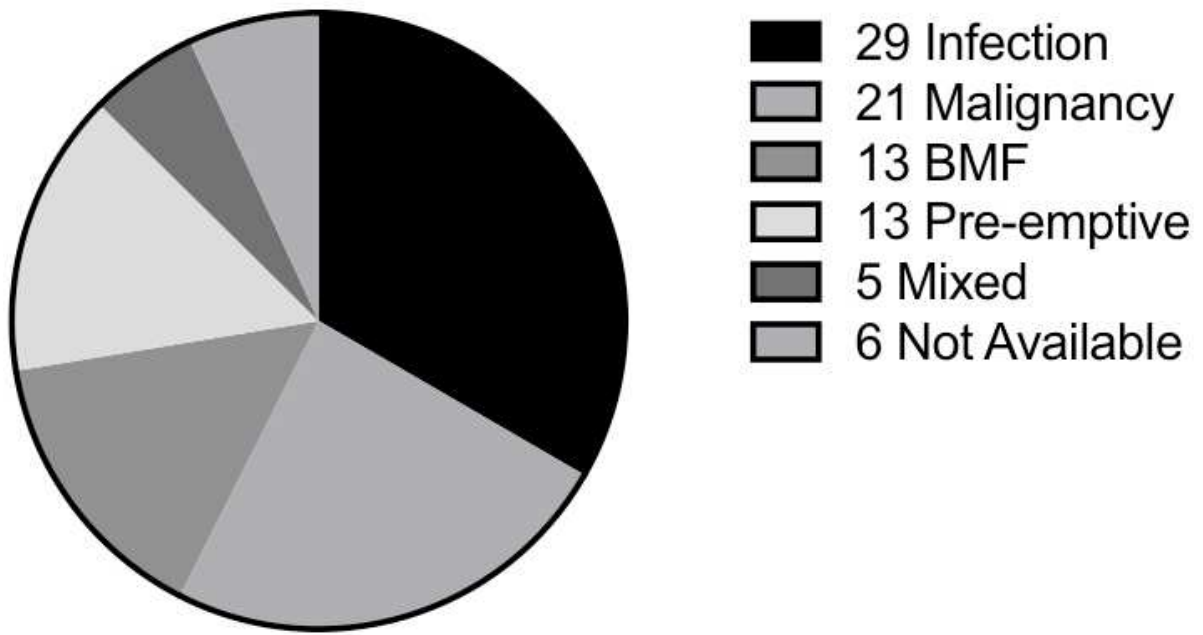
723 Figure 2. Probabilities of overall survival.

724 A. Kaplan Meier curve showing overall survival of 74 patients with with DNA
725 ligase 4, Cernunnos-XLF deficiency and Nijmegen Breakage Syndrome

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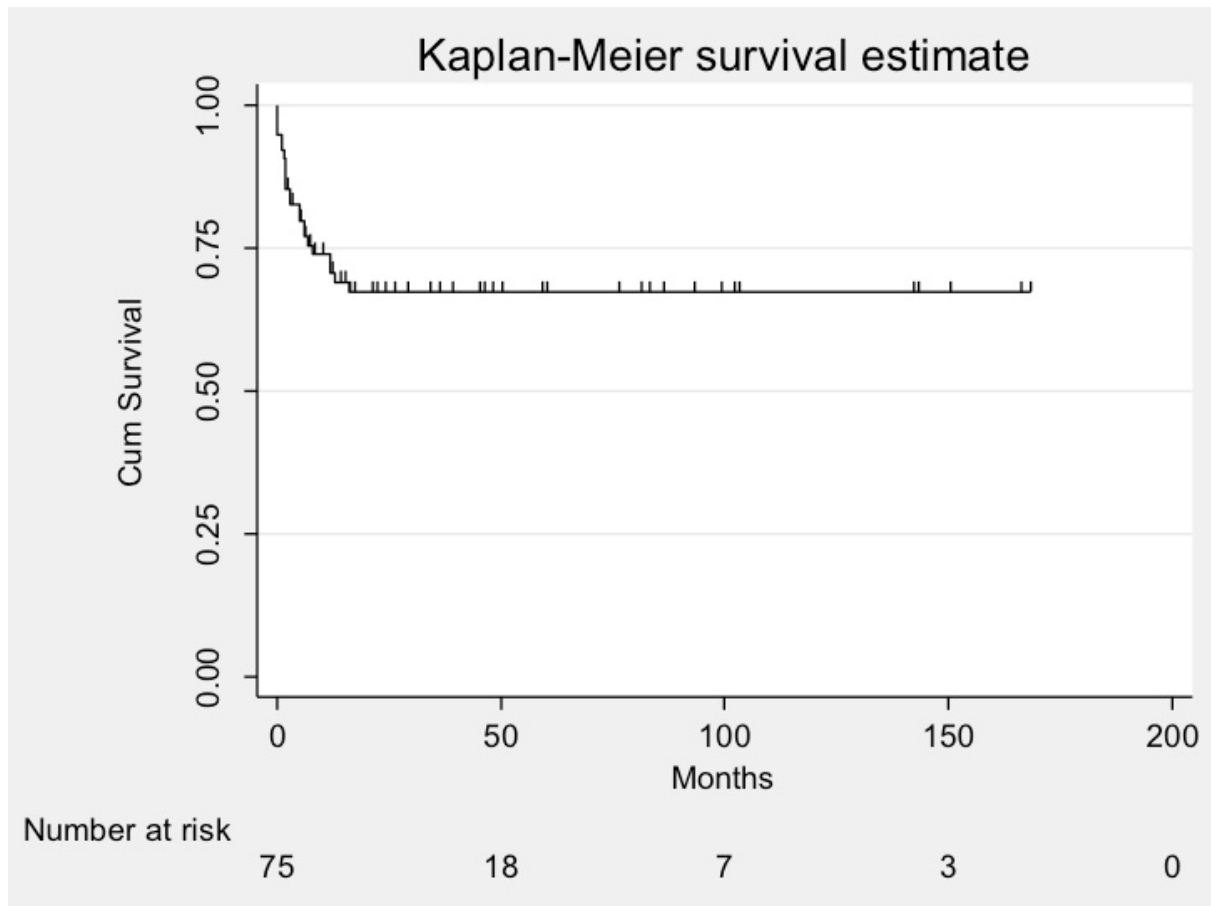
727 B. Kaplan Meier curve demonstrating differences in survival of 74 patients with
728 DNA ligase 4, Cernunnos-XLF deficiency and Nijmegen Breakage Syndrome
729 transplanted using a reduced intensity or myeloablative conditioning
730 regimens.

