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### ► To cite this version:

B. Delapierre, O. Reman, J. Dina, C. Breuil, M. Bellal, et al.. Low dose Rituximab for pre-emptive treatment of Epstein Barr virus reactivation after allogenic hematopoietic stem cell transplantation. Current Research in Translational Medicine, Elsevier-Masson SAS, 2019, 10.1016/j.retram.2019.03.001 . hal-02153833

**HAL Id: hal-02153833**

**<https://hal-normandie-univ.archives-ouvertes.fr/hal-02153833>**

Submitted on 20 Jul 2022

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**Title:** Low dose Rituximab for Pre-emptive Treatment of Epstein Barr Virus Reactivation after Allogenic Hematopoietic Stem Cell Transplantation

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02.31.27.20.73

## **Abstract**

**Introduction:** The most used preemptive therapy for Epstein Barr virus reactivation post allogenic hematopoietic stem cell (HSCT) transplant is **Rituximab**, 375 mg/m<sup>2</sup>, once weekly until EBV viremia negativity. There is no data suggesting such a high dose.

**Objective:** We hypothesized that a lower dose of **Rituximab** would be as efficient with less toxicity.

**Patients:** In a retrospective, monocentric study, we analyzed 16 consecutive patients treated preemptively with low dose **Rituximab** for EBV reactivation post HSCT. Patients were treated with low **Rituximab** dose of 100 mg/m<sup>2</sup> weekly. Success was defined by a decrease of EBV viremia of 1 log<sup>10</sup> and below 1000 UI/ml, and the absence of Post-transplant lymphoproliferative disorder (PTLD).

**Results:** Success rate was 93.4 % (15/16). One (1/16, 6%) PTLD was diagnosed after preemptive therapy, despite a negative viremia.

**Conclusion:** A low dose of **Rituximab** of 100 mg/m<sup>2</sup> per injection for pre-emptive therapy of EBV reactivation post HSCT is safe and effective for preventing PTLD. Prospective, randomized, multicentric trials with larger number of patient are needed to determine the best rituximab dose.

**Keywords:** Hematopoietic Stem Cell Transplantation; Epstein Barr Virus; Post Transplantation Lymphoproliferative Disorder; Rituximab

## **Introduction**

Post-transplant lymphoproliferative disorder (PTLD) is a severe, potentially fatal complication following allogeneic hematopoietic stem-cell transplantation (HSCT), with a historically overall 80% mortality. The introduction of EBV monitoring by PCR, pre-emptive therapy and treatment with Rituximab, permits considerable improvements. However, mortality remains high (30%) in diagnosed PTLT, which represents 3.2 % of transplanted patients<sup>1</sup>. The incidence of EBV viremia (EBV-DNA) is in the range of 0.1-63 %. The time from the onset of EBV-DNA ( $\geq 1000$  UI/mL) to EBV-PTLDs is less than a week<sup>2</sup>. EBV-PTLD develops 2-4 months following HSCT, and 60% are diagnosed in the first year after transplantation. Risk factors for PTLT are age, T-cell depletion, graft-versus-host disease (GVH), HLA mismatch, and a second HSCT. T cell depletion of the graft (notably ATG administration) is one of the main risk factors of EBV-PTLDs<sup>3</sup>. Rituximab post HSCT reduces the incidence of EBV-DNA but the impact on the incidence of PTLT is conflicting and there is no reduction of overall survival compared to a pre-emptive approach<sup>4</sup>. Positive outcome is obtained for approximately 90% patients treated pre-emptively with Rituximab.<sup>1</sup> However, Rituximab is not a harmless treatment with an may increase the risk of infections and non-relapse mortality related to prolonged immunodeficiency<sup>4</sup>. Preemptive Rituximab is usually used at the dose of 375 mg/m<sup>2</sup>, weekly until EBV viremia negativity. Usually, 1 to 4 doses are necessary and sufficient. <sup>1</sup> There is no data suggesting the necessity of such a high dose in the setting of pre-emptive therapy, without overt PTLT. Interestingly, in the Maloney *et al.* phase I study with Rituximab in 1994, a single 100 mg/m<sup>2</sup> injection was sufficient to deplete all CD 20 cells in the peripheral blood.<sup>5</sup> We hypothesized that a low dose of Rituximab of 100 mg/m<sup>2</sup> would be safe and efficient in this setting and report our experience.

## **Objectives**

The main objective was to evaluate the efficacy of low dose Rituximab for EBV reactivation after hematopoietic stem cell transplantation.

Secondary objectives were to determine the incidence of infection, GVHD (acute and chronic) and NRM.

## **Patients and Methods**

We retrospectively analyzed HSCT patients with EBV reactivation and treated with Rituximab at the Institut d'Hématologie de Basse Normandie between August 2012 and September 2017.

