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The optimal immunosuppressive therapy for aplastic anemia

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Abstract Immunosuppressive treatment (IST) has been the most effective therapeutic modality for patients with aplastic anemia (AA) who are not eligible for allogeneic stem cell transplantation from HLA-matched siblings because of donor unavailability, old age, or comorbidities. The combination of horse anti-thymocyte globulin (ATG) with cyclosporine A (CsA) has shown satisfactory results for these patients, and so it has been regarded as the standard IST regimen. However, treatment failure including unresponsiveness, relapse, and occurrence of clonal evolution remains a major problem, although the results of IST have been improved in the past two decades. Many studies have been conducted to overcome these problems; however, they have yet to show any satisfactory results. This review will discuss immune-mediated pathophysiology of AA, which is associated with therapeutic targets of immunosuppressive agents and clinical outcomes of most commonly used IST regimens. Several trials to improve IST including the addition of other immunosuppressive agents or growth factors to standard IST regimen, comparison between horse ATG/CsA and rabbit ATG/CsA as first-line treatment, and promising alternative agents including alemtuzumab and eltrombopag will also be discussed, focusing on recently published literatures.

Keywords Aplastic anemia · Immunosuppressive treatment · Anti-thymocyte globulin

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Introduction

Aplastic anemia (AA) is a clinical syndrome characterized by fatty replacement of the bone marrow (BM) with a near absence of hematopoietic precursor cells and peripheral blood pancytopenia [1]. In the past, severe AA (SAA) was almost uniformly fatal; nowadays, however, we can treat most patients effectively and expect long-term overall survival (OS) due to the improvement of therapeutic modalities. If available, allogeneic stem cell transplantation (SCT) from HLA-matched sibling donor is a preferred therapeutic option because of its curative nature. However, lack of a matched sibling donor, old age, and comorbidities limit allogeneic SCT in patients with SAA [2].

Immunosuppressive treatment (IST) is most widely used for patients with AA who are not eligible for allogeneic SCT from a matched sibling donor. Patients who show response to IST, especially anti-thymocyte globulin (ATG) and cyclosporine A (CsA), are able to achieve durable recovery from pancytopenia and excellent long-term survival that are not inferior to those of patients who received allogeneic SCT [3]. This review will discuss immune-mediated pathophysiology of AA, which is associated with therapeutic targets of immunosuppressive agents and clinical outcomes of most commonly used IST regimens. Several trials to improve IST including adding other immunosuppressive agent or growth factors to standard IST regimen, comparison between horse ATG/CsA and rabbit ATG/CsA as first-line treatment, and promising alternative agents including alemtuzumab and eltrombopag will also be discussed, focusing on recently published literatures.

Immune-mediated pathophysiology of aplastic anemia

AA is usually an immune-mediated disease, which is supported by the observations that removing lymphocytes