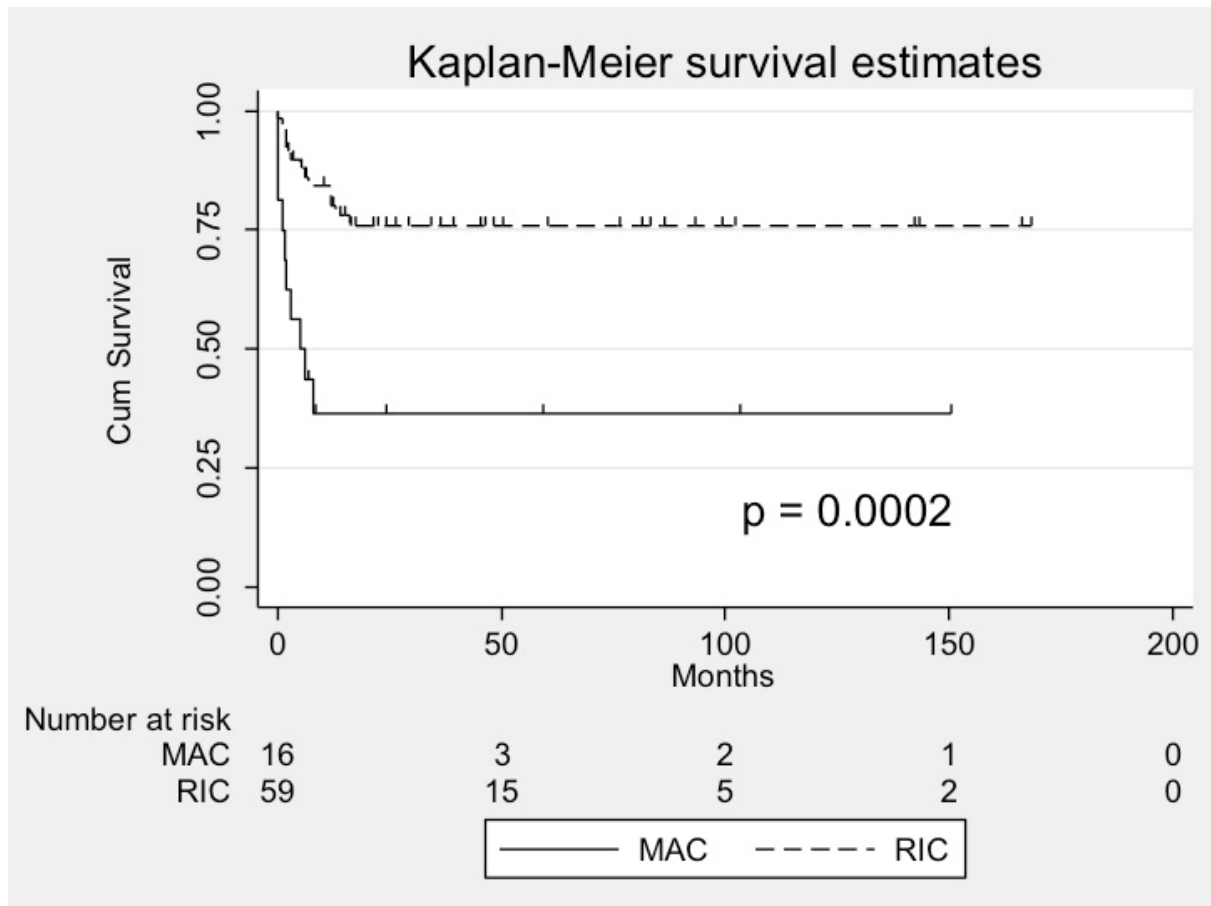
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Supplementary Tables and Figures legend.

