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ORIGINAL ARTICLE

An extension of the RITUX-ERAH study, multicenter randomized clinical trial comparing rituximab to placebo in acute antibody-mediated rejection after renal transplantation

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SUMMARY

The treatment of active antibody-mediated rejection (ABMR) is still a matter of debate, the place of rituximab remaining controversial. The French multicenter double-blind RITUX-ERAH study included 38 patients with ABMR in the first year of renal transplantation. All patients received plasma exchanges, intravenous immunoglobulins, and corticosteroids and were randomly assigned rituximab or placebo infusion at day 5. Additional rituximab infusions were allowed. In the intention-to-treat analysis, 12-month graft survival and renal function were not different between the rituximab and placebo groups. Long-term data are needed to conclude. Evaluation of the 7-year outcomes of the RITUX-ERAH study patients according to the rituximab or placebo treatment received. Eleven patients received placebo and 27 at least one infusion of rituximab. Seven years after ABMR, death-censored kidney allograft survival and renal function were not different between the groups. The evolution of anti-HLA sensitization was similar. There was no statistically significant difference in the incidence of infectious or neoplastic complications, but to be noted, seven cancers developed in six patients treated with rituximab (mean period of 44 months post-ABMR). In this cohort, there was no benefit 7 years after ABMR of rituximab in addition to plasma exchanges, intravenous immunoglobulins, and steroids.

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Extension study of the RITUX-ERAH study (ClinicalTrials.gov Identifier: NCT01350882, December 2011; EudraCT Number: 2007-003213-13).

Introduction

The phenotypic expression of alloimmune responses is broad along post-transplant course. This heterogeneity is multifactorial, involving the timing, the characteristics of the donor-specific antibodies (DSA) secreted, and the adaptive and innate cellular processes engaged. Currently, the standardization of a multimodal treatment that would be effective on clinical antibody-mediated rejection (ABMR) is an unmet need. The standard of care therapy includes plasmapheresis and high-dose intravenous immunoglobulins (IVIg), and additional therapeutics have been proposed, targeting B cells, T cells, plasma cells, distal or proximal complement molecules, or immunoglobulin G [1-4].

Donor-specific antibodies arise from long-lived plasma cells and also from the progeny of reactivated quiescent memory B cells after alloantigen re-exposure. B cells are essential actors in alloimmunity. Besides being the precursors of antibody-secreting cells, B cells are also very potent antigen-presenting cells, contribute to T-cell differentiation and memory T-cell development, to the development of tertiary lymphoid organs, and also secrete diverse cytokines [5,6]. Rituximab is a chimeric monoclonal antibody directed against the B cell-specific transmembrane molecule CD20. CD20 is a lineage-restricted molecule, expressed on B cells from early pre-B cell stage throughout B mature cell differentiation and downregulated at the point of terminal differentiation into plasma cells. Thus, rituximab mediates B-cell cycle arrest, and complement-dependent and antibody-dependent cell-mediated cytotoxicities [7,8].

To date, rituximab therapy has only been challenged in two randomized trials for (i) cellular or humoral acute rejection with B-cell infiltrate in 20 pediatric renal recipients treated with thymoglobulin and/or pulse steroids [9]; (ii) chronic ABMR in 25 kidney recipients in association with IVIg and versus placebo [10]. In the context of early active ABMR, the French prospective multicenter randomized controlled RITUX-ERAH study was conducted to compare the efficacy and safety of rituximab or placebo in addition to conventional therapy: plasma exchanges (PE) and IVIg [11]. In the intention-to-treat analysis, the composite criterion (graft loss or absence of improvement of renal function at day 12) was not different between the groups treated by rituximab or placebo. Graft survival, renal function, histological, and immunological features were not different at 12 months. There were trends for fewer chronic lesions at 6 months and for more frequent low MFI (≤1500) of the immunodominant DSA (iDSA) in the rituximab group. Long-term data as regards efficacy and safety are needed to conclude on the place of rituximab in the treatment of early ABMR.

