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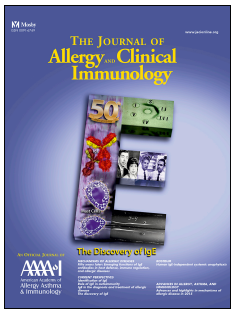

Outcome of Hematopoietic Cell Transplantation for DNA-Double Strand Breakage Repair Disorders

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Abstract

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

Methods: Data from 38 centres worldwide, including indication, donor, conditioning regimen, graft-versus-host disease (GvHD) and outcome were analyzed. Conditioning was classified as myeloablative (MAC) if it contained radiotherapy or alkylators and reduced intensity (RIC) if no alkylators and/or fludarabine ≤150 mg/m^2 and cyclophosphamide ≤ 40 mg/kg were used.

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

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

Conclusion: RIC-HCT resolves DNA repair disorder-associated immunodeficiency. Long-term follow-up is required for secondary malignancy surveillance. Routine HCT for ataxia-telangiectasia is not recommended.

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

Abbreviations:

AT - Ataxia-Telangiectasia

ATG – anti-thymocyte globulin

ATM - Ataxia-Telangiectasia mutated

Cernunnos-XLF – Cernunnos –XRCC4 like factor

CIBMTR - Center for International Blood and Marrow Transplant Research

CMC - cytomegalovirus

DNA-dsb – DNA double strand breaks

DNA-PKcs – DNA protein kinase catalytic subunit

EBMT - European Society for Blood and Marrow Transplantation

EBV - Epstein-Barr virus

GvHD - graft-versus-host disease

Gy - Gray

HCT - Hematopoietic cell transplantation

IEWP - Inborn Errors Working Party

LIG4 - DNA ligase 4 deficiency

MAC - Myeloablative conditioning

NBS – Nijmegen Breakage Syndrome

NHEJ - non-homologous end joining

NHEJ1 - Non-Homologous End Joining Factor 1

PIDTC - North American Primary Immunodeficiency Treatment Consortium

PTLD - post-transplant lymphoproliferative disorder

RAG1/2 - recombination activating gene 1/2

RIC - reduced intensity conditioning

SCETIDE - Stem CEll Transplant for primary Immune Deficiencies in Europe

SCID - severe combined immunodeficiency

XRCC4 - X-ray repair cross-complementing protein 4

Clinical implications

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

Capsule summary

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

Introduction

Maintenance of genomic stability requires repair of DNA, damaged through endogenous processes such as meiotic and mitotic replication errors, and exogenous processes including exposure to oxidising radicals, DNA-damaging chemicals, ultra-violet and ionizing radiation. Several repair pathways regulate the cell cycle, and recognize and repair DNA damage. One of the most serious events to threaten genomic stability, DNA-double strand breaks (DNA-dsb), if unchecked, lead to loss of genomic material, mutagenesis and oncogenesis or cell death[1]. Two pathways are employed to repair such damage: homologous recombination, which functions primarily in dividing cells and S phase, and requires a homologous template to maintain replication accuracy, and non-template dependent non-homologous end joining (NHEJ), which is particularly employed during phases of the cell cycle when a homologous template is not present. The latter is an especially error-prone process with some loss of DNA information at the site of the DNA-dsb[2].

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

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

Defects in the lymphoid-specific RAG1/2 proteins lead to T-lymphocyte negative, B-lymphocyte negative, natural killer lymphocyte positive (T-B-NK+) severe combined immunodeficiency (SCID) (5). Defects in Artemis, DNA-PKcs, DNA Ligase 4 and Cernunnos-XLF proteins also lead to T-B-NK+ SCID, and combined immunodeficiencies, often associated with other developmental anomalies, particularly microcephaly in patients with DNA Ligase 4 and Cernunnos-XLF deficiency, as a result of the ubiquitous expression of these proteins[6-14]. Hematopoietic cell transplantation (HCT) is curative for T-B-NK+ SCID, but best results with donor myeloid chimerism and long-term immune reconstitution are obtained if preparative chemotherapy is administered prior to transplantation[15]. However, in Artemis-deficient radiosensitive SCID patients, although overall survival is equivalent to patients with RAG-deficient SCID, significant long-term sequelae result from the administration of alkylating agents, which are required to gain donor stem cell engraftment with sustained, long-term thymopoiesis. The use of alkylating chemotherapy does not result in increased short-term toxicities or increased transplant-related mortality, but long-term effects on growth and development are observed, due to the effect of chemotherapy on other somatic cells that harbor the

