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EBV-ASSOCIATED POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE IN PATIENTS WHO RECEIVED ANTI-CD 20 AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION.

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Running head: Risk factors of PTLD after EBV infection in HSCT patients.

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Key point 1

Despite preemptive therapy with anti-CD20 for EBV-reactivation, PTLD still occurs. However, data on incidence risk and outcomes of PTLD in the preemptive era are scarce.

Key point 2

Despite anti CD20, the 2-year cumulative incidence of PTLD was 6.3 %. Multivariable analysis identified 4 risk factors associated with PTLD: HSCT from an unrelated donor, recipient HLA-DRB1*11:01, fever at diagnosis of EBV infection, and sex-mismatched

ABSTRACT

Post-transplant lymphoproliferative disease (PTLD) represents a severe complication associated with Epstein Barr Virus (EBV) infection after hematopoietic stem cell transplantation (HSCT). Although anti-CD-20 therapy is now used as a pre-emptive strategy for EBV reactivation, PTLD is still developed by some patients. Here we analyzed outcomes and risk factors associated with PTLD transformation in 208 transplanted patients, diagnosed with EBV DNAemia and receiving at last one course of rituximab.

The median age was 42.52 (8.35-74.77) years and median follow-up was 47.33 (3.18-126.20) months. Two-year overall survival (OS) of the entire cohort was 62.8 (95%CI 56.4-69.9) whereas 2-year cumulative incidence function (CIF) of PTLD was 6.3 % (95%CI 3.5-10.1%), for a median follow up of patients diagnosed with PTLD of 37.85 months. Multivariable analysis identified four risk factors associated with PTLD: HSCT from an unrelated donor, recipient human leukocyte antigen (HLA) DRB1*11:01, fever at diagnosis of EBV infection and sex-mismatched HSCT. Presence of more than two risk factors was associated with a higher risk of developing PTLD.

This retrospective study identifies risk factors associated with PTLD in EBV infected patients after HSCT and defines patient subgroups that may benefit from intensified pre-emptive strategies.

1. INTRODUCTION

Although there has been an improvement of supportive strategies of infectious complications in allogeneic stem cell transplantation (HSCT), Epstein-Barr virus (EBV)-related diseases remain a persistent issue associated with significant morbidity and mortality rate.^{1,2,3} Post-transplant lymphoproliferative disease (PTLD) represents the most fearsome complication associated with EBV infection in patients undergoing HSCT. The overall incidence of EBV-related PTLD varies among transplant centers: ranging between 1-14%, depending on donor type, intensity of conditioning regimen, use of T-cell depletion, and stem cell sources.⁴ Before the era of pre-emptive strategies based on anti-CD20 targeting, the incidence of early and late EBV-related PTLD was very high.⁵

The European Conference on Infections in Leukemia (ECIL) recently published guidelines for prevention and treatment of EBV-related diseases after HSCT, including PTLD.³ Assessment of pre-transplant EBV serology status and prospective monitoring of EBV-DNAemia by quantitative polymerase chain reaction (PCR) are highly recommended after high-risk allogeneic transplantations.³ Significant (or high-load) DNAemia is defined as EBV >10,000 copies/mL of plasma or whole blood.³ Persisting DNAemia can be associated with EBV-driven manifestations, such as EBV-driven organ disease or EBV-PTLD. Accepted histopathological criteria of EBV-PTLD are: the presence of lymphoid infiltrates, with or without disruption of underlying cellular architecture and evidence of EBV infection in many cells by in-situ hybridization (EBER-ISH).⁶ The World Health Organization (WHO) classification recognizes four types of morphological lesions: polyclonal early lesions (non-destructive lymphoid hyperplasia), polymorphic, monomorphic (B-cell or T/NK-cell) and classical Hodgkin lymphoma-type PTLD.⁷

Three main therapeutic approaches have been proposed for the prevention and treatment of EBV-PTLD: withdrawal of immunosuppression,⁸ administration of rituximab^{9,4,10,11,12} and use of EBV-specific cytotoxic T lymphocytes (CTLs).¹³ Rituximab therapy seems to be the

most effective strategy in the pre-emptive (decreasing the risk of PTLD transformation) and treatment settings. CTLs are not accessible to most transplant centers and the reduction of immunosuppression alone has shown limited efficacy and is not always possible in the context of graft-versus-host disease (GVHD). Therefore, prompt tapering of immunosuppression when possible in combination with pre-emptive rituximab treatment has been the mainstay in patients with EBV infection.⁴

New approaches to monitoring and pre-emptive treatment of EBV-DNAemia have reduced the incidence and improved outcomes of these patients.¹⁴⁻¹⁶ However, the mortality rate associated with this complication remains largely unsatisfactory⁴ and risk factors of EBV-PTLD transformation in patients already diagnosed with EBV-DNAemia and receiving pre-emptive rituximab remain poorly defined.

In this single center retrospective study, we analyzed the incidence, outcomes of EBV infection and risk factors associated with PTLD transformation despite the use of pre-emptive rituximab in patients after allogeneic HSCT.

2. PATIENTS AND METHODS

2.1 Patient population

We retrospectively analyzed all consecutive patients receiving at least 1 course of pre-emptive rituximab treatment for significant EBV DNAemia after HSCT in our center between 2010 and 2017.

The review of medical records was approved by the institutional ethical committee in agreement with the Helsinki Declaration of 1975, revised in 2008.¹⁷ Data collection was based on the European Society of Blood and Marrow Transplantation (EBMT) registry via the central data processing system "Project Manager Internet Server" (ProMISe). All patients had been regularly followed until April 2018 (or death). Pertinent clinical data including age, gender, disease diagnosis, comorbidities, type of transplant, human leukocyte antigen (HLA) matching, conditioning regimen, GVHD prophylaxis, acute and chronic GVHD, and other clinical complications were collected. Additional biological and clinical data such as EBV DNA viral load in whole blood, inflammatory markers, symptoms at onset of EBV infection and PTLD transformation, histopathological characteristics, phenotypic characterization of lymphocyte subsets at the onset of EBV infection, as well as number of courses of rituximab, and further treatment of PTLD were also retrospectively collected. Objective clinical data, including ancillary testing, laboratory results, and medical complications, and medication profiles, were abstracted through standardized chart review after each visit.

2.2 Transplant procedures

HLA-typing using high-resolution methods were used to select donors for allogeneic HSCT. Donor types included matched related donor (MRD), matched (MUD) and mismatched unrelated donor (MMUD), and haploidentical donors. Stem cell sources were peripheral blood (PB), bone marrow (BM), and cord blood (CB). Myeloablative conditioning regimens (MAC) included busulfan (3.2 mg/kg/day for 4 days) combined with cyclophosphamide (60 mg/kg/day for 2 days)¹⁸ or with fludarabine (30 mg/m²/day for 5 days) or total body irradiation (TBI) of 1200 cGy combined with cyclophosphamide (60 mg/kg/day for 2 days)¹⁹, or busulfan (3.2 mg/kg/day for 2-3 days) combined with Thiotepa (5 mg/kg/day for 2 days) and fludarabine (40 mg/m²/day for 4 days).²⁰ Reduced-intensity regimens (RIC) included Fludarabine-based protocols, according to the disease and age of the recipient. Standard

protocols of immunosuppression including cyclosporine and short-term methotrexate or cyclosporine with mycophenolic acid were used for GvHD prophylaxis. In addition, recipients of unrelated donor transplants received rabbit anti-thymocyte globulin or anti-lymphocyte globulin.²¹

2.3 EBV monitoring and EBV-PTLD diagnosis

Patients' EBV viral load was routinely monitored twice weekly by real-time quantitative PCR, as previously described²² until infection or EBV disease resolution and according to patients' risk profile.³ Of notice, a change in EBV load determination took place during the study period to standardize molecular quantification of EBV to requirements of the WHO Expert Committee on Biological Standardization (Abbott RealTime EBV assay, Abbott Molecular Inc., Des Plaines, IL, USA). A conversion factor was applied to convert results expressed in copies/milliliters (ml) to international units (IU)/ml.²² EBV infection (or reactivation) was defined as any positive detection of EBV DNA in blood. Significant EBV-DNAemia was characterized by an increase of EBV load above the threshold of 4.5 log₁₀ copies/ml or 3.8 log₁₀ IU/ml. For standardization, after conversion, all results were expressed in log₁₀ IU/ml.

