Regular Article

blood

TRANSPLANTATION

Hematopoietic stem cell transplantation in 29 patients hemizygous for hypomorphic *IKBKG*/NEMO mutations

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Key Points

- Global survival rate was 74% at a median follow-up after HSCT of 57 months.
- Preexisting mycobacterial infection and colitis were associated with poor HSCT outcome.

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X-linked recessive ectodermal dysplasia with immunodeficiency is a rare primary immunodeficiency caused by hypomorphic mutations of the *IKBKG* gene encoding the nuclear factor κB essential modulator (NEMO) protein. This condition displays enormous allelic, immunological, and clinical heterogeneity, and therapeutic decisions are difficult because NEMO operates in both hematopoietic and nonhematopoietic cells. Hematopoietic stem cell transplantation (HSCT) is potentially life-saving, but the small number of case reports available suggests it has been reserved for only the most severe cases. Here, we report the health status before HSCT, transplantation outcome, and clinical follow-up for a series of 29 patients from unrelated kindreds from 11 countries. Between them, these patients carry 23 different hypomorphic *IKBKG* mutations. HSCT was performed from HLA-identical related donors (n = 7), HLA-matched unrelated donors (n = 12), HLA-mismatched unrelated donors (n = 8), and HLA-haploidentical related donors (n = 2). Engraftment was documented in 24 patients, and graft-versus-host disease in 13 patients. Up to 7 patients died 0.2 to 12 months after HSCT. The global survival rate after HSCT among NEMO-deficient children was 74% at a median follow-up after HSCT of 57 months (range, 4-108 months). Preexisting mycobacterial infection and colitis were associated with poor HSCT outcome. The underlying mutation does not appear to have any influence, as patients with the same mutation had different outcomes. Transplantation did not appear to cure colitis, possibly as a result of cell-intrinsic disorders of the epithelial barrier. Overall, HSCT can cure most clinical features of patients with a variety of *IKBKG* mutations. (*Blood*. 2017;130(12):1456-1467)

Introduction

X-linked recessive ectodermal dysplasia with immunodeficiency (XR-EDA-ID) is a rare primary immunodeficiency (PID) caused by hypomorphic mutations of the *IKBKG* gene, encoding nuclear factor κB (NF-κB) essential modulator (NEMO) a key regulator of the canonical NF-κB signaling pathway (Figure 1).[1-3] NF-κB and NEMO are widely expressed and involved in many signal transduction pathways, including those downstream from interleukin 1 receptors, Toll-like receptors (TLRs), tumor necrosis factor receptors, vascular endothelial growth factor receptor-3, ectodysplasin-A receptor, and receptor activator of NF-κB (Figure 1).[4] Loss-of-function mutations of *IKBKG* underlie X-linked incontinentia pigmenti (IP), which is lethal in male fetuses.[4] In contrast, hypomorphic *IKBKG* mutations underlie XR-EDA-ID, which is characterized by diverse clinical manifestations, including EDA, life-threatening infections, and inflammatory diseases.[1,2,5-7] A few patients also have osteopetrosis and lymphedema (XR-OL-EDA-ID).[1,2,5-7] At least 3 nonhematopoietic pathways are affected: EDA results from alterations to the ectodysplasin/ectodysplasin-A receptor signaling pathway,[5] lymphedema from changes to the vascular endothelial growth factor-3 pathway,[1,2,5-7] and osteopetrosis from alterations to the receptor activator of NF-κB pathway.[5] Conversely, some NEMO-deficient patients display no signs of EDA.[1,2,5-10]

The pathogenesis of the inflammatory and infectious phenotypes remains more elusive. B-cell and antibody (Ab) deficiencies include hypogammaglobulinemia, hyperimmunoglobulin (hyper-Ig) M and Ab deficiency.[6,11,12] Most patients have normal T-cell counts and T-cell proliferation.[6,9-12] Impaired NK cell cytotoxicity has been reported.[6,11,12] The only immunologic abnormality consistently observed in these patients is a lack of glycan-specific Ab production, likely accounting for the high incidence of pneumococcal disease.[1,2,5-7] XR-EDA-ID is perhaps the most heterogeneous PID yet identified at the allelic, immunological, and clinical levels,[13] making clinical care of the patients particularly challenging. The precise mechanisms underlying the infectious and inflammatory phenotypes remain unknown, but hematopoietic stem cell transplantation (HSCT) has been performed in 29 children with *IKBKG* mutations[9,10,12,14-30] (personal communications [see supplemental Methods, available on the *Blood* Web site]). These children probably account for less than 10% of the children with XR-EDA-ID reported worldwide.[5] In contrast, 11 of the 14 reported patients with autosomal dominant EDA-ID, caused by gain-of-function mutations of *NFKBIA,* encoding IκBα, have undergone HSCT.[16,31-41] This higher proportion of patients undergoing transplantation reflects both the greater severity and the homogeneity of this condition. We collectively considered 29 NEMO-deficient patients who underwent HSCT.[1,9,10,12,14-28,30] This includes 10 previously unreported cases and has allowed for an updated analysis of the outcome of this procedure.

Methods

Patients and data collection

Patients with *IKBKG* hypomorphic mutations who underwent HSCT were studied.[1,9,10,12,14-28,30,31,33,42-44] We collected published and unpublished cases from other physicians around the world (Table 1; supplemental Data).

Statistical analysis

Descriptive statistics are presented in the supplemental Data.

Results

Hemizygous *IKBKG* mutation and NEMO protein expression

This series includes 19 patients for whom HSCT was reported in previous studies[1,9,10,12,14-28,30,31,33,42-44] and 10 unreported patients. These 29 unrelated patients originated from 11 countries and were living in 9 countries (France, Germany, Switzerland, United Kingdom, Mexico, Brazil, United States, New Zealand, and Japan) (Table 1). These patients were hemizygous for 23 different *IKBKG* mutations (Figure 2A). All mutations were private to this cohort, with the exception of the c.1167_1168insC mutation, which was found in 7 unrelated patients. Functional testing was carried out for a total of 14 mutations, all of which were shown to be hypomorphic (supplemental Data). The mutations were missense (n = 13), nonsense (n = 3), small insertion (n = 4), small deletion (n = 1), large duplication (n = 1), and splice (n = 1) mutations (Table 1). Half the mutations affected the zinc finger (ZF) domain (n = 17). Six mutations affected coiled coil domain 1 or 2, and 5 mutations affected the NEMO ubiquitin-binding domain (Figure 2A). Flow cytometry or western blotting showed NEMO protein levels to be abnormally low in 4 of the 6 patients tested (P8, P10, P26, P27). NEMO protein levels were normal in P19 and P28, but specific assays highlighted changes to NEMO function.[28,30] Functional assays were performed to test the NF-κB pathway specifically, in 17 patients (supplemental Data). Functional assays were not performed for 12 patients, 3 of whom carried previously described and tested mutations.

