BRIEF REPORT

Stem-Cell Gene Therapy for the Wiskott–Aldrich Syndrome

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SUMMARY

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The Wiskott–Aldrich syndrome (WAS) is an X-linked recessive primary immunode-
ficiency disorder associated with thrombocytopenia, eczema, and autoimmunity.
We treated two patients who had this disorder with a transfusion of autologous,
genetically modified hematopoietic stem cells (HSC). We found sustained expres-
sion of WAS protein expression in HSC, lymphoid and myeloid cells, and platelets
after gene therapy. T and B cells, natural killer (NK) cells, and monocytes were
functionally corrected. After treatment, the patients' clinical condition markedly
improved, with resolution of hemorrhagic diathesis, eczema, autoimmunity, and
predisposition to severe infection. Comprehensive insertion-site analysis showed
vector integration that targeted multiple genes controlling growth and immuno-
logic responses in a persistently polyclonal hematopoiesis. (Funded by Deutsche
Forschungsgemeinschaft and others; German Clinical Trials Register number,
DRKS00000330.)

THE WISKOTT–ALDRICH SYNDROME (WAS) IS A COMPLEX PRIMARY IMMU-
nodeficiency disorder that is characterized by recurrent infections, thrombo-
cytopenia, eczema, and autoimmunity[1,2] and caused by mutations in *WAS*.[3]
Its gene product, WAS protein (WASP), is a key regulator of actin polymerization in
hematopoietic cells, with domains involved in signaling, cell locomotion, and immu-
nologic-synapse formation.[4] The complex biologic features of this disease result
from multiple dysfunctions in different subgroups of leukocytes, including defec-
tive function of T and B cells, disturbed formation of the NK-cell immunologic
synapse, and impaired migratory responses in all leukocyte subgroups.[4,5] Severe
WAS leads to an early death from infection or bleeding.[6] Currently, the only curative
therapy consists of allogeneic HSC transplantation, which may be associated with
considerable risk of death or complications related to transplantation.[7]

HSC gene therapy has emerged as an innovative therapeutic strategy for various
primary immunodeficiency disorders.[8-12] WAS is a promising candidate disease for
gene-therapy approaches, since WASP expression is restricted to cells of the hemato-
poietic system, which grants a proliferative advantage over WASP-negative cells.[13,14]
Preclinical studies have shown efficient reconstitution of cellular function in vitro
and in vivo.[13] On the basis of our own preclinical in vitro and in vivo studies,[15,16]
we developed a clinical gene-transfer protocol.[13] Here we report the results in two
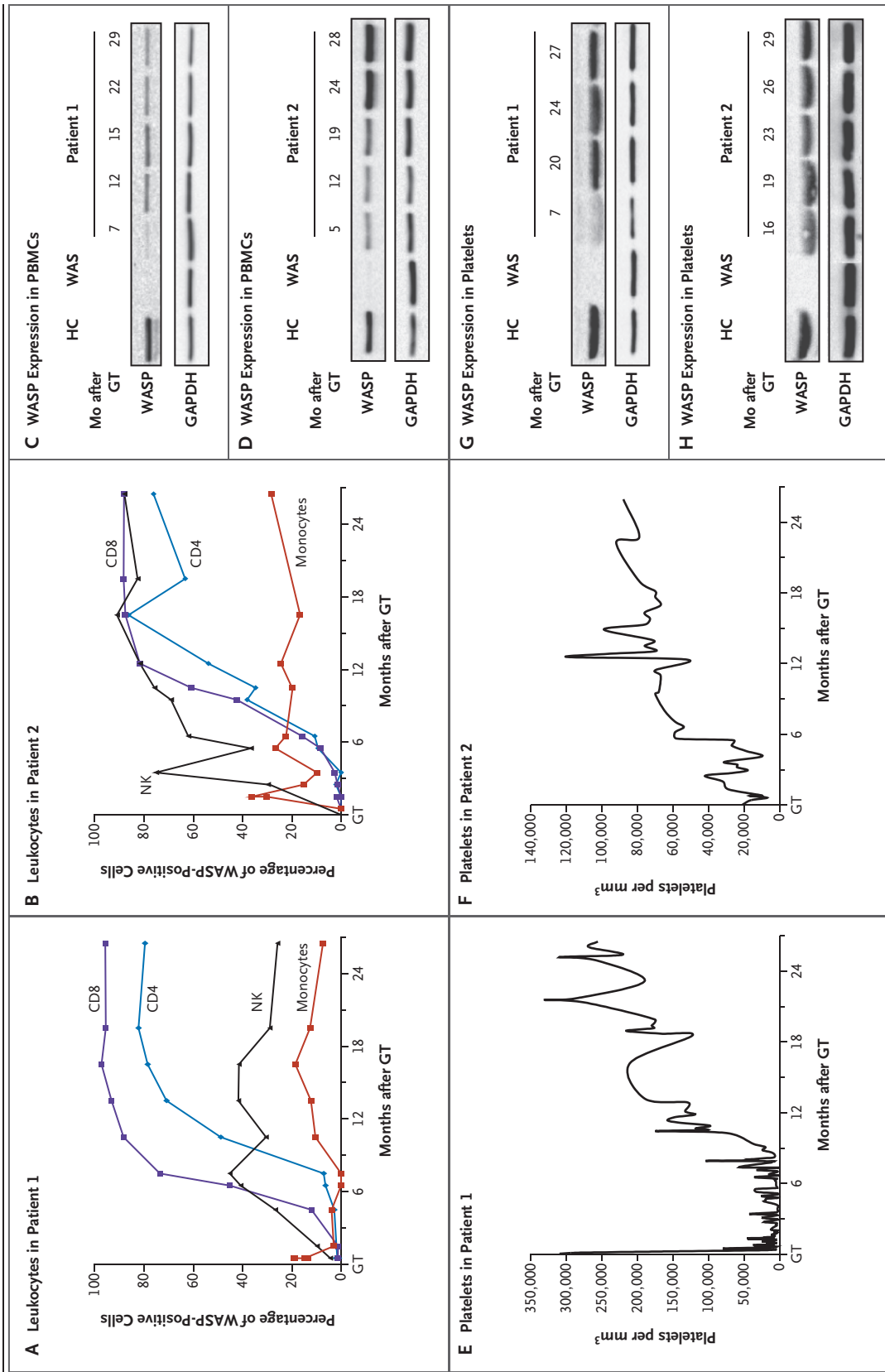young boys who were treated with HSC gene therapy.

