Recent advances in treatment of aplastic anemia

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Recent advances in the treatment of aplastic anemia (AA) made most of patients to expect to achieve a long-term survival. Allogeneic stem cell transplantation (SCT) from HLA-matched sibling donor (MSD-SCT) is a preferred first-line treatment option for younger patients with severe or very severe AA, whereas immunosuppressive treatment (IST) is an alternative option for others. Horse anti-thymocyte globuline (ATG) with cyclosporin A (CsA) had been a standard IST regimen with acceptable response rate. Recently, horse ATG had been not available and replaced with rabbit ATG in most countries. Subsequently, recent comparative studies showed that the outcomes of patients who received rabbit ATG/CsA were similar or inferior compared to those who received horse ATG/CsA. Therefore, further studies to improve the outcomes of IST, including additional eltrombopag, are necessary. On the other hand, the upper age limit of patients who are able to receive MSD-SCT as first-line treatment is a current issue because of favorable outcomes of MSD-SCT of older patients using fludarabine-based conditioning. In addition, further studies to improve the outcomes of patients who receive allogeneic SCT from alternative donors are needed. In this review, current issues and the newly emerging trends that may improve their outcomes in near futures will be discussed focusing the management of patients with AA.

Keywords: Anemia, aplastic; Allogeneic stem cell transplantation; Immunosuppressive treatment; Iron chelation therapy

INTRODUCTION

Aplastic anemia (AA) is an clinical syndrome characterized by fatty replacement and decreased hematopoietic precursors of the bone marrow (BM), which results in peripheral blood (PB) pancytopenia [1]. It has been suggested that immune-mediated destruction of hematopoietic stem cells by activated cytotoxic T-cells expressing inhibitory cytokines such as interferon-γ and tumor necrosis factor-α contribute to its occurrence [2]. These cytokines induces death of hematopoietic stem cells, at least partially through the Fas-dependent pathway of apoptosis [3]. The mechanism of activation of cytotoxic T-cells is unclear, but several potential factors which are associated with antigen recognition, susceptibility of immune response, and secretion of cytokines are found [4]. The annual incidence of AA is estimated to be 2 per 1,000,000 persons per year in Western countries [5,6], but it is relatively higher in the Far East with 4 to 7 per 1,000,000 persons per year [7-9]. When patients are diagnosed with AA, careful investigations to exclude other possible cause of pancytopenia with hypocellular BM, including hypocellular myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria, and late onset inherited BM failure disorders, are needed [10]. For their therapeutic decision making,

disease has been stratified to moderate, severe, and very severe AA based on the degree of pancytopenia and BM hypocellularity [11].

Four decades ago, when there were only a few effective treatment options, patients with severe or very severe aplastic anemia (SAA) died 1 to 2 years after diagnosis due to fatal infections and/or hemorrhagic complications. However, recent treatment options including allogeneic stem cell transplantation (SCT) and immunosuppressive treatment (IST), mainly anti-thymocyte globuline (ATG) with cyclosporin A (CsA), have helped them to expect a long-term survival [12]. Nevertheless, there are still many challenges in management of patients with AA; approximately half of patients fail to achieve hematologic response and experience relapse or clonal evolution associated with myelodysplasia and leukemia after IST. On the other hand, a substantial proportion of patients who need MSD-SCT, especially those who experienced failure to a first-line IST, are not able to receive transplantation due to donor unavailability or significant comorbidities. In addition, graft failure and serious complications including graft-vs-host disease (GVHD) and fatal infections after allogeneic SCT limit its more relevant applications [12].

In this review, current issues and the newly emerging trends that may improve their outcomes in near futures will be discussed focusing the management of patients with AA.

CURRENT TREATMENT SCHEME IN PATIENTS WITH SAA

Based on recent reports for the management of patients with SAA, a treatment algorithm is represented with Fig. 1. Allogeneic SCT from HLA-matched sibling donor (MSD-SCT) is the most preferred first-line treatment option for younger (≤ 50 years) SAA patients with a suitable donor and no significant comorbidities. Other patients can receive IST with ATG/CsA as their initial treatment. Allogeneic SCT from available HLA-matched donor should be considered as a salvage treatment in patients who fail to achieve response to first-line IST. If they cannot receive it, appropriate alternative treatment options, including novel IST regimen, allogeneic SCT from familial mismatched donor (FMD-SCT), androgen, and supportive care, should be offered according to their individual circumstances.
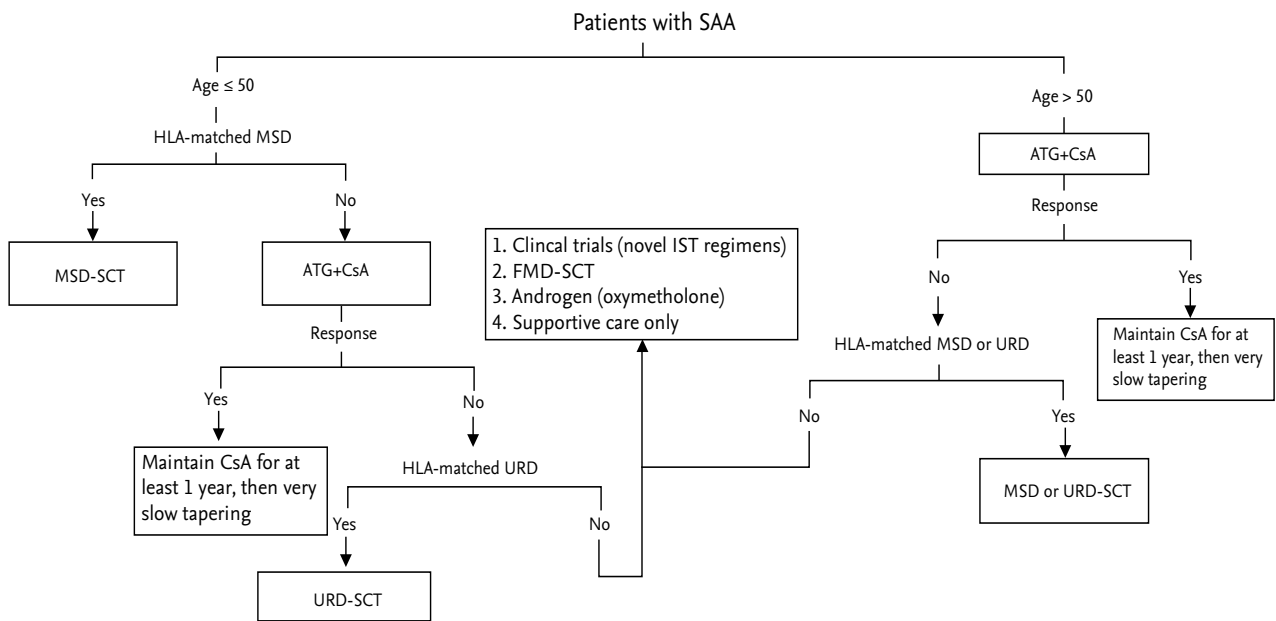

Figure 1. Treatment algorithm based on recent reports for the management of patients with severe aplastic anemia (SAA). MSD, matched sibling donor; URD, unrelated donor; ATG, anti-thymocyte globuline; CsA, cyclosporine A; FMD, familial mismatched donor; SCT, stem cell transplantation.

