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Low-dose rituximab induction therapy is effective in immunological high-risk renal transplantation without increasing cytomegalovirus infection

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Key Words:	rituximab, renal transplantation, renal function, cytomegalovirus, graft survival
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Methods
Ninety-two recipients undergoing living-donor renal transplantation in our institution from May 2009 to August 2018 were evaluated retrospectively. Indications for preoperative rituximab (200 mg/body) were the following: 1. ABO major mismatch, 2. ABO minor mismatch, 3. donor-specific anti-human leukocyte antigen antibody (DSA)-positive, 4. Focal segmental glomerulosclerosis (FSGS). We excluded 4 recipients who were followed less than 3 months, 5 who received over 200 mg/body rituximab and 7 who received prophylactic therapy for CMV.
Results
There were 59 patients in the rituximab group and 17 in the non-rituximab group. Groups differed significantly in age (median age, 53 vs 37 years, respectively; P=0.04), but not in sex (male, 64% vs 65% P=1.00), FSGS (3% vs 0%, P=1.00) or percentage of CMV-seronegative recipients of renal allografts from CMV-seropositive donors (12% vs 18%, P=0.68). Estimated glomerular filtration rate did not differ significantly between groups until 24 months after transplantation. CMV clinical symptoms (10% vs 24%, P=0.22), including fever≥38℃ (5% vs 12%, P=0.31) and gastrointestinal symptoms (5% vs 12%, P=0.31), and the 5-year survival rates of death-censored graft loss (90% vs 83%, P=0.43) did not differ significantly between groups.
Conclusions
Low-dose rituximab induction therapy is effective in immunological high-risk recipients without increasing CMV infection without valganciclovir prophylaxis.
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Low-dose rituximab induction therapy is effective in immunological high-risk renal transplantation without increasing cytomegalovirus infection

Running Title: Effect of low-dose rituximab on CMV infection

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Abstract

Objectives

Although rituximab expands the living donor pool for renal transplantation, it often increases the incidence of cytomegalovirus (CMV) infection. The aim of this study was to analyze the effect and impact of low-dose rituximab induction therapy on CMV infection in living-donor renal transplantation.

Methods

Ninety-two recipients undergoing living-donor renal transplantation in our institution from May 2009 to August 2018 were evaluated retrospectively. Indications for preoperative rituximab (200 mg/body) were the following: 1. ABO major mismatch, 2. ABO minor mismatch, 3. donor-specific anti-human leukocyte antigen antibody (DSA)-positive, 4. Focal segmental glomerulosclerosis (FSGS). We excluded 4 recipients who were followed less than 3 months, 5 who received over 200 mg/body rituximab and 7 who received prophylactic therapy for CMV.

Results

There were 59 patients in the rituximab group and 17 in the non-rituximab group. Groups differed significantly in age (median age, 53 vs 37 years, respectively; P=0.04), but not in sex (male, 64% vs 65% P=1.00), FSGS (3% vs 0%, P=1.00) or percentage of CMV-seronegative recipients of renal allografts from CMV-seropositive donors (12% vs 18%,

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Conclusions

Low-dose rituximab induction therapy is effective in immunological high-risk recipients without increasing CMV infection without valganciclovir prophylaxis.

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Key words: rituximab, renal transplantation, renal function, cytomegalovirus, graft survival

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Abbreviations

ABOi = ABO blood group-incompatible

ACR = acute cellular rejection

AMR = antibody-mediated rejection

BKV = BK virus

CMV = cytomegalovirus

DFPP = double-filtration plasmapheresis

DNA = deoxyribonucleic acid

eGFR = estimated glomerular filtration rate

ESRD = end-stage renal disease

FSGS = focal segmental glomerulosclerosis

G-CSF = granulocyte colony-stimulating factor

CREG = cross reactive group

HLAi = HLA antibody-incompatible

IVIG = intravenous immunoglobulin

LON = late-onset neutropenia

MMF = mycophenolate mofetil

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PCR = polymerase chain reaction

PEX = plasma exchange

sCr = serum creatinine

Tac-ER = extended-release tacrolimus

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Introduction

Worldwide, the number of patients with end-stage renal disease (ESRD) is increasing. The prevalence of ESRD treated in 2016 was 2,599 per million in Japan and 2,196 per million in the United States in the general population.[1] Renal replacement therapy includes kidney transplantation, hemodialysis and peritoneal dialysis. It is generally agreed that renal transplantation is superior to dialysis in survival, quality of life (QOL) and cost.[2-4]

A shortage of donors is a major problem worldwide and especially in Japan. In 2017 in Japan, there were approximately 330,000 dialysis patients and only 1,742 (0.5%) kidney transplants (1,544 from living donors, 65 from non-heart-beating donors and 133 from heart-beating donors).[5]

ABO blood group-incompatible (ABOi) and HLA antibody-incompatible (HLAi, HLA-sensitized) renal transplantation are effective strategies for expanding the donor pool. These types of transplantations require desensitization therapy to avoid antibody-mediated rejection (AMR). Plasma exchange, rituximab and intravenous immunoglobulin (IVIG) play an important role in desensitization therapy. Rituximab, a chimeric anti-CD20 monoclonal antibody, depletes B cells and is effective in ABOi and HLAi transplantation. Historically, splenectomy was used to reduce B cells, but rituximab

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has replaced splenectomy. Rituximab is also used in post-transplant lymphoproliferative disorder and in the treatment of acute rejection.[6-8] Rituximab is approved by national health insurance for ABO blood group major mismatch kidney transplantation and refractory nephrotic syndrome in Japan. Several studies demonstrated that rituximab is generally safe, but side effects include infectious complications, such as cytomegalovirus (CMV) infection and late-onset neutropenia (LON) with a normal dose of 375 mg/M.[2,6,9] The optimal dose for desensitization protocols remains uncertain.[6,9-11]

The aim of this study was to analyze the effect of low-dose rituximab (200 mg/body) as induction therapy in living-donor renal transplantation and its impact on CMV infection.