To rule out that patients with EBV-reativation did not already had developed PTLD, a careful clinical examination, laboratory assessment (including a complete hematological and biochemical profile, a serum immunoglobulin quantification and a lymphocyte immunophenotyping) and CT-scan were performed.

Together with significant EBV DNAemia, proven EBV-PTLD diagnosis was based on radiological and histological findings according to the 2018 revision of the WHO classification of lymphoid neoplasms.⁷ EBV-encoded RNA *in situ* hybridization (EBER-ISH) was performed in all cases to confirm the presence of EBV infection. Diagnosis of probable EBV-PTLD was established when the EBV-DNAemia was associated with lymphadenopathy, hepatosplenomegaly or any other organ involvement on computed tomography (CT) findings, without tissue biopsy and in the absence of other documented cause.

2.4 Pre-emptive therapy and treatment of PTLD

Rituximab was administered pre-emptively to all patients with significant EBV DNAemia at a dose of 375 mg/m² weekly until EBV load fell below 2.5 log₁₀ IU/ml.

Patients with rituximab-refractory EBV-DNAemia and therefore developing PTLD, received either an intensification of rituximab treatment (1000 mg total dose weekly until resolution of EBV-DNAemia and of clinical signs) associated with reduction of immunosuppression whenever feasible, or further systemic treatment such as cyclophosphamide, etoposide, doxorubicin, brentuximab or donor cellular products (donor lymphocytes or CD34+-selected stem cell boost infusions).

2.5 Statistical analysis

Data are presented as counts and percentage or median and range or inter-quartile range (IQR). Kaplan-Meier estimates²³ were used to determine the unadjusted probability of overall survival (OS), through 2-year post-transplant, with differences between the curves determined using log-rank tests, for univariate comparisons. OS was defined as the time from transplantation to death for any cause. In the case of a non-event, observations were censored at the time of last follow-up. Cumulative incidence functions (CIF) of acute and chronic GVHD, relapse and PTLD were calculated in a competing risk setting, where death was considered the competing event.^{24,25}

Predictive analyses for PTLD were based on the proportional hazard model for sub-distribution of competing risk. A set of independent predictors was constructed with a stepwise backward procedure. All predictors with a P-value below 0.10 were considered and sequentially removed if the P-value in the multivariable model was above 0.05.

Lymphocyte subgroups were analyzed as continuous and categorized variables, with cut-offs at the 50th percentile of the sample distribution (i.e. two equal size groups). Fisher's exact test was used for categorical variables and Kruskal-Wallis test for continuous variables. Groups were compared with the Mann-Whitney U non-parametric method or with Student's t-test. All tests were two-sided and P-values <0.05 were considered significant.

Statistical analyses were performed using R version 3.5.1.

3. RESULTS

3.1 Baseline characteristics

Among 1024 allogeneic HSCT performed at Saint Louis Hospital in Paris between 2010 and 2017, 208 patients presented a significant EBV DNAemia > 3.8 log₁₀ IU/ml in plasma following allogeneic HSCT. Patient characteristics and features of EBV infection are described in [table 1](#) and [2](#), respectively.

The median age of the 208 patients with EBV reactivation was 42.52 years (8.35-74.77), and the median follow-up for surviving patients was 47.33 (3.18-126.20) months. Diagnosis of EBV infection was made within a median of 35 days (range 6-713) after HSCT. Transplant indication was mainly acute leukemia (51%), while only 9% of the patients underwent HSCT for lymphoma. Conditioning regimens were more frequently of reduced intensity (57%) and fludarabine-based (57%). Most patients received an anti-T-cell serotherapy (80%) for GVHD prophylaxis. The majority of patients were transplanted with a donor matched for gender, ABO group, and cytomegalovirus (CMV) and EBV serology. Main stem cell source was PB (81%), whereas BM and CB were used respectively in 17% and 2% of cases.

3.2 EBV-PTLD cumulative incidence and subgroup description

Thirteen patients developed an EBV-related PTLD despite a pre-emptive anti-B cell therapy with rituximab. Calculated cumulative incidence was 6.3% (95% Confident Interval (CI) 3.5-10.1%, [Figure 1](#)). [Table 4](#) summarizes characteristics of patients diagnosed with a PTLD. The median age of patients with PTLD was 40.51 years (17.4-74.7), and their median follow up was 37.85 (24.85-52.59) months.

Patients were transplanted mainly from a non-relative donor (seven from a Mismatched, five from a matched unrelated donor) and only one patient received a graft from an HLA-identical sibling donor. Chimerism analysis showed a donor reconstitution in ten patients, a mixed chimerism in two cases and an autologous reconstitution in one patient. Seven patients had acute GVHD and nine were receiving a corticosteroid treatment at PTLD onset. An extra-nodal involvement was seen in ten patients with central nervous system involvement in two cases. At PTLD presentation, four patients (30%) had fever >38.5°Celsius, five (38%) had lymphadenopathy. Elevated lactate dehydrogenase levels (LDH ≥1.5x the upper limit of normal [ULN, 480 U/L]) and elevated ferritin (>3000 mg/L) serum levels, thrombocytopenia (<100x10⁹ platelets/L), anemia (hemoglobin < 10 g/dl), lymphopenia (<0.5 x10⁹/L), but not neutropenia, were frequent at disease onset. The median number of rituximab injection was five (2-11). EBV-DNAemia became negative within one month in 5 out of 13 patients. PTLD diagnosis was histologically proven in ten patients. Biopsies showed

a monomorphic PTLD in six cases and a polymorphic subtype in four patients. Immunohistochemistry analysis showed CD20 positivity in all patients with available histological samples. In three patients, the diagnosis was highly probable basing on CT-findings and tumor cytology. Patients developed PTLD within a median of 85 days after transplant (27-140) and 34 days (9-105) after EBV reactivation. Rituximab was initiated after a median of 14 days (3-32) after the onset of EBV infection. Median number of rituximab courses before PTLD transformation was 2 (1-4). In all patients developing a PTLD, immunosuppressive drugs were drastically withdrawn. In three patients the intensification of rituximab treatment, together with a reduction of immunosuppression, allowed achieving a complete response (CR) of PTLD. Five patients received other treatments including etoposide, cyclophosphamide, doxorubicin, intrathecal methotrexate, and brentuximab. In the other five patients, no further treatment was possible because of poor performance status. Donor lymphocyte infusion was performed in one case and one patient received a CD34-selected stem cell boost in the context of poor graft function. Overall response rate of all treatments for PTLD was 46%: six patients experienced a complete remission, but two of them died because of other complications (one for interstitial pneumonia and one for invasive aspergillosis). Overall, nine patients with PTLD diagnosis died for a mortality rate of 69%.

3.3 OS and cumulative incidence of relapse and of acute and chronic GVHD

Two-year OS of the entire cohort was 62.8 (95%CI [56.4-69.9]) (**Figure 2 A**). CIF of relapse from the original disease was 12.7% (95%CI [8.6-17.7]) at one year and 16.5 (95%CI [11.7-22.0]) at 2 years. The CIF of acute grade II-IV GVHD at day +100 was 59.9 (95%CI [52.9-66.2]) whereas that of grade III-IV acute GVHD was 25.1% (95%CI [19.4-31.2]). CIF of chronic GVHD was 34.7 (95%CI [28.2-41.2]) at 1 year and 41.4 (95%CI [34.5-48.2]) at 2 years (**Figure 2-B, -C, and D**).

