

Porcine antilymphocyte globulin (p-ALG) plus cyclosporine A (CsA) treatment in acquired severe aplastic anemia: a retrospective multicenter analysis

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Abstract Severe aplastic anemia (SAA) is a life-threatening bone marrow failure syndrome characterized by pancytopenia and hypocellular bone marrow. Antithymocyte globulin (ATG) is the drug of choice for immunosuppressive therapy (IST) in patients with SAA ineligible for allogeneic stem cell transplantation. This study aimed to evaluate the efficacy and safety profile of porcine antilymphocyte globulin (p-ALG) plus CsA in the treatment of acquired SAA. Clinical information of 69 SAA patients treated with p-ALG plus CsA was collected and retrospectively analyzed for early mortality, response rate, survival rate, side effects, and other complications. The median age at diagnosis was 27 years (range 14 to 52). The overall response rate was 76.8 % with a 90-day median response time (range 30–360 days). Overall response rates at 3, 6, 12, 18, and 24 months were 63.8, 73.9, 76.8, 75.4, and 75.4 %, respectively. The median follow-up time for sur-

viving patients was 24 months (range 4–44 months) and the 2-year overall survival (OS) rate was 88.4 %. The disease-free survival (DFS) rate at 2 years was 85.5 %. Older age (≥ 45 years), very (v)SAA subgroup, and lower baseline absolute lymphocyte count ($<1 \times 10^9/L$) were independent unfavorable predictors of overall survival ($p < 0.05$). Less than one third of patients had serum sickness or allergic reaction during ALG therapy, but symptoms could easily be relieved by steroid treatment; 27.54 % had mild hepatic impairment. Taken together, p-ALG showed similar efficacy and safety profiles to rabbit or horse ATG in IST of acquired SAA. It can be a suitable alternative preparation for rabbit ATG with the great advantage of lower medical expenses.

Keywords Severe aplastic anemia · Porcine ALG · Overall response · Overall survival

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Introduction

Severe aplastic anemia (SAA) is a serious condition caused by immune-triggered bone marrow failure. Delayed treatment is associated with high mortality [1]. Allogeneic bone marrow transplantation and immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine A (CsA) have dramatically changed the course of this illness, with the 10-year survival rate for either treatment now being about 70 % [2]. IST with ATG plus cyclosporine A has become the standard treatment for patients with SAA ineligible for allogeneic stem cell transplantation [3]. In western countries, two ATG preparations for IST are currently available: the historically first used preparation from horse and the later approved preparation from rabbit. The response rate for IST containing these two ATG preparations ranged from 60 to

80 % [4–6]. In recent randomized or prospective studies, it was found that rabbit ATG was inferior to horse ATG as a first-line treatment for severe aplastic anemia [6–8]. Horse ATG, however, is not available in China and most clinical centers use rabbit (r)-ATG with cyclosporine A as first-line treatment in SAA for patients not eligible for bone marrow transplantation. The response rate for this was reported between 75 and 85 % [9–11]. Although porcine antilymphocyte globulin (p-ALG) was approved in China to treat SAA in 2004, systematic investigation of this preparation in the treatment of SAA was seldom reported. Compared to horse or rabbit ATG, p-ALG is substantially cheaper, lowering the costs of therapy by 30 to 50 % per cycle of treatment (\$443 vs \$201/bottle). In this study, we have performed a multicenter retrospective analysis of p-ALG plus CsA in the treatment of acquired SAA.

Methods

Patients

Sixty-nine consecutive SAA patients who had no suitable related donor for allogeneic stem cell transplantation or were over 45 years old received IST from 2009 to 2013. The medical records of these patients who underwent IST were retrospectively reviewed in detail. The IST protocols described in this study were approved by the ethics committee of their own hospitals. Appropriate informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

All diagnoses were reviewed by at least three senior hematologists independently. Fanconi anemia, paroxysmal nocturnal hemoglobinuria (PNH), and other constitutive bone marrow failure syndromes were excluded before IST. Chromosomes were assayed after in vitro exposure of lymphocytes to diepoxybutane to exclude Fanconi anemia. In addition, all patients received Fanconi anemia mutation analysis including 43 exons of FANCA, 14 exons of FANCC, and 14 exons of FANCG by PCR and Sanger sequencing. The cytogenetic karyotypes, MDS FISH panel (–5/5q–, –7/7q–, +8, 20q–), bone marrow biopsy and immunohistology (including CD34, CD31, CD42, CD61, CD3, CD15, CD20, and others) were done prior to the ALG treatment.

