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Hematopoietic Stem Cell Transplantation in Severe Congenital Neutropenia

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Background. Severe congenital neutropenia (SCN) is an immunodeficiency characterized by disturbed myelopoiesis and an absolute neutrophil count (ANC) $<0.5 \times 10^9$/L. SCN is also a premalignant condition; a significant proportion of patients develop myelodysplastic syndrome or leukemia (MDS/L). Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for SCN. ***Procedure.*** Since 2004, eight HSCT have been performed in seven patients at our center. The indications were transformation to MDS/L (n = 2), granulocyte colony-stimulating factor receptor (*CSF3R*) mutation(s) (n = 2), granulocyte colony-stimulating factor (G-CSF) resistance (n = 2), and at the patient's own request (n = 1). ***Results.*** The mean age at transplantation was 13 years (2.8–28 years) (mean follow-up 32 months, range 21–60). Three patients harbored *ELANE* mutations, three *HAX1* mutations, and in one patient no causative mutation was identified. Two of the *ELANE* mutations were novel mutations. Three patients initially received myeloablative conditioning and four had reduced intensity conditioning (RIC). Three grafts were from HLA-identical siblings, three from matched unrelated donors and two were cord blood units. Engraftment occurred in all patients. Two of seven (29%) patients died; both had MDS/L and both were among the three that underwent myeloablative conditioning. One patient has chronic GVHD 2 years post-transplant. ***Conclusions.*** The role of HSCT should be explored further in patients with SCN. In particular, the influence of the conditioning regime needs to be evaluated in a larger cohort of patients. Pediatr Blood Cancer 2011;56:444–451.

Key words: chimerism; hematopoietic stem cell transplantation; myelodysplastic syndrome/leukemia; severe congenital neutropenia

INTRODUCTION

Severe congenital neutropenia (SCN) is a heterogeneous bone marrow disorder, characterized by a disturbed myelopoiesis and excessive apoptosis of myeloid progenitor cells [1,2]. SCN patients suffer from recurrent bacterial infections early in life, and display an absolute neutrophil count (ANC) below 0.5×10^9/L, and maturation arrest of the myelopoiesis in the bone marrow at the promyelocyte/myelocyte stage [3]. SCN was originally described 50 years ago by the Swedish pediatrician Rolf Kostmann as an autosomal recessive disorder in a large kindred in the north of Sweden [4,5]. More recent studies have identified mutations in *ELANE* (previously known as *ELA2*) in autosomal dominant inherited or sporadic SCN [6] while homozygous mutations of *HAX1* cause autosomal recessive SCN or Kostmann syndrome [7]. In addition, a few cases with a heterozygous mutation in the *GFI1* gene has been described [8]. In a number of cases with SCN mutation screening has been negative, suggesting additional genetic mechanisms.

The curative therapy for SCN is hematopoietic stem cell transplantation (HSCT), but most patients do not have an HLA-identical-related donor, and HSCT is associated with a considerable risk of both morbidity and mortality [9,10]. Transplant-related mortality is below 10% in patients with non-malignant disorders and when using HLA-identical sibling donors [11]. Today, the main treatment of SCN is granulocyte colony-stimulating factor (G-CSF), and over 90% of the patients respond to the treatment with increasing ANC and with decreasing frequencies of infections and improved quality of life [3]. SCN is considered as a premalignant condition; over 20% of the patients will develop myelodysplastic syndrome or leukemia (MDS/L) according to recent international studies [12,13]. Patients, who respond poorly to G-CSF and therefore require high doses develop MDS/L in about 40% of the cases [12]. Annual bone marrow examinations are recommended by the advisory board of the Severe Chronic Neutropenia International Registry (SCNIR) due to the risk of MDS/L [14]. Acquired mutations in the G-CSF receptor (*CSF3R*) and/or acquired cytogenetic aberrations, such as monosomy 7 and trisomy 21, can precede the evolution of leukemia, but these alterations are not seen in all cases and the true mechanism underlying the evolution to secondary malignancies in SCN patients thus remains to be understood [15,16]. Traditional chemotherapeutic treatment does not cure leukemia in this patient group and HSCT also often fails [17,18]. Here, we report our experiences with HSCT