from aplastic BM improves hematopoiesis, whereas adding them to normal BM inhibits hematopoiesis in vitro [4]. The effector cells were identified by immunophenotyping as activated CD8+ CD28− cytotoxic T cells expressing T-helper type 1 inhibitory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) [5]. Addition of antibodies to IFN-γ increased in vitro hematopoietic colony formation of BM cells in affected patients [6]. Long-term BM cultures which were manipulated to produce large amounts of IFN-γ markedly reduced long-term culture-initiating cells, which are surrogate markers of human hematopoietic stem cells [7]. IFN-γ and TNF-α reduced in vitro human hematopoietic progenitor cells, at least in part, through Fas-dependent apoptotic pathway [8]. INF-γ inhibits the transcription of cellular genes which enable to enter into the cell cycle through interferon regulatory factor 1 and induces nitric oxide synthase which produces toxic gas nitric oxide [8, 9]. These events ultimately lead to reduced cell cycling and cell death by apoptosis (Fig. 1). The cause of the activation of CD8+ cytotoxic T cells in patients with AA remains unclear. However, several studies showed higher prevalence of HLA-DR2 in patients with AA, suggesting a role for antigen recognition, nucleotide polymorphisms in genes which encode cytokines including IFN-γ and TNF-α, and constitutive expression of T-bet, a transcriptional regulator that is critical to T-helper type 1 cell polarization [10]. Subsequent investigations explored the oligoclonality of these CD8+ cytotoxic T cells, which showed the expression of T-cell receptor Vβ subfamilies and skewing of CDR3, as a marker of antigen-driven immune response [11–13]. Some groups have explored auto-antibodies against distinct proteins (kinectin, diazepam-binding inhibitor-related protein 1 and moesion) often detectable in patients with AA. However, the pathogenic role of these auto-antibodies should be further investigated, although there are some emerging evidence of their involvement in the pathogenesis of AA [14]. A recent study showed that a substantial proportion of patients with AA harbored clonal hematopoiesis characterized by the presence of acquired copy number-neutral loss of heterozygosity of the 6p arms (6pLOH), which represented escapes hematopoiesis from the autoimmunity. The missing HLA-alleles resulted from 6pLOH were conspicuously biased to particular alleles, including HLA-A*02:01, A*02:06, A*31:01, and B*40:02. This may provide the genetic basis of the high prevalence