3.4 Factors affecting overall survival in EBV infected transplanted patients

Univariable analysis showed several risk factors affecting OS of transplanted patients diagnosed with EBV infection: age >42 years (HR 2.69 [95%CI 1.69-4.28], $p < 0.001$), diagnosis of myelodysplastic syndrome and myeloproliferative neoplasm (MDS and MPN) (HR 2.37 [95%CI 1.47-3.81], $p < 0.001$), presence of grade III-IV acute GVHD (HR 2.42 [95%CI 1.56-3.77], $p < 0.001$). Absence of T-depleted therapy, RIC or MAC without TBI regimens, EBV viral load clearance within 1 month, and EBV serological status other than donor positive/recipient positive (D+/R+) represented protective factors for survival. In a multivariable model, only age, EBV serological status and presence of grade III-IV GVHD influenced OS. EBV clearance was withdrawn from the model because of a violation of assumption of proportional hazard. (**Table 3 and Figure 3**).

3.5 Risk factor analysis of PTLD transformation

The univariable analysis identified 3 risk factors associated with PTLD incidence despite pre-emptive rituximab: donor/recipient sex mismatch (HR 1.559, $p = 0.039$), presence of HLADRB11:01 allele (HR 4.850, $p = 0.004$), EBV viral load clearance after 1 rituximab course (HR 6.556, $p = 0.001$) (**table 4**).

In multivariable model EBV clearance after 1 rituximab course was removed because of a violation of assumption of proportionality basing on the scaled Schoenfeld's residuals.²⁶

Significant risk factors associated with PTLD incidence in multivariable analysis were: HSCT from an unrelated donor (HR 2.11 [95%CI 1.00-4.45], $p=0.05$), recipient HLA DRB1*11:01 (HR 4.85 [95%CI 1.57-14.97], $p=0.006$), fever at onset of EBV infection (HR 6.12 [95%CI 1.74-21.58], $p=0.005$), and sex-mismatched HSCT (HR 4.69 [95%CI 1.35-16.22] $p=0.015$) (**Figure 4**).

A risk factor index was created and **figure 5** illustrates the cumulative incidence of PTLD according to the number of risk factors of EBV-infected transplanted patients. At day +180 from transplant, incidence of PTLD was very low in case of 0-1 or 2 risk factors (1.9%, [95%CI 0.4-6.1%] and 4.6 [95%CI 1.5 - 10.5] respectively) and very high in case of contemporary presence of 3-4 risk factors (43.8 [95%CI 18.7 - 66.5]).

3.6 Analysis of lymphocyte subsets

Supplementary Figures 1 (a-e) and **2** show the distribution of CD4+, CD8+, B and NK cells and a heatmap of the absolute number of all the other lymphocyte subsets at the onset of EBV infection, in patients with resolved EBV infection and in patients developing PTLD. The absolute number of T-lymphocytes was significantly higher in patients resolving EBV, whereas NK cells were higher in patients developing PTLD ($p=0.016$ and $p=0.049$ respectively, not confirmed in multivariable analysis).

4. DISCUSSION

In the present study, we analyzed outcomes and risk factors associated with PTLD transformation in patients developing an EBV infection after HSCT despite pre-emptive administration of anti-CD20 therapy.

We were able to show that after pre-emptive rituximab, the cumulative incidence of PTLD after EBV infection was 6.3%, which is very close to previously reported incidence rates of PTLD.^{27,28} In multivariate analyses PTLD was associated with several risk factors: HSCT in unrelated and sex-mismatched context, recipient HLA allele DRB1*11:01 and fever at diagnosis of EBV infection. Presence of more than two risk factors was strongly associated with risk of developing PTLD (more than 40% at six months post-HSCT).

Sex-mismatched HSCT, especially HSCT of male recipients with female donors, is well known to be associated with a higher incidence of GVHD and inferior survival.^{29,30} Immune response against minor histocompatibility antigens encoded on the Y-chromosome of a male recipient (H-Y antigens) can be at the basis of this adverse effect.³¹ H-Y antigens have been shown to elicit a coordinated B-cell and T-cell response.³² In our cohort a sex-mismatched transplant was a factor associated with a higher risk of developing PTLD.

In multivariable analysis HLA DRB1*11:01 allele was an independent predictive factor of EBV-PTLD. **Supplementary figure 4** shows a dot chart including the allele frequency of more representative class I and class II HLA. DRB1*11:01 was the most frequent HLA allele in PTLD group (found in heterozygosis in 6 of 13 patients, 46%) patients. Possible associations between certain HLA types and the risk of developing PTLD have been reported in other studies, especially in PTLD occurring in solid organ transplant recipients.^{33,34,35,36} An association has also been found between some HLA class II molecules and other EBV related malignancies.³⁷ The most likely hypothesis is that HLA variants may affect the success of T-cell surveillance for EBV and thereby influence a transplant recipient's predisposition to PTLD. It has been shown that HLA-DR mediates interaction between EBV envelope glycoprotein gp42 and B cell participating to the viral envelope fusion necessary for virion entry in B cells.³⁸ The possibility that EBV might actively evade CD4+T lymphocytes through

some HLA-DR polymorphisms, has been suggested in some in vitro systems in which lytically infected B cells shed significant amounts of a soluble form of gp42 that can bind to mature HLA class II at the cell surface, protecting those cells from CD4+ recognition.³⁹ Other proteins, playing a key role in reactivation and viral genome persistence (such as Zta and EBV nuclear antigens [EBNAs]), also interact with HLA-DR to limit viral antigen presentation.^{40,41} Epitopes selected according to HLA-DR polymorphisms may trigger different patterns of immune responses.⁴⁰ The best-characterized components of these mechanisms are specific cytotoxic T cells (CTL) directed against viral gene products of the latent state, which include EBNAs the 1 to 6 and the latent membrane proteins (LMP) 1 and 2.⁴² It has been also reported that the lytic cycle of EBV correlates with the diminution of specific major histocompatibility complex (MHC) class I and class II molecules and *vice versa* the down-regulation of surface MHC class I and II expression is maintained throughout the lytic cycle of EBV, with a significant effect on Ag presentation.^{41,43} We can speculate that such mechanisms can be at the basis of HLA DRB1*11:01-associated risk of PTLD transformation after HSCT, but this obviously need confirmation from other studies.

Hyperthermia at the moment of EBV infection onset was the most clinically relevant risk factor associated with PTLD transformation (HR 6.12, p=0.005) in our cohort. This finding is not surprising if we consider fever as a clinical feature consistent with probable or proven EBV disease.⁴⁴ However, despite the presence of fever in 4 out of 13 patients further developing PTLD, none of those patients presented with clinical signs of lymphoproliferative disease at the beginning of EBV-DNAemia. In our study, fever at the onset of EBV-DNAemia also impacted on survival outcome in univariable and in multivariable analysis.

Unrelated donor, T-cell depletion, donor positive and recipient negative EBV serological status, RIC regimens, grade II-IV acute GVHD and splenectomy, represent several risk factors identified for EBV-related PTLD in general transplanted population.^{3,27,45} In our cohort of patients already infected with EBV and already receiving anti-CD20 pre-emptive treatment, T cell depletion, reduced intensity conditioning regimens and EBV serological status did not represent parameters predisposing evolution towards PTLD. Almost 80% of patients received a T-cell depleted treatment before transplant and all patients with PTLD had received ATG or ALG (thus it was impossible to include this factor in multivariable analysis). ATG may therefore be considered a risk factor for EBV DNAemia but not a risk factor for PTLD. Clearance of EBV-DNAemia after one month represents an independent factor impacting OS and PTLD incidence in the univariable analysis, but its consideration in the multivariable model violated the assumption of proportionality.⁴⁶ Indeed, PTLD transformation is *per se* strongly associated with EBV-DNAemia persistence, but statistically, the relative effect of this covariate on the hazard function seems to change over the time, probably due to the change in detection methods during the study period (see patients and methods).

Presence of a monoclonal immunoglobulin was not a risk factor of PTLD transformation in our study because it was also present frequently in patients with EBV-DNAemia solely.