Table S1. Characteristics of patients with DNA ligase 4.

Table S2. Characteristics of patients with defects in *NBN*.

Table S3. Characteristics of patients with defects in *NHEJ1*.

Table S4. Characteristics of patients with Ataxia Telangiectasia.

Figure S1. V(D)J Recombination

Figure 1A.

- A. DNA is uncoiled at transcription “factories” within the cell, where the associated recombination and repair proteins co-localize.
- B. The lymphoid specific recombinase activating gene 1 and 2 (RAG1/2) proteins recognize and bind the recombination signal sequences (RSS) that flank the V(D)J gene segments, and introduce site-specific DNA-DSBs.
- C. The phosphorylated blunt signal ends and the covalently sealed hairpin intermediate of the coding end are held together by the RAG complex.

Figure 1B.

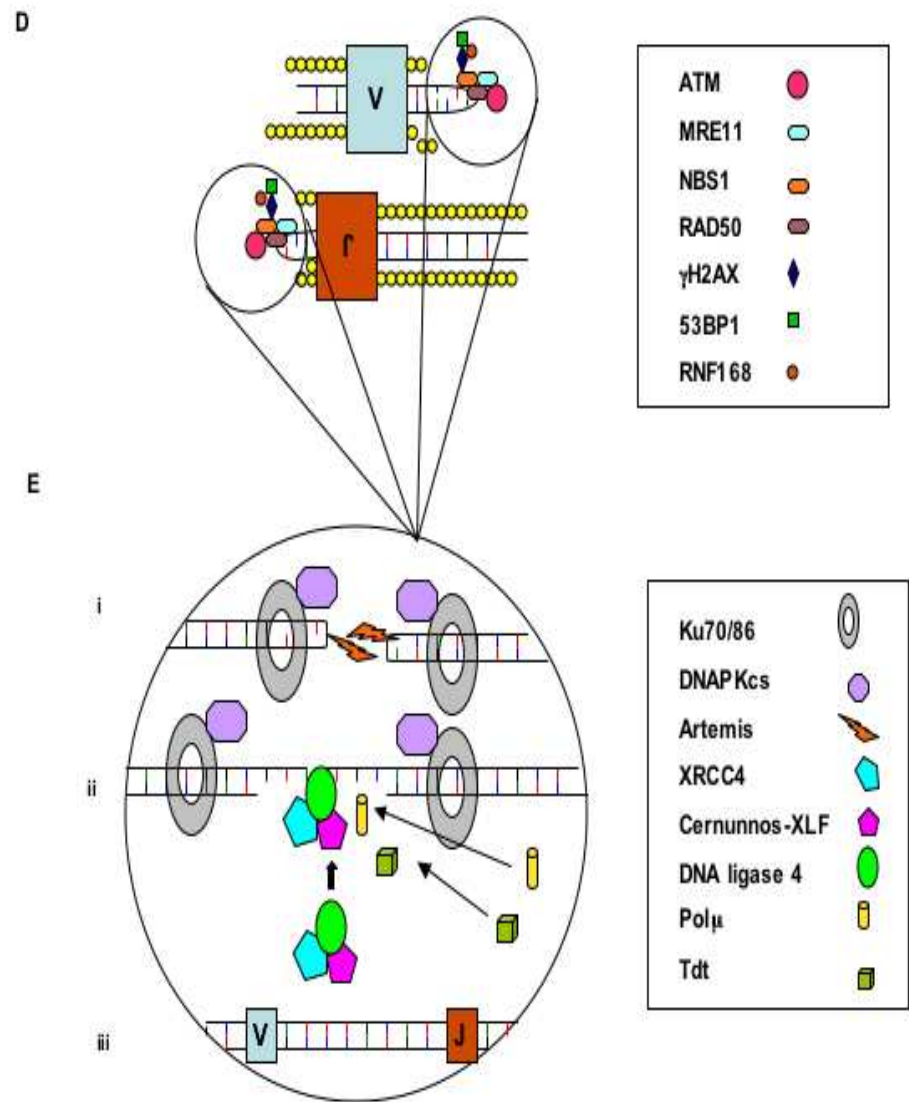
- D. The MRN complex binds the broken DNA ends and activates ATM which initiates cell cycle arrest and attraction of the repair proteins. H2AX, 53BP1 and RNF168, and with other proteins stabilize the damaged chromatin.
- Ei. Ku70/Ku80 heterodimer binds the coding ends and recruits DNA-PKcs and Artemis, which is required to open the hairpin intermediates. The covalently

sealed hairpin intermediate is randomly nicked by the DNA-Pkcs/Artemis complex, which generates a single stranded break with 3' or 5' overhangs.