Materials and methods

Study population and design

This extension study included all the patients from the RITUX-ERAH study (ClinicalTrials.gov Identifier: NCT01350882, December 2011; EudraCT Number: 2007-003213-13). It has been approved by the local ethical committee and the National Commission on Informatics and Liberty. There were no exclusion criteria.

The RITUX-ERAH study [11] was a multicenter (21 transplant centers) prospective, randomized, double-

blind, placebo-controlled, parallel-group trial. In brief, renal transplant adult recipients from an ABO-compatible living or deceased donor who were diagnosed with biopsy-proven active ABMR during the first year after transplantation between 2008 and 2011 were included. Active ABMR was defined as follows: deteriorated renal function (increase in serum creatinine level >20%, or no significant decrease in serum creatinine level in the first month post-transplantation), and at least two of the following criteria: (i) tissue damage according to Banff scores (1997 criteria, updated in 2007), (ii) C4d labeling of peritubular capillaries, and (iii) presence of anti-HLA DSA with mean fluorescence intensity (MFI) over 1500.

All patients received PE (plasma removal of 60 ml/kg and 5% albumin infusion), with at least 3 PE between day 1 and day 5, 48 h without PE after rituximab or placebo infusion at day 5, then 3 PE until day12; IVIg (100 mg/kg per day after each PE until day 4, then 1 g/kg per day on days 5 and 6); corticosteroids (CS; infusion of 500 mg/day methyl prednisolone during the first 3 days, then oral CS, 1 mg/kg per day); and daily immunosuppression with tacrolimus (trough level, 8–12 ng/ml) and mycophenolate mofetil (2 g/day). At day 5, patients randomly received intravenous infusions of rituximab (375 mg/m^2) or placebo. In case of insufficient efficacy of treatment for active ABMR, additional infusions of rituximab were possible after day 12, with a limit of 2 infusions of rituximab. Additional PE and IVIg infusions were possible. In the protocol, patients were followed for one year. After the end of the RITUX-ERAH protocol, follow-up and therapeutics were left at the discretion of the clinicians.

Given the fact that some patients randomized with placebo received rituximab "in rescue," the analyses in this extension study were per-protocol.

Clinical, biological data

Clinical and biological data from the end of the protocol (1 year after inclusion/ABMR) to the time of last follow-up were obtained. eGFR was calculated by the MDRD equation. The absence of sufficient histological data after the end of the protocol made analyses not possible.

Detection and characterization of anti-HLA donor-specific antibodies

The serum samples at the time of ABMR, 3, and 6 months after ABMR had been centrally tested retrospectively at the Histocompatibility and Immunogenetic Laboratory in Tours [12]. Two patients had no detected DSA at the time of ABMR (but still meeting inclusion criteria of the RITUX-ERAH study). After the end of the RITUX-ERAH protocol, anti-HLA antibody testing was left at the discretion of the clinicians in each center (minimum once a year).

The presence of circulating anti-HLA antibodies was determined by LABScreen® Mixed and if positive by single-antigen flow bead assay (Luminex LABScreen® Single Antigen assay; One Lambda, Canoga Park, CA, USA) according to the manufacturer's and local protocols. The data from ABMR to 5 years after are presented (insufficient data 7 years after ABMR).

Statistical analysis

Numbers and proportions were used for the description of categorical variables. Continuous variables were expressed as means with standard deviations. Variables were compared using Mann Whitney test and Fisher's exact test. All tests were two-sided, and the threshold of statistical significance was set at a P value of 5%. Allograft survival was analyzed from the time of ABMR to the date of last follow-up, with death-censored kidney allograft loss as the event of interest. Kaplan-Meier curves were used and compared with the use of the log-rank test according to the randomized treatment. For the analysis of long-term renal function, renal parameters were compared in the still functioning grafts. An analysis using the Last Observation Carried Forward (LOCF) imputation method for censored patients (by death or graft lost) was also used to describe eGFR evolution.

Statistical analyses were performed with R software (http://www.r-project.org) and GRAPHPAD PRISM 8.4.0.

Results

Study population

All 38 patients from the initial RITUX-ERAH study were included. In this per-protocol long-term analysis, 27 patients had received at least one infusion of rituximab, and 11 patients had only received placebo (Fig. 1).