genetic defect[15]. Similar significant effects of chemotherapy are seen in patients with Fanconi anaemia (OMIM 227650) and dyskeratosis congenita (OMIM 127550) – both DNA fragility syndromes[16,17]. Given the systemic nature of the DNA-dsb defect in other DNA-dsb repair disorders and the finding that the radiosensitivity is generally more severe than in Artemis-deficiency, it is possible that pre-administration of DNA-damaging chemotherapy prior to transplantation will lead to significant systemic morbidity and possible increased mortality.

Due to the primary immunodeficiency phenotype and the frequent occurrence of malignancy, a number of patients with DNA-dsb repair disorders have undergone HCT[10-13,18-28]. To assess outcome of HCT for DNA-dsb repair disorders, we surveyed patients transplanted for DNA ligase 4 deficiency (LIG4), Cernunnos-XLF deficiency (XLF or NHEJ1), Nijmegen Breakage Syndrome (NBS) and Ataxia-Telangiectasia (AT), using base line data from Stem CEll Transplant for primary Immune Deficiencies in Europe (SCETIDE), Inborn Errors Working Party (IEWP) of the European Society for Blood and Marrow Transplantation (EBMT) registry, the Center for International Blood and Marrow Transplant Research (CIBMTR) and the North American Primary Immune Deficiency Treatment Consortium (PIDTC) and supplemented with additional information from individual centers where available. Patients with mutations in *RAG1/2* and *DCLRE1C* (encoding Artemis) were excluded from the study, as HCT outcomes for these conditions have recently been reported[15].

Methods

Data collection

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

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

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

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

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

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

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

Analysis

Significance of results was determined by use of Fisher's exact test, utilising 2x2 contingency tables. A two-tailed p value of ≤ 0·05 was considered significant. Kaplan-Meier curves were created based on last known status at time of proforma received, cases where survival was not listed have been excluded from the survival analysis. All statistics were calculated using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, California).

Results

Data were collected from 38 centers worldwide, culminating in 55 newly identified patients, and 14 previously published patients with updated new information, giving new information on 69 patients. Available data from 18 previously published cases[10-14,18-28] were included where possible, totalling 87 cases. The median age of patients at HCT was 48 months (range 1.5 - 552 months), 47 were male (54%).

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

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

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

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

Survival

Of patients with DNA ligase 4, Cernunnos-XLF deficiency and NBS, there were survival data for 77, of whom 73 received conditioning. Overall survival was 53/77 (69%) (Figure 2A), of whom 2 died from relapse of malignancy giving a transplant-related survival of 71%. One patient with NBS rejected the graft, and is alive with disease. One rejected and succumbed to malignancy. Survival among those receiving myeloablative conditioning was significantly worse at 41% (7/17) compared with 79% (44/56) for those receiving reduced intensity conditioning, (p=0·006) (Figure 2B), describing 2 patients who died of malignancy relapse as survivors. There was no significant difference in transplanted-related mortality between those who received a modified Fanconi or other reduced intensity conditioning regimens (p=0·13). The Kaplan Meier curve demonstrates that the majority of deaths occur early in the course of transplant, particularly in those receiving myeloablative conditioning, suggesting poor tolerance of the conditioning regimen.