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; ANC, absolute neutrophil count; HSCT, hematopoietic stem-cell transplantation; G-CSF, granulocyte colony-stimulating factor; GVHD, graft-versus-host disease; MUD, matched unrelated donor; MDS/L, myelodysplastic syndrome/leukemia; RIC, reduced intensity conditioning, SCN, severe congenital neutropenia

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in patients with SCN belonging to the original Kostmann family as well as non-related patients with SCN with *ELANE* mutations or unknown mutations, all treated at the same clinical center during the past 5 years.

PATIENTS AND METHODS

Patients

Eight HSCT have been performed in seven patients with SCN since 2004 at the Karolinska University Hospital. Ethical approval for the current study was provided by the ethics committee at the Karolinska University Hospital. All patients fulfilled the criteria for SCN with ANC $<0.5 \times 10^9$/L prior to treatment with recombinant G-CSF, maturation arrest of the myelopoiesis in the bone marrow at the promyelocyte/myelocyte stage and a clinical picture typical of SCN. All patients were screened for known disease-causing mutations using standard methods previously described [2,7].

The Indications for HSCT

Transformation to MDS/L: Two patients were transplanted due to MDS/L. Patient 2 was diagnosed with pre-B-ALL (acute lymphoblastic leukemia) with aberrant expression of myeloid markers at an annual routine bone marrow examination. Prior to this occasion no cytogenetic aberrations or *CSF3R* mutations had been identified. Spectral karyotyping (SKY) analysis demonstrated a karyotype of 47,X,der(Y)t(Y;1),der(5)t(4;5),-der(7)t(6;7),+i(21)(q10). No *CSF3R* mutation was detected [19]. The treatment with G-CSF was discontinued and the percentage of leukemic blasts decreased. No chemotherapy was given. Two months later, at 12 years of age, the patient underwent allogeneic HSCT still with increased amount of leukemic blasts in the bone marrow (Table I).

Patient 3 had a *CSF3R* mutation and trisomy 21 detected in about 45% of mononuclear cells from the bone marrow upon an annual routine examination at 13 years of age. The complete blood count (CBC) and the bone marrow morphology showed no sign of MDS/L. Three months later a follow-up bone marrow examination was performed, but now the karyotype was normal and no *CSF3R* mutation could be detected. Approximately 6 months later the patient started to have respiratory problems and developed pulmonary infiltrates. A new bone marrow examination still did not show morphological features of MDS/L but an increased proportion of CD34+ cells were observed and again the *CSF3R* mutation and trisomy 21 were seen together with additional genetic aberrations. The karyotype was 47,XY,del(4), (q?), add(7)(q?), t(12;19)(q12;p13),+22[20]/48,idem,+21[8]/46,XY[2]. It was concluded that this was MDS or AML under development and the patient underwent allogeneic HSCT at 14 years of age. No chemotherapy was given prior to HSCT.

G-CSFR (*CSF3R*) mutation/s: Two patients (1 and 6) were transplanted, at 28 and 19 years of age, respectively, following detection of *CSF3R* mutations that were consistently detected at high levels several years before the HSCT.

G-CSF resistance: Two patients (4 and 5) were transplanted due to G-CSF resistance. Patient 4 was diagnosed at 12 months of age as a sporadic SCN. The patient did not respond to G-CSF treatment at doses up to 80 μg/kg/day; the ANC stayed below 0.2×10^9/L. The patient was transplanted twice, first at 2.8 and later at 3.2 years of age.

