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ARTICLE

Stem cell transplantation

European experience and risk factor analysis of donor cell-derived leukaemias/MDS following haematopoietic cell transplantation

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Abstract

Donor cell leukaemia (DCL) is a rare complication of allogeneic haematopoietic cell transplantation (HCT). We have investigated the prevalence and outcome of donor cell haematology malignancies within centres registered with the European Society of Blood and Marrow transplantation (EBMT). We have sought to identify risk factors to shed light on the pathogenesis of DCL as a model for leukaemogenesis. DCL cases were identified by questionnaire and a follow-up questionnaire requested detailed data. Control subjects from the EBMT registry who had not developed DCL were used for a matched pair analysis to identify risk factors. We identified 38 patients with DCL; the estimated prevalence was 80.5/100,000 transplants. Patients were predominantly treated for haematological malignancy. A clone was retrospectively identified in 7/25 (28%) donors for whom data was available. Overall survival was poor with 29/38 patients dead a median of 11 (range 0–91) months after DCL diagnosis. Matched case-pair analysis identified three factors on multivariate analysis as significantly associated with an increased risk for DCL: use of growth factors within the first 100 days after transplantation, in vivo T-cell depletion and multiple allografts. The risk factors identified, support reduced immune surveillance and replicative stress as pathogenic in the development of DCL.

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Introduction

Second malignancies are well described in survivors of allogeneic haematopoietic cell transplantation (HCT) and include solid cancers, post-transplant lymphoproliferative disease (PTLD) and donor related malignancies [1]. The development of leukaemia or myelodysplasia in donor cells within the recipient—'donor cell leukaemia' (DCL)—is rare but well described. The first published case of DCL in 1971 reported a 16-year old girl, transplanted for refractory ALL from her HLA (human leucocyte antigen) identical brother [2]. Leukaemia recurred 62 days after marrow transplantation with blasts showing a male genotype. Since then, several cases reports of DCL have been reported [2–11] and also some small case series [12–15]. Increased reporting in the last decade likely reflects the increasing number of HCT performed but also improved ability to identify donor cell origin. In 2005, the European Society for Blood and Marrow Transplantation (EBMT) initiated a retrospective survey collecting 14 cases of DCL from 91 centres reporting 10,489 transplants [12]. The incidence was estimated at 124 cases of DCL per 100,000 transplantations during the time period 1982–2003. DCL was diagnosed a median of 17 months after transplantation with no overt evidence of haematological malignancy in the donor with a median follow-up of 9 years. In 2006, Sala-Torra et al. [14] reported 12 cases of DCL [14]. In six of these, (pre-) malignant clones were retrospectively detected within the donor after transplantation had taken place. These authors proposed categorising of DCL into two groups: (1) a group in which a malignant clone is inadvertently transferred to the donor at the time of transplant and (2) a group in which donor cells become malignant in the new host environment. Since monoclonal lymphocytosis and lymphoid neoplasms increase with age, the risk of accidental transferred DCL might be expected to increase with increasing donor age [5, 16–19].

An alternative possibility is that undetected potential malignant clones are transferred from donor to patient and that these clones behave differently in the new host (transplant patient) environment compared to the donor from which they have come. Next generation sequencing has shed some light on the behaviour of pre-leukaemic clones transferred from donor in the context of allogeneic HCT. Berger et al. [20] describe a DDX41 kindred in which siblings shared heterozygous germline mutations in DDX41 and FANCD2. Development of post-transplant DCL in the patient transplanted for AML/MDS from an unaffected brother was accompanied by an expanding TET2 and TP53 clone from the unaffected brother associated with some additional mutations in DNMT3A and ASXL1 genes. Meanwhile the brother who donated the TET2/TP53 clone remained free from leukaemia highlighting the role of the environment in leukaemic development. Herold et al. [21] described a case of donor cell AML occurring 7 years after transplant for CLL, with AML occurring in the donor at a similar time. They used whole-exomic sequencing to chart the evolution of a donor cell demonstrating clonal haematopoiesis of indeterminate potential [22] in both the transplanted environment and in the original host. Although both patient and donor developed AML, different clones were demonstrated in each and the clinical outcome was different for each patient.