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Materials and Methods

Patients

We retrospectively collected data for all recipients who underwent living-donor renal transplantation in our institution between May 2009 and August 2018. The rituximab eligibility criteria for this study are shown in Figure 1. Briefly, they are the following: 1. ABO major mismatch, 2. ABO minor mismatch, 3. de novo donor-specific anti-human leukocyte antigen antibody (DSA)-positive status, 4. focal segmental glomerulosclerosis (FSGS). Rituximab was administered at a dose of 200 mg/body 1 to 2 weeks prior to the date of renal transplantation. Four recipients who were followed less than 3 months, 5 recipients who were administered over 200 mg/body and 7 recipients who received prophylactic therapy for CMV were excluded.

We divided the recipients into two groups: a rituximab group (Rit) and a non-rituximab group (non-Rit), and compared these groups in terms of renal function, graft loss (graft survival and death-censored graft survival), CMV infection including fever 38℃ or gastrointestinal symptoms, incidence of biopsy-proven acute rejection and granulocyte colony-stimulating factor (G-CSF) treatment. This retrospective study complied with the standards of the Declaration of Helsinki and current ethical guidelines, and it was approved by the Okayama University Institutional Review Board (Registration

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number: 1911-030).

Induction protocol

The immunosuppression protocol is shown in Figure S1-S5 (supplemental digital content). Triple therapy including extended-release tacrolimus (Tac-ER), mycophenolate mofetil (MMF), and prednisolone plus basiliximab was used in both groups. Basiliximab (20 mg/body) was administered on the day of transplantation and 4 days later. In addition to the immunosuppression protocol of the non-Rit group, the Rit group received a dose of rituximab 200 mg/body. All patients in the Rit group except those with ABO minor mismatch also received 2 sessions of double-filtration plasmapheresis (DFPP) and one of plasma exchange (PEX) prior to transplantation. A DPFF was undertaken 9 and 7 days before transplantation, and PEX was carried out one day before transplantation in the Rit group. Tac-ER was started at an initial dose of 0.1 mg/kg/day and controlled to achieve a target trough concentration of 5 ng/mL (4 days to 4 weeks before transplantation, depending on the immunological risk, 8-12 ng/mL during the first 2 weeks, 5-8 ng/mL during the first month, and 5 ng/mL thereafter). MMF was started at an initial dose of 500 mg/day from 4 days to 6 weeks before transplantation. Prednisolone 10 mg/day was given from 1 to 2 weeks before transplantation in the Rit group depending on immunological

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risk. On the day of transplantation, it was administered at a dose of 250 to 500 mg/day and gradually lowered to 5 mg/day over 6 months.

Renal function and graft loss

We compared the serum creatinine (sCr) concentration and estimated glomerular filtration rate (eGFR) between groups at 3, 6, 9, 12, 18 and 24 months. We also analyzed graft survival and death-censored graft survival.

CMV infection

Monitoring for CMV infection was performed by CMV antigenemia assay to detect CMV viremia. CMV antigenemia was routinely used because CMV polymerase chain reaction (PCR) is not approved by national health insurance in Japan. Patients were positive for CMV infection when CMV antigenemia was 5 or more by the CMV pp65 C10/C11 antigenemia assay. When the preoperative antibody test was negative, it was defined as positive if even one antigen-positive cell was observed. Furthermore, we documented fever ≥38℃ and gastrointestinal symptoms related to CMV. None of the patients received prophylaxis for CMV infection because prophylaxis was not approved by national health insurance in Japan until recently.

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G-CSF treatment

When neutrophil counts became 1000/mm^3 or less, defined as grade 3 neutropenia by the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0, G-CSF was administered, regardless of the presence of fever.

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Acute rejection

Whenever acute rejection was suspected, renal biopsy was performed. Per protocol, biopsy was performed at 1 year after transplantation. A 16-gauge biopsy gun was used to obtain 3 core biopsy samples under ultrasound guidance. Rejection was classified based on the Banff criteria.

Statistical Analysis

All statistical analyses were carried out with EZR version 1.36 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R.[12] Baseline background, adverse events and serum Cr concentrations were compared between the Rit and non-Rit groups with Fisher's exact test for categorical variables and the Mann–Whitney U-test for continuous variables. We considered *P* values less than 0.05

significant. Graft survival and death-censored graft survival were calculated by the log-rank test with the Kaplan-Meier method.