Eii. XRCC4, DNA ligase 4 and cernunnos-XLF (C-XLF) co-associate and are recruited to the ends. The signal ends are directly ligated by the XRCC4/DNA-LIG4/C-XLF complex. The opened hairpin intermediate is modified by polymerases, exonucleases and the lymphoid-specific terminal deoxynucleotidyl transferase (TdT), before

Eiii. being repaired and ligated by the XRCC4/DNA-LIG4/C-XLF complex

(Reproduced from reference 43 with permission)



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Table S1. Characteristics of patients with DNA ligase 4.

Patient	Age in months at HSCT Sex	Indication	Donor/ Stem cell source	conditioning	aGvHD	Complications	Follow up (months)	Outcome
New cases								
1 Transplant 1	5 M	Infection	MUD CB	Alemtuzumab 1mg/kg	nil	Initial graft failure chronic lung disease	-	Alive
2 Transplant 2	9 M	Graft failure	MUD BM	Flu 150mg/m ² * Melph 70mg/m ² Alemtuzumab 1mg/kg	nil	EBV viraemia and colitis, hypothyroidism, bronchiolitis obliterans	83	Alive
3	8 F	Autoimmunity Omenn phenotype	MMUD 5/6 CB	Flu 150mg/m ² ** Cy 20mg/kg Alemtuzumab 1mg/kg	Grade 2 Skin, liver	nil	48	Alive
4	17 M	Pre-emptive	MUD BM	Flu 150mg/m ² * Melph 70mg/m ² Alemtuzumab 1mg/kg	Grade 3, Skin and Gut	cGvHD, EBV & Adenovirus viraemia colitis, HTN, Cholecystitis, Dilated cardiomyopathy	36	Alive
5	18 M	Infection	MUD PBSC CD34+ selected	Flu 150mg/m ² * Melph 140mg/m ² ATG (dose n/a)	nil		83	Alive
6	18 M	n/a	MUD 8/8 BM	Flu 150mg/m ² * Melph 70 mg/m ² Alemtuzumab 1mg/kg	Grade 3	n/a	24	Alive

6	21	Infection	MMFD BM	Nil	nil	graft failure		Alive
Transplant 1	M							
Transplant 2	23	Graft failure	MMFD BM	Bu 12.9mg/kg * Flu 120mg/m ² Alemtuzumab 0.3mg/kg		mucositis, left arycartilage fracture, synechia of anterior vocal cord	143	Alive
7	20	Infection □ SCID phenotype	MFD □ BM	Flu 150mg/m ² * * Cy 20mg/kg Alemtuzumab 1mg/kg	nil	sepsis	2	alive
8	28	BMF	MUD BM	Flu 150mg/m ² * Melph 70mg/m ² Alemtuzumab 1mg/kg	nil	nil	46	Alive
9	28	n/a	MUD 8/8 BM	Flu 150mg/m ² * Melph 70 mg/m ² Alemtuzumab 1mg/kg	nil	n/a	36	Alive
10	31	Infection	MMFD BM	nil	Grade 3, Skin, Gut	Developmental delay	142	Alive
11	43	BMF	MUD BM, buffy coat enrichment, plasma reduction	Flu 150mg/m ² * * Cy 40mg/kg Alemtuzumab 1mg/kg	nil	nil	24	Alive
12	47	Infection	MFD BM	Flu 150mg/m ² * * Cy 20mg/kg ATG 30mg/kg	nil	nil	15	Alive

13	52 F	Infection	MMFD 9/10 BM	Flu 150mg/m ² * * Cy 20mg/kg Alemtuzumab 1mg/kg	nil	CMV viraemia, EBV-PTLD	50	Alive
14	54 M	Infection Autoimmunity BMF	MSD BM	Flu 150mg/m ² * * Cy 40mg/kg ATG 7.5mg/kg	Grade 1 skin	Limited cGvHD (resolved) Autoimmune hypothyroidism	45	Alive
15	75 F	BMF	MUD 9/10 BM	Bu 2.4mg/kg * * Flu 180mg/m ² Cy 40mg/kg Alemtuzumab 1.5mg/kg	nil	nil	22	Alive
16	83 F	BMF	MUD PBSC	Flu 150mg/m ² * * Cy 20mg/kg Alemtuzumab 1mg/kg	nil	nil	21	Alive
17	116 F	BMF	MMUD 5/6 BM	Flu 150mg/m ² * * Cy 40mg/kg ATG 10mg/kg	Grade 1 skin	PRES cGVHD - skin and mucosa Mixed chimerism	12	Alive
18	120 M	Infection	MSD BM	Flu 40mg/m ² * * Cy 24mg/kg ATG 3mg/kg	nil	nil	22	Alive
19	11 M	Infection SCID phenotype	MUD CB	Flu 90mg/m ² * Melph 114mg/m ²	nil	MOF	2	Dead
20	22 F	Infection	MRD BM	Bu 4mg/kg * Flu 120mg/m ² Melph 140mg/m ²	nil	Heart failure,multi- organ failure from D+1	5 days	Dead

