

Letter to the Editor

Successful hematopoietic stem cell transplantation for myelofibrosis in an adult with warts-hypogammaglobulinemia-immunodeficiency-myelokathexis syndrome

To the Editor:

Warts-hypogammaglobulinemia-immunodeficiency-myelokathexis (WHIM) syndrome is a rare autosomal-dominant disorder characterized by severe neutropenia, B-cell lymphopenia, hypogammaglobulinemia, and refractory *human papillomavirus* (HPV) infections. Contrasting with the neutropenia, abundant mature neutrophils with hypersegmented nuclei are retained in the bone marrow, a phenomenon named *myelokathexis*. Most cases of WHIM syndrome are caused by gain-of-function mutations in the C-X-C chemokine receptor type 4 (CXCR4). CXCR4 was first studied as a cell surface receptor for HIV. The interaction with its ligand, stromal cell-derived factor 1 or chemokine C-X-C motif ligand 12 (CXCL-12), is crucial for bone marrow homing, myelopoiesis, and lymphopoiesis. The reported mutations mostly affect the cytoplasmic C-terminal tail of the receptor, leading to increased production of inositol phosphate and prolonged ligand-stimulated release of intracellular calcium. This mechanism provides a rationale for the retention of neutrophils in the bone marrow.^{1,2}

The prognosis of patients with WHIM syndrome is hampered by pyogenic infections, the development of lymphoma, and HPV-related carcinoma, for example, cervical carcinoma and basal cell carcinoma.³ Fatal disseminated *Mycobacterium avium intracellulare* infection has also been reported.⁴ Plerixafor, a small molecule inhibitor of CXCR4 first studied as an anti-HIV drug and later used as a potent stem cell-mobilizing agent, is a potential treatment for WHIM syndrome. Interestingly, it is more effective in increasing peripheral blood lymphocyte numbers than neutrophil numbers.⁵

CXCR4 is expressed in the lung, the adrenal gland, and the salivary glands. Bone marrow endothelial cells and other stromal cells have also been shown to express both CXCR4 and its ligand. However, expression of CXCR4 is strongest on hematopoietic cells. Therefore, hematopoietic stem cell transplantation (HSCT) holds promise as a potential long-term treatment. There has been a single report in the literature of an unrelated cord blood transplantation in WHIM syndrome, however, with limited follow-up.⁶ A recent report on chromothripsis of chromosome 2 in a single hematopoietic stem cell leading to cure paves the way for gene therapy in WHIM syndrome.⁷

We report a patient with WHIM syndrome caused by a novel deletion in the C-terminal domain of CXCR4 complicated by myelofibrosis and cured by HSCT. The study was approved by the Ethics Committee of the University Hospitals Leuven; the patient gave written informed consent to report the case.

The patient presented at the age of 18 years with refractory cutaneous and genital warts and recurrent cutaneous herpes simplex virus infection. Her medical history included recurrent otitis media attributed to neutropenia. Blood analysis revealed severe neutropenia, B-cell lymphopenia, and hypogammaglobulinemia (see Table E1 in this article's Online Repository at

www.jacionline.org). Bone marrow aspiration and biopsy together with electron microscopic examination were compatible with myelokathexis showing abundant hypersegmented neutrophils (Fig E1, A) (Fig 1, A-C). Sequencing of CXCR4 revealed a de novo mutation c.956_957delCT (RefSeq NM_003467.2) resulting in a frame shift with a premature stopcodon (NP_003458.1; p.Ser319CysfsX24) in the C-terminal domain of the receptor (Fig 2).