Shin SH, et al. Advances in treatment of aplastic anemia

KJIM

IMMUNOSUPPRESSIVE TREAMTENT

Historical aspect of IST for patients with AA: horse ATG/CsA as a standard IST regimen

Before allogeneic SCT and IST were relevantly used to the treatment of the patients with AA, high-dose androgen and/or supportive care including blood transfusion were the only available treatments, but their efficacy was observed in only a small proportion of patients [12]. Based on the recovery of autologous BM function after receiving horse ATG or allogeneic BM infusion with horse ATG conditioning [13,14], many investigators evaluated the efficacy of horse ATG alone for patients with AA and reported that a substantial proportion of patients could achieve hematologic response [15-18]. After Stryckmans et al. [19] reported the ability to induce hematological improvement of CsA, a phase III prospective study conducted by Frickhofen et al. [20] showed that patients who received IST using a combination of horse ATG and CsA achieved a higher overall response rate compared to those using horse ATG alone (70% vs. 46% at 6 months, $p < 0.050$). In addition, a subsequent report for long-term outcomes of this study showed a higher failure-free survival rate (39% vs. 24% at 11 years, $p = 0.040$) in the horse ATG/CsA group, but similar overall survival rate (58% vs. 54% at 11 years, $p = 0.600$) between the two groups because most nonresponders received salvage therapy [21]. Thereafter, other studies validated the efficacy horse ATG/CsA, which showed satisfactory results with overall response and overall survival rates of approximately 60% to 70% and 60 to 90%, respectively. Based on these reports, horse ATG/CsA has been accepted as a standard IST regimen for patients with AA [22-24].

Pretreatment factors predicting response and relapse rates of ATG/CsA for patients with AA

Although several studies showed acceptable outcomes of ATG/CsA, there were only limited reports for pretreatment factors predicting response and relapse rates of patients with AA who received it. A large retrospective study by the US National Institutes of Health (NIH) group to determine whether pretreatment blood counts can predict response rate for patients receiving horse ATG/CsA showed that younger age, higher absolute reticulocyte count (ARC) and absolute lymphocyte

Table 1. Comparative studies of horse ATG/CsA and rabbit ATG/CsA as a front-line IST in patients with AA

Study	Study design	ATG preparation	No.	Dose, median (range), mg/kg	OR, %	CR, %	Relapse, %	OS, %
NIH group [33]	Phase III randomized prospective	ATGAM	60	40 for 4 day	68 at 6 mon[a]	NA	28 at 3 yr	96 at 3 yr[a]
		Thymoglobuline	60	3.5 for 5 day	37 at 6 mon		11 at 3 yr	76 at 3 yr
Brazilian group [34]	Retrospective	Lymphoglobuline	42	15 (10–21.4) for 5 day	60 at 6 mon[a]	12 at 6 mon	36	78 at 2 yr[a]
		Thymoglobuline	29	2.5 (1.5–3) for 5 day	35 at 6 mon	7 at 6 mon	30	55 at 2 yr
Cleveland Clinic [35]	Phase II prospective comparing with historical control	ATGAM	67	40 for 4 day	58 at 1 yr	8 at 1 yr	16	64 at 5 yr
		Thymoglobuline	20	3.5 for 5 day	50 at 1 yr	0 at 1 yr	5	65 at 5 yr
Korean group [36]	Retrospective	Lymphoglobuline	46	15 for 5 day	46 at 1 yr	17 at 1 yr	23 at 3 yr	84 at 5 yr
		Thymoglobuline	53	2.5 for 5 day	49 at 1 yr	11 at 1 yr	27 at 3 yr	83 at 5 yr
EBMT [37]	Phase II prospective comparing with historical control	Lymphoglobuline	105	NA	67 at best	44 at best	NA	86 at 2 yr[a]
		Thymoglobuline	35	3.75 for 5 day	60 at best	23 at best		68 at 2 yr
East Asian group [38]	Retrospective	Lymphoglobuline	297	15 for 5 day	60 at 6 mon	19 at 6 mon	7 at 10 yr[a]	92 at 10 yr[a]
		Thymoglobuline	158	2.3–5.0 for 5 day	55 at 6 mon	16 at 6 mon	15 at 10 yr	87 at 10 yr

ATG, anti-thymocyte globulin; CsA, cyclosporin A; IST, immunosuppressive treatment; AA, aplastic anemia; OR, overall response; CR, complete response; OS, overall survival; NIH, National Institutes of Health; NA, not available; EBMT, European Group for Blood and Marrow Transplantation.
[a]Significant difference between horse ATG group and rabbit ATG group ($p < 0.050$).

count (ALC) were associated with increased response rate. In this study, patients with higher ARC and ALC ($\geq 25 \times 10^9$/L and $\geq 1 \times 10^9$/L, respectively) achieved a higher overall response rate compared to those with lower ARC and ALC (83% vs. 41%, $p < 0.001$) [25]. In other studies, patients with HLA-DR15, normal cytogenetics, and paroxysmal nocturnal hemoglobinuria clone had higher overall response rate and quality of response compared to those without [26-28].

On the other hand, Saracco et al. [29] compared the relapse rate between those who responded to horse ATG/CsA that received rapid (0.8 mg/kg/month) and slow CsA (≤ 0.7 mg/kg/month) tapering after maintaining stable blood counts lasting 3 or more months, and reported that was the relapse rate was higher in the rapid tapering group (60% vs. 8% at 10 years, $p = 0.001$). This study suggests that tapering CsA should be initiated after at least 1 year from treatment and taper carefully by 10% every month. Late relapse also can be observed in several long-term responders, which may have resulted from suboptimal therapeutic range of CsA by intolerability or poor compliance [30]. In addition, the NIH group reported that shorter telomere length of PB leukocytes at diagnosis was associated with higher relapse (odds ratio [OR] 0.16; 95% confidence interval [CI], 0.03 to 0.69; $p = 0.010$) and clonal evolution (OR, 0.29; 95% CI, 0.11 to 0.76; $p = 0.010$) rates with a lower overall survival (OR, 0.35; 95% CI, 0.17 to 0.73; $p = 0.005$) rate, but was not associated with overall response rate [31]. Conversely, Sakaguchi et al. [32] reported that pediatric patients with shorter telomere length of PB lymphocytes at diagnosis was associated with a lower overall response rate (OR, 22.0; 95% CI, 4.19 to 115; $p < 0.001$), but not relapse, clonal evolution, and overall survival rates. Considering these conflicting results, further large prospective studies to validate the relation between the outcomes of ATG/CsA and telomere length at diagnosis are needed.

