

## Letter to the Editor

**Outcome of patients with NEMO deficiency following allogeneic hematopoietic cell transplant**

To the Editor:

Hypomorphic mutations in *IKBKG* gene lead to nuclear factor kappa B essential modulator (NEMO) deficiency; it is characterized by varying degrees of ectodermal dysplasia (ED) and immunodeficiency.<sup>1</sup> The immune defect in these patients leads to severe pyogenic bacterial infections, increased susceptibility to *Pneumocystis jirovecii* pneumonia, DNA viral infections, and atypical mycobacteria infections.<sup>1</sup> Attempts to correct the underlying immune defect with hematopoietic cell transplant (HCT) have resulted in variable outcomes.<sup>2-6</sup> The reported barriers for successful HCT in these patients include increased hepatotoxicity, potential disease-intrinsic barriers to engraftment with resulting poor engraftment,<sup>3</sup> and worsening of disease manifestations, especially colitis.<sup>2,3</sup> However, many of these observations were based on case reports with varying conditioning regimens, stem cell sources, and posttransplant management, thus precluding any definite conclusions.

We present a single-center experience of allogeneic HCT in 4 patients with NEMO deficiency who underwent unrelated bone marrow transplant with uniform myeloablative conditioning and supportive care (Table I). The details of their clinical presentation and underlying *IKBKG* mutation are available in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) (Table E1). All patients tolerated the myeloablative preparative regimen without sinusoidal obstruction syndrome or pulmonary hemorrhage. Neutrophil engraftment occurred in all patients between days +9 and +14 post-HCT. Based on our experience and other reports of successful engraftment,<sup>4-6</sup> there may not be a significant disease-intrinsic barrier to stem cell engraftment in this condition. Platelet engraftment ( $50 \times 10^9/L$ ) occurred from day +17 to +54 in 3 patients. In patient 4, despite good neutrophil engraftment, he had a poor platelet recovery. Peripheral blood chimerism was 100% donor at day +100 in all patients. However, in patient 2, donor chimerism decreased from 99.8% at 6 months post-HCT to 54.2% by the end of 2 years post-HCT. He received a stem cell boost because of declining donor chimerism. At last follow-up (7 years post-HCT), his whole blood donor chimerism was 53% and T-cell and B-cell chimerism was 71.4% and 64.1%, respectively.

Acute graft-versus-host disease (GvHD) of the skin occurred in 2 patients, and limited sclerodermatous chronic GvHD in another patient. In all, acute and chronic GvHD responded to medical management. The diagnosis of cutaneous GvHD in patients with NEMO deficiency can be challenging because the clinical and histological findings of both acute and chronic cutaneous GvHD and cutaneous manifestations of NEMO deficiency overlap. Histological findings of interface dermatitis with basal vacuolization of the dermal-epidermal junction, dyskeratotic keratinocytes, and a superficial lymphocytic infiltrate are common in both conditions. The limited data available suggest that successful HCT can result in resolution of the inflammatory eczematous/seborrheic dermatitis seen in some patients with NEMO deficiency.<sup>3</sup> Review of the literature captures 15 patients with NEMO deficiency who underwent HCT for the immune

defect; cutaneous GvHD was reported in 4 patients.<sup>6-9</sup> Of the 4 patients, 2 had acute stage 1 skin GvHD that resolved without the need for treatment with corticosteroids; in one patient GvHD responded to "standard therapy" and another patient responded well to extracorporeal photopheresis.<sup>6-9</sup> These reports suggest that the occurrence of GvHD in patients with NEMO deficiency who undergo HCT does not appear to be more frequent or more severe than seen with other HCT, and appears to respond to treatment appropriately. In our series, 2 patients had ED; post-HCT, no apparent improvement in ED was noted.

HCT in patients with NEMO deficiency has previously been reported to exacerbate pre-existing colitis or even augment a subclinical predisposition to colitis.<sup>2,3</sup> None of our patients had colitis before HCT; hence, we could not comment on the role of HCT in the resolution of colitis. However, in our cohort, after a median follow-up of 8 years, none developed new-onset colitis post-HCT, which is very reassuring.

Three of the 4 patients achieved T-cell immune reconstitution, and B-cell reconstitution was complete in 2 patients (Table II). Patient 2 developed mixed chimerism; he has normal lymphocyte subpopulations and good responses to mitogens and *Candida* and tetanus. Because of his decreasing donor chimerism, incomplete B-cell reconstitution, and breakthrough sinus infections, he continues to receive immunoglobulin replacement. Two patients with good B-cell reconstitution are currently doing well off immunoglobulin replacement therapy. In patient 4, despite maintaining 100% donor chimerism, he had poor immune reconstitution. He remained lymphopenic with decreased T- and B-cell numbers and function until his death. Natural killer cell function normalized in all surviving patients.

