# Long-term outcome of patients with acquired primary idiopathic pure red cell aplasia receiving cyclosporine A. A nationwide cohort study in Japan for the PRCA Collaborative Study Group

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## ABSTRACT

### Background and Objectives

Cyclosporine A (CsA) has become one of the leading agents for the treatment of pure red cell aplasia (PRCA). However, further studies are necessary to determine the relapse-free survival (RFS) and overall survival (OS) of patients treated with this drug, the minimum duration of therapy for induction of remission, and whether or not there is need for maintenance treatment.

### Design and Methods

We conducted a nationwide survey in Japan. From a total of 185 patients (with 73 primary idiopathic PRCA and 112 with secondary PRCA), we evaluated 62 patients with primary idiopathic PRCA for this report.

### Results

The remission induction therapy for these patients included CsA (n=31), corticosteroids (CS) (n=20) or other drugs (n=11). CsA and CS produced remissions in 23 (74%) and 12 (60%) patients, respectively. The salvage treatment produced remissions in 58 patients (94%). Forty-one and 15 patients were maintained on CsA±CS (CsA-containing group) or CS alone (CS group), respectively. The median RFS in the CsA-containing group was 103 months, longer than that seen in the CS group (33 months) ($p<0.01$). Of 14 patients whose CsA was discontinued, 12 patients (86%) relapsed after a median of 3 months (range 1.5 to 40 months), while only 3 of 27 patients (11%) relapsed during CsA-containing maintenance therapy. Thus, the discontinuance of maintenance therapy was strongly correlated with relapse ($p<0.001$). Four patients in the CsA-containing group died; however, the OS of this group was not significantly different from that of the CS-groups ($p=0.104$).

### Interpretation and Conclusions

CsA-containing regimens sustain prolonged RFS more effectively than CS in primary idiopathic PRCA and seem to be important to prevent relapse.

Key words: pure red cell aplasia, cyclosporine A, relapse-free survival, maintenance therapy.

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Pure red cell aplasia (PRCA) is characterized by severe normochromic, normocytic anemia associated with reticulocytopenia and absence of erythroblasts from an otherwise normal bone marrow.[1-4] The acquired form of chronic PRCA may present as a primary hematologic disorder in the absence of any other disease, or secondary to neoplasms, infections, collagen vascular diseases, chronic hemolytic anemias, or after exposure to a variety of drugs and chemicals. Primary or secondary PRCA not responding to treatment of the underlying diseases is treated as an immunologically-mediated disorder.[1-4] Remissions have been achieved by treatment with corticosteroids (CS), cyclophosphamide, cyclosporine A (CsA), anti-thymocyte globulin (ATG), splenectomy, and plasmapheresis.[1-7] More recently, the anti-CD20 monoclonal antibody rituximab[8,9] and the anti-CD52 monoclonal antibody alemtuzumab (campath-1H)[10] have been reported to induce the remission of therapy-resistant PRCA. In general, remission induction can be easily achieved in the majority of patients. However, in the era before CsA became available, Clark *et al*. clearly showed that 80% of patients relapsed during the 24 months after having achieved remission.[11] Up to the present, the efficacy of CS, cyclophosphamide and CsA for patients with primary or secondary PRCA has been reported to be between 30-56%, 7-20% and 75-87%, respectively.[1-7,11] CsA has become established as one of the leading agents for the treatment of PRCA since the first, successfully treated cases in 1984.[12] However, it is unclear how many patients treated with CsA achieve a sustained remission and how many relapse. Up to the present, very few studies on the long-term follow-up of patients treated with CsA have been reported. Moreover, comparing one therapeutic approach to another for the treatment of PRCA is almost impossible since this disease is so rare that controlled studies are practically impossible to perform. We, therefore, conducted a nationwide survey of PRCA cases in Japan to elucidate the current status of immunosuppressive therapy for PRCA.

## Design and Methods

### Patients

The first questionnaires were sent to hematology departments in Japan to estimate the number of patients, aged 15 years and over, with newly diagnosed acquired chronic PRCA, excluding those with human parvovirus B19 infection. Secondary questionnaires were sent to collect data on underlying diseases, laboratory findings (including peripheral blood cell count with reticulocyte count and leukocyte differentials), findings of bone marrow examinations, immunological and cytogenetic parameters and the efficacy and the side effects of immunosuppressive therapy. Secondary questionnaires did not collect information on the trough concentration of CsA. The recommended dose of CsA in Japan is 6 mg/kg, to provide a trough concentration ranging from 150 to 250 ng/mL. The first period of the survey was between January 1990 and December 2004 across 47 institutions and the second period was between January 1990 and March 2006 across 109 institutions, including a follow-up survey of the patients identified in the first period. All combined, a total of 273 patients were enrolled from 45 institutions in response to the first questionnaires. A total of 185 patients were enrolled in response to the second questionnaires.