Different pathogenic risk factors in the host have been postulated to be relevant to the development of DCL [23, 24]. Replicative stress and telomere shortening resulting in premature senescence, as well as cell cycle dysregulation and epigenetic reprogramming due to the high number of required cell divisions during HCT have been implicated. Furthermore, impaired immune surveillance by immunoregulatory dysfunction and immunosuppression may contribute. Viral reactivation is relevant to this as it can modify immune reconstitution after HCT. The importance of the stroma in supporting normal haematopoietsis is highlighted by experiments in mice where genetically altered mensenchymal osteoprogenitor cells are associated with the development of MDS/leukaemia [25]. Damaged marrow stroma and defective microenvironment and oncogenic transformation can be exacerbated by antigenic or viral stimulation. Another possible risk factor is use of exogenous GCSF, which could enhance leukaemic transformation through actions mediated via the GCSF receptor [26].

Due to the rarity of DCL very little is known about its clinical risk factors, outcome and optimal management. We have, therefore, updated a previous EBMT investigation of DCL with a view to identifying potential risk factors for its development and to evaluate outcome.

Methods

This was a multicentre cohort study. A questionnaire was sent to all 305 EBMT centres actively reporting to the EBMT on allogeneic transplant recipients. The EBMT is a voluntary working group of transplant centres that are required to report all consecutive stem cell transplantations and follow-ups once a year. All patients provide informed consent authorising the use of their personal information for research purposes and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

The questionnaire asked for reports of proven cases of DCL. A follow-up questionnaire requested data on initial disease, treatment, graft-versus-host disease (GvHD), immunosuppression and infectious complications. In addition details were sought regarding how donor cell origin was determined, the subsequent treatment and outcome for both patient and donor. Cumulative incidence of DCL was estimated from the date of the last HCT before DCL, taking

into consideration the competing risk of death due to other causes.

Controls who had not developed DCL were collected from the EBMT registry database and used in a nested case–control (two controls for one case) analysis to identify prognostic factors. Controls were matched for the following factors: age at HCT(+ /- 5 years), gender, diagnosis (malignant vs. non malignant), disease status at transplantation, year of transplantation (+ /- 5 years), type of donor (HLA-identical sibling, unrelated, haploidentical transplant), stem cell source (BM, PB or CB) and duration of follow-up (see Table 1). In one case it was not possible to get two controls and a single control only was used. It was not possible to identify a control for four of our DCL patients with the following diagnoses: Fanconi anaemia, sickle cell disease, ALL and aplastic anaemia.

Controls had a length of follow-up at least equal to the time from last HCT to DCL. The following parameters were investigated by univariate and multivariate analysis: donor age, previous type of transplantation (autologous/allogeneic), reduced-intensity conditioning regimen (RIC), total body irradiation (TBI ≥ 4 Gy), patient and donor CMV serology, use of G-CSF within 100 days post-transplant, in vivo T-cell depletion by anti-thymocyte globulin (ATG) or alemtuzumab and occurrence of acute or chronic GvHD.

Statistics

The two groups (cases and controls) were compared using Chi-square for categorical variables and Mann–Whitney test for continuous parameters. Multivariate analysis was performed using a conditional logistic regression. Then a stepwise backward and forward procedure was used with a cutoff significance level of 0.05 for deleting factors in the model. All *p*-values are two-sided with type I error rate fixed at 0.05. Statistical analyses were performed with SPSS 22 (SPSS Inc./IBM, Armonk, NY) and R 3.0.1 (R Development Core Team, Vienna, Austria) software packages.

Results

Prevalence and cumulative incidence of DCL

Of 305 EBMT centres, 80 participated in this study (26.2%) including data on 46,051 allogeneic transplants. Twenty-eight of 80 centres (35%) reported a total of 38 DCL patients. This gives an estimated DCL prevalence of 80.5 cases per 100,000 transplants and a cumulative incidence of DCL at 5, 10, and 25 years after the last HCT of 0.067%, 0.132% and 0.363%, respectively.