### **EBV-DNA Load Monitoring**

EBV viremia was monitored weekly or every 2 weeks depending on risk factors from d30 post transplantation to d100. Afterwards, EBV-DNA was monitored every two weeks (or more frequently in case of positive sample), until d180 and at every outpatient appointment until d365. The tests used are validated for the follow-up of EBV DNA quantification of immunocompromised patients. The whole blood samples were extracted by QIASymphony methods. The nucleic acids extraction was carry out using 400  $\mu$ L of sample and eluted in 90

µL of buffer AE (Qiagen). The kits provide all necessary reagents optimized for reliable EBV DNA detection and quantitation for in vitro diagnostic use, *artus* EBV. The *artus* EBV QS-RGQ Kit is part of the QIA symphony RGQ, with an automated workflow from sample to pathogen detection. The amplification was carry out on the Rotor-Gene Q real time PCR cyclers.

### **Preemptive treatment of EBV Viremia**

Only patients with detectable EBV-DNA and no symptoms were eligible for preemptive therapy. Patients with probable or proven PTLD were excluded. Patients with EBV-DNA > 1000 UI/ml have a reduction of immunosuppressive therapy if possible. Patients with persistent EBV-DNA > 1000 UI/ml or without possibility of reduction of immunosuppressive were treated preemptively with Rituximab (standard dose or low dose) weekly until decreased of EBV-DNA of 1 log 10 and below 1000 UI/ml.

### **Definitions**

Successful treatment was defined by a decreased of EBV-DNA by 1 log<sup>10</sup> and below 1000 UI/ml and the absence of PTLD. Side effects **were** graded according to Common Terminology for Adverse Event v4 (CTCAE v4). Bacterial, viral, fungal and parasitic infections were recorded. Acute Graft versus Host Disease (aGVHD) was analyzed according to 1994 criteria. Only grade 2 to 4 aGVHD were analyzed and patients with aGVHD before Rituximab **use** were excluded. Chronic GVHD (cGVHD) was diagnosed and graded according to National Institutes of Health consensus. Only moderate to severe cGVHD were analyzed and patients with cGVHD before **introduction of** Rituximab were excluded. Overall survival (OS) was defined as the time from HSCT to the last follow-up or death, regardless of the cause. Non-relapse mortality (NRM) was defined as the time from HSCT to death in patient without relapse. Proven PTLD was diagnosed according to WHO classification with a biopsy. <sup>1</sup>

### **Results**

One hundred patients had a detectable EBV-DNA among the 199 patients that were treated with HSCT during the same period. Viral load was > 1000 UI/ml in 75 patients (75%), and > 10000 UI/ml in 39 patients (39%). Thirty-four patients (34%) were treated preemptively with Rituximab. Thirty-three (33%), 33/75 (44%) and 33/39 (84%) patients were treated when they had a detectable viremia, > 1000 UI/ml viral load or > 10000 UI/ml viral load, respectively. Asymptomatic patients with viral load below the threshold were not treated according to local policy. A standard dose of 375 mg/m<sup>2</sup> weekly was administered to 18 patients (18 %) and low dose of 100 mg/m<sup>2</sup> was administered to 16 patients (16 %). Rituximab dose selection was practitioner discretion.

Patient characteristics are summarized in Table 1. Treatment with Rituximab was successful in 15/16 patients (93 %). One PTLD was diagnosed whereas EBV-DNA was decreased below 100 UI/ml. This PTLD was a monomorphic CD 20 positive lymphoproliferation, diagnosed 240 days after transplantation.

At the end of the follow up, OS was 69.7 % (11 / 16). NRM was 6.3 % (1/16). Relapse rate was 37.5 % (6/16). Ten patients (62.5 %) in the low dose group had a grade 3-4 infection and

5/16 (31.3 %) patients had grade 2-4 aGVHD. One patient had aGVHD before Rituximab injection. Six patients (37.5 %) developed cGVHD. One patient had cGVHD before Rituximab injection. (Table 2)

## **Discussion**

The present study is the first to evaluate a low Rituximab dose for preemptive therapy of EBV reactivation after allo HSCT.

The median number of injection was 2.3; which is comparable with others studies<sup>1</sup>, suggesting that the efficiency of low dose Rituximab is not linked with a larger number of injection. In our center, 18 others patients have received a median of Rituximab standard dose of 2.8 (data not shown).

These excellent success rate, superior to 90 % is comparable with others studies<sup>1</sup>. The interpretation of OS, NRM and relapse free survival has to be made with caution but seems similar to other studies. These retrospective study has some limitations: the limited number of patients, the heterogeneity in conditioning regimen and intensity, in diseases and in type of donor with inclusion of cord blood and haploidentical transplantation. The interpretation should be made with caution and prospective trials are needed to confirm or invalidate these results.