The comparison of efficacy between patients with AA who received horse ATG/CsA and rabbit ATG/CsA as a first-line IST

Horse ATG/CsA had been used as a regimen of choice for first-line IST, whereas rabbit ATG/CsA has been used as a second-line regimen for patients with refractory or relapsed AA [30]. However, Lymphoglobulin (Sangstat, Lyon, France), which had once been the most widely used preparation of horse ATG, was withdrawn from the market in 2007 due to manufacturing difficulties. Thereafter, horse ATG has become unavailable and replaced with rabbit ATG in most countries.

Until recently, there have been several studies comparing the outcomes of AA patients who received horse ATG/CsA and rabbit ATG/CsA as a first-line IST (Table 1). A phase III randomized prospective study conducted by the NIH group showed that the horse ATG/CsA group showed higher overall response (68% vs. 37% at 6 months, $p < 0.010$) and overall survival (96% vs. 76% at 3 years, $p = 0.040$) rates compared to the rabbit ATG/CsA group [33]. A retrospective study by Brazilian group also reported that horse ATG/CsA group showed higher overall response (60% vs. 35% at 6 months, $p < 0.050$) and overall survival (78% vs. 55% at 2 years, $p = 0.030$) rates [34]. However, a phase II prospective study comparing the historical horse ATG/CsA control group conducted by the Cleveland Clinic and a retrospective study by Korean group showed similar overall response (58% vs. 50% at 1 year, $p = 0.610$; and 46% vs. 49% at 1 year, $p = 0.740$, respectively) and overall survival (64% vs. 65% at 5 years, $p = 0.540$; and 84% vs. 83% at 5 year, $p = 0.460$, respectively) rates were observed between two groups [35,36]. On the other hand, another phase II prospective study comparing the historical horse ATG/CsA control group conducted by the European Group for Blood and Marrow Transplantation (EBMT) as well as a recent large pediatric retrospective study by East Asian Group, showed similar overall response (67% vs. 60%, $p > 0.050$; and 60% vs. 55% at 6 months, $p = 1.000$) rates, but the horse ATG/CsA group showed higher overall survival (86% vs. 68% at 2 years, $p = 0.010$; and 92% vs. 87%, $p < 0.010$, respectively) rates compared to the rabbit ATG/CsA group [37,38].

Compared to horse ATG, rabbit ATG evokes more prolonged and profound lymphocytopenia, which leads to a lower absolute number of CD4$^+$ regulatory T-cells [33,36]. These findings can explain lower response and survival rates of the rabbit ATG/CsA group in several studies. However, it is suggested that discrepancies of other factors including the dose and duration of CsA administration and ethnic factors may result in conflicting results [1]. Notably, there has not been any study that showed superior outcomes of rabbit ATG/CsA over

horse ATG/CsA. Therefore, horse ATG/CsA should be used preferentially as a first-line IST regimen and rabbit ATG/CsA should be considered only if horse ATG is unavailable.

Efforts to improve the outcomes of ATG/CsA by the addition of other agents

Because half of patients with AA experience failure to IST, there have been many efforts to improve its efficacy by adding other agents. However, prospective studies conducted by the NIH group failed to show that the addition of mycophenolate mofetil or silrolimus to horse ATG/CsA results in improved overall response rate and reduced relapse rate [39,40]. Meanwhile, a prospective study conducted by Teramura et al. [41] comparing the outcomes of patients who received horse ATG/CsA with or without granulocyte colony-stimulating factor (G-CSF) showed that patients who received G-CSF achieved higher overall response (77% vs. 57% at 4 years, $p = 0.030$) and lower relapse (15% vs. 42% at 4 years, $p = 0.010$) rates compared to those who did not, but there were no significant difference in overall survival rate (88% vs. 94% at 4 years, $p = 0.440$) and incidence of infectious complications (59% vs. 40%, $p = 0.070$). Conversely, similar overall response rate (73% vs. 66% at 6 years, $p = 0.540$) between the patients who received horse ATG/CsA with and without G-CSF was observed in another randomized study conducted by Tichelli et al. [42]. Because of conflicting results and insufficient data, many clinical centers have not included routine G-CSF administration with their IST protocol.

Eltrombopag, an oral form of nonpeptide thrombopoietin mimetic, was developed to induce platelet maturation and release by binding to thrombopoietin receptor (c-MPL) on megakaryocytes in patients with immune thrombocytopenic purpura [43]. However, a previous report, which showed that patients who lack c-MPL develop a form of congenital BM failure, suggested that thrombopoietin is critical to hematopoietic stem cell development and differentiation [44]. Based on this observation, a recent phase II prospective study was conducted to determine the eltrombopag can be effective for nonresponders to previous IST. In this study, 11 of 25 enrolled patients (44%) achieved hematological response in at least one-lineage and the normalization of BM tri-lineage hematopoiesis [45]. In its subsequent study, hematological response in at least one-lineage was observed in 17 of 43 enrolled patients (40%), including seven (16.3%) tri-lineage response. In addition, five patients (11.6%) who achieved sustained response maintained stable blood counts at a median 13 months (range, 1 to 15) after drug discontinuation [46]. These reports suggest that adding eltrombopag might improve the efficacy of horse ATG/CsA. A phase III randomized

Table 2. Reports of outcomes in patients with SAA who received MSD-SCT

Study	Study design	No.	Age, median (range), yr	Conditioning regimen	Graft failure, %	aGVHD at 100 days, %	cGVHD, %	OS, %
Gale et al. [59]	Retrospective	23	17 (3–43)	CY/TBI	4	30	13	61 at 1 yr
Seattle group [60]	Phase II prospective comparing with historical control	39	25 (2–52)	CY/ATG	5	15	34 at 3 yr	92 at 3 yr[a]
		39	25 (2–46)	CY alone	8	21	61 at 3 yr	72 at 3 yr[a]
Champlin et al. [61]	Phase III randomized prospective	60	26 (4–51)	CY alone	18	18	21 at 5 yr	74 at 5 yr
		70	23 (1–51)	CY + ATG	16	11	32 at 5 yr	80 at 5 yr
Ades et al. [62]	Retrospective	100	NA	CY + TAI	NA	42[a]	64 at 5 yr[a]	69 at 5 yr
		33		CY + ATG		0	42 at 5 yr	
Kahl et al. [63]	Retrospective	81	25 (2–63)	CY + ATG	4	24	26	88 at 9 yr

SAA, severe aplastic anemia; MSD-SCT, stem cell transplantation from HLA-matched sibling donor; aGVHD, acute graft-vs-host disease; cGVHD, chronic graft-vs-host disease; OS, overall survival; CY, cyclophosphamide; TBI, total body irradiation; ATG, anti-thymocyte globulin; NA, not available; TAI, thoraco-abdominal irradiation.
[a]Significant difference between horse ATG group and rabbit ATG group ($p < 0.050$).

study to validate this hypothesis is currently ongoing (www.clinicaltrials.gov as #NCT01623167).

