Allogeneic BK Virus–Specific T Cells for Progressive Multifocal Leukoencephalopathy

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SUMMARY

JC virus, the cause of progressive multifocal leukoencephalopathy (PML), and the BK virus are genetically similar and share sequence homology in immunogenic proteins. We treated three immunosuppressed patients with PML with ex vivo–expanded, partially HLA-matched, third-party–produced, cryopreserved BK virus–specific T cells. The immunosuppression in these patients was due to the conditioning regimen for cord-blood transplantation in one patient, a myeloproliferative neoplasm treated with ruxolitinib in another, and acquired immunodeficiency syndrome in the third. After T-cell infusion in two of the patients, alleviation of the clinical signs and imaging features of PML was seen and JC virus in the cerebrospinal fluid (CSF) cleared. The other patient had a reduction in JC viral load and stabilization of symptoms that persisted until her death 8 months after the first infusion. Two of the patients had immune reconstitution syndrome. Donor-derived T cells were detected in the CSF after infusion. (Funded by the M.D. Anderson Cancer Center Moon Shots Program and the National Institutes of Health; ClinicalTrials.gov number, NCT02479698.)
Hyperintense signals on T2-weighted fluid-attenuated inversion-recovery (T2-FLAIR) images in the middle cerebellar peduncles extending toward the pons (yellow arrows) and involvement of the left cerebellum (red arrow). Images from day 21 to day 258 after virus-specific T-cell infusion show reduction in the size of the white-matter lesions and atrophic changes. The image from day 258 shows prominence of cerebellar sulci (red arrow) and increased size of fourth ventricle.