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Results

Seventy-seven patients were included in our study (Figure 1). Of these, 60 were in the Rit group, and 17 were in the non-Rit group. Table 1 shows patient background characteristics. Between the two groups, there were significant differences in age (median; 53 vs 37, *P*=0.04), proportions of ABO compatible (20% vs 100%), ABO minor mismatch (29% vs 0%), ABO major mismatch (51% vs 0%) (*P*<0.01), and DSA positive patients (29% vs 0%, *P*=0.01) and in the presence of a cross-reactive group (CREG) (46% vs 12%, *P*=0.01). However, no statistically significant differences were observed in other variables including sex (65% vs 65%, *P*=1.00), FSGS (3% vs 0%, *P*=1.00), percentage of CMV-seronegative recipients of renal allografts from CMV-seropositive donors (12% vs 18%, *P*=0.68), and percentage of CMV-seronegative recipients of renal allografts from CMV-seronegative donors (3% vs 6%, *P*=0.54). No cases were repeat renal transplantation. Among donors, there were no differences in age (median, 59 vs 61, *P*=0.57) and preoperative sCr (median, 0.69 vs 0.70 mg/dL, *P*=0.85).

Figure 2 shows long-term renal function after transplantation. A significant difference in sCr concentration was observed only at 3 months (median, 1.37 vs 1.08 mg/mL, *P*=0.03). sCr concentrations did not differ significantly between groups after 3 months. sCr concentrations were 1.48 vs 1.32 mg/mL (*P*=0.16), 1.45 vs 1.23 mg/mL (*P*=0.30),

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1.44 vs 1.23 mg/mL (*P*=0.31), 1.44 vs 1.28 mg/mL (*P*=0.38), and 1.38 vs 1.48 mg/mL (*P*=0.49) at 6, 9, 12, 18, 24 months, respectively. There was no significant difference between two groups in eGFR at any time point.

There was no significant difference in graft survival (log-rank, *P*=0.79) and death-censored graft survival (log-rank, *P*=0.43) (Figure 3). The 5-year survival rates were 90% vs 83% in graft survival and 90% vs 83% in death-censored graft survival. In the Rit group, graft loss occurred in 3 recipients. Each was related to acute cellular rejection (ACR) at 23 months, the recurrence of tubular acidosis associated with eating disorders at 36 months, and BKV nephropathy at 37 months. In addition, one patient died of an accident not related to transplantation. In the non-Rit group, there were 2 cases of graft loss. One was due to the suspected recurrence of FSGF at 24 months, and the other was due to ACR at 32 months after transplantation.

There was no significant difference in the incidence of CMV antigenemia >5 (24% vs 47%, *P*=0.07), CMV clinical symptoms (10% vs 24%, *P*=0.22) including fever ≥38℃ (5% vs 12%, *P*=0.31) and gastrointestinal symptoms (5% vs 12%, *P*=0.31), G-CSF administration (31% vs 24%, *P*=0.77) and acute rejection (7% vs 6%, *P*=1.00) (Table 2).

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Discussion

Our data suggest that low-dose rituximab provides excellent graft survival in immunological high-risk kidney transplantation without increasing CMV infection without valganciclovir prophylaxis.

Rituximab is a monoclonal chimeric human-murine antibody against the protein CD20 and is composed of 1382 amino acids. CD20 is located mainly on the surface of immune system B cells, except for terminally differentiated plasma cells. Antibody binding to the surface of CD20 is the trigger for the death of cells through complement-dependent cytotoxicity, antibody-dependent-cellular-cytotoxicity, apoptosis of CD20-positive cells, and other mechanisms.[13,14]

Rituximab was originally developed as an antitumor agent for CD20-positive B cell lymphoma. Since then, adaptation of rituximab has expanded to the field of blood cancers, autoimmune diseases, and organ transplantation. In renal transplantation, rituximab has been used for the treatment of AMR and post-transplant lymphoproliferative disorder, or as an induction therapy for ABOi and HLAi renal transplantation.[15] A reduction in B cells in the peripheral blood occurs within 1–3 days after administration, and B-cell depletion lasts for over 15 months.[16] Therefore, we administered rituximab 1 to 2 weeks before renal transplantation in terms of B cell reconstitution (autoimmune reset). Some adverse

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events related to rituximab have been reported, such as infectious complications, LON, progressive multifocal leukoencephalopathy, and cardiovascular events.[6] In a prospective, double-blind, randomized, placebo-controlled study of rituximab as an induction therapy, there was no significant difference in the incidence of infections caused by CMV (4% vs 1%) or in severe leukopenia (4% vs 1%).[9] However, the background characteristics of the patients in their study were different from ours in many ways: 1. One dose of rituximab 375 mg/m^2 body surface area or one dose of placebo was administered within 24 hr before revascularization. 2. Immunological high-risk patients, such as ABO incompatible recipients, were not included. 3. No patient received immunological agents more than one day before transplantation. 4. No patient received induction therapy with basiliximab. 5. All patients received antiviral prophylaxis with valganciclovir if CMV incompatible otherwise with valaciclovir. Although the incidence of CMV infection seems to be higher in our study than their study, there was no significant difference between groups without antiviral prophylaxis, suggesting that low-dose rituximab does not increase CMV infection even without antiviral prophylaxis.