Table 1 shows the baseline characteristics of the recipients and donors at the time of transplantation and inclusion in the RITUX-ERAH protocol.

To be noted as for the severity of ABMR at the time of inclusion, three patients had oliguria. Fourteen out of 38 patients (37%) had a creatininemia higher than

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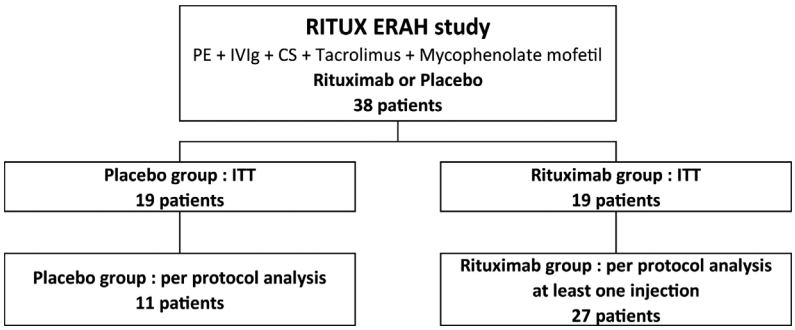

Figure 1 Patient flow chart. CS, corticosteroids; ITT, intention to treat; IVIg, intravenous immunoglobulins; PE, plasma exchanges.

Table 1. Baseline characteristics of study population.

Characteristics	Rituximab (*N* = 27)	Placebo (*N* = 11)	All patients (*N* = 38)	*P* value
Recipients				
Age (year)	48 ± 16	40 ± 15	45 ± 16	0.20
Male gender, *n* (%)	15 (55.6%)	6 (54.5%)	21 (55.3%)	0.96
Retransplantation, *n* (%)	10 (37%)	5 (45.5%)	15 (39.5%)	0.63
Transfusions, *n* (%)	13 (48.1%)	5 (45.5%)	18 (47.4%)	0.88
Pregnancies, *n* (%)	8 (29.6%)	4 (36.4%)	12 (31.6%)	0.70
Donors				
Age (year)	50 ± 18	37 ± 16	46 ± 19	0.05
Male gender, *n* (%)	15 (55.6%)	7 (63.6%)	22 (57.9%)	0.65
Deceased, *n* (%)	27 (100%)	10 (90.9%)	37 (97.4%)	0.29
Non-heart beating donor, *n* (%)	1 (3.7%)	1 (9.1%)	2 (5.3%)	0.50
Expanded criteria donor, *n* (%)	9 (33.3%)	1 (9.1%)	10 (26.3%)	0.23
Cold ischemia time (h)	18 ± 6	17 ± 8	18 ± 7	0.85
Post-transplantation regimen				
Anti-thymocyte globulin, *n* (%)	19 (70.4%)	6 (54.5%)	25 (65.8%)	0.35
Basiliximab, *n* (%)	7 (25.9%)	3 (27.3%)	10 (26.3%)	1.00
Cyclosporin, *n* (%)	7 (25.9%)	1 (9.1%)	8 (21.1%)	0.40
Tacrolimus, *n* (%)	19 (70.4%)	10 (90.9%)	29 (76.3%)	0.24
MMF or EC-MPS, *n* (%)	25 (92.6%)	11 (100%)	36 (94.7%)	1.00
Corticosteroids, *n* (%)	26 (96.3%)	11 (100%)	37 (97.4%)	1.00
Clinical & biological data—ABMR				
Time to rejection, *n* (%)				
<1 month	13 (48%)	6 (55%)	19 (50%)	0.72
1–3 month	2 (7%)	1 (9%)	3 (8%)	1.00
>3 month	12 (44%)	4 (36%)	16 (42%)	0.73
Serum creatinine, µmol/l	282 ± 192	288 ± 168	284 ± 183	0.90
Patients with serum creatinine >250 µmol/l, *n* (%)	10 (37%)	4 (36.4%)	14 (36.8%)	1.00
Proteinuria, g/day	1.0 ± 1.8	1.0 ± 1.4	1.0 ± 1.7	0.78

ABMR, active antibody-mediated rejection; EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil.