Treatment consisted of immunoglobulin substitution, G-CSF, and prophylactic antibiotics. The HPV lesions were treated with imiquimod, laser therapy, and excision. She was immunized with Gardasil. G-CSF treatment was not successful in increasing neutrophil counts and warts persisted. Progressive pancytopenia was noticed 1.5 years after presentation. New bone marrow biopsy showed myelofibrosis grade 2/3 (Fig E1, B; Fig 1, D-G).⁸ There was no evidence of hairy cell leukemia, myelodysplasia, or chronic myeloid leukemia. There was subtle erythrophagocytosis. Bone marrow HHV-6 PCR and Parvovirus B19 PCR were negative. The patient did not carry the JAK2 V617F mutation typical of primary myelofibrosis and karyotype was normal. Because of refractory pancytopenia with transfusion dependence and due to the patient's wish for definitive cure, HSCT with peripheral blood stem cells from a 10/10 matched unrelated donor (MUD) was performed following conditioning with ATG 10 mg/kg, treosulfan 14 g/m², and Fludarabine 150 mg/m². Graft-versus-host-disease prophylaxis consisted of cyclosporine and methotrexate. The graft contained 7.5×10^6 CD34(+) cells/kg. Engraftment of neutrophils ($>1.5 \times 10^9$ /L) and platelets ($>50 \times 10^9$ /L) occurred at day 16. There was no acute or chronic graft-versus-host-disease. However, there was a flare of HPV lesions with increase in both plantar and genital warts and appearance of interdental papillomata treated with imiquimod. Finally, the lesions resolved completely at 1.5 years post-HSCT.

Bone marrow examination showed disappearance of myelofibrosis and normal hematopoiesis without features reminiscent of WHIM syndrome as early as 1 month post-HSCT (Fig E1, C). She received immunoglobulin substitution until 1 year post-HSCT. Bone marrow biopsy 1 year post-HSCT was normal (Fig 1, H and I). Chimerism was full donor at 1 year post-HSCT. Split chimerism as determined by PCR on cells sorted by fluorescence-activated cell sorting was full donor in B, T, and natural killer cells. At latest follow-up, 4 years posttransplant, chimerism is still full donor. The patient is off all treatment and mounts protective antipolysaccharide (antipneumococcal, anti-*Haemophilus influenzae*) and antiprotein antibody responses to immunization antigens (antitetanus, antipertussis). She has persistent mild CD4(+) T-cell lymphopenia and partial IgA deficiency with IgG and IgM in the lower normal range (Table E1). This incomplete immune reconstitution may well fit in the context of a MUD donor; alternatively, it may be explained by the preceding myelofibrosis although scarce data on HSCT in myelofibrosis preclude definite conclusions. Finally, the expression of CXCR4 on stromal cells may play a role.

The novel mutation reported most likely results in increased calcium fluxes, like the reported truncating mutations in the C-terminal region although we were not able to prove this.

The development of myelofibrosis in this patient is intriguing and has never been reported in WHIM syndrome.