A shortage of donors is a serious problem in Japan. The average waiting time for deceased donor renal transplantation is 17 years, and deceased donor renal transplantations account for only 11% of all renal transplantations.[5] To expand living

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donor resources, ABO minor and major mismatch renal transplantation is routinely performed. ABO major mismatch renal transplantation accounts for 29.3% of living donor renal transplantation in Japan.[5] The biggest task for ABO major mismatch renal transplantation is to prevent graft loss by AMR with anti-A antibody and anti-B antibody, and rituximab is helpful in that regard.[17,18] Splenectomy had been used but was replaced with rituximab as an induction therapy in ABO major mismatch transplantation.[19]

DSA negatively impacts graft survival. The mainstream of general desensitization in these renal transplantations is B cell depletion by rituximab, and antibody removal by blood purification therapy and IVIG. Since national health insurance does not cover rituximab and IVIG in Japan, only rituximab is used in most institutions after IRB approval, and IVIG is avoided because of the cost. Therefore, the mainstream approach to general desensitization in renal transplantation is B cell depletion by rituximab and antibody removal by blood purification therapy including DFPP or PEX in Japan.

In our facility, we used rituximab as induction therapy, not only for ABO major mismatch, but also for ABO minor mismatch, for the following reasons: 1. ABO minor mismatched renal transplantation is immunologically higher risk than ABO matched. 2. To avoid passenger lymphocyte syndrome in ABO minor mismatched renal transplantation.[20] It is known that hemolytic anemia is sometimes caused because of graft

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versus host disease in the acute phase of ABO minor mismatched renal transplantation.[21-23] Renal graft irradiation can prevent hemolytic anemia, but there is a risk of secondary carcinogenesis by irradiation.[22,24]

In this study, we recognized the excellent efficacy of rituximab as an induction therapy. Graft survival and death-censored graft survival were not different between groups, even though low-dose rituximab was used in immunological high-risk patients. There was no difference in long-term renal function between groups until 24 months. Furthermore, there were no significant differences between groups in the incidence of CMV infection, G-CSF treatment and acute rejection. Rituximab has the potential to improve graft survival without increasing CMV infection.

Several studies have assessed rituximab as an induction therapy. Tyden et al. reported that the administration of rituximab results in fewer and milder cases of rejection without increasing infectious complications and leukopenia during the first 6 months after transplantation.[9] Takagi reported that cytokine release syndrome may result in T-cell activation and increase in the risk of acute rejection. They suggested that the dose and timing of rituximab administration may be important to prevent acute rejection.[25] We administered a rituximab dose of 200 mg/body 1 to 2 weeks before renal transplantation based on a previous study, which seems to be appropriate.[25]

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This study has some limitations. First, it was a retrospective small study in one single center, and thus has the potential for selection bias. Second, we administered rituximab 1 to 2 weeks before renal transplantation in our studies. Many studies have shown that rituximab was given as induction therapy at the day of renal transplantation.[8] However, as mentioned above, we think that rituximab should be administered more than 3 days before living-donor renal transplantation from the view point of autoimmune reset.

Despite these limitations, these findings demonstrate that low-dose rituximab induction therapy is effective in immunological high-risk recipients without increasing adverse events. In conclusion, low-dose rituximab induction therapy is effective in immunological high-risk recipients. It does not increase CMV infection in patients who do not receive valganciclovir prophylaxis.

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Conflict of interest

None declared.

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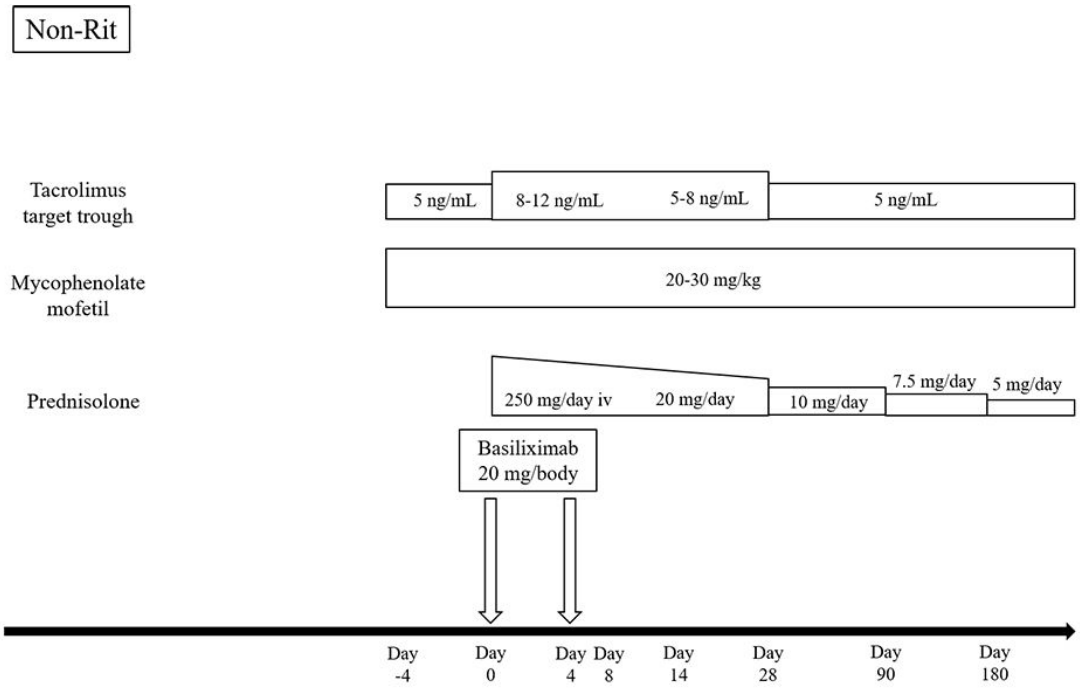