PNH clone was detected with flow cytometric analysis (BD Bioscience, San Jose, USA) of CD55 and CD59 expression (BD Immunocytometry Systems, San Jose, CA) on red blood cells and glycosyl-phosphatidylinositol expression on neutrophils. The cutoff value used to characterize the PNH clone was 3.0 % in erythrocyte lineage and 0.1 % in granulocyte lineage [12, 13]. No baseline PNH clone was found by flow cytometry in our series. The diagnosis of SAA required bone marrow cellularity less than 25 % and at least two of the following three criteria from peripheral blood counts: platelet counts

$\leq 20 \times 10^9/L$, neutrophil count $\leq 0.5 \times 10^9/L$, and corrected reticulocyte count ≤ 1 %. Very severe aplastic anemia was defined as meeting the SAA criteria and having neutrophil counts less than $0.2 \times 10^9/L$ [14].

Immunosuppressive regimens

All patients underwent skin test to assess allergic hypersensitivity. p-ALG® (Wuhan Institute of Biological Products, China, State Medical Permit No. S10830001) was approved by the State Food and Drug Administration (SFDA) in 2004. All patients were treated with p-ALG intravenously 25 mg/kg/day followed by 1.5 mg/kg/day methylprednisolone on days 1–5 and then 1 mg/kg/day prednisolone for 30 days and tapered gradually to prevent serum sickness. CsA 3–5 mg/kg/day was used for at least 2 years. The CsA dose was adjusted to achieve a whole-blood trough level of 200 ng/mL. Complete blood count as well as biochemistry parameters was monitored accordingly. Granulocyte colony-stimulating factor (G-CSF) was administered for all patients at 5 µg/kg/day subcutaneously everyday until day 30 and then administered if neutropenia occurred, and the dose was adjusted with neutrophil counts. As prophylaxis against *Pneumocystis carinii* pneumonia, all patients received TMP-SMX during the treatment period. Oral acyclovir and voriconazole were given for viral and fungal prophylaxis.

Response criteria

Complete response was defined as independence from transfusion, associated with normal hemogram parameters. Partial response was defined as no longer meeting the criteria for SAA without normalization of blood counts and no transfusion dependence for platelet and/or red blood cells. Continuous transfusion dependency was classified as no response. Relapse was defined as a decrease in blood counts to values either requiring transfusions or needing reinstitution of IST or HSCT [15]. Clonal evolution was defined as newly diagnosed clonal cytogenetic abnormalities or characteristic dysplastic or leukemic changes in the bone marrow. Early mortality was defined as death within the first 60 days after ATG treatment [16].

Follow-up

Overall survival (OS) was measured from the date of IST to either death from any cause or the date of last follow-up through June 30, 2014. Disease-free survival (DFS) was calculated from the date of IST to the first day of either recurrence or clonal transformation or death. Follow-up information consisted of a complete blood count, blood biochemistry, the assessment of bone marrow changes, and clinical or molecular evidence of clonal disease.

Statistical methods

Software SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was applied to perform all statistical analyses. The overall survival and disease-free survival was analyzed by the Kaplan-Meier method. Log rank test and chi-square test were applied to calculate the significance of differences between risk groups with a level of significance of $p < 0.05$. Cox regression was used to assess independent prognostic predictors in multivariate analysis with a stepwise selection algorithm of important covariates.

Results

Patients' characteristics

A total of 69 SAA patients received p-ALG plus CsA as IST protocol. The male/female ratio was 1.56. Median age at diagnosis was 27 years (range 14 to 52). Median time from diagnosis to IST was 18 days (range 4–583 days). At diagnosis, median neutrophil count was $0.32 \times 10^9/L$ (range $0 \sim 2.87 \times 10^9/L$); median hemoglobin count was 62 g/L (range 31.4–140 g/L); median platelet count was $9 \times 10^9/L$ (range $1 \sim 41 \times 10^9/L$). Of the patients, 89.9 % were classified as idiopathic SAA; 7.2 % were hepatitis-related SAA and 2.9 % drug-induced SAA. All patients received only one course of p-ALG treatment. The patients' characteristics are shown in Table 1.