P values are for the comparison between patients treated by rituximab and patients who received the placebo. Categorical variables are expressed as numbers and percentages, no. (%) and were compared by Fisher's exact tests. Continuous variables are expressed as mean ± SD and were compared by Mann–Whitney tests.

250 µmol/l. Mean creatinine was 284 (±183) µmol/l (median 201 µmol/l, min/max 113/976 µmol/l). Five patients (13%) had a proteinuria above 1 g/24 h. Mean proteinuria was 0.96 (±1.71) g/day (median 0.34 g/day, min/max 0.05/7.8 g/day). Mean microvascular inflammation (glomerulitis + peritubular capillaritis scores) was 2.4 (±1.6). Eight patients had vascularitis, and four patients had transplant glomerulopathy.

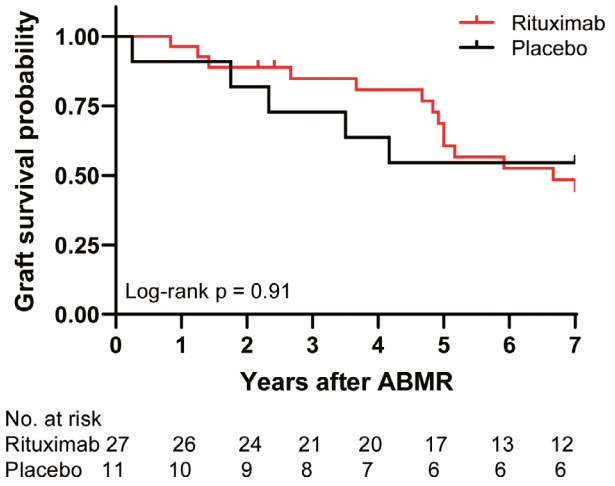

Figure 2 Death-censored kidney allograft survival according to rituximab or placebo randomized treatment after antibody-mediated rejection (ABMR). Kaplan-Meier curves for death-censored kidney allograft survival are shown for patients who received at least one infusion of rituximab and for patients who received only placebo. The result of the log-rank test is shown. ABMR, active antibody-mediated rejection.

Survival and renal outcomes

The mean follow-up time after ABMR diagnosis was 64.2 (3–109) months. Two deaths occurred in the rituximab group, one because of melanoma, one of severe diffuse tuberculosis infection and none in the placebo group.

Death-censored kidney allograft survival was not different between the patients treated by rituximab or by placebo. At 7 years, graft survival was 55% after placebo and 44% after rituximab ($P = 0.91$ by log-rank test; Fig. 2). In the placebo group, graft losses were attributed to chronic ABMR and one chronic dysfunction was not histologically documented. In the rituximab group, causes of graft loss were chronic ABMR (31.3%), BK virus nephropathy (12.5%), chronic dysfunction with no histologic evaluation (43.8%), and patient's death.

At the time of ABMR, there were not statistical trends for higher frequencies of older donors, deceased donors, expanded criteria donor donors in the rituximab group and of non-heart beating donor in the placebo group. Graft loss was not associated with those parameters.

For the still functioning grafts, the comparison of renal function between ABMR and 7 years after ABMR was not statistically different in terms of creatinine levels (Fig. 3a) and proteinuria (Fig. 3b). At 7 years after ABMR, mean serum creatinine was 148 µmol/l (eGFR 46 ml/min/1.73 m^2) in the placebo group and 194 µmol/l (37 ml/min/1.73 m^2) in the rituximab