Figure 1. Restored Expression of Wiskott–Aldrich Syndrome (WAS) Protein (WASP) after Gene Therapy.

Shown is the restoration of WASP expression after gene therapy (GT) in different subgroups of leukocytes, as determined by fluorescence-activated cell sorting, in Patient 1 (Panel A) and Patient 2 (Panel B). WASP protein expression is confirmed in both patients on Western blot analyses in peripheral-blood mononuclear cells (PBMCs) (Panels C and D) and platelet protein lysates (Panels G and H), as compared with a sample from a healthy control subject (HC) and from a patient with WAS who did not undergo gene therapy. There is also a marked increase in platelet counts in Patients 1 and 2 (Panels E and F).

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METHODS

STUDY DESIGN

The clinical gene-therapy protocol was reviewed by local and federal authorities, in accordance with current regulations of the European Community. Inclusion criteria for the study included an age of at least 12 months, a diagnosis of severe WAS (as documented by molecular and clinical phenotype), no indication of malignant disease before gene therapy, documentation of informed consent or informed assent, and isolation of a sufficient number of vital CD34+ cells from bone marrow or leukapheresis. Two 3-year-old boys who had been referred for gene therapy fulfilled the inclusion criteria, and their parents provided written informed consent.

STUDY OVERSIGHT

The trial was conducted in compliance with the Good Clinical Practice guidelines of the European Clinical Trials Directive and the provisions of the Declaration of Helsinki. A detailed description of the technical methods that were used in the study is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

RESULTS

PATIENTS

Patient 1 presented with hematoma and petechiae after birth, and within the first year of life, hemorrhagic colitis, eczema, and therapy-refractory autoimmune hemolytic anemia developed. In addition, he had autoimmune neutropenia and recurrent infections (bronchitis, otitis media, and enterocolitis). Molecular analysis revealed a splice site mutation in *WAS,* IVS6+1. No residual protein or somatic chimerism was detectable (Fig. 1 in the Supplementary Appendix).

Patient 2 presented with thrombocytopenia and hemorrhagic diathesis soon after birth. He had recurrent episodes of upper respiratory tract infection, life-threatening salmonella septicemia, and eczema that was not responsive to local and systemic therapy. Molecular studies revealed a known mutation in *WAS* (c. G257A, p. Arg86His).[3,6] No residual protein or somatic chimerism was detectable (Fig. 1 in the Supplementary Appendix).