Supplementary Figure 1

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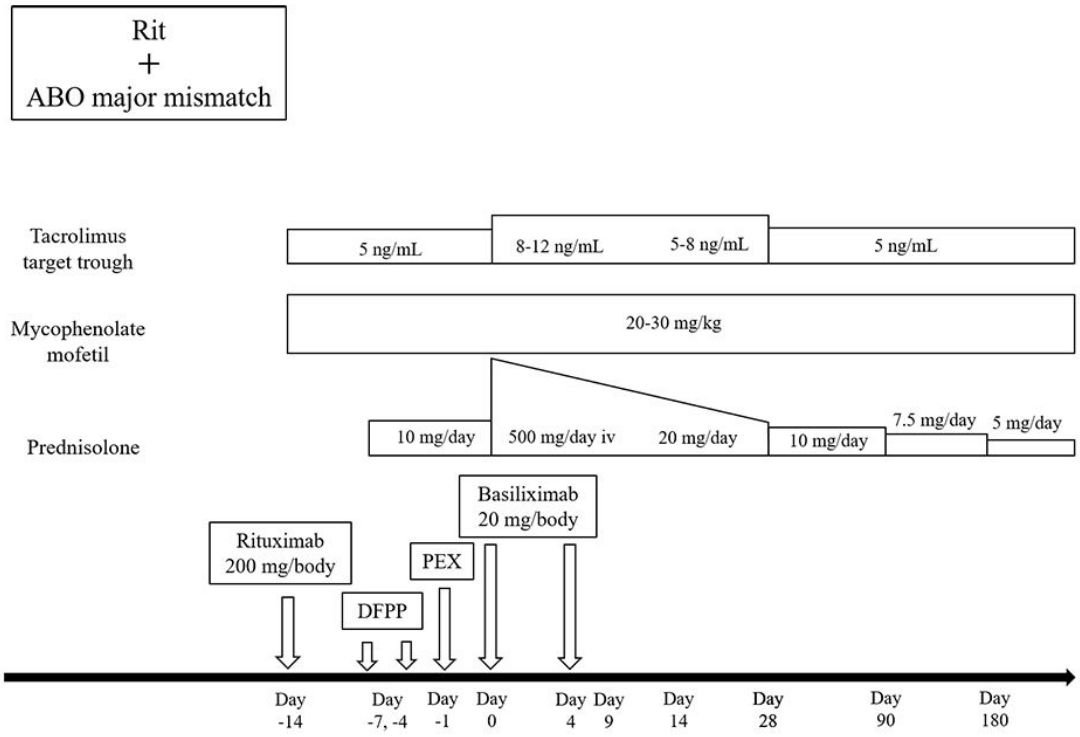

Supplementary Figure 2

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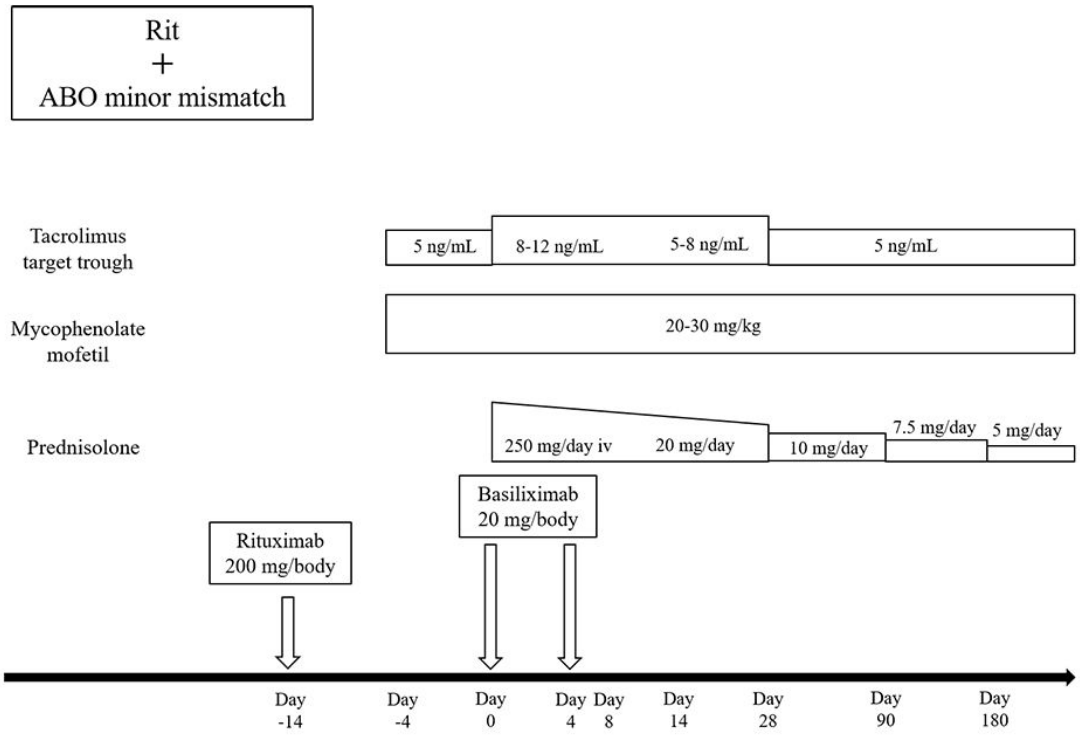

