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LETTER TO THE EDITOR

Sirolimus addition to tacrolimus-based immunosuppression induces complete remission of post-transplant lymphoproliferative disorder in a liver transplant recipient

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Post-transplant lymphoproliferative disorders (PTLDs) represent the major cause of malignancy-related mortality after solid organ transplantation [1,2]. Primary or reactivated Epstein–Barr virus (EBV) and cytomegalovirus (CMV) infection, treatment with T-cell antibodies, and especially the amount of overall immunosuppression are known risk factors [3,4]. Reduction or withdrawal of immunosuppressive medication, particularly of calcineurin inhibitors (CNIs), is effective, but carries the risk of graft rejection [5]. Additionally, monoclonal lymphomas can be treated with B-cell antibodies (rituximab) and/or chemotherapy, radiation or occasionally surgical resection [6]. Recently, *in vitro* studies and case reports have suggested that mammalian target of rapamycin (mTOR) inhibitors may lead to regression of lymphomas while offering safe prevention of graft rejection because of their immunosuppressive properties. The majority of cases describe PTLDs in kidney transplant recipients, who received mTOR inhibitors with or without rituximab while CNIs were stopped [7–9]. Data on the treatment of PTLDs after liver transplantation are very sparse [4].

Here we present the first liver transplant recipient with complete remission of a highly malignant monoclonal B-cell lymphoma after sirolimus add-on to a triple immunosuppressive regimen without CNI withdrawal: A 20-year-old woman underwent liver transplantation in 1998 for cirrhosis because of primary sclerosing cholangitis (PSC). The diagnosis of PSC and ulcerative colitis (UC) was obtained 10 years beforehand. At the time of transplantation, UC was inactive. The patient was positive for EBV and CMV antibodies indicating past infection. As a result of negative CMV antibodies of the donor, no CMV prophylaxis was given. Serologies for hepatitis B, hepatitis C, and human immunodeficiency virus were negative throughout. Initial immunosuppression after transplantation comprised of cyclosporine A and steroids. Neither antilymphocyte nor IL-2 receptor antibodies were given at any time.

Within the first year after transplantation, but also thereafter in total seven biopsy proven, acute rejection episodes occurred always responding promptly to steroid pulse therapy. Therefore, maintenance immunosuppression was switched to tacrolimus, mycophenolate mofetil (MMF), and steroids in 2000. In September 2004, MMF was substituted by azathioprine (AZA) because of active UC.

In September 2007, an ulcerative lesion of about 7 cm in diameter was incidentally found in the terminal ileum during routine screening colonoscopy for UC. Biopsy revealed a monoclonal diffuse large B-cell lymphoma infiltrating the small intestine (Fig. 1). Immunohistochemistry showed CD20 positive blasts and a slight bcl2-positivity. The proliferation rate was 70% determined by the proportion of MiB1-positive cells diagnostic for a highly malignant lymphoma. Epstein–Barr virus early RNA *in-situ* hybridization showed a moderate amount of EBV-positive cells. EBV-PCR in the peripheral blood, however, was just slightly positive and was stable throughout without antiviral therapy. Tumor staging including whole body scan did not reveal any other manifestations of the lymphoma.

Immediately after diagnosis, sirolimus was added (median achieved trough level 7.7 ng/ml, range: 4.5–14.2 ng/ml) to the immunosuppressive regimen with tacrolimus, AZA and steroids. As a result of the multiple previous rejection episodes in the past, tacrolimus was not stopped, but high-dose maintenance therapy was continued (median achieved trough level within 3 years prior to diagnosis: 13.9 ng/ml, range: 9.6–21.4 ng/ml; median achieved trough level after diagnosis: 10.3 ng/ml, range: 6.3–17.8 ng/ml). Nevertheless, 3 months later, control colonoscopy revealed complete macroscopic and histological remission of the lymphoma. Over the following months, tacrolimus was gradually tapered and eventually stopped 6 months after the diagnosis of PTLD.