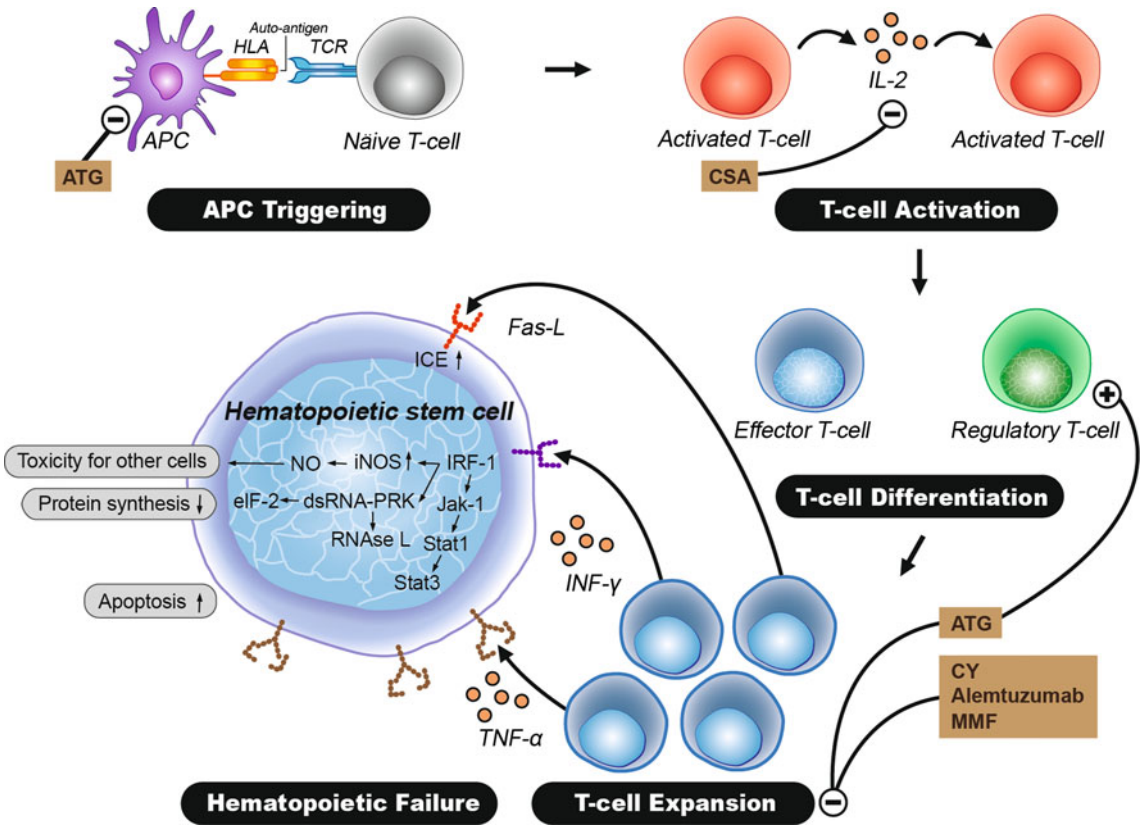

Fig. 1 The immune-mediated pathophysiology of aplastic anemia and associated therapeutic targets of immunosuppressive agents. Reproduced and modified with permission from Young et al. [10]. *ATG* anti-thymocyte globulin, *CY* cyclophosphamide, *MMF* mycophenolate mofetil, *CsA* cyclosporine A

of AA in East Asia [15]. These findings which involve the immune-mediated pathophysiology of AA are associated with therapeutic targets of immunosuppressive agents (Fig. 1). For example, ATG plays the role of depleting T cells through complement-dependent lysis and apoptosis, interference with functional properties of antigen-presenting cells, and induction of regulatory T and natural killer T cells which are profoundly decreased in patients with AA [16–19]. CsA inhibits IL-2 production of activated T cells and prevents expansion of cytotoxic T cells in response to IL-2 [20].

Current standard immunosuppressive regimen; horse ATG with cyclosporine A

Since pioneering trials which showed immunosuppression by horse ATG alone without infusing allogeneic BM as the effective treatment for patients with AA [21, 22], subsequent studies which showed response rates of 30–70 % confirmed that horse ATG was a feasible therapeutic option for patients with AA who are not eligible for allogeneic SCT [23–26]. The combination of horse ATG and CsA was investigated by several studies to improve these results. The long-term follow-up of a randomized trial in Germany comparing the results between patients who received horse ATG/CsA and horse ATG alone reported that the horse ATG/CsA arm showed superior overall response rate (OR) at 4 months (70 versus 41 %, $P = 0.02$) and failure-free survival (39 versus 24 %, $P = 0.04$) at 11 years in comparison to those of horse ATG alone [27]. OS (58 versus 54 %, $P = 0.6$) at 11 years between the two arms was similar because most patients who failed to respond to IST received salvage treatments including repeated IST. Subsequent trials validated horse ATG/CsA as standard IST regimen by showing satisfactory results with a 60–80 % probability of response and 60–90 % OS (Table 1) [28–30].

Predicting factors of response and relapse after immunosuppressive treatment

Although there have been advances in understanding the immune-mediated pathophysiology of AA, the predicting factors of response and relapse in patients who had showed response to IST are not distinctly defined and there are only a few limited studies. US National Institutes of Health (NIH) conducted a retrospective analysis in a large number of patients with SAA who were treated with horse ATG-based IST to determine whether pretreatment blood counts discriminate patients with SAA who have a higher likelihood of response. It was found that younger age, higher baseline absolute reticulocyte count (ARC), and absolute lymphocyte count (ALC) were highly predictive of response at 6 months in multivariate analysis [31]. Patients with baseline ARC $\geq 25 \times 10^9$/l and ALC $\geq 1 \times 10^9$/l had a much higher probability of OR at 6 months following IST compared to those with lower ARC and ALC (83 versus 41 %, $P < 0.001$). Some investigators showed that the patients who had HLA-DR15 or presence of paroxysmal nocturnal hemoglobinuria (PNH) clone, which had been implicated in the immune-mediated pathophysiology of AA, had higher response rate and quality of response to IST than those who did not [32, 33]. A recent retrospective study reported that patients with abnormal cytogenetics at diagnosis showed significantly lower response rate to IST in comparison to that of patients with normal cytogenetics [34].