Interestingly, immunohistochemistry analysis of available samples showed a CD-20 positivity in all patients diagnosed with PTLD. This is an important finding, which leads to the speculation that the resistance to rituximab treatment in our cohort is merely clinical and should be considered as a “breakthrough” mechanism, occurring with the tumor mass progression and EBV proliferation under immunosuppression, rather than a change in molecular pathways of infected and transformed B-cells. Undoubtedly, these results must be

confirmed with further invitro or invivo studies aiming to elucidate the mechanisms of resistance to anti-CD20 therapy in this particular setting.

EBV-PTLD incidence in our study is quite low if we consider that analysis has been conducted on a potentially high-risk population. We can argue that this result depends on the pre-emptive rituximab therapy that is considered the best way to optimize outcomes in HSCT patients with significant EBV DNAemia.³ The overall survival rate of patients not developing PTLD was nearly 70% at 3 years that is similar to a survival rate expected in an HSCT population.⁴⁷ Therefore we can assume that EBV infection after HSCT, when occurred in absence of risk factors of PTLD transformation, is not associated with poor outcomes. However, in our analysis, PTLD occurrence is still associated with a high mortality rate (close to 70%), even if the overall response rate of patients developing an EBV-PTLD was nearly 50%.

To date, no standard therapy has been accepted for rituximab-resistant EBV-PTLD. In the setting of rituximab failure, second-line therapy options include cellular therapy (DLI or CTLs) or rituximab associated with chemotherapy. Unselected DLI from an EBV-positive donor is employed to restore broad T-cell reactivity, including EBV-specific responses; however, this procedure can be associated with severe GVHD. CHOP-like⁴⁸ or dose-adjusted-EPOCH-R⁴⁹ regimens have been proposed especially in the context of solid-organ PTLD. However, in the setting of HSCT chemotherapy regimens are difficult to manage. Brentuximab has been reported as a possible agent for PTLD with evidence of CD30+ expression.^{50,51,52} In our series only 4 patients with PTLD are alive at last follow-up time: 1 after a chemotherapy regimen including etoposide and high dose of intrathecal methotrexate, 3 without receiving second-line chemotherapy, but with an intensification of rituximab treatment and drastic reduction of immunosuppressive therapy. Patients receiving other chemotherapy regimens (CHOP-like) died with persistent disease. Brentuximab was associated with chemotherapy in 2 cases without success. One patient received DLI, and one other received a CD34+ selected boost in a context of poor graft function. These patients experienced a complete remission, but they died some months later because of other infectious complications ([Table 5](#)). Our group already showed that pre-emptive rituximab is an important strategy to decrease risk of EBV-related PTLD, however, the associated prolonged and profound B-cell deficiency is responsible for an increased bacterial infection and mortality risk.⁵³

The retrospective nature and the limited number of PTLD cases are definitive drawbacks of this study and need further validation on larger cohorts. Despite these limitations, we believe that our results may be useful for a better management of this post-transplant complication and implementation of pre-emptive strategies (such as the escalation of rituximab doses or virus-specific T-cell products) for selected patients presenting with multiple risk factors.

Authorship and Disclosures

Conception and design: GS, SP, CB

Data collection and assembly: CB, SP

Data analysis and interpretation: SP

Manuscript writing: SP, CB & GS

Final approval of manuscript: All authors

Conflict-of-interest disclosure:

The authors declare no competing financial interests.

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Table 1: Patient characteristics			
	Frequency (%), median (range)		
	All patients	PTLD+	PTLD-
	208	13	195
Age in years, median (range)	42.52 (8.35-74.77)	40.51 (17.49-74.77)	42.52 (8.35-69.13)
Follow-up, months (range)	47,33(3.18-126.20)	37.85 (24.85-52.59)	47.80 (3.18-126.20)
SEX			
Male	123 (58%)	5 (38%)	118 (60%)
Female	84 (42%)	8 (62%)	76 (40%)
Diagnosis			
AML	61 (30%)	1 (8%)	60 (31%)
ALL	43 (21%)	4 (31%)	39 (20%)
MPN; CML	32 (15%)	2 (14%)	30 (15%)
MDS	20 (10%)	1 (8%)	19 (10%)
Lymphoma and CLL	19 (9%)	1 (8%)	18 (9%)
BMF	24 (11%)	4 (31%)	20 (10%)
Hemoglobinopathy	5 (2%)		5 (3%)
MM and Plasma Cell disorders	4 (2%)		4 (2%)
Conditioning regimen			
MAC with TBI	46 (22%)	5 (38%)	41 (21%)
MAC without TBI	44 (21%)	1 (8%)	43 (22%)
RIC	118 (57%)	7 (54%)	111 (57%)
Busulfan contained regimen	102 (49%)	3 (23%)	99 (50%)
Fludarabine contained regimen	118 (57%)	7 (54%)	111 (57%)
Cyclophosphamide contained regimen	113 (54%)	10 (77%)	103 (53%)
Splenectomy before conditioning regimen	7 (13%)	0	7 (14%)
T-cell depleting therapy			
Thymoglobuline	124 (60%)	10 (77%)	114 (59%)
Lymphoglobuline	43 (20%)	3 (23%)	40 (20%)
Alemtuzumab	3 (1%)	0	3 (2%)
GVHD prophylaxis			
CSA-MTX	90 (43%)	6 (46%)	84 (43%)
CSA-MMF	112 (54%)	7 (54%)	105 (54%)
PTCy and CSA-MMF	6 (3%)		6 (3%)
Type of donor			
MRD	48 (23%)	1 (8%)	47 (24%)
MUD	59 (28%)	5 (38%)	54 (27%)
MMUD	95 (46%)	7 (54%)	88 (45%)
Haploidentical	6 (3%)		6 (3%)
Stem cell source			
PB	169 (81%)	9 (70%)	160 (82%)
BM	35 (17%)	2 (15%)	33 (17%)

UCB	4 (2%)	2 (15%)	2 (1%)
CMV serological status			
D+ / R+	67 (33%)	4 (31%)	63 (33%)
D- / R -	58 (28%)	5 (39%)	53 (27%)
D+ / R -	32 (15%)	2 (15%)	30 (15%)
D - / R+	51 (24%)	2 (15%)	49 (25%)
EBV serological status			
D+ / R+	175 (84%)	10 (77%)	165 (84%)
D- / R -	4 (2%)		4 (2%)
D+ / R -	6 (3%)	1 (8%)	5 (3%)
D - / R+	23 (11%)	2 (15%)	21 (11%)
Mismatch sex (D/R)			
M/M	88 (42%)	2 (15%)	86 (45%)
F/F	28 (13%)	2 (15%)	26 (13%)
M/F	57 (28%)	6 (46%)	51 (26%)
F/M	35 (17%)	3 (24%)	32 (16%)
ABO matching			
ABO matched	123 (58%)	5 (33%)	118 (59%)
ABO major Mismatch	36 (17%)	3 (25%)	33 (17%)
ABO minor Mismatch	36 (18%)	5 (42%)	31 (17%)
ABO mixed incompatibility	13 (7%)		13 (7%)

ABBREVIATIONS : ALL: Acute Lymphoblastic Leukemia ; AML : Acute Myelogenous Leukemia ; MDS : Myelodysplastic syndrome ; MPN : Myeloproliferative neoplasms ; CLL: Chronic Lymphocytic Leukemia; BMF: Bone Marrow Failure; MAC: Myeloablative;conditioning; RIC: Reduced intensity conditioning ; TBI: Total body irradiation; CSA: Cyclosporine; MTX: Methotrexate; MMF: Mycophenolic acid; PTCy: Post-transplant cyclophosphamide; PB : peripheral blood stem cells , BM : Bone Marrow, CB : Cord Blood ; MUD : Matched unrelated donor ; MMUD : mismatched unrelated donor ; MM: Mismatched ; D: Donor; R: Recipient; CMV: Cytomegalovirus; EBV: Epstein Barr Virus; M: Male; F: Female; PTLD: Post-transplant lymphoproliferative disorders.