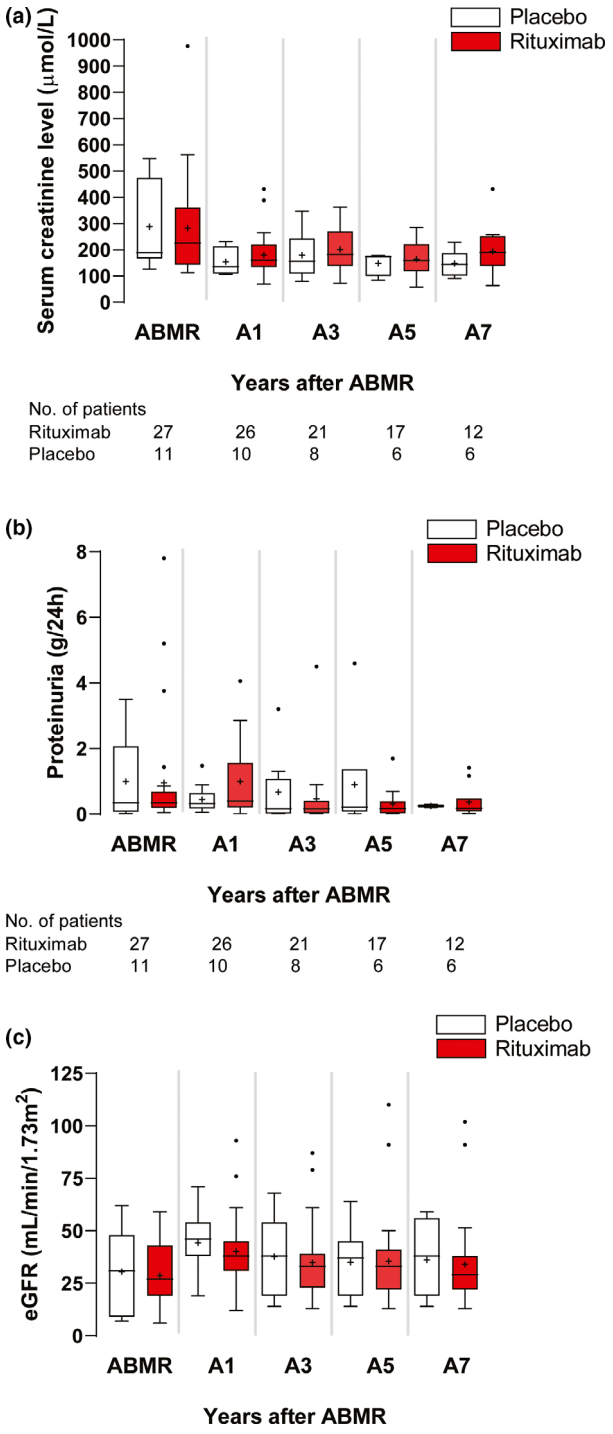

Figure 3 Evolution of kidney graft function from the day of antibody-mediated rejection (ABMR) to 7 years after ABMR. (Panel a) Represents the evolution of mean serum creatinine (µmol/l). (Panel b) Represents the evolution of mean proteinuria (g/24 h) from the time of ABMR to 7 years after. (Panel c) Represents the evolution of eGFR (ml/min/1.73 m^2) from the time of ABMR to 7 years after using LOCF imputation method for missing data (from censored patients). Box and whiskers (Tukey method, showing means as "+" and outliers as points). A1; A3; A5; A7, 1, 3, 5, 7 years after ABMR.

group ($P = 0.33$); mean proteinuria 0.25 g/l in the placebo group and 0.37 g/l in the rituximab group ($P = 0.27$). With the LOCF imputation method, eGFR analysis showed no statistical difference of evolution from 1 to 7 years post-ABMR (Fig. 3c).

Also, additional perfusions of rituximab were allowed at the discretion of the clinicians. In an additional analysis, three groups were compared according to the treatment received: only placebo ($n = 11$), only randomized rituximab ($n = 20$), and rescue rituximab in the first month post-ABMR ($n = 7$) that could reflect severe or worse evolution of ABMR. Death-censored graft survival at 7 years post-ABMR was not significantly different (55% after placebo, 48% after randomized rituximab, 33% after rescue rituximab, $P = 0.98$ by log-rank test). Renal function (serum creatinine levels, proteinuria) was not different (data not shown).

Fourteen patients had late renal biopsies (more than 12 months after ABMR): four patients in the placebo group and 10 patients in the rituximab group. 30% and 25% of those patients had transplant glomerulopathy in the rituximab and the placebo group, respectively. All patients with cg scores higher or equal to 2 at any time point lost their graft.