Table 1 Characteristics of patients and their matched control subjects

		Controls	DCL patients
		Controls	DCL patients
		$n = 67$	$n = 34$
Median age		42.4 (6.9–69.3)	41.2 (5–70)
Median year of HCT		2002 (87–2011)	2003 (85–2010)
Gender	Male	36 (54%)	18 (53%)
	Female	31 (46%)	16 (47%)
Type of donor	Sibling	41 (61%)	21 (62%)
	Unrelated	24 (36%)	12 (35%)
	Haploidentical	2 (3%)	1
Status at HCT	CR	46 (69%)	23 (68%)
	No CR	19 (28%)	10 (29%)
	n/a	2 (3%)	1 (3%)
Source of stem cells	BM	27 (40%)	14 (41%)
	PB	36 (54%)	18 (53%)
	CB	4 (6%)	2 (6%)
Diagnosis	AML	23 (34%)	10 (29%)
	ALL	10 (15%)	4 (12%)
	CML	12 (18%)	8 (24%)
	CLL	3 (4%)	3 (9%)
	MDS	6 (9%)	4 (12%)
	NHL	7 (10%)	3 (9%)
	MM	4 (6%)	1 (3%)
	SAA	2 (3%)	1 (3%)

33/38 patients had two matched controls and one additional patient had a single matched control

DCL donor cell leukaemia, *HCT* haematopoietic cell transplantation, *CR* complete remission, *n/a* non-applicable because diagnosis was aplastic anaemia, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood, *AML* acute myeloid leukaemia, *ALL* acute lymphoid leukaemia, *CML* chronic myeloid leukaemia, *CLL* chronic lymphocytic leukaemia, *MDS* myelodysplasia, *NHS NHL* non Hodgkins lymphoma, *MM* multiple myeloma, *SAA* severe aplastic anaemia

Patient characteristics

Characteristics of patients affected by DCL and their controls are summarised in Table 2. The majority were transplanted for underlying malignant disease. Twenty-four patients (63%) had related (matched sibling $n = 22$, haploidentical $n = 2$) donors and in 14 cases (37%) the donor was unrelated. Most patients (37/38) received treatment prior to the HCT preceding DCL diagnosis: 26/38 patients received chemotherapy, in combination with immuno- (3/26) or radiotherapy (6/26). One patient received immunotherapy only. Ten in 38 patients were heavily pretreated; five patients underwent high-dose chemotherapy and autologous transplantation and five patients received one ($n = 4$) or two ($n = 1$) previous allogeneic HCT (three of them with

Table 2 Characteristics of patients affected by DCL

Patient #	Age at dx of DCL (years)	Primary diagnosis	Disease status prior to HCT	Donor	TBI dose (Gy)	Source of stem cells	T-cell depletion	Previous HCT	GVHD prophylaxis	Acute GVHD	Chronic GVHD	Viral reactivation from D+100	Time from last allograft to developing DCL (years)
1	13	ALL	CR	Sib	12	BM	No		CsA + Corticosteroids	Grade II	No	No	8.03
2	55	ALL	CR	Sib	No	BM	ATG		CsA + MTX + ATG	Grade I	No	VZV/ HBV	9.65
3	45	ALL	CR	Haplo	No	CB	No		CsA + Corticosteroids	Absent	No	CMV	1.33
4	17	ALL	Refractory	Haplo	8	PB	ATG		ATG	Absent	No	No	0.13
5	36	ALL	CR	VUD	12	BM	No		CsA	Absent	No	Missing	9.98
6	17	AML	CR	MMUD	No	CB	No	1 Allo	CsA + MMF	Grade II	Limited	CMV	4.54
7	47	AML	Unknown	Sib	10	BM	No		CsA + Corticosteroids	Grade II	Extensive	Missing	23.31
8	41	AML	CR	VUD	No	PB	ATG		TAC + ATG + MMF	Grade I	No	CMV	2.13
9	47	AML	CR	Sib	12	PB	Alemtuzumab		CsA + Alemtuzumab	Grade III	Extensive	No	3.06
10	65	AML	PR	VUD	4	PB	ATG		CsA + ATG + MMF	Absent	Limited	CMV/ HBV	2.68
11	56	AML	CR	Sib	No	PB	No		CsA + MTX	Absent	No	Missing	1.05
12	30	AML	CR	Sib	No	PB	No		CsA + MTX	Absent	Limited	Missing	5.92
13	70	AML	CR	Sib	No	PB	ATG		CsA + MTX + ATG	Absent	No	No	0.67
14	59	AML	CR	Sib	2	PB	No		CsA + TAC + MMF	Absent	No	Missing	10.02
15	40	AA	Unknown	VUD	No	PB	Alemtuzumab	2 Allos	Sirolimus + Alemtuzumab	Grade II	No	CMV	0.82
16	66	AA	Progression	VUD	No	PB	No		CsA + MTX	Grade I	No	CMV	2.53
17	56	CLL	CP	Sib	9	BM	No		CsA	Grade I	Limited	VZV	9.85
18	55	CLL	PR	Sib	12	PB	No		CsA + MTX	Absent	Limited	No	5.77
19	50	CLL	PR	Sib	No	PB	ATG		TAC + ATG + MMF	Absent	No	No	0.28
20	41	CML	CP	VUD	No	BM	ATG		CsA + MTX + ATG	Grade I	No	CMV	1.17
21	35	CML	CP	MMUD	No	CB	Alemtuzumab		CsA + MMF + Alemtuzumab	Absent	No	CMV/ RSV/ HSV/	1.44