Early death and overall response

Of the 69 patients, 7 (10.14 %) died within 60 days. The highest early death rates in single center was 33.3 % (4/12), followed by 13.04 % (3/23). Six of them died of severe infection (four died of severe sepsis and two died of invasive fungal infection) and all six had preexisting infections. One died of cranial hemorrhage (Table 4). Fifty-three patients (76.8 %) became transfusion independent after one course of p-ALG with 56.5 % complete response and 20.3 % partial response. The median response time was 90 days (range 30–360 days). The overall response rates at 3, 6, 12, 18, and 24 months were 63.8, 73.9, 76.8, 75.4, and 75.4 %, respectively. The median neutrophil counts ($\times 10^9/L$) at 3, 6, 12, 18, and 24 months were 1.89 (0.1–14.5), 2.01 (0.51–13.19), 2.35 (0.54–4.13), 2.09 (0.48–3.25), and 2.07 (0.47–3.25), respectively. The median hemoglobin levels (g/L) at 3, 6, 12, 18, and 24 months were 105 (37–149), 122 (31–158), 138 (22–164), 140 (39–156), and 121 (32–149), respectively. The median platelet counts ($\times 10^9/L$) at 3, 6, 12, 18, and 24 months were 52 (1.4–213), 125 (1–251), 201 (2–324), 198 (2–236), and 215 (7–273), respectively. No prognostic factors were correlated with the overall response rate in our study (Table 2).

Table 1 Clinical characteristics of the 69 patients

Patient characteristics	No. of patients (%)
Male/female	1.56
Median age at diagnosis (range)	27 years (14–52)
Cause of aplastic anemia	
Idiopathic	62 (89.9 %)
Hepatitis-related	5 (7.2 %)
Drug-induced	2 (2.9 %)
Median time from diagnosis to IST (range)	18 days (4–583 days)
Response to IST	
Complete response	39 (56.5 %)
Partial response	14 (20.3 %)
No response	16 (23.2 %)
Overall response at 3 months*	44 (63.8 %)
Overall response at 6 months*	51 (73.9 %)
Overall response at 12 months*	53 (76.8 %)
Overall response at 18 months*	52 (75.4 %)
Overall response at 24 months*	52 (75.4 %)
Median time of follow-up (range)	24 months (4–44 months)
Median neutrophil count, $10^9/L$ (range)	0.32 (0–2.87)
Median hemoglobin, g/L (range)	62 (31.4–140)
Median platelet count, $10^9/L$ (range)	9 (1–41)
Median reticulocyte count, $10^9/L$ (range)	9.7 (2.4–82.6)

*Overall response= Complete response + Partial response

Overall survival and relapse

The median follow-up time for surviving patients was 24 months (range 4–52 months) and the 2-year OS rate was 88.4 % (Fig. 1a). The estimated DFS rate at 2 years was 85.5 % (Fig. 1b). In univariate analysis of prognostic factors for overall survival, older age (≥ 45 years), very (v)SAA subgroup, preexisting infection, and lower absolute lymphocyte count ($< 1 \times 10^9/L$) predicted inferior survival rate ($p < 0.05$). In multivariate Cox regression analysis, older age (≥ 45 years), vSAA subgroup, and lower baseline absolute lymphocyte count (ALC) ($< 1 \times 10^9/L$) were independent predictors of OS rate ($p < 0.05$, Fig. 2 and Table 3). One patient showed clonal evolution (1.4 %) and transformed to myelodysplastic syndrome (RAEB-I) during the follow-up period. Only one patient relapsed (from partial response to transfusion dependent) after 23 months (Table 4).

Kinetics of lymphocyte depletion after porcine ALG

The kinetic lymphocyte depletion after porcine ALG was depicted in Fig. 3. During the first week after ALG, the lymphopenia was profound. The lymphocytes gradually recovered after 2 weeks of ALG treatment.

Table 2 The univariate analysis of prognostic factors for overall survival and overall response

Factors	Subtype	Number (percentage)	Mean OS (months)	<i>p</i> value ^a	Overall response (%)	Nonresponse (%)	<i>p</i> value ^b
Sex	Male	42 (60.9)	46.3	0.955	83.3	16.7	0.129
	Female	27 (39.1)	36.1		66.7	33.3	
Age	<45 years old	63 (91.3)	47.26	0.048	77.8	22.2	0.157
	≥45 years old	6 (8.7)	27.39		66.7	33.3	
SAA subtypes	vSAA	17 (24.6)	33.82	0.013	58.8	41.2	0.090
	SAA	52 (75.4)	49.04		82.7	17.3	
Time intervals	<60 days	58 (84.1)	46.77	0.463	81	19	0.129
	≥60 days	11 (15.9)	28.61		54.5	45.5	
Preexisting infection	None	48 (69.6)	43.27	0.035	82.0	18.0	0.181
	Existed	21 (30.4)	40.06		63.2	36.8	
Baseline ALC	≥1×10 ⁹ /L	36 (52.2)	50.61	0.019	86.1	13.9	>0.05
	<1×10 ⁹ /L	33 (47.8)	36.78		66.7	33.3	
Baseline ARC	≥25×10 ⁹ /L	16 (23.2)	—	0.11	75.5	24.5	>0.05
	<25×10 ⁹ /L	53 (76.8)	—		81.3	18.7	