Immunological outcomes

No difference was observed after rituximab or placebo administration in terms of number of DSA (Table 2), MFI of iDSA (Fig. 4), and sums of the DSAs' MFI. MFI of iDSA tended to decrease after ABMR treatment. The number of patients having only weak MFI (<2000) iDSA at 5 years post-ABMR was not different between the two groups (80% after placebo, 73% after rituximab in still functioning grafts, $P = 0.9$).

Table 2. Allosensitization evolution after randomized treatment of antibody-mediated rejection (ABMR).

Characteristics	Rituximab	Placebo	*P* value
No. of IgG DSAs			
ABMR	2.7 ± 1.7	2.3 ± 1.9	NS
1 year after ABMR	0.8 ± 0.7	0.6 ± 0.7	
3 years after ABMR	0.3 ± 0.6	0.6 ± 0.7	
5 years after ABMR	0.5 ± 0.7	1.2 ± 1.3	

ABMR, active antibody-mediated rejection; DSA, Donor-specific antibodies; NS, Nonsignificant.

Continuous variables are expressed as mean ± SD and were compared by Mann–Whitney tests. *P* values are for the comparison between patients treated by rituximab and patients who received the placebo. At the time of ABMR, all patients were tested and 36 had DSA; at 1 year after ABMR, 36 patients were tested (still functioning grafts) and 21 had DSA; at 3 years after ABMR, 27 patients were tested (still functioning grafts and available data) and 9 had DSA; at 5 years after ABMR, 20 patients were tested and 8 had DSA.

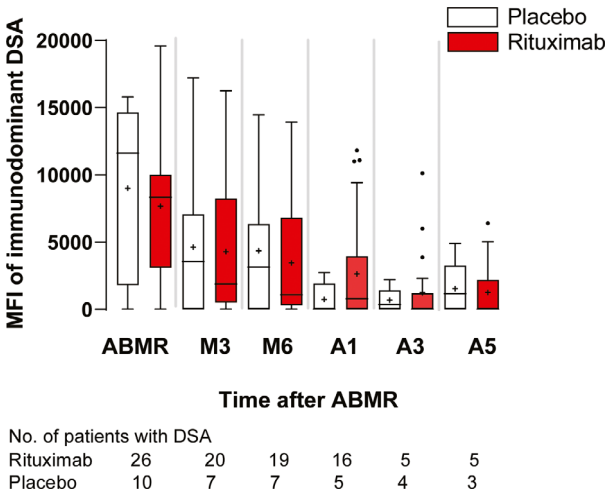

Figure 4 MFI evolution of immunodominant DSA (iDSA) from the time of active antibody-mediated rejection (ABMR) to 5 years after ABMR. Box and whiskers (Tukey method, showing means as "+" and outliers as points).M3; M6, 3, 6 months after ABMR;A1; A3; A5, 1, 3, 5 years after ABMR. 7 years after ABMR is not described because of insufficient data.

Safety

Table 3 describes the infectious and neoplastic complications in the period of follow-up. There was no difference of incidence of bacterial, viral, and parasitic infections between the placebo and the rituximab groups. No neoplasms occurred after placebo. In six patients treated with rituximab (22.2%), seven cancers developed (mean period of 44 months post-ABMR): five cutaneous cancers and 2 non-cutaneous cancers. The incidence was not statistically significant ($P = 0.154$) between the two groups. One patient had before ABMR a history of renal cancer and recurrence after ABMR treatment; one developed a prostate adenocarcinoma; one patient developed a melanoma. Two patients with a history of basal-cell carcinoma developed either another basal-cell carcinoma or a squamous cell carcinoma. Five patients with neoplastic history before ABMR had no neoplastic complications post-ABMR treatment.

Discussion

In the RITUX-ERAH trial, national double-blind randomized controlled trial, the addition of rituximab to

Table 3. Numbers of complications (bacterial, viral, parasitic infections, neoplasms) after rituximab or placebo treatment from the time of antibody-mediated rejection to 7 years after.