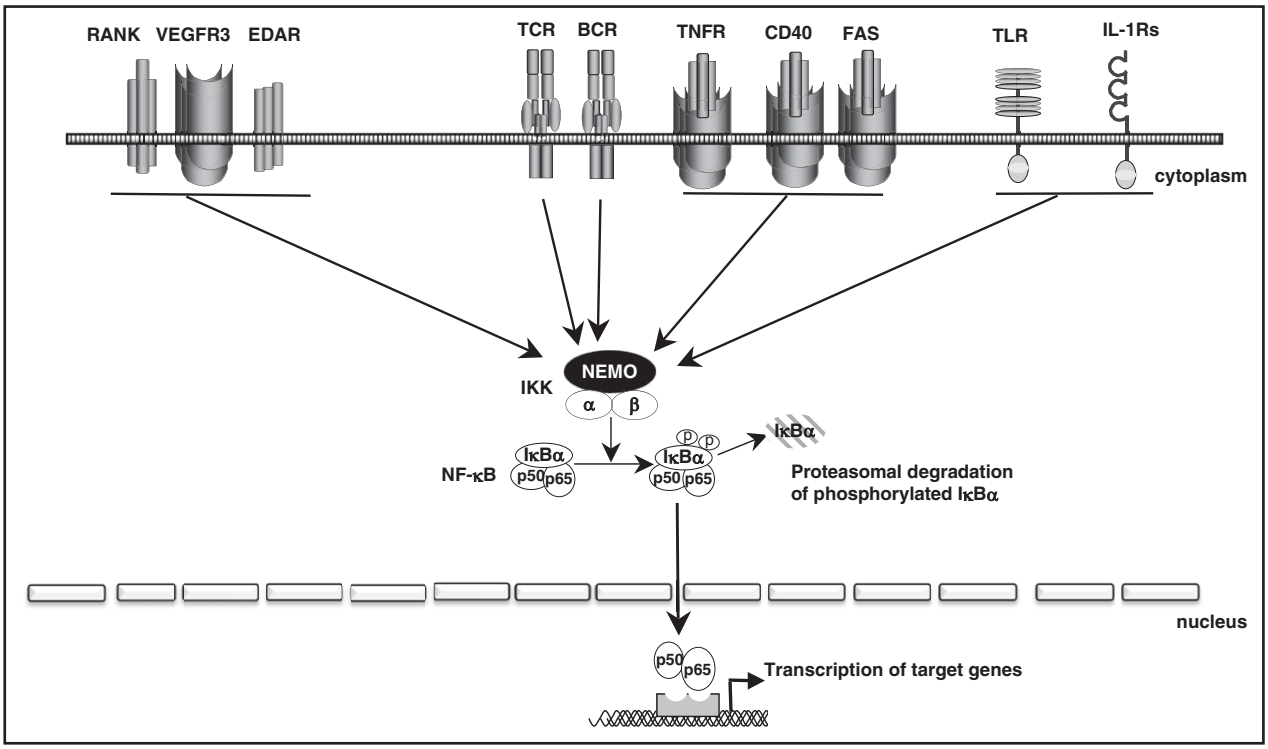

Figure 1. NF-kB pathway. Immune receptor signaling pathways leading to NF-κB activation can be grouped into 4 categories on the basis of the surface receptors involved: developmental receptors (receptor activator of NF-κB, vascular endothelial growth factor receptor-3, and ectodysplasin-A receptor), antigen receptors (T-cell receptor [TCR] and B-cell receptor [BCR]), members of the TNF receptor superfamily (TNF-Rs: tumor necrosis factor receptors, CD40, FAS, etc), and members of the Toll-interleukin receptors superfamily (TIR: IL-1 receptors and TLRs). The protein of the NF-kB signaling pathway (NEMO) responsible for EDA-ID is shown in black.

Carrier mothers and the developmental phenotype of the patients

Genetic analysis of the *IKBKG* coding region in the patients' mothers found the same *IKBKG* mutation as in their affected sons, implying that none of the mutations occurred de novo, except in P24, who displayed mosaicism and whose mother did not carry the *IKBKG* mutation. When assessed, more than half these mothers presented mild IP symptoms. Almost all the women with IP symptoms carried mutations affecting the NEMO ZF domain. However, not all women carrying ZF mutations presented IP symptoms, suggesting phenotypic heterogeneity among women carrying the same mutation, possibly because of sporadic X inactivation.[45-47] Mothers of P5 and P8 had IP. Five had inflammatory diseases: arthralgia (P1's mother), colitis (P2's mother), and recurrent mouth ulcers (the mothers of P3, P7, and P11). P6's mother had recurrent bacterial infections. Twenty-four patients presented signs of EDA (Table 1). Only 3 patients (P12, P16, P21) had no signs of EDA. These patients were 7, 2, and 13 years old, respectively, at the time of HSCT. Consistent with these findings, the mutations of P12 and P16 had previously been described as associated with an absence of EDA in other patients.[6] P21, whose mutation affects the ZF domain, does not have EDA either.[24] Data were not available for P18 and P20. The 7 patients carrying the c.1167_1168insC mutation all had EDA. Six children presented with osteopetrosis (P2, P3, P6, P8, P22, P25): 5 patients with lymphedema (P3, P8, P9, P11, P25) and 3 patients with both features (Table 1).

Infectious phenotype of the patients

Median age at symptom onset was 2 months (range, birth-30 months) (Table 1). All patients except P2 and P29 had recurrent infections before HSCT (Table 2). P2 was diagnosed at birth because 1 of his older brothers had previously died of an invasive *Pseudomonas aeruginosa* infection in the context of EDA-ID with an *IKBKG* mutation. He thus received antibiotic prophylaxis and IV IgG (IVIG) from birth. P29 was diagnosed with hypogammaglobulinemia and low naive T-cell count, and thus received IVIG and antibiotic prophylaxis from age 1 month. No data were available for P16 and P20. The 25 remaining patients displayed severe recurrent bacterial infections, mostly as a result of pyogenic bacteria (*Staphylococcus aureus* [n = 9; 33%] and *Streptococcus pneumoniae* [n = 10; 37%]), *Haemophilus influenzae b* (n = 4; 14.8%), and other Gram-negative bacteria (n = 12; 44.4%). Sixteen patients had mycobacterial infection before HSCT: *Mycobacterium avium* (9/16), *M fortuitum* (2/16), *M kansasii* (1/16), *M szulgai* (1/16), and Bacille Calmette-Guérin vaccine (3/16). The mycobacterial species responsible for infection was not specified for P20. Severe viral infections were common (16/28) and were mostly caused by *Herpesviridae sp* (CMV [n = 6], EBV [n = 5], VZV [n = 2], HSV [n = 2], and HHV6 [n = 2]). Six patients developed hypoxic *Pneumocystis* pneumonia before the implementation of prophylaxis. Finally, 8 patients had other fungal infections, most commonly mucocutaneous candidiasis (n = 6).

Inflammatory and autoimmune diseases

Thirteen patients had inflammatory skin involvement before HSCT, with early skin rash resulting from eczematous dermatitis. Skin biopsy was performed in 8 patients, revealing spongiotic dermatitis with superficial perivascular inflammatory lymphocytes and eosinophil infiltrates. Skin involvement was treated with topical corticosteroids, but systemic corticosteroids or cyclosporin (P1, P25) treatment were administered in severe cases. Fifteen patients had symptoms of inflammatory bowel disease (IBD) before HSCT. Eight of these

Table 1. Genetic and clinical features of the 29 NEMO-deficient patients before HSCT

Patient	Age at first symptom (months)	*IKBKG* mutation	Origin (country)	EDA	Rash	Colitis	O	L	Other symptoms	References
1	0	*c.1167_1168insC*	USA	Yes	Yes	Yes	No	No		16, 18; A. J. Mancini (pc)
2	2	*c.1237insT*	France	Yes	No	Yes	Yes	No		C. Picard (pc)
3	0	p.Q157P	USA	Yes	No	No	Yes	Yes	Anemia	25; A. Jain (pc)
4	6	p.F312L	UK	Yes	Yes	No	No	No		W. Qasim (pc)
5	0	p.L153R	USA	Yes	No	Yes	No	No		12, 20, 41
6	2	*c.1167-1168insC*	Switzerland	Yes	Yes	No	Yes	No	HLH, developmental delay,* MGUS	21; T. Gungor (pc)
7	0	p.G205X	Germany	Yes	Yes	No	No	No		29; S. Ehl (pc)
8	2	p.X420W	UK	Yes	No	Yes	Yes	Yes		1, 14
9	0	*c.768+5G>A*	USA	Yes	No	Yes	No	Yes		16, 19; J. Orange (pc)
10	30	p.D311E	Japan	Yes	No	No	No	No		22
11	1.5	*c.1167_1168insC*	USA	Yes	Yes	No	No	Yes	Delayed umbilical cord separation	24; A. Filipovitch (pc)
12	12	p.R254Q	USA	No	No	No	No	No	AIHA	24; A. Filipovitch (pc)
13	9	*c.1245insT*	UK	Yes	Yes	No	No	No		S. Jolles and M. Abinun (pc)
14	1.5	*c.1182_1183delTT*	UK	Yes	No	NR	No	No	Erosive arthritis	W. Qasim (pc)
15	9	p.V146G	USA	Yes	Yes	Yes	No	No		9, 27; E. W. Gelfand (pc)
16	6	p.D113N	USA	No	No	No	No	No		9, 10; E. W. Gelfand (pc)
17	12	p.G348X	Japan	Yes	No	Yes	No	No		K. Imai and T. Okano (pc)
18	2	*c.1167-1168insC*	USA	NR	NR	NR	No	No		J. Orange (pc)
19	1	p.H413P	Brazil	yes	yes	yes	no	no	Ichthyosis vulgaris	30; A. Condino (pc)
20	NR	p.Q391X	Japan	NR	NR	NR	NR	NR		K. Imai (pc)
21	3	p.M407V	USA	No	No	NR	No	No	AIHA, neurological problem	24; A. Filipovitch (pc)
22	NR	p.E315A	USA	Yes	No	No	Yes	No	Seizures (*M avium* brain lesion), lupus anticoagulant	24; A. Filipovitch (pc)
23	3	p.A162P	New Zealand	Yes	No	Yes	No	No	Hashimoto thyroiditis	N. Cole (pc)
24	4	*c.1167-1168insC*	UK	Yes	Yes	Yes	No	No		A. Gennery (pc)
25	1.5	*c.1167_1168insC*	Greece/Italy	Yes	Yes	Yes	Yes	Yes		42
26	0	Duplication intron 3 to exon 6	Japan	Yes	No	Yes	No	No		23
27	0	*c.1167_1168insC*	Japan	Yes	Yes	Yes	No	No	Colic polyps	15, 17; R. Nishikomori, T. Kawai (pc)
28	1	p.D306N	Mexico	Yes	Yes	Yes	No	No	ITP, intestinal vasculitis†	28; L. Blancas (pc)
29	1	p.Q304-A305insDLP	France	Yes	Yes	Yes	No	No		S. Blanche (pc)

AIHA, autoimmune hemolytic anemia; EDA, ectodermal anhidrotic dysplasia; HLH, hemophagocytic lymphohistiocytosis; ITP, immune thrombocytopenic purpura; L, lymphedema; MGUS, monoclonal gammopathy of undetermined significance; NR, not reported; O, osteopetrosis; pc, personal communication (see supplemental Data).