Salvage treatment for patients with AA refractory or relapsed to a first-line IST

Not only MSD-SCT but also allogeneic SCT from HLA-matched unrelated donor (URD-SCT) can be a considerable treatment option for SAA patients who failed to first-line IST. It is supported by a recent phase III prospective study conducted by Kosaka et al. [47], which showed a higher failure-free survival rate of pediatric patients who received URD-SCT compared to those who received horse ATG/CsA (84% vs. 10% at 5 years, $p < 0.010$) after experiencing failure to first-line IST. However, alternative treatment options, including an additional course of ATG/CsA or other IST regimens, also can be considered for patients who are ineligible for allogeneic SCT.

Studies for second course of ATG/CsA were not relatively abundant and reported a wide range of response rates. A phase II prospective multicenter study conducted by Di Bona et al. [48] reported that nonresponders to first-line horse ATG/CsA who received a second course rabbit ATG/CsA showed acceptable overall and complete response rates (70% and 30%, respectively). In addition, a retrospective study by the NIH group showed that overall response rates of a second course rabbit ATG/CsA at 6 months in patients who failed to achieve response or experienced relapse to first-line horse ATG/CsA were 30% and 65%, respectively [49]. However, a retrospective study by the NIH group showed that non-responders to first-line rabbit ATG/CsA who received a second course horse ATG/CsA achieved relatively poor overall response rate (21% at 3 months) [50]. Based on this result, It is not recommended second course of ATG/CSA in patients who failed achieve response to first-line rabbit ATG/CsA.

Alemtuzumab, which is a humanized anti-CD52 IgG1 monoclonal antibody with lymphocytopenic activity, or high-dose cyclophosphamide (CY) has been also used in patients with refractory or relapsed AA. A pilot study conducted by Risitano et al. [51] for 19 patients with AA (including 13 refractory) showed an overall response rate of 58% and complete response rate of 26% after receiving alemtuzumab (a total dose of 103 mg subcutaneously) and low-dose CsA. In addition, the NIH group investigated the role of alemtuzumab monotherapy (test dose of 1 mg followed by 10 mg/kg/day for 10 days) for patients with refractory or relapsed SAA, which showed overall response rates at 6 months of 37% and 56% with overall survival rates at 3 years of 83% and 86%, respectively [52]. In these studies, clinically significant infectious complications including cytomegalovirus reactivation and Epstein-Barr virus-related disease was not problematic. On the other hand, a recent report for long-term outcomes of CY (50 mg/kg for 4 days) in 23 patients with refractory AA showed feasible overall and complete response rates (47.8% and 21.7% at 10 years, respectively) with overall survival rate (61.8% at 10 years) [53]. However, CY for patients with AA should be used with caution because frequent fatal infectious complications followed by delayed neutrophil recovery in patients who received it as a first-line treatment were observed in a previous study [54].

ALLOGENEIC SCT FOR PATIENTS WITH SAA

Allogeneic MSD-SCT

MSD-SCT is the most preferred treatment option for younger SAA patients with a suitable donor and no significant comorbidities. Until recently, many investigators have explored optimal conditioning regimen for SAA patients who receive MSD-SCT to achieve sustained engraftment with minimal fatal complications (Table 2). In the initial experiences of MSD-SCT using CY alone conditioning, high incidence of graft failure was a major challenge, particularly in previous heavily transfused patients [55]. Subsequently, MSD-SCT using radiation, including local-field and total-body irradiation (TBI), plus CY conditioning was attempted and resulted in a lower incidence of graft failure, but was associated with relatively higher incidences of long-term regimen-related morbidities and mortality [56-59]. Thereafter, several investigators attempted MSD-SCT using CY plus ATG conditioning, which might induce both effective immunoablation and lymphoablation [60-63]. In a phase II prospective study conducted by the Seattle group, the incidence of neutrophil engraftment, acute and chronic GVHD for patients who received MSD-SCT using CY (200 mg/kg) plus horse ATG (90 mg/kg) conditioning were 95%, 15%, and 34%,

Shin SH, et al. Advances in treatment of aplastic anemia

KJIM

respectively. Their overall survival rate was significantly higher compared to that of historical control group who received CY alone conditioning (92% vs. 72% at 3 years, $p = 0.043$) [60]. In addition, a retrospective study by Ades et al. [62] comparing the outcomes of patients who received MSD-SCT using CY (200 mg/kg) plus rabbit ATG (10 mg/kg) conditioning and thoraco-abdominal irradiation (TAI, 600 cGy) plus CY (150 mg/kg) conditioning showed that the CY/ATG group was associated with a lower incidence of acute (0% vs. 42%, $p < 0.001$) and chronic GVHD (42% vs. 64%, $p = 0.025$) with higher overall survival rate (OR, 6.20; 95% CI, 1.50 to 25.90; $p < 0.001$) compared to the TAI/CY group.

On the other hand, minimizing the incidence of GVHD is essential for SAA patients who receive allogeneic SCT, considering that they do not need its graft-versus-leukemia effect. A large registry-based study conducted by EBMT/Center for International Blood and Marrow Transplant Research (CIBMTR) comparing the outcomes of patients who received MSD-SCT using peripheral blood stem cells (PBSCs) and BM as a graft source showed that higher incidence of chronic GVHD (27% vs. 12%, $p = 0.002$) and lower overall survival rate (73% vs. 85% at 5 years, $p = 0.024$) were observed in the PBSC group compared to the BM group, especially in patients younger than 20 [64]. In addition, a phase III randomized study conducted by Gruppo Trapianto di Midollo Osseo/EBMT comparing the outcomes of patients who received MSD-SCT using CsA plus methotrexate (MTX) and CsA alone GVHD prophylaxis showed that the incidence of trans-

plant-related mortality (3% vs. 15% at 1 year, $p = 0.070$) and overall survival rate (94% vs. 78% at 5 years, $p = 0.050$) were significantly superior in the CsA/MTX group compared to the CsA alone group [65]. Therefore, BM and CsA/MTX are widely used as the preferable stem cell source and GVHD prophylactic regimen for SAA patients who receive MSD-SCT.

On the other hand, the upper age limit of SAA patients who are able to receive MSD-SCT remains an unsolved challenge. In a large registry analysis conducted by CIBMTR, the impact of patients' age on transplant outcomes was analyzed. The incidence of neutrophil engraftment was not significantly different in all age groups, whereas patients older than 40 showed a significantly lower incidence of platelet engraftment compared to those younger than 20 (OR, 0.45; 95% CI, 0.25 to 0.83; $p = 0.010$), whereas not compared to those aged between 20 and 40 (OR, 0.60; 95% CI, 0.33 to 1.10; $p = 0.098$). The incidence of mortality for patients older than 40 was significantly higher compared to those of patients younger than 20 (OR, 2.70; 95% CI, 1.89 to 3.87; $p < 0.001$) and aged between 20 and 40 (OR, 1.60; 95% CI, 1.13 to 2.26; $p = 0.008$) [66]. This study showed that the incidence of mortality increased with age for patients who received MSD-SCT although it should be interpreted with caution because patients in this study cohort received heterogeneous conditioning regimens with mostly receiving CY (200 mg/kg) with or without ATG (71.4%). However, the difficulties of determining the most appropriate first-line treatment for older patients still remain because lower overall response and