Table 2 (continued)

Patient #	Age at dx of DCL (years)	Primary diagnosis	Disease status prior to HCT	Donor	TBI dose (Gy)	Source of stem cells	T-cell depletion	Previous HCT	GVHD prophylaxis	Acute GVHD	Chronic GVHD	Viral reactivation from D+100	Time from last allograft to developing DCL (years)
												Parainfluenza type 3	
22	36	CML	CR	Sib	12	BM	No		CsA + MTX	Absent	No	No	0.94
23	46	CML	CP	VUD	12	BM	ATG		CsA + MTX + ATG	Grade III	Extensive	No	7.34
24	44	CML	CP	VUD	No	BM	No	1 Auto	CsA + MTX + MMF	Absent	Extensive	CMV	6.63
25	47	CML	CR	Sib	14,4	BM	No		CsA	Grade II	Extensive	Missing	9.15
26	57	CML	CP	Sib	10	BM	No	1 Allo	CsA	Absent	No	No	17.91
27	63	CML	CP	Sib	No	BM	No		CsA + MTX	Grade I	Limited	VZV	17.78
28	21	Fanconi	Stable disease	VUD	2	CB	ATG	1 Allo	CsA + ATG	Grade I	No	CMV	2.12
29	38	Granulocytic sarcoma	CR	VUD	12	BM	Alemtuzumab		CsA + Alemtuzumab	Grade I	No	CMV	2.30
30	62	MDS	Never treated	Sib	No	PB	ATG	1 Allo	CsA + MTX + ATG	.	Limited	CMV	2.17
31	57	MDS	Never treated	Sib	9	PB	No		CsA	Grade I	Extensive	VZV	4.41
32	16	MDS	Treatment not aimed at remission	Sib	No	BM	No		CsA + Corticosteroids	Grade III	Limited	VZV	3.37
33	73	MDS	CR	Sib	No	PB	No		CsA + MTX	Absent	Limited	No	2.38
34	63	Multiple myeloma	PR	VUD	No	PB	No	2 Autos	CsA + MMF	Grade II	Extensive	No	4.00
35	58	Follicular lymphoma	PR	Sib	No	PB	Alemtuzumab		CsA + MTX + MMF	Absent	No	VZV	5.63
36	55	NHL	PR	VUD	2	PB	No	1 Auto	TAC + MMF	Grade III	Limited	Missing	4.93
37	48	NHL	CR	Sib	No	PB	No	1 Auto	CsA	Absent	No	No	6.00
38	19	Sickle cell disease	Stable disease	Sib	No	PB	No	1 Auto	CsA	Grade I	No	CMV	0.30

DCL donor cell leukaemia, *dx* diagnosis, *ALL* acute lymphoid leukaemia, *AML* acute myeloid leukaemia, *CLL* chronic lymphoid leukaemia, *CML* chronic myeloid leukaemia, *MDS* myelodysplasic syndrome, *NHL* non Hodgkins lymphoma, *AA* aplastic anaemia, *HCT* haematopoietic cell transplant, *CR* complete remission, *PR* partial remission, *CP* chronic phase, *Sib* sibling donor, *VUD* volunteer unrelated donor, *MMUD* mismatched unrelated donor, *Haplo* haploidentical, *PB* peripheral blood, *BM* bone marrow, *CB* cord blood, *ATG* anti-thymocyte globulin, *allo* allogeneic HCT, *auto* autologous HCT, *CsA* cyclosporine, *MTX* methotrexate, *MMF* mycophenolate mofetil, *TAC* tacrolimus, *VZV* varicella-zoster virus, *HBV* Hepatitis B Virus, *CMV* Cytomegalovirus, *HSV* Herpes Simplex, *RSV* respiratory syncytial virus