*Subependymal calcifications on brain MRI.

†Diagnosed on gut angiography.

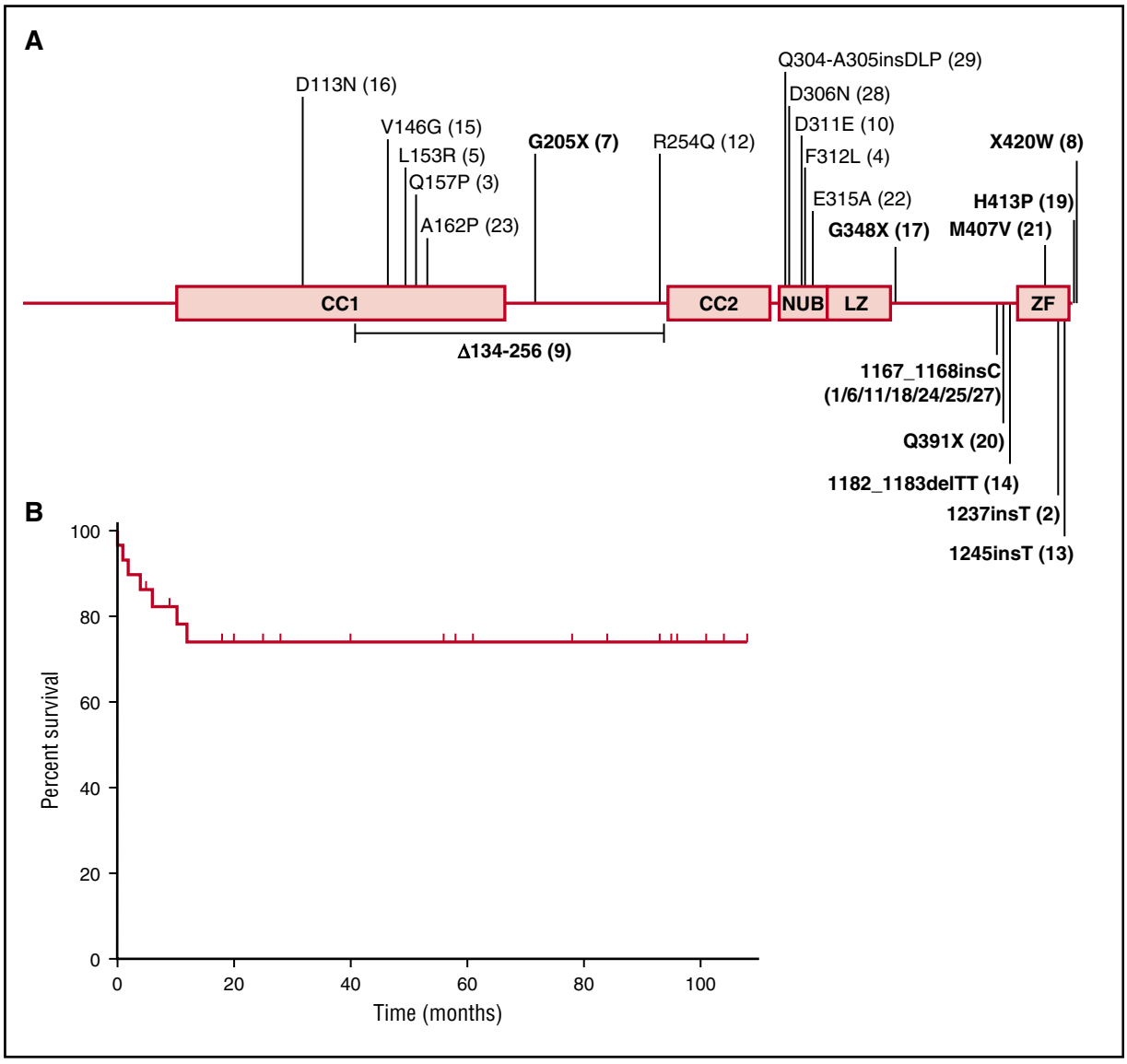

Figure 2. *IKBKG* mutations of the patients of this cohort and outcome after HSCT. (A) Schematic representation of the NEMO protein, with the various locations of the mutations found in the patients indicated. Patients with mutations affecting the zinc finger domain are shown in bold. The genetic defect of patient 26 (duplication of *IKBKG* intron 3 to exon 6) is not shown. (B) Overall survival rate of the 29 NEMO-deficient patients after HSCT. CC1, coiled coil domain 1; CC2, coiled coil domain 2; LZ, leucine zipper domain; NUB, NEMO ubiquitin-binding domain.

cases were confirmed by a gut biopsy showing nonspecific inflammation characterized by neutrophilic infiltrates without granuloma, crypt abscesses, glandular destruction, and crypt regeneration. Four children received immunosuppressive treatment before HSCT (P1, P5, P25, P29). P6 developed secondary hemophagocytic lymphohistiocytosis concomitantly with bacterial sepsis, which resolved with recovery from infection. The following autoimmune additional features were observed: cytopenia (hemolytic autoimmune anemia for P12 and P21, and immune thrombocytopenic purpura for P28), lupus anticoagulant (asymptomatic, P22), and Hashimoto's thyroiditis (P23; Table 1).

Immunological phenotype

Immunological investigations before HSCT revealed heterogeneous abnormalities. Hypogammaglobulinemia was common among these patients (19/24). Six patients presented with hyper-IgM (P6, P9, P11, P19, P24, P25). These patients carried the Δ134-256 (P9), c.1167_1168insC (P6, P11, P24, P25), or H413P mutations (P19), leading to changes to NEMO protein domains already implicated, to various extents, in hyper-IgM phenotype.[6] Posttetanus immunization-specific Abs were below protective levels in half of those assayed (7/12), and low memory $CD27^+/CD19^+$ B-cell counts persisted in all assessed patients (7/7). CD4 or CD8 T lymphocytosis was also observed in 7 patients. Normal results were obtained in tests of lymphocyte proliferation in response to mitogens in all but 3 of the children (P4, P11, P26; 3/22). The cytotoxic activity of NK cells was poor (11/12) (Table 2).

Management before HSCT

Most children needed nutritional support before HSCT (13/18), and 5 required parenteral nutrition. Twenty-four patients received IVIG, and 22 received trimethoprim-sulfamethoxazole prophylaxis against bacterial infections. Ten patients received azithromycin or clarithromycin for prophylaxis against mycobacterial infection. All patients with mycobacterial disease received combined antimycobacterial antibiotic

Table 2. Clinical and immunological phenotype of the 29 NEMO-deficient patients before HSCT