TABLE I. Patient and Donor Characteristics

Patient number	Age at HSCT (years)	Sex	Age at diagnosis (months)	Mutation	Associated findings	Indication of HSCT	Donor	Donor age (years) and sex	Match	Stem cell source
1	28	F	0.5	*ELANE* (p.Leu121His) het[19] *CSF3R* (p.Gln741X) and (p.Gln749X)		*CSF3R* mutations	Sibling	23/M	ABDR id	PBSC
2	12	M	5	*HAX1* (p.Gln190X) Hom[7]	Neurological deficits	ALL	MUD	22/F	ABDR id	BM
3	14	M	3	*ELANE*(p.His87del) Het[a]		MDS/AML	MUD	36/F	ABDR id	PBSC
4	2.8	F	12	wt for *ELANE*, *HAX1*, *GFI1*, and *CSF3R*		G-CSF resistance	Sibling	0/M	ABDR id	Cord
4	3.2	F	12	wt for *ELANE*, *HAX1*, *GFI1*, and *CSF3R*		Re-transplantation due to rejection	Same sibling	0.7/M	ABDR id	BM + MCS
5	7	M	1 week	*ELANE* (p.Cys151Ser) Het[a]		G-CSF resistance and MDS	MUD	0/M	B mismatch	Cord
6	19	F	12	*HAX1* (p.Gln190X) Hom[31] *CSF3R* (p.Gln749X)	Neurological deficits	*CSF3R* mutation	MUD	22/F	ABDR id	BM
7	18	M	18	*HAX1* (p.Trp44X) Hom[7]		Patient's own request	Sibling	5/M	ABDR id	BM

Het, heterozygous mutation; Hom, homozygous mutation; PBSC, peripheral blood stem cell source; BM, bone marrow; MCS, mesenchymal stem cells from matched unrelated donor (bone marrow) with the dose 1×10^6/kg. [a]These mutations have not been previously reported.

Patient 5 was diagnosed at 1 week of age due to purulent infections in the eye and nose and is considered as a sporadic SCN. The parents are of Arabic origin, but not related. The boy needed high doses of G-CSF, up to 72 μg/kg/day, to reach ANC $>1.0 \times 10^9$/L. An increased number of leukemic blasts in the bone marrow were detected in a bone marrow examination performed just prior to the start of the conditioning treatment and MDS development was suspected. No chemotherapy was given prior to HSCT, which was performed at the age of 7 years.

At the patient's own request: patient 7 was very distressed by the injection of the G-CSF preparation and the compliance of the treatment was poor. He and the parents requested transplantation. The patient was on G-CSF treatment for 14 years prior to HSCT, which was performed at the age of 18 years.

Conditioning and GVHD Prophylaxis

The conditioning regimens and the graft-versus-host disease (GVHD) prophylaxis were individualized in an attempt to address specific clinical situations (SCN and/or MDS/L), available donor, and stem cell source. The standard conditioning regimens were changed during these years. Three patients received myeloablative conditioning consisting of busulphan (16 mg/kg) in combination with cyclophosphamide (120 mg/kg) and with the addition of melphalane (140 mg/m^2) in two patients [20]. Four patients received reduced intensity conditioning (RIC). One patient received fludarabine (30 mg/m^2) for 6 consecutive days, combined with busulphan (8 mg/kg) [21]. Three patients received fludarabine (30 mg/m^2) for 5 days combined with treosulphan (14 mg/kg) for 3 days. For retransplantation, fludarabine (30 mg/m^2/day) for 5 days was combined with cyclophosphamide (120 mg/kg). Two patients received cyclosporine (CsA) combined with four doses of methotrexate (MTX) [22]. Two patients with MDS/L were given low-dose CsA combined with MTX [23]. The goal for low-dose CsA was serum levels of 100–150 ng/ml. The standard therapeutic levels are 200–300 ng/ml. The recipients of cord blood transplants received CsA combined with prednisolone. Two patients were given rapamycin, one in combination with CsA, and the other combined with tacrolimus (Table II).