Table 3 Donor cell leukaemia characteristics and outcome for patient and donor

Patient #	Primary diagnosis	DCL diagnosis	Tests to confirm donor origin	DCL treatment	Patient outcome	Survival time from DCL (months)	Donor haematologcal malignancy	Time from donation of stem cells to donor malignancy (years)	Donor follow-up (years)
1	ALL	AML	Cytogenetics	Chemotherapy	Dead	7	No	n/a	8.60
2	ALL	AML	FISH	HCT	Dead	31	No	n/a	12.22
3	ALL	AML	VNTR or STR	Chemotherapy	Dead	7	No	n/a	1.90
4	ALL	AML	VNTR or STR	HCT	Dead	5	Unknown	Unknown	Unknown
5	ALL	CLL	VNTR or STR	Watch and wait	Alive	120	Unknown	Unknown	Unknown
6	AML	AML	VNTR or STR	HCT	Dead	13	No	n/a	5.60
7	AML	AML	Cytogenetics	Chemotherapy	Alive	32	No	n/a	25.96
8	AML	AML	Cytogenetics / VNTR or STR / FISH	HCT planned	Alive	62	Unknown	Unknown	Unknown
9	AML	AML	VNTR or STR	Chemotherapy	Dead	10	No	n/a	3.87
10	AML	AML	BM histology	Chemotherapy	Dead	4	Unknown	Unknown	Unknown
11	AML	AML	Cytogenetics / VNTR or STR / FISH	HCT	Dead	14	No	n/a	2.18
12	AML	CML	HLA typing	Chemotherapy	Dead	15	No	n/a	7.17
13	AML	CLL	Cytogenetics	Watch and wait	Dead	22	CLL	2.23	2.48
14	AML	CLL	VNTR or STR / FISH	Watch and wait	Alive	41	CLL	9.07	13.46
15	AA	MDS	VNTR or STR	HCT	Dead	31	No	n/a	3.43
16	AA	MDS	Cytogenetics / FISH	HCT	Dead	7	Unknown	Unknown	Unknown
17	CLL	MDS	VNTR or STR	HCT	Dead	17	AML	9.81	11.29
18	CLL	CLL	VNTR or STR	Chemotherapy	Alive	62	CLL B-cells/ Small lymphocytic lymphoma	4.91	10.96
19	CLL	CLL	HLA typing	Chemotherapy	Dead	91	CLL	1.42	2.97
20	CML	AML	VNTR or STR/FISH	HCT	Alive	196	Unknown	Unknown	Unknown
21	CML	AML	HLA typing/FISH	Unknown	Dead	1	Unknown	Unknown	Unknown
22	CML	AML	Cytogenetics	HCT	Dead	8	No	n/a	1.61
23	CML	AML	VNTR or STR	HCT	Alive	96	Unknown	Unknown	Unknown
24	CML	AML	HLA typing	Chemotherapy	Dead	15	Unknown	Unknown	Unknown
25	CML	AML	VNTR or STR	HCT	Dead	8	No	n/a	9.85
26	CML	MDS	Cytogenetics	HCT	Dead	11	No	n/a	18.80
27	CML	ALL	VNTR or STR	Chemotherapy	Dead	2	No	n/a	17.95
28	Fanconi	AML	Cytogenetics	HCT	Dead	40	Unknown	Unknown	Unknown
29	Granulocytic sarcoma	AML	VNTR or STR	Chemotherapy	Dead	6	Unknown	Unknown	Unknown
30	MDS	AML	Cytogenetics / FISH	HCT	Dead	8	No	n/a	2.84

Table 3 (continued)