Patient	Pyogenic bacterial infection	Fungal infection	Mycobacterial infection	Viral infection	B cells			T cells LPT to PHA	NK cells cytotoxic activity
					IgG/A/M levels	Posttetanic vaccine serology	$CD19^+$ $CD27^+$ B-cell count		
1	Recurrent	No	*M fortuitum*	No	Low	Negative	NR	Normal	NR
2	No	No	No	No	Low	NR	NR	Normal	NR
3	Recurrent	*P jirovecii*	No	CMV	Low	NR	NR	NR	NR
4	Recurrent	No	*M avium*	HSV	Normal	NR	NR	Low	NR
5	Recurrent	No	No	CMV, MCV	Low IgG/M, hyper IgA	Normal	NR	Normal	Low
6	Recurrent	*P jirovecii, Candida sp*	*M avium*	CMV	Hyper IgM	NR	NR	Normal	Low
7	Recurrent	No	No	No	Low	NR	NR	Normal	Low
8	Recurrent	*P jirovecii, C albicans*	*M kansasii*	ADV	Low IgG	Normal	NR	Normal	Low
9	Recurrent	No	*M avium*	VZV (vaccine strain)	Low IgG, hyper IgM	Negative	Low	Normal	NR
10	Recurrent	No	*M bovis*	EBV	Low IgG	NR	NR	Normal	Normal
11	Recurrent	*Metarhizium anisopliae*	No	No	Low IgG, hyper IgM	NR	Low	Low	Low
12	Recurrent	No	*M fortuitum*	No	Low	Negative	Low	Normal	Low
13	Recurrent	No	*M avium*	ADV	Hyper IgA	NR	Low	Normal	Low
14	Recurrent	No	Yes (not specified)	No	Low	NR	NR	NR	NR
15	Recurrent	*C parapsilosis*	No	CMV	Low IgG, hyper IgA	Normal	NR	Normal	NR
16	NR	*P jirovecii*	No	CMV, rotavirus	Low IgA	Normal	NR	Normal	Low
17	Recurrent	*Aspergillus niger*	*M avium, M bovis*	No	NR	Negative	NR	Normal	NR
18	Recurrent	No	*M avium*	HHV6	NR	NR	NR	NR	NR
19	Recurrent	*C parapsilosis*	*M bovis*	no	Low IgG, hyper IgM	Negative	NR	NR	NR
20	NR	NR	NR	NR	NR	NR	NR	NR	NR
21	Recurrent	No	No	EBV	Low	Normal	Low	Normal	Low
22	Recurrent	No	*M avium*	No	NR	Negative	Low	NR	NR
23	Recurrent	*P jirovecii*	No	EBV, VZV	NR	NR	NR	NR	NR
24	Recurrent	*P jirovecii*	*M avium*	Sapovirus, EBV, HHV6	Low IgG, hyper IgM	NR	NR	Normal	NR
25	Recurrent	No	No	No	Low IgG, hyper IgM	NR	NR	Normal	Low
26	One episode of sepsis	No	*M szulgai*	No	Low	NR	NR	Low	NR
27	Recurrent	*C glabrata*	No	No	Low	NR	NR	Normal	Low
28	Recurrent	*C albicans*	*M avium*	EBV, HSV	Normal	NR	NR	Normal	NR
29	Cutaneous abscess	No	No	CMV	Low IgG	Negative	Low	Normal	NR

ADV, adenovirus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV6, human herpesvirus 6; HSV, herpes simplex virus; LPT, lymphocyte proliferation test; MCV, *Molluscum contagiosum* virus; NR, not reported; *P jirovecii*, *Pneumocystis jirovecii*; VZV, varicella zoster virus.

therapy, and 4 of them received interferon (IFN)-γ (P8, P13, P18, P22). Ten children were treated with antiviral drugs. Two patients were receiving cyclosporin A and corticosteroids for suspected maternofetal graft-versus-host disease (GVHD) during the neonatal period (P25, P27). Four patients received immunosuppressive drugs to treat colitis (corticosteroids and infliximab for P1, azathioprine and sulfalazine for P5, and infliximab for P29), and P28 was treated with corticosteroids, cyclosporin A, rituximab, and thalidomide for refractory immune thrombocytopenic purpura.

HSCT donor and conditioning regimens

Most of the HSC donors were unrelated donors (UDs; 20/29; 68.9%). Twelve were HLA-matched unrelated donors (MUDs), and 8 were mismatched unrelated donors. Seven donors were matched sibling donors (MSDs), including 5 sisters, 3 of whom carried *IKBKG* mutation (P5-P7; Table 3). Two donors had symptoms (IP for P6's donor; recurrent mouth ulcers, uveitis, and erythema nodosum for P7's donor). P28 and P29's donors were their mother, haploidentical carriers (T-depleted graft for P28 and T-replete graft for P29). The sources of HSCs used were bone marrow (n = 13), cord blood (n = 6), peripheral blood SC (n = 3), and not specified (n = 5 patients). The conditioning regimens (supplemental Data) for the first HSCT were mostly classical myeloablative regimens (15/28). A reduced intensity conditioning (RIC) regimen was used in 13 children (Table 3). Eight cases of severe conditioning toxicity were reported: tubulopathy (P6), severe venoocclusive disease (P7), severe hypotension during antithymocyte globulin infusion followed by fatal venoocclusive disease (P8), profuse diarrhea (P9), fatal intracranial bleeding (P18), *P aeruginosa* septicemia (P24), hepatic toxicity after methotrexate infusion (P27), and severe mucositis

Table 3. HSCT of the 29 NEMO-deficient patients

Patient	Age at HSCT (y)	HLA match	Source	MNC ($\times 10^8$ cells/kg)	Conditioning regimen	Day ANC>500	GVHD prophylaxis	Donor chimerism (%)
1	0.41	MSD	PSC	6.2	Flu-Bu-rATG	17	NR	19
1bis	1.25	MSD†	PSC	6	Flu-Mel-Alz	16	NR	98
2	0.33	MSD	BM	4.03	Flu-Bu-rATG	19	CsA-MMF	85
3	0.41	MSD	BM	NR	Flu-TBI	NR	NR	86
4	5.4	MSD	NR	NR	Bu-Flu-rATG	NR	Csa-CST	100
5	5.41	MSD*	BM/CB	3.63	Bu-Cy	14	CST-CsA	98
6	6.33	MSD*	BM/CB	NR	Flu-Bu-Alz	29	CsA	99
7	0.75	MSD*	BM	NR	Bu-Cy- rATG	NR	CST-CsA	100
8	1.41	MUD	BM	NR	Bu-Cy- rATG	None	NR	NR
9	3.41	MUD	BM	0.25	Bu-Cy- rATG	None	None	65
9bis	3.58	MUD†	PSC	0.1	Flu	12	None	100
10	4.9	MUD	BM	5.9	Flu-Mel-rATG	10	Tacro-MMF-CST	100
11	3.3	MUD	BM	6.12	Bu-Cy-eATG	9	CST-CsA	100
12	7.75	MUD	BM	7.2	Bu-Cy- rATG	11	CST-CsA	60
12bis	12.5	MUD†	PSC	NR	No	NR	No	53
13	6	MUD	BM	6.6	Flu-Mel-rATG	14	CsA-MMF	100
14	8	MUD	PSC	19.2	Flu-Mel-Alz	NR	NR	82
15	2.4	MUD	CB	NR	Bu-Cy- rATG	15	CsA-MMF	100
16	2.1	MUD	CB	NR	Bu-Cy-eATG	NR	CsA-MMF	100
17	12.25	MUD	NR	NR	Flu-Bu-rATG	NR	NR	100
18	NR	MUD	NR	NR	Bu-Cy	NR	NR	NR
19	2.25	MUD	NR	NR	NR	NR	Yes (unspecified)	80
19bis	2.5	MUD†	PSC	NR	NR	NR	No	NR
20	1	MUD 9/10	NR	NR	Bu-Flu-rATG	NR	Tacro-MTX	NR
21	13.5	MUD 7/8	BM	3.33	Bu-Cy-eATG	11	CST-CsA	100
22	18.83	MUD 7/8	BM	3.94	Bu-Cy-eATG	14	CST-CsA	100
23	3.8	MUD 5/6	CB	6.9	Bu-Cy-eATG	NR	CsA-MMF	100
24	4.5	MUD 8/10	BM	5.9	Flu-Bu-Alz	15	CsA-MMF	100
25	0.62	MUD 7/10	CB	1.19	Bu-Cy-eATG	20	CST-CsA	100
26	2.16	MUD 5/8	CB	0.85	Flu-Mel-rATG	13	Tacro-then CsA	30
27	3	MUD 2/6	CB	0.6	Flu-Mel-rATG	19	MTX J1/3-tacro	100
28	13.83	Haplo id*	PSC	4	Flu-Mel-rATG	10	CST-CsA	NR
29	0.9	Haplo id*	BM	13	Flu-Bu-Alz	37	Cy-CsA-MMF	100

The "bis" for patients 1, 9, 12 and 19 corresponds to the second HSCT. MUD mismatches are specified.

Alz, alemtuzumab; ANC >500, absolute neutrophil count >500/mm^3; ATG, antithymocyte globulin (e, horse; r, rabbit); BM, bone marrow stem cell graft; Bu, busulfan; CB, cord blood stem cell graft; CsA, cyclosporin A; CST, corticosteroids; Cy, cyclophosphamide; Flu, fludarabine; haplo-id, haplo-identical donor; Mel, melphalan; MMF, mycophenolate mofetil; MNC, mononuclear cell count; MTX, methotrexate; PSC, peripheral blood stem cells; Tacro, tacrolimus; TBI, total body irradiation; y, years.

