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A pilot dose-escalating study of alemtuzumab plus cyclosporine for patients with bone marrow failure syndrome

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Abstract

The pathogenesis of bone marrow failure syndrome (BMFS) involves both T- and B-cells. Since alemtuzumab (ALM) is a monoclonal anti-CD52 antibody that targets both cell types, we assessed the effects of treatment with ALM and cyclosporine (CS) on 19 patients with BMFS (median age 48 years; range, 16–74 years), including 14 with severe/very severe aplastic anemia (AA), 3 with transfusion-dependent AA and 1 each with myelodysplastic syndrome (MDS) and pure red cell aplasia (PRCA). The dose of ALM was escalated from dose cohort I (10 mg on day 1, 20 mg on day 2, and 30 mg on day 3) to dose cohort II (30 mg/d for 3 days) plus CS for at least 6 months. Thirteen patients were in dose cohort I and 6 were in dose cohort II. Five patients (23.5%) had a complete response (CR), 2 (11.8%) had a partial response (PR), and 12 (64.7%) had no response, making the overall response rate 36.8% (7/19). The overall response rates in dose cohorts I and II were 46.2% (6/13) and 16.7% (1/6), respectively. Among the 17 patients with AA, the ORR was 35.3% (6/17), 50.0% (6/12) in dose cohort 1 and 0 (0/5) in dose cohort II. Most responsive patients responded within 3 months. Among responders, median time to initial response was 2.07 months (95% CI, 1.40–2.75 months) and median time from initial response to complete response in complete responders was 9.33 months (95% CI, 0.0–31.71 months). The 2-year survival rate was 81.6%. These findings indicate that ALM-CS should be one option for IST in BMFS, and that 60 mg of ALM may be sufficient compared with the higher dose (90 mg).

Keywords: Alemtuzumab; Cyclosporine; Bone marrow failure syndrome

1. Introduction

Bone marrow failure syndrome (BMFS) is a disease in which peripheral blood cytopenia(s) occurs as a result of suppressed production of hematopoietic cells. Although BMFS includes highly heterogeneous hematological disorders, from aplastic anemia (AA) to myelodysplastic syndrome (MDS), its common pathogenesis is related to immunological dysregulation [1–5].

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a definitive therapeutic option for patients with BMFS. In alloHSCT, hypoproductive hematopoietic cells can be replaced by normal hematopoietic cells from a healthy donor. Despite its treatment-related mortality, alloHSCT is the treatment of choice for patients with severe AA. Due to donor limitations, however, alloHSCT is not an option for some patients with BMFS. Another option for these patients is immunosuppressive therapy (IST), since pathologic T-cells play an important role in the pathogenesis of AA [6–11]. The combination of antithymocyte globulin (ATG) and cyclosporine (CS) is regarded as standard IST [12,13]. Although other drugs have been tested in addition to or as substitutes for ATG–CS, most of them failed to show improved efficacy over ATG–CS [14–19].

Alemtuzumab (ALM), a humanized anti-CD52 monoclonal antibody, can effectively remove both T- and B-cells, including pathologic lymphocytes, from peripheral blood [20–22], suggesting that ALM may be a candidate drug for IST in BMFS. In addition to controlling lymphocytes, ALM is

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an immune modulating drug that can be used to treat patients with autoimmune cytopenia and pure red cell aplasia (PRCA) [23–26]. Small trials have suggested that ALM can play a regulatory role similar to that of ATG in modifying abnormal T-cells in patients with BMFS [27]. However, whether ALM can act as an IST in these patients is not known, and the optimal dose of ALM has not yet been established. We therefore assessed the effectiveness of ALM-CS as an IST in patients with BMFS, including those with AA, MDS and PRCA. Using dose escalation, we assessed the optimal dose of ALM for IST, as well as evaluating early and late treatment-related toxicities.

2. Methods

2.1. Patients

Patients with BMFS over 15 years of age were eligible for this study. BMFS included patients with severe aplastic anemia (SAA), defined as a hypocellular bone marrow and two or more of the following: granulocyte count <500/μL, platelet count <20,000/μL, and corrected reticulocyte count <1.0%; very severe aplastic anemia (VSAA), defined as the criteria for SAA plus a granulocyte count <200/μL; and patients who did not meet the criteria for SAA or VSAA, but who had a granulocyte count <500/μL, a platelet count <20,000/μL, or red cell transfusion-dependency. Other patients included those with MDS, defined as <10% bone marrow blasts, and a granulocyte count <500/μL, a platelet count <20,000/μL, or red cell transfusion-dependency; and those with transfusion-dependent PRCA. The absence of a suitable sibling donor or patient refusal of alloHSCT from a matched sibling donor was required for enrollment, as was a Karnofsky performance scale of 60 or over. Patients were excluded if they were pregnant or lactating, if they had congenital cytopenias such as Fanconi anemia, and if they had hypoplastic marrow along with a PNH clone. Patients were excluded if they had a psychiatric disorder or a mental deficiency severe enough to make compliance with treatment unlikely or informed consent impossible; if they had a major illness or organ failure; if they had been treated with ATG, antilymphocyte globulin (ALG), CS, or corticosteroids at methylprednisolone dose ≥2 mg/kg within the previous 12 months; if they had ever received ALM prior to this study; or if they had a systemic reaction to intravenous testing with ALM. Patients with prior malignancies, other than basal or squamous cell skin cancer or in situ cervical cancer, had to be cancer-free for at least 5 years. National and international public registries were searched for matched unrelated donors (MUDs) at the time of enrollment.