Two patients developed mycobacterial infections before HCT. In 1 patient, mycobacterial infection was adequately treated before HCT; post-HCT, he did not reactivate mycobacterial infection. However, patient 4 was taken to HCT with active *Mycobacterium avium* complex (MAC) disease because he had failed to respond to multidrug therapy including immune modulation with IFN- $\gamma$ , IFN- $\alpha$ , and GM-CSF. Post-HCT, he continued to have disseminated MAC infection and he died on day +367 because of refractory disseminated MAC infection, herpes simplex virus viremia, and pneumonitis. Our observation of poor immune reconstitution in a setting of active disseminated mycobacterial disease supports previous reports of similar findings in a setting of disseminated mycobacterial infection.<sup>10</sup> Disseminated mycobacterial disease in the bone marrow hamper immune reconstitution by adversely affecting early lymphoid development in the bone marrow, and later in the thymus and spleen. Hence, adequate control of mycobacterial disease before HCT or planning HCT before the development of mycobacterial disease may be critical for good HCT outcome in patients with NEMO deficiency.

In summary, we report long-term HCT outcome of 4 patients with NEMO deficiency. We conclude that HCT can be an effective modality for correction of the immunodeficiency associated with NEMO deficiency. Critical genetic and immune studies aimed at identifying patients with NEMO deficiency at risk of mycobacterial infection are needed. Once a patient with NEMO deficiency is deemed high risk for mycobacterial infection, HCT should be considered before the development of difficult to control mycobacterial disease. Also, the active

**TABLE I.** HCT characteristics and post-HCT course

| Characteristics         | Patient ID         |                          |                    |                             |
|-------------------------|--------------------|--------------------------|--------------------|-----------------------------|
|                         | 1                  | 2                        | 3                  | 4                           |
| Year of HCT             | 2006               | 2007                     | 2007               | 2007                        |
| Age at HCT              | 3 y 4 mo           | 7 y 9 mo                 | 13 y 6 mo          | 18 y 10 mo                  |
| Follow-up duration (y)  | 9                  | 8                        | 8                  | 1                           |
| Donor HLA match         | 8/8 URD            | 8/8 URD                  | 7/8 URD            | 7/8 URD                     |
| Donor age (y)/sex       | 25/F               | 38/F                     | 26/F               | 22/F                        |
| Stem cell source        | BM                 | BM                       | BM                 | BM                          |
| Conditioning            | BU/CY/ATG(Horse)   | BU/CY/ATG(Rabbit)        | BU/CY/ATG(Rabbit)  | BU/CY/ATG(Rabbit)           |
| GvHD prophylaxis        | CSA, prednisone    | CSA, prednisone          | CSA, prednisone    | CSA, prednisone             |
| SOS prophylaxis         | Ursodiol           | Ursodiol                 | Ursodiol           | Ursodiol                    |
| TNC/kg                  | $6.12 \times 10^8$ | $7.2 \times 10^8$        | $3.33 \times 10^8$ | $3.94 \times 10^8$          |
| Acute GvHD              | —                  | Grade II skin and liver  | —                  | Grade II skin               |
| Viral reactivations     | Adenovirus         | EBV, CMV, and adenovirus | EBV                | HSV                         |
| Mycobacterial Infection | —                  | —                        | —                  | Refractory disseminated MAC |
| Chronic GvHD            | Limited cutaneous  | —                        | —                  | —                           |
| Chimerism               | 100                | 53*                      | 100                | 100                         |
| Outcome                 | Alive              | Alive                    | Alive              | Died at day +367            |

Conditioning regimen: busulfan 16 mg/kg from day −10 to day −7, cyclophosphamide 200 mg/kg from day −5 to day −2, rabbit ATG or horse ATG on day −2, day −1, and day +1 and day +2.

BU/CY/ATG, Busulfan/cytoxan/antithymocytic-globulin; CSA, cyclosporine-A; F, female; HSV, herpes simplex virus; MAC, *Mycobacterium avium* complex; SOS, sinusoidal obstruction syndrome; TNC, total nucleated cells; URD, unrelated donor.

\*Received stem cell boost 5 years after the initial HCT.