Figure 2 (facing page). **Correction of Leukocyte Function after Gene Therapy (GT).**
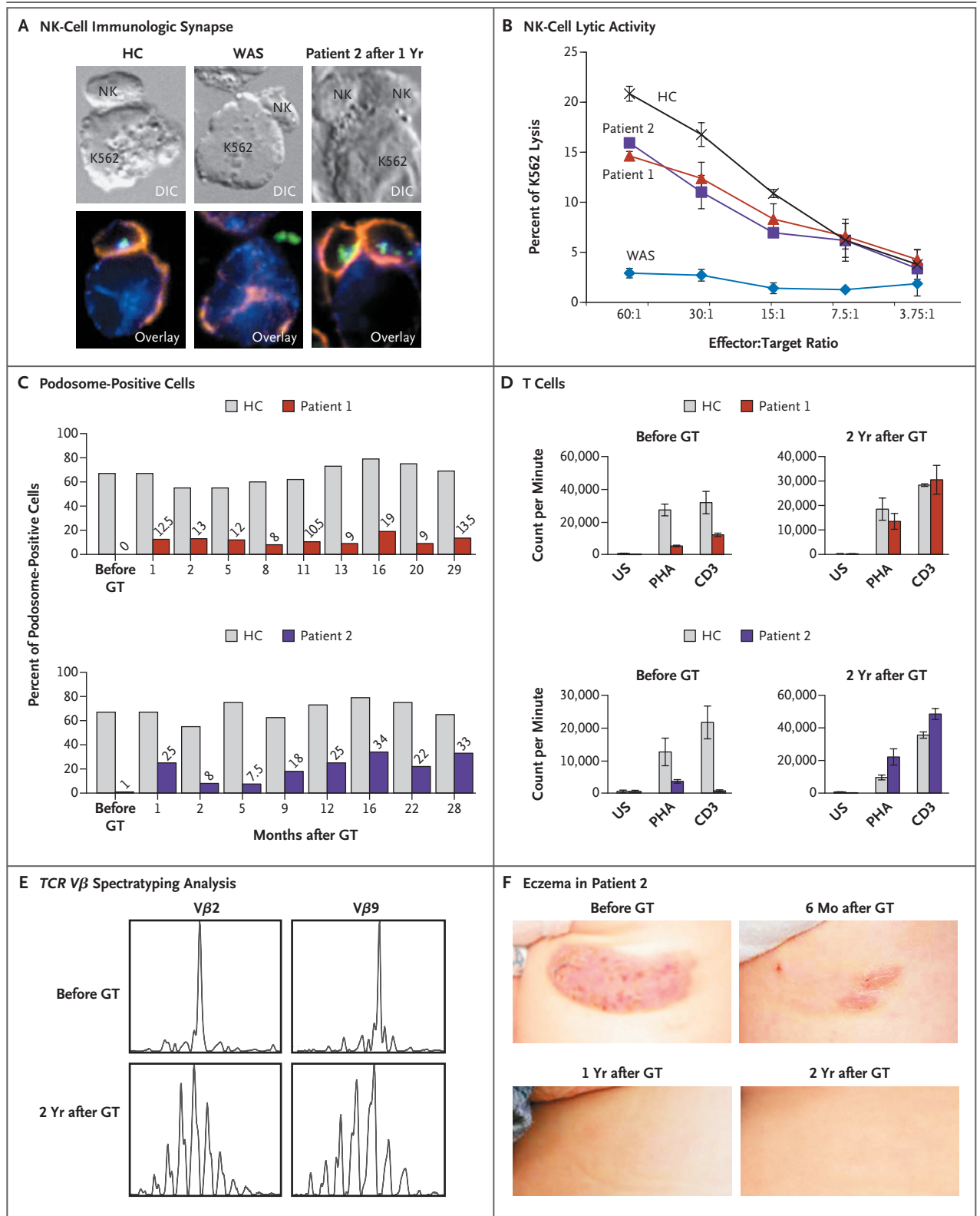
Formation of the immunologic synapse between natural killer (NK) cells and tumor cells (K562), which is impaired in patients with the Wiskott–Aldrich syndrome (WAS), is restored after GT. The localization of perforin is restored 1 year after GT in Patient 2, with an immunologic synapse similar in appearance to that in a sample from a healthy control subject (HC) and in contrast to that from a patient with WAS who did not undergo GT (Panel A). Differential interference contrast (DIC) microscopy shows normal formation of cellular conjugates. However, an overlay view of the immunofluorescence analysis shows that the perforin staining is localized close to the adjacent cells in a healthy control and in Patient 2 after therapy, in contrast to the findings in a patient with untreated WAS. NK-cell lytic activity is at least partially restored after GT in Patients 1 and 2, as compared with an untreated patient with WAS and a control subject (Panel B). Podosome formation is restored in a fraction of monocytes from both patients after GT, as confirmed at multiple time points, in comparison with values in a control sample (Panel C). T-cell proliferative responses are normalized 2 years after GT, after stimulation with phytohemagglutinin (PHA) or CD3, as compared with unstimulated (US) samples (Panel D). The skewed Vβ family repertoire for T-cell receptors (TCRs), which was observed in both Patients 1 and 2, has normalized after GT (Panel E). (For a complete set of TCR Vβ spectratyping, see Fig. 9 in the Supplementary Appendix.) The severe and therapy-refractory eczema in Patient 2 shows complete regression 2 years after GT (Panel F). The I bars indicate standard errors.

GENE TRANSFER

We collected autologous CD34+ HSCs by leukapheresis. The cells were then transduced with WASP-expressing retroviral vectors that were created with backbone vector CMMP and pseudotyped with gibbon ape leukemia virus (GALV) envelope protein; the cells were then reinfused 4 days later (Table 1 in the Supplementary Appendix). Before reinfusion of CD34+ cells, busulfan was administered at a dose of 4 mg per kilogram of body weight per day on days 3 and 2 before the procedure. Transient myelosuppression and partial alopecia were observed as therapy-associated side effects. For details regarding the vector that was used and the clinical gene-therapy protocol, see the Supplementary Appendix.

RESTORATION OF WAS EXPRESSION

After gene therapy, WASP-positive cells in various leukocyte subgroups were detected on flow cytometry (Fig. 1A and 1B). The frequency of WASP-

A NK-Cell Immunologic Synapse

HC   WAS   Patient 2 after 1 Yr

B NK-Cell Lytic Activity

C Podosome-Positive Cells

D T Cells

E *TCR Vβ* Spectratyping Analysis

F Eczema in Patient 2

positive monocytes, initially detected soon after gene therapy, has remained stable at 7 to 28%. An increased percentage of lymphoid cells were WASP-positive, consistent with a known proliferative advantage conferred by WASP expression in that lineage.[14] The level of WASP-positive NK cells ranged from 25 to 90% over time. An increase in the percentage of WASP-positive CD4+ and CD8+ T cells was seen 6 to 12 months after gene therapy and has remained stable ever since. Restoration of WASP expression in peripheral-blood mononuclear cells was confirmed on Western blot analyses (Fig. 1C and 1D).

