Efficacy of Recombinant Human Interleukin 7 in a Patient With Severe Lymphopenia-Related Progressive Multifocal Leukoencephalopathy

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In this study, we report the case of a patient with profound lymphopenia after allogenic bone marrow transplantation who developed severe progressive multifocal leukoencephalopathy. Single-agent recombinant human interleukin-7 therapy was associated with restoration of anti-John Cunningham polyomavirus (JCV) T-cell responses, JCV clearance from cerebrospinal fluid, and a dramatic clinical improvement.

Keywords. bone marrow transplantation; JC virus; progressive multifocal leukoencephalopathy; recombinant interleukin-7; T-cell lymphopenia.

Progressive multifocal leukoencephalopathy (PML) is a rare, devastating demyelinating disease of the central nervous system (CNS) that is caused by oligodendrocyte infection by John Cunningham polyomavirus (JCV) [1, 2]. Conditions associated with cellular immunodeficiency, such as human immunodeficiency virus (HIV) infection, hematological malignancies, transplantation, and immunosuppressive therapy with monoclonal antibodies such as natalizumab, which block trafficking across the brain–blood barrier, can cause JCV reactivation in the CNS, leading to PML [1, 2]. No specific antiviral treatment has shown clear clinical efficacy in PML [1, 2]. The lipid formulation hexadecyloxypropyl-cidofovir (CMX001) has shown activity against JCV in vitro [3]. One PML patient has also been treated successfully with a combination of CMX001 and recombinant human interleukin-7 (rhIL-7) [4], but additional studies are required to confirm the antiviral effect of CMX001 in vivo. The only therapeutic approach that has shown proven clinical efficacy in PML remains the restoration of systemic or local anti-JCV T-cell responses [1, 2]. This restoration can be achieved in patients with acquired immune deficiency syndrome (AIDS) through effective antiretroviral treatment, and in patients with natalizumab-treated multiple sclerosis through natalizumab withdrawal and plasma exchange [1, 2]. In contrast, there are no effective therapeutic options for patients who are HIV-seronegative with lymphopenia-associated PML.

Interleukin-7 is a cytokine essential for T-cell development and homeostasis [5]. Administration of rhIL-7 to normal and lymphopenic mice, nonhuman primates and humans results in widespread T-cell proliferation, increased T-cell numbers and function, modulation of peripheral T-cell subsets, and increased T-cell receptor repertoire diversity [5]. These effects raise the prospect that IL-7 might be beneficial in the treatment of lymphopenia and its complications, including PML [4–6]. Progressive multifocal leukoencephalopathy secondary to severe lymphopenia after bone marrow transplantation is associated with a high mortality rate [7, 8].

In this study, we report the case of a woman diagnosed with PML after allogenic bone marrow transplantation for secondary myelofibrosis, in whom single-agent rhIL-7 therapy was associated with an improvement in anti-JCV T-cell responses, JC virus clearance from cerebrospinal fluid (CSF), and a dramatic clinical improvement.

A 53-year-old woman was diagnosed in 1992 with polycythaemia vera after portal vein thrombosis. She was successively treated with hydroxycarbamide, mercaptopurine, and pipobroman. In 2000, she gradually developed secondary myelofibrosis associated with splenomegaly and profound pancytopenia. In 2005, clonal homozygous mutation (V617F) of the Janus kinase 2 (JAK2) was identified in peripheral blood myeloid lineage. However, the patient was not eligible for JAK2 inhibitor treatment. In February 2011, she received allogenic bone marrow transplantation from a mismatched unrelated donor (9 of 10 matched pairs) using peripheral blood stem cells. The myeloablative conditioning