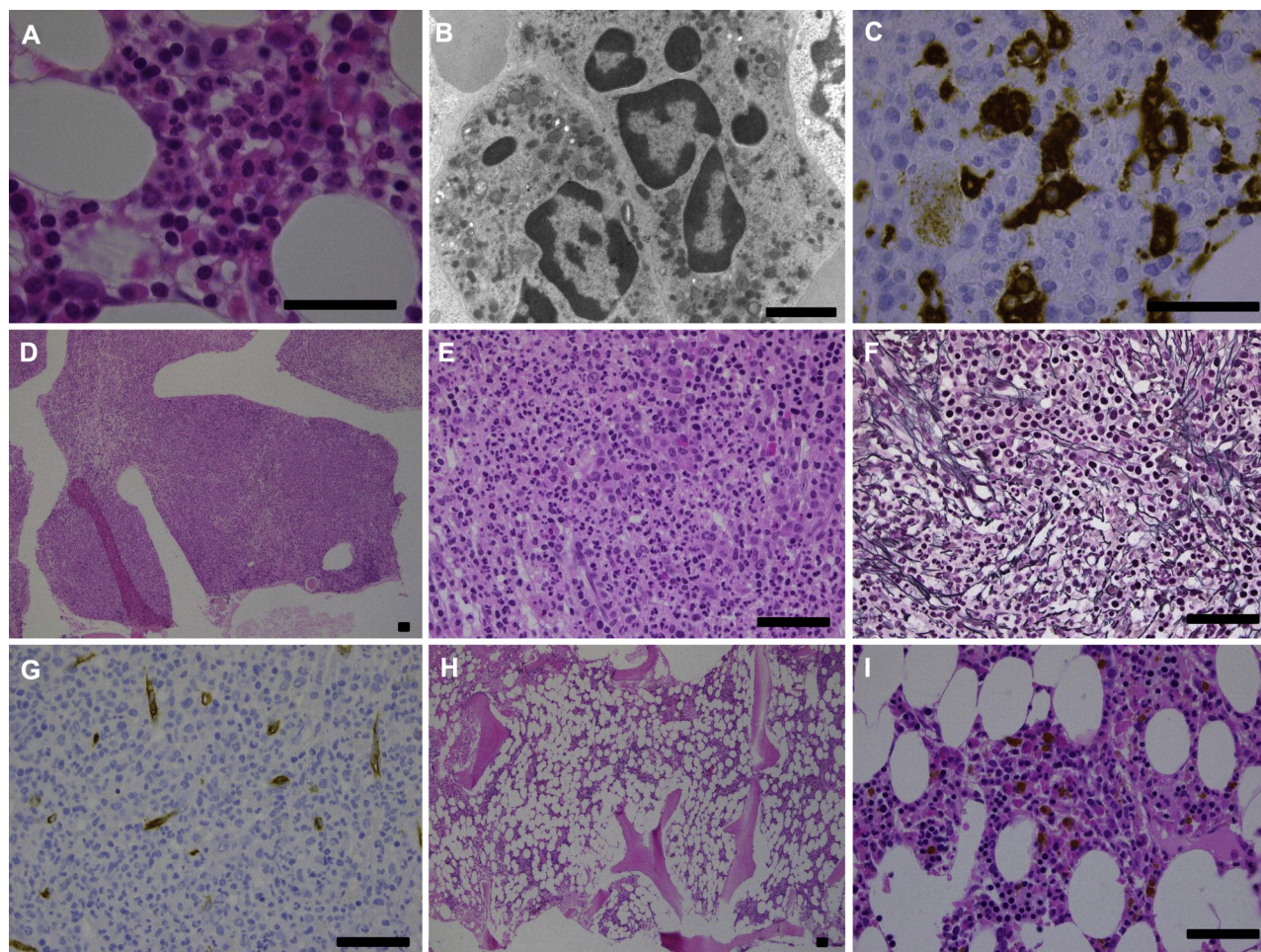


FIG 1. WHIM syndrome patient's bone marrow trephine findings at presentation, at diagnosis of myelofibrosis, and post-HSCT. **A-C**, Histological section of the bone marrow trephine (a hematoxylin-eosin [HE] stain at 630 $\times$) at diagnosis shows normal cellularity and normal distribution of the 3 hematopoietic lineages with a prominent myeloid lineage with aberrant hyperlobulation in the granulocytes, confirmed by transmission electron microscopy (Fig 1, **B**, 30,000 $\times$). A moderate degree of erythrophagocytosis is present (Fig 1, **C**, immunohistochemistry against CD68, 630 $\times$). **D-G**, Histological staining of the bone marrow trephine (Fig 1, **E** and **F**, HE stain at 50 $\times$ and 400 $\times$, respectively) in the context of pancytopenia shows an increased cellularity, the intertrabecular fat being replaced by an overrepresented myeloid series. The myeloid series is composed of a mixture of prominent neutrophils in conjunction with focal groups of myeloid precursors, contained within grade 2/3 myelofibrosis according to Tefferi et al⁸ (Fig 1, **F**, reticulin silver staining, 400 $\times$). The megakaryocytes are inconspicuous and the erythroid series are organized in erythroid islands. CD34(+) blasts are not increased (Fig 1, **G**, immunohistochemistry, 400 $\times$). **H** and **I**, Histological staining of the bone marrow biopsy 1 y post-HSCT showing normalization of cellularity and marrow composition after stem cell transplantation (Fig 1, **H** and **I**, HE 50 $\times$, HE 400 $\times$, respectively). (Scale bars **A**, **C**–**I**: 50 μ m; **B**: 1.5 μ m.)