**TABLE II.** Profile of immune reconstitution post-HCT

| Immune evaluation                                      | Patient ID                       |                                  |                                     |                                  |
|--------------------------------------------------------|----------------------------------|----------------------------------|-------------------------------------|----------------------------------|
|                                                        | 1                                | 2                                | 3                                   | 4*                               |
| CD3 (cells/ $\mu$ L)                                   | 2453                             | 1676                             | 1852                                | 255 (L)                          |
| CD4 (cells/ $\mu$ L)                                   | 1566                             | 908                              | 945                                 | 56 (L)                           |
| CD8 (cells/ $\mu$ L)                                   | 749                              | 709                              | 832                                 | 190 (L)                          |
| CD4/CD8 ratio                                          | 2                                | 1.3                              | 1.1                                 | 0.3 (L)                          |
| CD19 (cells/ $\mu$ L)                                  | 2705                             | 220                              | 807                                 | 0 (L)                            |
| CD 16/56 (cells/ $\mu$ L)                              | 314                              | 142                              | 115                                 | 20 (L)                           |
| CD4 naive % (CD4 <sup>+</sup> CD45RA <sup>+</sup> )    | 40.9(L)                          | 53.4                             | 51.1                                | ND                               |
| CD8 naive % (CD8 <sup>+</sup> CD45RA <sup>+</sup> )    | 69.8                             | 54.1                             | 67.5                                | ND                               |
| Mitogen                                                | Normal PHA, ConA, & PWM          | Normal PHA, ConA, & PWM          | Normal PHA, ConA, & PWM             | Decreased PHA & ConA, normal PWM |
| Antigen                                                | Normal tetanus<br>Normal Candida | Normal tetanus<br>Normal Candida | Normal tetanus<br>Decreased Candida | ND                               |
| NK lytic units (>2.6)                                  | 11.7                             | 4.1                              | 6.4                                 | 13.5                             |
| Class-switched B cells % of CD27 <sup>+</sup> B cells† | 22%                              | 24%                              | 19%                                 | ND                               |
| IgA                                                    | 123                              | 46.9(L)                          | 49.5 (L)                            | 185                              |
| IgG                                                    | 803                              | 1090                             | 602                                 | 1010                             |
| IgM                                                    | 143                              | 40 (L)                           | 39 (L)                              | 39(L)                            |
| Pneumococcal titers‡                                   | 13 of 14                         | ND                               | 5 of 14                             | ND                               |
| Tetanus titers                                         | Protective                       | ND                               | Protective                          | ND                               |
| Rubella titers                                         | Protective                       | ND                               | Protective                          | ND                               |
| IVIG/SCIG                                              | —                                | +                                | —                                   | +                                |

ConA, Concanavalin A; IVIG, intravenous immunoglobulin; (L), values below lower limit of normal for age; ND, not done or not indicated; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SCIG, subcutaneous immunoglobulin replacement.

\*Died at day +367.

†CD19<sup>+</sup>CD27<sup>+</sup>IgD<sup>−</sup>IgM<sup>−</sup> cells.

‡Indicates the number of serotypes against which there is protective titers.

mycobacterial disease might constitute a barrier to successful immune reconstitution and stem cell function. Further studies are warranted to fully understand the risk and benefits and help delineate the role of HCT in correcting the immune defect in NEMO deficiency.

Shanmuganathan Chandrakasan, MD<sup>a</sup>

Rebecca A. Marsh, MD<sup>b</sup>

Gulbu Uzel, MD<sup>c</sup>

Steven M. Holland, MD<sup>c</sup>

Kara N. Shah, MD, PhD<sup>d</sup>

Jack Bleesing, MD, PhD<sup>b</sup>

From <sup>a</sup>the Division of Bone Marrow Transplantation, Aflac Cancer and Blood Disorder Center, Department of Pediatrics, Emory University School of Medicine, Atlanta, Ga; <sup>b</sup>the Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; <sup>c</sup>the Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md; and <sup>d</sup>the Division of Dermatology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio. E-mail: [Shanmuganathan.chandrakasan@emory.edu](mailto:Shanmuganathan.chandrakasan@emory.edu).

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## CLINICAL MANIFESTATION AND PRE-HCT COURSE

Patient 1 was born to a mother with *Incontinentia pigmenti*. His course was complicated by *Klebsiella pneumoniae* sepsis and meningitis at age 6 weeks, *Pneumocystis jiroveci* pneumonia at age 3 months, and later by *Metarhizium anisopliae* leg ulcer. He had been on intravenous immunoglobulin (IVIG) replacement and antimicrobial prophylaxis since age 3 months. Later, when his teeth erupted they were noted to be conical and wide spaced. In addition, he had a skin rash and sparse hair consistent with ED. Toll-like receptor signaling showed no response through any of the Toll-like receptors tested. Later, genetic testing revealed 1167insC in the *IKBKG* gene, resulting in a premature stop codon before the zinc finger-binding domain of the protein. He was transplanted at age 3 years for underlying immune defects predisposing to severe infections highlighted above.