2.2. Treatment

Prior to treatment, patients were assessed for allergy to ALM by intravenous infusion of a test dose of 1 mg. Patients who had systemic reactions (e.g. urticaria, tachycardia, dyspnea, rigor, uncontrolled fever, hypotension or anaphylaxis) were excluded from treatment. Patients were premedicated 30 min before ALM infusion with acetaminophen 650 mg p.o. and pheniramine maleate 45.5 mg iv and methyl prednisolone 40 mg iv. Patients were treated with prophylactic Bactrim (160 mg bid twice weekly) and acyclovir (200 mg bid daily) for the first 2 months. Patients in dose cohort I received 10 mg ALM on day 1, 20 mg on day 2, and 30 mg on day 3, whereas patients in dose cohort II received 30 mg/d for 3 consecutive days. The CS dose was 1.5 mg/kg bid per day for 6 months, with adjustments for plasma concentration and changes in renal function. CS was started at the same time as ALM, and the plasma concentration of CS was monitored twice weekly until it reached a therapeutic level and was maintained between 100 and 300 ng/mL. Thereafter CS blood concentration was monitored once a month. Menstruating women were given norethindrone 10 mg p.o. daily. Patients received full supportive care, including transfusion of blood products, antibiotics, and anti-emetics, when appropriate. The reason(s) for treatment, dosage, and the dates of treatment were recorded. Cellular blood products were transfused, after leukocyte filtration and irradiation, to maintain hematocrit >30% or platelet >20 K/μL.

The study protocol was approved by the Institutional Review Board of Ulsan University Hospital, and all patients provided written informed consent.

2.3. Evaluation procedures, definitions of disease status and response criteria

Bone marrow biopsy and aspiration, including cytogenetics and aplastic anemia FISH panel, were performed before treatment. Other pre-treatment tests included flow cytometry for CD55 and CD59, anti-nuclear antibody, abdominal sonogram, and serologic tests for hepatitis B/C viruses, human immunodeficiency virus and Epstein-Barr virus. Cytomegalovirus (CMV) antigenemia was performed before treatment and monthly thereafter for 1 year.

Definitions of disease status and response criteria have been described previously [28]. Hematological response to ALM-CS therapy was assessed 3, 6 and 12 months after treatment. Response criteria were not sustained by transfusions or growth factors and were confirmed by a minimum of two observations at least 4 weeks apart. Complete response (CR) was defined as transfusion-independence and normal cell types and concentrations for age and gender. Partial response (PR) included transfusion-independence, plus, for patients with SAA/VSAA, the absence of criteria for SAA, and, for patients with MDS or PRCA, a ≥3 mg/dL increase in hemoglobin concentration (if initially <6 g/dL); a ≥500/μL increase in granulocyte count (if <500/μL) or a doubled or normal granulocyte count (if previously ≥500/μL); a ≥20,000/μL increase in platelet count (if previously <20,000/μL) or a doubled or normal platelet count (if previously ≥20,000/μL). Patients with transfusion-dependence or peripheral blood counts not meeting PR criteria were regarded as having no response. Relapse was defined as a decrease in any of the peripheral blood cell counts to <50% of the median sustained count during response or becoming transfusion-dependent. Treatment failure was assessed 6 months after ALM-CS, and salvage treatment was considered. However, patients without response 3 months after ALM-CS were eligible for alloHSCT from MUD and cases where the patient was still unresponsive following alloHSCT were classified as treatment failure. Laboratory tests for response evaluation were regularly performed at least once monthly for the first year. Toxicities were evaluated for the first 3 weeks (short-term toxicity) and for the period from 3 weeks to 6 months (long-term toxicity) and graded according to the National Cancer Institute common toxicity criteria for adverse events version 3.

2.4. Statistical considerations

This study was designed as a prospective, dose-escalating trial. We planned to enroll 12 patients in each dose cohort. Response rates were evaluated with Fisher's exact test. Blood cell counts on different treatment days were compared using Student's paired *t*-test. Survival curves were computed according to the Kaplan–Meier method. Overall survival (OS) was defined as the time interval between start of ALM-CS treatment and death from any cause. Factors prognostic of response were assessed using stepwise multiple logistic regression, and factors prognostic of survival were assessed using the stepwise Cox proportional hazards model.

3. Results

3.1. Patients

Starting in April 2005, we enrolled 19 patients (13 female, 76.5%), of median age 48 years (range, 16–74 years). Seventeen patients had aplastic anemia, with 1 each having small portion MDS and PRCA. All patients were transfusion-dependent and idiopathic. Sixteen patients were dependent on red blood cell transfusions and 14 on platelet transfusions. Prior to ALM-CS, the mean amounts of transfused red cells, platelet concentrates and platelet apheresis were 9.2 units (range, 0–41 units), 16.3 units (range, 0–172 units) and 4.7 units (range, 0–22 units), respectively. Median time from diagnosis to ALM-CS was 1.4 months (range, 0.2–149.3 months). Fifteen patients (78.9%) had received no prior therapy, 3 (15.8%) had received antithymoglobulin, and 1 (5.3%) had been treated with danazol. All dose cohort II patients were IST-naïve. Mean WBC, absolute neutrophil, hemoglobin and platelet concentrations were 3100/μL (range, 390–2570/μL), 1237/μL (range, 107–4257/μL), 7.4 g/dL (range, 3.4–13.5 g/dL) and 55,000/μL (range, 3000–334,000/μL), respectively. Two patients were positive for hepatitis B surface antigen and one was positive for hepatitis C virus. Mean bone marrow cellularity was 10.8% (range, 0.8–40%). Table 1 presents the details of the patient characteristics.

