blood

To the editor:

Gene-corrected human Munc13-4–deficient CD8$^+$ T cells can efficiently restrict EBV-driven lymphoproliferation in immunodeficient mice

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Familial hemophagocytic lymphohistiocytosis type 3 (FHL3) is a hyperinflammatory disease caused by mutations in the *UNC13D* gene (coding for the Munc13-4 protein); FHL3 accounts for 30% to 35% of FHL cases.[1] In cytotoxic T cells (CTLs) and natural killer cells, Munc13-4 helps to prime perforin-containing, cytotoxic granules before they fuse with the plasma membrane at the immunological synapse.[2,3] In the absence of effective cytotoxicity, antigen-presenting cells continue to stimulate CTLs, leading to massive proliferation of the latter, the excessive production of interferon γ (IFNγ), macrophage hyperactivation, and tissue infiltration by activated cells. The clinical phenotype is characterized by prolonged fever, hepatosplenomegaly, lymphadenopathy, rashes, and edema.[4] The condition often appears to be triggered by a viral infection (Epstein-Barr virus [EBV], in particular).[5-7]

Hematopoietic stem cell transplantation (HSCT) is the only available curative option for FHL3; however, the posttreatment overall survival rate is not satisfactory and depends on (1) the disease state prior to HSCT and (2) the availability of a matched sibling donor.[4] In addition to HSCT, gene therapy of hematopoietic stem cells has been tested as a treatment of FHL2 in a murine model.[8,9] Given that the main defect in FHL disease is cytotoxic dysfunction of mature T cells, the latter constitute a potentially valuable target for gene therapy approaches. The genetic modification of T cells has produced remarkable clinical outcomes in cancer immunotherapy and in cases of adenosine deaminase deficiency.[10,11] To investigate the feasibility and efficacy of gene transfer into human FHL3 T cells as a means of gene therapy for FHL3, we transduced prestimulated patients' T cells with a measles virus H and F glycoprotein-pseudotyped lentiviral vector (H/F-LV) prior to adoptive transfer in a NSG mouse model bearing EBV-induced lymphoma. The cytotoxic activity of gene-corrected T cells was restored, as evidenced by an increase in their in vitro degranulation capacity and a progressive regression in the mass of EBV lymphoma (due to the efficient homing of functional cytotoxic T cells in vivo). Transduction of T cells from patients with FHL3 with a conventional vesicular stomatitis virus-G lentiviral vector (VSVG-LV) also restored the degranulation capacity (albeit with a lower transduction efficiency than H/F-LV). T memory stem cells (T_{SCM}) were also successfully transduced, and maintained their stem characteristics throughout the culture period. The present work is the first to highlight the potential of engineered T-cell gene therapy in a context of FHL.

Activated peripheral blood mononuclear cells (PBMCs) from FHL3 patients were transduced with either H/F-LV at multiplicity of infection (MOI) of 5 or with VSVG-LV at MOIs of 5 and 100. Both vectors code for a human Munc13-4/cyan fluorescent protein (CFP) fusion protein (supplemental Figure 1A, available on the *Blood* Web site).

An evaluation of the transduction efficacy in bulk transduced cells (6 days after transduction) revealed that the mean vector copy number was higher for H/F-LV than for VSVG-LV (2.22 for H/F-LV; 0.15 and 0.35 for VSVG-LV at MOIs of 5 and 100, respectively) and that Munc13-4 messenger RNA (mRNA) (Figure 1A) and protein (Figure 1B) expression levels were higher in bulk-H/F-LV–transduced cells. The CFP expression on CD8$^+$ gated cells reached a value of 47.8% ± 13% for H/F-LV but only 7.06% ± 2.4% for VSVG-LV at an MOI of 5 and 12.5% ± 2.5% at an MOI of 100 (Figure 1C).

The surface expression of CD107a/b after T-cell receptor (TCR) stimulation (which accompanies the release of cytotoxic granules) indicated that transduced CD8$^+$ T cells expressing Munc13-4/CFP (Munc13-4/CFP$^+$) had recovered normal (healthy control) levels of granule release capacity (Figure 1D). This result also suggested that the fusion of CFP with Munc13-4 did not alter the protein's functionality.

