onset breast cancer in two aunts. The patient's mother had a cerebellar astrocytoma, two vaginal tumors, and a malignant nevus (Fig. 2A).

The germ-line *PTEN* genes of the child and her father had a base change, c.334C→G. Although this change putatively encodes an L112V missense mutation, *PTEN* messenger RNA (mRNA) analysis instead revealed activation of a cryptic splice site (Fig. 2B). The germ-line *TP53* gene in the proband, her sister, and her mother contained an R282W deleterious missense mutation. Thus, the proband had inherited deleterious mutations in both *TP53* and *PTEN*.

In tumors from patients with the Li–Fraumeni syndrome or Cowden's syndrome, there is often somatic mutation or silencing of the second copy of the tumor-suppressor gene.[3] The granulosa-cell tumor, xanthoastrocytoma, and multiple liposarcoma samples from our proband revealed no somatic mutations in *TP53* or *PTEN*. Loss of heterozygosity was not detected in *TP53*, but it was detected in *PTEN* in the granulosa-cell tumor and the liposarcoma specimen after chemotherapy (but not in specimens from the initial resection and lung metastasis) (Fig. 2C). The finding that several of the patient's tumors did not have loss of heterozygosity or somatic mutations was also reported for tumors from a mouse model that was doubly heterozygous for *p53* and *Pten* mutations.[4]

The types and numbers of tumors that developed in the proband by 4 years of age are not typical of the Li–Fraumeni syndrome or Cowden's syndrome, and four malignant conditions are expected to develop in only 2% of patients with the Li–Fraumeni syndrome.[1,5] This tumor spectrum may reflect the intricate coregulation of the TP53 and PTEN proteins.[4] Clinically, it may be useful to sequence in parallel multiple cancer-associated genes of patients with unusual cancer phenotypes.

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Cytomegalovirus Immunity after Vaccination with Autologous Glioblastoma Lysate

TO THE EDITOR: Glioblastoma is a malignant brain tumor with overall survival rates of less than 3.3% at 5 years.[1] Few effective treatments are available. The durability of a radiographically defined response to treatment is limited, and median survival is less than 2 years.

We are conducting a phase 1 trial of autologous dendritic-cell vaccination as adjunctive therapy in glioma, a study that has been approved by the institutional review board at the University of California, Los Angeles. All patients are treated with surgery, standard radiotherapy, and temozolomide, followed by vaccination with dendritic cells that are pulsed with an autologous tumor lysate. To date, we have enrolled 14 patients with newly diagnosed glioblastoma (World Health Organization grade IV).

Here we describe Patient 4-908 with glioblastoma who was enrolled in the trial and in whom a robust CD8+ T-cell response to the pp65 immunodominant epitope of human cytomegalovirus (CMV) began immediately after one injection of