Engraftment and Chimerism Analysis

Definition of engraftment was ANC $>0.5 \times 109$/L or complete donor engraftment based on ≥95% CD3+, CD19+, and CD 33+ donor cells in peripheral blood (PB). For chimerism analysis, PB samples were collected from the donor and recipient before transplant and from the recipient on days +14, +21, +28, and usually every other week up to 3 months and monthly thereafter. Bone marrow was also analyzed in some of the patients. DNA from donor and recipient pretransplantation samples was extracted, using standard protocols (MagNA Pure, Roche, Basel, Switzerland). To evaluate lineage-specific chimerism, CD3, CD19, and CD33-positive cells were selected from PB, using immunomagnetic beads (Dynal, Oslo, Norway). The methodology and sensitivity of chimerism analysis in the various cell lineages is described elsewhere [24].

RESULTS

The mutations found that cause the underlying SCN are described in Table I. Of the *ELANE* mutations identified in this study, two mutations were novel (H87del and C151S). The mean age at transplantation was 13 years (range 2.8 and 28 years). The post-transplantation mean follow-up was 32 months (range 21–60 months). Four grafts were from HLA-identical siblings (one cord blood). Three grafts were from MUD and one was from an HLA-mismatched unrelated cord blood unit (Table I).

Engraftment and Hematological Recovery After HSCT

Donor engraftment occurred in all HSCT procedures. ANC $>0.5 \times 10^9$/L was achieved after seven of eights transplantations (Table II). The ANC count reached $>0.5 \times 10^9$/L at a mean of 28 days (range 12–54 days). Patient 5 did not reach ANC $>0.5 \times 10^9$/L but complete donor chimerism in PB was documented at 1 month after HSCT. Moreover, the patient developed acute GVHD grade IV and therapy-resistant septicemia, from which the patient died on day 40. Three patients received G-CSF post-transplantation; one (patient 4) due to a rejection (the patient was re-transplanted) and two (patients 6 and 7) received a short course of G-CSF because of relatively long neutropenia post-HSCT. One patient (patient 4) is mixed chimera in all cell lineages but with normal or near normal ANC values and currently does not require G-CSF treatment. This patient had ANC above 0.5 at day +54, this could be related to a G-CSF effect on autologous neutrophils. At +90 days 20% of the CD 33+ cells were of donor origin.

Chimerism Data

Three patients became complete donor chimeras at 3 months and four were mixed chimeras in blood. At 12 months, only patient 4 had a domination of recipient cells in CD3+, CD19+, and CD33+ cells (Table III). Recipient cells also dominated in the bone marrow at 1 year post-transplant in this patient. However, at 33 months after the second HSCT the patient is >95% donor in CD33+ cells, but 35% and 20% donor in, CD19 and CD3+ cells, respectively.

GVHD

Three of seven patients developed acute GVHD grades II–IV (Table II). Patient 5, who had received a cord blood graft, developed grade IV GVHD with therapy-resistant septicemia and died. One of six patients (patient 3) who survived for more than 3 months developed chronic GVHD in lungs, gut, and skin 2 years post-transplant and remains on immunosuppressive treatment.

Donor Lymphocyte Infusion (DLI)

Patient 2 received a total of four DLIs with increasing numbers of CD3+ cells (0.2×10^6 to 1×10^7 CD3+ cells/kg) due to increasing proportions of recipient cells. The patient responded and become a donor chimera.

Patient 4 received DLI, 1×10^6 CD3+ cells/kg 2 months after due to a threatening rejection. She went on and rejected completely and was retransplanted at day 119 after the first HSCT with bone marrow from the same sibling. There was mixed chimerism after the second transplant and she again received DLI, 5×10^6 CD3+ cells/kg a month after the second HSCT. Donor chimerism has thereafter gradually increased with >95% donor cells in the myeloid fraction almost 3 years after the second transplant.

Patient 7 received DLI at a dose of 1×10^6 CD 3+ cells/kg at day +45 after HSCT due to an increasing proportion of recipient cells.