Supplementary Figure 3

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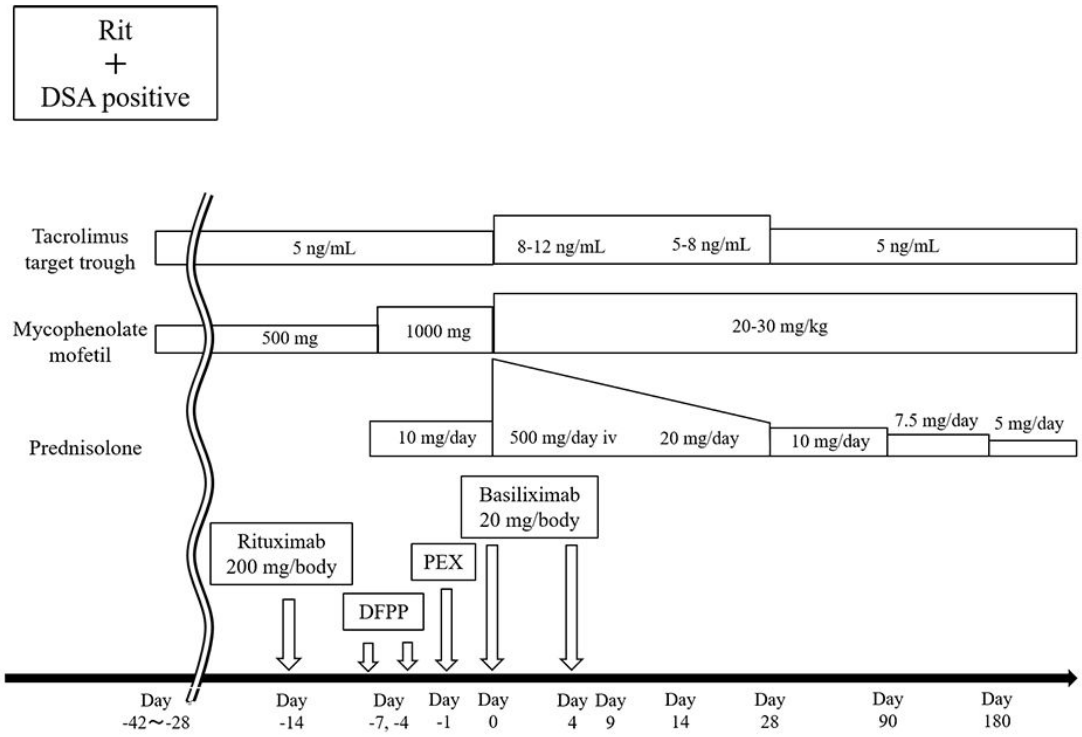

Supplementary Figure 4

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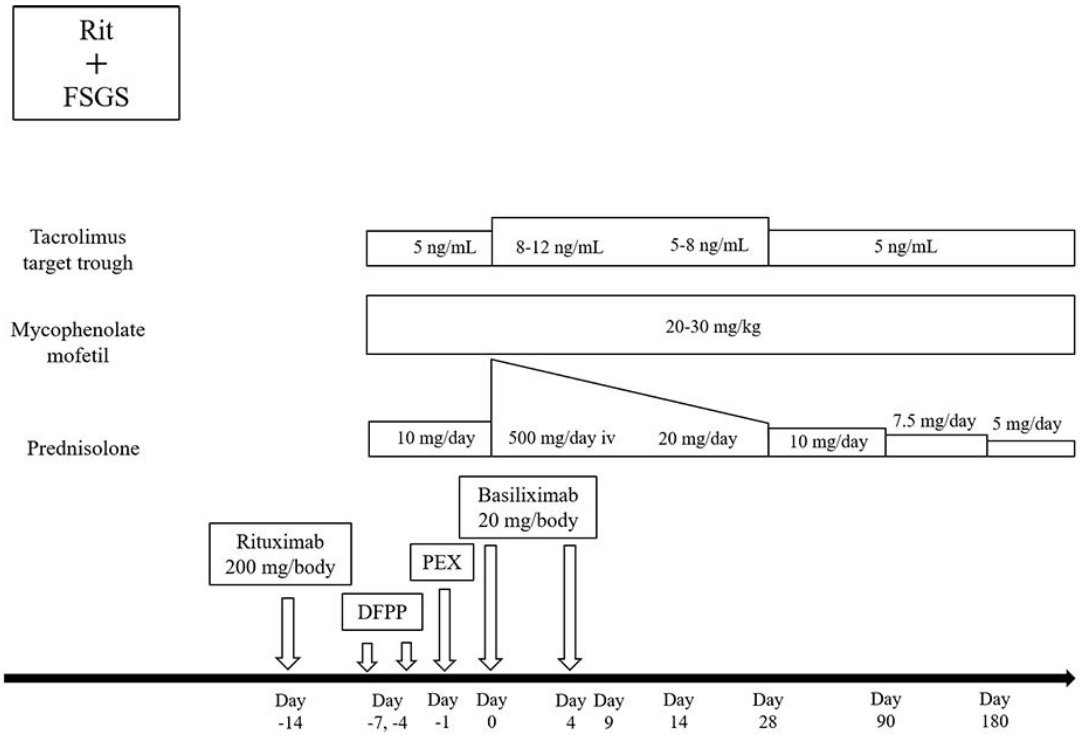

Supplementary Figure 5

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Figure legends

Figure 1. Study flow chart.

