

cytotoxic T cells) returned to normal levels.<sup>2</sup> In the two patients described by Clatworthy et al., the number of CD8+ T lymphocytes was low (190 and 80 cells per cubic millimeter; normal range, 200 to 900), but the number of CD19+ cells was within the normal range. Since alemtuzumab could eliminate about 77% of CD8+ T lymphocytes by means of non-complement-mediated mechanisms,<sup>3</sup> persistently suppressed CD8+ T cells could have influenced the development of anti-glomerular basement membrane antibodies in their patients.

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**THE AUTHORS REPLY:** Shin and Lee suggest that the development of anti-glomerular basement membrane disease in two patients treated with alemtuzumab was due to a deficiency of CD8 suppressor T cells. The speculation is based on a description of a patient with atypical, spontaneously resolving, anti-glomerular basement membrane disease in which low levels of so-called CD8 suppressor T cells were observed.<sup>1</sup> The OKT8 antibody that was used to define suppressors in this article merely identifies CD8 T cells, with no demonstra-

tion of a deficiency of suppressor function within this subgroup. Aside from the limitations of this article, CD8 T-cell depletion actually prevents or ameliorates disease in animal models of anti-glomerular basement membrane disease.<sup>2</sup> In addition, data suggest that CD8 regulatory T cells in humans reside in the CD8+CD28- T-cell compartment.<sup>3</sup> After treatment with alemtuzumab, this regulator-containing CD8+CD28- subgroup is enriched within the reconstituted CD8 T-cell compartment.<sup>4</sup>

Since acceptance of our letter, we have become aware that one of our two patients was previously mentioned in the adverse-effects section of an article on alemtuzumab in multiple sclerosis.<sup>5</sup>

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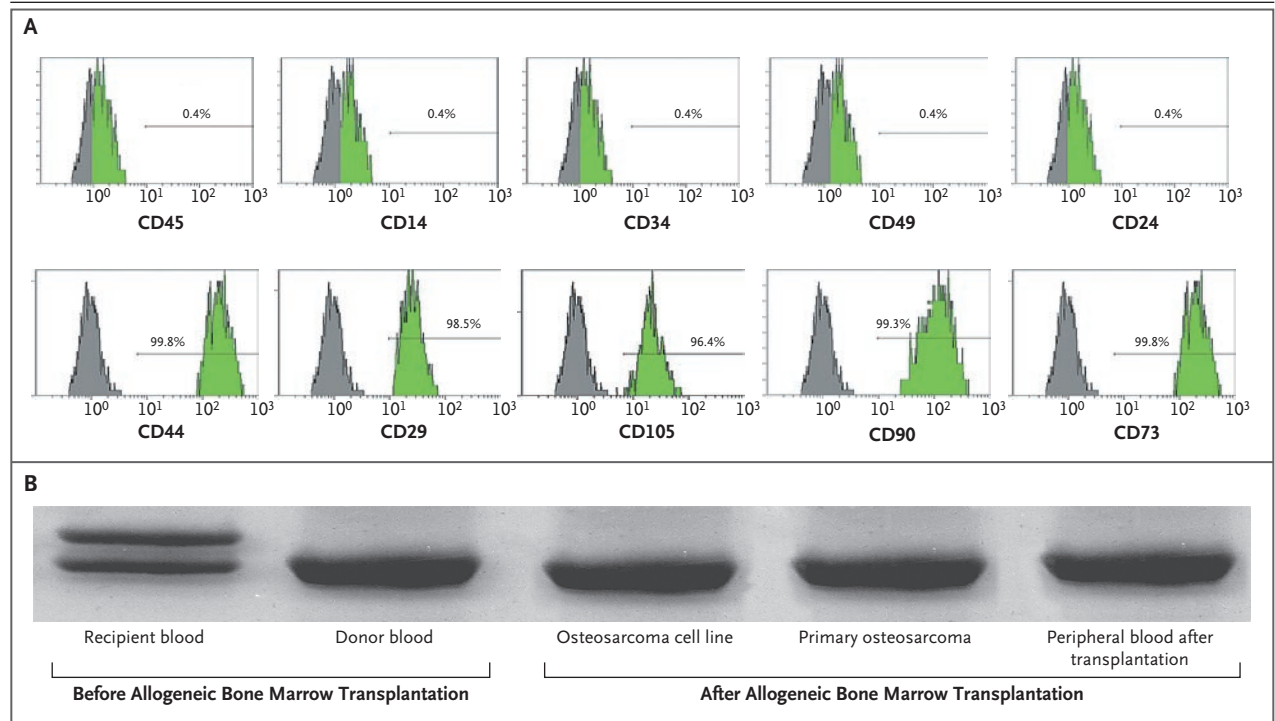
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## Osteosarcoma Derived from Donor Stem Cells Carrying the Norrie's Disease Gene

**TO THE EDITOR:** There have been few reports of osteosarcoma after allogeneic bone marrow transplantation.<sup>1</sup> We report the development of osteosarcoma in a recipient 17 years after stem-cell transplantation.