### Classification of PRCA

There are several proposed classifications of PRCA. One is based on pathophysiology[5] and another, on underlying diseases.[2] The prognosis of patients with PRCA, which is one of the most important end-points of this study, depends on the nature of their underlying diseases.[11] We, therefore, classified our patients with PRCA based on their underlying diseases, according to the classification proposed by Dessypris and Lipton[2] with some modifications. In this classification, primary PRCA comprises preleukemic, autoimmune and idiopathic forms. The patients with definite cytogenetic abnormalities were classified as having secondary PRCA as either myelodysplastic syndrome (MDS) or preleukemia. Primary autoimmune PRCA is defined as the cases in which an immune pathogenic mechanism can be established by *in vitro* assay. Secondary questionnaires did not collect information on *in vitro* assays, therefore, cases of idiopathic PRCA in this study may include primary autoimmune PRCA.

### Data analysis

The secondary questionnaires collected data on the reticulocyte count and a bone marrow examination at onset of aplasia but not at recovery. Remission was defined as no need for any further transfusions, whereas relapse was defined as the need to receive transfusions. The period to achieve maximum response varied from patient to patient; therefore, the date of remission was defined as that of the last transfusion after the initiation of remission induction therapy. Complete remission (CR), partial remission (PR) and no response (NR) were defined as the achievement of normal hemoglobin levels without transfusion, the presence of anemia without transfusion dependence, and the continued need for transfusions, respectively. It is difficult to determine the efficacy of each agent precisely when the patients are either concomitantly or sequentially treated with several agents. Moreover, the first agent(s) given may contribute to the efficacy of the agent(s) given subsequently. Therefore, in this study, the efficacy of the agent(s) reported in secondary questionnaires was re-evaluated according to the following criteria. In a simultaneous combination, the efficacies of all of the agents were determined as the same. In sequential administration and in a later on combination, the efficacy of each agent was determined depending on the response obtained during the period of administration, except for ATG and methylprednisolone. ATG and methylprednisolone usual-

ly do not produce immediate remission; therefore, the efficacies of these agents were evaluated together with the agent(s) used concomitantly and/or sequentially. The minimum period required for an evaluation of the response of an agent was defined as 2 weeks; therefore, an agent combined later on, within 2 weeks, was, for the purposes of the analysis, considered a simultaneous combination with the preceding agent(s).

Regarding maintenance treatment, the patients were classified according to the agent used for maintenance therapy as receiving CsA±CS (CsA-containing group) or CS alone (CS group) regardless of the agent(s) used for successful remission induction. The agents for remission induction and salvage therapy were defined as those used initially and those used either sequentially or in a later on combination, respectively. The agent for maintenance therapy was defined as that used or tailed off after successful remission induction. The RFS was estimated as transfusion-free survival. The overall survival and RFS were estimated by the Kaplan-Meier method and statistical differences were calculated by the log-rank test and $\chi^2$ test.

## Results

### Classification of PRCA

According to the criteria of Dessypris and Lipton,[2] of the total of 185 collected patients with PRCA, 73 (39%) were classified as having primary idiopathic PRCA and 112 (61%) as having secondary PRCA (Table 1). From the 73 patients with primary idiopathic PRCA, 11 patients were excluded from further analysis because of insufficient data (nine patients) or too short an observation period after initiation of immunosuppressive therapy (two patients; 1 and 8 days of observation). Finally, 62 patients with primary PRCA were eligible for further analysis. The patients' age at the onset of anemia ranged from 18 to 89 years (55±18, mean ± standard deviation, SD) with a 23:39 (1:1.7) male to female ratio (Figure 1). The year at onset of PRCA was 1998±5 (mean±SD), ranging from 1990 to 2005.

### Rate of response to the remission induction therapy

The remission induction therapy for these patients included CsA (n=31), CS (n=20), cyclophosphamide (n=3), anabolic steroids (n=1), or a simultaneous combination of CsA and anabolic steroids or CS (n=7) (Figure 1 and Table 2). CsA, as a remission induction therapy, produced CR or PR in 23/31 patients (74%). The initial dose of CsA for the responding patients was 4.8±1.2 mg/kg (mean±SD, n=23) with a range of 2.9 to 7.6 mg/kg body weight, which was higher than that for non-responding patients (3.9±1.3 mg/kg with a range of 2.1 to 5.6 mg/kg, n=8), although the difference was not statistically significant. When the patients who were treated with CsA alone were evaluated (n=23), the time for transfusion-independence from the start of therapy was 82±200 days (range, 0 to 910 days). Fifteen patients (65%) achieved transfusion-independence within 2 weeks, 17 patients (74%) within 1 month, 18 patients (78%) within 3 months and 20 patients (87%) within 6 months. CS, as a remission induction therapy, produced a CR or PR in 12/20 patients (60%). The initial dose of prednisolone in patients who responded to CS was 0.8±0.2 mg/kg (mean±SD, n=12) with a range of 0.5 to 1.0 mg/kg. There was no significant difference in the dose between the responders and non-responders. When the patients who were treated by CS alone were evaluated (n=9), the time for transfusion-independence from the start of therapy was 65±101 days (range, 0 to 311 days). Three patients (33%) achieved transfusion-independence within