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

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

and 32% (7/22) following myeloablative conditioning (p= 0·0006). There was no significant difference in outcome between those who underwent HCT for malignancy (12/22 survivors) or for other indications (37/57 survivors, p=0·44). There was no significant difference in survivors for those receiving RIC (11/17) or MAC (1/5) conditioning when malignancy was the reason for HSCT (p=0·14). There was also no significant difference in survivors for those receiving RIC (5/25) or MAC (4/9) conditioning when infection was the reason for HSCT (p=0·09). There were too few cases who were transplanted for bone marrow failure to make a similar comparison.

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

Graft versus host disease

Data on presence or absence of acute (a) GvHD was available for 83 patients; in 41 of these, aGvHD was present (49%). Of the reported patients with aGvHD, 24 (59%) had mild (grade 1- 2), and 15 (37%) had severe (grade 3-4) aGvHD (a grade was unavailable for 2 patients). Rates of aGvHD were lower in the RIC group, 26/56 cases (46%) for which data were available, compared to the MAC group, in whom 12/21 cases (57%) experienced aGvHD although this was not statistically significant (p=0·45). Three of 4 patients who received infused stem cells with no pre-conditioning experienced aGvHD grade 1, 3 and 4 respectively. There was no significant difference in survival between those experiencing grades 0-1 compared with grades 2-4 aGvHD (p=0·22).

Mortality

Overall mortality was 29/85 (34%) - information was unavailable for 2 previously published patients[24]. Two patients died of multi-organ failure during the conditioning process – both received full myeloablative conditioning. Eleven others died of multi-organ failure post-transplantation, making multi-organ failure the most common cause of death (45%). Eleven deaths were predominantly infectious (38%)

Other Complications

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

Follow up

Given the retrospective and multi-institutional nature of the study, detailed information regarding long term (> 5years) follow up was scarce. Median length of follow up was 35 months (range 2-168 months). No secondary malignancies were reported during the follow up period, which although short overall, does include 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.

426

427

Discussion

Many patients with DNA-dsb repair defects exhibit immunodeficiency, ranging from mild to severe combined immunodeficiency. They are at increased risk of developing lymphoid malignancy. Allogeneic HCT is curative for many immunodeficiencies[33]. Establishment of effective DNA repair mechanisms in lymphoid progenitors leading to restoration of functional adaptive immunity may prevent the future development of lymphoid malignancy in this cohort of patients. Lymphoid malignancy is difficult to treat effectively when established because of the aggressive nature of the tumours and poor tolerance of patients to cytotoxic radio- and chemotherapy[34]. It is therefore a reasonable strategy to consider HCT in these patients. However, as most patients have some residual immunity, and even in the SCID phenotype, natural killer cells are present, rejection and poor stem cell engraftment are likely without some preparative cytoreductive pre-conditioning. The systemic nature of the genetic defect, however, increases the risk of substantial morbidity or mortality from chemotherapy or ionising radiation administered prior to transplantation. Only a few small case series of patients with DNA-dsb repair defects undergoing HCT have been published. To date there has been no formal large case series from which to gauge experience.

We now report a multi-institutional retrospective survey on outcome of HCT for 55 previously unpublished patients and update information for 18 previously reported patients with DNA-dsb repair defects. We have demonstrated that HCT can correct the hematopoietic defect and underlying immunodeficiency. Furthermore we have demonstrated that survival is significantly superior when reduced intensity conditioning is used. It is likely that chemotherapy agents, especially alkylating 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 immunodeficiencies[35-38]. 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 PTLD in 6

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

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

Within this patient cohort there are few data on long-term follow up. Transplantation, unsurprisingly given the systemic nature of the defect, appears not to improve the effects of the primary disease on growth or neurological development. It may be, as in patients with Artemis-SCID, that use of any alkylating agent leads to long-term sequalae[15]. It will be difficult to predict whether growth or development has been improved or deteriorated as a result of chemotherapy, given the scarce data available on the natural history of these diseases, and the variability of phenotype already reported. However, determining the long-term and late beneficial and adverse effects of HCT in DNA-dsb defects will be important to inform about the utility of this treatment approach. A recent report on a cohort of patients with mutations in *NBN* documented poor survival in those developing malignancy[24]. Given the good survival outcome in this cohort amongst those who received reduced intensity conditioning regimens – a pre-emptive approach to transplant may be considered. Of particular importance, therefore, will be long-term follow up to determine frequency of secondary malignancies – not reported so far in other primary immunodeficiency transplant series, but a well-recognised complication in patients transplanted for Fanconi anemia[39].