At the level of hematopoietic progenitor cells, a stable chimerism was found in the bone marrow of both patients, with 9% of CD34+ cells expressing the *WASP* transgene in Patient 1 and 20% in Patient 2 (Fig. 2 in the Supplementary Appendix). The presence of vector-positive cells in all leukocyte subgroups at different time points after gene therapy was confirmed on quantitative polymerase-chain-reaction (PCR) assay (Fig. 3 in the Supplementary Appendix). PCR analysis of colony-forming units from both patients confirmed vector integrations in 3 of 101 colonies in Patient 1 and in 23 of 103 colonies in Patient 2 (data not shown).

An increase in platelet counts was noted, starting 6 to 9 months after gene therapy, which marked the end of episodes of clinical bleeding (Fig. 1E and 1F). On the most recent follow-up, 2.5 years after gene therapy, platelet counts were 256,000 for Patient 1 and 88,000 for Patient 2. WASP expression in platelet-rich plasma from both patients was confirmed on Western blot and flow cytometric analysis (Fig. 1G and 1H, and Fig. 4 in the Supplementary Appendix).

CORRECTION OF DEFECTIVE LEUKOCYTE FUNCTION

We next analyzed whether restored WASP expression would lead to improved leukocyte function. In NK cells, WASP deficiency leads to a decreased accumulation of F-actin and perforin at the NK-cell immunologic synapse,[17] which reduces NK-cell–mediated cytotoxicity. After gene therapy, substantial proportions of the NK-cell immunologic synapses were restored in both patients (Fig. 2A, and Fig. 5 and 6 in the Supplementary Appendix), and NK-cell lytic activity was reconstituted (Fig. 2B). WASP-deficient monocytes and dendritic cells are characterized by the inability to form podosomes.[4] The fraction of podosome-

Figure 3 (facing page). Sustained Polyclonal Hematopoietic Repopulation after Gene Therapy.

In Panels A and B, retroviral insertion sites (RISs) were detected by linear-amplification–mediated polymerase-chain-reaction (LAM-PCR) analysis of transduced hematopoietic cells at various days after gene therapy from bone marrow cells and primary blood leukocytes obtained from Patients 1 and 2. CD3 denotes T cells, G granulocytes, HC healthy control subject, IC internal vector control, and M 100-bp marker. In Panels C and D, the relative contribution of individual clones retrieved from bone marrow cells and primary blood leukocytes is estimated in sequenced polyclonal-cell samples from Patients 1 and 2. The presence of individual gene-corrected cells is given as the percentage of all LAM-PCR amplicon sequence reads identified in a particular sample. The 10 most frequently sequenced RISs are ranked from 1 to 10 according to retrieval frequency. Genes that are highlighted in gray carry a RIS that was detected at more than one time point within the 10 most frequently sequenced sites. Also listed are the numbers of other unique, mappable RISs that were sequenced less frequently than the first 10 in the respective sample analyzed, which are labeled "others."

positive monocytes after gene transfer ranged from 8 to 34% (Fig. 2C, and Fig. 7 in the Supplementary Appendix), indicating that restored WASP expression reconstituted the capacity to rearrange the cytoskeleton in myeloid cells.

T-cell abnormalities in patients with WAS include diminished T-cell proliferative responses and a skewed antigen-recognition repertoire for T-cell receptors.[4] After gene therapy, T-cell proliferative responses were reconstituted at multiple time points in both patients (Fig. 2D, and Fig. 8 in the Supplementary Appendix). *Vβ* gene spectratyping of T-cell receptors showed increasing polyclonality of *TCR* usage in both patients after gene therapy, as shown by a distribution pattern that was consistent with decreasing oligoclonal T-cell receptor *Vβ* rearrangements (Fig. 2E, and Fig. 9 in the Supplementary Appendix).