*Carrier sister or mother.

†Same donor as for the first HSCT.

(P7, 13). Almost all patients with severe conditioning regimen adverse effects carried mutations affecting the ZF domain (7/8), resulting in a higher frequency of conditioning regimen toxicity in patients with such mutations than in patients with mutations affecting other domains (39% vs 9%; $P = .1$). Four children underwent a second HSCT because of graft failure (P1, P9, P12, P19). The second HSCT was performed with peripheral blood SC from the same donor as for the first HSCT in all cases, using RIC for P1, fludarabine alone for P9, and no conditioning for P12 (data not available for P19; Table 3).

HSCT engraftment

The median count of first graft mononuclear cells was 4.96×10^8/kg (range, 0.25-19.2 $\times 10^8$/kg). Donor leukocyte chimerism after first HSCT mostly exceeded 90% (20/23). Chimerism could not be evaluated in 4 children who died less than 2 months after HSCT (P8, P18, P19, P26), and data were not available for 2 patients. The median time between the first graft and the attainment of an absolute neutrophil count above 500/mm^3 was 14 days (range, 9-37 days), excluding the cases of early death (P8 died on day 6) and early graft failure (P9). Among the 4 patients who suffered graft rejection, 2 patients required an early boost infusion of CD34$^+$ cells less than 3 months after the first HSCT (P9, P19); they both died after the second HSCT because of respiratory viral infection (P9, partial lymphoid engraftment) or septic shock (P19). The other 2 children, P1 and P12, received boosts 10 and 57 months after the first HSCT, respectively. The second HSCT was successful in both cases. The type of conditioning regimen did not influence graft failure occurrence (7.7% among RIC conditioned patients vs 13.3% among classical myeloablative regimens conditioned children group; $P = .55$; data not available for P19). All patients received post-HSCT GVHD prophylaxis except P9, who suffered graft failure, and P28, who received a T-depleted haplo-identical graft. The GVHD prophylaxis regimens consisted of corticosteroids and cyclosporin A (9/20) or mycophenolate mofetil and cyclosporin A (7/20). P29 received cyclophosphamide after his T-replete haploidentical graft. Acute GVHD occurred in half of the patients (13/27), but had no effect on survival rate (supplemental Figure 1A). Most cases of involved the skin (8/10), with progression to chronic skin GVHD in 2 patients, as well as the gut (3/9) and liver (1/9). Most cases were unipolar (9/11) and of low grade (grade I or II in 7 of 11 specified cases; Table 4).

Outcome after HSCT

Median age at the time of first HSCT was 3.35 years (range, 4 months-18.8 years). The overall survival rate was 74% at 108 months after transplantation (Figure 2B), with a median follow-up of 57 months in

Table 4. HSCT outcome of the 29 NEMO-deficient patients

Patient	GVHD	Symptoms after HSCT	Status	Cause of death
1	Yes	Graft failure after first HSCT	Alive	
1bis	No	Partial cutaneous improvement, colitis, brain pseudotumor		
2	No	Warts	Alive	
3	Yes	Skin rash	Alive	
4	Yes	None	Alive	
5	Yes	Colitis, CMV reactivation	Alive	
6	No	VZV encephalitis and bacterial infections	Alive	
7	No	Severe colitis	Alive	
8	No	VOD	Dead	VOD
9	No	Graft failure after first HSCT	Dead	ARDS (parainfluenza virus infection)
9bis	No	Bacterial and viral infections, colitis		
10	Yes	Multiple papillomas virus	Alive	
11	Yes	Bacterial and viral infections	Alive	
12	Yes	Graft failure after first HSCT	Alive	
12bis	No	CMV, EBV, and ADV reactivation		
13	No	Warts, VZV encephalopathy	Alive	
14	No	Colitis, chronic lung disease, arthropathy, arteriopathy*	Alive	
15	No	None	Alive	
16	No	Allergies to sulfamides and walnuts	Alive	
17	NR	BK virus cystitis	Alive	
18	NR	Intracranial bleeding	Dead	Intracranial bleeding
19	No	Graft failure after first HSCT	Dead	Septic shock
19bis	No	Bacterial septic shock		
20	No	Transient idiopathic intracranial hypertension	Alive	
21	No	EBV viremia	Alive	
22	No	Mycobacterial disseminated infection, HSV pneumonitis	Dead	Disseminated mycobacterial infection
23	Yes	Bacterial infection	Alive	
24	Yes	Colitis, *Pseudomonas* infection	Alive	
25	No	Severe RSV infection 1 y post HSCT	Alive	
26	Yes	Bacterial septic shock	Dead	Septic shock
27	Yes	None	Alive	
28	Yes	Bacterial septic shock	Dead	Septic shock
29	Yes	CMV reactivation, colitis	Alive	

The "bis" for patients 1, 9, 12 and 19 corresponds to the second HSCT.
ARDS, acute respiratory distress syndrome; RSV, respiratory syncytial virus.
*Unexplained cerebral arteriopathy of the circle of Willis requiring surgical bypass.

surviving children (range, 4-108 months). Surprisingly, age at transplantation did not seem to influence the survival rate (supplemental Figure 1B). All patients receiving HSCs from a MSD were still alive at the end of follow-up, whereas only 68.5% of children undergoing HSCT with HSCs from an UD survived. Overall, there were no statistically significant differences regarding the type of HSC donor (survival rate among MSD graft: 100% at 108 months; MUD graft: 67.7% at 108 months; UCB graft: 75% at 108 months; haplo-identical graft: 50% at 5 months; $P = 0.31$; Figure 3A). There was also no difference in survival between children receiving grafts from MUDs and children receiving grafts from mismatched UDs (66.7% at 101 months and 70% at 108 months, respectively [$P = .72$]; data not shown). The choice of a classical myeloablative regimen or RIC regimen had no significant effect on survival rate after HSCT (63.5% at 101 months vs 92.3% at 108 months, respectively, $P = .14$; Figure 3B). Seven patients died after HSCT, a median of 2 months after the last HSCT (range, 0.2-12 months). Severe infection was a major cause of death in this population: 3 patients died of bacterial septic shock, 1 from a severe viral respiratory infection, and another from disseminated *M avium* complex infection (documented before HSCT). Two other children died of conditioning regimen toxicity (venoocclusive disease and intracranial bleeding; Table 4). The children who died received grafts with significantly lower mononuclear cell counts than those of the other children (2.39×10^8/kg vs 6.01×10^8/kg [$P = .02$]; data not shown). Mycobacterial infection before HSCT seemed to decrease survival rate, as only 60% of the children with mycobacterial infection were alive after HSCT vs 92.3% of those without (Figure 3C; $P = .07$). In contrast, viral and fungal infections before HSCT did not influence survival rate after HSCT (supplemental Figure 1D-E). Bacterial infections recurred after HSCT in 30% of the patients with successful engraftment (8/27), but only 2 of these patients had infections more than 6 months after HSCT (P6: pneumococcal meningitis and *H influenzae* infection; P12: sinusitis).

IBD after HSCT

Five of the 15 patients presenting with IBD before transplantation died after HSCT, 4 patients had persistent or intermittent colitis (P1, P5, P24, P29), and IBD symptoms disappeared in 6 patients. IBD occurred de novo post-HSCT in 2 previously unaffected patients without pathological evidence of gastrointestinal GVHD (P7, P14). Gut biopsies were performed in P7, highlighting cell tufting in the small intestine and multiple large ulcers in the large intestine. Moreover, gut-infiltrating immune cells were strongly stained for nuclear p65, indicating normal