**Table 1. Classification of 185 patients with aquired pure red cell aplasia.**

| *Causes of pure red cell aplasia* | *Patients* *Number* | *Percent* |
|---|---|---|
| Primary | | |
| Idiopathic | 73 | 39.5% |
| Secondary, associated with | | |
| Thymoma | 42 | 22.7% |
| Hematologic malignancies | | |
| Chronic lymphocytic leukemia | | |
| B-cell type | 1 | 0.5% |
| Large granular lymphocyte leukemia | 14 | 7.6% |
| Macroglobulinemia | 3 | 1.6% |
| Malignant lymphoma | 8 | 4.3% |
| Myelodysplastic syndrome | 11 | 5.9% |
| Acute myeloblastic leukemia | 1 | 0.5% |
| Preleukemic | 1 | 0.5% |
| Solid tumors | 5 | 2.7% |
| Autoimmune, collagen vascular diseases | | |
| Rheumatoid arthritis | 7 | 3.8% |
| Systemic lupus erythematosus | 1 | 0.5% |
| Systemic sclerosis | 1 | 0.5% |
| Sjögren's syndrome | 2 | 1.1% |
| Polymyalgia rheumatica | 1 | 0.5% |
| Autoimmune hemolytic anemia | 1 | 0.5% |
| Evans' syndrome | 1 | 0.5% |
| Type 1 diabetes mellitus | 1 | 0.5% |
| Myasthenia gravis | 1 | 0.5% |
| Chronic thyroiditis | 1 | 0.5% |
| Autoimmune hepatitis | 2 | 1.1% |
| Drugs | 2 | 1.1% |
| Chronic renal failure | 5 | 5.7% |

**Table 2. Response to remission induction therapy.**

| *Initial agent(s)* | *No. of patients* | *Response, No. (%)* *CR* | *PR* | *CR+PR* | *NR* |
|---|---|---|---|---|---|
| CsA | 31 | 10 (32%) | 13 (42%) | 23 (74%) | 8 (26%) |
| CS | 20 | 4 (20%) | 8 (40%) | 12 (60%) | 8 (40%) |
| CY | 3 | 0 | 0 | 0 | 3 (100%) |
| AS | 1 | 0 | 0 | 0 | 1 (100%) |
| CsA+CS | 4 | 0 | 4 (100%) | 4 (100%) | 0 |
| CsA+AS | 1 | 0 | 1 (100%) | 1 (100%) | 0 |
| CS+AS | 2 | 1 (50%) | 1 (50%) | 2 (100%) | 0 |
| Total | 62 | 15 (24%) | 27 (44%) | 42 (68%) | 20 (32%) |

*CsA: cyclosporine A; CS: corticosteroid including methyl-prednisolone and prednisolone; CY: cyclophosphamide; AS: anabolic steroid; CR: complete remission; PR: partial remission; NR: no response.*