Complications	Rituximab	Placebo
Infections		
Pyelonephritis and urinary tract infections	16	19
Other bacterial complications	4	10
CMV infection	3	1
BK virus infection	3	0
Pneumocystosis	0	1
Neoplasms		
Non-cutaneous cancer	2 (kidney, prostate)	0
Cutaneous cancer	5	0

standard of care therapy for early ABMR in renal transplantation did not improve long-term outcomes. At 7 years after ABMR, death-censored kidney allograft survival was not different between the groups (55% after placebo and 44% after rituximab). This rate of graft survival was similar to the published literature [13]. For the patients with a functional graft, renal function was not different, and it should be noted that the long-term renal function was correct with weak proteinuria in those patients. Anti-HLA sensitization globally decreased over time in the remaining patients, but its evolution remained comparable in the two groups, suggesting that among therapeutics PE and IVIg were the main reasons of these observations. In the study by Moreso *et al.* [10], the immunologic evolution was also not different in 25 chronic ABMR treated randomly by IVIg and rituximab versus placebo.

Factors contributing to the lack of efficacy of rituximab may be multiple. First, DSA originate from long-lived plasma cells and from the progeny of reactivated quiescent memory B cells. Despite the profound B-cell depletion, overall antibody levels are relatively maintained after rituximab B depletion. The prevalence of hypogammaglobulinemia is moderate, variable owing to disease-specific dosing and treatment duration [14]. The fact that rituximab has poor effect on antibody-producing plasma cells might account as one of the explanations of the absence of significant effect on already established injuries. Bortezomib, as a proteasome inhibitor, induces depletion of alloreactive short- and long-lived plasma cells, modulates MHC-dependent antigenic presentation, and reduces antibody titers. Nevertheless, the BORTEJECT trial [15] found no benefit of randomized treatment by bortezomib in 44 patients with late ABMR in terms of eGFR, DSA characteristics, histology or molecular rejection phenotypes.

Secondly, CD20-positive cells sensitivity to rituximab depletion may vary upon cell subsets, circulatory dynamics, survival factors from the microenvironment, accessibility of the drug, and effectors cells responsible for antibody-dependent cell-mediated cytotoxicity (ADCC) to the tissues [16,17]. For instance, Kamburova and coworkers showed that a single infusion of 375 mg/m^2 of rituximab could not completely deplete B-cell population located in secondary lymphoid nodes [18]. After treatment, the B cell resistant population was enriched in CD19+ CD20− switched memory B cells, with a modified Ig-isotype secretion. In renal allograft, interstitial tertiary lymphoid organs are a site of persistent alloantibody production after rituximab, despite rituximab-related efficient depletion of circulating B cells [19]. Elevated levels of the prosurvival B cell-activating factor (BAFF) have been shown during the initial period of profound B-cell depletion, later decreasing during effective B-cell re-expansion [20]. The immediate post-depletion increase of this B lymphocyte stimulator may also contribute to the survival and regeneration of alloreactive B cells in the circulation and in lymphoid organs. Elevated levels of BAFF pretreatment may lead to a shorter duration of B-cell depletion. Flow cytometric analyses determined that 80% of the residual B cells post-depletion have phenotypic markers of CD27+ memory B cells. Memory B cells are characterized by a high expression of activation and prosurvival molecules [21]. Patients displaying higher post-treatment levels of CD27+ B cells could be more likely to experience rapid clinical relapse [17].

Moreover, insufficient dosing and schedule may play a role in rituximab resistance. The period of depletion is highly variable between patients. The optimal dose of rituximab to induce sufficient durable B-cell depletion is uncertain, and particularly in a context of massive proteinuria. In glomerulonephritis, the dose of 1 g instead of 375 mg/m^2, designed initially in lymphomas, has been proposed. The optimal number of infusions and the possible necessity of a maintenance therapy are also unknown. In autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, a longer duration of B-cell depletion obtained by extra doses is associated with better clinical outcomes [22].