Table 2: EBV Infection Characteristics			
	All patients	PTLD+	PTLD-
Time between transplant and EBV infection (days, range)	35 (6-713)	35 (18-118)	36 (6-713)
EBV maximal viral load (PCR median log UI/ml, IQR1st and 3rd)	4.68 (4.31-5.144)	5.55 (4.98-6.05)	4.64 (4.30-5.08)
EBV viral load before rituximab treatment (PCR median log UI/ml, IQR1st and 3rd)	4.55 (4.19-5.05)	5.37 (4.95-6.05)	4.53 (4.17-5.02)
Time between EBV infection and PTLT transformation (median, range)		34 (9-105)	
Time between transplant and PTLT transformation (days, range)		85 (27-140)	
Time between EBV infection and rituximab initiating (days, range)	14 (0-387)	14 (3-32)	14 (0-133)
Time between rituximab initiating and PTLT transformation (days, range)		19 (2-73)	
EBV viral load after 1st course of rituximab (PCR median log, IQR1st and 3rd)	2.8 (0-4.17)	4.66 (3.95-5.10)	2.68 (0-2.42)
Fever*	38 (18%)	4 (30%)	34 (17%)
Adenopathy*	10 (5%)	5 (45%)	5 (2%)
CRP (mg/L, median IQR1st and 3rd, normal level <2 mg/L)*	3.0 (2.0-12.0)	7 (2-22.5)	3 (2-10)
LDH (U/L, median IQR1st and 3rd, normal range 240 – 480 U/L)*	604 (399-702)	662 (452-819)	525 (400-688)
Ferritine (µg/L, median IQR1st and 3rd, normal range 13-150 µg/L)*	2406 (1723-4953)	3092 (2390-5706)	2379 (1684-4930)
Hemoglobin (g/dL, median IQR1st and 3rd, normal range 12 – 16 g/dL)*	10 (9.2-11.1)	9.2 (8.7-10.3)	10 (9.3-11.1)
Gammaglobulin (g/dL, median IQR1st and 3rd)*	5.40 (3.75-7.9)	5.40 (4.40-5.70)	5.40 (3.75-7.55)
Presence of monoclonal gamma protein*	32 (15%)	3 (23%)	29 (14%)
Lymphocytopenia (<0.5 x 10 ⁹ /L)*	88 (50%)	7 (63%)	81 (44%)
Neutropenia (<1.0 x 10 ⁹ /L)*	17 (9%)	1 (9%)	16 (9%)
Thrombocytopenia (<100 x 10 ⁹ /L)*	115 (61%)	8 (72%)	107 (61%)
Median number of rituximab injections	2 (1-11)	5 (2-11)	2 (1-6)
Negative EBV viral load after 1 month of treatment (Nb of patients, %)	184 (88%)	5 (38%)	179 (91%)

* At the onset of EBV infection.

Table3: Univariable analysis factor influencing OS		
	OS at 2 years	
	HR (95%CI)	p-value
Overall		
Age≤42 years		
Age>42 years	2.69 (1.69-4.28)	<0.001
Diagnosis		
Acute leukemia		
MDS and MPN	2.37 (1.47-3.81)	<0.001
Lymphoma and CLL	1.06 (0.47-2.39)	0.878
Non malignant disease	0.68 (0.30-1.52)	0.351
MM and Plasma Cell disorders	0.70 (0.09-5.15)	0.731
Conditioning regimen		
MAC with TBI		
MAC without TBI	0.49 (0.27-0.90)	0.022
RIC	0.51 (0.28-0.92)	0.026
T-cell depleting therapy		
Thymoglobuline		
Lymphoglobuline	0.88 (0.53-1.46)	0.629
Non T- depleteed	0.40 (0.18-0.86)	0.019
Type if donor		
MRD		
MUD	0.98 (0.54-1.78)	0.956
MMUD	1.04 (0.60-1.80)	0.861
Haploidentical	0.93 (0.21-4.01)	0.934
Stem cell source		
PB		
BM	0.55 (0.25-1.03)	0.062
UCB	0.29 (0.04-2.15)	0.994
Serological status CMV		
D+ / R+		
D- / R -	0.70 (0.39-1.25)	0.234
D+ / R -	0.78 (0.40-1.54)	0.489
D -/ R+	1.13 (0.65-1.94)	0.651
Serological status EBV		
D+/ R+		
Other EBV serological Status	0.33 (0.14-0.76)	0.009
Mismatch sex (D/R)		
M/M		
F/F	0.65 (0.32-1.29)	0.225
M/F	0.70 (0.41-1.17)	0.179
F/M	0.44 (0.21-0.90)	0.025
ABO matching		
ABO matched		

ABO major mismatch	1.46 (0.83-2.56)	0.188
ABO minor mismatch	1.59 (0.90-2.80)	0.104
ABO mixed incompatibility	1.48 (0.62-3.49)	0.368
Fever at EBV infection onset		
No		
Yes	1.90 (1.12-3.23)	0.017
EBV-DNAemia clearance within 1 month		
Yes		
No	2.78 (1.58-4.87)	<0.001
Reduction of EBV-DNAemia		
>1 log UI/ml		
<1 log UI/ml	1.923 (1.20-3.07)	0.006
Rituximab treatment		
<2 courses		
>3 courses	1.28 (0.76-2.16)	0.344
Presence of acute grade III-IV GVHD		
No		
Yes	2.42 (1.56-3.77)	<0.001
Presence of extensive chronic GVHD		
No		
Yes	0.54 (0.31-0.94)	0.032

ABBREVIATIONS: ALL: Acute Lymphoblastic Leukemia; AML: Acute Myelogenous Leukemia; MDS: Myelodysplastic syndrome; MPN: Myeloproliferative neoplasms; CLL: Chronic Lymphocytic Leukemia; BMF: Bone Marrow Failure; MM: multiple myeloma; MAC: Myeloablative conditioning; RIC: Reduced intensity conditioning; TBI: Total body irradiation; PB: peripheral blood stem cells, BM: Bone Marrow, CB: Cord Blood; MUD: Matched unrelated donor; MMUD: mismatched unrelated donor; MM: Mismatched; D: Donor; R: Recipient; CMV: Cytomegalovirus; EBV: Epstein Barr Virus; M: Male; F: Female; PTLD: Post-transplant lymphoproliferative disorders; GVHD: Graft versus host disease.

Table 4 : Univariable analysis of factors influenced CI of PTLD*			
	HR	95%CI	p-value
Age	1.030	(0.349-3.071)	0.950
Diagnosis	1.290	(0.855-1.960)	0.220
Donor	1.295	(0.760-2.200)	0.140
Stem cell source	2.430	(0.976-6.070)	0.056
CMV serological status	0.873	(0.565-1.350)	0.450
EBV serological status	1.640	(0.461-5.830)	0.689
Sex mismatch	1.559	(1.020-2.360)	0.039
Conditioning regimen	1.330	(0.670-2.631)	0.420
T-cell depletion	1.760	(0.968-3.210)	0.064
Cy based conditioning	2.982	(0.804-9.622)	0.198
Flu based conditioning	0.550	(0.168-1.805)	0.323
Grade III-IV AGVHD	0.534	(0.119-2.382)	0.413
Presence of HLADRB11:01 allele	4.850	(1.660-14.20)	0.004
Fever at onset of EBV infection	2.810	(0.924-8.531)	0.069
Presence of polyclonal Ig	1.006	(0.323-3.463)	0.932
Presence of monoclonal Ig	1.820	(0.491-6.760)	0.370
Absence of EBV-DNAemia clearance within 1 month after rituximab initiating	2.660	(4.750-43.40)	<0.001
Rituximab initiating <7 days from diagnosis of EBV infection	0.395	(0.122-1.281)	0.120
T Lymphocyte number at onset of EBV infection	0.996	(0.991-1.124)	0.063

