Remission Induction Therapy with Rituximab for Microscopic Polyangiitis: A Feasibility Study

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Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is systemic vascular inflammation. Microscopic polyangiitis (MPA) is a major type of AAV in Japan. MPA often affects the kidneys and lungs, leading to death if untreated. Induction therapy (i.e., initial treatment) for MPA has not been optimized, although methylprednisolone and cyclophosphamide are commonly used. Recently, rituximab (RTX) (a monoclonal antibody against the protein CD20) has also been used to treat refractory AAV. RTX at 375 mg/m²/week for 4 weeks (i.e., the conventional lymphoma dosing schedule) is used, but the optimal dosing schedule is controversial. Indeed, a single-dose of RTX successfully controlled nephrotic syndrome. However, to date, the effectiveness of a single RTX dose in treating MPA has not been fully investigated in Japan. This was a retrospective observational study. Six newly diagnosed patients with MPA were initially treated with methylprednisolone and a single dose of RTX (375 mg/m²). We investigated the patients' clinical features, as well as the efficacy and safety of RTX treatment. All patients attained remission on a tapered prednisolone dose of < 10 mg/day during the first 12 months. One patient relapsed after 12 months whereas another required hospitalization owing to infective spondyloarthritis. Adverse reactions to RTX infusion and late-onset neutropenia were not observed. Therefore, a single-dose treatment with RTX induced remission with few complications, and allowed tapering the prednisolone treatment. We conclude that a single dose of RTX is a promising induction therapy for MPA, reducing the cost associated with multiple doses.

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Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)associated vasculitis (AAV) is a potentially life-threatening autoimmune disorder (Serra et al. 1984; Savage et al. 1985). AAV is classified into four types according to clinical symptoms, clinical presentation, and laboratory findings: eosinophilic granulomatosis with polyangiitis (EGPA), granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and polyarteritis nodosa (PAN) (Jennette et al. 1994; Wiik 2002; Jennette et al. 2013). Among these types of AAV, the incidence of MPA is higher in Japan than it is in the US and Europe (Fujimoto et al. 2006, 2011; Shiraki et al. 2007; Watts et al. 2008). The effects of AAV are often restricted to small- and medium-sized vessels, resulting in kidney and lung pathologies in patients with MPA. Intensive immunosuppressive therapy has been used to improve the prognosis of patients with MPA (Mukhtyar et al. 2009a), including first-line drugs such as glucocorticoids (GC) and cyclophosphamide (CY) (Yamagata et al. 2005, 2012). Other advances in the development of AAV therapeutics have also improved prognosis (Koyama et al. 2009). Recently, rituximab (RTX), a monoclonal antibody against CD20 (which is primarily found on the surface of B cells), has been used in the treatment of AAV (Greco et al. 2015) and has been reported to be as effective as CY (Stone et al. 2010; Geetha et al. 2015). However, approximately 36-60% of patients receiving RTX treatment develop infections (Stone et al. 2010; Geetha et al. 2015).

Nagafuchi et al. (2015) reported that Japanese patients receiving RTX for the treatment of refractory AAV showed beneficial outcomes; however, these patients also developed various infections. We suggest that the incidence of infections in RTX-treated patients with AAV may be attributable to the dosage used, which is equivalent to that used to treat B-cell lymphoma (four consecutive weekly doses of 375 mg/m²). Furthermore, B-cell depletion after RTX administration lasts for 6-9 months. AAV is not a B-cell lymphoproliferative disease and, therefore, four weekly doses of RTX may excessively suppress normal immune functions.

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Studies have shown that a single dose of RTX is effective in the treatment of some types of nephrotic syndromes including minimal change of nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS) (Kamei et al. 2009; Ochi et al. 2012). These reports suggest that relapses develop simultaneously with the recovery of B-cells. Turner-Stokes et al. (2014) reported that a single dose of RTX is a reasonable therapy for inducing remission in patients with AAV.

Taken together, these results suggest that a single dose of RTX is a viable option for induction therapy in patients with AAV. However, the effects of a single dose of RTX for AAV induction therapy have not been fully investigated in Japan. Therefore, to clarify the efficacy, safety, and adverse effects of RTX therapy, we describe a single center experience of treating MPA using single-dose RTX.