Table 3. Large-study reports of outcomes in patients with SAA who received URD-SCT

Study	Study design	No.	Age, median (range), yr	Conditioning regimen	Graft failure, %	aGVHD at 100 days, %	cGVHD, %	OS, %
EBMT [70]	Registry-based	38	14 (3–37)	FLU/CY/ATG	18	11	27	73 at 2 yr
EBMT [71]	Registry-based	52	13 (3–51)	FLU/CY/ATG	17	17	23	73 at 5 yr
		48	27 (7–53)	FLU/CY/ATG + TBI	17	19	38	79 at 5 yr
Maury et al. [73]	Retrospective	89	17 (0–52)	FLU-based	9	11	32	77 at 4 yr
EBMT [74]	Registry-based	349	19 (1–65)	Various conditioning	11	28	22	57 at 5 yr
Lee et al. [76]	Retrospective	50	28 (15–53)	TBI 800/CY	2	46	50 at 5 yr	88 at 5 yr

SAA, severe aplastic anemia; URD-SCT, stem cell transplantation from HLA-matched unrelated donor; aGVHD, acute graft-vs-host disease; cGVHD, chronic graft-vs-host disease; OS, overall survival; EBMT, European Group for Blood and Marrow Transplantation; FLU, fludarabine; CY, cyclophosphamide; ATG, anti-thymocyte globulin; TBI, total body irradiation.

survival rates were also observed when they received IST [25].

On the other hand, a recent phase II prospective study conducted by EBMT which analyzed the outcomes of patients who received MSD-SCT using fludarabine (FLU, 120 mg/m²) plus CY (1,200 mg/m²) with rabbit ATG (15 mg/kg) showed that no significant difference in overall survival rate was observed between patients older than 40 and those aged between 30 and 40 [67]. We also reported the outcomes of 82 SAA patients who received MSD-SCT using FLU (180 mg/kg) plus CY (100 mg/kg) with rabbit ATG (10 mg/kg) conditioning. In this study, there was no significant difference in overall survival rate among patients with age lesser than 20, 20 to 39, 40 to 49 and 50 to 59 (88%, 97%, 92%, and 86% at 3 years, respectively, $p = 0.426$) [68]. Consequently, these reports suggest that FLU-based conditioning may be a promising option to overcome relatively poor outcomes of MSD-SCT in older patients. Therefore, MSD-SCT using FLU-based conditioning should be considered as a feasible first-line treatment for older patients.

Allogeneic URD-SCT
URD-SCT can be considered as a treatment option for SAA patients who failed first-line IST or require emergent allogeneic SCT if they have no suitable MSD. Two decades ago, only about one-third of patients who received URD-SCT experienced long-term survival [69]. Recently, however, relevant applications of high-resolution HLA typing for donor selection and more optimized conditioning regimen improved overall survival rate to approximately 70 to 80% [70-74].

The conditioning regimen for URD-SCT for SAA patients that could minimize the incidence of graft failure and regimen-related toxicities still remains uncertain. An initial phase II prospective study conducted by EBMT which analyzed the outcomes of patients who received URD-SCT using FLU (120 mg/m²) plus CY (1,200 mg/m²) with rabbit ATG (7.5 mg/kg) conditioning showed that the incidence of graft failure was 18% with an overall survival rate of 73% at 2 years. In this study, patients older than 14 showed a significantly higher incidence of graft failure (32% vs. 5%, $p = 0.030$) with a lower trend of overall survival rate (61% vs. 84% at 2 years, $p = 0.200$) [70], which suggested that the modification of conditioning was required. In addition,

an EBMT registry-based analysis for the outcomes of patients who received URD-SCT using FLU (120 mg/m²) plus CY (1,200 mg/m²) with rabbit ATG (7.5 mg/kg) ± low-dose TBI (200 cGY) conditioning showed that there was no significant difference in overall survival rate between the patients older and younger than 27 when they received low-dose TBI containing regimen (78% vs. 79% at 5 years, $p > 0.050$). However, relatively high incidence of mortalities by graft failure (7%), posttransplant lymphoproliferative disease (4%) and GVHD (4%) of this study remained to be a challenge to overcome [71]. On the other hand, the Seattle group conducted a dose-escalating study to find the most appropriate TBI dose, when used with CY (200 mg/kg) plus horse ATG (120 mg/kg) conditioning. After escalating or de-escalating 200 cGy based on the development of graft failure or regimen-related toxicities from staring 600 cGy, they found that 200 cGy was the most effective dose in minimizing regimen-related toxicities without sacrificing sustained engraftment [72]. In addition, we conducted a prospective study of de-escalating TBI to find optimal dose, when used with CY (120 mg/kg), which showed higher overall survival rate in the 800 cGy group was observed compared to the 1,000 and 1,200 cGy groups (92% vs. 40% vs. 44% at 3 years, $p < 0.001$) [75]. In a subsequent report of the long-term results for patients who received URD-SCT using TBI (800 cGy) plus CY (120 mg/kg) conditioning, all enrolled patients achieved sustained myeloid engraftment after a median of 13 days (range, 8 to 30) with acceptable incidences of acute and chronic GVHD (46% and 50%, respectively) and overall survival rate (88% at 5 years) [76]. The current available reports of outcomes for SAA patients who received URD-SCT are summarized in Table 3.

Because patients who received URD-SCT experience more frequent and severe GVHD compared to those who received MSD-SCT [77], more effective GVHD prophylaxis, especially for SAA patients, is needed. A matched-pair analysis based on the Japan Marrow Donor Program registry comparing the outcomes of patients who received URD-SCT using GVHD prophylaxis with FK506 and CsA along with MTX showed that the FK506/MTX group showed a higher overall survival rate (83% vs. 50% at 5 years, $p = 0.012$) with a lower trend for incidence of chronic GVHD (13% vs. 36%, $p = 0.104$) [78]. On the other hand, several patients unavoidably

Shin SH, et al. Advances in treatment of aplastic anemia

KJIM

receive URD-SCT using PBSC as stem cells source and/ or from partially HLA-URD. Because they are associated with a higher incidence of GVHD or lower survival rate [76,79], we conducted a phase II prospective study of adding low-dose rabbit ATG (2.5 mg/kg) to TBI (800 cGy) plus CY (120 mg/kg) conditioning in patients who received URD-SCT from these donors and compared the historical control group who did not receive additional low-dose rabbit ATG. In this study, the incidence of acute GVHD was significantly decreased (21% vs. 64%, p = 0.028), but not chronic GVHD (31% vs. 57%, p = 0.247) in patients who received low-dose rabbit ATG compared to the historical control group [80]. However, further studies are needed to determine whether this strategy is effective for SAA patients.

Allogeneic FMD-SCT

FMD-SCT had been considered a treatment option for patients with aggressive hematologic malignancies and lacking HLA-matched donors. Although there are several advantages including universal donor availability without delay, high incidences of graft failure and GVHD due to bidirectional barrier of HLA incompatibilities have been considered as a great concern [81,82]. However, recent advances in controlling these challenges extended its application to patients with less aggressive diseases, including SAA.