On the basis of these in vitro data, we measured the in vivo cytotoxic activity of Munc13-4/CFP–transduced T cells from FHL3 patients. EBV is one the major infectious triggers of hemophagocytic lymphohistiocytosis (HLH). We therefore decided to test the ability of Munc13-4/CFP–transduced T cells to eliminate EBV-induced B-cell lymphoma in vivo. Briefly, NSG mice were transplanted with a luciferase-expressing, EBV-transformed B- lymphoblastoid cell line (B-LCL) derived from a FHL3 patient (P5). When tumors were palpable (around 7 days later), NSG mice were transplanted with EBV-specific T cells from a single FHL3 patient (bulk-transduced with either H/F-LV–expressing Munc13-4/CFP or H/F-LV–expressing CFP) (Figure 2A). Measurements of CFP expression 7 days after transduction (ie, before the infusion of EBV-T cells into the mice) showed that up to 55.2% of the cells had been transduced (Figure 2B) and that the Munc13-4 mRNA level was 5 times higher in bulk-transduced cells than in healthy controls (data not shown). As shown in Figure 2C-D, and as observed previously[12] using the same xenograft model but with a healthy control as a donor, the tumor mass declined progressively and significantly in NSG mice transplanted with Munc13-4/CFP–transduced T cells. This contrasted with the outcomes in nontransplanted mice and in mice transplanted with CFP-transduced T cells, in which the tumors continued to grow up to