Materials and Methods

Patients and methods

In this retrospective, observational, single-center study, six patients with AAV receiving RTX as an induction therapy were monitored. According to the algorithm used, the conditions of all patients were classified as MPA (Tables 1 and 2). All six patients were primarily administered methylprednisolone followed by a single dose of RTX (375 mg/m²) (Table 3). All patients were observed for a 12-month follow-up period. Then, 500 mg of methylprednisolone was administered intravenously. CY (10 mg/kg) was also administered intravenously. The frequency of administration is shown in Table 3. RTX (357 kg/m²) was administered only once.

Diagnostic algorithm

The types of vasculitis were classified using an algorithm (Watts et al. 2007). The diagnostic criteria of vasculitis differ slightly between Western countries and Japan. The validity of the diagnosis was confirmed according to the Japanese diagnostic criteria (Ozaki 2007).

The types of vasculitis were based on severity and organ involvement, as reported previously (Ozaki et al. 2012) with the following definitions. (1) The severe form consisted of a generalized type (MPA with the involvement of more than two organs), a pulmonary-renal type (glomerulonephritis plus either limited pulmonary hemorrhage or extended interstitial pneumonia), and a rapidly progressive glomerulonephritis (RPGN) type. (2) The most severe form was defined as that with diffuse alveolar hemorrhage, intestinal perforation, acute pancreatitis, cerebral hemorrhage, or concurrent antiglomerular basement membrane antibodies. This form also included patients with the severe form who were resistant to the severity-based treatment protocol described below. (3) The mild form included a renal-limited type (except for RPGN), a pulmonary-limited type (except for pulmonary hemorrhage), and other mild forms.

Assessments of severity of vasculitis

Clinical classification, clinical severity score, and grade were classified according to a previous report (Ozaki et al. 2012). Briefly, the scoring system of severity was as follows. Grades 1, 2, 3, and 4 were equivalent to the sums of the scores 0-2, 3-5, 6-7, and 8-9, respectively. The grading system consisted of four elements: the value of serum creatinine, age, the presence of lung involvement, and

value of serum C-reactive protein (CRP). The sum of the scores was the severity score.

A serum creatinine level < 3.0 mg/dL was scored 0, \ge 3.0 and < 6.0 mg/dL was scored 1, and \ge 6 mg/dL was scored 2. If the patient was receiving renal replacement therapy, the score was 4.

Furthermore, age < 60 years was scored $0, \ge 60$ and < 69 years was scored 1, and age ≥ 70 years was scored 2. The absence and presence of lung involvement were scored 0 and 2, respectively. A serum CRP value < 2.6 mg/dL was scored 0, ≥ 2.6 mg/dL and < 10 mg/dL was scored 1, and ≥ 10 mg/dL was scored 2.

The disease severity, which was used to evaluate vasculitis activity, was assessed based on previous reports and the Birmingham vasculitis activity score (BVAS, 2003 ver. 3.0). The BVAS is associated with vasculitis and represents the whole-body signs of damage in eight organs (skin, mucous membrane/eye, ear/nose/throat, chest, cardiovascular, abdominal, renal, and nervous system). BVAS is usually used for GPA diagnosis, but is useful in Japanese patients with MPA (Yumura et al. 2014).

The vasculitis damage index (VDI) was scored at 3, 6, and 12 months (Mukhtyar et al. 2009a). VDI is an indicator of chronic disorders due to vasculitis and side effects due to treatment (Exley et al. 1997).

Definition of remission and relapse

We used a previously reported definition of remission and relapse (Mukhtyar et al. 2009b). Briefly, complete remission was defined as the absence of clinical manifestations of active vasculitis and a BVAS2003 of 0-1. Remission was defined as the absence of clinical manifestations, but with a BVAS2003 score > 1; relapses were defined as the recurrence or development of at least one manifestation of vasculitis. Major and minor relapses required more intensive immunosuppressive drug treatment and an increase in oral steroids to control the vasculitis.