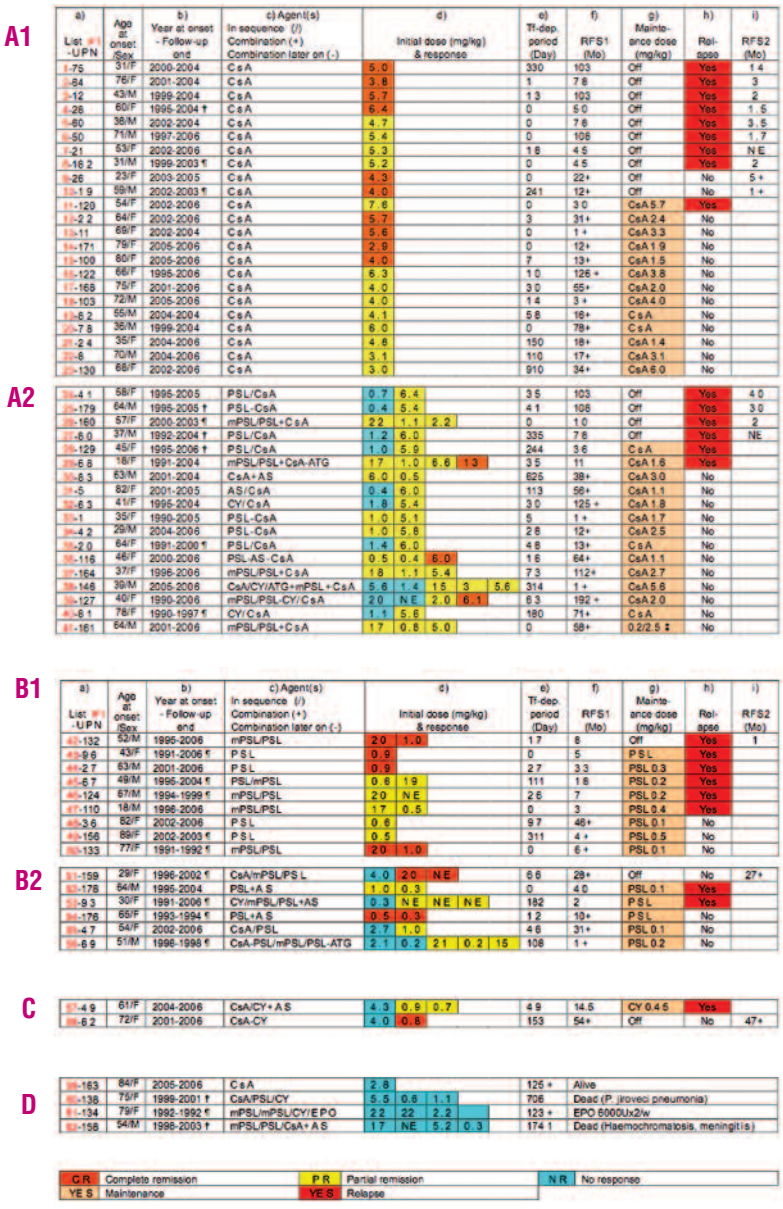


**Figure 1.** Immunosuppressive therapy in patients with primary idiopathic PRCA. (A) Cyclosporine A (CsA)-containing group: (A1) CsA alone, (A2) CsA in combination with other agents. (B) corticosteroid (CS) group: (B1) CS alone, (B2) CS in combination with other agents. (C) cyclophosphamide (CY) group. (D) Transfusion-dependent patients (non-responders). Abbreviations in each column; a) List #1; list number in Figure 1 and UPN (unspecified patient's number), b)¶Year at end of follow-up; †Death, c) agents are listed in order, (/); in sequential administration, (+); in simultaneous combination, (-); in combination later on, CsA; cyclosporine A, PSL; prednisolone, mPSL; methylprednisolone pulse therapy; ATG; antithymocyte globulin, CY; cyclophosphamide, AS; anabolic steroid, d) The initial dose and response to the agent; the order of agents corresponds to that shown in column c) and doses indicated are in mg/kg body weight/day, the color of each box shows response as indicated in the figure, e) Transfusion-dependent period (days) after the initiation of remission induction therapy, NE; not evaluable, f) RFS1; relapse-free survival (months) estimated as transfusion-free survival is shown as the period before the discontinuation of maintenance therapy, g) Off; tapered off, ‡doses of prednisolone/CsA in order, h) Relapse was defined as reappearance of transfusion requirement, i) RFS2; RFS after the discontinuation of maintenance therapy. EPO; erythropoietin.

2 weeks, six patients (67%) within 1 month and eight patients (89%) within 6 months. A simultaneous combination of CS and CsA produced remission in 4/4 patients. Cyclophosphamide was tried in three patients at a dose of 0.3 to 1.8 mg/kg, but no obvious responses were observed.

### Salvage therapy

Twenty patients failed to respond to remission induction therapy. The effective salvage therapies for these patients are summarized in Figure 2 in which the period of administration of the initial agent(s) is also shown. The remission induction agent was rapidly discontinued in several patients. Among eight patients who failed to respond to initial CsA, six patients responded to CS (Figure 1-B2, 51-159, 55-47 and 56-69), cyclophosphamide (Figure 1C, 57-49 and 58-62), or a simultaneous combination of ATG+methylprednisolone+CsA (Figure 1-A2, 38-146). Of the remaining two CsA non-responders, one patient (Figure 1D, 59-163) was treated with CsA at a dose of 2.8 mg/kg/day but was still transfusion-dependent after 125 days, and the other did not respond to salvage therapies with a sequential administration of prednisolone and cyclophosphamide and eventually died due to *Pneumocystis jiroveci* pneumonia (Figure 1D, 60-138). Eight patients did not respond to CS; five patients responded to CsA (Figure 1-A2, 24-41, 25-179, 27-80, 28-129 and 35-20) and one other responded to cyclophosphamide (Figure 1-A2, 39-127). Of the remaining two CS non-responders, one patient was lost to the follow-up during the administration of erythropoietin (Figure 1D, 61-134), and the other did not respond to salvage therapies with a combination of CsA and anabolic steroids, received 240 units of red blood cell transfusion, and eventually died due to bacterial meningitis (Figure 1D, 62-158). There were three patients who did not respond to cyclophosphamide; two patients responded to CsA (Figure 1-A2, 32-63 and 40-81) and the other