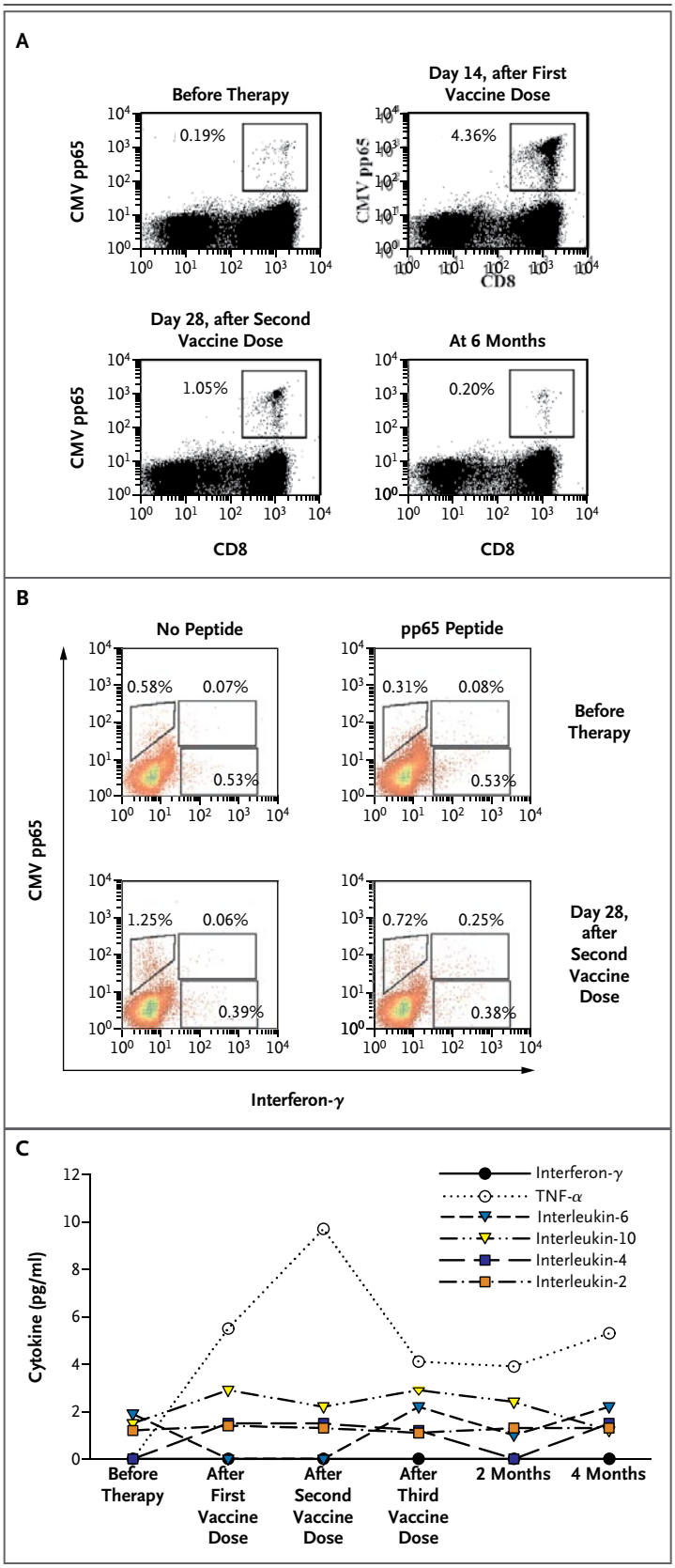

Figure 1. Induction of Immune Responses Specific for Human Cytomegalovirus (CMV) after Vaccination with Dendritic Cells Pulsed with Autologous Tumor Lysate.

In Panel A, histograms show human CMV–specific CD8+ T cells (CD4–, CD13–, and CD19–, CD3+CD8+ CMV pp65+) before treatment, after the first and second dendritic-cell vaccinations, and at 6 months after vaccination. The percentages shown are those of all CD3+CD8+ T cells. In Panel B, combined tetramer and intracellular cytokine staining of peripheral-blood mononuclear cells (PBMCs) from Patient 4-908 is shown before treatment and after two vaccinations with dendritic cells. PBMCs were restimulated for 6 hours, with and without the immunodominant human CMV peptide, in the presence of Brefeldin A, followed by surface-antibody labeling, fixation and permeabilization, and labeling of intracellular interferon-γ. In a clockwise fashion, the percentages in each gate reflect the frequency of CD3+CD8+ T cells that were positive for CMV pp65 and negative for interferon-γ, positive for both CMV and interferon-γ, and negative for CMV but positive for interferon-γ . In Panel C, serum cytokine levels are shown during the initial dendritic-cell vaccination regimen once every 2 weeks, in which 50 μl of serum was tested for the presence of cytokines related to type 1 helper T cells (interleukin-2, tumor necrosis factor α [TNF-α], and interferon-γ) and type 2 helper T cells (interleukin-4, -6, and -10) by cytometric bead array at the indicated times.

the vaccine (Fig. 1A and 1B). We found that after vaccination, 4.4% of the patient's CD8+ T cells were specific for pp65. The vaccination was not associated with any systemic symptoms. Local lymphadenopathy was observed at the time of the expansion of the human CMV–specific T cells.[2]

The patient's tumor appeared to be infected with CMV. Immunohistochemical staining of tumor tissue showed focal expression of human CMV pp65 in the tumor but not in a tumor from Patient 2-261 with an HLA-A0201–positive glioblastoma, in whom a CMV-specific immune response did not occur after vaccination. In Patient 4-908, serum cytokine measurements revealed increased levels of tumor necrosis factor α after one dendritic-cell vaccination, a response that peaked 2 weeks after the second vaccination (Fig. 1C).

An association has been discovered between human CMV and malignant gliomas.[3-5] We found that a CMV-specific CD8+ T-cell response was induced in a patient with glioblastoma after therapeutic vaccination with dendritic cells pulsed with an autologous tumor lysate. Our results suggest that the presence of human CMV in glial cells could serve as an immunotherapeutic target in

glioblastoma. The relative ease of eliciting an immune response against viral antigens contrasts with the difficulty of immunization against "self" tumor antigens. This case highlights the potential for targeted vaccination strategies with the use of dendritic cells as an adjunct to standard treatments for glioblastoma, although multicenter, randomized studies will be necessary to demonstrate clinical efficacy.

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