A 23-month-old boy with  $\beta$ -thalassemia received a bone marrow transplant from his HLA-identical 11-year-old brother in September 1989.

Norrie's disease had been diagnosed in the donor at 12 months of age, after enucleation of the right eye was performed because a bilateral retinoblastoma was suspected. No genetic analysis was conducted at the time of surgery. Norrie's disease is an X-linked recessive disease caused by mutations in the *NDP* gene on Xp11.4.<sup>2</sup> It primarily affects the eye and almost always leads to blind-



**Figure 1. Osteosarcoma Cell-Line Immunophenotype and Chimerism.**

Panel A shows the results of flow-cytometric analysis and the osteosarcoma cell-line immunophenotype. A total of  $2 \times 10^5$  to  $5 \times 10^5$  cells were stained for 20 minutes with monoclonal antibodies against CD45, CD14, CD34, CD49, CD24, CD44, CD29, CD105, CD90, and CD73 (Beckman Coulter) and 0.5 mg of propidium iodide per milliliter (Sigma) for the viability analysis. Appropriate combinations of fluorescein isothiocyanate-conjugated, phycoerythrin-conjugated, and peridinin chlorophyll protein-conjugated monoclonal antibodies were used. Labeled cells were analyzed on an Epics XL cytometer (Beckman Coulter) with the use of the XL2 software program. Gray denotes negative controls, and green denotes positive cells. The horizontal lines indicate the percentages of positive cells in the samples. Panel B shows chimerism. Genomic DNA was extracted from the patient's peripheral blood, pelvic-biopsy specimen, and osteosarcoma cell line and from the donor's peripheral blood with the use of standard procedures (the phenol-chloroform method). To evaluate chimerism, a semiquantitative approach based on polymerase-chain-reaction amplification of polymorphic genes (DNA variation sequences with a frequency of more than 1% in humans, such as variable numbers of tandem repeats) was used. The primers were 5'GCCCATAGGTTTT-GAACTCA3' (forward primer) and 5'TGATTTGTCTGTAATTGCCAGC3' (reverse primer).

ness. The chemotherapy regimen before the bone marrow transplantation consisted of high-dose cyclophosphamide and busulfan. From day 30, the recipient was a complete hematopoietic chimera. Seventeen years later, a metastatic chondroblastic osteosarcoma of the pelvis developed in the 18-year-old recipient. Despite aggressive treatment, the patient died of tumor progression 22 months after the diagnosis of osteosarcoma and 19 years after stem-cell transplantation.

An osteosarcoma cell line that was established from the recipient's tumor had a doubling time of 168 hours (7 days) (the normal doubling time in a generated osteosarcoma cell line is 1.4 to 37.0 days). The cells expressed mesenchymal stem-cell markers (CD44, CD29, CD105, CD90, and CD73)

at a very high level (Fig. 1), but they did not express CD45, CD14, CD34, CD49, or CD24. Cells from the osteosarcoma cell line were examined by means of cytogenetic analysis and fluorescence in situ hybridization (FISH) with the use of a specific probe for the *p53* gene.<sup>3</sup> Fifty metaphases were analyzed after GTG banding. Two distinct karyotypes were found: 47XY,-5,+22,+m and 45XY,-12,-15,+m. Blood samples from the recipient and donor had wild-type copies of chromosome 17, whereas the osteosarcoma cell line showed trisomy 17 in 22 of 100 interphase nuclei. Neither the cytogenetic analysis nor *p53* FISH analysis of the primary tumor was performed because of an inadequate specimen, and for ethical reasons, donor mesenchymal stem cells were not

analyzed at the time of the diagnosis of osteosarcoma. A semiquantitative method based on polymerase-chain-reaction amplification of polymorphic genes showed complete donor chimerism of recipient hematopoietic cells, the cells from the osteosarcoma-biopsy specimen, and the osteosarcoma cell line (Fig. 1).<sup>3</sup> Moreover, the blood samples from the donor and the recipient, the osteosarcoma-biopsy specimen, and the osteosarcoma cell line had a mutation of L15R of exon 2 of the *NDP* gene, whereas swab cells from the recipient's oral cavity did not.

Our data suggest the donor origin of the osteosarcoma. The mutated *NDP* in the osteosarcoma cells was most likely a marker of chimerism, because cancer did not develop in the donor, and Norrie's disease is not associated with an increased risk of cancer.

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