Table 1
Patient characteristics

Characteristic	No. of patients (%)
Gender	
Male	5 (26.3)
Female	14 (73.7)
Disease	
Severe/very severe AA	14 (73.7)
Transfusion-dependent AA	3 (15.8)
Hypoplastic MDS	1 (5.3)
PRCA	1 (5.3)
Transfusion-dependency	
Any	19 (100)
Red blood cells	16 (84.2)
Platelets	14 (73.7)
Treatment prior to ALM	
None	15 (78.9)
ATG–CS	3 (15.8)
Danazol	1 (5.3)
Age (years) (median (range))	48 (16–81)
Time from diagnosis to ALM (months) (median (range))	1.4 (0.2–149.3)
Amount of transfusion prior to ALM (mean (range))	
Red blood cells (units)	9.2 (0–41)
Platelets (units)	16.3 (0–172)

Abbreviations: AA, aplastic anemia; MDS, myelodysplastic syndrome; PRCA, pure red cell aplasia; ALM, alemtuzumab-cyclosporine; ATG–CS, antithymoglobin-cyclosporine.

3.2. Treatment

Thirteen patients were in dose cohort I and six in dose cohort II. All patients completed the assigned scheduled dose of ALM. There were reductions or delays in ALM infusion dose. CS was started in all patients and adjusted to maintain adequate serum concentration. Three patients could not receive the full scheduled 6 months of CS because 1 died of infection, 1 developed acute renal failure and 1 underwent alloHSCT within 6 months after CS. Another 2 patients required CS dose reductions. After interim analysis, we decided not to enroll additional patients into dose cohort II.

3.3. Responses

Median follow-up for response evaluation was 18.3 months (range, 3.06–28.42 months). Maximal responses to ALM-CS were CR in 5 patients (26.3%), PR in 2 (10.5%), and no response in 12 (63.2%), making the overall response rate (ORR) 36.8% (7/19) (Table 2). ORR in dose cohorts I and II were 46.2% (6/13) and 16.7% (1/6), respectively ($p = 0.144$). Among the 17 patients with AA, the ORR was 35.3% (6/17), 50.0% (6/12) in dose cohort I and 0 (0/5) in dose cohort II. Detailed information on the AA patients is listed in Table 3.

Of the 7 responsive patients, all responded within 3 months. Among responders, median time to initial response was 2.07 months (95% CI, 1.40–2.75 months) and median time from initial response to complete response in complete responders was 9.33 months (95% CI, 0.0–31.71 months (Fig. 1). Response to ALM-CS was not affected by type of diagnosis ($p = 0.301$), fever ($p = 0.482$), or skin rash ($p = 0.793$) during treatment. Age, prior treatment, time to treatment, severity of disease and gender did not have a significant effect on maximal response. Of the 3 patients who failed to respond to prior ATG–CS, 1 showed CR and 1 showed PR. When we assessed variables relative to WBC changes during follow-up, we found that only percentage of lymphocytes at 3 months ($p = 0.020$) after ALM-CS affected maximal response (Table 4).

3.4. Changes in peripheral white blood cell counts

ALM-CS treatment resulted in significant decreases in white blood cell counts (WBC) for the first 6 months ($p = 0.033$), returning to pretreatment levels after 9 months ($p = 0.184$) (Fig. 2). Although absolute neutrophil counts (ANCs) were significantly higher on treatment days 2 ($p = 0.003$), 3 ($p = 0.001$) and 4 ($p = 0.001$) than on day 1, rapid recovery to pre-treatment levels was achieved after 1 month ($p = 0.231$). The initial mean lymphocyte percentage was 54% (range, 10.6–92.6%). The mean absolute lymphocyte count (ANC) on treatment day 1 was 1754/μL (range, 617–6358/μL). The percentage of lymphocytes was almost depleted, beginning on treatment day 2 ($p < 0.001$) until 6 months ($p = 0.008$) after treatment. The percentage of lymphocytes significantly increased between months 1 and 3 ($p < 0.001$), and between months 3 and 6 ($p = 0.024$). ALC was significantly decreased, beginning on treatment day 2 ($p < 0.001$) until 6 months ($p = 0.027$), but increased significantly between months 1 and 3 ($p < 0.001$).

3.5. Safety profiles

Toxicity during ALM included fever in 5 patients (26.3%), skin rash in 5 (26.3%), ALT elevation in 9 (G1 in 7, G2 in 2), G1 AST elevation in 5 (26.3%), and hyperbilirubinemia in 4 (G1 in 3, G3 in 1). Adverse events were similar in dose cohorts I and II. There were no incidents of anaphylaxis, serum sickness or CMV reactivation. Toxicities during CS were also mild. During CS treatment, G1 ALT elevation was observed in 5 patients (26.3%), G1 hyperbilirubinemia in 6 (31.6%), and G1 azotemia in 6 (31.6%). Acute renal failure developed in 1 patient, who recovered after supportive care and discontinuation of acyclovir and CS, suggesting that azotemia was caused by either acyclovir or CS. There was no significant gingival hypertrophy or hypertension during CS treatment. One year after ALM-CS, 1 patient with AA had converted to refractory cytopenia with multilineage dysplasia.

3.6. Relapse and salvage therapies

Only one patient relapsed. This patient, who was diagnosed with AA, initially showed PR to ALM-CS but relapsed afterward and was transformed to MDS after 22.7 months. Eight patients received salvage therapies after the failure of ALM-CS. There was no second ALM-CS trial. Three patients received ATG–CS but none responded. Two patients (1 with MDS converted from AA and 1 with de novo MDS) were treated with azacytidine; the latter patient showed CR to azacytidine. Five patients underwent alloHSCT after ALM-CS treatment; of these, 4 patients received alloHSCT from matched unrelated donors and 1 from a matched sibling donor. The latter patient had initially refused alloHSCT and participated in this trial as a second-line therapy. All patients who underwent alloHSCT were not responsive to ALM-CS. There was no graft failure or CMV reactivation. Three patients tried second-line ATG–CS, but all failed.