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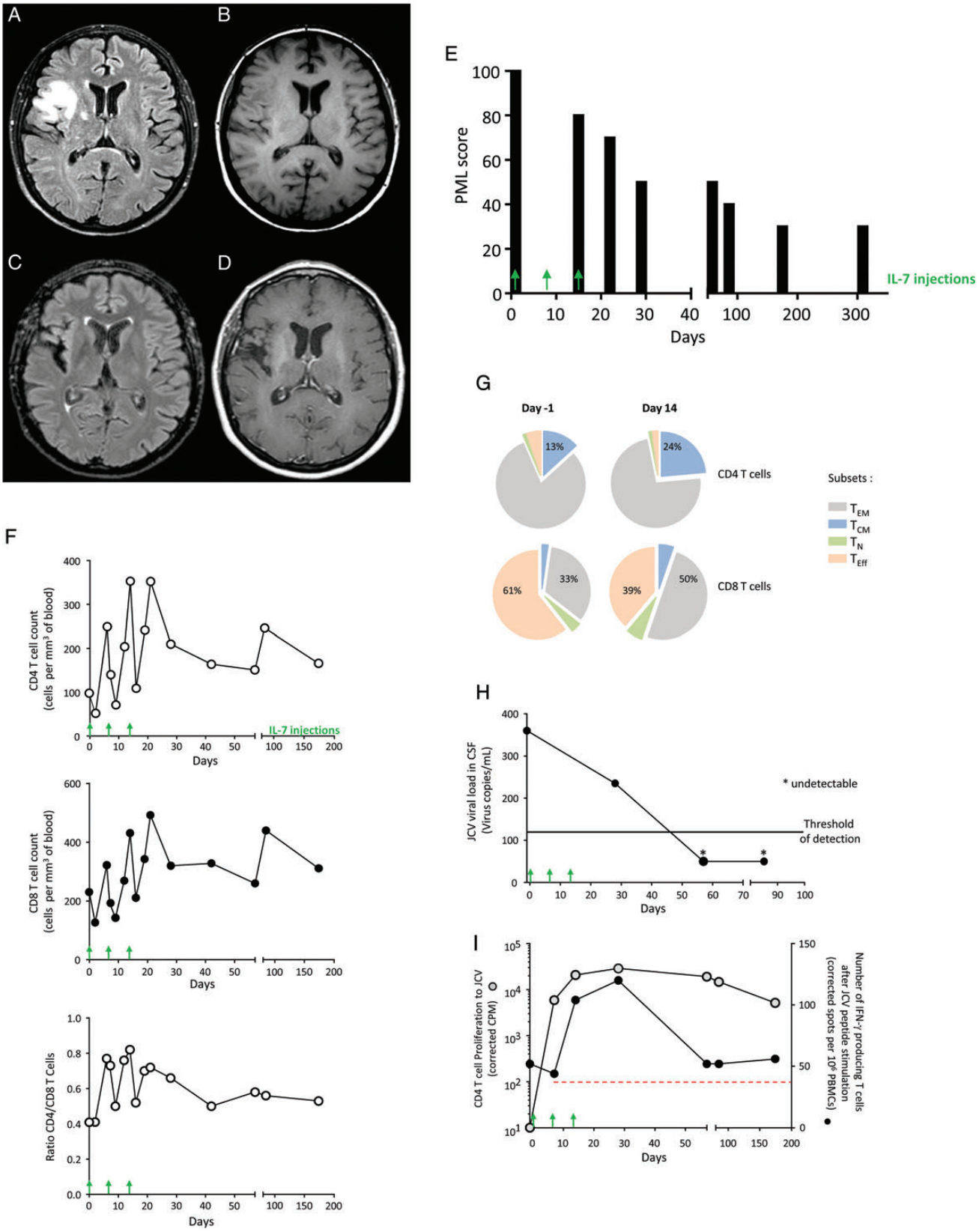

Figure 1. Magnetic resonance (MR) images, neurological score, and immunovirological parameters. A and B show T2-weighted and T1-weighted MR images 2 weeks before recombinant human interleukin-7 (rhIL-7) treatment. C and D show T2-weighted and T1-weighted MR images 18 months after rhIL-7 treatment. E shows the time course of the progressive multifocal leukoencephalopathy neurological score[9] after the 3 injections of rhIL-7 (green arrows). F shows the CD4 T-cell count (top), CD8 T-cell count (middle), and CD4/CD8 T-cell ratio (bottom) at various times after the 3 IL-7 injections (green arrows).

regimen included fludarabine, busulfan, and antithymocyte globulin. Graft-versus-host (GVH) disease prevention included short-term methotrexate and cyclosporine. In August 2011, she developed a grade 2 cutaneous GVH disease that resolved after 2 months of corticosteroid treatment. Cyclosporine was stopped in October 2011. Therefore, the patient was no longer on therapeutic immunosuppression. In November 2011, a full donor chimerism was obtained, and the patient became JAK2-mutation negative. However, hematological recovery was incomplete, with 11 g/dL hemoglobin, 850 neutrophils/μL, 600 lymphocytes/μL, and 30 000 platelets/μL. The patient received antimicrobial prophylaxis with phenoxymethylpenicillin, acyclovir, pyrimethamine-sulfadoxine, and pentamidine inhalation.