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

Declarations:

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Author contributions:

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

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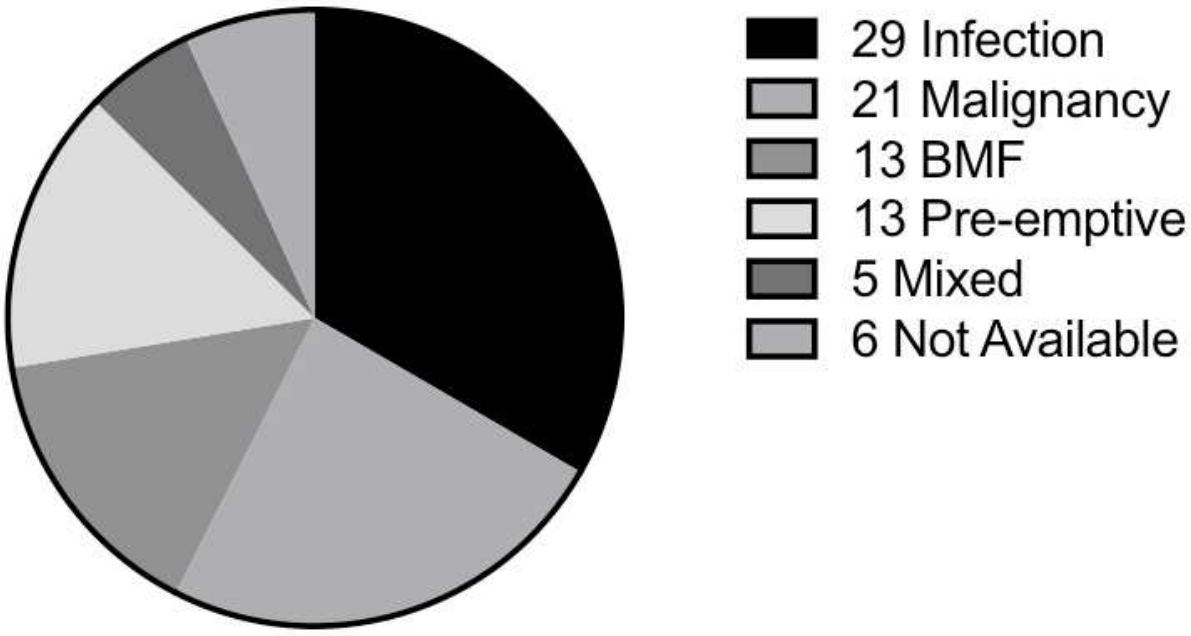
Figure 1. Indication for hematopoietic cell transplantation

Figure 2. Probabilities of overall survival.