Significance of values in bold is $p < 0.05$

Time intervals was calculated from time of diagnosis to time of IST; – no statistics are computed since in the group absolute reticulocyte at diagnosis greater than $25 \times 10^9/L$, all cases are censored

ALC absolute lymphocyte count, ARC absolute reticulocyte count

^a *p* value was calculated by the Kaplan-Meier method

^b *p* value was calculated by chi-square test

Side effects

Of the total 69 patients, 15 patients (21.73 %) had serum sickness that could be relieved by steroid treatment. Twenty-three patients (33.33 %) had allergic reaction (including fever and skin rash) during ALG therapy that could be relieved by antiallergic treatment. Nineteen patients (27.54 %) had mild elevated liver enzymes. No elevated serum creatinine levels, fatal serum sickness, or anaphylactic shock was reported in this cohort.

Discussion

IST with ATG and CsA is an effective front-line treatment for SAA when there is no histocompatible sibling donor or the patient's age is generally over 45 years old. In western countries, standard initial IST is horse ATG plus CsA, which induces hematologic recovery in 60 to 70 % of cases and excellent long-term survival among responders, as shown in several large prospective studies in the USA, Europe, and Japan [17–20]. A more lymphocytotoxic agent, rabbit ATG, was

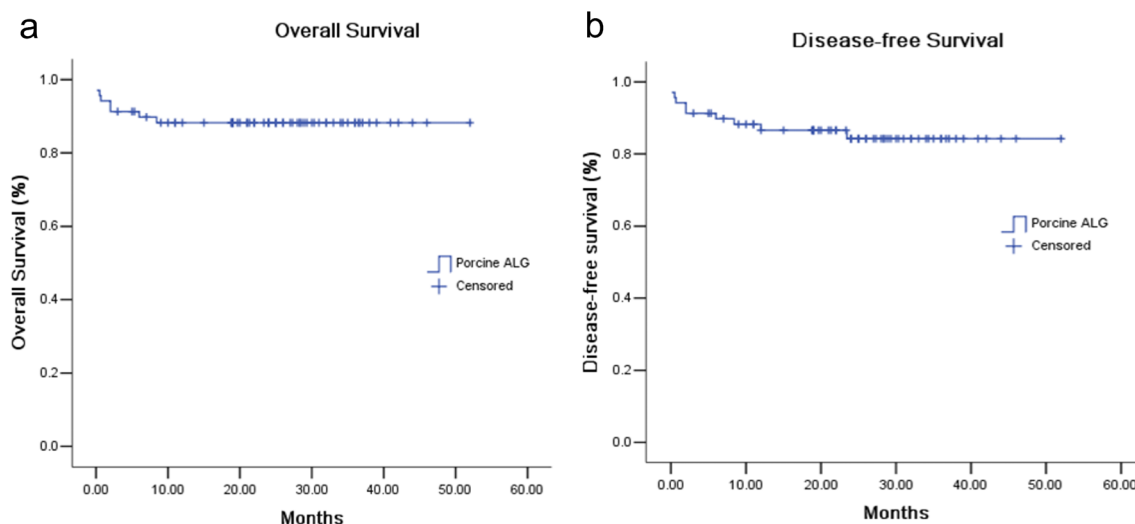


Fig. 1 Kaplan-Meier curve of overall survival (OS) and disease-free survival (DFS) of 69 patients. The 2-year OS rate was 89.86 %; the estimated survival time was 46.833 ± 1.851 months (a). The DFS rate at 2 years was 85.5 % (b)