3.7. Survival

Three patients (15.8%) died of bacterial infection ($n = 2$) or after alloHSCT ($n = 1$). The latter patient did not recover from infection due to severe neutropenia just prior to scheduled alloHSCT and received alloHSCT from MUD. Despite successful engraftment, this patient died of an uncontrolled infection. The median overall survival was not yet reached. The 2-year survival rate was 81.6%.

4. Discussion

The failure of hematopoiesis in AA appears to involve cytotoxic T lymphocytes, both directly and indirectly [29,30]. Analysis of T-cell receptors has shown that oligoclonal expansion of T-cells may be responsible for myelosuppression and stem cell failure [2,3]. CS inhibits the transcription of genes encoding IL-2, γ-IFN and several other cytokines,

Table 2
Responses according to dose cohort and time

Dose cohort	Evaluation time	No. of patients	Response			ORR (%)
			CR	PR	NR	
Total	Maximal	19	5	2	12	36.8
	3 months	19	1	5	13	31.6
	6 months	17	1	6	10	41.2
	12 months	12	1	4	7	41.7
I	Maximal	13	4	2	7	46.2
	3 months	13	0	5	8	38.5
	6 months	13	0	6	7	46.2
	12 months	9	1	4	4	55.6
II	Maximal	6	1	0	5	16.7
	3 months	6	1	0	5	16.7
	6 months	4	1	0	3	25
	12 months	3	0	0	3	0

Abbreviations: ORR, objective response rate; CR, complete response; PR, partial response; NR, no response.

Table 3
Detailed information of patients with aplastic anemia

UPN	Age	Gender	Disease state	Prior therapies	Time from Dx to Tx (M)	ALM dose (mg)	R_{Int}	Time to R_{Int} (M)	R_{Max}	Time to R_{Max} (M)	Salvage therapies	Relapse	Clonal evolution	Long-term FU
1	56	Female	SAA	Danazol	7.0	60	PR	0.95	PR	0.95	NR to AZA	Relapse after 22 M,	MDS	Alive
2	17	Male	SAA	ATG-CsA	149.3	60	NR	–	NR	–	CR after alloSCT	No	No	Alive
3	19	Male	SAA	None	0.6	60	NR	–	NR	–	CR after alloSCT	No	No	Alive
4	16	Female	SAA	ATG-CsA	138.5	60	PR	2.07	CR	4.90	No	No	No	Alive
5	38	Female	TDAA	None	1.4	60	PR	5.69	PR	5.69	No	No	No	Alive
6	57	Male	SAA	None	4.3	60	PR	2.17	CR	16.71	No	No	No	Alive
7	56	Female	SAA	None	1.2	60	NR	–	NR	–	NR to ATG-CsA	–	No	Alive
8	48	Female	SAA	None	1.6	60	PR	1.81	CR	15.99	No	No	No	Alive
9	19	Female	TDAA	None	0.2	60	PR	2.34	CR	13.42	No	No	No	Alive
11	66	Female	SAA	None	0.6	60	NR	–	NR	–	NR to ATG-CsA	–	No	Alive
12	24	Female	SAA	None	1.6	60	NR	–	NR	–	CR after alloSCT	–	No	Died of infection
13	56	Female	SAA	ATG-CsA	14.5	60	NR	–	NR	–	No	–	No	Died of infection
14	57	Male	TDAA	None	1.0	90	NR	–	NR	–	No	–	No	Died of infection
15	31	Female	SAA	None	108.0	90	NR	–	NR	–	CR after alloSCT	No	No	Alive
16	58	Female	SAA	None	0.3	90	NR	–	NR	–	NR to ATG-CsA	–	No	Alive
18	43	Male	VSAA	None	21.7	90	NR	–	NR	–	CR after alloSCT	–	No	Alive
19	81	Female	VSAA	None	0.3	90	NR	–	NR	–	No	–	No	Alive

Abbreviations: UPN, unique patient number; ALM, alemtuzumab; Dx, diagnosis; Tx, alemtuzumab-cyclosporin; M, months; ALM, alemtuzumab; R_{Int}, initial response; R_{Max}, maximal response; FU, follow-up; TDAA, transfusion-dependent non-severe aplastic anemia; SAA, severe aplastic anemia; VSAA, very severe aplastic anemia; CR, complete response; PR, partial response; NR, no response; AZA, azacytidine; MDS, myelodysplastic syndrome; ATG-CsA, antithymoglobulin antibody + cyclosporine A; alloSCT, allogeneic hematopoietic stem cell transplantation.

Table 4
Factors affecting maximal response

Characteristic	No. of patients	Response			ORR (%)	*p*-Value
		CR	PR	NR		
Age						0.350
<50 years	10	4	1	5	50.0	
≥50 years	9	1	1	7	22.2	
Dose cohort						0.333
I	13	4	2	7	46.2	
II	6	1	0	5	16.7	
Time from Dx to ALM						1.000
<1 year	14	3	2	9	35.7	
≥1 year	5	2	0	3	40.0	
Gender						0.603
Male	5	1	0	4	20.0	
Female	14	4	2	8	42.9	
Prior treatment						
None	15	4	1	10	33.3	0.603
Any	4	1	1	2	50.0	
Disease severity						0.237
Severe/very severe	14	3	1	10	28.6	
Non-severe	5	2	1	2	60.0	
WBC at M1						0.377
<1.2 K/μL	11	3	0	8	27.3	
≥1.2 K/μL	8	2	2	4	50.0	
ANC at M1						0.057
<1.0 K/μL	9	1	0	8	11.1	
≥1.0 K/μL	10	4	2	4	60.0	
% Lymphocytes at M1						0.350
<8%	10	3	2	5	50.0	
≥8%	9	2	0	7	22.2	
ALC at M1						1.000
<70/μL	10	3	1	6	40.0	
≥70/μL	9	2	1	6	33.3	
% Lymphocytes at M3						0.020
<20%	9	5	1	3	66.7	
≥20%	10	0	1	9	33.3	
Disease						
AA	17	4	2	9	35.3	
MDS	1	0	0	1	0.0	
PRCA	1	1	0	0	100.0	