Patient 2 was referred at age 7 years with the presumptive diagnosis of common variable immunodeficiency. He had developed recurrent sinopulmonary infections at age 1 year. Further evaluation showed hypogammaglobulinemia with inadequate response to pneumococcal vaccine. He was started on IVIG replacement at 3 years. At the age of 4 years, he developed autoimmune hemolytic anemia requiring high-dose steroids, IVIG, and rituximab. At 5 years, he developed disseminated *Mycobacterium fortuitum* infection. He was treated with amikacin, azithromycin, and ciprofloxacin. Immune evaluation at our institution revealed a marked decrease in memory B cells (CD27<sup>+</sup>). History of nontuberculous mycobacteria with hypogammaglobulinemia prompted us to evaluate him for a NEMO defect. He was found to have a

c.761G>A *IKBKG* mutation, resulting in p.R25Q. He had no clinical evidence of ED. He was transplanted at age 7 years for correction of underlying immune defect predisposing him to disseminated mycobacterial infections.

Patient 3 was a 13-year-old boy referred for further evaluation of immune deficiency. At age 2 years, he had recurrent sinus infections. Diagnosis of common variable immunodeficiency was made on the basis of hypogammaglobulinemia with absent pneumococcal antibody titers after vaccination and autoimmune hemolytic anemia managed with high-dose steroids. He had markedly decreased memory B cells (CD27<sup>+</sup> B cells). The combination of developmental abnormalities with hypogammaglobulinemia prompted an evaluation for NEMO defect. He had no ED. *IKBKG* gene sequencing revealed a c.1219A>G, p.M407V. He was transplanted at age 13 years for underlying immune deficiency and prevent mycobacterial infection.

Patient 4 was a 17-year-old boy referred for evaluation of HCT. His older brother and 2 maternal uncles had histories of disseminated MAC infections. He had been symptomatic since age 2 years. Significant infectious complications included chronic sinusitis and *H influenzae* bacteremia at age 2 years, and recurrent MAC bacteremia and disseminated infection since age 15 years. He had mild ED. He also developed lupus-like symptoms characterized by a lupus anticoagulant, nephritis, and livido reticularis. His MAC was managed with azithromycin, rifampin, ethambutol, clofazimine and moxifloxacin, IFN- $\gamma$ , IFN- $\alpha$ , and GM-CSF with incomplete response. At the time of transplant, he had multiple MAC liver lesions. On sequencing the *IKBKG* gene, he had c.761G>A, p.E315A amino acid change. He was transplanted at age 18 years for his underlying immune defect leading to refractory mycobacterial disease.

**TABLE E1.** Genotype-phenotype characteristics for the transplanted patients with NEMO deficiency

| Characteristics                  | Patient ID |            |                  |            |
|----------------------------------|------------|------------|------------------|------------|
|                                  | 1          | 2          | 3                | 4          |
| Mutation                         | 1167insC   | R254Q      | M407V            | E315A      |
| EDA                              | Yes        | No         | No               | Yes        |
| IBD                              | No         | No         | No               | No         |
| SGA/prematurity                  | No         | No         | One of triplets* | No         |
| Viral infection                  | No         | No         | Probable         | No         |
| Pyogenic infection               | Yes        | Yes        | Yes              | Yes        |
| Mycobacterial infection          | No         | Yes        | No               | Yes        |
| Fungal infection                 | Yes        | No         | No               | No         |
| Lymphedema                       | No         | No         | No               | No         |
| Skin manifestations              | Yes        | No         | No               | Yes        |
| Neurological problems            | No         | No         | Yes              | Yes        |
| Bone defects/osteopetrosis       | No         | No         | No               | Yes        |
| Specific Ab. defect/autoimmunity | No         | Yes (AIHA) | No               | Yes (AIHA) |
| Hypo-IgG                         | Yes        | Yes        | Yes              | No         |
| Hyper-IgM                        | No         | No         | No               | No         |
| Hyper-IgA                        | No         | No         | No               | No         |
| Hyper-IgE                        | No         | No         | Yes              | No         |

Ab., Antibodies; AIHA, autoimmune hemolytic anemia; EDA, ectodermal dysplasia with anhidrosis; IBD, inflammatory bowel disease; SGA, small for gestational age.

\*Was born at 33 weeks of gestational age.