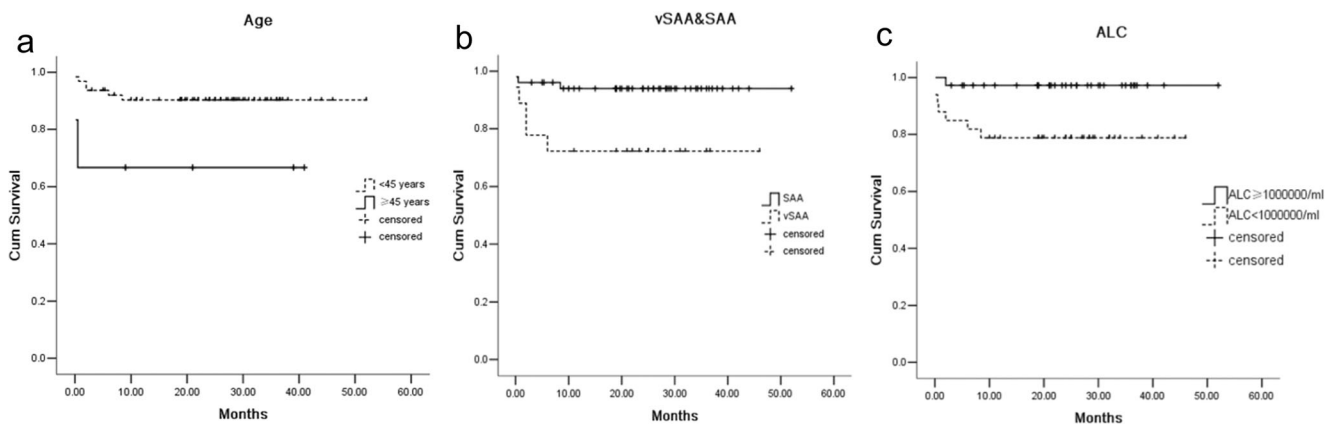


Fig. 2 Comparison of overall survival in relation to age (a), SAA subgroup (b), and ALC count (c). The older age (≥ 45 years), vSAA subgroup, and low ALC count ($< 1 \times 10^9/L$) had a significantly shorter OS rate compared to the SAA group ($p < 0.05$)

successful in salvaging patients with refractory or relapsed SAA after initial treatment with horse ATG [21, 22]. Whether horse (h)-ATG was still superior to r-ATG was controversially discussed [6, 23–27]. In China, rabbit ATG was the only available agent before 2004, but most patients could not afford this expensive drug which is not covered by any insurances. In recent years, some centers in China tried p-ALG to substitute r-ATG in IST. Bing et al. used p-ALG and results showed 83.3 % overall response with a 90-day median response time and 87.5 % 1.5 years survival rate [28]. Despite biological similarities in manufacturing, there may exist many differences in pharmacokinetics and effects on neutrophils, lymphocyte subsets, and cytokine release between the three types of ATG [29]. Any one of these differences might be related to their clinical efficacy in suppressing the immune system and restoring hematopoiesis in SAA patients. Table 5 has summarized the clinical efficacy of IST with different ATG preparations in recent published data. Our retrospective data shows that the response rate and 2-year OS rate are not inferior to the results reported with rabbit ATG in China [9–11] and horse/rabbit ATG in western countries [6, 15–18, 20].

In this study, 7 of 69 patients died within 60 days. Patients who had preexisting infections were more likely to develop

fatal infection during or after IST. Platelet refractoriness was the cause of fatal cranial hemorrhage in another patient. The above results indicate that, irrespective of the stage of IST, infections are the most common cause of death. More than one third of patients treated in one center have encountered early death which is obviously higher than other centers. This suggests that IST should be performed in centers with expertise in managing it to avoid early death. According to our study, no serious side effects were observed. Common side effects including skin rash, serum sickness, and mildly elevated liver enzymes were also observed in studies investigating horse or rabbit ATG.

In our series, the relapse rate was lower than other published data in western countries but quite similar with other Chinese patients [9–11, 28, 30] irrespective of the type of ATG. There may have two reasons for the lower relapse rate. First, all patients in our cohort received G-CSF support. G-CSF was previously proven that it had some benefits in terms of the promotion of hematopoietic recovery and suppression of relapse rate [31], but further follow-up is required to elucidate whether it increases the risk of clonal evolution. Second,

Table 3 The multivariate analysis of prognostic factors of OS

Multivariate variables	Odds ratio	95 % CI	<i>p</i> value ^a
Age (≥ 45 / < 45 years old)	5.268	1.352–45.849	0.022
SAA subtype (vSAA/SAA)	5.720	1.385–26.585	0.017
Baseline ALC (≥ 1 / $< 1 \times 10^9/L$)	4.879	1.318–102.285	0.027