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

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



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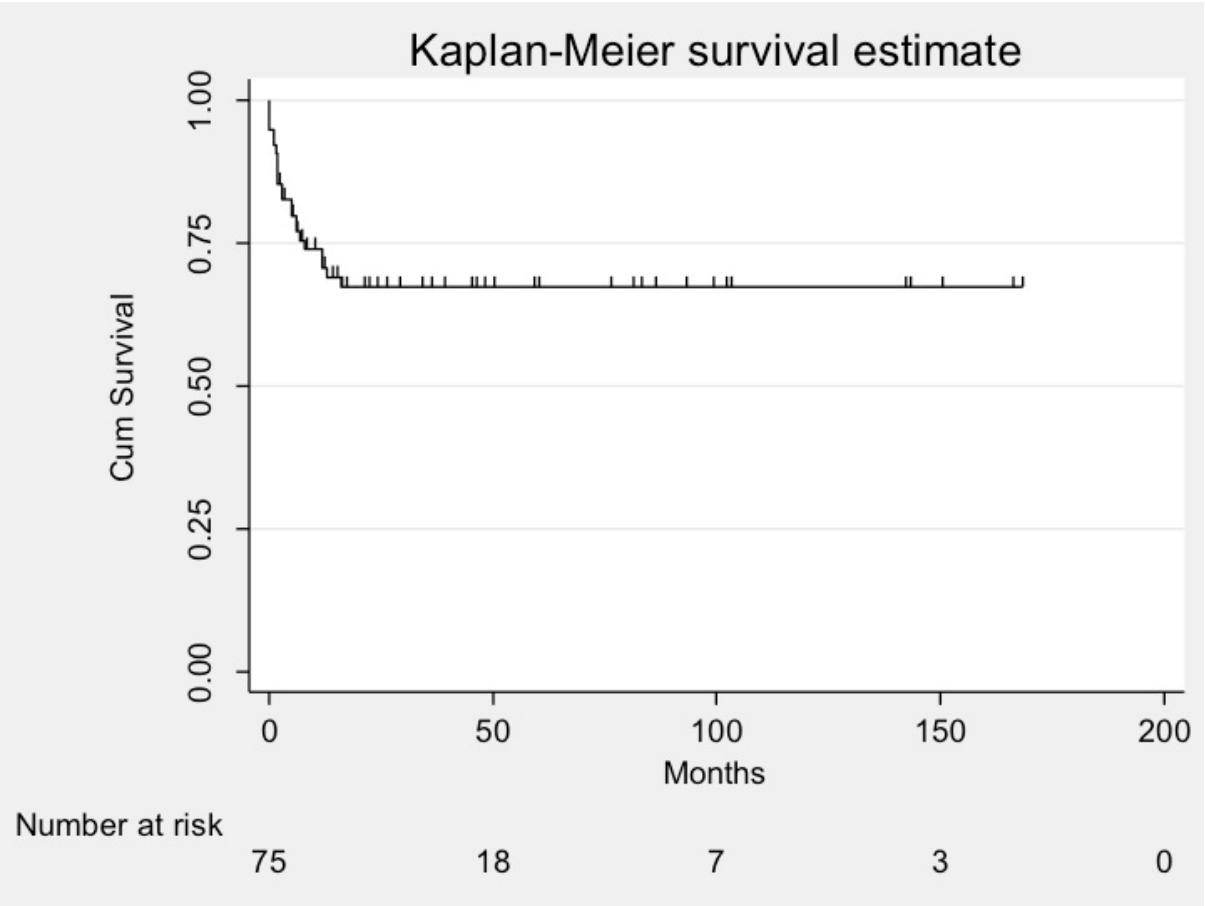
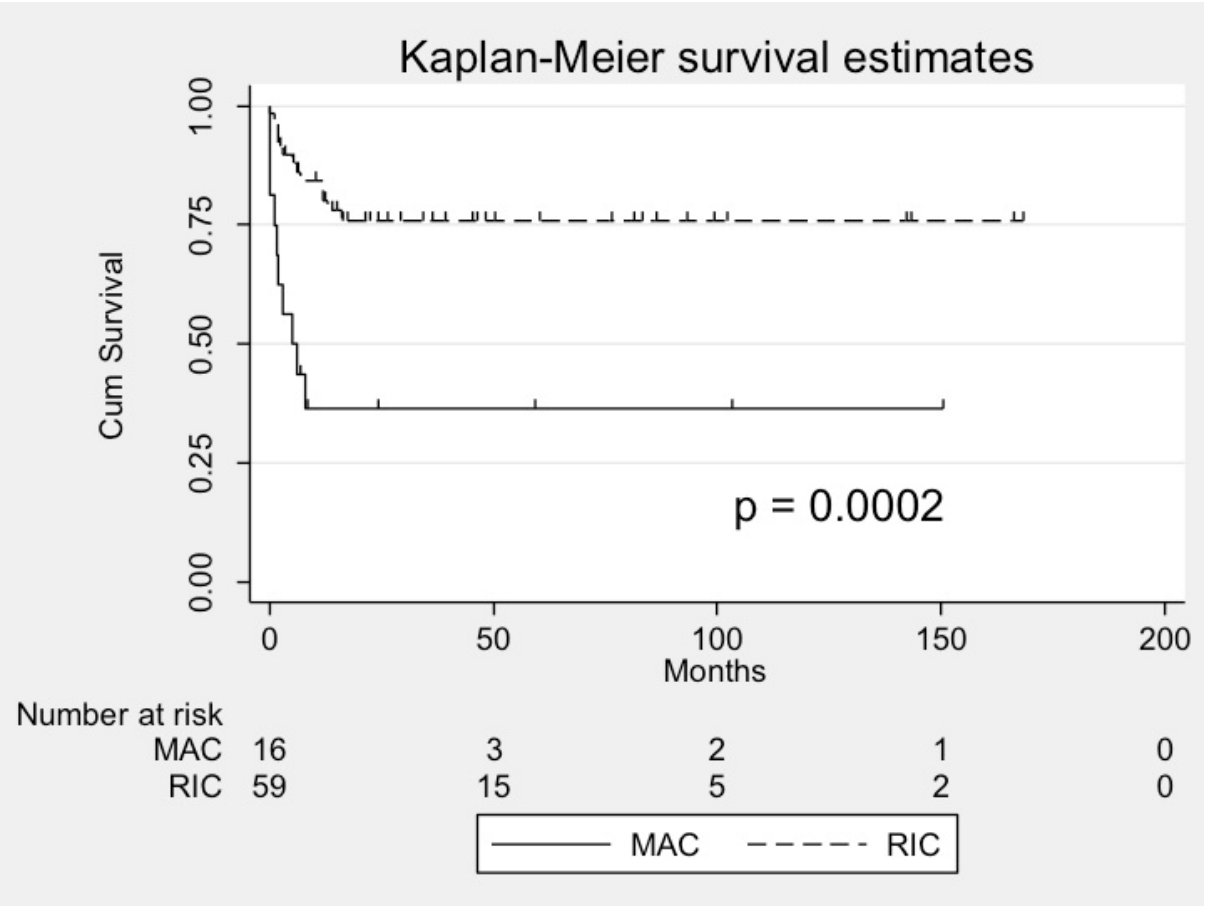