The percentage of WASP-expressing B cells increased over time after gene therapy, with proportions of WASP+CD19+ cells of 57% in Patient 1 and 69% in Patient 2 2.5 years after gene therapy. Abnormal immunoglobulin levels (increased IgM in Patient 1 and decreased IgG in Patient 2) normalized after gene therapy. Although normal immunoglobulin levels have remained stable in Patient 2, IgG levels have not remained at a protective level in Patient 1, who had undergone earlier splenectomy (Table 2 in the Supplementary Appendix). Both patients were immunized
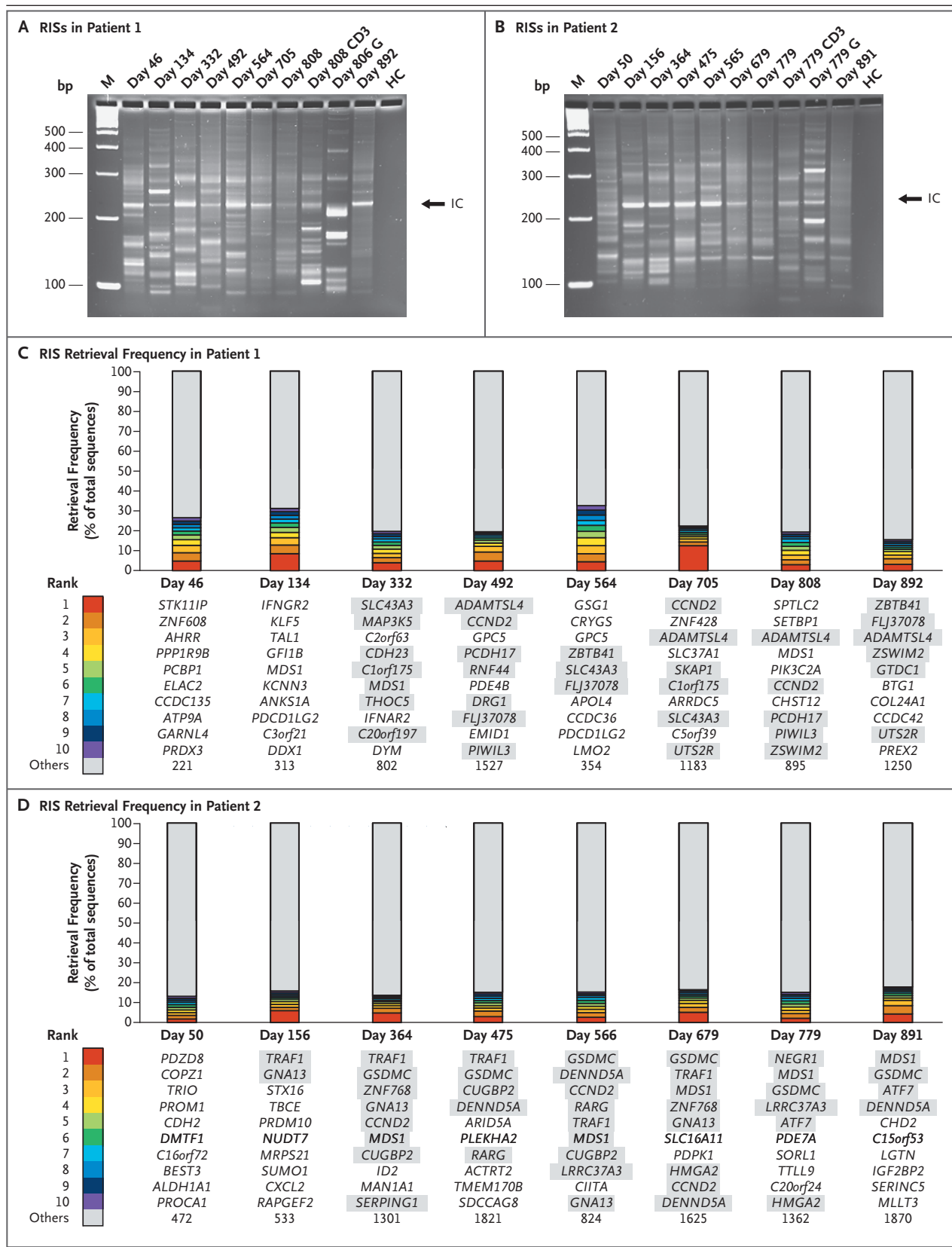
with hexavalent vaccine 12 months after gene therapy and successfully produced specific antibodies to tetanus, diphtheria, and *Haemophilus influenzae* (Table 3 in the Supplementary Appendix).

INFECTIONS AND AUTOIMMUNITY

After gene therapy, the frequency and severity of infections decreased in both patients. However, 2 years after gene therapy, Patient 1 had an episode of pneumococcal meningitis, which was responsive to intravenous antibiotic treatment. Contributing factors may have been his previous splenectomy or insufficient B-cell reconstitution.