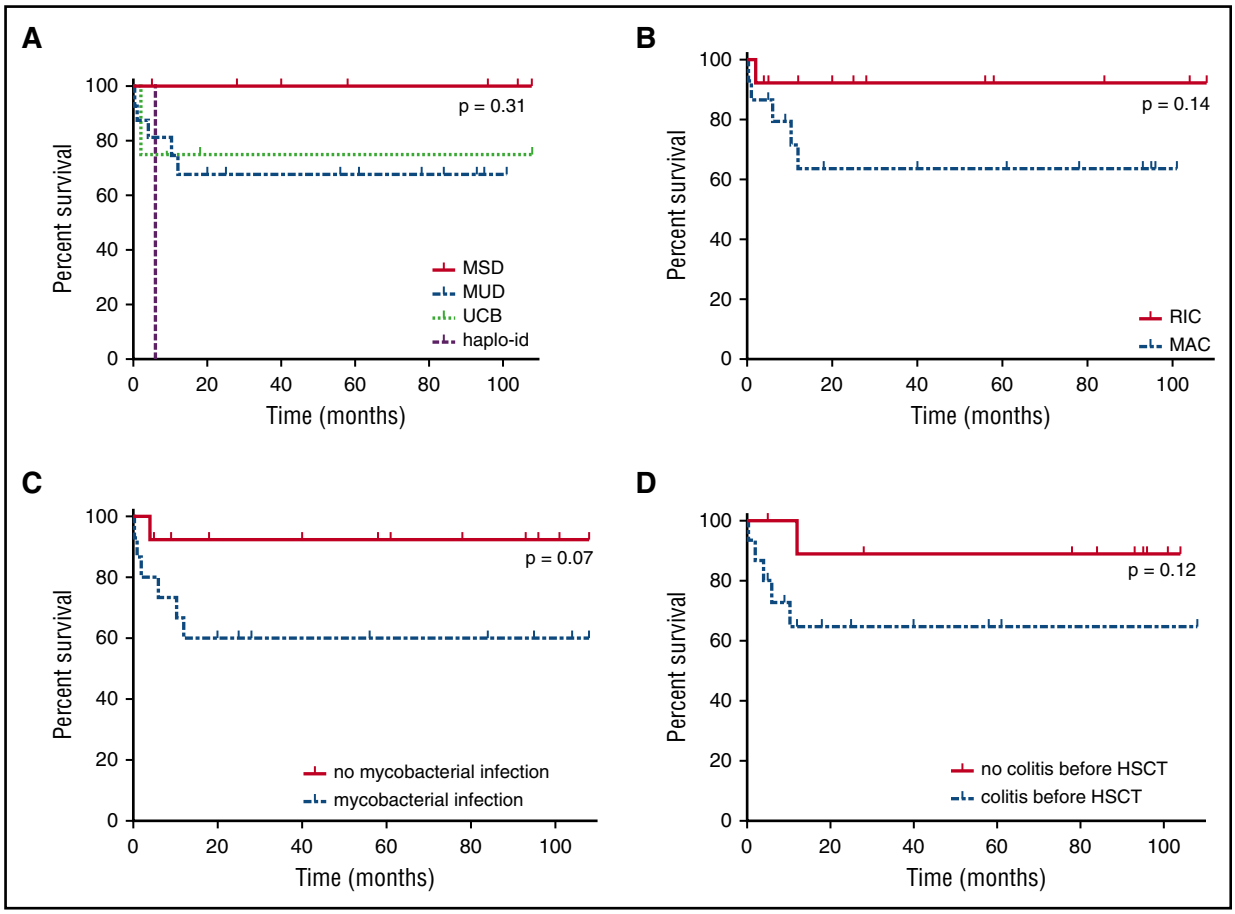

Figure 3. Survival rate of NEMO-deficient patients after HSCT. (A) Survival rate after HSCT, by donor type. MUD, blue dotted line; MSD, red full line; UCB, matched unrelated cord blood donor, green regular small dotted line; haplo-id: haploidentical donor, purple regular large dotted line. (B) Survival rate after HSCT by conditioning regimen. MAC, blue dotted line; RIC, red full line. (C) Survival rate after HSCT, by presence or absence of mycobacterial infection before HSCT. The red full line corresponds to patients with no mycobacterial infection, and the blue dotted line corresponds to patients with mycobacterial infection before HSCT. (D) Survival rate after HSCT, by presence or absence of colitis before HSCT. The red full line corresponds to patients without colitis, and the blue dotted line corresponds to patients with colitis before HSCT.

NEMO function, whereas intestinal epithelial cells had no nuclear p65, suggesting the possible involvement of an intrinsic NF-κB signaling defect in intestinal epithelial cells.[29] No biopsy results were reported for P14. P7 and P14 were both treated with corticosteroids and infliximab (with high doses of infliximab for P7). Post-HSCT outcome seemed to be worse among children suffering from IBD before HSCT, only 64.6% of whom were still alive after HSCT vs 88.9% of the patients without gastrointestinal symptoms before the graft. However, this difference was not statistically significant (Figure 3D; $P = .12$).

Immunological reconstitution after HSCT

Immunological reconstitution was assessed in the 22 surviving patients. Data were unavailable for 7 patients (P1, P3, P4, P17, P20, P24, P27) or cannot be assessed (P29, last follow-up only few months after graft). NEMO protein levels in PBMCs were restored to normal (2/2), as was the production of cytokines by PBMCs in response to TLR ligands (3/3) and NK cell cytotoxicity (3/3). Complete B-cell reconstitution (B-cell subsets and Ig levels) occurred in almost all children (11/13). B-cell proliferation in response to CD40 stimulation recovered after HSCT (3/3). Only 2 patients were still on IVIG 95 and 37 months after HSCT (P12, P14), 1 of whom was treated with immunosuppressive drugs for a recurrence of severe inflammatory colitis (P14, corticosteroids and infliximab). Both have partial chimerism on B cells.

Discussion

We report HSCT outcome for an international cohort of 29 NEMO-deficient patients with various forms of EDA-ID, representing the largest cohort assembled to date. Because data were retrospectively collected, some patient characteristics and complications may be underestimated, and the follow-up of the children may be heterogeneous, leading to missing data. In this cohort, 59% of the patients had mutations affecting the ZF domain of NEMO. This domain is a highly conserved structural motif involved in the recognition of upstream regulators of the IKK complex; it is essential for NF-κB activation in various immune cell types and in ectoderm-derived cells.[48,49] Mutations affecting the ZF domain have been associated with a more severe EDA-ID phenotype, including a higher frequency of EDA, osteopetrosis, IBD, pyogenic bacterial infections, and mycobacterial susceptibility.[5,6] As HSCT is generally considered only for the sickest patients, this may account for the high proportion of patients with ZF domain mutations in this cohort. Nevertheless, a comparison of this cohort with the patients reported by Hanson et al revealed a similar proportion of patients with ZF domain mutations, but a significantly higher frequency of inflammatory symptoms, IBD, and chronic viral infections among our patients (supplemental Table 1). These

observations suggest that the *IKBKG* genotype is important, but that other individual or environmental cofactors may modify individual inflammatory and infectious complications. The *IKBKG* genotype may be considered when discussing the case for HSCT in NEMO-deficient patients, but is not sufficient to be an indication for HSCT in itself.

A previous report suggested there were intrinsic difficulties with HSCT engraftment in patients with EDA-ID.[50] We did not find this to be the case in this study, for which the global engraftment rate was 93%. Five patients received grafts from a carrier sister or mother. One of these patients had recurrent infections resulting from encapsulated bacteria after HSCT (P6), 2 had persistent intermittent colitis (P5, P29), and the third developed de novo colitis (P7). In contrast, no bacterial infections occurred in the 4 children receiving HSCs from MSDs without *IKBKG* mutations; only 1 of these patients had persistent colitis (P1). This suggests HSCs from a carrier sister or mother may only partially correct the susceptibility to bacterial infections, possibly as a result of random X-linked *IKBKG* inactivation in some types of leukocytes, leading to a deficiency in half the donor immune cells.[45-47] Moreover, X inactivation may be skewed in the leukocytes of the *IKBKG* mutation carriers, potentially amplifying the deficiency. Consistent with this hypothesis, skewed X inactivation favored the mutant allele in the carrier sister acting as the donor for P7 (only 26% PBMCs expressed the wild-type allele), and this skewed X inactivation was more pronounced in the recipient, P7, in whom only 10% of PBMCs expressed the wild-type allele.[29] We report only 4 cases, too small a number of patients for any firm conclusions to be drawn. However, it would be interesting to study X inactivation in the PBMCs of the carrier sisters acting as donors and in the recipients, to determine whether this process affects HSCT outcome (Bustamante et al in preparation).