On the other hand, a prospective study in Italy found that patients with rapid CsA tapering after response showed significantly higher relapse rate in comparison to that of patients with slow CsA tapering (60.0 versus 7.6 %, $P = 0.001$) [35]. This suggests that very slow tapering of CsA by 10 % every 3 months after at least 1 year from IST may reduce relapse in patients who showed response to IST [36]. Late relapse may also be

Table 1 Results of immunosuppressive treatment with horse ATG/CsA in the patients with SAA

Study	IST regimen	*N*	OR (%)	CR (%)	Relapse (%)	CE (%)	OS (%)
German [27][a]	hATG/CsA	43	70 at 4 months*	7 at 4 months	45 at 11 years	25 at 11 years[b]	58 at 11 years
	hATG	41	41 at 4 months*	12.2 at 4 months	30 at 11 years		54 at 11 years
EBMT [28][b]	hATG/CsA	54	74 at 6 months	57 at 6 months	0 at 1 years	4 at 1 years	91 at 3 years
US NIH [29]	hATG/CsA	122	61 at 6 months	NA	35 at 5 years	11 at 5 years	55 at 7 years
Japan [30][c]	hATG/CsA	44	70.4 at 6 months	31.8 at 6 months	0 at 10 years	5 at 10 years	88 at 10 years

OR overall response, *CR* complete response, *CE* clonal evolution, *OS* overall survival, *hATG* horse ATG, *CsA* cyclosporine A, *NA* not available

* Significant difference between horse ATG/CsA group and horse ATG alone group ($P < 0.05$)

[a] Randomized prospective study comparing between horse ATG/CsA and horse ATG

[b] Randomized prospective study comparing between horse ATG/CsA and CsA for patients with non-severe aplastic anemia

[c] Patients with hepatitis-associated aplastic anemia only

attributed to a suboptimal therapeutic range of CsA in long-term responders due to intolerability or poor compliance [14]. In a prospective study conducted by US NIH, short telomere length was not related to response, whereas it was associated with higher relapse rate (hazard ratio = 6.25, $P = 0.01$), clonal evolution (hazard ratio = 3.45, $P = 0.01$) and survival (hazard ratio = 2.86, $P = 0.005$) [37].

Management of patients who have refractory or relapsed aplastic anemia after immunosuppressive treatment

Until recently, there have been only limited data for the management of patients with refractory or relapsed AA after IST with horse ATG/CsA. A multicenter study in Italy for the second course of rabbit ATG/CsA showed OR of 77 %, including 30 % complete response rate (CR), in patients refractory to one course of horse ATG/CsA [38]. US NIH also analyzed the outcomes of rabbit ATG/CsA for patients who were refractory or relapsed after one course of horse ATG/CSA. This study showed OR of 30 % in refractory patients and 65 % in relapsed patients [39]. A third course of ATG-containing IST showed benefit only in previous responders, but not in patients who were refractory to previous IST [40]. On the other hand, cyclophosphamide (CY) or alemtuzumab is used as alternative IST regimen in these patients [41–46].

For patients who received IST as a first-line treatment because of old age or comorbidities, allogeneic SCT from a matched sibling using fludarabine-based reduced intensity conditioning may be an available therapeutic option, as recent studies have reported low treatment-related mortality in it [47, 48]. Allogeneic SCT from an alternative donor may also be a considerable option for patients who do not have a HLA-identical sibling, considering recent improving results [10].

Attempts to improve immunosuppressive treatment by the addition of other immunosuppressants or growth factor