Signs and symptoms of autoimmunity disappeared in both patients within the first year after gene therapy. In Patient 1, severe autoimmune hemolytic anemia (with polyspecific anti-Jka antibodies), autoimmune thrombocytopenia (with antibodies directed against membrane glycoproteins IIb/IIIa, Ib/IX, and V), and autoimmune neutropenia (with antibodies against HNA-1a) disappeared (Table 4 in the Supplementary Appendix), and severe eczema resolved in Patient 2 (Fig. 2F). Since recent studies have highlighted the role of regulatory T cells (Tregs) in controlling autoimmunity in WASP deficiency,[18] we measured the relative proportion of WASP-expressing regulatory T cells. The percentage of WASP-positive cells in CD4+CD25+FOXP3+ cells was higher than in the nonregulatory T-cell population (CD4+CD25−FOXP3−) in both patients (Fig. 11 in the Supplementary Appendix).
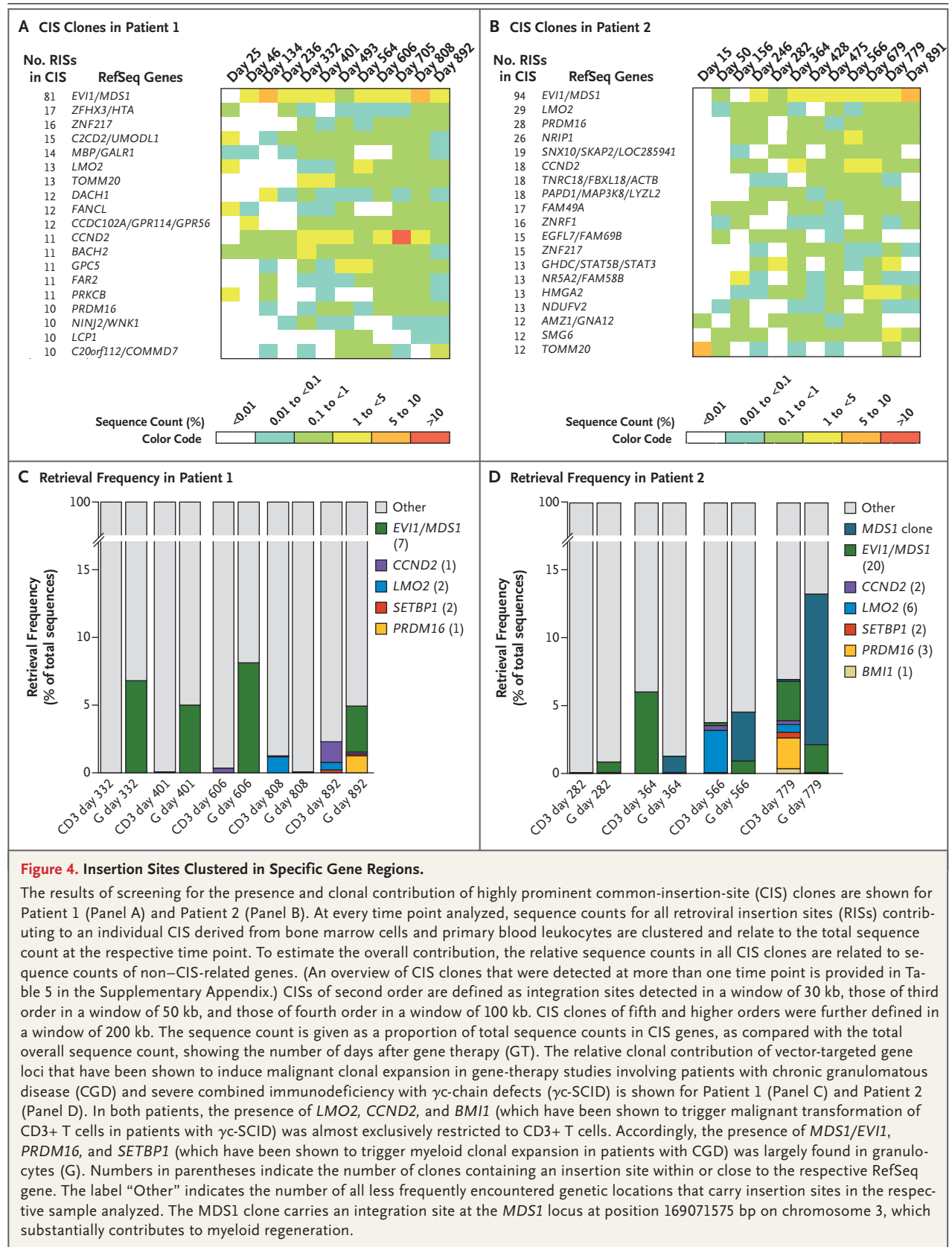
HIGH-RESOLUTION ANALYSIS OF INSERTION SITE

No morphologic or cytogenetic aberrations were seen in bone marrow aspirates after gene therapy. The clonal distribution and fate of gene-corrected cells in vivo were monitored by large-scale analyses of retroviral vector insertion sites with linear-amplification–mediated PCR (LAM-PCR),[19] followed by 454 pyrosequencing. In both patients, a highly polyclonal reconstitution pattern was seen in bone marrow cells, primary blood leukocytes, lymph-node cells (analyzed in Patient 1), sorted lymphoid CD3+ T cells, and myeloid granulocytes (Fig. 3A and 3B). Of approximately 400,000 raw LAM-PCR amplicon sequence reads, 5709 unique retroviral insertion sites (in Patient 1) and 9538 sites (in Patient 2) were retrieved from bone marrow, lymph nodes, and primary blood leukocytes and could be mapped to a single position in the human genome. Vector integration occurred preferentially in the vicinity of transcription start

sites and into gene coding regions of the human genome, reflecting a typical gammaretroviral target-site distribution (Fig. 12 in the Supplementary Appendix).

The abundance of individual gene-corrected cells and their clonal progeny in the hematopoietic system was estimated by the retrieval frequency of individual retroviral insertion site sequences generated by deep sequencing (Fig. 3C and 3D). In both patients, several clones appeared repeatedly within the 10 most abundant clones in each analyzed time point, indicating that clonal skewing had emerged among active hematopoietic gene-corrected clones. Ingenuity pathway analyses in both patients revealed a substantial enrichment of genes that are involved in biologic processes regulating cellular development, growth, and proliferation, as well as hematopoiesis. More specifically, a substantial enrichment of vector insertions in or near genes regulating immune and autoimmune responses was observed in both patients (Fig. 13 in the Supplementary Appendix).