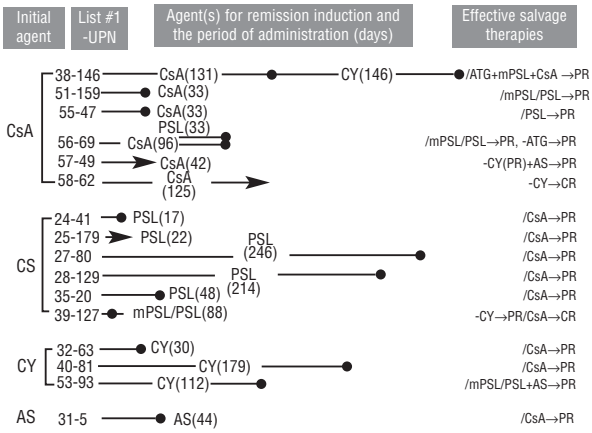


**Figure 2.** Effective salvage therapies for patients who failed to respond to the remission induction therapy. The initial agent(s) that failed to produce remission was discontinued (-○) or continued (→). Agents for salvage therapy were started in combination later on with the initial agent (–), simultaneously (+) or sequentially (/). The abbreviations are the same as those in the legend to Figure 1.

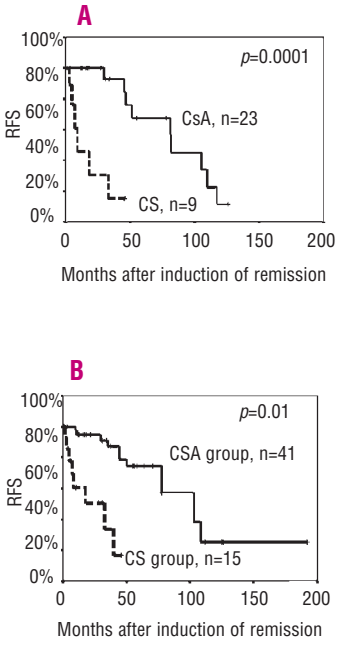


**Figure 3.** Relapse-free survival (RFS) of patients with primary idiopathic PRCA. RFS after induction of first remission was estimated as transfusion-free survival. **A.** The RFS of the patients treated with cyclosporine A (CsA) alone (solid line) (Patients listed in Figure 1-A1, n=23) is compared to that of the patients treated with corticosteroids (CS) alone (broken line) (Patients listed in Figure 1-B1, n=9). **B.** The RFS in the CsA-containing group (solid line) (patients listed in Figure 1-A1+A2, n=41) is compared to that of the CS group (broken line) (patients listed in Figure 1-B1+B2, n=15). There was a statistically significant difference between the duration of remission in the two groups based on the generalized Wilcoxon's test ($p<0.0001$ for A and $p<0.01$ for B).

responded to a combination of CS and anabolic steroids (Figure 1-B2, 53-93). A patient who was refractory to anabolic steroids responded to CsA (Figure 1-A2, 31-5). Finally, 58/62 patients (94%) with primary idiopathic PRCA responded to immunosuppressive therapy.

### Relapse-free survival

Figure 3A illustrates the duration of RFS of the patients treated with CsA alone or CS alone (Figure 3A) after the first remission was induced. Among the 23 patients in CsA alone group, the estimated median RFS was 82 months, with a median observation period of 34 months (range, 1 to 126 months). On the other hand, among the nine patients in the group treated with CS alone, the estimated median RFS was 9 months, with a median observation period of 7 months (range, 3 to 46 months). The duration of initial remission was, therefore, longer after CsA than after CS, and the differences was statistically significant ($p<0.0001$).

Exposure to other agents might affect the efficacy of CsA to sustain remission. Figure 3B illustrates the duration of RFS among patients in the CsA-containing group (Figure 1, A1 plus A2) and the CS-group (Figure 1, B1 plus B2) after the first remission had been induced. Among the 41 patients in the CsA-containing group, the estimated median RFS was 103 months, with a median observation period of 45 months (range, 1 to 196 months). On the other hand, among the 15 patients in the CS group, the estimated median RFS was 33 months, with a median observation period of 9 months (range, 1 to 55 months). The group of patients who achieved remission with a CsA-containing regimen had a longer duration of initial remission in comparison to the CS group, with the difference being statistically significant ($p<0.01$). There was no difference in the age at onset between the CsA-containing group and CS group, which was 54±18 years old (mean±SD), with a range from 18 to 82 and 56±20 years old with a range from 18 to 89, respectively. It was difficult to derive any conclusions on RFS in the cyclophosphamide group because there were only two patients in this group.