Because 20–40 % of patients with SAA fail to show hematologic response after IST, several attempts to improve outcomes by adding other immunosuppressants including mycophenolate mofetil (MMF) and silrolimus, or granulocyte colony stimulating factor (G-CSF) to standard horse ATG/CsA were conducted (Table 2). A large prospective study carried out by the US NIH reported the results of horse ATG/CsA plus MMF [49]. The OR at 6 months was 62 % (including CR of 16 %) and the cumulative incidence of relapse at 4 years was 37 %, which were not superior in comparison to those of conventional horse ATG/CsA. The US NIH also conducted a prospective randomized study comparing horse ATG/CsA with silrolimus and without silrolimus, which did not show superior OR at 6 months (51 versus 62 %), cumulative incidence of relapse at 3 years (28 versus 25 %, $P = 0.77$), and clonal evolution at 3 years (10 versus 9 %, $P = 0.85$) [50]. A randomized study in Japan reported that adding G-CSF to horse ATG/CsA showed the benefit of superior OR at 6 months (77 versus 57 %, $P = 0.03$) and lower relapse rate at 4 years (15 versus 42 %, $P = 0.01$) in comparison to horse ATG/CsA without G-CSF, although it did not show differences in OS at 4 years (94 versus 88 %, $P = 0.44$) and incidence of infectious episodes (58.8 versus 40.4 %, $P = 0.07$) [51]. Another randomized controlled study of treatment-naïve patients with SAA receiving horse ATG/CsA with or without G-CSF was conducted by the European Blood and Marrow Transplant (EBMT) group [52]. It did not show the significant differences in OS at 6 years (76 versus 77 %, $P = 0.642$), event-free survival at 6 years (46 versus 39 %, $P = 0.343$), and best response rate (73 versus 66 %, $P = 0.535$), whereas patients who received G-CSF showed benefits of lower incidence of infectious episodes within the first

Table 2 Results of the addition of immunosuppressants or G-CSF to horse ATG/CsA

Study	Groups	*N*	OR (%)	CR (%)	Relapse (%)	CE (%)	OS (%)
US NIH [49]	hATG/CsA + MMF	104	62 at 6 months	16 at 6 months	37 at 4 years	9 at 4 years	79 at 4 years
US NIH [50]	hATG/CsA + Sirolimus	35	51 at 6 months	0 at 6 months	28 at 3 years	10 at 3 years	97 at 3 years
	hATG/CsA	42	62 at 6 months	12 at 6 months	25 at 3 years	9 at 3 years	90 at 3 years
Japan [51]	hATG/CsA + G-CSF	51	77 at 6 months*	4 at 6 months	15 at 4 years*	5 at 4 years	94 at 4 years
	hATG/CsA	50	57 at 6 months*	7 at 6 months	42 at 4 years*	3 at 4 years	88 at 4 years
EBMT [52]	hATG/CsA + G-CSF	97	73[a]	12[a]	32 at 6 years	19 at 6 years	76 at 6 years
	hATG/CsA	95	66[a]	9[a]	33 at 6 years	28 at 6 years	77 at 6 years

OR overall response, *CR* complete response, *CE* clonal evolution, *OS* overall survival, *hATG* horse ATG, *CsA* cyclosporine A, *MMF* mycophenolate mofetil, *NA* not available

* Significant difference between horse ATG/CsA + G-CSF group and horse ATg/CsA group ($P < 0.05$)

[a] Best response

90 days (24 versus 36 %, $P = 0.006$) and fewer hospitalization days (82 versus 87 %, $P = 0.0003$) between patients who received G-CSF and who did not. Therefore, current practices of many centers have not included routine G-CSF with IST protocols because of insufficient evidence and controversial results.

The comparison of outcomes between horse ATG/CsA versus rabbit ATG/CsA as a first-line treatment

Rabbit ATG, which is more lymphocytotoxic than horse ATG, has been mainly used in treating the patients who failed in initial IST with horse ATG [38, 39]. Recently, rabbit ATG has been the only ATG formulation available for use in many countries due to difficulties in manufacturing horse ATG. Some investigators compared the efficacy of IST with horse ATG/CsA and rabbit ATG/CsA, but the results were controversial (Table 3). In a large randomized prospective study carried out by the US NIH, OR at 6 months (68 versus 37 %, $P < 0.001$) and OS at 3 years (96 versus 76 %, $P = 0.04$) were significantly superior in the horse ATG/CsA arm compared to those of the rabbit ATG/CsA arm [53]. A retrospective analysis in Brazil also reported that patients who received IST with horse ATG/CsA showed significantly superior OR at 6 months (59.5 versus 34.5 %, $P = 0.05$) and OS at 2 years (78.4 versus 55.4 %, $P = 0.03$) [54]. However, a phase 2 prospective study of the Cleveland Clinic reported that the similar OR at 1 year (58 versus 50 %, $P = 0.61$) and OS (64 versus 65 %, $P = 0.54$) were not significantly different between the two groups [55]. A recent retrospective analysis in Korea showed that OR at 1 year (45.7 versus 49.1 %, $P = 0.735$) and OS at 5 years (83.5 versus 82.7 %, $P = 0.46$) were similar between the two groups [56]. On the other hand, a phase 2 prospective study carried out by the EBMT showed acceptable best response rates in both groups (67 versus 60 %, respectively), but OS at 2 years in the horse ATG/CsA group was significantly higher than that of the rabbit ATG group (86 versus 68 %, $P = 0.009$) [57].