Abbreviations: CR, complete response; PR, partial response; NR, no response; NE, not evaluable; ORR, overall response rate; Dx, diagnosis; ALM, alemtuzumab-cyclosporine; WBC, white blood cell; M1, 1 month after treatment; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; M3, 3 months after treatment; AA, aplastic anemia; MDS, myelodysplastic syndrome; PRCA, pure red cell aplasia.

thereby blocking pivotal steps in the evolution of the immune response. These findings suggest that IST may be beneficial in patients with BMFS.

CD52 is a costimulatory molecule involved in the induction of CD4+ regulatory T-cells, and CD52 ligation induces CD4 and CD8 down modulation in vivo and in vitro [31,32]. Following its binding to CD52, ALM mediates lymphocyte lysis via complement-mediated cytolysis and antibody-dependent cell mediated cytotoxicity, but it does not mediate the lysis of hematopoietic stem cells or progenitor cells [33–36]. Lymphocyte depletion was found to continue for 6 months after ALM-CS treatment, with significant lymphocyte recovery observed beginning 1 month after treatment. Although we did not perform lymphocyte subset analysis, it is speculated that not only T-cell depletion but also the reconstitution of the T-cell repertoire and changes in the peripheral dendritic cell repertoire can cause induction of CR [37,38].

Several previous case reports and small sized studies have shown that ALM can be effective for patients with PRCA and immune cytopenia, including immune hemolytic anemia and immune thrombocytopenia purpura [23–26]. Our find-

ing, of a 36.8% ORR to ALM-CS, was lower than the ORR in response to ATG–CS (61–77%) [14–16,39], although ORRs to ATG–CS in Korea (45% and 47%) were not as high as in Western countries [40,41]. These findings suggested that ATG–CS is insufficient as a standard treatment for patients with BMFS. Low response rates to IST suggest that the latter can play a bridging role prior to alloHSCT from MUD. The ORR of 46.2% in dose cohort I suggests that ALM-CS may be useful as bridging therapy. In responders, the response to ALM-CS was very rapid, a median of 2.07 months (95% CI, 0.1.40–2.75 months), although one patient achieved PR at 7 months. This rapid response may be caused by the effective elimination of lymphocytes, especially pathogenic T-cells, the main target of IST. Median time from PR to CR in responders was 9.33 months (95% CI, 0.0–31.71 months). AlloHSCT from MUD after 3 months was permitted to avoid delaying alloHSCT. Two of the 3 patients who failed prior ATG–CS showed response to ALM-CS. Therefore the rapid responsiveness and good rescue result of ALM-CS suggest that it may be useful for patients waiting for MUD alloHSCT from a public registry, either as bridging or second-line IST.

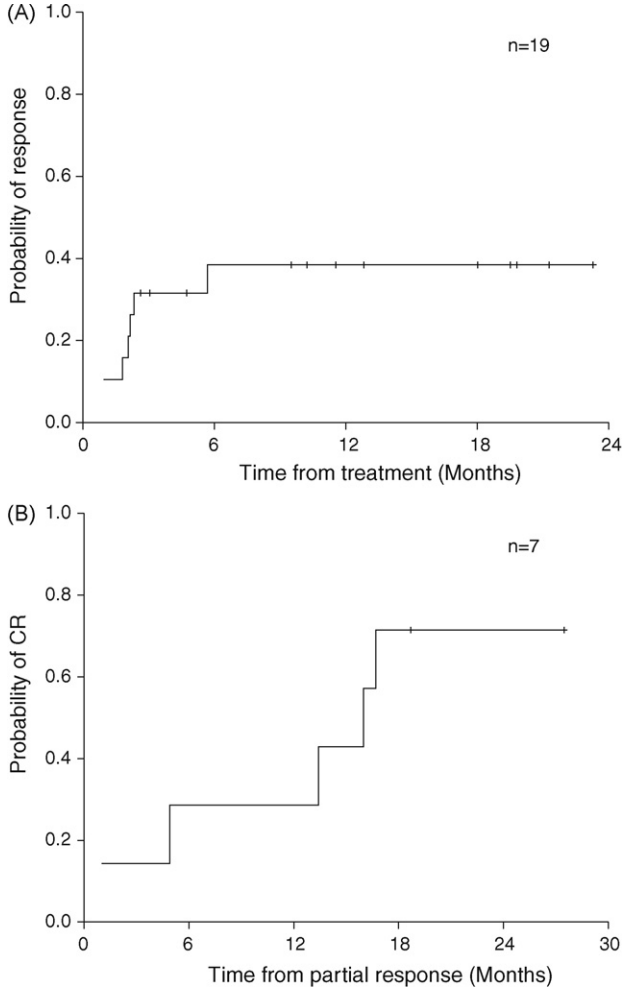

Fig. 1. Time to initial response in all patients (A) and time from initial response to complete response among responders (B). Among responders, median time to initial response was 2.07 months (95% CI, 1.40–2.75 months) and median time from initial response to complete response in complete responders was 9.33 months (95% CI, 0.0–31.71 months).