ABBREVIATIONS : CD3: CD3+ Lymphocytes; TNC: Total nucleated cells; CD34: CD34+ cells; CMV: Cytomegalovirus; EBV: Epstein Barr Virus; Cy: Cyclophosphamide; Flu: Fludarabine; AGVHD: Acute Graft versus host disease; Ig: Gammaglobulins

Table 5: Characteristics of patients developing PTLD

Patient	Sex	Initial diagnosis	Age at HSCT	Stem cell source	Type of donor // donor sex	aGvHD present (maximum grade)	Time between rituximab initiation and PTLD transformation	Use of corticosteroids before significant EBV-DNAemia	Time between HSCT and PTLD (days)	PTLD classification on biopsy	Positive Immunohistochemistry	Extranodal involvement	PCR max UI/mL	EBV (log)	Nb of Ritux	Other treatments	Outcome PTLD	Status
#196	F	BMF	55	BM	MMUD // M	No aGvHD	73	Yes	124	Monomorphic PTLD B	CD20, CD79a	Liver, Spleen	6.05	11	No		NR	Dead
#197	F	PTCL-NOS	34	PB	MUD // M	Grade II	27	Yes	90	Monomorphic PTLD B	CD20, CD79a, CD30,	Liver, Spleen	5.65	4	Cyclophosphamide, etoposide, boost	CR	Dead	
#198	M	ALL-B	17	PB	MUD // F	No aGvHD	9	No	47	No Biopsy	N/A	CNS	6.54	4	Etoposide, Methotrexate IT	CR	Alive	
#199	F	AML	57	PB	MMUD // M	No aGvHD	7	No	62	Monomorphic PTLD B	CD20, CD79a, MUM1	Liver, CNS	6.92	5	Etoposide, Methotrexate IT	NR	Dead	
#200	F	MDS	62	PB	MUD // F	Grade II	52	yes	140	Monomorphic PTLD B	CD20, CD79a	Colon	4.78	2	No	NR	Dead	
#201	M	BMF	40	CB	MM // F	No aGvHD	2	no	139	Only cytoaspiration	CD20	No	4.30	2	No	CR	Alive	
#202	M	ALL-B	33	PB	MUD // M	Grade II	28	yes	83	Monomorphic PTLD B	CD20, CD79a, CD30	Liver, Spleen, Kidney	5.55	4	Cyclophosphamide, Doxorubicine, Etoposide, Brentuximab, DLI	CR	Dead	

#203	M	ALL-B	19	PB	MMUD // F	Grade III	50	yes	87	Polymorphic PTLD	CD20, CD79a	Tonsils, Cavum, Kidney	5.37	7	No	CR	Alive
#204	M	MPN	74	PB	MMUD // M	Grade I	9	yes	49	Polymorphic PTLD	CD20, CD79a	No	4.98	2	No	NR	Dead
#205	F	MPN	56	PB	MMUD // M	No aGvHD	60	yes	95	Monomorphic PTLD B	CD20, CD79a	Liver, Bone	5.26	5	No	NR	Dead
#206	F	ALL-B	36	PB	MMUD // M	Grade III	19	yes	63	Polymorphic PTLD	CD20, CD79a, CD30, MUM1	Liver, Cavum	6.94	8	Cyclophosphamide, etoposide, aracytine, brentuximab	NR	Dead
#207	F	BMF	34	CB	MM // F	No aGvHD	7	no	85	Polymorphic PTLD	CD20, CD30, CD79a, CD138	Cavum	4.15	5	No	CR	Alive
#208	F	BMF	52	BM	Identical sibling // M	Grade II	6	yes	27	No biopsy	N/A	No	5.93	5	No	NR	Dead

ABBREVIATIONS : M : male, F : Female ; ALL-B : B-cell Acute Lymphoblastic Leukemia ; AML : Acute Myelogenous Leukemia ; MDS : Myelodysplastic syndrome ; MPN : Myeloproliferative neoplasms ; PTL Nos: Peripheral T-cell lymphoma not otherwise specified PB : peripheral blood stem cells , BM : Bone Marrow, CB : Cord Blood ; MUD : Matched unrelated donor ; MMUD : mismatched unrelated donor ; MM: Mismatched ; DLI : Donor lymphocyte infusion ; IT :intratecal ; CR : Complete remission; NR : Non responder.

Figure 1:

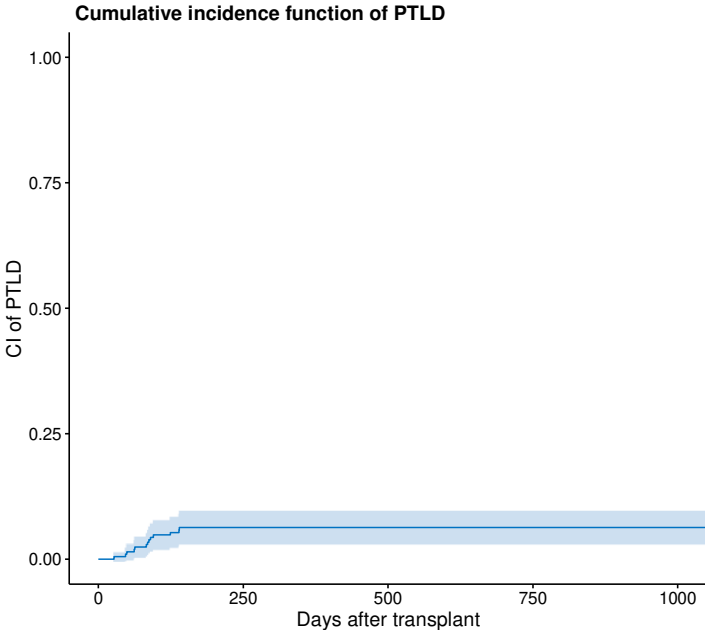
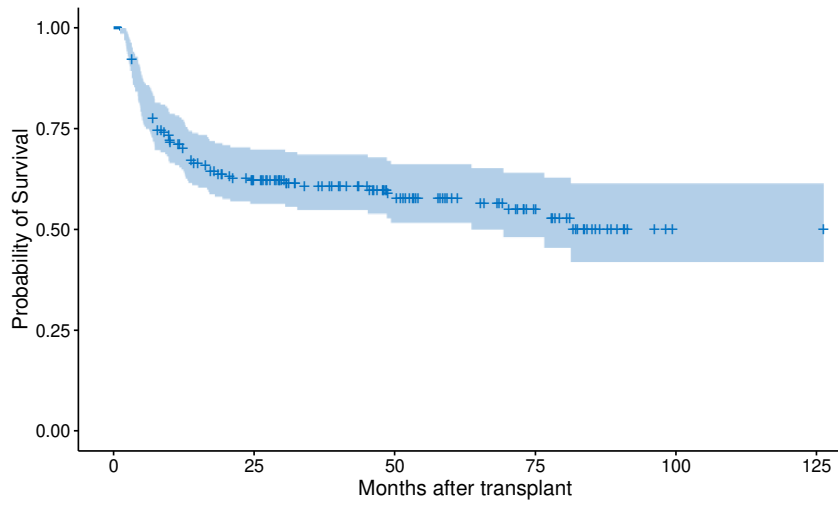
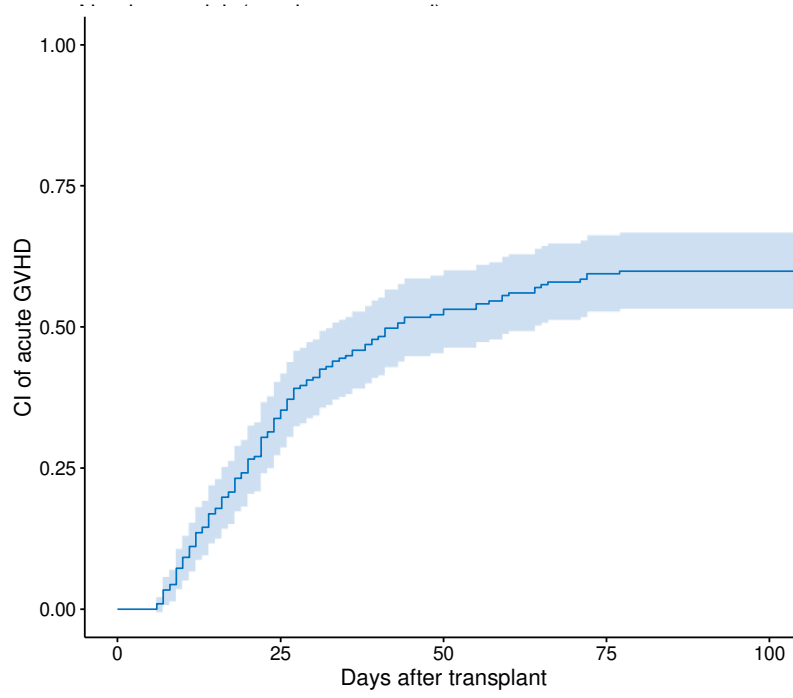