Hypermethylation of the CXCR4 promoter with diminished CXCR4 expression was noted in primary myelofibrosis.⁹ There are some reports to show that CXCR4-CXCL-12 signaling directly activates JAK2 signaling cascades, leading to Ras/Raf-MAPK and PI3K/Akt activation and resulting in cell proliferation.¹⁰ Also, in tumor cells, increased CXCL-12/CXCR4 signaling has been shown to lead to inhibition of apoptosis via altered nuclear factor kappa B and BCL2 signaling.¹¹ Because WHIM syndrome is usually caused by CXCR4 gain-of-function mutations, this may lead to increased JAK2 signaling with increased proliferation. Most patients with primary myelofibrosis carry the activating mutation JAK2 (V617F); in this context, exactly the same pathways (MAPK-PI3K/Akt) are upregulated. Thus, we hypothesize that enhanced CXCR4/CXCL-12 signaling

as in WHIM syndrome may result in a phenocopy of the JAK2 gain-of-function mutation in primary myelofibrosis. Although the exact role of activating CXCR4 mutations in the development of myelofibrosis is at present unstudied, we speculate that fibrosis may be an additional explanation for the neutropenia/pancytopenia sometimes observed in WHIM syndrome. Because not all features of WHIM are as penetrant as neutropenia, it seems appropriate to consider the underlying WHIM syndrome in any patient presenting with primary myelofibrosis especially because standard treatment of the myelofibrosis with JAK-2 inhibitors may be hazardous.

We illustrate that WHIM syndrome can be cured by HSCT. Follow-up data 4 years post-HSCT are provided. Importantly, during the preengraftment phase and until partial lymphocyte