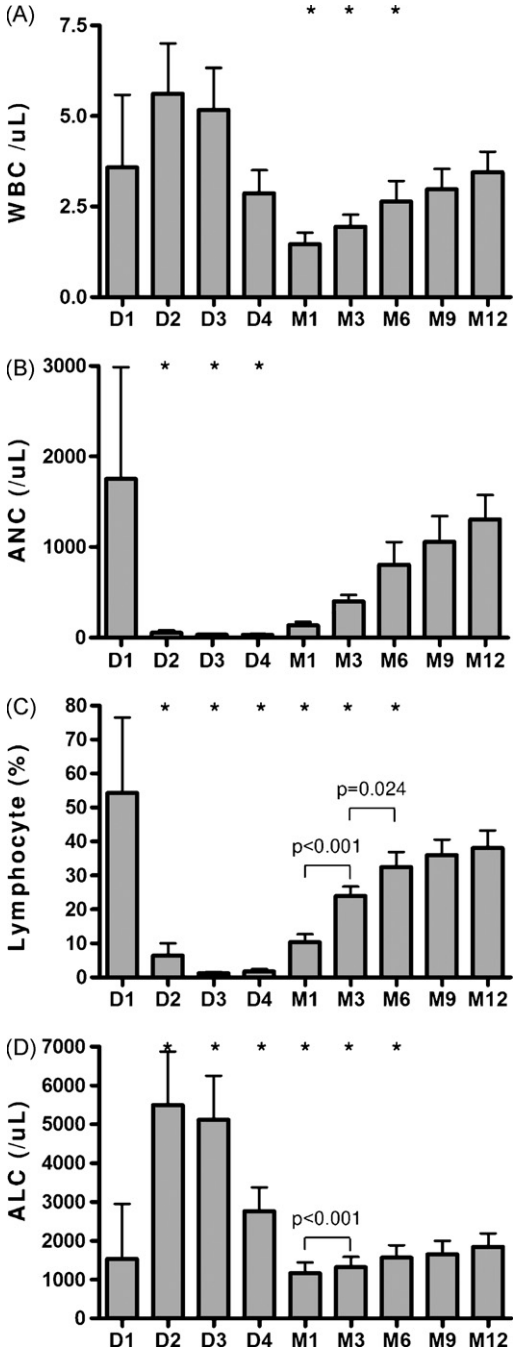

Fig. 2. Changes in white blood cell counts (WBC), absolute neutrophil count (ANC), percentage of lymphocytes, and absolute lymphocyte count (ALC) during treatment and follow-up period up to 12 months. Each solid bar and error bar represent mean and standard deviation, respectively. Dates from treatment include day 1 (D1, $n = 19$), day 2 (D2, $n = 19$), day 3 (D3, $n = 19$), day 4 (D4, $n = 19$), month 1 (M1, $n = 19$), month 3 (M3, $n = 19$), month 6 (M6, $n = 17$), month 9 (M9, $n = 13$), and month 12 (M12, $n = 12$). Asterisks indicate statistically significant changes compared with D1. Specified p-values indicate the statistical differences between 2 time points.

Interim analysis showed that dose cohort I had a better response rate than dose cohort II, but the difference did not attain statistical significance ($p = 0.333$). Only 1 patient in dose cohort II showed a response at 3 months. Among patients with AA, none treated with dose cohort II responded at 3 months, compared with the 50% (6/12) ORR in dose cohort I. Because almost all patients who were responsive to ALM-CS responded within the first 3 months, we concluded that further enrollment in dose cohort II would show little advantage when compared with dose cohort I. Therefore we did not accrue the planned 12 patients into dose cohort II. Although this decision may have affected the relatively poor response rate observed in dose cohort II, our results indicate that dose cohort I may result in sufficient immune suppression in patients with BMFS. We do not know why the lower dose showed a higher ORR.

The role of IST is still debatable in MDS treatment. The similarity between hypoplastic MDS and AA sometimes makes discrimination difficult, despite some typical features such as dysplasia. Some hypoplastic MDS patients obviously respond to IST [42,43], suggesting that pancytopenia in MDS may be in part lymphocyte-mediated. Although we therefore included patients with low risk MDS, the number was too small to determine whether ALM-CS has a role as IST in MDS; however, the one patient with MDS was not responsive to ALM-CS. Additional studies may determine whether ALM-CS is effective in patients with MDS. The one PRCA patient recovered rapidly after ALM-CS, in agreement with several case reports. Further studies are required to establish the efficacy of ALM for patients with PRCA.

ALM may produce moderate to severe dose related hypotension accompanied by slight tachycardia, ALM Fab binding to lymphoid tissues and the mononuclear phagocyte system, and significant Fab binding in the male reproductive tract (epididymis, sperm, seminal vesicle) and the skin. We found that our patients developed mild fever and rashes. Laboratory abnormalities included elevation of ALT and creatinine. Overall toxicities were excellent. Because >90% of Koreans are seropositive for CMV, we were concerned about the possibility of CMV reactivation after incorporation of ALM into the conditioning regimen for alloHSCT. We therefore assessed CMV antigenemia regularly and all patients received prophylactic acyclovir for 2 months. None of our patients was positive for CMV antigenemia or CMV disease, in agreement with previous findings [23]. The absence of CMV reactivation may be due to the low dose of ALM used here, which were lower than that used in alloHSCT studies. There may be a correlation between ALM dose and the rate of CMV reactivation. In addition, CMV reactivation may occur only if ALM is combined with alloHSCT, in which immune reconstitutions were delayed. Finally rapid recovery of ALC between months 1 and 3 may protect against CMV reactivation.

All patients who received alloHSCT achieved successful engraftment. Only one patient died of infection after alloHSCT. Thus, prior ALM-CS did not affect engraftment, CMV reactivation or infection. One MDS patient, who failed to respond to ALM-CS, was responsive to subsequent azacytidine therapy and finally achieved CR. Thus, ALM-CS may not have a negative effect on epigenetic therapy. However, the effect of ALM-CS on salvage treatment should be verified by studies in larger number of patients. Two of the three patients who had failed to respond to ATG–CS were responsive to ALM-CS, suggesting that ALM-CS may be effective as second-line IST after failure of ATG.