These controversies could be explained by some causes. The preparation and dose of ATG varied according to each study. The more lymphocytotoxic nature of rabbit ATG resulted in more prolonged and protracted lymphocytopenia in patients who received IST with rabbit ATG/CsA in comparison to those who received IST with horse ATG/CsA (Fig. 2) [53, 56]. The EBMT study suggests that the inferior survival in studies that used higher doses of rabbit ATG (3.75 mg/kg/day for 5 days) may be attributed to early mortality due to infectious complications despite similar best response. A phase 3 prospective study which compares the rabbit ATG doses of 2.5 and 3.5 mg/kg/day is now being conducted because the optimal dose of rabbit ATG has yet to be established. The differences in the duration of CsA administration may also have resulted in varying outcomes, although they were not described in most studies. In addition, ethnic factors may have partially contributed to different results because the results of two retrospective studies from Asia showed similar OR and OS between the two groups [58, 59].

Table 3 Studies comparing horse ATG/CsA and rabbit ATG/CsA as a front-line IST in patients with SAA

Study	ATG preparation	*N*	Dose, median (range)	OR (%)	CR (%)	OS (%)
US NIH [53][a]	ATGAM	60	40 mg/kg for 4 days	68* at 6 months	NA	96* at 3 years
	Thymoglobulin	60	3.5 mg/kg for 5 days	37* at 6 months	NA	76* at 3 years
Brazil [54][b]	Lymphoglobuline	42	15 mg/kg (10–21.4) for 5 days	59.5* at 6 months	11.9 at 6 months	78.4* at 2 years
	Thymoglobulin	29	2.5 mg/kg (1.5–3) for 5 days	34.5* at 6 months	6.9 at 6 months	55.4* at 2 years
Cleveland Clinic [55][c]	ATGAM	67	40 mg/kg for 4 days	58 at 1 years	8 at 1 years	64 at 5 years
	Thymoglobulin	20	3.5 mg/kg for 5 days	50 at 1 years	0 at 1 years	65 at 5 years
Korea [56][b]	Lymphoglobuline	46	15 mg/kg for 5 days	45.7 at 1 years	17.4 at 1 years	83.5 at 5 years
	Thymoglobulin	53	2.5 mg/kg for 5 days	49.1 at 1 years	11.3 at 1 years	82.7 at 5 years
EBMT [57][c]	Lymphoglobuline	105	NA	67[d]	44[d]	86* at 2 years
	Thymoglobulin	35	3.75 mg/kg for 5 days	60[d]	23[d]	68* at 2 years

ATG anti-thymocyte globulin, *OR* overall response, *CR* complete response, *OS* overall survival, *NA* not available

* Significant difference between horse ATG group and rabbit ATG group ($P < 0.05$)