### Factors related to first relapse

Twenty-four out of 58 patients (41%) have had at least one relapse (Figure 1). Fifteen out of these 24 relapsed patients were in the CsA-containing group (Figure 1A). When the rate of first relapse was evaluated in relation to maintenance CsA therapy, it was found that of the 14 patients whose CsA was discontinued, 12 (86%) relapsed after a median period of 3 months (range, 1.5 to 40 months), while only 3 of 27 patients (11%) relapsed during maintenance therapy (Figure 1A). This indicates that maintenance CsA therapy prevents relapse ($p<0.001$, $\chi^2$ test). The other agents used for remission induction might have affected the efficacy of CsA as maintenance therapy. However, the efficacy of CsA at preventing relapse was also noted in the patients who were treated with CsA alone (Figure 1-A1) ($p<0.01$) as well as in the patients who were treated with CsA and the other agents (Figure 1-A2) ($p<0.05$). In contrast, 8/15 patients in the CS group (53%) relapsed within 2 to 40 months after remission and 7/8 patients (88%) relapsed during maintenance prednisolone therapy, thus suggesting the difficulty of maintaining remission with prednisolone.

### Relapse-free period after discontinuation of CsA

The relapse-free period after discontinuation of CsA therapy (shown as RFS2 in Figure 1) was 10±14 months (n=10), with a range of 1.5 to 40 months, indicating that

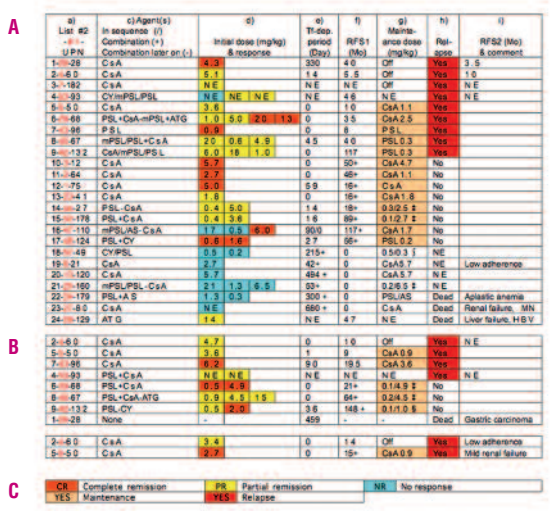

Figure 4 (*left*). **Patients in first relapse (A), second relapse (B) and third relapse (C). Abbreviations in each column are the same as those shown in the legend to Figure 1 except for a) list #2; list number in this figure followed by the list number shown in Figure 1 (#1) and UPN, g) ‡doses of prednisolone/CsA in order, /doses of cyclophosphamide/prednisolone in order, §doses of prednisolone/cyclophosphamide in order, i) MN; membranous nephropathy, HBV; hepatitis B virus infection.**

relapse can occur even 3 years after the discontinuation of CsA. Two patients have maintained remission after discontinuation of CsA therapy (Figures 1A, 9-26 and 10-19); however, the relapse-free periods after discontinuation of CsA therapy are only 1 and 5 months.

### *Duration of CsA therapy*

The mean duration of CsA therapy in patients who relapsed after discontinuation of CsA was 76±32 months, with a range of 10 to 108 months (n=12). In contrast, the mean duration of CsA therapy in patients who are in remission under CsA therapy was 45±48 months (n=24), with a range of 1 to 192 months. The mean dosage of CsA in patients who are in continuing remission for more than 24 months was 2.2±0.8 mg/kg (n=10), 40% of the beginning dose, with a range from 1.1 to 3.8 mg/kg (Figure 1A), excluding one patient (23-130) whose dose of CsA had gradually been increased.

### *Response of patients in first relapse to different therapies*

All patients who had a first relapse were re-treated in an attempt to re-induce remission, and this treatment was successful in 18/24 patients (75%) (Figure 4A, 1-26-28 to 17-46-124 and 24-28-129; corresponding to list No (#2) in Figure 4-list No(#1) in Figure 1-UPN in order). In the 15 relapsed patients in the CsA-containing group, CsA alone was again tried as the initial re-induction therapy for 11 patients, and this treatment was successful in eight of these 11 patients (73%). Three patients did not respond to CsA; one patient with low adherence (frequent self-discontinuation of CsA) (19-7-21), one patient whose dose of CsA was low due to renal dysfunction associated with membranous nephropathy (23-27-80), and one patient who seemed to be resistant to CsA (20-11-120). The remaining four patients were retreated by sequential administration of immuran and ATG (24-28-129), CS concomitantly with anabolic steroids (22-25-179) or CsA (6-29-68), or CS in combination later on with CsA (21-26-160). The two patients treated with ATG (24-28-129) or with a combination of CS and CsA (21-26-160) responded to therapy.