21	33 M	Infection	MUD BM	Flu 150mg/m ^{2*} Melph 140mg/m ² ATG (dose n/a)	Grade 2 Skin, gut	VOD, mucositis, died multi-organ failure, GI bleeding	n/a	Dead
22	49 F	Infection BMF	MMUD CB	Flu 150mg/m ² * * Cy 40mg/kg ATG 10mg/kg	Grade 3 gut	pericardial effusion, SVT, MAS	7	Dead
23	8 M	Pre-emptive	MUD BM	Treo 42g/m ^{2***} Flu 150mg/m ² ATG 10mg/kg	Grade 3 Skin, gut	Norovirus, TPN dependence, graft failure, osteopaenic fractures, HTN, rhinovirus, MOF GI and pulmonary haemorrhage	8	Dead
24	10 F	Infection	Paternal haplo- identical PBSC CD34+ selected	Flu 120mg/m ² *** Melph 140mg/m ² TT 10mg/kg	Grade 1 skin	GI and pulmonary haemorrhage	1	Dead
25	13 M	Infection SCID phenotype	MMUD 9/10 BM	Flu 150mg/m ^{2***} TT 15mg/kg ATG 10mg/kg	Grade 3, skin, gut, liver	P. aeruginosa, RSV, EBV, CMV, capillary leak syndrome Pneumopathy	5	Dead
26	60 M	BMF	Maternal CD34+ haplo	Flu 200 mg/m ^{2***} Cy 20mg/kg TT 5mg/kg ATG 3.gmg/kg	nil	Rejection Fungal pneumonia	6	Dead
Updated Cases 27²⁰	M 49	Infection	MUD PBSC	Flu 150mg/m ² * * Cy 40mg/mg Alemtuzumab	Grade 2 Skin, gut	Autoimmune hypothyroidism	93	Alive

28 ¹⁹	F 6	Infection SCID phenotype	MUD BM	0.6mg/kg YTH24/54 1.6mg/kg Bu 16mg/kg*** Cy 200mg/kg	Grade 4 Skin, gut	cGvHD resp failure, cardiac hypertrophy, renal failure, EBV, developmental delay, raised ICP, tube feed, optic neuritis	103	Alive
29 ¹⁰	M 552	BMF MDS	MSD BM	Bu 12.8mg/kg*** Cy 120mg/kg	nil	Severe mucositis, CMV cGvHD	?	Alive
30 ¹¹	F 19	Infection SCID phenotype	MMUD BM (TCD)	Bu 16mg/kg*** Cy 200mg/kg, ATG 10mg/kg	nil	EBV-PTLD	2	Dead
31 ¹¹	F 2.5	Pre-emptive SCID phenotype	MMUD BM 3/6 TCD	Bu 15mg/kg*** Cy 200mg/kg ATG 10mg/kg	nil	VOD Pneumopathy	1.5	Dead
32 ⁴¹	M 212	BMF	TCR□/□ PBSC haploidentical mother	Flu 180mg/m ² * Cy 60mg/kg ATG 2.5mg/kg	Grade 3 GI	Poor immunoreconstitution, BK viral infection acute renal failure	12	Dead
Published 33 ¹⁶	F 132	BMF	MSD BM	Flu 120mg/m ² * * Cy 40mg/kg ATG 60mg/kg	nil	Delayed puberty	60	Alive
34 ¹²	F 4	SCID phenotype	MUD BM	Flu (dose n/a)*** TT (dose n/a)	nil	severe HUS, with renal impairment	8	Alive
35 ¹³	F 18	Infection	MSD cord	Bu 20mg/kg *** Cy 200mg/kg	nil	Died before HSCT, VOD, resp arrest	-	Dead

36 ¹²	F 24	Infection Autoimmunity Malignancy	Myeloablative*** No details available	Died during conditioning MOF aspergillosis	-	Dead
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* Reduced intensity conditioning regimen, ** Fanconi or modified Fanconi regimen, *** Myeloablative conditioning regimen. ATG, anti-thymocyte globulin; Bu, busulphan; Cy, cyclophosphamide; Flu, fludarabine; Melph, melphalan; Treo, treosulphan; TT, thiotepa; YTH24/54, anti-CD45 monoclonal antibodies

BM, bone marrow; BMF, bone marrow failure; CB, cord blood; CMV, cytomegalovirus; EBV, Epstein-Barr virus; (a)(c)GvHD, (acute) (chronic) graft-versus-host disease; GI, gastrointestinal; HSCT, haematopoietic stem cell transplant; HTN, hypertension; HUS, haemolytic uraemic syndrome; MAS, macrophage activation syndrome; MFD, matched family donor; MMFD, mismatched family donor; MOF, multi-organ failure; MUD, matched unrelated donor; n/a, not available; PBSC, peripheral blood stem cells; PRES, posterior reversible encephalopathy syndrome; PTLD, post-transplant lymphoproliferative disease; RSV, respiratory syncytial virus; SCID, severe combined immunodeficiency; SVT, supra-ventricular tachycardia; TCD, T cell depleted; TCRa/b, T cell receptor alpha/beta depletion; TPN, total parenteral nutrition; VOD, veno-occlusive disease

Table S2. Characteristics of patients with defects in *NBN*.