We further monitored the genomic distribution of insertion sites that were clustered in subgenomic regions and associated with particular reference sequence (RefSeq) genes over time as an indicator of in vivo selection of gene-corrected cells. In both patients, the same multiple vector targeted genes that were previously found to expand clones in gene-corrected myelopoiesis (*MDS1/EVI1*, *PRDM16*, and *SETBP1*) and lymphopoiesis (*LMO2*, *CCND2*, and *BMI1*) were noted[12,20-23] (Fig. 3E and 3F). The most active cell clone in Patient 1 showed a vector integrant upstream of *CCND2*, and in Patient 2, the site of insertion was *MDS1/EVI1* (Fig. 4A and 4B, and Table 5 in the Supplementary Appendix).

Insertion-site analyses in sorted CD3+ lymphocytes and myeloid granulocytes revealed that the clonal contribution of cells with insertions in coincident insertion sites that have been found to trigger clonal expansion in gene-corrected lymphopoiesis and myelopoiesis was almost exclusively related to either lymphoid cells or myeloid cells (Fig. 4C and 4D). In contrast, retroviral insertion sites that were identical between sorted lymphoid or myeloid cell fractions were also retrieved from both patients, at least 9000 times as frequently as expected by statistical chance alone. This marking indicates the initial transduction of cells with multilineage capacity (Table 6 in the Supplementary Appendix). Prospective monitoring

Figure 4. Insertion Sites Clustered in Specific Gene Regions.

The results of screening for the presence and clonal contribution of highly prominent common-insertion-site (CIS) clones are shown for Patient 1 (Panel A) and Patient 2 (Panel B). At every time point analyzed, sequence counts for all retroviral insertion sites (RISs) contributing to an individual CIS derived from bone marrow cells and primary blood leukocytes are clustered and relate to the total sequence count at the respective time point. To estimate the overall contribution, the relative sequence counts in all CIS clones are related to sequence counts of non–CIS-related genes. (An overview of CIS clones that were detected at more than one time point is provided in Table 5 in the Supplementary Appendix.) CISs of second order are defined as integration sites detected in a window of 30 kb, those of third order in a window of 50 kb, and those of fourth order in a window of 100 kb. CIS clones of fifth and higher orders were further defined in a window of 200 kb. The sequence count is given as a proportion of total sequence counts in CIS genes, as compared with the total overall sequence count, showing the number of days after gene therapy (GT). The relative clonal contribution of vector-targeted gene loci that have been shown to induce malignant clonal expansion in gene-therapy studies involving patients with chronic granulomatous disease (CGD) and severe combined immunodeficiency with γc-chain defects (γc-SCID) is shown for Patient 1 (Panel C) and Patient 2 (Panel D). In both patients, the presence of *LMO2*, *CCND2*, and *BMI1* (which have been shown to trigger malignant transformation of CD3+ T cells in patients with γc-SCID) was almost exclusively restricted to CD3+ T cells. Accordingly, the presence of *MDS1/EVI1*, *PRDM16*, and *SETBP1* (which have been shown to trigger myeloid clonal expansion in patients with CGD) was largely found in granulocytes (G). Numbers in parentheses indicate the number of clones containing an insertion site within or close to the respective RefSeq gene. The label "Other" indicates the number of all less frequently encountered genetic locations that carry insertion sites in the respective sample analyzed. The MDS1 clone carries an integration site at the *MDS1* locus at position 169071575 bp on chromosome 3, which substantially contributes to myeloid regeneration.

for signs of clonal dominance in gene-corrected hematopoiesis will continue in both patients.

DISCUSSION

Previous clinical studies of hematopoietic gene therapy have reported sustained or temporary correction of lymphocytes in patients with severe combined immunodeficiency or adrenoleukodystrophy[8,9,24] and of myeloid cells in patients with chronic granulomatous disease or adrenoleukodystrophy.[12,24] Our study shows that gene therapy for the Wiskott–Aldrich syndrome is feasible and has not been associated with treatment-limiting adverse events up to 3 years after gene therapy. These findings indicate the successful correction of early hematopoietic progenitor or stem cells that contributed quantitatively to all cell lineages of blood. We also confirmed correction of platelets on transplantation of retrovirus-transduced HSCs in humans.

In line with a proliferative advantage of WASP-positive cells,[14] our results show increasing proportions of corrected lymphocytes over time. Since the reconstitution of the lymphoid system reaches a plateau, there continues to be homeostatic control. Engraftment of progenitor cells was stable, similar to the results achieved in a recent trial of lentivirus-based gene therapy for adrenoleukodystrophy,[24] in which a completely myeloablative conditioning regimen was used.

The pathophysiology of thrombocytopenia in WAS is complex and involves decreased production of platelets and increased immune and non-immune mechanisms of platelet destruction.[25] This study provides evidence that gene transfer can correct thrombocytopenia in humans, a major cause of death and complications in patients with WAS.