Patient #	Primary diagnosis	DCL diagnosis	Tests to confirm donor origin	DCL treatment	Patient outcome	Survival time from DCL (months)	Donor haematologcal malignancy	Time from donation of stem cells to donor malignancy (years)	Donor follow-up (years)
31	MDS	MDS	VNTR or STR	HCT	Alive	63	AML	4.36	5.07
32	MDS	MDS	VNTR or STR	HCT	Dead	82	No	n/a	10.25
33	MDS	MDS	FISH	Watch and wait	Dead	13	Unknown	Unknown	Unknown
34	Multiple myeloma	AML	Cytogenetics	HCT	Dead	17	No	n/a	5.38
35	Follicular lymphoma	AML	VNTR or STR	Chemotherapy	Dead	5	No	n/a	6.02
36	NHL	AML	VNTR or STR	Chemotherapy	Dead	6	Unknown	Unknown	Unknown
37	NHL	ALL	Cytogenetics / VNTR or STR	Chemotherapy	Dead	0	No	n/a	6.01
38	Sickle cell disease	CML	Cytogenetics	Chemotherapy	Alive	191	CML	0.3	9.59

Survival data given to 1 April 2018, or until last follow-up for three patients (patients # 7,14,18) who were lost to follow-up prior to this date

DCL donor cell leukaemia, *ALL* acute lymphoid leukaemia, *AML* acute myeloid leukaemia, *CLL* chronic lymphoid leukaemia, *CML* chronic myeloid leukaemia, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkins lymphoma, *cytogenetics* conventional microscopic cytogenetics, *FISH* fluorescence in situ hybridisation, *VNTR* variable number of tandem repeats, *STR* short tandem repeats, *BM* bone marrow, *HLA* human leucocyte antigen, *HCT* hematopoietic cell transplant

different donors and for two this information is not known). Disease status prior to last HCT before DCL diagnosis was available for 36/38 patients (Table 1). Conditioning regimens were intended to be myeloablative in 26/38 patients and 17/38 patients received TBI with a total dose of ≥ 8 Gy (13/17) or ≤ 4 Gy (4/17). Sixteen in 34 cases (47%) received GCSF in the first 100 days and 9/34 cases (26%) received ATG. Acute GvHD was observed in 20/37 patients and chronic GvHD in 17/38 patients: 10/17 with mild symptoms and 7/17 with moderate or severe symptoms according to the NIH classification [27].

Cytomegalovirus (CMV) reactivation within the first 100 days after transplantation was reported for 15/38 patients and over day + 100 for 13/38 patients. Details of reactivation of other viral pathogens are given in Table 2.

DCL diagnosis and demonstration of donor cell origin/chimaerism analysis

Median time from last allogeneic transplantation to DCL diagnosis was 44 months (range, 2–279 months). Donor cell leukaemia was diagnosed as AML ($N = 22$) in 58% of the cases, MDS ($N = 7$), ALL ($N = 2$), CML ($N = 2$) and CLL ($N = 5$). Information on cytogenetic and molecular analyses were available for 32/38 patients and 29/38 patients, respectively. Aberrations included monosomy 7 in 5 patients (3 AML; 1 MDS; 1 CML), trisomy 8 in 3 patients (2 AML; 1 MDS), RUNX1 mutation in two patients (two AML), WT1 mutation in two patients (one MDS, one AML) and immunoglobulin gene rearrangements in two patients (CLL). Donor origin of disease was confirmed by cytogenetics (FISH and/or conventional cytogenetics) in 11 patients, by molecular testing (STR/VNTR and/or HLA typing) in 20 patients and by both techniques in six patients; in one patient the method of donor type confirmation remains unclear. Donor chimaerism was indicated as full (33/38) or mixed (3/38; two conventional cytogenetics, one STR/VNTR analysis) at the point of DCL diagnosis; for two patients there were no detailed data available on the results of chimaerism analysis (performed by HLA typing and conventional cytogenetics).

DCL outcome and treatment

Treatment depended on the nature of the disease (Table 3). Four did not receive further treatment (three CLL, one MDS). Fourteen of 38 patients received conventional chemotherapy and 18/38 were re-treated with HCT. 14/18 received a transplant from a new donor and two from the same geno-identical donor. Three patients receiving HCT for AML also received donor lymphocyte infusions (DLI) post-transplant. Two in 38 patients had disease specific treatment (CLL—lenalidomide, CML—imatinib).

