Fatal Epstein-Barr virus-associated lymphoproliferative disorder following treatment with a novel mTOR Inhibitor for relapsed chronic lymphocytic leukemia

We report on a patient with relapsed chronic lymphocytic leukemia (CLL) treated with the novel mTOR inhibitor RAD001 within a phase II clinical trial. Although the patient initially responded to therapy, RAD001 was discontinued after 32 weeks due to progression and fludarabine-based chemotherapy was started. The patient subsequently developed a rapidly fatal Epstein-Barr-virus-associated lymphoproliferative disorder, clonally unrelated to the CLL. The clinical course suggests caution when using newer immunosuppressive drugs for treatment of CLL, especially in the context of additional purine analog therapy.

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Inhibition of mammalian target of rapamycin (mTOR) represents an attractive target in chronic lymphocytic leukemia (CLL).[1,2] RAD001 is a novel oral mTOR inhibitor. It is used as an immunosuppressant in solid organ transplants[3] and is being developed as an anti-cancer agent.[4] We report the case of a rapidly fatal EBV-associated lymphoproliferative disorder following treatment with RAD001 within a phase II clinical trial. A 70-year old woman with B-CLL was previously treated with chlorambucil/prednisone, cyclophosphamide/doxorubicin/vincristine and splenectomy for autoimmune hemolytic anemia. The patient had no other relevant medical conditions or medication. Treatment with RAD001 (5 mg daily) was begun at a white blood cell (WBC) count of 150,000/μL. WBC levels initially dropped to 100,000/μL and then stabilized (Figure 1A). The study medication was well tolerated with no side effects. After 24 weeks, leukocyte counts rose consistently and RAD001 was discontinued at a WBC count of 200,000/μL after 32 weeks. The patient was subsequently treated with one course of fludarabine/cyclophosphamide administered four days after discontinuation of RAD001, and WBC counts immediately dropped. Three weeks later the patient presented to the emergency unit with a temperature of 39°C and fatigue. Tests showed elevated serum C-reactive protein and a chest X-ray confirmed interstitial pulmonary infiltrates. The patient was admitted into hospital, i.v. antibiotics were administered and antiviral prophylaxis with valciclovir continued. Two days later, a new chest x-ray revealed increasing pulmonary infiltrates. EBV-DNA was detected in the bronchoalveolar fluid and at extremely high levels in plasma and peripheral blood lymphocytes (Figure 1B and C). Despite broadened antiviral and antifungal therapy, patient died of multi-organ failure. At autopsy, the lungs revealed extensive infiltrates with widespread necrosis. Histologically, the lungs, liver, bone marrow, kidney and lymph nodes showed infiltrates of small lymphocytes consistent with B-CLL. A polymorphous large cell infiltrate with Reed-Sternberg-like giant cells and necrosis was identified in the lungs and in other organs (Figure 2A). EBV-DNA was detected by PCR in these tissues (Figure 1C) and in-situ hybridization revealed abundant expression of Epstein-Barr-virus encoded early RNAs (Figure 2B). The large cells showed expression of CD20, CD30, and latent membrane protein-1 of EBV (Figure 2 C-D). PCR from the splenectomy specimen and lung tissues with EBV-positive large cell proliferation showed an identical monoclonal B-cell rearrangement in both specimens, representing the B-CLL clone. Two additional clonal bands were identified in the lung, indicative of a second monoclonal B-cell proliferation (not shown).