Supplementary Tables and Figures legend.

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

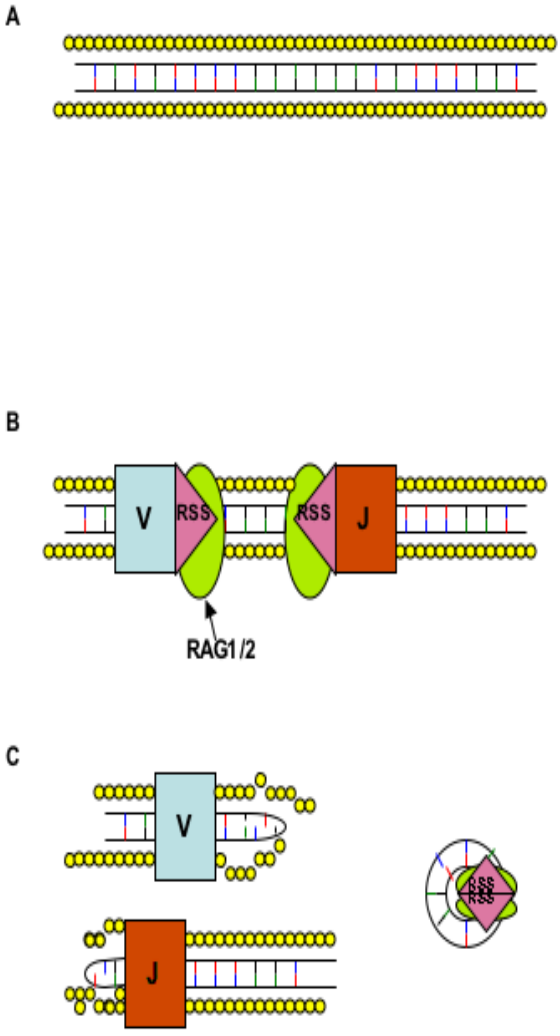
sealed hairpin intermediate is randomly nicked by the DNA-Pkcs/Artemis complex, which generates a single stranded break with 3' or 5' overhangs.