Outcome

Primary outcomes of this study included the ratio and length of remission and incidence of vasculitis relapse. Secondary outcomes included safety and adverse effects of RTX treatment as well as adverse events resulting in hospitalization. The kidney function was determined using the formula for estimated glomerular filtration rate (eGFR) for Japanese individuals (Matsuo et al. 2009).

Informed consent

All procedures in this study were performed in accordance with the ethical standards of the Red Cross Ishinomaki Hospital (Approval Number 15-39) and the 1964 Helsinki Declaration as well as its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

Results

Patient characteristics

Patients were newly diagnosed with MPA, and their characteristics at the time of enrollment are shown in Table 1. All patients were consulted at our department for further examination of RPGN.

Patient 1 was initially suspected to have infective endocarditis, and then developed cerebral infarction during

Patient	Age (years)	Sex	Height	Weight	Diagnosis	Туре	Clinical classification	Clinical severity score	Grade	BVAS	Pulmonary finding	Berden classification	Other involved organ
			(cm)	(kg)									
1	55	М	167	68.9	MPA	RPGN	Severe	2	Ι	34	None	Mixed	Heart, CNS
2	53	F	164	40.7	MPA	Pulmo- Renal	Severe	5	Π	18	Interstitial pneumonia	Crescentic	Joint
3	79	М	153	54	MPA	RPGN	Severe	2	II	12	None	Mixed	None
4	76	F	141	40.6	MPA	Pulmo- Renal	Most Severe	5	Π	23	Alveolar hemorrhage	Crescentic	None
5	73	М	169	51.7	MPA	RPGN	Severe	3	II	12	None	Sclerotic	None
6	78	F	153	64.1	MPA	RPGN	Severe	4	II	16	None	Focal	None

Table 1. S	summary of	patients.
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MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis.

RPGN, rapid progressive glomerulonephritis; CNS, central nervous system; BVAS, Birmingham vasculitis activity score.

the course with fever that was unresponsive to antibiotic therapy. The patient showed gradually increasing serum creatinine levels accompanied by microhematuria and proteinuria. Renal biopsy showed pauci-immune crescentic glomerulonephritis, and the patient was finally diagnosed with MPA.

Patient 2 was initially admitted for suspected interstitial pneumonia. After admission, the serum creatinine level rose, accompanied by microhematuria and proteinuria. The condition of patient 2 was very complicated, and the patient did not show any E symptoms (E symptoms include nose, eyes, ears, and throat symptoms). These investigations were conducted by otolaryngologists and ophthalmologists. Patient 2 showed interstitial pneumonia but no evidence of a nodule or cavity in the lung field. Renal biopsy showed necrotizing glomerulonephritis without immune complex deposits and no granuloma in the kidney. We subsequently diagnosed the condition as MPA. However, it is difficult to classify the various types of vasculitis, which often overlap (Khan and Watts 2013). The research team on intractable vasculitis reported that 2.3% of patients with MPA were serum proteinase 3 (PR3)-ANCA-positive (Japan Intractable disease information center, http://www.nanbyou. or.jp/entry/245, last access 2017/4/13). We followed up the patient for over 2 years, and no E or additional lung symptoms were observed.

Patients 3, 4, and 5 were positive for ANCA. They showed decreased renal function accompanied by microhematuria and proteinuria, and they were diagnosed with MPA.

Patient 6 developed renal dysfunction with microhematuria and proteinuria and was ANCA-negative. Renal biopsy showed pauci-immune crescentic glomerulonephritis, and the condition was diagnosed as MPA.

In summary, all patients presented with renal dysfunction (creatinine, 1.29-4.05 mg/dL) accompanied by microhematuria (urinary red blood cell count > 21-30/high power field) and proteinuria (0.8-4.3 g/g creatinine). Patients had anemia (hemoglobin (Hb), 7.1-10.7 g/dL), low serum albumin level (2.4-3.0 g/dL), and slightly increased CRP level (0.27-2.26 mg/dL). These data suggest glomerulonephritis with systemic inflammation.