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TABLE II. Hematopoietic Stem Cell Transplantation (HSCT) and Outcomes

Patient number	Cell dose (CD34+ cells/10^6/kg)	Conditioning regime	ATG	GVHD prophylaxis	Engraftment	ANC $>0.5 \times 10^9$/L Day	Platelets $>50 \times 10^9$/L Day	G-CSF post-SCT	Acute GVHD grade	Chronic GVHD grade	Rejection	Donor lymphocyte infusion	Follow-up time (months)	Outcome/clinical status
1	19.1	Flu + Bu	Camp	Fk + Rapa	Yes	22	14	No	0	0	0		60	Well hyperthyreosis (radiojod and levothyroxin). Normal ANC
2	4.48	Bu + Cy	ATG	CsA + MTX	Yes	17	18	No	II	0	0	Yes		Died 10 months post-HSCT of unknown cause
3	26.8	Bu + Cy + -Mel	ATG	LowCsA + MTX	Yes	12	13	No	I	Yes	0		31	Severe cGVHD. Normal ANC
4	0.6	Flu + Treo	ATG	CsA + Pred	Yes	54	26	Yes	0	0	Yes	Yes	Retranspl day 119	
4	9.0	Flu + Cy	ATG	LowCsA + MTX	Yes	25	1	No	0	0	Partial	Yes	26	Well recipient chimerism, subnormal ANC
5	0.07	Bu + Cy + -Mel	ATG	CsA + Pred	Yes	No	No	No	IV	0	0			Died day 40 of aGVHD
6	2.4	Flu-Treo	ATG	CsA + MTX	Yes	20	25	Yes	I	0	0		24	Quite well. Nutrition and respiratory problems. Valproat. Normal ANC
7	1.0	Flu-Treo	ATG	CsA + Rapa	Yes	23	170	Yes	II after DLI	0	0	Yes	21	Well. Normal ANC

ATG, anti-thymoglobulin was given 6–8 mg/kg day −4/−3 to day −1; Camp, Campath (alemtuzumab); Flu, fludara; Bu, busulfan; Cy, cyclophosphamid; Mel, melphalan; Treo, treosulfan; Fk, tacromilus; Rapa, rapamycin; CsA, cyclosporine; MTX, methotrexate; Pred, prednisolon; GVHD, graft-versus-host disease; ANC, absolute neutrophil count; G-CSF, granulocyte colony-stimulating factor. Donor lymphocyte infusion was done due to chimerism. Pat 2: d129 0.2×10^6/kg and d150 1×10^6/kg. Pat 4a: d61 1×10^6/kg. Pat 4b: d35 5×10^6/kg. Pat 7: d45 1×10^6/kg and d76 5×10^6/kg.

TABLE III. Chimerism Data in Bone Marrow (BM) and Blood (% Donor Cells)

Patient number	BM 3 months				BM 12 months				Blood 3 months			Blood 12 months		
	CD34	CD33	CD19	CD3	CD34	CD33	CD19	CD3	CD33	CD19	CD3	CD33	CD19	CD3
1	NE	NE	95	80–90	76	99	99	71	90–95	80–85	10–15	98	98	86
2	75–80	90–95	40–50	30–40	89[b]	>99[b]	95[b]	62[b]	NE	85–90	40	>99[b]	>99[b]	77[b]
3	NE	>99	>99	>99	ND	ND	ND	ND	>99	>99	>99	>99	NE	>99
4	5–10	10–15	5–10	10					20	15–20	5–10			
4	ND	ND	ND	ND	NE[b]	40–45[b]	45[b]	20[b]	25–30	20	5–10	20	30–35	15–20
5	ND	ND	ND	ND					95[d]	NE[d]	>99[d]			
6	ND	ND	ND	ND	ND	ND	ND	ND	>99	>99	>99	>99	>99	98.5
7	NE[a]	>95[a]	90[a]	50[a]	85[c]	>95[c]	NE[c]	70[c]	70	<5	30	>99	NE	>99

NE, not evaluable; ND, not done. All values given are single values at 3 or 12 months after HSCT if available, other time points are shown in footnote [b]. [a]4 months after SCT; [b]9 months after SCT; [c]7 months after SCT; [d]1 month after SCT.