Lastly, another factor that may inter-individually interfere in the responsiveness to rituximab is genomic polymorphisms, probably of a mild impact. The polymorphisms of Fcγ Ig receptors (FcγR) may impact

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rituximab-induced depletion by ADCC. Polymorphisms of FcγRIIIA have been shown to be associated with the degree of B-cell depletion, clinical response in autoimmune diseases and toxicity [23-27]. Internalization of rituximab is regulated by FcγR especially FcγRIIB. It results in a lower amount on the target cell surface which reduces the ability to activate FcγR-dependent ADCC functions. It correlates with impaired B-cell depletion [28]. Besides, there is evidence that the clinical efficacy of rituximab in cancer is in part dependent on complement. In patients with lymphoma, *C1qA [276A/G]* polymorphism or complement regulatory gene *CFH* polymorphism have been associated with higher clinical response and duration of response to rituximab therapy of follicular lymphoma [29-31].

While we know that ABMR treatment should target multiple pathophysiologic pathways, depleting B cell might not be enough in the process of antibody formation, and in this aim, the combination of rituximab and belimumab therapies is under evaluation in some autoimmune disorders but not yet in transplantation. A pilot trial is currently recruiting to study in late ABMR the blockade of IL-6, pro-inflammatory cytokine which promote differentiation and survival of B cell, plasmablasts, T follicular helper cell differentiation, and germinal center formation. Daratumumab, anti-CD38 humanized monoclonal antibody, has sporadically been administrated in resistant ABMR but with the disadvantage of depleting CD38+ immunoregulatory B and T cells alongside CD38+ NK cells, memory B cells, plasmablasts, and plasma cells [32]. To target plasma cells, second-generation proteasome inhibitor (carfilzomib) has been tried in refractory pulmonary allograft ABMR [33].

Considering the properties of DSA and interfering with related mechanisms of tissue injury is another crucial therapeutic approach. Terminal complement blockade by anti-C5 therapy may benefit as primary therapy in very selected patients with early active ABMR and complement-activating anti-HLA DSA [34-36]. Proximal complement molecule blockade is currently investigated [37]. Extended immunologic and histologic phenotype and transcriptomic analyses will hopefully in a near future help designing better therapeutic strategies and identify profiles of patients likely to respond.

In this cohort, there was a trend for a higher incidence of neoplasms after rituximab infusion. Seven neoplastic complications were observed in 22.2% of the group treated by rituximab and none after placebo. Most were cutaneous cancers (71.4%) including a melanoma, in addition to a prostate adenocarcinoma and a recurrence of renal cancer.

In a large hematologic cohort of almost 23 900 patients, Tao *et al.* [38] found an elevated incidence of some neoplastic events (including melanoma) after the beginning of the rituximab era for the treatment of diffuse large B-cell lymphoma. The literature remains however contradictory. In large cohorts of rituximab-exposed patients with rheumatoid arthritis (e.g., Emery et al with a 409 700 patients' database; Lopez-Olivo *et al.* in a meta-analysis of 29 400 patients), no evidence of increased risk of malignancy of any organ-specific type has been identified [39, 40] or more specifically of melanoma [41].

A causal relation remains difficult to establish in the context of polymedication including concomitant immunosuppressive regimen and comorbidities. Close and specific follow-up is necessary in particular for skin cancer and neoplastic recurrences.

Our study has limitations. As written in the initial manuscript, the number of patients included was lower than the planned sample size because at this time rituximab was often used at the time of transplantation to prevent ABMR. A high rate of rituximab conversion in the placebo group was noted, constraining to a per-protocol analysis to report long-term outcomes. The potential development of chronic ABMR in the cohort could not be assessed as histological data were rare after the end of the one-year trial.

In conclusion, with this designed protocolled treatment, we could not evidence a long-term benefit of rituximab versus placebo in the treatment of early ABMR.

Authorship

EB: participated in research design, performance of the research, data analysis, and writing of the article. SV, GB, EM, JB, SC, VC, PM, JT, VV, DA, DB, PG, FH, MH, NK, PM, CM, VP, CP-N, RP, JS, P-FW: participated in performance of the research. PG and MB: participated in the research design, performance of the research, data analysis, and writing of the article.

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Conflict of interest

The authors have no conflict to interest to declare.

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