The mean patient age was 69 years (range, 55-79 years) and the type of AAV, severity, clinical severity score, and grade are shown in Table 1. All patients underwent percutaneous renal biopsy. The Berden classification and histopathologic classification of ANCA-associated glomerulonephritis are presented in Table 2 (Berden et al. 2010). The Berden pathologic classification of renal pathology was used to determine the renal outcome. According to the flowchart, renal pathology was classified into four groups: (1) renal biopsy revealed that more than 50% of the glomeruli were sclerotic, classified as sclerotic class; (2) renal biopsy did not meet condition 1 and more than 50% of the glomeruli were normal, classified as focal class; (3) renal biopsy did not meet conditions 1 and 2, while more than 50% of the glomeruli showed cellular crescents, classified as crescentic class; and (4) renal biopsy did not meet any of the above conditions, classified as mixed class.

Furthermore, the type of pulmonary disease is described in Table 1. A summary of the laboratory data at diagnosis is shown in Table 2, while details of the induction/combination treatments and adverse events are shown in Table 3.

Primary outcomes

The time course of the peripheral cluster of differentiation 20 (CD20) and CD19 counts is shown in Fig. 1. CD20-positive blood cell counts were decreased quickly after RTX administration in the five patients, except for Patient 1 (Fig. 1A), and CD20 counts remained lowered during the course of 12 months in all patients. These results suggest the efficacy of the RTX treatment. Likewise, CD19-positive blood cell counts were decreased in two patients (Patient 4 and 6) after RTX administration (Fig. 1B), and CD19 counts were maintained at low levels in all

Table 2. Summary of laboratory data at diagnosis.

Patient	Hb	WBCs	Neutrophils	Eosinophils	Lymphocytes	Albumin	Cre	eGFR	Proteinuria	uRBC	CRP	MPO-ANCA	PR3-ANCA
	(g/dL)	(/µL)	(/µL)	(/µL)	(/µL)	(g/dL)	(mg/dL)	(mL/min/1.73 m ²)	(g/gCre)	(/HPF)	(mg/dL)	(U/mL)	(U/mL)
1	8.7	7900	5680	95	2645	3.0	1.62	36.2	0.8	31-50	0.27	<1	<1
2	10.1	3700	2879	48	363	2.9	4.05	9.9	2.6	>100	1.16	<1	50.3
3	7.1	14000	13020	70	560	2.6	1.83	28.6	0.8	21-30	1.18	>300	<1
4	8.8	13300	10068	173	1902	3.0	1.29	31.3	1.5	>100	2.26	116	<1
5	10.7	12900	11739	65	587	2.5	3.19	15.9	0.9	>100	0.3	26.8	<1
6	8.3	10700	8624	33	1455	2.4	3.01	12.3	4.3	>100	1.21	<1	<1

Hb, hemoglobin; WBC, white blood cell; RBC, red blood cell; HPF, high power field; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; MPO, myeloperoxidase; PR3, proteinase 3; ANCA, anti-neutrophil cytoplasmic antibody; Cre, creatinine.

Table 3. Summary of induction/combination treatment and adverse effects.

Patient	Induction treatment 1	Induction treatment 2	Combination immunosuppressant	Adverse event during 12 months
1	mPSL pulse ×1		none	Exacerbation of CHF
2	mPSL pulse ×3	IVCY	none	none
3	mPSL pulse ×2		none	Infective spondyloarthritis, angina pectoris, intermittent claudication.
4	mPSL pulse ×1		none	none
5	mPSL pulse ×2		Mizoribine	none
6	mPSL pulse ×1		none	none

mPSL, methylprednisolone; IVCY, intravenous cyclophosphamide; CHF, congestive heart failure.

patients during the course of 12 months.

The mean BVAS value, which was 19 (range, 12-34) at study entry, was lower at 6 months after RTX treatment, compared with the value at baseline (see Table 1), and remission was observed in all patients at 6 months (Fig. 2). However, the BVAS values were increased from 6 to 12 months in two patients (Patient 4 and 5).