In the eight relapsed patients in the CS group, CS was again tried as an initial re-induction therapy for six patients. CS alone was again tried as the initial re-induction therapy for two patients, and this treatment was successful (7-43-96, 14-44-27). Three patients responded to a combination of CS and CsA (8-45-67, 15-52-178) or CS and cyclophosphamide (17-46-124). One patient responded to CsA (9-42-132). The remaining two patients failed to respond to cyclophosphamide (4-53-93) or methylprednisolone followed by anabolic steroids (16-47-110), but responded to CS and CsA, respectively. As a result, 8/8 relapsed patients in CS responders achieved remission and CsA or cyclophosphamide was newly introduced in 4/8 patients as maintenance therapy. A combination of cyclophosphamide and CS was tried for one relapsed patient (18-57-49) in the cyclophosphamide group but this patient remained transfusion-dependent. Three patients were lost to the follow-up after successful re-induction (3-8-182 and 17-46-124) or during re-induction therapy (21-26-160).

### *Recurrent relapses*

A second relapse occurred in 9/17 patients (Figure 4A). Three out of nine patients experienced a second relapse after discontinuation of CsA therapy (1-4-28, 2-5-60 and 3-8-182). One patient was lost to the follow-up (3-8-182). Seven out of the remaining eight patients were re-induced to a third remission (Figure 4B). One patient was treated by transfusion alone because of the presence of gastric carcinoma (1-4-28). CsA with or without concomitant CS was tried in 6/7 patients and induced remission in all six patients. One patient who had responded to CS achieved complete remission with a later on combination of cyclophosphamide (9-42-132). Thus, no patient treated with CS alone was present after the second relapse. A third relapse occurred in 4/7 patients (Figure 4B). One patient autonomously decided to discontinue CsA and relapsed (2-5-60). Two patients were lost to the follow-up after the third relapse (4-53-93 & 7-43-96). The remaining two patients were successfully re-induced into remission by CsA alone (Figure 4C) but have been experiencing frequent relapses up to the present due to self-discontinuation of CsA (2-5-60) and the limitation of dose escalation due to mild renal failure (5-6-50).

### *Mortality and overall survival (OS)*

Six out of 62 patients (9.7%) died and the estimated 10-year OS after the onset of PRCA was 95%; the median OS

has not yet been reached. Two patients did not respond to remission induction therapy and died from infections (Figure 1D, 60-138 and 62-158). After the first relapse, three patients in the CsA-containing group died (Figure 4A, 22-25-179, 23-27-80 and 24-28-129). One patient (22-25-179) eventually developed aplastic anemia and died from a serious infection, one patient (23-27-80) died from renal failure associated with membranous nephropathy, and the other (24-28-129) died due to liver failure caused by cirrhosis of the liver after hepatitis B virus infection. After a second relapse, one patient (Figure 4B, 1-4-28) in the CsA-containing group, died; the cause of death was gastric carcinoma found 4 years after the onset of PRCA. All four of these patients were in the CsA-containing group who had experienced relapse at least once; however, the OS was not significantly different between patients in the CsA-containing group and those in the CS group ($p$=0.104).

## Discussion

Primary idiopathic PRCA is a clinical disorder defined by the absence of any other disease and is pathogenetically heterogeneous. The most frequent disease underlying secondary PRCA is large granular lymphocyte leukemia (LGL),[6] also referred to as lymphoproliferative disease of granular lymphocytes[13] or granular lymphocyte proliferative disorders.[14] This often has unique clinical features such as autoimmune diseases including rheumatoid arthritis, aplastic anemia, PRCA, neutropenia and thrombocytopenia, and sustained remission may be achieved by treatment with CsA or cyclophosphamide, with or without prednisolone.[6,13,14] The diagnosis of LGL is somewhat difficult in patients without lymphocytosis. Although 14/185 patients were classified as having LGL and secondary PRCA in this study, it remains possible that some patients with LGL are included in this series of cases with supposedly primary idiopathic PRCA. In addition, the data of the current study are derived from a retrospective analysis and the responses cannot be attributed to CsA alone but must, more appropriately, be attributed to CsA-containing regimens, which include both CsA alone and CsA plus other drugs. In this study, we showed, for the first time, that the median RFS of patients in the CsA-containing group was 103 months, which is longer than that seen in the CS group (33 months) ($p$<0.01). In the CsA-containing group, the discontinuation of CsA was strongly correlated with relapse ($p$<0.001). Two patients have maintained remission after the discontinuation of CsA; however, the relapse-free periods after the discontinuation are only 1 and 5 months. Considering that a relapse can occur even 40 months after the discontinuation of CsA, these observation periods may be insufficient to conclude that some patients can be cured by CsA. In contrast, 88% of the relapses in the CS group occurred during maintenance prednisolone therapy. Therefore, CsA-containing therapy can sustain a longer duration of initial remission than CS and seems to be important to prevent relapse. Although vigorous and continuous immunosuppressive treatment is capable of inducing and maintaining remission in a majority of patients, it carries an increased risk of serious infections,[16] malignancy,[17,18] and sterility.[19] In our series, two patients died during remission induction due to opportunistic infections (*Pneumocystis jiroveci* pneumonia and bacterial meningitis), which suggests that adequate prevention and treatment of infection are requisites for successful management of patients. After achieving the first remission, four patients died and all of them were CsA responders who relapsed at least once. The causes of death were the development of aplastic anemia, renal failure with membranous nephropathy, liver failure associated with hepatitis B virus infection and gastric carcinoma. The relationship of CsA with the former three diseases is unclear because CsA is one of the effective treatments for aplastic anemia,[20] membranous nephropathy[21] and probably for hepatitis B virus infection as well.[22] Although immunosuppressive therapy enhances viral replication, it has been shown that CsA by itself impairs hepatitis B virus replication by blocking cytosolic calcium signaling.[22] Gastric carcinoma was found in one patient 4 years after the onset of PRCA, but the relationship of this neoplasm to the pathogenesis of PRCA or its treatment with CsA is not clear. Organ transplant experiences have shown that long-term immunosuppression is associated with post-transplant malignancies.[18,19] Therefore, continuous and careful follow-up is required for patients receiving long-term CsA therapy. In addition, the mean maintenance dosage of CsA in Japanese patients who are continuing in first remission for more than 24 months was 2.2±0.8 mg/kg, 40% of the initial dose, suggesting that it would be difficult to reduce the dose of CsA under this level while maintaining remission. One important question is whether or not the maintenance of patients in remission may have a beneficial influence on survival. In the era when CsA was not yet available, Clark *et al.* showed that the treatment of relapses was almost equally successful in 10/13 patients entering a second or third remission, and that the median survival of patients with primary PRCA was 14 years.[11] In our cohort the estimated 10-year OS was 95% and the median OS has not yet been reached; furthermore, we found that CsA-containing regimens can sustain remission for more than 10 years as continuous maintenance therapy. The decreased probability of relapse and the resulting decreased requirement of blood transfusions reduces the dangers of hemolysis, infections and iron overload with possible superoxide damage to body tissues. Although CsA-containing regimens are more expensive than prednisolone, CsA-containing regimens seem to be important to prevent relapse.