**CASE REPORTS**

Patient 1 was a 32-year-old woman who underwent double cord-blood transplantation with myeloablative conditioning for FLT3-positive acute myeloid leukemia that was in first remission. Immunosuppression was discontinued 7 months after transplantation. At 20 months after transplantation, 13 months after discontinuation of prophylaxis for graft-versus-host disease, she presented with weakness on her left side, slurred speech, and confusion. She had dysarthria, a severely ataxic gait, and an inability to stand unaided. Her CD4 cell count had been 100 to 150 per cubic millimeter since engraftment of the stem-cell transplant. Magnetic resonance imaging (MRI) examination of the head revealed hyperintense signals on T2-weighted fluid-attenuated inversion-recovery (T2-FLAIR) images in the middle cerebellar peduncles, left cerebellum, right midbrain, right internal capsule, and pons, findings consistent with PML (Fig. 1A, and Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Lumbar puncture was performed, and a JC virus DNA load of 130 copies per milliliter was found in the cerebrospinal fluid (CSF) in association with normal cytologic findings, which met the criteria for a diagnosis of PML. Mirtazapine was administered. Three weeks later, a repeat MRI showed progression of the lesions (Fig. 1A and 1B), and the JC viral load had increased to 700 copies per milliliter.
### Table 1. Clinical Courses in the Three Patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At screening, before first infusion</strong></td>
<td>32-Yr-old woman with high-risk acute myeloid leukemia, presented 20 mo after cord-blood transplantation</td>
<td>73-Yr-old woman with a JAK2-positive myeloproliferative neoplasm, presented after 8 yr of ruxolitinib therapy</td>
<td>35-Yr-old man with 7 yr history of HIV seropositivity, discontinued HAART 5 yr earlier</td>
</tr>
<tr>
<td><strong>Age, sex, and underlying diagnosis</strong></td>
<td>Intermittent double vision, nystagmus, dysarthria, weakness on left side, dysmetria, ataxia, and difficulty ambulating independently</td>
<td>Confusion, decreased attention span, expressive-speech difficulty, nystagmus, and right homonymous hemianopia, ataxia</td>
<td>Dysarthria, dysphagia, bilateral hyperreflexia, ataxia, and inability to ambulate or sit unaided</td>
</tr>
<tr>
<td><strong>Symptoms and signs</strong></td>
<td>Extensive confluent bilateral asymmetric T2-FLAIR hyperintensity within the cerebellar hemispheres, middle cerebellar peduncles, pons, right midbrain, and posterior limb of the right internal capsule</td>
<td>Multifocal disease with dominant lesion in the left posterior parietal subcortical white matter extending to the posterior temporal lobe with central area of leukomalacia; no associated enhancement</td>
<td>T2-FLAIR hyperintensity in bilateral middle cerebellar peduncle, pons, medulla, and medial cerebellar hemispheres and right parietal subcortical white matter; no associated enhancement</td>
</tr>
<tr>
<td><strong>MRI findings</strong></td>
<td>JC viral load — copies/ml</td>
<td>Cross-sectional and imaging data are summarized in the following table.</td>
<td>JC viral load — copies/ml</td>
</tr>
<tr>
<td>CSF</td>
<td>700</td>
<td>230,000</td>
<td>4,300</td>
</tr>
<tr>
<td>Blood</td>
<td>Not assessed</td>
<td>4,800</td>
<td>&lt;40</td>
</tr>
<tr>
<td><strong>At last follow-up</strong></td>
<td><strong>Total no. of infusions</strong></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Symptoms and signs</strong></td>
<td>Had complete resolution of all symptoms, fully recovered, returned to work.</td>
<td>Patient and family elected for transfer to hospice on day 42 after the infusion. Patient died in hospice at day 252.</td>
<td>Able to walk with a cane. Speech remains slightly slurred.</td>
</tr>
<tr>
<td><strong>MRI findings</strong></td>
<td>T2-FLAIR hyperintensity within the brain stem and cerebellum persisted with atrophic changes; complete resolution of enhancement</td>
<td>T2-FLAIR hyperintensity in left parietal lobe, extending to posterior temporal lobe; enhancement of the splenium and the periventricular white-matter lesions; small areas of enhancement noted in the frontal lobes, coronal radiate, and centrum semiovale, suggestive of IRIS</td>
<td>Decreased edema associated with right parietal subcortical white-matter T2-FLAIR hyperintensity and resolution of enhancement; T2-FLAIR hyperintensity of the brain stem and cerebellum persisted, but with atrophic changes</td>
</tr>
<tr>
<td><strong>JC viral load — copies/ml</strong></td>
<td>CSF</td>
<td>Not detectable</td>
<td>800</td>
</tr>
<tr>
<td>Blood</td>
<td>Not detectable</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

* CSF denotes cerebrospinal fluid, T2-FLAIR T2-weighted fluid-attenuated inversion-recovery, HAART highly active antiretroviral therapy, and IRIS immune reconstitution inflammatory syndrome.
Mirtazapine treatment was discontinued, and the patient was treated with BK virus–specific T cells.

Patient 2 was a 73-year-old woman with JAK2-positive polycythemia rubra vera that had been treated with ruxolitinib for 8 years; she presented with a 6-month history of progressive confusion, expressive aphasia, blurred vision, and ataxia (Table S1 in the Supplementary Appendix). An MRI examination revealed parieto-occipital subcortical signal abnormalities in the left cerebral hemisphere that extended to the posterior temporal lobe and, to a lesser extent, to the left cerebellar hemisphere — findings consistent with PML (Fig. 1C and 1D). The JC viral load in the CSF was 230,000 copies per milliliter. Ruxolitinib treatment was discontinued, and 2 months later, the patient was treated with BK virus–specific T cells.