Patient	Age in months at HSCT Sex	Indication	Donor/ Stem cell source	conditioning	aGvHD	Complications	Follow up (months)	Outcome
New cases								
37	F 45	Autoimmunity	MSD BM	Alemtuzumab* * 1mg/kg Flu 150mg/m ² Cy 20mg/kg	Grade 2 Skin	AIHA	14	Alive
38	M 69	Malignancy	TCRa/b PBSC MUD 9/10.	Bu 4mg/kg, * Flu 150mg/m ² , Cy 30mg/kg, ATG 5mg/kg, Rituximab 100mg/m ²	nil	Hepatitis CMV viraemia	5	Alive
39	M 71	Infection	TCRa/b PBSC 9/10 sibling donor	Bu 4mg/kg, * Flu 150mg/m ² , Cy 20mg/kg, ATG 5mg/kg, Rituximab 100mg/m ²	nil	nil	2	Alive
40	M 90	Malignancy	TCRa/b PBSC MUD 10/10	Bu 4mg/kg, * Flu 150mg/m ² , Cy 40mg/kg, ATG 5mg/kg, Rituximab 100mg/m ²	nil	hepatitis	6	Alive
41	F 107	Pre-emptive	MUD 9/10 PBSC CD34+ with T cell add- back 1*10 ⁸ /kg	Bu 2mg/kg* * Flu 180mg/kg Cy 20mg/kg Alemtuzumab 0.5mg/kg	nil	Secondary graft loss	14	Alive with disease

42	M 144	Malignancy	MSD BM	Bu 4mg/kg, * Flu 150mg/m ² , Cy 20 mg/kg, ATG 5mg/kg	Grade 1 skin	Norovirus, adenovirus enterocolitis	16	Alive
43	F 205	EBV- associated LPD	MSD BM	Bu 4mg/kg, * Flu 150mg/m ² , Cy 30mg/kg, ATG, Rituximab	nil	EBV-PTLD	6	Alive
44	M 228	n/a	MUD 8/8 PBSC	TBI(2Gy), *** Flu 150mg/m ²	Grade 2	cGvHD	59	Alive
45	F 60	Malignancy	MUD PBSC TCRa/b	Bu 4mg/kg,* Flu 150mg/m ² , Cy 40mg/kg, ATG 5mg/kg, Rituximab 100mg/m ²	Grade 1 skin	Mucositis Grade 2, relapse PTCL	3	Dead
46	F 136	Malignancy	MMFD PBSC	Melph 140mg/m ² * Flu 120mg/m ² , Alemtuzumab 1mg/kg	nil	VOD, MOF, sepsis	2	Dead
47	F 204	BMF	MUD PBSC TCRa/b	Bu 4mg/kg, * Flu 150mg/m ² , Cy 40mg/kg, ATG 5mg/kg, Rituximab 100mg/m ²	nil	Rejected 10 months Developed T cell lymphoma	16	Dead
Updated cases 48^{21, 34}	F 27	Infection	MSD BM	Alemtuzumab* * 1mg/kg Flu 150mg/m ² Cy 20mg/kg	nil	Autoimmune hyperthyroidism	102	Alive
49 ^{21, 34}	M 42	Pre-emptive	MFD 10/10 BM	Thoracoabdominal*** irradiation 5 Gy	nil	ADV, CMV Mucositis	150	Alive

				Cy 20mg/kg Alemtuzumab 1mg/kg		Mixed chimerism			
50 ³⁴	F 77	Malignancy	MUD BM	Flu 150mg/m ² , * * Cy 20mg/kg, Alemtuzumab 1mg/kg	Grade 1 Skin	nil	29	Alive	
51 ³⁴	M 110	Malignancy	MFD BM	Flu 150mg/m ² , * * Cy 40mg/kg, ATG 70mg/kg, Rituximab 750mg/m ²	Grade 3 Skin	cGvHD skin, liver CMV reactivation	48	Alive	
52 ²¹	M 240	Malignancy	MUD PBSC	Melph 140mg/m ² , * Flu 125mg/m ² , ATG 60mg/kg	Grade 1 skin	nil	99	Alive	
53 ⁴⁰	M 185	Malignancy	MSD BM	Melph 140mg/m ² * Flu 150mg/m ² , Alemtuzumab 1mg/kg	Grade 1 Skin /gut	Toxoplasmosis	1	Dead	
Published cases									
54 ²²	F 19	Infection	MUD CB	ATG 10mg/kg* * Flu 150mg/m ²	nil	nil	34	Alive	
55 ³⁴	F 46	Infection	MUD PBSC	Cy 20mg/kg Flu 150mg/m ² , ** Cy 20mg/kg Alemtuzumab 1mg/kg	Grade 2 Skin, gut	nil	48	Alive	
56 ³⁴	72 F	Malignancy	MUD PBSC	Bu 2mg/kg** Flu150mg/m ²	nil	nil	17	Alive	
57 ^{21, 34}	M 165	Malignancy	MUD PBSC	ATG 7,5mg/kg Bu 2mg/kg** Flu 150mg/m ² ,	Grade 2 Skin	cGvHD Mild	81	Alive	