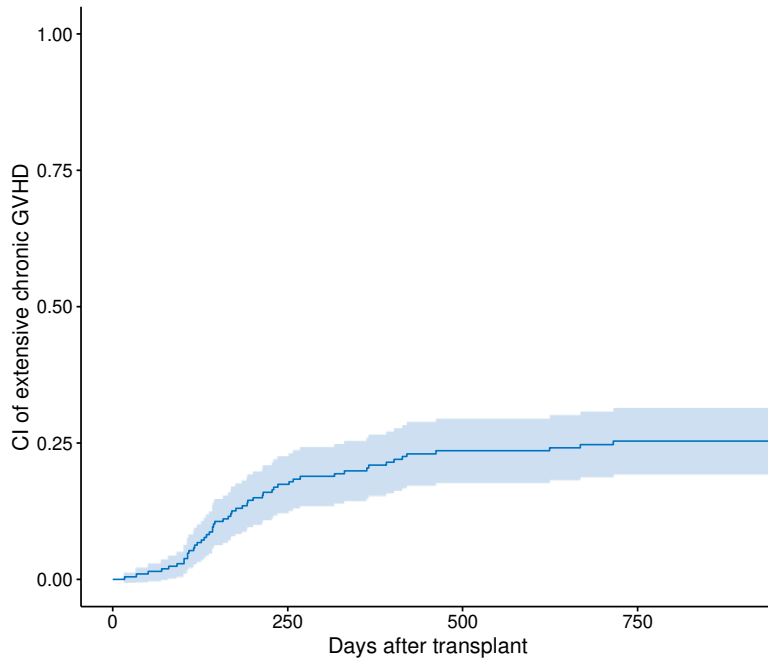
Figure 2



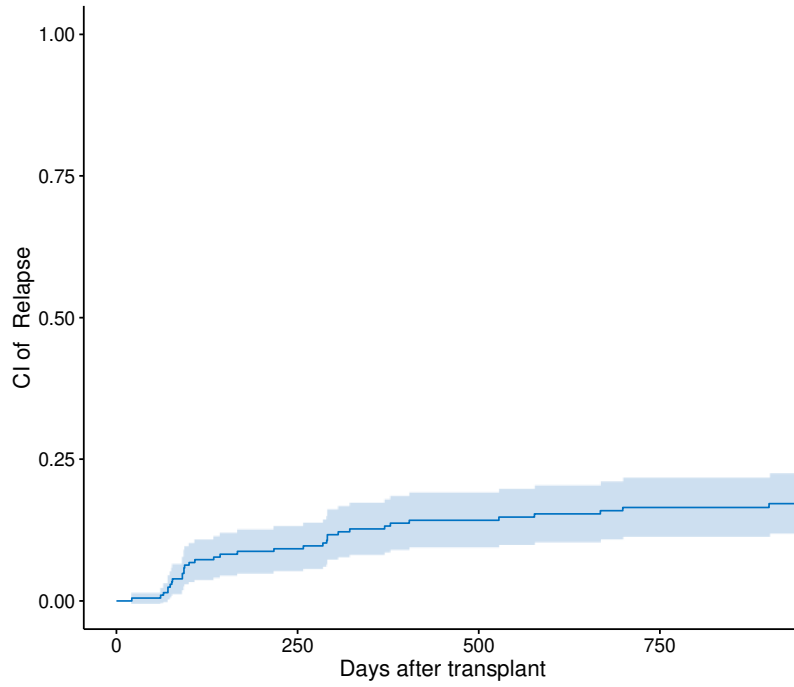
a)



b)

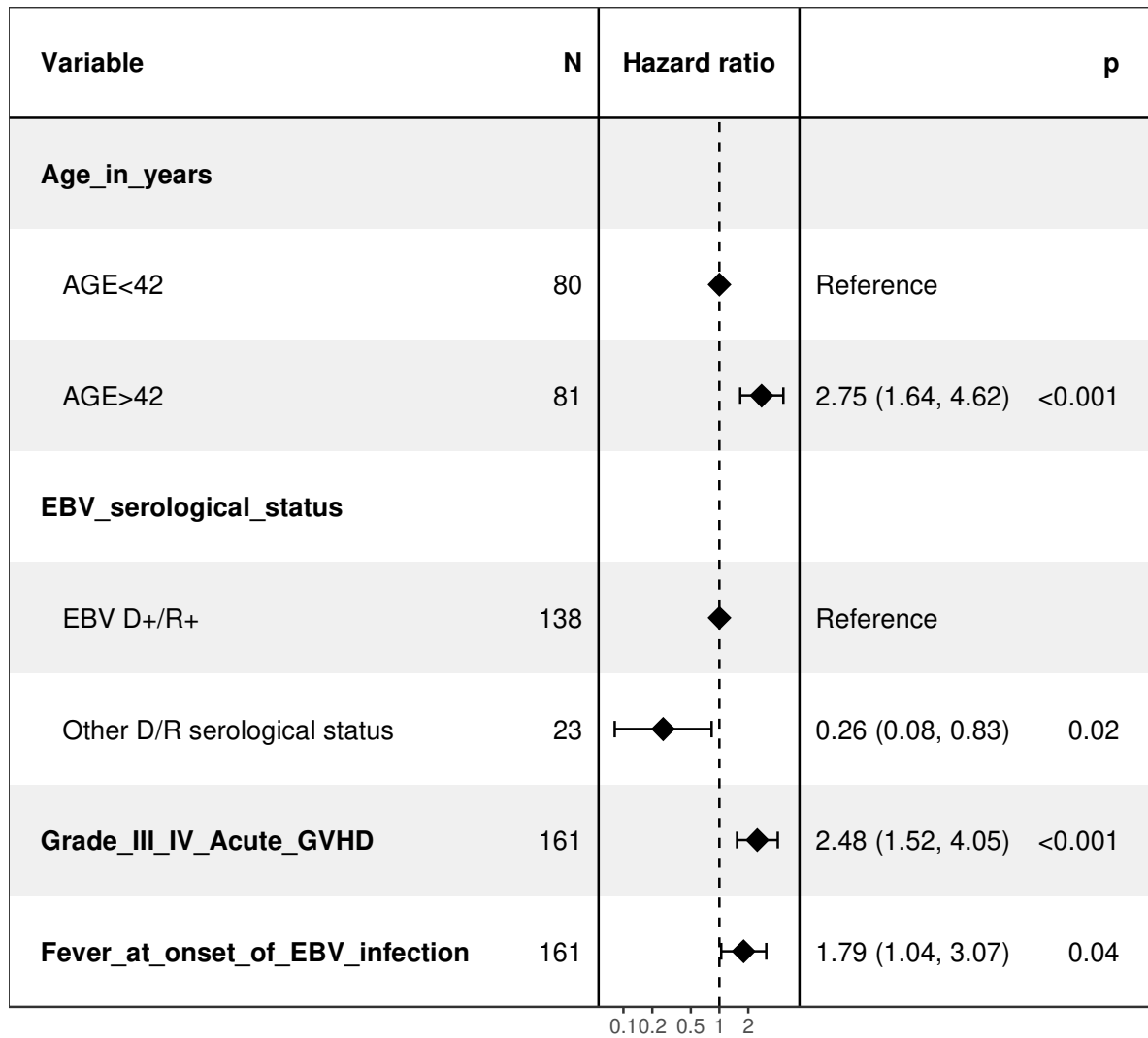


c)