[a] Randomized prospective study

[b] Retrospective study

[c] Prospective study comparing with historical control

[d] Best response

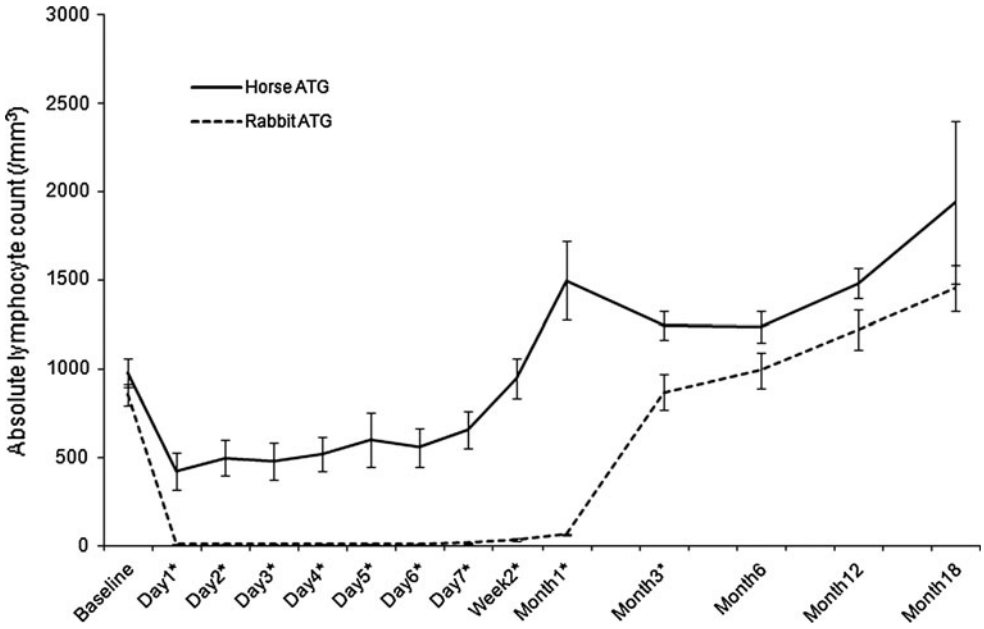

Fig. 2 The kinetics of absolute lymphocyte count between patients who received immunosuppressive treatment with horse ATG and rabbit ATG. Reproduced with permission from Shin et al. [56]. *Significant difference between horse ATG group and rabbit ATG group ($P < 0.05$)

Alternative immunosuppressive regimens

High-dose CY is the most extensively investigated immunosuppressive agent as an alternative IST regimen. A pilot study for a small series of patients who received high-dose CY (45 mg/kg for 4 consecutive days) reported an excellent CR of 70 % and long-term OS of 60 % without relapse or evidence of clonal evolution [45]. After this promising pilot study, a phase 3 prospective trial to compare response rates to IST with either high-dose CY/CsA or ATG/CsA in treatment-naïve patients with SAA was conducted, which is terminated prematurely because of excessive early mortality related to fatal infectious complications followed by delayed neutrophil recovery of the CY/CsA arm [41]. However, a recently published long-term follow-up report of 67 SAA patients (44 treatment-naïve and 23 refractory) who were treated with high-dose CY (50 mg/kg for 4 consecutive days) showed acceptable response rates of 70.5 % (including CR of 43.2 %) and 47.8 % (including CR of 21.7 %), while the actual OS at 10 years was 88 and 61.8 % in treatment-naïve and refractory patients, respectively [46]. Early mortality rate was acceptable (7.5 %), but the cumulative incidence of fungal infections in the early post-treatment period at 2 months was 21 % in treatment-naïve and 39 % in refractory patients.