The immunosuppression was discontinued at day +47. Due to continuously increasing recipient cells a second DLI was given at day +72, 5×10^6 CD3+ cells/kg and a third DLI, 8.4×10^6 CD3+ cells/kg, at day +98. Thereafter the patient developed GVHD of the liver which responded to steroids and cyclosporine A treatment. The patient responded to DLI and is today 100% donor in all hematopoietic cell lineages.

Outcome

Two out of seven (29%) patients have died—both with MDS/L (Table II). One patient died of acute GVHD and the other at 10 months post-transplant of unknown cause [25]. Thus, five out of seven patients (71%) survived. One patient has a mixed chimerism with a high proportion of recipient cells. However, the patient has a normal or slightly subnormal ANC without G-CSF treatment and no problems of bacterial infections. Four of the five surviving patients have a good quality of life.

DISCUSSION

SCN is a heterogeneous bone marrow failure syndrome with different modes of inheritance. The two major causative genetic defects in SCN are heterozygous *ELANE* mutations and homozygous *HAX1* mutations. Out of 114 patients tested and registered to the European branch of the SCNIR, 65 (57%) patients harbor *ELANE* mutations and 14 (12%) *HAX1* mutations, while 35 patients (31%) were negative for both mutations [16]. We and others have shown that patients belonging to the original Kostmann family harbor mutations in the *HAX1* gene located on chromosome 1q22 [7,26]. Here, we provide a retrospective analysis of HSCT performed in patients belonging to the original Kostmann family as well as in other unrelated patients with SCN who were treated at the same clinical center. The patient cohort is small and heterogeneous, but since the disease is rare and there are very few reports on HSCT in these patients with SCN, we find it important to report our experiences.

Patients with both *ELANE* and *HAX1* mutations appear to have a similar phenotype and a risk of MDS/L [7,16]. However, there are a few patients with SCN without detectable mutations in *ELANE* or *HAX1*, and in this group of patients MDS/L has also occurred [13]. Most patients with SCN respond to G-CSF treatment and have a good quality of life, but it does not cure the disease. The only cure for SCN is HSCT but due to the potential toxicity, HSCT is only recommended for certain indications, that is, development of MDS/L and unresponsiveness to G-CSF treatment. The cumulative risk of developing MDS/L is currently estimated to be >20% after 10 years treatment with G-CSF and in certain subgroups of patients it is over 40% [12]. This raises the question whether patients with an HLA-identical sibling should be recommended to undergo HSCT prior to any signs of MDS/L. The discussion as to whether HSCT should be an alternative is preferably done early after diagnosis. Factors to base the decision on include the response to G-CSF therapy, the long-term costs of the respective treatments, the risk of developing MDS/L (as determined based on the acquisition of *CSF3R* mutations and/or acquired cytogenetic aberrations such as monosomy 7 and trisomy 21 [15,16]) and the suitability of the donor versus the risk of mortality and morbidity of the HSCT procedure itself. With regard to the response to G-CSF therapy, obviously non-response to therapy is an indication for HSCT; however, since as