Until recently, several investigators have attempted to search optimal conditioning regimen and strategy of graft manipulations for SAA patients who receive FMD-SCT. An initial retrospective study by the Seattle group showed that patients who received unmanipulated FMD-SCT using more intensified conditioning consisted of TBI (1,200 cGy) and CY (120 mg/kg) achieved a higher incidence of sustained engraftment (83% vs. 29%, p < 0.050) and overall survival rate (50% vs. 0%, p < 0.050) compared to those using CY alone (200 mg/kg) conditioning [83]. Another retrospective study by Tzeng et al. [84] showed that no graft failure was observed in six patients who received unmanipulated FMD-SCT using TBI (800 cGY) plus CY (200 mg/kg) conditioning. In a recent retrospective study by the Peking group for patients who received FMD-SCT, all patients achieved sustained engraftment by using intensified conditioning that consisted of CY (200 mg/kg) plus rabbit (2.5 mg/ kg) or horse ATG (20 mg/kg) with intravenous busufu-

lan (6.4 mg/kg) following G-CSF primed BM and mobilized PBSC infusion, with acceptable overall survival rate (65% at 2 years) and incidences of acute and chronic GVHD (42% and 56%, respectively) [85]. In addition, various novel strategies including using posttransplant CY and selective CD3$^+$ T-cell depleted grafts have been also attempted to improve the outcomes of patients who receive FMD-SCT [86,87].

IRON CHELATION THEARPY FOR PATIENTS WITH AA

A substantial proportion of patients with AA unavoidably receive blood transfusions as supportive care after receiving available treatments. Long-term regular transfusion may lead to iron overload, which is associated with significant morbidities and mortality [88,89]. Deferasirox is an oral iron chelator, whose efficiency has been proven for patients with various transfusion-dependent hematological diseases [90,91]. A recent sub-group analysis for 116 patients with AA, conducted by Lee et al. [92], of a large phase II prospective study which evaluated its efficacy and safety showed that their serum ferritin level significantly decreased (3,254 to 1,854 ng/mL, p < 0.001) at 1 year after deferasirox without significant toxicities except mild renal toxicities. A subsequent *post hoc* analysis of this study showed hematologic improvement was observed in a subset of patients (46%) who received deferasirox without concomitant IST with a significant difference in serum ferritin level change from baseline between those who achieved response and those who did not (–46% vs. –28%, p = 0.017) [93]. Other investigators also reported that a substantial proportion of patients who received deferasirox achieved hematological improvement [94-96], but further investigations are required to determine which mechanism was involved.

CONCLUSIONS

Although recent advances have helped most AA patients to achieve long-term survival, there are many unsolved challenges in their appropriate management. About half of patients who received IST as a first-line

treatment still experience failure. Therefore, further studies are needed to improve the response rate and reduce the incidence of relapse after IST, especially considering the unavailability of horse ATG in many countries. Eltrombopag may be a promising additional agent to improve the efficacy of IST, but further validations for its beneficial effects are needed. In addition, more effective treatment for patients who are ineligible for allogeneic SCT after experiencing failure to first-line IST should be explored.

On the other hand, the upper age limit of SAA patients who are able to receive MSD-SCT as a first-line treatment should be re-determined, considering the favorable outcomes of older patients who received MSD-SCT using FLU-based conditioning. It may contribute to determine the most appropriate treatment option for them along with further studies to investigate pretreatment factors predicting response and relapse after fist-line IST. In addition, more optimized conditioning and effective GVHD prophylaxis should be also developed to improve the outcomes of allogeneic SCT from alternative donors.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Shin SH, Lee JW. The optimal immunosuppressive therapy for aplastic anemia. Int J Hematol 2013;97:564-572.
2. Maciejewski J, Selleri C, Anderson S, Young NS. Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro. Blood 1995;85:3183-3190.
3. Risitano AM, Maciejewski JP, Green S, Plasilova M, Zeng W, Young NS. In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing. Lancet 2004;364:355-364.
4. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 2006;108:2509-2519.
5. Incidence of aplastic anemia: the relevance of diagnostic criteria: by the International Agranulocytosis and Aplastic Anemia Study. Blood 1987;70:1718-1721.
6. Mary JY, Baumelou E, Guiguet M. Epidemiology of aplastic anemia in France: a prospective multicentric study: the French Cooperative Group for Epidemiological Study of Aplastic Anemia. Blood 1990;75:1646-1653.
7. Issaragrisil S. Epidemiology of aplastic anemia in Thailand: Thai Aplastic Anemia Study Group. Int J Hematol 1999;70:137-140.
8. Yang C, Zhang X. Incidence survey of aplastic anemia in China. Chin Med Sci J 1991;6:203-207.
9. Yong AS, Goh AS, Rahman M, Menon J, Purushothaman V. Epidemiology of aplastic anaemia in the state of Sabah, Malaysia. Med J Malaysia 1998;53:59-62.
10. Marsh JC, Ball SE, Cavenagh J, et al. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol 2009;147:43-70.
11. Rozman C, Marin P, Nomdedeu B, Montserrat E. Criteria for severe aplastic anaemia. Lancet 1987;2:955-957.
12. Scheinberg P. Aplastic anemia: therapeutic updates in immunosuppression and transplantation. Hematology Am Soc Hematol Educ Program 2012;2012:292-300.
13. Speck B, Gluckman E, Haak HL, van Rood JJ. Treatment of aplastic anaemia by antilymphocyte globulin with and without allogeneic bone-marrow infusions. Lancet 1977;2:1145-1148.
14. Speck B, Gratwohl A, Nissen C, et al. Treatment of severe aplastic anaemia with antilymphocyte globulin or bone-marrow transplantation. Br Med J (Clin Res Ed) 1981;282:860-863.
15. Champlin R, Ho W, Gale RP. Antithymocyte globulin treatment in patients with aplastic anemia: a prospective randomized trial. N Engl J Med 1983;308:113-118.
16. Gluckman E, Devergie A, Poros A, Degoulet P. Results of immunosuppression in 170 cases of severe aplastic anaemia: report of the European Group of Bone Marrow Transplant (EGBMT). Br J Haematol 1982;51:541-550.
17. de Planque MM, Bacigalupo A, Wursch A, et al. Long-term follow-up of severe aplastic anaemia patients treated with antithymocyte globulin: Severe Aplastic Anaemia Working Party of the European Cooperative Group for Bone Marrow Transplantation (EBMT). Br J Haematol 1989;73:121-126.
18. Young N, Griffith P, Brittain E, et al. A multicenter trial

Shin SH, et al. Advances in treatment of aplastic anemia

KJIM

of antithymocyte globulin in aplastic anemia and related diseases. Blood 1988;72:1861-1869.

19. Stryckmans PA, Dumont JP, Velu T, Debusscher L. Cyclosporine in refractory severe aplastic anemia. N Engl J Med 1984;310:655-656.