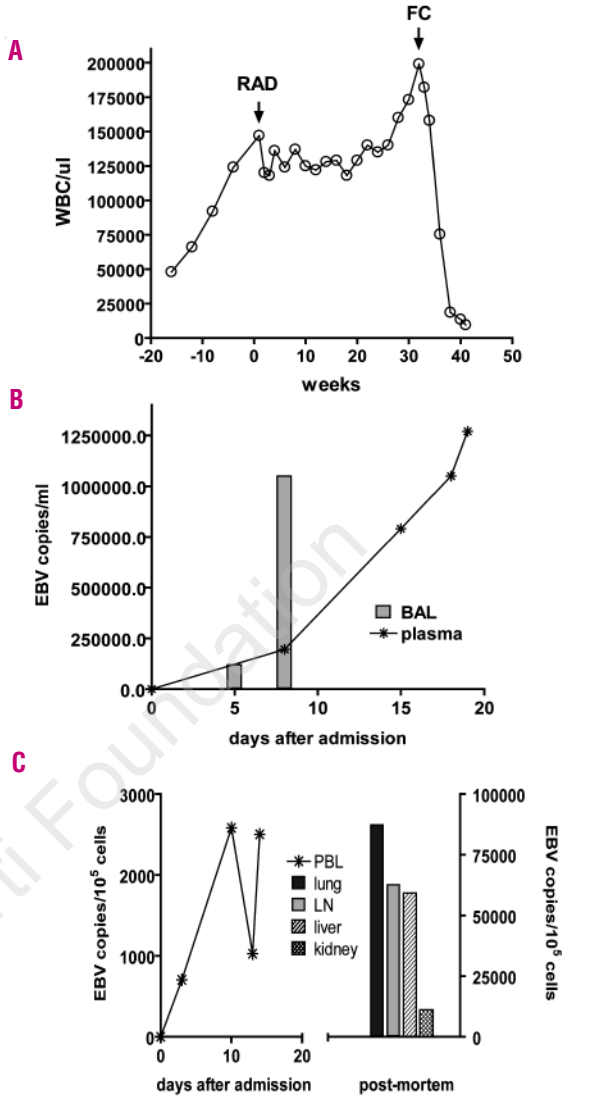

Figure 1. Range of WBC levels and EBV viral load during the course of the patient's illness. (A) Peripheral blood WBC levels during the course of the patient's disease. The beginning of treatment with RAD001 and the time point of chemotherapy with fludarabine and cyclophosphamide (FC) are indicated. (B) EBV-DNA load in the bronchoalveolar fluid (BAL, grey bars) and blood plasma as detected by PCR during the final phase of the patient's illness. (C) EBV-DNA detected by PCR in the cellular fraction during the final phase of the disease and at autopsy. Left panel: EBV-DNA levels in peripheral blood lymphocytes (PBL); right panel: EBV-DNA detected post-mortem in lungs, lymph nodes, liver and kidneys.

EBV-associated lymphoproliferative disorder (LPD) is a complication arising in solid-organ transplant patients and patients with hematologic malignancies receiving allogeneic stem cell transplantation.[5] The disorder results from uncontrolled proliferation of EBV-transformed B-cells in the setting of chronic immunosuppression.[5] The degree of immunosuppression, especially with respect to T-cell function, determines the risk of developing LPD. Fludarabine, a

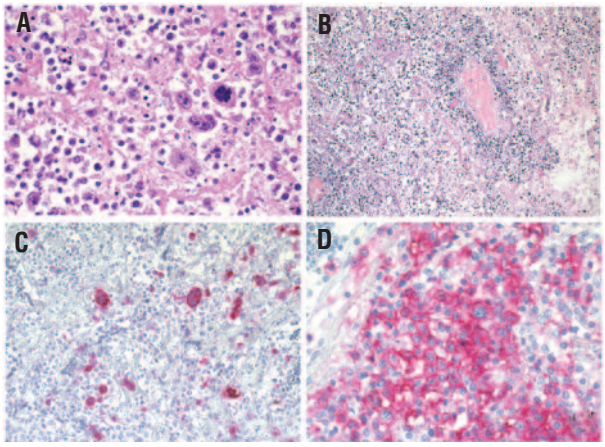

Figure 2. Histology, immunohistochemistry and *in situ* hybridization of EBV-associated lymphoproliferation in lung tissues. A. The lungs show a polymorphic lymphoid infiltrate with a mixture of small and large cells, occasional Reed-Sternberg like cells and apoptotic figures. Tissue was stained with hematoxylin and eosin (H&E) and examined through a 40×/0.75 numerical objective lens using a Zeiss Axioskop 2 plus microscope fitted with a Zeiss Axiocam camera (Carl Zeiss AG, Germany). Images were acquired using AxioVision software (Carl Zeiss AG, Germany). B. *In situ* hybridization for Epstein-Barr early RNAs (EBERs) reveals strong nuclear staining of most cells. Note angiocentric distribution of positive cells, 100× total magnification using a 10×/0.30 objective lens. C. Many large cells show strong expression of Epstein Barr latent membrane protein 1 (LMP-1). ABC technique, 200× total magnification using a 20×/0.50 objective lens. D. The neoplastic cells show strong expression of CD20. ABC technique, 400x total magnification using a 40×/0.75 objective lens.

purine analog effective in CLL, leads to T-cell dysfunction by inducing a marked decrease in $CD4^+$ and $CD8^+$ lymphocytes. The use of fludarabine in the treatment of CLL has resulted in increased opportunistic infections, including herpes viruses. However, EBV-positive lymphoma following fludarabine-based chemotherapy is rare.[6] T-cell counts were not evaluated within our clinical trial, so there is no data available concerning $CD4^+$ and $CD8^+$ lymphocyte levels during the course of therapy. However, before starting treatment with RAD001, the patient's absolute T-cell counts were within normal range. RAD001 is well-tolerated in the setting of solid-organ transplantation.[3] However, patients with CLL suffer from an immune deficiency which may make them increasingly susceptible to LPD which can be induced by altered T-cell function. It is possible that administration of fludarabine within a short interval after discontinuation of RAD001 contributed to patient vulnerability for EBV-reactivation. The combination of RAD001 followed by fludarabine is likely to induce profound T-cell depletion similar to that observed with alemtuzumab.[7] The patient was also heavily pretreated which contributed to impaired immune function. On the other hand, mTOR seems to be important for EBV transformation. Inhibition of mTOR prevents proliferation of EBV-transfected transformed B-lymphocytes *in vitro* and *in vivo*.[8] Therefore, the LPD in our patient could also have been a consequence of insufficient mTOR suppression due to low serum levels of RAD001. In fact, mTOR inhibition might have controlled the malignant EBV-positive B-cell clone with disease progressing only after termination of RAD001. EBV-positive B-cell clones are dependent on IL-10[9]. They produce high levels of IL-10 by an autocrine feedback loop while mTOR inhibition suppresses IL-10 production.[10] Indeed, in our patient, IL-10 was undetectable in the serum during therapy with RAD001 but increased dramatically during the last days of the patient's life (*not shown*).

In conclusion, while mTOR inhibitors could have a promising role in cancer therapy, they should be used with caution in patients with pre-existing immune disorders such as heavily pretreated CLL.

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