TABLE IV. Published HSCT in SCN

Refs.	No. of patients	Indication(s)	Donor	Conditioning	Outcome and notes
Zeidler et al. [28]	11	Non-malignant. Non-responder or partial responder to G-CSF	MRD (8), single ag-mm (2), haploid father (1)	Bu-Cy (4), Bu-Cy-ATG (3), Bu-Cy-ATG-Thiot (1), Cy (1), Bu-Cy-Thiot (1), Bu-Cy-Mel (1)	1 graft rejection; 2 dead (GVHD grade IV and multiorgan failure); 1 GVHD grade IV and failure to thrive; 1 GVHD grade III; 1 cystectomy due to hemorrhagic cystitis; 5 no major problems
Toyoda et al. [36]	1	G-CSF resistance	MUD	TBI-Cy-Etop-ATG	Live 23 months post-txp. Pretranspl lung abscesses
Dallorso et al. [37]	1	MDS/AML	MUD	Bu-Cy-Mel-ATG	Live 33 months post-txp. Pretranspl fungal infection of the lung; 1 FLAG and 1 IDA-FLAG prior SCT had no effect of the AML
Ferry et al. [29]	9	MDS/acute leukemia (4). G-CSF refractory (4). Bone marrow failure (1)	MUD (3), UCB (4), RD (2)	Bu-Cy-ATG (5), Bu-Cy (1), TBI-Cy-Etop-ATG, TBI-Cy (1), TBI-Thiot-Cy-ATG (1), Flu-ATG (1)	3 dead/6 alive. 3/4 with MDS/AL is alive. Cause of death: 2 septic chock, 1 aspergillus. 2 SCT twice. 1 malignant relapse after 6 months
Mino et al. [38]	1	Non-responder to G-CSF	UMCB	Bu-Cy-ATG	Alive
Nakazawa et al. [39]	1	Non-responder to G-CSF + cytogenetic aberration	UCB with HLA-DRB1 mismatch	TBI-Flu-Cy	Alive
Choi et al. [17]	6	MDS (2), MDS/AML (2), AML (2)	MUD (4), URD single ag mm (2)	TBI-Cy (1), Bu-AraC-Cy (5)	4 dead/2 alive. Cause of death: 2 CGVHD, 1 graft failure, 1 relapsed AML
Fukano et al. [32]	1	Non-responder to G-CSF	UCB single mismatch	Flu-Mel-ATG (RIC)	Alive. Full donor chimerism
Cojean et al. [40]	1	Non-responder to G-CSF	MRD	Bu-Cy-ATG	Alive 8 months post-txp. Pretranspl inflammatory pseudotumor of the liver. Full donor chimerism
Thachil et al. [41]	1	Non-responder to G-CSF	MUD	Flu-Campath-Thiot	Alive 30 months post-txp. Full donor chimerism
Markel et al. [42]	2	Non-responder to G-CSF	UCB	Unknown	Both alive. One patient underwent 3 UCB SCT due to inadequate conditioning
Yesilipek et al. [43]	2	Non-responder to G-CSF	UCB 6/6 and 5/6 HLA match	Bu-Cy	Both alive. Full donor chimerism
Total	37				28 alive/37 patients (76%)

MDS/AML, myelodysplastic syndrome/acute myeloid leukemia; MRD, mixed related donor; MUD, mixed unrelated donor; UCB, unrelated cord blood; RD, related donor; UMCB, unrelated matched cord blood; Bu, busulfan; Cy, cyclophosphamid; Flu, fludarabin; Mel, melphalan; Thiot, thiotepa; Etop, etoposid; ATG, anti-thymoglobulin; TBI, total body irradiation; AraC, cytarabin; Campath, alemtuzumab; IDA-FLAG, a chemotherapy regimen with idarubicin, fludarabine, Ara-C and G-CSF; RIC, reduced intensity conditioning. The search was done in Pubmed. The search terms were: SCN and transplantation, severe chronic neutropenia and transplantation and Kostmann syndrome and transplantation.

many as 40% of less-responsive patients are at risk of developing MDS/L [12], such patients may also be strong candidates for HSCT.