The mean VDI at 3 months was 4.2 (range, 2-6), and three patients (Patient 2, 4, and 5) showed improved VDI (Fig.3). Patient 2 and 4 showed diminished proteinuria (data not shown), and Patient 4 also showed the increased Hb level (> 10 g/dL). Likewise, Patient 5 showed decreased levels of proteinuria and increased Hb (data not shown). These factors might have contributed to the improved VDI (Fig. 3). On the other hand, the mean VDI remained unchanged in four patients (Patient 1-4) at 6 and 12 months (Fig. 3). In particular, Patient 1 showed no noticeable change in the VDI during 12 months. Two patients showed the increased VDI at 6 months compared with the value at 3 months (Patient 3 and 6). The VDI remained elevated at 12 months in Patient 3, but was decreased to the value at 3 months in Patient 6. Such a difference may be related to the fact that Patient 3 suffered from infective spondylolysis, angina pectoris, and intermittent claudication (see Table 3).

All patients exhibited low eGFR (< 50 mL/min/1.73 m²) (Fig. 4) and proteinuria (> 0.5 g/g creatinine) at diagnosis (see Table 2). The eGFR was increased in five patients, except for Patient 1 with the continuous decrease in eGFR (Fig. 4). Patient 1 had advanced diabetic complications (retinopathy and nephropathy) with a persistent non-nephrotic range of proteinuria (1.0-2.0 g/g creatinine). RTX administration did not affect the glycemic control, and renal dysfunction progressed. We assume that the renal function declined due to diabetes but not vasculitis.

We succeeded in gradually reducing the dose of prednisolone in all patients during 12 months (Fig. 5). However, one patient relapsed 367 days after starting immunosuppressive therapy (Patient 4), which was associated with the increased dose of prednisolone.

There were four ANCA-positive patients (see Table 2). ANCA titers were well suppressed after treatment of these patients (Patient 2, 3, 4 and 5) (Fig. 6). However, Patient 4 relapsed after 12 months (see Fig. 5).

Adverse effects

During the 12-month follow-up period, two patients were admitted to the hospital (Table 3). One patient was



Fig. 1. Change of peripheral B cell count.

A. Time course change of peripheral differentiation 20 (CD20) count after single dose of rituximab (RTX). CD20-positive blood cell counts were determined in five patients prior to RTX administration (Pre RTX) and then over the course of 12 months after a single dose of RTX. CD20 counts were decreased quickly after RTX administration in the five patients. CD20 counts remained lowered over the duration of monitoring. B. Time course change of peripheral differentiation 19 (CD19) count after single dose of rituximab (RTX).

diagnosed with exacerbated heart failure that was not a vasculitis-related or treatment-related event; this patient was discharged after conservative therapy (Patient 1). The second patient, who was diagnosed with infective spondyloarthritis, was administered antibiotics and discharged; we determined that this was a treatment-related event (Patient 3).

Discussion

The results of this feasibility study suggest that singledose RTX therapy is a viable option for induction therapy of patients with MPA. All the patients achieved remission 6 months after commencing treatment. We understand that the study population size was too small to determine the effectiveness and incidence of adverse effects. However,



Fig. 2. Changes in Birmingham vasculitis activity score (BVAS) over time.



Fig. 3. Changes in vasculitis damage index (VDI) over time.

other valuable information was revealed in this study, which will be the focus of the rest of the discussion.

The remission ratio observed in this study was similar to that previously observed after four consecutive weekly administrations of RTX (Stone et al. 2010). One patient, Patient 4, with exacerbated general fatigue required urinalysis and intensive immunosuppressive therapy during the first 12 months, and methylprednisolone pulse therapy successfully induced a complete remission. The relapse incidence was also similar to that previously reported using four consecutive weekly administrations of RTX (Specks et al. 2013). This patient developed recurring vasculitis despite having a CD19 and CD20 count that had not yet reverted to baseline values. A previous report has shown that a single dose of RTX completely suppresses B-cells (Turner-Stokes et al. 2014), which is supported by the results of our present study (Fig. 1). These data suggest that a single dose of RTX sufficiently suppressed peripheral B-cell counts.

Specks et al. (2013) suggested that RTX therapy is inferior to CY therapy for refractory myeloperoxidase-ANCA. However, our data suggest that single dose RTX therapy is equivalent to a CY regimen for induction in patients newly diagnosed with AAV (Geetha et al. 2015).



Fig. 4. Changes in estimated glomerular filtration rate (eGFR) over time.