The patient presented with neurological symptoms in February 2012 and gradually developed left brachiofacial paresthesia. Computed tomography (CT) of the brain showed 2 hypodense white-matter lesions in the right hemisphere, whereas magnetic resonance imaging (MRI) revealed multiple subcortical white-matter lesions (hyperintense on T2-weighted images, hypointense on T1-weighted images, with no gadolinium enhancement) in the right frontal and parietal lobes. The lesions were consistent with demyelination and suggestive of PML (data not shown). Polymerase chain reaction (PCR) revealed JCV DNA in CSF (360 copies/mL), confirming the diagnosis of PML. The patient had been JCV-seropositive before bone marrow grafting (STRATIFY JCV Antibody, Focus Diagnostics). At PML onset, she had moderate lymphopenia (902/μL) but severe CD4 T-cell lymphopenia (135/μL) and a normal absolute CD8 T-cell count (CD4/CD8 ratio: 0.37). The immunoglobulin G level was normal (11.2 g/L). Human immunodeficiency virus serostatus determined before (January 2011) and after (May 2011, August 2011, and June 2012) bone marrow transplantation was negative. Thoracic CT performed for nonproductive cough with fever showed an interstitial syndrome. Bronchial lavage revealed *Pneumocystis jiroveci* cysts (prophylaxis with pyrimethamine-sulfadoxine and pentamidine was stopped in February 2012), and she was treated with atovaquone. She became afebrile 3 days later but rapidly developed progressive left-sided sensory-motor impairment. Physical examination showed severe hemiparesis (rating 1/5) with multimodal sensory impairment on the left side of the body. Her neurological status rapidly worsened, with the onset of swallowing disorders and dysarthria, and she became totally bedridden. Myoclonus occurred in the left upper limb but resolved, without recurrence, after the introduction of levetiracetam. Magnetic resonance imaging performed on 14 March 2012 showed progression of the brain lesions (Figure 1A and B).

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY TREATMENT

Agreement was obtained from the French health authorities Agence Nationale de Sécurité du Médicament et des Produits de Santé for compassionate use of CYT107, a rhIL-7, and the patient gave her fully informed consent for this treatment. The drug was provided by Cytheris (France). A single cycle of CYT107 was administered, consisting of 3 subcutaneous injections on day 0 (April 4, 2012), day 7 (April 11, 2012), and day 14 (April 18, 2012). Each injection delivered 20 μg/kg, for a total dose of 1200 μg per injection. Each injection was well tolerated, except for "flu-like" symptoms that were treated with paracetamol. We used a published PML-specific clinical score to assess neurological changes [9]. Her clinical neurological status stabilized on day 7. A motor improvement in the left upper limb was noted on day 14, along with an improvement in dysarthria. Her neurological status continued to improve gradually (Figure 1E).

Her CD4 and CD8 T-cell counts in blood rose with a sawtooth pattern, ranging from 52 to 353 and 232 to 492 cells/μL, respectively (Figure 1F). Six months after rhIL-7 administration, her absolute CD4 and CD8 T-cell counts were 166 and 311 cells/μL, respectively (Figure 1F). The CD4/CD8 T-cell ratio also increased but remained inverted (Figure 1F). Within CD4 T-cells, expansion of the central memory subset (T_{CM}) was noted (Figure 1G). Changes were also observed in the CD8 T-cell pool, with expansion of the effector memory subset (T_{EM}) and a decrease in the proportion of cells with a terminally differentiated effector phenotype (T_{EFF}) (Figure 1G). JC virus load in CSF fell below the detection limit within 2 months after the first rhIL-7 injection (Figure 1H). Because JCV-specific memory CD4 T-cells play a critical role in controlling JCV replication in the CNS and in preventing PML [9, 10], we examined the patient's memory CD4 T-cell proliferation to JCV [9, 10]. Strong proliferation to recall antigens is the feature of CD4

Figure 1 continued. G shows the distribution of CD4 and CD8 T-cell subsets determined by flow cytometry the day before the first rIL-7 injection and at the posttreatment peak blood CD4 T-cell count (day 14): T_{CM}, central memory T cells (CD45RA$^-$ CCR7$^+$ CD4$^+$ CD3$^+$); T_{EM}, effector memory T cells (CD45RA$^-$ CCR7$^-$ CD4$^+$ CD3$^+$); T_N, naive T cells (CD45RA$^+$ CCR7$^+$ CD4$^+$ CD3$^+$); T_{EFF}, effector T cells (CD45RA$^+$ CCR7$^-$ CD4$^+$ CD3$^+$). (H), JC virus (JCV) load in cerebrospinal fluid was measured by quantitative polymerase chain reaction with a detection limit of 125 copies/mL [9]. I shows proliferative CD4 T-cell responses to purified JCV (MAD-4 strain, ATCC) after 6 days of exposure [9, 10]. Proliferation was measured by means of ^{3}H-thymidine incorporation [9, 10]. Results (gray dots) are expressed as median counts per min (CPM) of activated wells (in quadruplicate) minus median CPM in untreated wells (corrected CPM). With the exception of day 0, all JCV-specific CD4 T-cell proliferative responses were positive. Criteria of positivity were previously published [9]. Peripheral blood mononuclear cells (PBMCs) were also stimulated overnight with a pool of JCV peptides overlapping JCV VP1 protein, and interferon-γ secretion was measured with an enzyme-linked immunospot assay performed in duplicate (black dots) [9, 11]. Results are expressed as mean spots per 10^6 activated PBMCs minus the mean spots obtained in untreated wells (corrected spots). The threshold of positivity was 40 spots per 10^6 PBMCs [10] (red line in I). Abbreviations: CSF, cerebrospinal fluid; IFN, interferon; PML, progressive multifocal leukoencephalopathy.