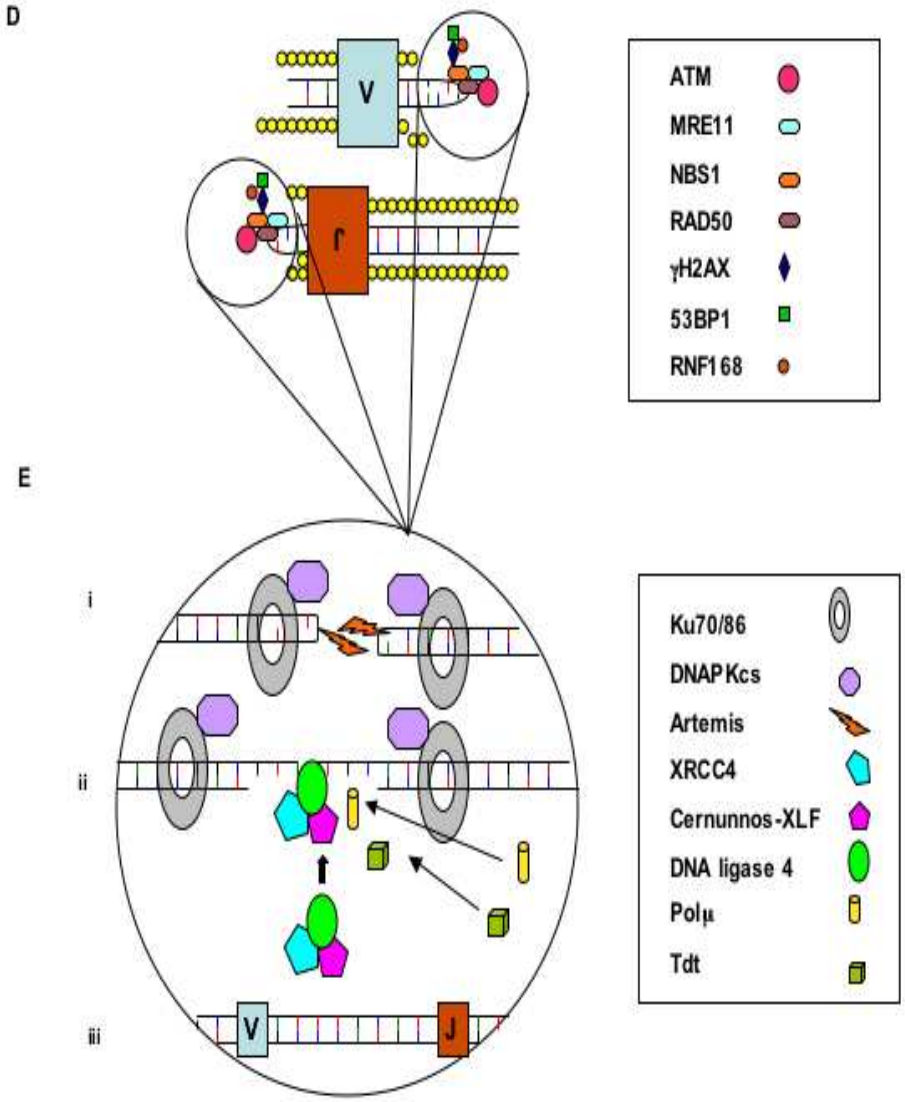
Eii. XRCC4, DNA ligase 4 and cernunnos-XLF (C-XLF) co-associate and are recruited to the ends. The signal ends are directly ligated by the XRCC4/DNA-LIG4/C-XLF complex. The opened hairpin intermediate is modified by polymerases, exonucleases and the lymphoid-specific terminal deoxynucleotidyl transferase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