20. Frickhofen N, Kaltwasser JP, Schrezenmeier H, et al. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine: the German Aplastic Anemia Study Group. N Engl J Med 1991;324:1297-1304.

21. Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmeier H; German Aplastic Anemia Study Group. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. Blood 2003;101:1236-1242.

22. Marsh J, Schrezenmeier H, Marin P, 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;93:2191-2195.

23. 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;289:1130-1135.

24. Osugi Y, Yagasaki H, Sako M, et al. Antithymocyte globulin and cyclosporine for treatment of 44 children with hepatitis associated aplastic anemia. Haematologica 2007;92:1687-1690.

25. Scheinberg P, Wu CO, Nunez O, Young NS. Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. Br J Haematol 2009;144:206-216.

26. Maciejewski JP, Follmann D, Nakamura R, et al. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and the PNH/aplastic anemia syndrome. Blood 2001;98:3513-3519.

27. Sugimori C, Chuhjo T, Feng X, et al. Minor population of CD55-CD59- blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia. Blood 2006;107:1308-1314.

28. Kim SY, Lee JW, Lee SE, et al. The characteristics and clinical outcome of adult patients with aplastic anemia and abnormal cytogenetics at diagnosis. Genes Chromosomes Cancer 2010;49:844-850.

29. Saracco P, Quarello P, Iori AP, et al. Cyclosporin A response and dependence in children with acquired aplastic anaemia: a multicentre retrospective study with long-term observation follow-up. Br J Haematol 2008;140:197-205.

30. Risitano AM. Immunosuppressive therapies in the management of immune-mediated marrow failures in adults: where we stand and where we are going. Br J Haematol 2011;152:127-140.

31. Scheinberg P, Cooper JN, Sloand EM, Wu CO, Calado RT, Young NS. Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. JAMA 2010;304:1358-1364.

32. Sakaguchi H, Nishio N, Hama A, et al. Peripheral blood lymphocyte telomere length as a predictor of response to immunosuppressive therapy in childhood aplastic anemia. Haematologica 2014;99:1312-1316.

33. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. N Engl J Med 2011;365:430-438.

34. Atta EH, Dias DS, Marra VL, de Azevedo AM. Comparison between horse and rabbit antithymocyte globulin as first-line treatment for patients with severe aplastic anemia: a single-center retrospective study. Ann Hematol 2010;89:851-859.

35. Afable MG, 2nd, Shaik M, Sugimoto Y, et al. Efficacy of rabbit anti-thymocyte globulin in severe aplastic anemia. Haematologica 2011;96:1269-1275.

36. Shin SH, Yoon JH, Yahng SA, et al. The efficacy of rabbit antithymocyte globulin with cyclosporine in comparison to horse antithymocyte globulin as a first-line treatment in adult patients with severe aplastic anemia: a single-center retrospective study. Ann Hematol 2013;92:817-824.

37. Marsh JC, Bacigalupo A, Schrezenmeier H, et al. Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party. Blood 2012;119:5391-5396.

38. Jeong DC, Chung NG, Cho B, et al. Long-term outcome after immunosuppressive therapy with horse or rabbit antithymocyte globulin and cyclosporine for severe aplastic anemia in children. Haematologica 2014;99:664-671.

39. Scheinberg P, Wu CO, Nunez O, et al. Treatment of

severe aplastic anemia with a combination of horse antithymocyte globulin and cyclosporine, with or without sirolimus: a prospective randomized study. Haematologica 2009;94:348-354.

40. 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;133:606-611.

41. Teramura M, Kimura A, Iwase S, 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;110:1756-1761.

42. Tichelli A, Schrezenmeier H, Socie G, et al. A randomized controlled study in patients with newly diagnosed severe aplastic anemia receiving antithymocyte globulin (ATG), cyclosporine, with or without G-CSF: a study of the SAA Working Party of the European Group for Blood and Marrow Transplantation. Blood 2011;117:4434-4441.

43. Kuter DJ. The biology of thrombopoietin and thrombopoietin receptor agonists. Int J Hematol 2013;98:10-23.

44. Geddis AE. Congenital amegakaryocytic thrombocytopenia. Pediatr Blood Cancer 2011;57:199-203.

45. Olnes MJ, Scheinberg P, Calvo KR, et al. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. N Engl J Med 2012;367:11-19.

46. Desmond R, Townsley DM, Dumitriu B, et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. Blood 2014;123:1818-1825.

47. Kosaka Y, Yagasaki H, Sano K, et al. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. Blood 2008;111:1054-1059.

48. Di Bona E, Rodeghiero F, Bruno B, et al. Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatment for aplastic anaemia patients unresponsive to a first course of intensive immunosuppressive therapy: Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Br J Haematol 1999;107:330-334.

49. Scheinberg P, Nunez O, Young NS. Retreatment with rabbit anti-thymocyte globulin and ciclosporin for patients with relapsed or refractory severe aplastic anaemia. Br J Haematol 2006;133:622-627.

50. Scheinberg P, Townsley D, Dumitriu B, et al. Horse antithymocyte globulin as salvage therapy after rabbit antithymocyte globulin for severe aplastic anemia. Am J Hematol 2014;89:467-469.

51. Risitano AM, Selleri C, Serio B, et al. Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anaemia and single-lineage marrow failure: a pilot study and a survey from the EBMT WPSAA. Br J Haematol 2010;148:791-796.

52. Scheinberg P, Nunez O, Weinstein B, Scheinberg P, Wu CO, Young NS. Activity of alemtuzumab monotherapy in treatment-naive, relapsed, and refractory severe acquired aplastic anemia. Blood 2012;119:345-354.

53. Brodsky RA, Chen AR, Dorr D, et al. High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up. Blood 2010;115:2136-2141.

54. Tisdale JF, Dunn DE, Geller N, et al. High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial. Lancet 2000;356:1554-1559.

55. McCann SR, Bacigalupo A, Gluckman E, et al. Graft rejection and second bone marrow transplants for acquired aplastic anaemia: a report from the Aplastic Anaemia Working Party of the European Bone Marrow Transplant Group. Bone Marrow Transplant 1994;13:233-237.

56. Champlin RE, Horowitz MM, van Bekkum DW, et al. Graft failure following bone marrow transplantation for severe aplastic anemia: risk factors and treatment results. Blood 1989;73:606-613.

57. Gluckman E, Horowitz MM, Champlin RE, et al. Bone marrow transplantation for severe aplastic anemia: influence of conditioning and graft-versus-host disease prophylaxis regimens on outcome. Blood 1992;79:269-275.

58. Deeg HJ, Socie G, Schoch G, et al. Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. Blood 1996;87:386-392.

59. Gale RP, Ho W, Feig S, et al. Prevention of graft rejection following bone marrow transplantation. Blood 1981;57:9-12.

60. Storb R, Etzioni R, Anasetti C, et al. Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with

aplastic anemia. Blood 1994;84:941-949.