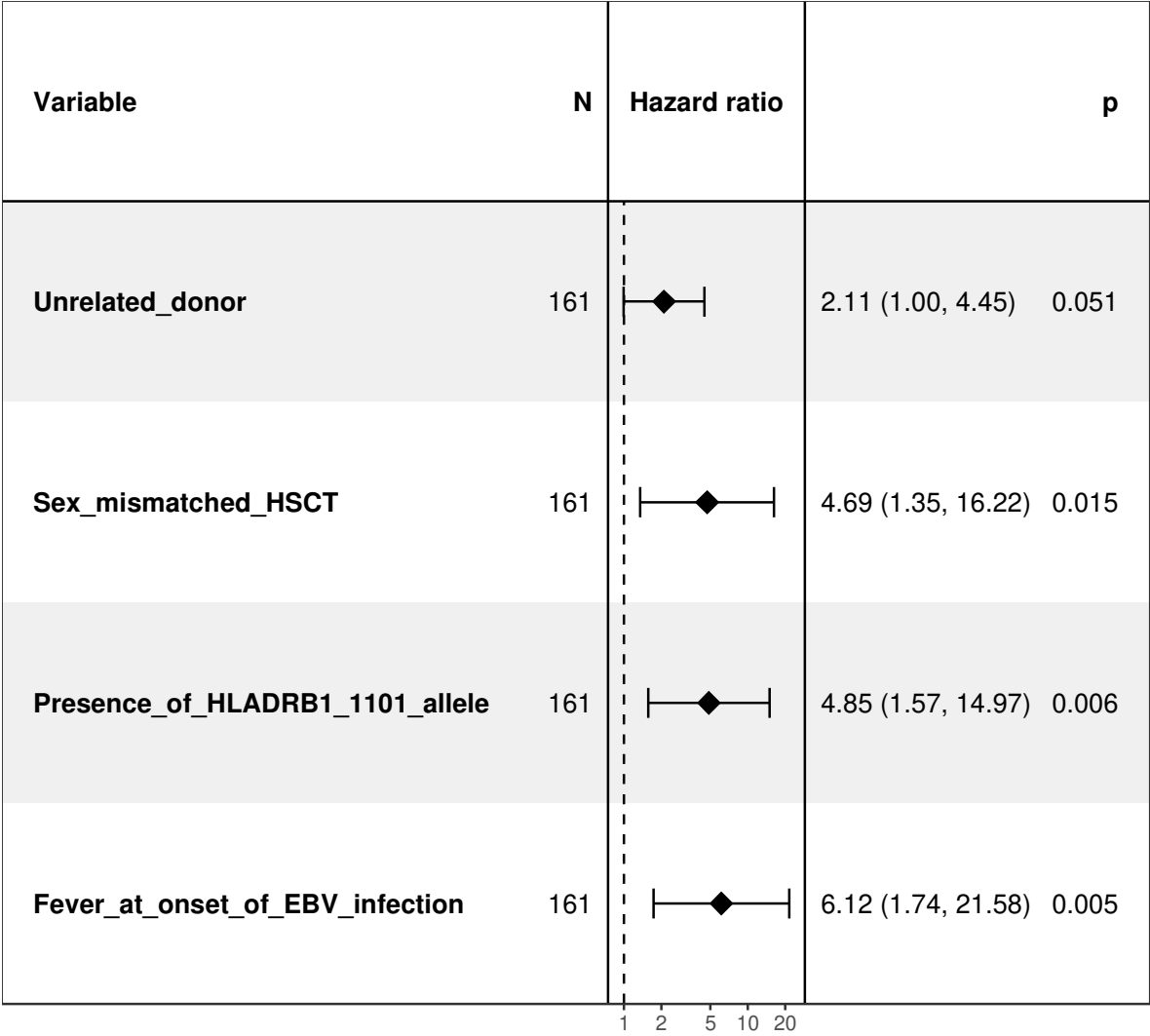
d)

Figure 3:



Number of events: 69; Global p-value (Log-Rank): 2.0256-08; AIC 615.11; Concordance Index: 0.73

Figure 4:



Number of events: 13; Global p-value (Log-Rank): 0.0014497; AIC 120.12; Concordance Index: 0.78

Figure 5

Cumulative incidence of PTLD according risk factors

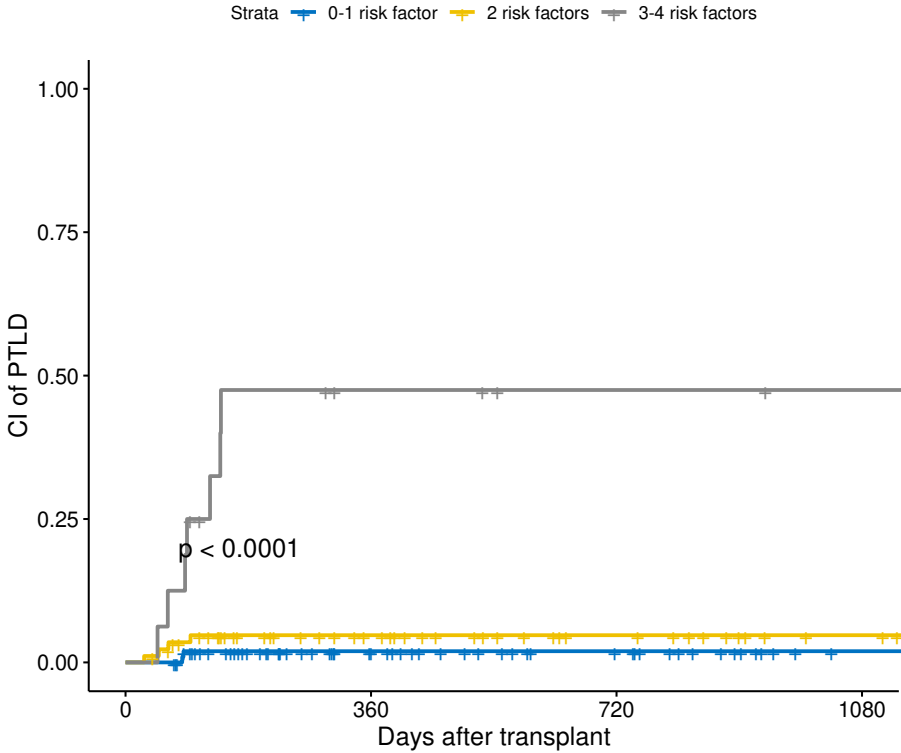


Figure legend

Figure 1: Cumulative incidence of PTLD in transplanted patients with significant EBV-DNAemia receiving Rituximab pre-emptive therapy

Figure 2: Outcomes of patients receiving at least 1 course of rituximab for an significant EBV-DNAemia A) Overall survival B) Cumulative incidence of relapse C) Cumulative incidence of grade III-IV acute GVHD D) Cumulative incidence of extensive chronic GVHD.

Figure 3: Forest Plot Multivariable Analysis of factors influencing OS.

Figure 4: Forest Plot Multivariable Analysis Risk Factors of CIF of PTLD.

Figure 5: PTLD cumulative incidence in transplanted patients with significant EBV-DNAemia receiving rituximab pre-emptive therapy according to number of risk factors: 1) HSCT from an unrelated donor, 2) Recipient HLA DRB1*11:01, 3) Fever at diagnosis of EBV infection, 4) Sex-mismatched HSCT.