One patient had a proven PTLD at day 240 despite preemptive treatment of EBV Reactivation from day 60 to day 90 (four Rituximab injections) and a successful decreased of EBV-DNA below 100 UI/ml. This PTLD was a monomorphic, angiocentric CD 20+ PTLD and was successfully treated with surgical removal and reduction of immunosuppressive therapy. EBV-DNA at PTLD diagnosis was 2.71 UI/mL. PET-TDM show unique localized PTLD. Eighteen months after, patient is alive and well. EBV-DNA has an excellent negative predictive value for PTLD but these datas confirms that physiopathology of PTLD is not fully understood.

The indication of preemptive treatment is debated. There is no threshold value of EBV DNA for preemptive therapy in ECIL and SFGM-TC recommendations<sup>1,6</sup>. In Raberahona *et al* study, no EBV viremia threshold was associated with an overall survival difference. Long-term survival was similar in Rituximab-treated and non-treated in patients with detectable EBV-DNA, except for patients with EBV-DNA  $\geq 50,000$  UI/ml.<sup>7</sup> In Kalra *et al* study, authors suggest a potential threshold value DNA of 100,000–500,000 UI/mL.<sup>8</sup> On the other hand, in a large study of 319 allo-HSCTs, short and long term survival were not inferior in the patients who were treated with Rituximab for EBV DNA or PTLD compared with the other transplanted patients.<sup>9</sup> In two other studies, there was no statistically significant difference in term of non-relapse mortality or overall survival between patients who had an EBV reactivation and those who had not.<sup>10,11</sup>

Eventually, in another study, patients with EBV reactivation had longer PFS and OS than those without. In this study, number of circulating NK cells were significantly increased in patients with an EBV reactivation, suggesting a potential graft versus leukemia effect linked to NK cell proliferation.<sup>12</sup>

Rituximab should be limited to patients at highest risk of EBV-PTLD, because its use is associated with an increased risk of cytopenias and bacterial infections that can lead to non-relapse mortality.<sup>1</sup> In Petropoulou *et al* study, bacterial infection incidence was significantly higher in Rituximab patients compared with controls (55 % vs 35 %). The incidence of bacterial, viral, and fungal infections was 64%, 59%, and 23%, respectively. A slower B cells reconstitution was observed. Rituximab patients had higher non relapse mortality (NRM)

(35% vs. 15%) than controls, but these results were not significant<sup>4</sup>. Rituximab patients had significantly lower CD19 B at day 30 and day 60. Hypogammaglobulinemia was more frequent in Rituximab patients and received more immunoglobulin therapy<sup>4</sup>. Cases of persistent hypogammaglobulinemia associated with infections after Rituximab post HSCT have been described. Nishio *et al* suggested a higher toxicity of Rituximab when immunosuppression is ongoing such as Human immunodeficiency Virus (HIV) or HSCT. Flow cytometry revealed a decrease in B cell expressing CD 27. These data suggested that, in the setting of underlying immunosuppression such as HSCT, Rituximab may induce a similar immunocompromised situation as describe in common variable immunodeficiency (CVID)<sup>13</sup>. Infection rate in our study (62 %) was similar to Petropoulou *et al* study<sup>4</sup> and similar to patients treated with a standard dose of Rituximab of 375 mg/m<sup>2</sup> in our center (61 %, data not shown). Infections observed were mainly bacterial or presumed bacterial: 3 bacteraemias (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*), 3 pneumonitis, 1 severe colitis and 1 perineal abscess. One case of pneumocystis and 1 case of CMV disease were also observed.

A protective effect of Rituximab on Chronic Graft-Versus-Host Disease (cGVHD) has been suggested in patients treated with Rituximab for EBV replication post HSCT<sup>14</sup>. Several groups reported that Rituximab had a therapeutic effect on cGVHD<sup>15</sup>. The link between cGVHD and Graft versus leukemia (GVL) effect has been already demonstrated following HSCT. Ji S-M *et al* studied cumulative incidence of relapse (CIR) to evaluate the possible inhibiting role of Rituximab in graft versus leukemia effect. There was no increase in the CIR in the Rituximab group.<sup>14</sup>

Our study has limitations because of its retrospective nature; the limited number of patients included and because the study was not powered to determine differences in GVHD incidence. However, it was an exploratory study and these endpoints should be tested in another well-designed comparative study. Some of patients already suffered from GVHD when EBV replication occurred. The effect of Rituximab on aGVHD, cGVHD and the Graft versus Leukemia effect could not be evaluated. aGVHD, cGVHD and Relapse rate were similar to other studies. Relapse rate in patients treated with standard dose of Rituximab was lower (28 %, data not shown), but because the population was heterogeneous and not comparable with patients treated with low Rituximab dose, it is difficult to draw any conclusion on the relapse risk and the use of Rituximab.