Patient 3 was a 35-year-old man with acquired immunodeficiency syndrome (AIDS) who had discontinued highly active antiretroviral therapy (HAART) 5 years earlier because of the side effects. He presented with progressive dysarthria, dysphagia, and ataxia (Table S1 in the Supplementary Appendix). MRI examination of the head revealed white-matter lesions in the cerebellum, pons, medulla, and medial cerebellar hemispheres, findings consistent with PML (Fig. 1G, 1H, 1I, and 1J). The CSF contained JC virus, which was not quantified; the CD4 cell count was 19 per cubic millimeter, and the HIV viral load was greater than 1,500,000 copies per milliliter. HAART was reintroduced, and the CD4 cell count increased to 182 per cubic millimeter and the HIV viral load decreased to 276 copies per milliliter. The symptoms of PML progressed over a period of 4 months despite CD4 cell counts remaining between 147 and 182 per cubic millimeter and the HIV viral load remaining low (Table 1). A repeat MRI examination revealed enlargement of lesions in the brain stem, cerebellum, and parietal subcortical white matter (Fig. 1G and 1H). The JC viral load in the CSF was 4300 copies per milliliter, the CD4 cell count was 116 per cubic millimeter, and the HIV viral load decreased to 66 copies per milliliter. The symptoms of PML progressed over a period of 4 months despite CD4 cell counts remaining between 147 and 182 per cubic millimeter and the HIV viral load remaining low (Table 1). A repeat MRI examination revealed enlargement of lesions in the brain stem, cerebellum, and parietal subcortical white matter (Fig. 1G and 1H).

To determine the persistence of donor-derived, virus-specific T cells in the peripheral blood and their trafficking to the CSF, we used the mismatch in the HLA-Bw genotype between Patient 1 (who was HLA-Bw4–positive) and her virus-specific T-cell donor (who was HLA-Bw6–positive). We developed a flow-chimerism assay using fluorochrome-conjugated antibodies against HLA groups Bw4 and Bw6. Flow cytometry was performed on a BD LSRFortessa X-20 instrument, and data were analyzed with the use of FlowJo software, version 10.0.8 (TreeStar). The gating strategy for the detection of HLA-Bw6–positive virus-specific T cells is shown in Figure S1 in the Supplementary Appendix.

Methods

Study Design

We performed a phase 2, protocol-driven study, involving three patients with PML, to evaluate whether cryopreserved, third-party–produced, viral-specific T cells that had been designed for the treatment of patients with BK virus infection after stem-cell transplantation could be used to treat PML. The patients’ CSF was examined before each infusion to measure the JC viral load. The patients received BK virus–specific T-cell infusions every 4 weeks until JC virus was cleared from the CSF.

The bank of BK virus–specific T cells was generated from 27 healthy donors by a method described in the Supplementary Appendix. The cryopreserved cells were thawed, and for each patient, the most closely HLA-matched T-cell line (meaning that at least one HLA-A and one HLA-DRB1 allele matched; see Table S2 in the Supplementary Appendix) was selected and administered at a dose of 2 × 10⁸ T cells per kilogram of body weight. The characteristics of the infused products for each patient are shown in Table S3 in the Supplementary Appendix.

The study was approved by the institutional review board of the M.D. Anderson Cancer Center, and all the patients provided written informed consent. The study was designed by the second and last authors. All the authors vouch for the accuracy of reported data, analyses, and adverse events and for the adherence of the study to the protocol, available at NEJM.org. There was no industry involvement in the study.

Phenotyping and Tracking of Donor-Derived, Virus-Specific T Cells

To determine the persistence of donor-derived, virus-specific T cells in the peripheral blood and their trafficking to the CSF, we used the mismatch in the HLA-Bw genotype between Patient 1 (who was HLA-Bw4–positive) and her virus-specific T-cell donor (who was HLA-Bw6–positive). We developed a flow-chimerism assay using fluorochrome-conjugated antibodies against HLA groups Bw4 and Bw6. Flow cytometry was performed on a BD LSRFortessa X-20 instrument, and data were analyzed with the use of FlowJo software, version 10.0.8 (TreeStar). The gating strategy for the detection of HLA-Bw6–positive virus-specific T cells is shown in Figure S1 in the Supplementary Appendix.