58 ²¹	M 174	Malignancy	MMFD TCD PBSC	ATG 60mg/kg Flu 160mg/m ² ,*** TT 10mg/kg	nil	haemorrhagic cystitis Mucositis, ITP, Sepsis, Adeno cryptosporidiosis	24	Alive
59 ³⁴	M 102	Malignancy	MSD BM	Melph 70mg/m ² Flu (dose n/a)* Cy (dose n/a)	Nil	Rejected	11	Alive
	113	Malignancy relapse	MSD BM	Bu 12mg/kg*** Cy 120mg/kg	Gut	sepsis	3	Dead
60 ³⁴	F 110	Malignancy	MSD BM	Bu 2mg/kg** Flu 150mg/m ² , ATG (dose n/a)	nil	Lymphoma relapse	2	Dead
61 ^{21, 34}	M 192	Malignancy	MSD PBSC	Bu 10mg/kg *** Cy 120mg/kg TT 25mg/kg	nil	nil	Sepsis D+5	Dead
62 ³⁴	218 M	Malignancy	MUD PBSC	Flu150mg/m ² * Melph 140mg/m ² ATG (dose n/a)	nil	sepsis	6	Dead

Reduced intensity conditioning regimen, ** Fanconi or modified Fanconi regimen, *** Myeloablative conditioning regimen.
ATG, anti-thymocyte globulin; Bu, busulphan; Cy, cyclophosphamide; Flu, fludarabine; Melph, melphalan; TBI, total body irradiation; TT, thiotepa;

ADV, adenovirus; AIHA, autoimmune haemolytic anaemia; BM, bone marrow; BMF, bone marrow failure; CB, cord blood; CMV, cytomegalovirus; EBV, Epstein-Barr virus; (a)(c)GvHD, (acute) (chronic) graft-versus-host disease; GI, gastrointestinal; HSCT, haematopoietic stem cell transplant; ITP, idiopathic thrombocytopenia; LPD, lymphoproliferative disease; MFD, matched family donor; MMFD, mismatched family donor; MOF, multi-organ failure; MUD, matched unrelated donor; n/a, not available; PBSC, peripheral blood stem cells; TCD, T cell depleted; TCRa/b, T cell receptor alpha/beta depletion; VOD, veno-occlusive disease

Table S3. Characteristics of patients with defects in *NHEJ1*.

Patient	Age in months at HSCT Sex	Indication	Donor/ Stem cell source	conditioning	GvHD	Complications	Follow up (months)	Outcome
New cases								
63	M 5	Infection	MUD PBSC	Flu 150mg/m ² * Cy 20 mg/kg Alemtuzumab 1mg/kg	Grade 4 skin	ADV	10	Alive
64	M 10	Infection	MSD BM	Flu 150mg/m ² * Cy 20 mg/kg	nil	CMV	3	Alive
65	F 12	SCID-like Infection	MSD BM	nil	Grade 1 aGvHD skin	nil	76	Alive
66	M 17	Infection	MSD BM	Cy 200mg/kg* ATG 60mg/kg	nil	nil	168	Alive
67	M 28	Infection	MSD BM	Cy 200mg/kg * ATG 60mg/kg	nil	idiopathic pneumonitis	166	Alive
68	F 48	Infection	MMUD CB	Flu 150mg/m ² * * Cy 40mg/kg ATG 60mg/kg	Grade 2 skin	CMV reactivation	24	Alive
69	M 100	BMF	MUD 6/6 PBSC	Flu 150mg/m ² * * Cy 4mg/kg ATG 7.5mg/kg	Grade 2, Skin	Severe skin cGvHD with scleroderma and joint deformation Cachexia, oesophageal stenosis	86	Alive
70	F 112	Infection, cytopenia	MSD BM	Flu 150mg/m ² * * Cy 40mg/kg	Grade 1 aGvHD	lung cGvHD obstructive lung	39	Alive