In conclusion, we have demonstrated for the first time that CsA-containing regimens, in comparison to CS, sustain a more prolonged RFS in patients with primary idiopathic PRCA. Furthermore, maintenance CsA-containing regimens seem to be important to prevent relapse. Nevertheless, an individualized approach to the manage-

ment of primary PRCA is suggested, and other therapeutic modalities may be required to cure primary PRCA. Prospective randomized studies are needed to identify agents and/or strategies that can cure primary idiopathic PRCA and to determine whether or not maintenance treatment is necessary. It should be appreciated that such studies must last decades considering the recurrent nature of this disorder.

### Appendix

*The following institutions participated in the Collaborative Study Group: Aichi Medical School, Akita University, Asahikawa Medical School, Chiba University, Dokkyo Medical School, Ehime University, Fujita Health University, Fukui University, Fukui National Hospital, Fukuoka University, Fukushima Medical University, Gifu University, Gunma University, Hamamatsu Medical School, Hirosaki University, Hiroshima University, Hokkaido University, Hyogo Medical University, Iwate Medical School, Jichi Medical School, Jikei University, Juntendo University, Kagawa Childrens' Hospital, Kagawa University, Kagoshima University, Kanazawa University, Kanazawa Medical School, Kansai Medical University, Kawasaki Medical School, Keio University, Kinki University, Kitazato University, Kobe University, Kochi University, Kumamoto University, Kurume University, Kyoto Prefectural University, Kyoto University, Kumamoto Medical Center, Kyushu University, Mie University, Nagasaki University, Nagoya City University, Nagoya Medical Center, Nagoya University, Nara Medical University, National Cancer Center, National Institute of Infectious Diseases, Niigata University, Nishi Sapporo National Hospital, Nippon Medical School, Nippon University, NTT Kanto Medical Center, Oita University, Okayama Medical Center, Okayama University, Osaka City University, Osaka Medical School, Osaka National Hospital, Osaka University, Ryukyu University, Saga University, Saitama Medical School, Sapporo Medical School, Sendai Medical Center, Shimane University, Shinsyu University, Showa University, St. Marianna University, Teikyo University, Toho University, Tohoku University, Tokai University, Tokushima University, Tottori University, Tokyo Medical Center, Tokyo Medical School, Tokyo Medical and Dental University, Tokyo University, Tokyo Women's Medical School, Tsukuba University, University of Occupational and Environmental Health, Wakayama Medical University, Waseda University, Yamagata University, Yamaguchi University, Yamanashi University, Yokohama City University.*

### Authors' Contributions

*KS: designed the research, analyzed the data and wrote the paper. MH: analyzed data and contributed to writing the paper. NF: analyzed data. MT, MB, KD, HT, SN, AU, MO, and KO: designed the research and contributed to the organization of this collaborative study.*

### Conflict of Interest

*The authors reported no potential conflicts of interest.*

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