The assessment of the clonal inventory in patients whose disease has been successfully corrected uncovers vector influence on key regulators of hematopoiesis. In addition, we observed preferential targeting of genes implicated in autoimmunity control, suggesting an unexpected, disease-related gene targeting. Most interestingly,

insertion-site recovery in sorted lymphoid and myeloid fractions provides novel molecular evidence that the molecular signatures contributing to long-term production of hematologic cells are distinct in both cell lineages of WAS patients. These data suggest that distinctive mechanisms control selective proliferative advantage or differentiation of myeloid and lymphoid cells.

Despite targeting potential oncogenes, no persistent clonal imbalance has yet been observed. Deep sequencing revealed a fluctuating pattern of molecularly defined hematopoietic clones and evidence of initial transduction of HSCs with multilineage capacity, suggesting that hematopoiesis continues to follow a cyclic pattern. Clinically, both patients had marked improvement in their susceptibility to infection, autoimmunity, and bleeding. Longer follow-up and in-depth analyses in additional patients are warranted in light of the considerable clinical benefit derived from genetic correction of this disease. Hematopoietic stem-cell gene transfer may achieve complete immunologic correction without producing the toxic effects implicit in the concept of retrovirus-based stem-cell gene transfer.

REFERENCES

1. Puck JM, Candotti F. Lessons from the Wiskott–Aldrich syndrome. N Engl J Med 2006;355:1759-61.
2. Notarangelo LD, Miao CH, Ochs HD. Wiskott-Aldrich syndrome. Curr Opin Hematol 2008;15:30-6.

3. Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. Cell 1994;78:635-44.
4. Thrasher AJ, Burns SO. WASP: a key immunological multitasker. Nat Rev Immunol 2010;10:182-92.

5. Bosticardo M, Marangoni F, Aiuti A, Villa A, Grazia Roncarolo M. Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome. Blood 2009;113:6288-95.
6. Imai K, Morio T, Zhu Y, et al. Clinical

course of patients with *WASP* gene mutations. Blood 2004;103:456-64.

7. Ozsahin H, Cavazzana-Calvo M, Notarangelo LD, et al. Long-term outcome following hematopoietic stem-cell transplantation in Wiskott-Aldrich syndrome: collaborative study of the European Society for Immunodeficiencies and European Group for Blood and Marrow Transplantation. Blood 2008;111:439-45.

8. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 2000;288:669-72.

9. Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med 2002; 346:1185-93.

10. Aiuti A, Slavin S, Aker M, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science 2002;296:2410-3.

11. Aiuti A, Cattaneo F, Galimberti S, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 2009;360:447-58.

12. Ott MG, Schmidt M, Schwarzwaelder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 2006;12:401-9.

13. Boztug K, Dewey RA, Klein C. Development of hematopoietic stem cell gene

therapy for Wiskott-Aldrich syndrome. Curr Opin Mol Ther 2006;8:390-5.

14. Westerberg LS, de la Fuente MA, Wermeling F, et al. WASP confers selective advantage for specific hematopoietic cell populations and serves a unique role in marginal zone B-cell homeostasis and function. Blood 2008;112:4139-47.

15. Klein C, Nguyen D, Lui C-H, et al. Gene therapy for Wiskott-Aldrich syndrome: rescue of T-cell signaling and amelioration of colitis upon transplantation of retrovirally transduced hematopoietic stem cells in mice. Blood 2003;101: 2159-66.

16. Dewey RA, Avedillo Diez I, Ballmaier M, et al. Retroviral WASP gene transfer into human hematopoietic stem cells reconstitutes the actin cytoskeleton in myeloid progeny cells differentiated in vitro. Exp Hematol 2006;34:1161-9.

17. Orange JS, Ramesh N, Remold-O'Donnell E, et al. Wiskott-Aldrich syndrome protein is required for NK cell cytotoxicity and colocalizes with actin to NK cell-activating immunologic synapses. Proc Natl Acad Sci U S A 2002;99:11351-6.

18. Humblet-Baron S, Sather B, Anover S, et al. Wiskott-Aldrich syndrome protein is required for regulatory T cell homeostasis. J Clin Invest 2007;117:407-18.

19. Schmidt M, Schwarzwaelder K, Bartholomae C, et al. High-resolution insertion-site analysis by linear amplification-mediated PCR (LAM-PCR). Nat Methods 2007;4:1051-7.

20. Hacein-Bey-Abina S, von Kalle C, Schmidt M, et al. LMO2-associated clonal T-cell proliferations in two patients after gene therapy for SCID-X1. Science 2003; 302:415-9. [Erratum, Science 2003;302: 568.]

21. Hacein-Bey-Abina S, Garrigue A, Wang GP, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008;118: 3132-42.

22. Howe SJ, Mansour MR, Schwarzwaelder K, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 2008;118:3143-50.

23. Stein S, Ott MG, Schultze-Strasser S, et al. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med 2010;16:198-204.

24. Cartier N, Hacein-Bey-Abina S, Bartholomae CC, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science 2009;326:818-23.

25. Sabri S, Foudi A, Boukour S, et al. Deficiency in the Wiskott-Aldrich protein induces premature proplatelet formation and platelet production in the bone marrow compartment. Blood 2006;108:134-40.

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