T_{CM}, and this subset helps memory CD8 T cells to become efficient secondary effectors [9]. The patient had no detectable JCV-specific proliferative CD4 T-cell response before rhIL-7 injection (Figure 1I), but sustained recovery of JCV-specific CD4 T-cell proliferation was noted within 1 week after the first rhIL-7 injection (Figure 1I). In addition, an increased frequency of interferon (IFN)-γ-producing JCV-specific T-cells was observed in response to overnight activation with overlapping peptides covering JCV VP1 protein (ex vivo IFN-γ enzyme-linked immunospot assay) [9, 11], for up to 30 days after the first rhIL-7 injection (Figure 1I). As previously shown, these IFN-γ-producing cells, induced by short-term activation, mainly corresponded to effector memory CD8 and CD4 T-cells [11]. The frequency of JCV-specific IFN-γ producing cells then declined as JCV was cleared from CSF (Figure 1H and I). Four months after rIL-7 treatment, the patient's only persistent symptom was a proprioceptive deficit in the left lower limb, and she was able to walk with a cane. Eighteen months after rhIL-7 administration, she was only slightly restricted in her activities of daily living, and brain MRI showed regression of the initial white-matter abnormalities, leaving focal cerebral atrophy (Figure 1C and D).

Thus, rhIL-7 was associated with effective anti-JCV T-cell recovery and virus clearance from CSF, along with a rapid and dramatic clinical improvement. This result contrasts sharply with the poor neurological outcomes and high mortality generally associated with PML secondary to allogenic bone marrow grafting [7, 8]. Rapid restoration of effective anti-JCV T-cell immunity, thereby blocking JCV replication in the CNS and limiting the extension of demyelinating lesions, is crucial to improve PML outcome. In our patient, the 3 rIL-7 injections led to a sawtooth pattern of circulating CD4 and CD8 T-cell recovery, with peak counts reaching up to 3.5 times the pretreatment values. The fall in the number of circulating T-cells observed just after rhIL-7 administration might be related to tissue entry, possibly after up-regulation of chemokine receptors and tissue homing receptors by rhIL-7, as suggested by several previous observations in humans and experimental models [12, 13]. Those observations also suggested that T-cell proliferation in response to IL-7 occurs within tissues, including lymph nodes, followed by partial migration to the blood compartment. Therefore, blood counts may not fully reflect the level of T-cell expansion triggered by rhIL-7 [12, 13]. In our patient, relative stabilization of the circulating T-cell count occurred 2 weeks after the third rhL-7 injection, at a level higher than before treatment, although the patient remained lymphopenic. A single cycle of 3 injections of rhIL7 was associated with an improvement in anti-JCV memory CD4 T-cell responses and control of JCV replication in the brain. Recombinant human interleukin-7 was well tolerated, with no immune reconstitution inflammatory syndrome (IRIS) and no episodes of graft-versus-host disease during 18 months of follow-up. However, in PML patients, IRIS, which may lead to neurological deterioration, is clearly a potential adverse effect of most therapeutic approaches aiming to restore immune function and should therefore be considered in future studies of rIL-7 administration. Note that the risk of IRIS appears lower in patients with profound lymphopenia, such as AIDS patients with PML [9], in comparison with patients with natalizumab-associated PML, who have no CD4 or CD8 T-cell depletion and in whom plasma exchange leads to massive afflux of activated autoimmune and anti-JCV T-lymphocytes [1]. The spectacular clinical response observed in our patient warrants a controlled trial of rhIL-7 in PML associated with profound lymphopenia.

Acknowledgment

Potential conflicts of interest. T. C. was employee and shareholder of Cytheris from December 2, 2007 to October 15, 2013.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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