## **Conclusion**

In this retrospective, monocentric study, the use of a low Rituximab dose (100 mg/m<sup>2</sup>) for pre-emptive therapy of EBV reactivation post HSCT was efficient with a success rate over 90 %. A prospective randomized multicentric trial with larger number of patient is needed to determine the best Rituximab dose.

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**Table1. Patients characteristics**

Characteristics	Low dose Rituximab 100 mg/m <sup>2</sup>
Number of patients (n)	16
Age at transplantation, median (range) , year	48 (17 ; 68)
Disease, n (%)	
<i>AML / MDS</i>	9 (57 %)
<i>ALL</i>	4 (25 %)
<i>Aplastic anemia</i>	1 (6%)
<i>Myelofibrosis</i>	1 (6 %)
<i>Lymphoma</i>	1 (6 %)
Donor type, n (%)	
<i>HLA-identical sibling</i>	3 (17 %)
<i>HLA-matched unreleated</i>	8 (46 %)
<i>Haploidentical</i>	4 (31 %)
<i>Cordblood</i>	1 (6 %)
Type, n (%)	
<i>PBSC</i>	10 (63 %)
<i>BM</i>	5 (31 %)
<i>CB</i>	1 (6 %)
Conditioning, n (%)	
<i>RIC</i>	7 (44 %)
<i>MAC</i>	9 (56 %)
EBV Viremia> 1000 (UI/ml), n (%)	16 (100 %)
EBV Viremia> 10000 (UI/ml), n (%)	14 (88 %)
Time to EBV Viremia from HSCT , median (range)	98.7 (26 ; 406)
Received ATG during conditioning, n (%)	
<i>Yes</i>	11 (68%)
<i>no</i>	5 (32%)

*AML: Acute Myeloid Leukemia; MDS: Myelodysplastic Syndrome; ALL: Acute Lymphoid Leukemia; PBSC: Peripheral Blood Stem Cell; BM: Bone Marrow; CB: Cord Blood; RIC: Reduced Intensity Conditioning; MAC: Myelo Ablative Conditioning; ATG: Antithymoglobuline*

**Table 2: Outcome**

<b>Outcome</b>	<b>Low dose Rituximab 100 mg/m<sup>2</sup></b>
Follow up, days, median (range)	396 (27 ; 1040)
Rituximab date from transplantation, days (range)	89 (29 ; 406)
Number of injection, n (%)	
<i>1 or 2</i>	9 (56%)
<i>3 or 4</i>	7 (44%)
EBV Viremia J0, median, UI/ml (range)	270000 (550; 3311000)
EBV Viremia J8, median, UI/ml (range)	7415 (0 ; 46700)
EBV Viremia J15, median, UI/ml (range)	230 (0 ; 2130)
EBV Viremia J22, median, UI/ml (range)	93 (0 ; 1400)
EBV Viremia J29, median, UI/ml (range)	0
PTLD, n (%)	
<i>Yes</i>	1 (6.2%)
<i>No</i>	15 (93.8 %)
Hemophagocytic syndrome, n (%)	
<i>Yes</i>	1 (6.2%)
<i>No</i>	15 (93.8 %)
<b>Success, n (%)</b>	
<b><i>Yes</i></b>	<b>15 (93%)</b>
<b><i>no</i></b>	<b>1 (7%)</b>
Death, n (%)	
<i>Yes</i>	5 (31.3 %)
<i>No</i>	11 (68.7%)
Non-relapse mortality, n (%)	
<i>Yes</i>	1 (6%)
<i>No</i>	15 (94 %)
Grade 3-4 Infection, n(%)	
<i>Yes</i>	10 (62%)
<i>No</i>	6 (38%)
Relapse, n (%)	
<i>Yes</i>	6 (38%)
<i>No</i>	10 (62 %)
Grade 2-4 aGVHD, n (%)	
<i>Yes</i>	5 (32 %)

	No	10 (63 %)
<i>aGVHD before Rituximab</i>		1 (6 %)
<hr/>		
Moderate to Severe cGVHD, n,%)		
	Yes	6 (38%)
	No	9 (56 %)
<i>cGVHD before Rituximab</i>		1 (6 %)

*PTLD: Post Transplant Lymphoproliferative Disorder; aGVHD: acute Graft versus host disease; cGVHD: chronic graft versus host disease*

### **List of abbreviations**

PTLD: Post Transplantation Lymphoproliferative Disorder

HSCT: Hematopoietic Stem Cell Transplantation

EBV: Epstein Barr Virus

EBV-DNA: Epstein Barr Virus viremia

aGVHD / cGVHD : acute/chronic Graft Versus Host disease

HLA: Human Leucocyte Antigen

ATG: Anti Thymocyte Globulin

OS: Overall Survival

NRM: Non relapse mortality

RFS: Relapse free survival