Fig. 5. Changes in dose of prednisolone administered over time.

In the present study, one patient, Patient 3, developed a serious infection, which is an important finding. As mentioned above, a traditional RTX dose of 375 mg/m^2 for 4 consecutive weeks resulted in a total infection rate of 36-60% (Stone et al. 2010; Geetha et al. 2015), suggesting that single-dose RTX therapy may reduce complications from infections. Lowering the incidence of infectious complications is a key factor in controlling vasculitis.

Single-dose RTX therapy was successfully used to taper methylprednisolone treatment. All patients achieved remission on < 10 mg/day methylprednisolone during the first 12 months, which was an important factor in the low

incidence of infective complications.

The European League Against Rheumatism-European Dialysis and Transplant Association (EULAR-EDTA) task force recommends between 7.5 and 10 mg prednisolone after 5 months of treatment (Yates et al. 2016). In this study, the average prednisolone dose was 10.4 and 6.4 mg after 3 and 5 months, respectively. Optimal prednisolone reduction regimens after single-dose RTX therapy should be investigated in future studies.

Another advantage of RTX therapy is that dose adjustments to compensate for changes in renal function are unnecessary. The RiCRAV trial reported that two out of



Fig. 6. Changes in anti-neutrophil cytoplasmic antibody (ANCA) titer over time.

seven patients with AAV who were treated with RTX were diagnosed with cancer. However, while these patients were previously treated with other drugs, such as CY, methotrexate, cyclosporine, azathioprine, mizoribine, and infliximab, these factors should not be dismissed because the duration of AAV treatment is 1-7 years. Whether RTX is a human carcinogen or not remains controversial. Patients with AAV already have an increased risk of developing malignancies (Pankhurst et al. 2004; De Groot et al. 2005), but there are reports that RTX does not induce carcinogenesis (Mahr et al. 2013; Fleury et al. 2016). Consequently, careful attention should be focused on monitoring the onset of malignancy and follow-up of patients with RTX-treated vasculitis.

Late-onset neutropenia (LON) was not observed in this study. Knight et al. (2016) reported that 11.9% of patients with AAV developed LON after a median time of 86 days (range 56-168 days) following their last RTX treatment. As mentioned above, the size of our study is likely too small to detect LON, and therefore, further studies should be conducted to determine if a single dose of RTX could reduce the incidence of LON.

This study has some limitations, which are worth mentioning. For instance, this was a small, single-center, observational study, with limited detection power for defining complications. In addition, the enrolled patients were limited to those with low-grade severity, which prevented us from analyzing the effects of RTX in patients with highgrade severity. These points should be clarified in future, large randomized studies.

The levels of VDI appeared extremely high in this study. In addition to the description in the results section, patient 3 was administered medication for complications

but was not hospitalized, and this could have contributed to the rise in VDI levels.

CY sometimes causes pancytopenia. GC worsens the control of diabetes and induces osteoporosis and cataracts. GC use also increases VDI levels. RTX is not considered to affect blood sugar control, and is expected to be beneficial to avoid these problems (e.g., GC toxicity). Therefore, RTX is a promising treatment for AAV that is expected to produce fewer adverse effects.

Finally, we would like to mention the cost effectiveness of single-dose RTX. Specifically, the drug cost of a single dose of RTX therapy is one-fourth that of the conventional 4-week protocol. In this study, we could not investigate the length of hospital stay and other expenses. RTX is a very expensive drug (500 mg cost \pm 213,815). Therefore, the single dose is a promising cost-saving therapy compared to the conventional regimen.

In summary, single-dose RTX induction therapy for AAV is a promising induction regimen. Especially for patients to whom we want to avoid administering high-dose steroids or CY, single-dose RTX therapy would be more cost effective compared to the conventional weekly fourdose RTX therapy.

In conclusion, our study revealed that single-dose RTX is a promising induction therapy for MPA, and that its therapeutic effects were equivalent to those observed with four doses. Furthermore, single-dose RTX therapy sufficiently depleted B-cells, induced remission with few infectious complications, and succeeded in tapering GC treatments.

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Conflict of Interest

The authors declare no conflict of interest.

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