In conclusion, we have shown here that patients with BMFS showed a reasonable and rapid response to ALM-CS. This treatment regimen was associated with minimal toxicities and was well tolerated. These findings suggest that ALM-CS may be an option for possible IST in patients with BMFS, at least as a second-line therapy. Moreover, the 60 mg schedule of ALM may be sufficient compared with the higher dose (90 mg).

Conflict of interest

All authors have no conflict of interests.

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Contributions. H. Kim designed this study, analyzed data and wrote the paper. Y.J. Min, J.H. Baek, S.J. Shin and J.H. Park performed study. E.H. Lee and E.K. Noh contributed the management of data and patients. M.Y. Lee analyzed data.

References

[1] Sugawara T, Endo K, Shishido T, Sato A, Kameoka J, Fukuhara O, et al. T cell-mediated inhibition of erythropoiesis in myelodysplastic syndromes. Am J Hematol 1992;41:304–5.

[2] Manz CY, Dietrich PY, Schnuriger V, Nissen C, Wodnar-Filipowicz A. T-cell receptor beta chain variability in bone marrow and peripheral blood in severe acquired aplastic anemia. Blood Cells Mol Dis 1997;23:110–22.

[3] Melenhorst JJ, Fibbe WE, Struyk L, van der Elsen PJ, Willemze R, Landegent JE. Analysis of T-cell clonality in bone marrow of patients with acquired aplastic anaemia. Br J Haematol 1997;96:85–91.

[4] Molldrem JJ, Jiang YZ, Stetler-Stevenson M, Mavroudis D, Hensel N, Barrett AJ. Haematological response of patients with myelodysplastic syndrome to antithymocyte globulin is associated with a loss of lymphocyte-mediated inhibition of CFU-GM and alterations in T-cell receptor Vbeta profiles. Br J Haematol 1998;102:1314–22.

[5] Kochenderfer JN, Kobayashi S, Wieder ED, Su C, Molldrem JJ. Loss of T-lymphocyte clonal dominance in patients with myelodysplastic syndrome responsive to immunosuppression. Blood 2002;100:3639–45.