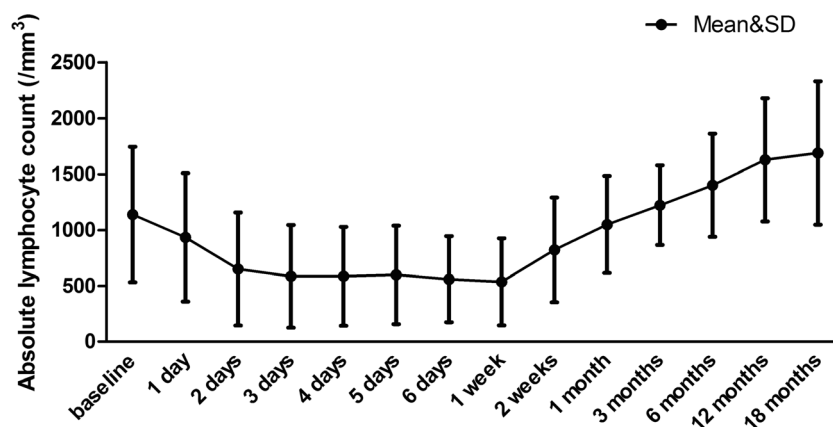
ALC absolute lymphocyte count

^a Forward and backward Wald test

Table 4 Information of early death and other late events

	Number (percentage)	Cause
Early death	7 (10.14)	Infection (85.7 %); hemorrhage (14.3 %)
Late death	1 (1.4)	Infection (100 %)
Clonal evolution	1 (1.4)	MDS (RAEB-I) after 3 months of IST
Relapse	1 (1.4)	Become transfusion dependent after 23 months of IST
Late infection events	5 (7.2)	Invasive fungal infection (60 %); bacteremia (40 %)

Fig. 3 Kinetics of absolute lymphocyte counts after p-ALG treatment



most patients (95.7 %) are still receiving CsA at the time of analysis. It has been also demonstrated that the combination of ATG and CsA is superior to ATG or CsA alone in terms of hematologic response [18, 32]. This may be another reason for the low relapse rate. Although clonal evolution may occur in approximately 10 to 15 % of patients [24], some clonal abnormalities appear to be transient and may be omitted. In addition, most clonal evolution occurred as the late event of SAA. More clonal evolution may be observed if the follow-up time prolonged.

Although our study was limited by its retrospective nature and the short time of follow-up, the 88.4 % 2-year OS rate showed excellent efficacy of p-ALG. Thus, p-ALG may serve as a potential alternative for r-ATG. In this study, vSAA subgroup, older age, and low ALC count were three independent unfavorable predictors of overall

survival. A higher baseline absolute reticulocyte count (ARC) and ALC may indicate better residual marrow function and the presence of a sufficient number of stem cells to sustain blood cell production after IST [33]. These results are similar to previous studies with SAA patients treated with horse/rabbit ATG [33]. Most studies on horse/rabbit ATG also showed that patients with vSAA had higher morbidity due to infection and higher early mortality [34]. However, more prospective studies are required to validate our conclusions.

In summary, our retrospective results show the great potential of p-ALG plus CsA as the first-line IST in the treatment of acquired SAA. It shows similar efficacy to rabbit/horse ATG while the costs of treatment are substantially lower. Thus, it may prove to be a cheaper and more reliable alternative to rabbit ATG.

Table 5 Comparison of clinical efficacy of IST with different protocols in published data

Countries/study	Study type	Study number	ATG preparation	OS rate	OR rate (%)
China/current study	Retrospective	69	p-ALG	88.4 % (2 years)	76.8
China [28]	Retrospective	48	p-ALG	87.5 % (1.5 years)	83.3
USA [6]	Prospective	60	r-ATG	96 % (3 years)	37
		60	h-ATG	76 % (3 years)	68
Korea [25]	Retrospective	46	h-ATG	83.5 % (5 years)	45.7
		53	r-ATG	82.7 % (5 years)	49.1
Brazil [14]	Retrospective	42	h-ATG	78.4 % (2 years)	59.5
		29	r-ATG	55.4 % (2 years)	34.5
EBMT [8]	Prospective	105	h-ATG	86 % (2 years)	67
		35	r-ATG	68 % (2 years)	60
Japan [23]	Retrospective	12	r-ATG	NA	67
		14	h-ATG	NA	75

p-ALG porcine antilymphocyte globulin, *r-ATG* rabbit antithymocyte globulin, *h-ATG* horse antithymocyte globulin, *OS* overall survival, *OR* overall response, *NA* not available

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Conflict of interest All authors declare no conflicts of interest.

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Informed consent was obtained from all patients for being included in the study.