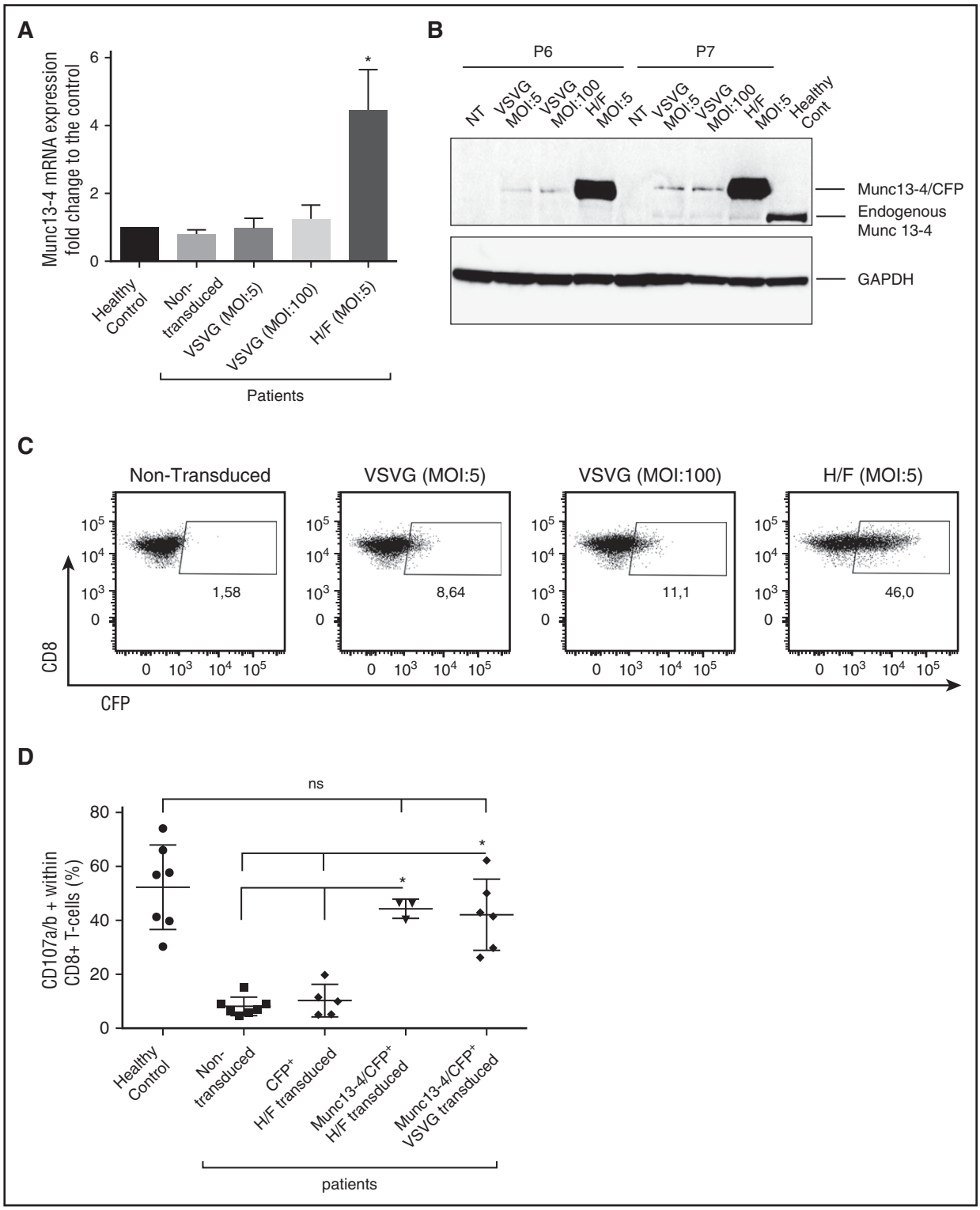

Figure 1. In vitro restoration of Munc13-4 expression and cytotoxic function after gene transfer into Munc13-4–deficient T cells. (A) The level of Munc13-4 mRNA expression was 4.4 to 5.5 times higher in the patients' H/F-LV-bulk–transduced cells than in healthy controls and nontransduced FHL3 cells, and was 1.5 times higher in VSVG-LV–transduced cells (MOI, 100) than in nontransduced cells. The experiments were performed on 3 different FHL3 samples (P5, P6, and P7). The data were normalized against *GAPDH* and are expressed as a fold-change relative to healthy control T cells. It is noteworthy that mRNA levels were similar in nontransduced patient cells and healthy control cells. (B) Munc13-4 protein expression 6 days after transduction (in the same 3 samples as described for panel A; only P6 and P7 are presented here). (C) Dot plots represent (for $CD8^+$ gated cells) the number of Munc13-4/CFP^+ cells as a percentage of the $CD8^+$ population 6 days after transduction with H/F-LV at an MOI of 5 or with VSVG-LV at MOIs of 5 and 100; 1 representative experiment of 3 (same samples as in panel A) is shown. (D) The number of CD107a/b^+ cells as a percentage of the $CD8^+$ T-cell population after stimulation with 30 μg/mL anti-CD3. In transduced samples, the percentage of CD107a/b^+ cells are presented with respect to CFP^+ or Munc13-4/CFP^+ gated cells. n = 7 different FHL3 samples for nontransduced cells (P1-P7); n = 5 for H/F-CFP as control (P3-P7); n = 3 for H/F (P5-P7); n = 6 for VSVG experiments (P1-P3, P5-P7). Data are presented as the mean ± standard deviation (SD). *P* values were calculated using an unpaired Student *t* test for mRNA expression and a 2-sided Mann-Whitney test for CD107a/b surface expression in the degranulation assay. *$P < .05$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ns, nonsignificant; NT, nontransduced.