61. Champlin RE, Perez WS, Passweg JR, et al. Bone marrow transplantation for severe aplastic anemia: a randomized controlled study of conditioning regimens. Blood 2007;109:4582-4585.

62. Ades L, Mary JY, Robin M, et al. Long-term outcome after bone marrow transplantation for severe aplastic anemia. Blood 2004;103:2490-2497.

63. Kahl C, Leisenring W, Deeg HJ, et al. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long-term follow-up. Br J Haematol 2005;130:747-751.

64. Schrezenmeier H, Passweg JR, Marsh JC, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood 2007;110:1397-1400.

65. Locatelli F, Bruno B, Zecca M, et al. Cyclosporin A and short-term methotrexate versus cyclosporin A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: results of a GITMO/EBMT randomized trial. Blood 2000;96:1690-1697.

66. Gupta V, Eapen M, Brazauskas R, et al. Impact of age on outcomes after bone marrow transplantation for acquired aplastic anemia using HLA-matched sibling donors. Haematologica 2010;95:2119-2125.

67. Maury S, Bacigalupo A, Anderlini P, et al. Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a comparison with conventional conditioning regimen. Haematologica 2009;94:1312-1315.

68. Shin SH, Yoon JH, Yahng SA, et al. Influence of recipient's age on the outcome of HLA-matched sibling transplants in adult patients with severe aplastic anemia who conditioned with f ludarabine-based regimen. Paper presented at: 54th ASH Annual Meeting and Exposition; 2012 Dec 8-11; Atlanta: 1921-1922.

69. Passweg JR, Perez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. Bone Marrow Transplant 2006;37:641-649.

70. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. Bone Marrow Transplant 2005;36:947-950.

71. Bacigalupo A, Socie G, Lanino E, et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA Working Party. Haematologica 2010;95:976-982.

72. Deeg HJ, O'Donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. Blood 2006;108:1485-1491.

73. Maury S, Balere-Appert ML, Chir Z, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica 2007;92:589-596.

74. Viollier R, Socie G, Tichelli A, et al. Recent improvement in outcome of unrelated donor transplantation for aplastic anemia. Bone Marrow Transplant 2008;41:45-50.

75. Kim SY, Lee JW, Lim J, et al. Unrelated donor bone marrow transplants for severe aplastic anemia with conditioning using total body irradiation and cyclophosphamide. Biol Blood Marrow Transplant 2007;13:863-870.

76. Lee JW, Cho BS, Lee SE, et al. The outcome of unrelated hematopoietic stem cell transplants with total body irradiation (800 cGy) and cyclophosphamide (120 mg/kg) in adult patients with acquired severe aplastic anemia. Biol Blood Marrow Transplant 2011;17:101-108.

77. Yakoub-Agha I, Mesnil F, Kuentz M, et al. Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: a prospective study from the French Society of Bone Marrow Transplantation and Cell Therapy. J Clin Oncol 2006;24:5695-5702.

78. Yagasaki H, Kojima S, Yabe H, et al. Tacrolimus/Methotrexate versus cyclosporine/methotrexate as graft-versus-host disease prophylaxis in patients with severe

aplastic anemia who received bone marrow transplantation from unrelated donors: results of matched pair analysis. Biol Blood Marrow Transplant 2009;15:1603-1608.

79. Baldomero H, Gratwohl M, Gratwohl A, et al. The EBMT activity survey 2009: trends over the past 5 years. Bone Marrow Transplant 2011;46:485-501.

80. Lee SE, Yoon JH, Shin SH, et al. In vivo T-cell depletion with low-dose antithymocyte goblulin to reduce acute GVHD in urenlated donor stem cell transplant for patients with severe aplastic anemia. Paper presented at: 18th EHA 2013 Annual Meeting; 2013 Jun 13-16; Stockholm: 380-381.

81. Wang Y, Liu DH, Liu KY, et al. Long-term follow-up of haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for the treatment of leukemia: nine years of experience at a single center. Cancer 2013;119:978-985.

82. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol 2005;23:3447-3454.

83. Wagner JL, Deeg HJ, Seidel K, et al. Bone marrow transplantation for severe aplastic anemia from genotypically HLA-nonidentical relatives: an update of the Seattle experience. Transplantation 1996;61:54-61.

84. Tzeng CH, Chen PM, Fan S, Liu JH, Chiou TJ, Hsieh RK. CY/TBI-800 as a pretransplant regimen for allogeneic bone marrow transplantation for severe aplastic anemia using HLA-haploidentical family donors. Bone Marrow Transplant 1996;18:273-277.

85. Xu LP, Liu KY, Liu DH, et al. A novel protocol for haploidentical hematopoietic SCT without in vitro T-cell depletion in the treatment of severe acquired aplastic anemia. Bone Marrow Transplant 2012;47:1507-1512.

86. Dezern AE, Luznik L, Fuchs EJ, Jones RJ, Brodsky RA. Post-transplantation cyclophosphamide for GVHD prophylaxis in severe aplastic anemia. Bone Marrow Transplant 2011;46:1012-1013.

87. Im HJ, Koh KN, Choi ES, et al. Excellent outcome of haploidentical hematopoietic stem cell transplantation in children and adolescents with acquired severe aplastic anemia. Biol Blood Marrow Transplant 2013;19:754-759.

88. Takatoku M, Uchiyama T, Okamoto S, et al. Retrospective nationwide survey of Japanese patients with transfusion-dependent MDS and aplastic anemia highlights the negative impact of iron overload on morbidity/mortality. Eur J Haematol 2007;78:487-494.

89. Kushner JP, Porter JP, Olivieri NF. Secondary iron overload. Hematology Am Soc Hematol Educ Program 2001;2001:47-61.

90. Porter J, Galanello R, Saglio G, et al. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-yr prospective study. Eur J Haematol 2008;80:168-176.

91. Cappellini MD, Cohen A, Piga A, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood 2006;107:3455-3462.

92. Lee JW, Yoon SS, Shen ZX, et al. Iron chelation therapy with deferasirox in patients with aplastic anemia: a subgroup analysis of 116 patients from the EPIC trial. Blood 2010;116:2448-2454.

93. Lee JW, Yoon SS, Shen ZX, et al. Hematologic responses in patients with aplastic anemia treated with deferasirox: a post hoc analysis from the EPIC study. Haematologica 2013;98:1045-1048.

94. Lee SE, Yahng SA, Cho BS, et al. Improvement in hematopoiesis after iron chelation therapy with deferasirox in patients with aplastic anemia. Acta Haematol 2013;129:72-77.

95. Lee JW, Jang PS, Chung NG, Cho B, Jeong DC, Kim HK. Iron chelation therapy with deferasirox results in recovery of hematopoiesis in a child with aplastic anemia. Pediatr Hematol Oncol 2011;28:718-720.

96. Koh KN, Park M, Kim BE, Im HJ, Seo JJ. Restoration of hematopoiesis after iron chelation therapy with deferasirox in 2 children with severe aplastic anemia. J Pediatr Hematol Oncol 2010;32:611-614.