Overall survival following DCL was poor with 29/38 patients dead at a median time of 11 months after diagnosis (range, 0–91 months). The main causes of death were relapse/progression of DCL (12 patients), relapse/progression of primary disease (3 patients), transplant related causes of death (10 patients), secondary malignancy ($n = 1$), sepsis ($n = 1$), myocardial infarction ($n = 1$) and 1 patient unknown.

Of 9/38 patients remaining alive (median follow-up 63 months, range 32–196), four had chronic leukaemia (three CLL, one CML), four had AML in CR (three receiving HCT, one treated with chemotherapy) and one had MDS (treated with HCT).

Donors' characteristics and donor follow-up

The median donor age at HCT was 38 years [0–72]; 25/38 donors were male. Prior to HCT, all passed the standard pre-HCT laboratory evaluation and had no clinical evidence of disease. Follow-up data was available for 25/38 donors (22 related and three unrelated donors). At the time of data collection, all donors were alive a median of 6 years (range 1.6–26 years) post donation of their stem cells.

Detailed follow-up data was available for 25 donors; of these seven developed overt evidence of haematological malignancy during the follow-up time of this study (median 52 months, range 3.5–117). Five donors (HLA-identical siblings) developed the same chronic leukaemia as their recipient (four CLL, one CML). The donor/recipient pair who developed CML were both diagnosed at a similar time point 4 months after transplant. Where the donor had CLL the time course of the recipient developing CLL varied from 1.4–9.1 years. A malignant clone was identified retrospectively within the graft in two donors [18].

Two donors (HLA-identical siblings) developed AML 4.4 and 9.8 years after donating stem cells; their recipients developed MDS with a similar time course.

Case–control study/pathogenetic risk factor analysis

Thirty-four DCL cases were matched with 67 controls (Table 4). In univariate analysis, the following factors were identified as being significantly associated with an increased risk for development of DCL ($p < 0.05$): the use of growth factor within the first 100 days after transplantation ($p = 0.015$), and previous allograft ($p = 0.01$). By multivariate analysis, three factors were significantly associated with a higher risk of DCL: the use of growth factor within the first 100 days after transplantation (HR = 2.43; 95% CI: 1.15–5.13; $p = 0.020$), in vivo T-cell depletion by either alemtuzumab or ATG (HR 2.59; 95% CI: 1.21–5.56; $p = 0.014$) and previous allograft (HR 4.08; 95% CI: 1.37–12.19; $p = 0.012$). There was no significant association between acute or chronic GvHD with DCL.

Table 4 Characteristics of control subjects compared to patient group for non-matching factors

		Control $n = 67$	Case $n = 34$	p-value
Donor age	>35	43 (64%)	18 (64%)	0.97
Previous auto HCT	Yes	5 (7%)	4 (12%)	0.48
Previous allo HCT	Yes	0	4 (12%)	0.01
Reduced-intensity HCT	Yes	26 (39%)	12 (35%)	0.73
TBI	Yes	41 (61%)	16 (47%)	0.18
Growth factor < 100 days	Yes	17 (25%)	16 (50%)	0.015
in vivo T-cell depletion	Yes	20 (30%)	14 (41%)	0.26
Acute GvHD grade II+	Yes	16 (24%)	10 (30%)	0.49
Chronic GvHD	Yes	36 (59%)	17 (50%)	0.40
Alemtuzumab	Yes	5 (7%)	5 (15%)	0.25
Anti-thymocyte globulins	Yes	15 (22%)	9 (26%)	0.65

HCT haematopoietic cell transplant, *GvHD* graft-versus- host disease, *TBI* total body irradiation

Discussion

This European multi-centre survey has characterised a series of 38 DCL patients, which demonstrates the heterogeneity of this rare complication. The estimated prevalence from our data is 80.5 cases per 100.000 transplants. This is of similar magnitude albeit lower than the 124 cases/100,000 transplants reported by the first EBMT centre survey on DCL [12]. Other case series of DCL found a distinctly higher prevalence with 4 cases/841 transplants [15] and up to 2 cases/40 transplants [11]. For all studies, including the present study, we cannot exclude under-reporting (no systematic search for DCL) or over-reporting because of lack of involvement of centres without cases of DCL. Despite the improvement in molecular diagnostic tools for detection of donor type malignant cells, the frequency of cases between 1985 and 2011 has not changed substantially from the 20-year period 1982–2002 [12]. The median time interval from HCT to DCL diagnosis reported in our study is similar to that observed by Sala-Torra et al. [14].