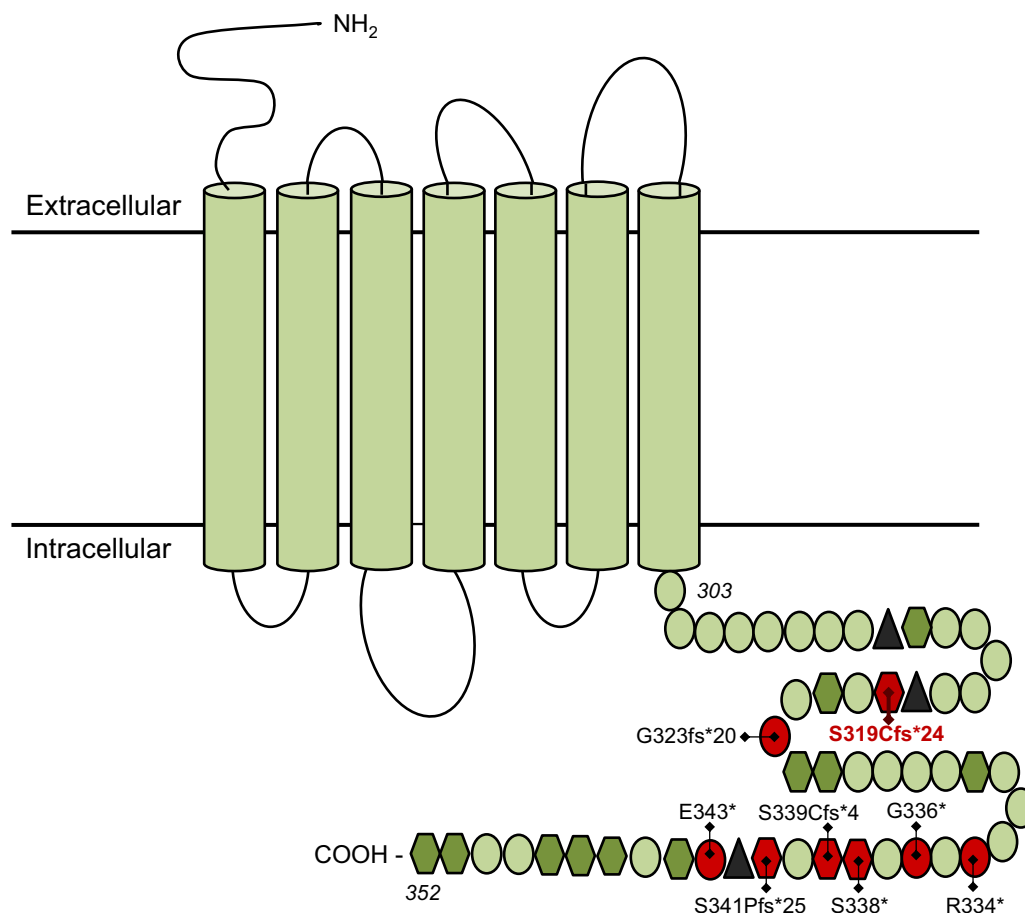


FIG 2. Indication of the mutation in the C-terminal domain of CXCR4 in relation to other mutations described in CXCR4. The mutation reported here is indicated in red. Within the C-terminal domain, red dots denote the positions of previously reported mutations. Threonine is represented by a black triangle, serine by a green hexagon. Other amino acids are represented by red dots. Figure modified from Al Ustwani et al.²

immune reconstitution was achieved, there was a flare of genital and cutaneous warts and condylomata. However, with T cells rising, the warts and condylomata subsided. Four years post-HSCT, chimerism is full donor. Moreover, bone marrow examination at 1 year post-HSCT was normocellular and free of myelofibrosis.

At the time of HSCT, plerixafor was not available, so we cannot hypothesize on the potential cure of myelofibrosis by plerixafor treatment. Given the risks of mortality and morbidity associated with HSCT and the absence of major side effects in the first trials, a trial with plerixafor is likely to be indicated. Indeed, it has been shown that daily intramuscular plerixafor injections resulted in substantial increases in both peripheral blood lymphocyte and neutrophil numbers (although even at the highest doses the neutrophil counts never normalized). However, the long-term risks and benefits of plerixafor treatment in WHIM syndrome are unknown, especially in the context of myelofibrosis. Moreover, WHIM syndrome carries a substantial risk of potentially fatal infection and of development of lymphoma and cancerous skin/genital lesions. It is not clear at this point how penetrant these features are in WHIM syndrome and to what extent long-term plerixafor therapy will be protective in this context. Clearly, the merits and risks of different therapeutic options should be

considered for the management of each individual case. The data presented in this report are encouraging for a definitive cure by HSCT.

In conclusion, we describe the development of pancytopenia due to myelofibrosis in a patient with WHIM syndrome, caused by a novel mutation affecting the C-terminal domain of the intracellular part of the CXCR4 receptor. Moreover, we report the successful outcome following HSCT from a 10/10 MUD. Four years post-HSCT she is off all treatment.