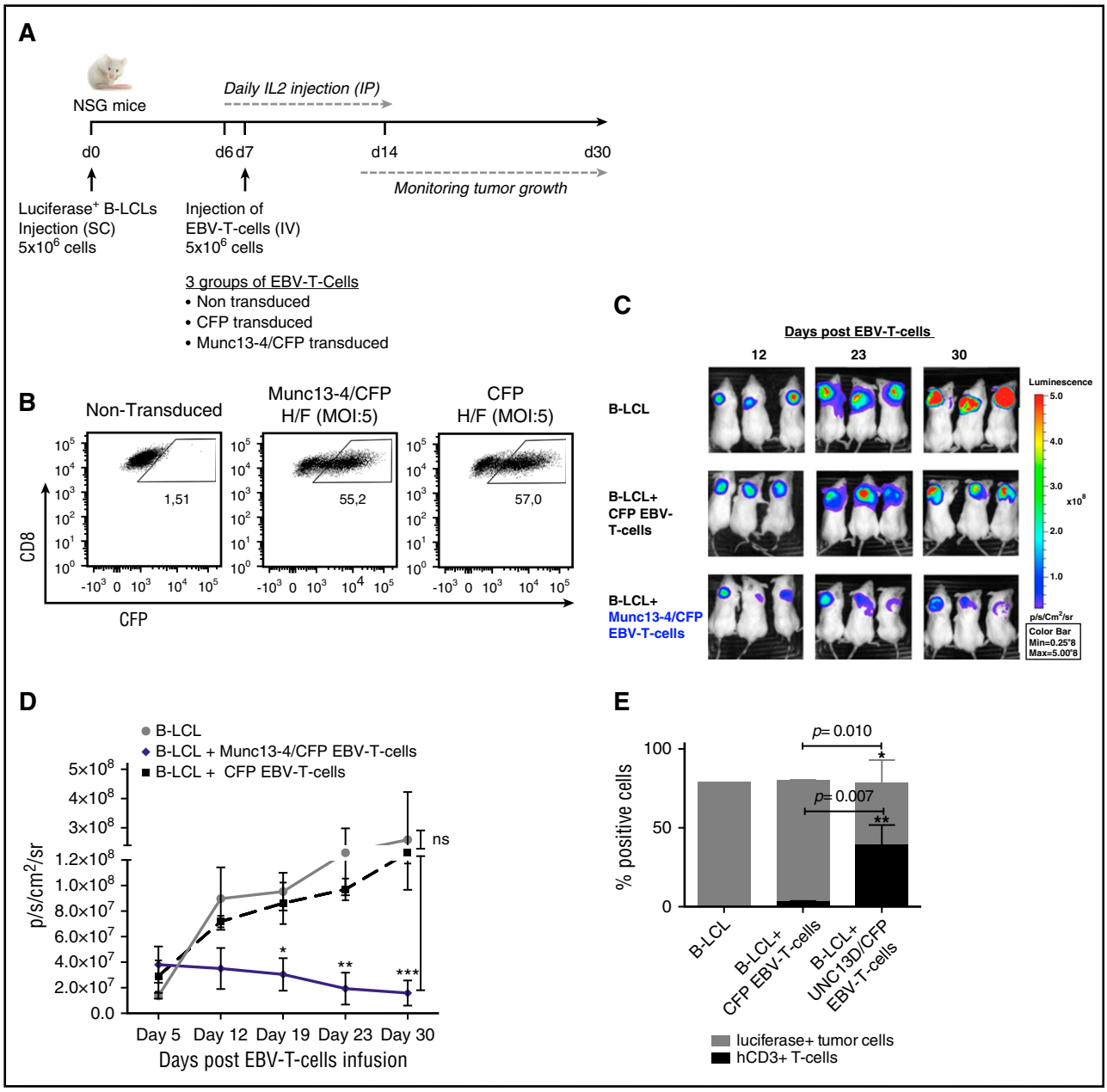

Figure 2. Adoptive transfer of Munc13-4–corrected EBV-specific T cells induces the regression of EBV B-cell lymphoma in NSG mice. (A) Experimental design for the xenograft NSG mouse model. The experiment was performed with PBMCs from P5 (an EBV-seropositive patient). N = 3 mice per group. (B) The dot plot represents the number of Munc13-4/CFP$^+$ or CFP$^+$ cells as a percentage of the CD8$^+$-gated population 7 days after transduction. (C) Bioimmunoluminescence imaging using the IVIS in vivo imaging system (Xenogen; Caliper Life Sciences, Hopkinton, MA) for NSG mice bearing EBV B-cell lymphoma. EBV-T cells, EBV-specific cytotoxic T cells. (D) The time course of tumor growth. Photon emission from luciferase-positive tumor cells was quantified as the peak photons per second per cm^2 per steradian (p/s/cm^2/sr). Error bars represent the mean ± standard error of the mean (SEM). The *P* value was calculated in a 2-way analysis of variance with a Bonferroni posttest. (E) The percentage of human CD3$^+$ (hCD3$^+$) T cells and luciferase-expressing lymphoma cells in digested tumors, as measured by flow cytometry. The mean cell count ± SD is shown. The *P* value was calculated in an unpaired Student *t* test. **P* < .05; ***P* < .01; ****P* < .001. IP, intraperitoneal; SC, subcutaneous.

30 days posttransplantation. Immunostaining and flow cytometry analysis revealed massive lymphocytic tumor infiltration in NSG mice transplanted with Munc13-4/CFP–transduced T cells (Figure 2E; supplemental Figure 1B). These results clearly demonstrate that transduction with a LV containing the *UNC13D* gene restores not only the expression of Munc13-4 protein but also the in vitro granule release capacity and in vivo cytotoxic function of T cells from FHL3 patients.

In a clinical trial of T-cell gene therapy for adenosine deaminase deficiency, the transduced T cells persisted in the circulation for up to 12 years after infusion[13] as a result of efficient transduction, in vitro expansion, and the in vivo persistence of T_{SCM}. In the present study, we confirmed the presence of T_{SCM} within the T-cell pool from FHL3 patients (supplemental Figure 2A). We also demonstrated that T_{SCM} were transduced by both H/F- and VSVG-LVs (supplemental Figure 2B). The proportion of T_{SCM} increased 48 hours after TCR stimulation and decreased between days 2 and 8 of culture as a result of differentiation into other memory cells. However, a fraction of these T_{SCM} was still detectable at day 8 in both nontransduced and transduced conditions, whereas no naive cells were found in the culture (supplemental Figure 2C).

Our study is the first to highlight the potential of T-cell gene therapy in a context of FHL. Targeting mature T cells (rather than hematopoietic stem cells) is associated with a lower risk of insertional mutagenesis and cell transformation.[14] However, one of the limitations of this strategy relates to the generally low transductional efficacy of VSVG-LV. The post-HSCT outcome in HLH patients shows that remission is maintained as long as the level of T-cell donor chimerism reaches 10% to 15%.[15,16] The conventional VSVG-LV strategy must therefore be tested in a preclinical murine model, in order to establish whether or not poor in vitro T-cell transduction prevents the achievement of this level of chimerism in vivo. Another limitation relates to the availability of T cells as a result of pancytopenia and the manifestations of HLH. Novel anti-inflammatory modalities for IFNγ blockade (such as the JAK1/2 inhibitor ruxolitinib) can reduce inflammation and correct certain manifestations of FHL (such as blood cytopenia) in murine models of FHL.[17,18] Ruxolitinib has already demonstrated clinical efficacy in other inflammatory conditions[19,20]; if approved in HLH patients, this compound could be used as an anti-inflammatory agent for reducing immune system imbalance prior to T-cell immunotherapy.

*J.R. and I.R. contributed equally to this study.

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