[6] Kaulen DR, Golovanova TA, Pyatikhina DP, Khorobrikh VV. Inhibition of haemopoietic stem cells by syngeneic lymphocytes treated with antilymphocyte serum. Folia Biol (Praha) 1975;21:95–102.
[7] Jansen J, Veenhof WF, Goselink HM. Anti-thymocyte globulin effective in the treatment of aplastic anemia does not stimulate granulocyte precursor cells. Exp Hematol 1981;9:1028–34.
[8] Doney K, Martin P, Storb R, Whitehead J, Smith A, Hansen JA, et al. A randomized trial of antihuman thymocyte globulin versus murine monoclonal antihuman T-cell antibodies as immunosuppressive therapy for aplastic anemia. Exp Hematol 1985;13:520–4.
[9] Huang AT, Mold NG, Zhang SF. Antithymocyte globulin stimulates human hematopoietic progenitor cells. Proc Natl Acad Sci USA 1987;84:5942–6.
[10] Tong J, Bacigalupo A, Piaggio G, Figari O, Marmont A. Effect of antilymphocyte globulin (ALG) on bone marrow T/non-T cells from aplastic anaemia patients and normal controls. Br J Haematol 1989;73:546–50.
[11] Franzke A, Geffers R, Hunger JK, Pfortner S, Piao W, Ivanyi P, et al. Identification of novel regulators in T-cell differentiation of aplastic anemia patients. BMC Genom 2006;7:263.
[12] Gluckman E, Esperou-Bourdeau H, Baruchel A, Boogaerts M, Briere J, Donadio D, et al. Multicenter randomized study comparing cyclosporine-A alone and antithymocyte globulin with prednisone for treatment of severe aplastic anemia. Blood 1992;79:2540–6.
[13] Marsh J, Schrezenmeier H, Marin P, Ilhan O, Ljungman P, McCann S, et al. Prospective randomized multicenter study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of patients with nonsevere aplastic anemia: a report from the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anaemia Working Party. Blood 1999;93:2191–5.
[14] Bacigalupo A, Bruno B, Saracco P, Di Bona E, Locasciulli A, Locatelli F, et al. Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midolio Osseo (GITMO). Blood 2000;95:1931–4.
[15] Kojima S, Hibi S, Kosaka Y, Yamamoto M, Tsuchida M, Mugishima H, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. Blood 2000;96:2049–54.
[16] Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmeier H. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. Blood 2003;101:1236–42.
[17] Scheinberg P, Nunez O, Wu C, Young NS. Treatment of severe aplastic anaemia with combined immunosuppression: anti-thymocyte globulin, ciclosporin and mycophenolate mofetil. Br J Haematol 2006;133:606–11.
[18] Teramura M, Kimura A, Iwase S, Yonemura Y, Nakao S, Urabe A, et al. Treatment of severe aplastic anemia with antithymocyte globulin and cyclosporin A with or without G-CSF in adults; a multicenter randomized study in Japan. Blood 2007;110:1756–61.
[19] Champlin RE, Ho WG, Feig SA, Winston DJ, Lenarsky C, Gale RP. Do androgens enhance the response to antithymocyte globulin in patients with aplastic anemia? A prospective randomized trial. Blood 1985;66:184–8.
[20] Greenwood J, Gorman SD, Routledge EG, Lloyd IS, Waldmann H. Engineering multiple-domain forms of the therapeutic antibody CAMPATH-1H: effects on complement lysis. Ther Immunol 1994;1:247–55.
[21] Williams RJ, Clarke E, Blair A, Evely R, Hale G, Waldmann H, et al. Impact on T-cell depletion and CD34+ cell recovery using humanised CD52 monoclonal antibody (CAMPATH-1H) in BM and PSBC collections; comparison with CAMPATH-1M and CAMPATH-1G. Cytotherapy 2000;2:5–14.
[22] Nuckel H, Frey UH, Roth A, Duhrsen U, Siffert W. Alemtuzumab induces enhanced apoptosis in vitro in B-cells from patients with chronic lymphocytic leukemia by antibody-dependent cellular cytotoxicity. Eur J Pharmacol 2005;514:217–24.
[23] Willis F, Marsh JC, Bevan DH, Killick SB, Lucas G, Griffiths R, et al. The effect of treatment with Campath-1H in patients with autoimmune cytopenias. Br J Haematol 2001;114:891–8.
[24] Ru X, Liebman HA. Successful treatment of refractory pure red cell aplasia associated with lymphoproliferative disorders with the anti-CD52 monoclonal antibody alemtuzumab (Campath-1H). Br J Haematol 2003;123:278–81.
[25] Au WY, Lam CC, Chim CS, Pang AW, Kwong YL. Alemtuzumab induced complete remission of therapy-resistant pure red cell aplasia. Leuk Res 2005;29:1213–5.
[26] Schutzinger C, Gaiger A, Thalhammer R, Vesely M, Fritsche-Polanz R, Schwarzinger I, et al. Remission of pure red cell aplasia in T-cell receptor gammadelta-large granular lymphocyte leukemia after therapy with low-dose alemtuzumab. Leukemia 2005;19:2005–8.
[27] Lee JH, Lee JH, Choi SJ, Lee KH. Alemtuzumab treatment for aplastic anemia and low risk myelodysplastic syndrome: preliminary results. Proc Kor Soc Hematol 2007;42:88.
[28] Frickhofen N, Rosenfeld SJ. Immunosuppressive treatment of aplastic anemia with antithymocyte globulin and cyclosporine. Semin Hematol 2000;37:56–68.
[29] Young NS, Maciejewski J. The pathophysiology of acquired aplastic anemia. N Engl J Med 1997;336:1365–72.
[30] Dufour C, Corcione A, Svahn J, Haupt R, Battilana N, Pistoia V. Interferon gamma and tumour necrosis factor alpha are overexpressed in bone marrow T lymphocytes from paediatric patients with aplastic anaemia. Br J Haematol 2001;115:1023–31.
[31] Watanabe T, Masuyama J, Sohma Y, Inazawa H, Horie K, Kojima K, et al. CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. Clin Immunol 2006;120:247–59.
[32] Shah A, Lowenstein H, Chant A, Khan A. CD52 ligation induces CD4 and CD8 down modulation in vivo and in vitro. Transpl Int 2006;19:749–58.
[33] Rowan WC, Hale G, Tite JP, Brett SJ. Cross-linking of the CAMPATH-1 antigen (CD52) triggers activation of normal human T lymphocytes. Int Immunol 1995;7:69–77.
[34] Gilleece MH, Dexter TM. Effect of Campath-1H antibody on human hematopoietic progenitors in vitro. Blood 1993;82:807–12.
[35] Wing MG, Moreau T, Greenwood J, Smith RM, Hale G, Isaacs J, et al. Mechanism of first-dose cytokine-release syndrome by CAMPATH 1-H: involvement of CD16 (FcgammaRIII) and CD11a/CD18 (LFA-1) on NK cells. J Clin Invest 1996;98:2819–26.
[36] Rowan W, Tite J, Topley P, Brett SJ. Cross-linking of the CAMPATH-1 antigen (CD52) mediates growth inhibition in human B- and T-lymphoma cell lines, and subsequent emergence of CD52-deficient cells. Immunology 1998;95:427–36.
[37] Rezvany MR, Tehrani MJ, Karlsson C, Lundin J, Rabbani H, Osterborg A, et al. Reconstitution of the T-cell repertoire following treatment with alemtuzumab (anti-CD52 monoclonal antibody) in patients with B-cell chronic lymphocytic leukaemia. Br J Haematol 2006;135: 475–85.
[38] Kirsch BM, Haidinger M, Zeyda M, Bohmig GA, Tombinsky J, Muhlbacher F, et al. Alemtuzumab (Campath-1H) induction therapy and dendritic cells: Impact on peripheral dendritic cell repertoire in renal allograft recipients. Transpl Immunol 2006;16:254–7.
[39] Rosenfeld S, Follmann D, Nunez O, Young NS. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. JAMA 2003;289:1130–5.
[40] Kim I, Yoon SS, Park S, Kim BK, Kim NK. The treatment of severe aplastic anemia: outcomes of bone marrow transplantation and immunosuppressive therapy in a single institution of Korea. J Kor Med Sci 2003;18:365–71.
[41] Ahn MJ, Choi JH, Lee YY, Choi IY, Kim IS, Yoon SS, et al. Outcome of adult severe or very severe aplastic anemia treated with

immunosuppressive therapy compared with bone marrow transplantation: multicenter trial. Int J Hematol 2003;78:133–8.
[42] Tichelli A, Gratwohl A, Wuersch A, Nissen C, Speck B. Antilymphocyte globulin for myelodysplastic syndrome. Br J Haematol 1988;68:139–40.
[43] Young N, Griffith P, Brittain E, Elfenbein G, Gardner F, Huang A, et al. A multicenter trial of antithymocyte globulin in aplastic anemia and related diseases. Blood 1988;72:1861–9.