Global survival was 74%, a rate similar to those reported for HSCT in other non-SCID PIDs (79% at 3 years).[51] Remarkably, all deaths occurred early, during the first year after HSCT, and most were a result of severe infections, suggesting a role of induced immunosuppression post-HSCT and infectious status before grafting. Mycobacterial infection has been shown to be associated with poor prognosis in patients not undergoing transplantation.[6,50] In this series, mycobacterial infection before HSCT seemed to be associated with greater post-HSCT mortality, although this difference was not statistically significant. This observation may reflect an inability to clear mycobacterial infections effectively before HSCT. Four of the 16 patients with mycobacterial infections before HSCT received IFN-γ therapy, including 3 patients who died after HSCT, 1 from disseminated mycobacterial disease. The number of patients treated was too small to determine whether IFN-γ therapy itself influenced the prognosis. Surprisingly, 1 patient died of fatal intracranial bleeding soon after transplantation, even though he did not have severe thrombocytopenia (platelet count remained above 20 000/mm^3). It is possible that this bleeding results from vascular instability caused by aberrant NF-κB pathways in the vascular endothelium. Indeed, fundamental studies have suggested the NF-κB pathway is involved in promoting the proliferation of endothelial progenitor cells.[52] Moreover, NF-κB signaling is essential for the TNF-α activation of endothelial cells and the resulting regulation of vascular permeability and control of inflammatory responses in endothelial cells.[53] Consistent with this hypothesis, P20 developed an unexplained cerebral arteriopathy of the circle of Willis after HSCT, and P26 had intestinal vasculitis before HSCT, suggesting the involvement of endothelial cells. These findings suggest more attention should be paid to platelet levels during HSCT for NEMO-deficient patients. It seems to be important to keep a higher threshold for platelet transfusion in NEMO-deficient patients undergoing HSCT to limit hemorrhagic complications if there are clinical, biological, and radiological elements suggesting central nervous system vasculitis.

Patients with IBD before HSCT appeared to have a poorer prognosis after HSCT, although this difference was not statistically significant. The integrity of the intestinal epithelium is altered in NEMO deficiency-related colitis, potentially favoring bacterial translocation and increasing the risk for life-threatening systemic infections. IBD persisted in 4 patients and appeared de novo in another 2, after HSCT. These observations suggest that the pathogenesis of NEMO deficiency-related colitis may involve a nonhematopoietic component, and that HSCT does not correct IBD. This may reflect the importance of the NF-κB pathway in intestinal epithelial cells (IECs) for controlling epithelial gut integrity and interactions with the mucosal immune system and gut microflora. Indeed, in a mouse model, the inhibition of *IKBKG* gene expression in IECs induces the apoptosis of colonic epithelial cells, impairs antimicrobial peptide production, and favors the translocation of bacteria into the mucosa.[54] This epithelial defect triggers a chronic inflammatory response in the colon, leading to severe chronic intestinal inflammation.[54] Similar results were obtained when the production of other proteins of the IKK complex was disrupted, highlighting the key role of the NF-κB pathway in gut homeostasis.[54,55] HSCT corrects the NEMO defect in immune cells (ie, cells derived from hematopoietic stem cells), but not in IECs, potentially accounting for the persistence of colitis after HSCT in some patients, and consistent with the late onset of colitis, after and despite HSCT. The impairment of antimicrobial peptide production in NEMO-deficient IECs may also account for the persistence of bacterial infections after HSCT in some patients. Moreover, some authors have suggested that immune reconstitution after engraftment may favor IBD because altered NEMO-deficient IECs may stimulate donor immune cells, which are responsive to TLR ligands.[29] This might lead to the secretion of large amounts of TNF-α by the donor immune cells, worsening IBD. This hypothesis is supported by the observed beneficial effects of anti-TNF-α therapy in patients with postgraft colitis (P1, P7, P14, P29).

In conclusion, we show here that the overall survival of NEMO-deficient patients after HSCT is similar to that of patients with other non-SCID PIDs; pre-HSCT mycobacterial infection and IBD seem to be associated with a poorer post-HSCT prognosis; and HSCT does not appear to cure IBD. Studies of a larger cohort of patients are required to confirm these results and to compare the outcomes of patients undergoing and not undergoing HSCT, to establish consensual guidelines for the management of NEMO-deficient patients. Indeed, the decision as to whether NEMO-deficient patients should undergo HSCT is a major clinical challenge. Our results suggest the most life-threatening clinical manifestations, at least, can be cured by HSCT.

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Authorship

Contribution: C.M., K.I., J.S.O., and C.P. collected, analyzed, and interpreted data and wrote the paper; C.I., A.J.M., Z.Y.K., S.D.G., T.K., R.N., E.I., I.P., S.S., J.P.S., T.O., E.C., S.C., A.O.-V., W.Q., M.Y.-N., S.E.P., A.K., N.C., S.J., J.B., A.R.G., M.A., T.G., P.V., D.M., B.N., S.B., S.M.H., G.U., B.C.-C., A.C.-N., S.E., R.D., S.Y.P., E.W.G., C.P., A.J., and T.V. provided clinical information from patients; C.M., J.R., A.P., J.B., J.-L.C., and C.P. analyzed genetic and immunological data; K.I., J.-L.C., J.S.O., and C.P. provided essential clinical information from patients, interpreted data, and wrote the paper; and C.P. designed the research, interpreted data, and wrote the paper.