The flow chart shows the derivation of the two groups.

Figure 2. Mean renal function transition over 24 months in the two groups.

Regarding serum creatinine, a significant difference was observed only at 3 months (median; 1.37 vs 1.08 mg/mL, *P*=0.03). For the estimated glomerular filtration rate, no significant difference was observed at any time point.

Figure 3. Kaplan-Meier (A) graft survival and (B) death-censored graft survival curves in Rit and Non-Rit groups.

Both showed no statistically significant differences (A: p=0.79, B: 0.43). Number at risk indicates the number of active patients at each time interval.

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Table 1. Background characteristics

	Total	Rit	Non-Rit	P-value
Total cases, n (%)	76 (100)	59 (77)	17 (23)	
Recipients				
Age, median (IQR)	46 (31 - 58)	53 (33 - 60)	37 (30 - 50)	0.04
Sex (male), n (%)	49 (64)	38 (64)	11 (65)	1.00
ABO, n (%)				<0.01
Match	30 (39)	12 (20)	17 (100)	
Minor mismatch	17 (22)	17 (29)	0 (0)	
Major mismatch	30 (39)	30 (51)	0 (0)	
DSA (+), n (%)	17 (22)	17 (29)	0 (0)	0.01
CREG (+), n (%)	29 (38)	27 (46)	2 (12)	0.01
FSGS, n (%)	2 (3)	2 (3)	0 (0)	1.00
CMV D+R-, n (%)	10 (13)	7 (12)	3 (18)	0.68
CMV D-R-, n (%)	3 (4)	2 (3)	1 (6)	0.54
Donors				
Age, median (IQR)	59 (53 - 64)	59 (54 - 65)	61 (48 - 64)	0.57
Preoperative Cr, median (IQR)	0.69 (0.59 – 0.79)	0.69 (0.60 - 0.80)	0.70 (0.58 - 0.77)	0.85

IQR, interquartile range; DSA, donor specific anti-HLA antibodies; CREG, cross reactive group; CMV D+R-, cytomegalovirus-seronegative recipients of renal allografts from CMV-seropositive donors; CMV D-R-, cytomegalovirus-seronegative recipients of renal allografts from CMV-seronegative donors; Cr, creatinine.

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Table 2. Adverse events

	Total	Rit	Non-Rit	P-value
Adverse event, n (%)				
Total cases	76 (100)	59 (77)	17 (23)	
CMV antigenemia ≥ 5	22 (29)	14 (24)	8 (47)	0.07
CMV clinical symptoms	10 (13)	6 (10)	4 (24)	0.22
Fever ≥ 38℃	5 (7)	3 (5)	2 (12)	0.31
Gastrointestinal symptoms	5 (7)	3 (5)	2 (12)	0.31
G-CSF administration	22 (29)	18 (31)	4 (24)	0.77
Acute rejection	5 (7)	4 (7)	1 (6)	1.00

CMV, cytomegalovirus; G-CSF, granulocyte-colony stimulating factor.