Currently, the patient receives sirolimus, AZA, and steroids. Multiple biopsies of the intestine and lymph nodes during a laparotomy for hernia repair in 2008 were benign. So far, 2 years later, colonoscopies and imaging studies performed every 3 months did not reveal recurrence of PTLD. Additionally, no further rejection episodes have occurred.

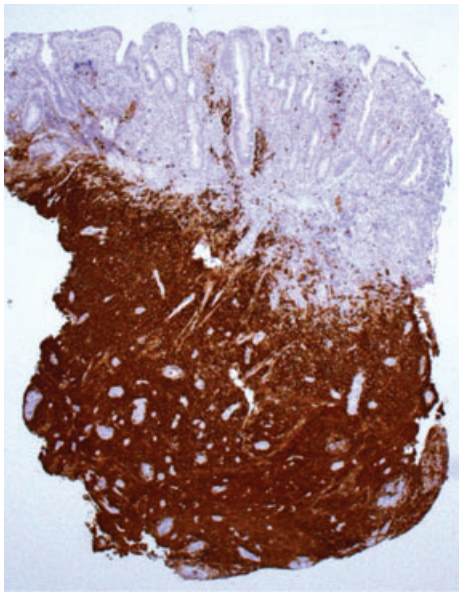

Figure 1 Biopsy of ileal mucosa. Immunohistochemical staining on CD20 shows brown staining of dense submucosal and focally intramucosal atypically located on lymphatic infiltrates of B-cells. These, stained with the proliferation antibody MIB1, revealed high proliferative activity indicating the diagnosis of high grade malignant B-cell lymphoma.

As a result of their antiproliferative, antiangiogenic, and proapoptotic effects, mTOR inhibitors have been suggested to be a therapeutic option in several malignancies. The mTOR signaling pathway is constitutively activated not only in the most solid tumors, but also in PTLDs, regardless of EBV genome expression [10]. It was further demonstrated that rapamycin directly inhibits the *in vitro* and *in vivo* proliferation of EBV-infected B lymphoblastoid cell lines from patients with PTLD by arresting cells in the G1 phase of the cell cycle [11]. Few case reports of solid organ transplant recipients have demonstrated the efficacy of sirolimus in PTLD also *in vivo* [4,5,7–9]. However, according to the current hypothesis in all cases published so far, CNIs were stopped or significantly reduced the same time mTOR inhibitors were started. Furthermore, surgical resection was performed when applicable or when rituximab and/or chemotherapy was added. In contrast, in our patient, solely the addition of sirolimus to a high-dose CNI-based triple immunosuppressive regimen led to a complete remission. Based on monoclonality, high proliferative activity and the presence of CD20 positive blasts therapy with rituximab and chemotherapy with a combination of cyclophosphamid, doxorubicin, vincristine and prednisolone (R-CHOP) were initially planned. However, because of the patient's preference and the localized manifestation of the lesion, therapy was not directly started. Furthermore, as a consequence of multiple rejection episodes in the past, tacrolimus was continued. Thus, complete remission because of sirolimus add-on treatment was achieved within 3 months. Given that sirolimus induced B-cell growth inhibition is reversed by the addition of tacrolimus *in vitro*, this was an unexpected clinical course [12]. However, recent data indicate that rapamycin enhances the number and quality of virus-specific memory $CD8^+$ T cells in mice [13]. As the function of EBV-specific $CD8^+$ memory T cells may be impaired in transplant recipients, this finding could be an explanation for the efficacy of sirolimus in PTLD – particularly since, rapamycin reverses HHV-8 positive Kaposi sarcomas and demonstrates significantly lower CMV infections in renal transplant recipients [14,15]. Another favorable factor for the rapid and complete response in our patient might have been the localized manifestation of the PTLD. In conclusion, sirolimus add-on therapy to a tacrolimus-based immunosuppressive regimen demonstrated to be an effective therapeutic option in PTLD. Complete withdrawal of CNIs is still recommended, but as demonstrated, a slow taper is possible and preferable to avoid rejection episodes. In addition, in patients with limited PTLD, start of chemotherapy may be delayed under strict surveillance to await response to mTOR inhibitors.

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