Alemtuzumab is a humanized monoclonal antibody that specifically kills CD52-bearing cells via both antibody-dependent cellular cytotoxicity and complement-mediated lysis [44]. A pilot study conducted by the EBMT group which analyzed 19 AA patients (6 treatment-naïve and 13 refractory) who were subcutaneously injected with a total of 103 mg alemtuzumab and low dose oral CsA (1 mg/kg) reported a response rate of 58 % (including CR of 26 %) at a median time of 3 months [43]. Infectious complications were not frequent and no cytomegalovirus (CMV) disease except subclinical CMV reactivations and Epstein–Barr virus (EBV)-related diseases was observed. The recently updated data of this study were reported, which showed OS of 74 %, whereas failure-free survival was only 40 % due to refractory relapse rate of 15 % and clonal evolution rate of 15 % [60]. Another dose-escalating pilot study, which analyzed 17 patients with AA including 15 treatment-naïve patients, reported an OR of 35.5 % [61]. In this study, patients who received alemtuzumab of 60 mg with CsA showed an OR of 50.0 %, whereas all patients who received alemtuzumab of 90 mg with CsA failed to achieve response. It suggests that the lower dose of alemtuzumab (60 mg) may be sufficient compared with the higher dose (90 mg). In addition, the US NIH investigated the efficacy of alemtuzumab (test dose of 1 mg followed by 10 mg/kg/day for 10 days) in 88 patients with treatment-naïve, relapsed, and refractory SAA [42]. Patients were tolerable and considerably safe to use alemtuzumab with acceptable infectious events. Subclinical EBV and CMV reactivations were common, whereas no case of EBV or CMV disease was observed and no patient received preemptive therapy. The OR was 37 and 56 %, while OS at 3 years was 83 and 86 % for refractory and relapsed disease, respectively. However, the alemtuzumab in treatment-naïve patients arm was closed early because the response rate for treatment-naïve patients was only 19 % with 3 early deaths. These data suggest that alemtuzumab is more effective in refractory or relapsed patients than treatment-naïve patients.

Eltrombopag in refractory patients with severe aplastic anemia

Eltrombopag is an oral, small molecule, non-peptide thrombopoietin receptor (c-MPL) agonist which promotes megakaryopoiesis and releases platelets from mature megakaryocytes [62]. Hematopoietic stem cells and progenitor cells also had c-MPL on their cell surfaces, and the addition of recombinant thrombopoietin expands the pool of hematopoietic stem cells in in vivo culture [63]. The patients with congenital amegakaryocytic thrombocytopenia who have MPL mutations eventually experienced multilineage marrow failure [64]. These findings suggest that the stimulation of c-MPL signaling pathways improves the depletion of hematopoietic stem and progenitor cells in AA. A phase 2 study for 25 patients with refractory AA was conducted to determine whether eltrombopag can be of benefit to these patients with minimal toxicities [65]. Hematologic responses were achieved in 11 (44.0 %) patients in at least one lineage; 9 (36.0 %) in platelet counts, 6 (24.0 %) in red blood cells counts, and 9 (36.0 %) in neutrophil counts. In addition, the normalization of trilineage hematopoiesis in the BM of responsive patients was observed. These results suggest that further studies are required to determine whether the addition of eltrombopag to first-line IST improves response rate and survival.

Conclusions

Immunosuppression, especially with horse ATG/CsA, has been the most effective therapeutic modality for patients with AA who are not eligible for allogeneic SCT, showing satisfactory OR of 60–80 % and OS of 60–90 %. However, treatment failures such as unresponsiveness, relapse, and clonal evolution remain a major problem, although the results of IST have been improved in the past two decades. Unfortunately, many studies which conducted intensified IST by adding other immunosuppressants failed to show noteworthy results to improve response and survival. However, the addition of G-CSF may be beneficial in some selected patients, although it is not recommended routinely. The results of salvage IST for refractory or relapsed patients are limited and unsatisfactory. Therefore, further efforts are required to improve their outcomes, including adopting novel alternative agents. Rabbit ATG is currently the only available formulation due to manufacturing difficulties of horse ATG in many countries. However, the results of IST with rabbit ATG/CsA as a first-line treatment remain controversial when compared with those of horse ATG/CsA. One prospective study showed superior results of horse ATG/CsA, whereas other studies reported similar results. These controversies could be attributed to some reasonable factors, including the preparation and dose of rabbit ATG which evokes more profound and protracted lymphocytopenia compared to horse ATG, differences in the duration of CsA maintenance between studies, and ethnic factors. Although other alternative agents such as CY, alemtuzumab, and eltrombopag have shown potential activities, further studies are needed for appropriate clinical applications. In conclusion, further studies to improve initial response rate and reduce relapse rate, and to develop effective therapeutic modalities for refractory or relapsed patients are required.

Conflict of interest The authors declare that they have no conflict of interest.

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