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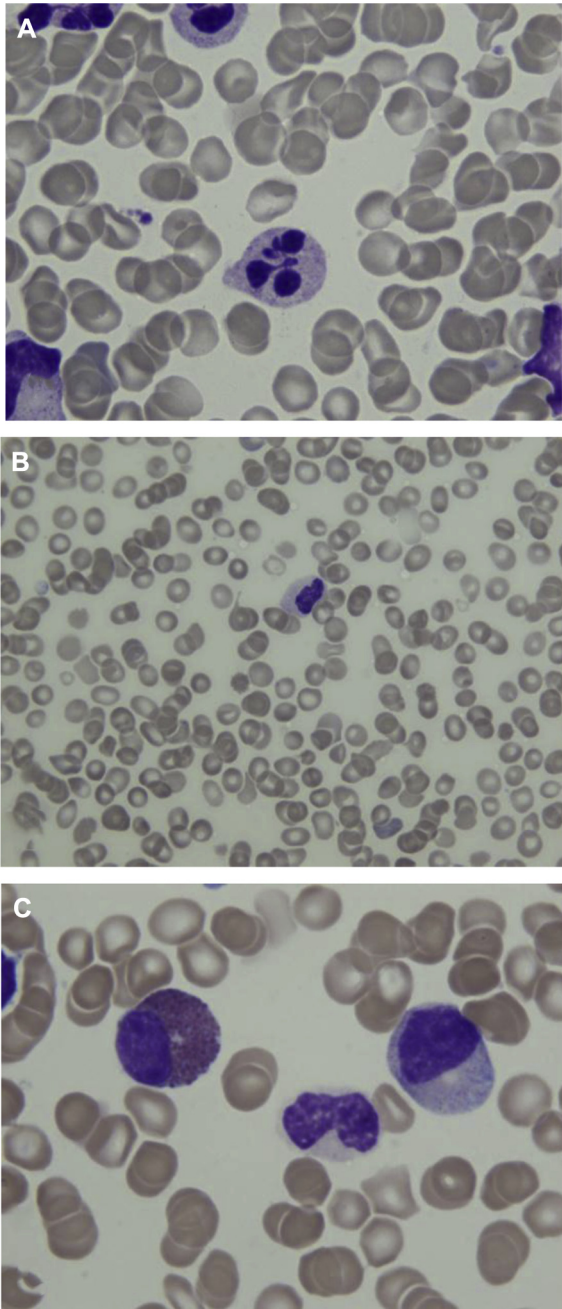


FIG E1. **A**, Bone marrow aspirate stained with May-Grünwald-Giemsa showing hypersegmented neutrophils ($\times 100$). **B**, Bone marrow aspirate showing a hypocellular bone marrow with teardrop cells consistent with myelofibrosis ($\times 50$, May-Grünwald-Giemsa stain). **C**, Bone marrow aspirate shows normal hematopoiesis at 1 month post-HSCT ($\times 100$ May-Grünwald-Giemsa stain).

TABLE E1. Peripheral blood cell count, immunoglobulin levels, and immunophenotyping at diagnosis and at latest follow-up 4 years post-HSCT

Stage	ANC ($\times 10^9/L$)	ALC ($\times 10^9/L$)	AMC ($\times 10^9/L$)	Hemoglobin (g/dL)	Platelet ($\times 10^9/L$)	IgG (g/L)	IgA (g/L)	IgM (g/L)
At diagnosis	0.59	0.6	0.3	13.3	308	5.7	0.53	0.62
At latest follow-up	2.60	1.5	0.5	13.9	201	7.68	0.62	0.55
Normal range	2.5-7.8	1.2-3.6	0.2-0.8	12-16	150-450	7.5-15.6	0.82-4.5	0.46-3.0

Stage	CD19(+) ($\times 10^9/L$)	CD3(+) ($\times 10^9/L$)	CD4(+) ($\times 10^9/L$)	CD8(+) ($\times 10^9/L$)	CD3(-)/CD56(+) and/or CD16(+) ($\times 10^9/L$)
At diagnosis	0.025	0.424	0.308	0.089	0.125
At latest follow-up	0.254	1.071	0.443	0.474	0.119
Normal range	0.082-0.476	0.798-2.823	0.338-0.079	0.219-1.124	0.075-0.838

Values in boldface are outside the normal range. Lymphocyte proliferation with PHA stimulation index 619.

CD(3+)CD27(+)CD45RA(+) T cells: 30% of CD3+ T cells.

ANC, Absolute neutrophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count.