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References

1. Döffinger R, Smahi A, Bessia C, et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nat Genet*. 2001;27(3):277-285.
2. Zonana J, Elder ME, Schneider LC, et al. A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). *Am J Hum Genet*. 2000;67(6):1555-1562.
3. Zhang Q, Lenardo MJ, Baltimore D. 30 years of NF-κB: a blossoming of relevance to human pathobiology. *Cell*. 2017;168(1-2):37-57.
4. Smahi A, Courtois G, Vabres P, et al; The International Incontinentia Pigmenti (IP) Consortium. Genomic rearrangement in NEMO impairs NF-kappaB activation and is a cause of incontinentia pigmenti. *Nature*. 2000;405(6785):466-472.
5. Puel A, Picard C, Ku CL, Smahi A, Casanova JL. Inherited disorders of NF-kappaB-mediated immunity in man. *Curr Opin Immunol*. 2004;16(1):34-41.
6. Hanson EP, Monaco-Shawver L, Solt LA, et al. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. *J Allergy Clin Immunol*. 2008;122(6):1169-1177.
7. Picard C, Casanova JL, Puel A. Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. *Clin Microbiol Rev*. 2011;24(3):490-497.
8. Mooster JL, Cancrini C, Simonetti A, et al. Immune deficiency caused by impaired expression of nuclear factor-kappaB essential modifier (NEMO) because of a mutation in the 5′ untranslated region of the NEMO gene. *J Allergy Clin Immunol*. 2010;126(1):127-132.
9. Abbott JK, Quinones RR, de la Morena MT, Gelfand EW. Successful hematopoietic cell transplantation in patients with unique NF-κB essential modulator (NEMO) mutations. *Bone Marrow Transplant*. 2014;49(11):1446-1447.
10. Salt BH, Niemela JE, Pandey R, et al. IKBKG (nuclear factor-kappa B essential modulator) mutation can be associated with opportunistic infection without impairing Toll-like receptor function. *J Allergy Clin Immunol*. 2008;121(4):976-982.
11. Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W. Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. *Nat Immunol*. 2001;2(3):223-228.
12. Orange JS, Brodeur SR, Jain A, et al. Deficient natural killer cell cytotoxicity in patients with IKK-gamma/NEMO mutations. *J Clin Invest*. 2002;109(11):1501-1509.
13. Picard C, Al-Herz W, Bousfiha A, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. *J Clin Immunol*. 2015;35(8):696-726.
14. Dupuis-Girod S, Corradini N, Hadj-Rabia S, et al. Osteopetrosis, lymphedema, anhidrotic ectodermal dysplasia, and immunodeficiency in a boy and incontinentia pigmenti in his mother. *Pediatrics*. 2002;109(6):e97.
15. Tono C, Takahashi Y, Terui K, et al. Correction of immunodeficiency associated with NEMO mutation by umbilical cord blood transplantation using a reduced-intensity conditioning regimen. *Bone Marrow Transplant*. 2007;39(12):801-804.
16. Fish JD, Duerst RE, Gelfand EW, Orange JS, Bunin N. Challenges in the use of allogeneic hematopoietic SCT for ectodermal dysplasia with immune deficiency. *Bone Marrow Transplant*. 2009;43(3):217-221.
17. Minakawa S, Takeda H, Nakano H, et al. Successful umbilical cord blood transplantation for intractable eczematous eruption in hypohidrotic ectodermal dysplasia with immunodeficiency. *Clin Exp Dermatol*. 2009;34(7):e441-e442.
18. Mancini AJ, Lawley LP, Uzel G. X-linked ectodermal dysplasia with immunodeficiency caused by NEMO mutation: early recognition and diagnosis. *Arch Dermatol*. 2008;144(3):342-346.
19. Keller MD, Petersen M, Ong P, et al. Hypohidrotic ectodermal dysplasia and immunodeficiency with coincident NEMO and EDA mutations. *Front Immunol*. 2011;2:61.
20. Orange JS, Levy O, Brodeur SR, et al. Human nuclear factor kappa B essential modulator mutation can result in immunodeficiency without ectodermal dysplasia. *J Allergy Clin Immunol*. 2004;114(3):650-656.
21. Pachlopnik Schmid JM, Junge SA, Hossle JP, et al. Transient hemophagocytosis with deficient cellular cytotoxicity, monoclonal immunoglobulin M gammopathy, increased T-cell numbers, and hypomorphic NEMO mutation. *Pediatrics*. 2006;117(5):e1049-e1056.
22. Imamura M, Kawai T, Okada S, et al. Disseminated BCG infection mimicking metastatic nasopharyngeal carcinoma in an immunodeficient child with a novel hypomorphic NEMO mutation. *J Clin Immunol*. 2011;31(5):802-810.
23. Kawai T, Nishikomori R, Heike T. Diagnosis and treatment in anhidrotic ectodermal dysplasia with immunodeficiency. *Allergol Int*. 2012;61(2):207-217.
24. Chandrakasan S, Bleesing J, Bellman D, Marsh RA, Davies S, Filipovich A. Hematopoietic cell transplant for patients with NEMO defect: a single center experience. *Biol Blood Marrow Transplant*. 2013;19(2):S233-S256.
25. Carlberg VM, Lofgren SM, Mann JA, et al. Hypohidrotic ectodermal dysplasia, osteopetrosis, lymphedema, and immunodeficiency in an infant with multiple opportunistic infections. *Pediatr Dermatol*. 2014;31(6):716-721.
26. Temmerman ST, Ma CA, Zhao Y, et al. Defective nuclear IKKα function in patients with ectodermal dysplasia with immune deficiency. *J Clin Invest*. 2012;122(1):315-326.
27. Devora GA, Sun L, Chen Z, et al. A novel missense mutation in the nuclear factor-κB essential modulator (NEMO) gene resulting in impaired activation of the NF-κB pathway and a unique clinical phenotype presenting as MRSA subdural empyema. *J Clin Immunol*. 2010;30(6):881-885.
28. Ramírez-Alejo N, Alcántara-Montiel JC, Yamazaki-Nakashimada M, et al. Novel hypomorphic mutation in IKBKG impairs NEMO-ubiquitylation causing ectodermal dysplasia, immunodeficiency, incontinentia pigmenti, and immune thrombocytopenic purpura. *Clin Immunol*. 2015;160(2):163-171.
29. Klemann C, Pannicke U, Morris-Rosendahl DJ, et al. Transplantation from a symptomatic carrier sister restores host defenses but does not prevent colitis in NEMO deficiency. *Clin Immunol*. 2016;164:52-56.
30. Khan TA, Schimke LF, Amaral EP, et al. Interferon-gamma reduces the proliferation of M. tuberculosis within macrophages from a patient with a novel hypomorphic NEMO mutation. *Pediatr Blood Cancer*. 2016;63(10):1863-1866.
31. Courtois G, Smahi A, Reichenbach J, et al. A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J Clin Invest*. 2003;112(7):1108-1115.
32. Janssen R, van Wengen A, Hoeve MA, et al. The same IkappaBalpha mutation in two related individuals leads to completely different clinical syndromes. *J Exp Med*. 2004;200(5):559-568.
33. Dupuis-Girod S, Cancrini C, Le Deist F, et al. Successful allogeneic hemopoietic stem cell transplantation in a child who had anhidrotic ectodermal dysplasia with immunodeficiency. *Pediatrics*. 2006;118(1):e205-e211.
34. McDonald DR, Mooster JL, Reddy M, Bawle E, Secord E, Geha RS. Heterozygous N-terminal deletion of IkappaBalpha results in functional nuclear factor kappaB haploinsufficiency, ectodermal dysplasia, and immune deficiency. *J Allergy Clin Immunol*. 2007;120(4):900-907.
35. Lopez-Granados E, Keenan JE, Kinney MC, et al. A novel mutation in NFKBIA/IKBA results in a degradation-resistant N-truncated protein and is associated with ectodermal dysplasia with immunodeficiency. *Hum Mutat*. 2008;29(6):861-868.

36. Ohnishi H, Miyata R, Suzuki T, et al. A rapid screening method to detect autosomal-dominant ectodermal dysplasia with immune deficiency syndrome. *J Allergy Clin Immunol*. 2012;129(2): 578-580.

37. Giancane G, Ferrari S, Carsetti R, Papoff P, Iacobini M, Duse M. Anhidrotic ectodermal dysplasia: a new mutation. *J Allergy Clin Immunol*. 2013;132(6):1451-1453.

38. Schimke LF, Rieber N, Rylaarsdam S, et al. A novel gain-of-function IKBA mutation underlies ectodermal dysplasia with immunodeficiency and polyendocrinopathy. *J Clin Immunol*. 2013;33(6): 1088-1099.

39. Yoshioka T, Nishikomori R, Hara J, et al. Autosomal dominant anhidrotic ectodermal dysplasia with immunodeficiency caused by a novel NFKBIA mutation, p.Ser36Tyr, presents with mild ectodermal dysplasia and non-infectious systemic inflammation. *J Clin Immunol*. 2013; 33(7):1165-1174.

40. Lee AJ, Moncada-Vélez M, Picard C, et al. Severe mycobacterial diseases in a patient with goF IκBα mutation without EDA. *J Clin Immunol*. 2016; 36(1):12-15.

41. Boisson B, Puel A, Picard C, Casanova JL. Human IκBα gain of function: a severe and syndromic immunodeficiency. *J Clin Immunol*. 2017;37(5):397-412.

42. Pai SY, Levy O, Jabara HH, et al. Allogeneic transplantation successfully corrects immune defects, but not susceptibility to colitis, in a patient with nuclear factor-kappaB essential modulator deficiency. *J Allergy Clin Immunol*. 2008;122(6): 1113-1118.

43. Permaul P, Narla A, Hornick JL, Pai SY. Allogeneic hematopoietic stem cell transplantation for X-linked ectodermal dysplasia and immunodeficiency: case report and review of outcomes. *Immunol Res*. 2009;44(1-3):89-98.

44. Marsh RA, Lucky AW, Walsh TJ, et al. Cutaneous infection with Metarhizium anisopliae in a patient with hypohidrotic ectodermal dysplasia and immune deficiency. *Pediatr Infect Dis J*. 2008; 27(3):283-284.

45. Fusco F, Pescatore A, Conte MI, et al. EDA-ID and IP, two faces of the same coin: how the same IKBKG/NEMO mutation affecting the NF-κB pathway can cause immunodeficiency and/or inflammation. *Int Rev Immunol*. 2015;34(6): 445-459.

46. Nelson DL. NEMO, NFkappaB signaling and incontinentia pigmenti. *Curr Opin Genet Dev*. 2006;16(3):282-288.

47. Ørstavik KH, Kristiansen M, Knudsen GP, et al. Novel splicing mutation in the NEMO (IKK-gamma) gene with severe immunodeficiency and heterogeneity of X-chromosome inactivation. *Am J Med Genet A*. 2006;140(1):31-39.

48. Cordier F, Vinolo E, Véron M, Delepierre M, Agou F. Solution structure of NEMO zinc finger and impact of an anhidrotic ectodermal dysplasia with immunodeficiency-related point mutation. *J Mol Biol*. 2008;377(5):1419-1432.

49. Shifera AS. The zinc finger domain of IKKγ (NEMO) protein in health and disease. *J Cell Mol Med*. 2010;14(10):2404-2414.

50. Kawai T, Nishikomori R, Izawa K, et al. Frequent somatic mosaicism of NEMO in T cells of patients with X-linked anhidrotic ectodermal dysplasia with immunodeficiency. *Blood*. 2012; 119(23):5458-5466.

51. Cavazzana M. Hematopoietic stem cell gene therapy: progress on the clinical front. *Hum Gene Ther*. 2014;25(3):165-170.

52. Li W, Wang H, Kuang CY, et al. An essential role for the Id1/PI3K/Akt/NFkB/survivin signalling pathway in promoting the proliferation of endothelial progenitor cells in vitro. *Mol Cell Biochem*. 2012;363(1-2):135-145.

53. Kempe S, Kestler H, Lasar A, Wirth T. NF-kappaB controls the global pro-inflammatory response in endothelial cells: evidence for the regulation of a pro-atherogenic program. *Nucleic Acids Res*. 2005;33(16):5308-5319.

54. Nenci A, Becker C, Wullaert A, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature*. 2007;446(7135):557-561.

55. Zaph C, Troy AE, Taylor BC, et al. Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. *Nature*. 2007;446(7135): 552-556.