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

(Reproduced from reference 43 with permission)

1

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XRCC4
Cernunnos-XLF
DNA ligase 4
Polμ
Tdt

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
Transplant 2	9	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
2	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
3	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,	36	Alive
4	18 M	Infection	MUD PBSC CD34+ selected	Flu 150mg/m^2* Melph 140mg/m^2 ATG (dose n/a)	nil	Dilated cardiomyopathy	83	Alive
5	18 M	n/a	MUD 8/8 BM	Flu 150mg/m^2* Melph 70 mg/m2 Alemtuzumab 1mg/kg	Grade 3	n/a	24	Alive

6 Transplant 1	21 M	Infection	MMFD BM	Nil	nil	graft failure		Alive
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 F	Infection□SCID phenotype	MFD □BM	Flu 150mg/m^2* * Cy 20mg/kg Alemtuzumab 1mg/kg	nil	sepsis	2	alive
8	28 M	BMF	MUD BM	Flu 150mg/m^2* Melph 70mg/m^2 Alemtuzumab 1mg/kg	nil	nil	46	Alive
9	28 F	n/a	MUD 8/8 BM	Flu 150mg/m^2* Melph 70 mg/m2 Alemtuzumab 1mg/kg	nil	n/a	36	Alive
10	31 F	Infection	MMFD BM	nil	Grade 3, Skin, Gut	Developmental delay	142	Alive
11	43 F	BMF	MUD BM, buffy coat enrichment, plasma reduction	Flu 150mg/m^2 * * Cy 40mg/kg Alemtuzumab 1mg/kg	nil	nil	24	Alive
12	47 M	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	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								
27[20]	M 49	Infection	MUD PBSC	Flu 150mg/m^2 * * Cy 40mg/mg Alemtuzumab	Grade 2 Skin, gut	Autoimmune hypothyroidism	93	Alive

				0.6mg/kg YTH24/54 1.6mg/kg				
28[19]	F 6	Infection SCID phenotype	MUD BM	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

* 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 Cy 20mg/kg	nil	nil	34	Alive
55[34]	F 46	Infection	MUD PBSC	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 ATG 7,5mg/kg	nil	nil	17	Alive
57[21, 34]	M 165	Malignancy	MUD PBSC	Bu 2mg/kg** Flu 150mg/m^2,	Grade 2 Skin	cGvHD Mild	81	Alive

				ATG 60mg/kg		haemorrhagic cystitis		
58[21]	M 174	Malignancy	MMFD TCD PBSC	Flu 160mg/m^2,*** TT 10mg/kg Melph 70mg/m^2	nil	Mucositis, ITP, Sepsis, Adeno cryptosporidiosis	24	Alive
59[34]	M 102	Malignancy	MSD BM	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

				ATG 60mg/kg	skin	disease		
71	M 172	Infection BMF	MUD 10/10 BM	ATG 15mg/kg*** Treo 42mg/m^2 Flu 160mg/m^2	nil	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/m2 ATG 60 mg/kg	Grade 3 Skin, liver	reactivation fulminant hepatic failure, gammopathy, EBV reactivation, encephalopathy	10	Dead

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; PTLD, post-transplant lymphoproliferative disease; VOD, veno-occlusive disease