The New England Journal of Medicine

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the Supplementary Appendix. We also used a 40-parameter mass-cytometry panel to characterize donor and recipient T cells (Table S4 in the Supplementary Appendix).

RESULTS

CHANGES AFTER FIRST INFUSION
After the first infusion, all three patients had a reduction in the JC viral load in the CSF; from 700 to 78 copies per milliliter in Patient 1, from 230,000 to 5200 copies per milliliter in Patient 2, and from 4300 to 1300 copies per milliliter in Patient 3 (Table S1 in the Supplementary Appendix). The CD4 cell counts before the T-cell infusion as compared with the counts after the first infusion were 153 versus 77 per cubic millimeter, 625 versus 743 per cubic millimeter, and 96 versus 111 per cubic millimeter, respectively. In Patient 1, there was complete resolution of neurologic symptoms with the exception of slight dysarthria, and a reduction in the size of the white-matter lesions was detected on MRI examination (Fig. 1A). In Patient 2, the neurologic symptoms and signs stopped progressing, and findings consistent with immune reconstitution inflammatory syndrome (IRIS) were seen on MRI (Fig. 1E and 1F). Patient 3 had clinical improvement and was able to sit unaided, which had not been possible before the infusion; he also had less dysarthria and better coordination, and findings consistent with IRIS were seen on MRI (Fig. 1G and 1H).

CHANGES AFTER SUBSEQUENT INFUSIONS
Patient 1 received two additional infusions, which resulted in complete clearance of JC virus in the CSF and resolution of clinical and imaging findings. She remained asymptomatic at the most recent follow-up, 27 months after the first infusion and 24 months after the last infusion (Table 1). Patient 2 received a second infusion that was associated with further reduction in JC viral load in the CSF, to 800 copies per milliliter; her symptoms and signs remained static, but there was no improvement in clinical status or findings on imaging, and she pursued hospice care and died 8 months after the first infusion (Table 1). Patient 3 received three additional infusions, which were followed by complete clearance of JC virus in the CSF. The patient regained independent mobility, and there was a reduction in the size of MRI signal changes (Fig. 1I and 1J). Nine months after the first infusion, the patient was able to walk with a cane and had minimal dysarthria (Table 1).

ADVERSE EVENTS
No infusion-related reactions occurred. Patient 2 had MRI evidence of IRIS after the first infusion, without clinical manifestation. Patient 3 had worsening numbness and ataxia 1 week after the first infusion, which resolved within 2 weeks. An MRI examination revealed new enhancement overlying the pons and the superior cerebellum, a finding suggestive of IRIS. A description of the adverse events is provided in Table S5 in the Supplementary Appendix.

PERSISTENCE AND TRAFFICKING OF DONOR T CELLS TO THE CSF IN PATIENT 1
The HLA-Bw mismatch between Patient 1 and her T-cell donor allowed us to study the trafficking of virus-specific T cells to the CSF. Both cord units used in her transplantation were HLA-Bw6–negative, whereas the virus-specific T cells were HLA-Bw6–positive (Table S2 in the Supplementary Appendix). The population of donor
virus-specific T cells grew to 294 times its original size in the peripheral blood by 14 days after the first infusion, and approximately 20% of the patient's CD3 cells in the CSF were of donor origin, which suggested that successful transit of virus-specific T cells to the central nervous system was occurring (Fig. 2A). Donor virus-specific T cells with an effector memory phenotype were detected in the recipient's CSF for at least 250 days (Fig. 2B, and Fig. S2 in the Supplementary Appendix), which suggested that activated type 1 helper T cells homed to the CSF.
Mass cytometry performed with blood samples that were obtained at multiple time points after adoptive transfer showed that the donor virus-specific T cells were predominantly CD4 cells and that they expanded and differentiated in vivo to give rise to multiple subpopulations with phenotypic characteristics of both memory and activated T cells, a finding consistent with an antiviral response (Figs. S3 and S4 in the Supplementary Appendix). We verified that BK virus–specific T cells recognize JC virus (Fig. S5 in the Supplementary Appendix) and identified individual epitopes, against which the virus-specific T cells are directed (Table S6 in the Supplementary Appendix).