HSCT has been reported as a possible treatment of SCN prior to the introduction of G-CSF treatment [27]. The main indication for HSCT has been malignant transformation to MDS/L [17], but the procedure has also been performed in patients without leukemic transformation [28]. The French SCN register reported their experience of HSCT in nine cases with different indications for the transplant [29]. In the literature we found altogether 37 patients with SCN that had underwent HSCT (Table IV). In our series, seven patients were transplanted, one patient twice, between 2004 and 2008. Different stem cell sources were used based on availability. In two patients cord blood were used. One recipient was grafted with cord blood from a sibling but rejected and was retransplanted with bone marrow from the same donor. Another patient received unrelated cord blood, the patient did not show neutrophil engraftment; however, chimerism analysis showed more than 95% donor cells in the blood. This patient developed GVHD and died 40 days after transplantation. As experienced in our cord blood grafted patients and data from other reports [30,31], rejection or poor engraftment are problems more commonly seen after cord blood as compared to bone marrow or PB transplantations. Three patients were transplanted with MDS/L. Two of these patients have died, while one is alive but has a severe chronic GVHD. This is in accordance with previous experiences showing that the prognosis is poor if leukemia has developed. Patients with MDS have a better outcome [17]. Choi et al. [17] reported SCT in six patients with MDS/L. All four patients who received induction chemotherapy

died, while two patients with MDS survived. In the present study, all four patients with other indications for HSCT are alive. This illustrates the need of yearly bone marrow controls since leukemia evolution is preceded in many cases by the acquisition of *CSF3R* mutations [16] and/or cytogenetic aberrations in mononuclear bone marrow cells [15].

One of five surviving patients in our cohort has mixed chimerism with the majority of recipient cells in CD3+ and CD19+ cell fractions, but >95% donor in CD33+ cells in blood. The goal is obviously to achieve 100% donor cells, but one should also aim to avoid an undesirable degree of toxicity such as acute GVHD. Mixed chimerism is acceptable in many non-malignant cases. In patients transplanted for SCN, we do not yet know if having remaining recipient cells with the underlying mutation poses a risk for development of secondary malignancies. Two MDS/AML relapses are reported but no data regarding chimerism were given for these patients (Table IV) [17,29]. Fukano et al. [32] have suggested RIC to avoid toxicity both in the short and in the long-term, but they did not discuss the risk of mixed chimerism. We cannot exclude that patients with mixed chimerism are at risk of developing MDS/L. The remaining cells with the underlying mutation could still be prone to develop into a malignant clone, and it might therefore be logical to try to achieve complete donor chimerism. This can either be achieved by intensifying the conditioning regimen or to give post-transplant DLI. Both of these strategies are associated with an increased risk for transplant-related toxicity. Longer follow-up and larger patient series might help to resolve this issue.

The role of continuous G-CSF treatment for the malignant evolution is not fully understood. The present view is that G-CSF, a growth factor with known anti-apoptotic properties, might stimulate an established malignant clone but may not trigger the emergence of such a clone. Leukemia has been reported in patients with SCN prior to the introduction of treatment with G-CSF [33], while MDS/L has not been reported in G-CSF-treated patients with cyclic neutropenia. Moreover, transgenic mice harboring SCN patient-derived *CSF3R* mutations show a hyperresponsiveness to G-CSF, yet do not develop leukemia, despite prolonged administration of large doses of G-CSF [34,35]. Acquired mutations in the *CSF3R* gene may thus not be the driving event in leukemogenesis but could be reflective of an inherent genetic instability in cells from these patients. It has been suggested that the pathogenesis of SCN and its evolution to secondary malignancies may involve an underlying genomic instability and pharmacological doses of G-CSF could potentially stimulate the stepwise acquisition of genetic changes in bone marrow cells in these patients and proliferation of a potential malignant clone of cells [16].

In summary, despite recent improvements in transplantation, HSCT in SCN is associated with significant mortality and morbidity. The poor prognosis reported for patients with SCN who have developed leukemia [17] was also recapitulated in our single-center experience concerning both morbidity and mortality. SCN is a rare condition and international cooperation to optimize a protocol for HSCT in SCN patients is desired to achieve a more unified approach and hopefully improved outcomes. Moreover, the role of mixed chimerism for the development of MDS/L in cells harboring the underlying mutation has to be studied further. Finally, if mixed chimerism is demonstrated to convey a risk of malignant transformation, it will be necessary to avoid this by employing fully myeloablative conditioning and grafts with high cell counts.

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