71	M 172	Infection BMF	MUD 10/10 BM	ATG 60mg/kg ATG 15mg/kg*** Treo 42mg/m ² Flu 160mg/m ²	skin nil	disease nil	7	Alive
72	15 M	Autoimmune (AIHA)	MUD CB	Bu 6.4mg/kg* Flu 120mg/m ² ATG 10mg/kg	Grade 2, Skin	Sepsis, EBV, myocarditis	5	Dead
73	41 M	Infection	MUD BM	Flu 150mg/m ² , ** Cy 40mg/kg, Alemtuzumab 1mg/kg	Grade 3, Skin and Gut	Pancreatitis, CMV, renal failure, HTN, seizures, myelofibrosis, hyperglycaemia	12	Dead
74	F 108	BMF	MUD 4/6 CB x2	Flu 150mg/m ² * Melph 140mg/m ² Alemtuzumab 1.5mg/kg	Grade 3 Gut	cGvHD EBV, ADV	13	Dead
Published cases								
75 ²⁵	M 10	Infection	MMUD PBSC	Flu 120mg/m ² ** Cy 40mg/kg ATG 15mg/kg	Grade 2, Skin aGvHD	cGvHD EBV-PTLD	26	Alive
76 ²⁶	F 15	Infection AIHA	MSD BM	nil	nil	nil	83	Alive
77 ²⁶	F 18	Infection	MSD BM	nil	Grade 4 Gut	nil	6	Alive
78 ²⁴	F 22	Infection	BM	n/a	n/a	n/a	n/a	n/a
79 ²⁴	M 101	Infection	BM	n/a	n/a	n/a	n/a	n/a

Reduced intensity conditioning regimen, ** Fanconi or modified Fanconi regimen, *** Myeloablative conditioning regimen.
ATG, anti-thymocyte globulin; Bu, busulphan; Cy, cyclophosphamide; Flu, fludarabine; Melph, melphalan; Treo, treosulphan;

ADV, adenovirus; AIHA, autoimmune haemolytic anaemia; BM, bone marrow; BMF, bone marrow failure; CB, cord blood; CMV, cytomegalovirus; EBV, Epstein-Barr virus; (a)(c)GvHD, (acute) (chronic) graft-versus-host disease; HTN, hypertension; MFD, matched family donor; MOF, multi-organ failure; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; n/a, not available; PBSC, peripheral blood stem cells; PTLD, post-transplant lymphoproliferative disease;

Table S4. Characteristics of patients with Ataxia Telangiectasia.

Patient	Age in months at HSCT Sex	Indication	Donor/ Stem cell source	conditioning	aGvHD	Complications	Follow up (months)	Outcome
New cases								
80	156 M	Malignancy	MSD BM	Bu 1.6mg/kg* *, Flu 180mg/m ² , Cy 40mg/kg, rituximab (dose n/a)	nil	haemorrhagic cystitis, VOD, Septicaemia, GI bleed	27	Alive
81	8 M	Infection	MUD BM	Treo 36mg/m ² *** Flu 150mg/m ² Alemtuzumab 1mg/kg	Grade 1- 2 skin	EBV-PTLD	6	Dead
82	22 F	BMF	MFD BM	Treo 46g/m ² *** Flu 150mg/m ²	Grade 3, liver and skin	PTLD, Hepatic failure	20	Dead
83	101 F	n/a	MSD n/a	Bu (dose n/a)*** Cy (dose n/a)	Grade 2, skin and gut	MOF	4	Dead
84	138 M	Malignancy	MFD PBSC	Flu 150mg/m ² ,* * Cy 0.3mg/kg	Grade (n/a) skin	Extensive cGvHD skin Interstitial pneumonitis	11	Dead
85	144 M	Malignancy	MSD BM	Bu (dose n/a)*** Cy (dose n/a)	Grade 2 skin	Pericardial effusion Hemorrhagic cystitis	3	Dead
Updated publication								
86 ²⁷	54 M	ALL-T	MSD BM	Bu 2mg/kg,* * Flu 150mg/m ²	nil	haemorrhagic cystitis, CMV	48	Alive

87 ⁴²	22 M	Infection	MFD BM	ATG 80mg/kg, OKT3 (dose n/a)* Treo 36g/m ² *** Flu 150mg/m ² ATG 60 mg/kg	Grade 3 Skin, liver	reactivation fulminant hepatic failure, gammopathy, EBV reactivation, encephalopathy	10	Dead
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Reduced intensity conditioning regimen, ** Fanconi or modified Fanconi regimen, *** Myeloablative conditioning regimen. ATG, anti-thymocyte globulin; Bu, busulphan; Cy, cyclophosphamide; Flu, fludarabine; OKT3, Muromonab-CD3; Treo, treosulphan

BM, bone marrow; BMF, bone marrow failure; CMV, cytomegalovirus; EBV, Epstein-Barr virus; (a)(c)GvHD, (acute) (chronic) graft-versus-host disease; GI, gastrointestinal; MFD, matched family donor; MMFD, mismatched family donor; MOF, multi-organ failure; MUD, matched unrelated donor; n/a, not available; PBSC, peripheral blood stem cells; PTLN, post-transplant lymphoproliferative disease; VOD, veno-occlusive disease