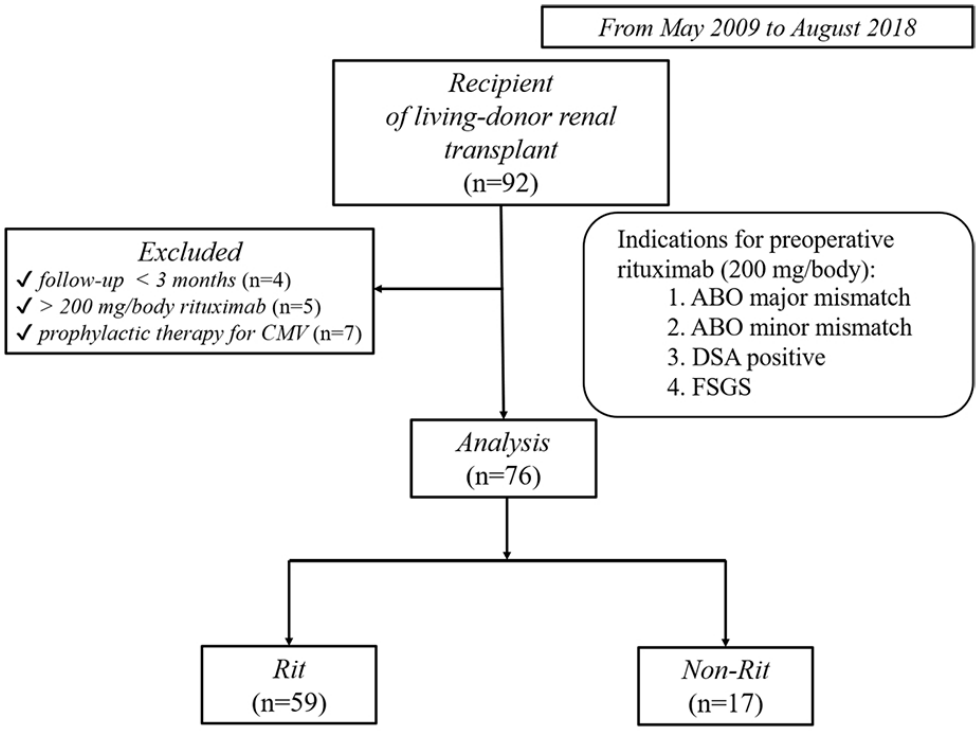
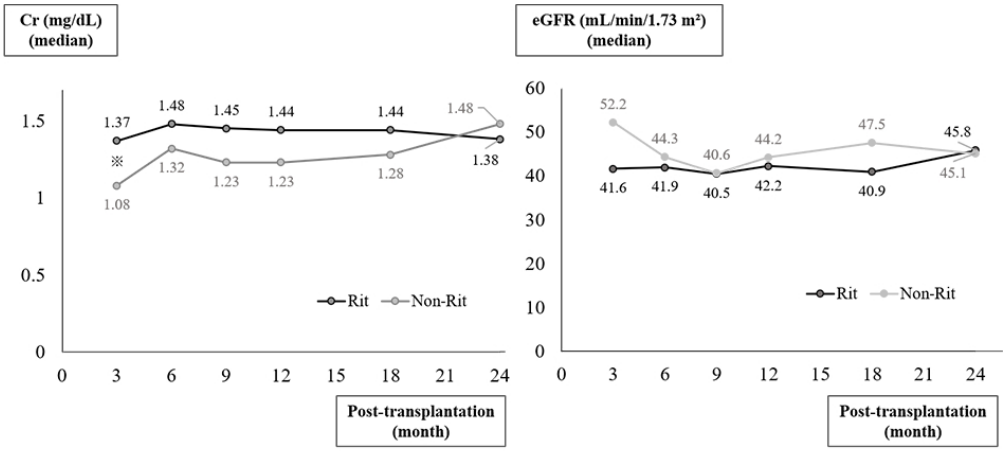


Figure 1

Figure 1. Study flow chart.

80x64mm (300 x 300 DPI)



Month	3	6	9	12	18	24
P-value	0.03	0.16	0.30	0.31	0.38	0.49

Month	3	6	9	12	18	24
P-value	0.24	0.83	0.78	0.93	0.28	0.71

Figure 2

Figure 2. Mean renal function transition over 24 months in the two groups.

80x51mm (300 x 300 DPI)

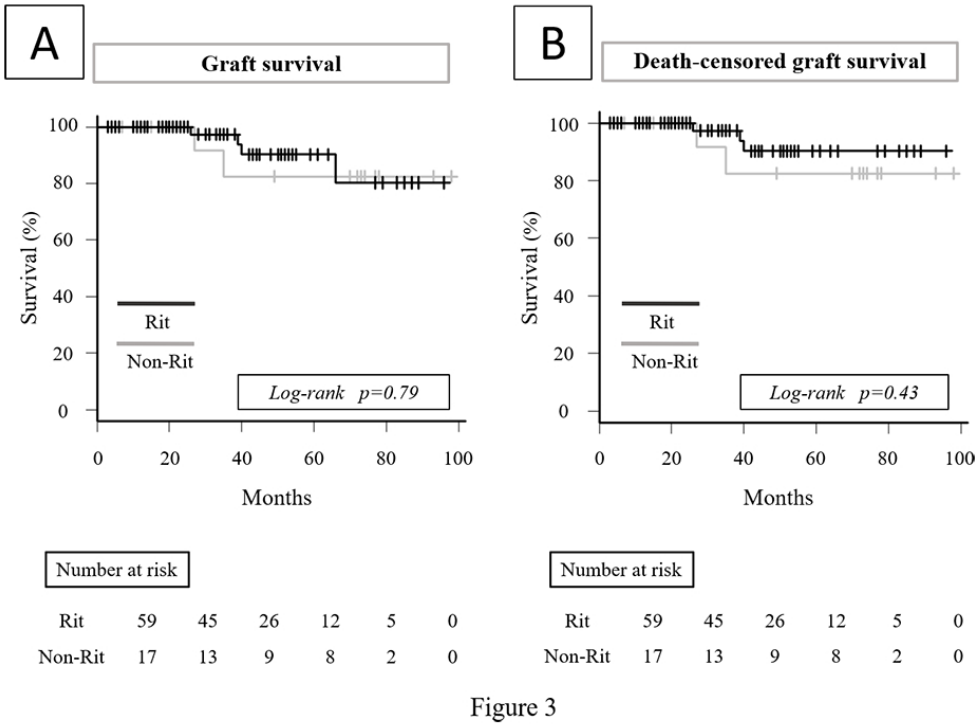


Figure 3. Kaplan-Meier (A) graft survival and (B) death-censored graft survival curves in Rit and Non-Rit groups.

80x59mm (300 x 300 DPI)