**Discussion**

In this proof-of-principle study, we took advantage of shared epitopes among the members of the *Polyomaviridae* family by using third-party, ex vivo–expanded, virus-specific T cells directed against BK virus to treat three patients with PML. In one patient, ruxolitinib treatment was discontinued when PML became evident, but there was continued neurologic deterioration. In the patient with HIV infection, HAART was initiated after the diagnosis of PML, and the CD4 cell count increased, but his clinical condition worsened. A role for the recovery of inherent immune response as the cause of clinical improvement in patients with PML cannot be ruled out, but improvement in all three patients coincided with virus-specific T-cell infusion. After infusion of virus-specific T cells, Patients 1 and 3 had clinical improvement in association with the disappearance of JC virus from the CSF. These responses occurred despite persistent T-cell immunodeficiency, which supports the possibility that the response was mediated by the adoptively infused virus-specific T cells. Patient 2 had a 2.5-log reduction in the JC viral load and a cessation of the progression of symptoms of PML; however, because of her neurologic disability, she chose to enter hospice and died there. In view of the viral response, the lack of clinical recovery in this patient may have been related to irreversible central nervous system damage from PML or to an inadequate effect of the virus-specific T-cell treatment.

The development of IRIS in two of the patients suggests that the inflammatory response to the infection may have been induced by the infused T cells, since the T-cell infusion did not alter absolute T-cell counts in peripheral blood. Moreover, there was no enhancement of whitematter lesions seen on MRJ at the time of diagnosis of PML, and IRIS initially manifested as enhancement in imaging performed within 4 weeks after the first T-cell infusion.

We confirmed trafficking of the infused virus-specific T cells to the central nervous system, as reflected by the surrogate of their appearance in the CSF of Patient 1, in whom HLA-Bw6–positive donor T cells persisted in the CSF for more than 250 days. This finding shows that third-party HLA-mismatched allogeneic T cells can cross the blood–brain barrier, survive, proliferate, and putatively mediate an antiviral response. Moreover, we verified that BK virus–specific T cells recognize JC virus, and we identified individual epitopes against which they are directed. These immunogenic epitopes were predominantly HLA class II–restricted. The infused cells may have survived because of the underlying immunosuppressed state in these patients.

Although the infused virus-specific T cells were only partially HLA matched to the patient, we did not observe graft-versus-host disease in these patients, as has previously been reported in association with this therapeutic approach. The use of ex vivo–expanded, cryopreserved, and banked virus-specific T cells allows for the rapid selection of virus-specific T cells on the basis of the most closely HLA-matched third-party donor. An alternative approach would be to generate autologous or allogeneic HLA-matched virus-specific T cells. However, the generation of a patient-specific product could impede widespread clinical use, and it is not always possible to generate T cells from patients who have marked immunosuppression. The use of cytokines (interleukin-2 and -7) and vaccines has been tried as treatment for PML. However, their efficacy depends on the presence of precursor JC virus–specific T cells, and the time required to induce an effective T-cell response may limit clinical recovery.

Third-party–produced, “off-the-shelf,” partially HLA-matched, BK virus–specific T cells may serve as therapy for PML. Further study in a larger group of patients is required to determine the success rate, durability, and longer-term adverse events associated with this treatment.
Supported in part by an M.D. Anderson Cancer Center AML Moonshot Grant. The flow studies were supported by a grant from the National Institutes of Health (5R01CA061508-21) to Drs. Rezvani and Shpall and were performed in the Flow Cytometry and Cellular Imaging Facility, which is supported in part by the National Institutes of Health through a support grant to M.D. Anderson Cancer Center (CA016672). Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

**REFERENCES**


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