Our data lends some support to the categorisation of DCL into two different groups as already proposed [14] because in some of our cases it was possible to demonstrate accidental transfer of pre-existing malignant clones, whereas in others it was not. However, it is recognised that leukaemia is a multistep process and although 7/28 donors had evidence of an abnormal clone/disease, we cannot exclude the possibility that additional donors had pre-leukaemic clones despite not developing overt disease within the time frame of the study. This is statistically more likely in donors who already had family members with leukaemia (sibling transplants) but could also apply to unrelated donors. It is also possible that different mechanisms are relevant depending on whether the patient developed a chronic or acute leukaemia.

In spite of modern techniques in molecular diagnostics, it remains a challenge to confirm beyond doubt the donor origin of malignant cells in DCL. Different methods for analysing chimaerism are available, and while results of cytogenetic analysis might be impaired by slowly dividing leukaemic clones or missing spontaneous cell division, further details like genomic instability with potential gain and loss of the Y chromosome or clonal evolution still have to be noted for FISH analysis [28]. The gold standard for chimaerism analysis especially for verification of DCL are STR- and VNTR analysis [29, 30]. The prerequisite is that the collected material belongs to the malignant cell population only. Nonetheless FISH analysis remains an accepted technique to assess chimaerism in sex-mismatched transplantations [24]. Recent use of whole-exome sequencing to demonstrate emergence of AML in donor cells after allogeneic HCT also highlights the value of this technique in demonstrating the donor origin of a leukaemic clone [20].

Cytogenetic abnormalities including monosomy 7 and trisomy 8 were relatively frequent in patients developing AML/MDS. Both are known to be associated with therapy-related MDS and leukaemia after treatment with alkylating agents. Several case reports also demonstrate the occurrence of monosomy 7 and trisomy 8 in DCL [10, 15, 31]. In our cohort 36/38 patients received an alkylating agent-containing conditioning regimen. As these agents are given sufficiently far ahead to avoid toxicity to incoming cells, detection of therapy-associated chromosomal aberrations implicates residual effects of the previous chemotherapy on marrow stroma rather than a direct effect on donor stem cells. The suggestion of a (pre-) damaged or mutagenic microenvironment is further supported by a DCL case after transplantation for Fanconi anaemia, a condition in which the stroma is known to be defective [6, 32].

Two aims of the study were to identify potential risk factors for DCL and shed light on the pathogenesis of DCL as a model for leukaemogenesis. Three factors were identified as being significantly associated with an increased risk for DCL development on multivariate analysis: the use of growth factors within the first 100 days after transplantation, in vivo T-cell depletion and multiple allografts. Telomere shortening and replicative stress play a pivotal role in cell senescence induced genomic instability as characterised in AML [30]. Significant telomere shortening has been observed in recipients after HCT especially during the first year after transplantation. Application of growth factors during the first 100 days after transplantation might further enhance replicative stress leading to greater genomic instability and thereby contributing to malignant transformation.

Expansion of a malignant clone is usually prevented by functional immune surveillance mediated by cytotoxic T-lymphocytes. Despite the existence of donor T-cells, however, immune function remains impaired immediately post transplant not least because of the immunosuppressive agents administered to prevent graft failure and GvHD. In our patient cohort, in vivo T-cell depletion was found to be significantly associated with DCL development supporting the hypothesis of impaired immune surveillance by reduced functional T-lymphocytes.

In this analysis, prior allogeneic transplantation was associated with an increased risk of developing DCL while prior autologous transplantation was not. One could argue that this lends support to the relevance of reduced immune surveillance in DCL development.

Prognosis of DCL was poor and most of the patients died of progression or relapse of the DCL. Transplantation-related death was common. Nonetheless, in addition to three cases of donor cell-derived chronic leukaemias, long-term survival was also reported for five cases of MDS/AML, four of whom underwent a second HCT from an alternative donor.

Summary and conclusion

In summary the data presented in this paper, representing the largest series to date of patients developing DCL, demonstrates that the outlook of DCL is poor. The risk factor analysis supports reduced immune surveillance and replicative stress as pathogenic in the development of DCL.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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