Biol Blood Marrow Transplant 21 (2015) 959–970

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Ask the Expert

Rabbit Anti–T Cell Globulin in Allogeneic Hematopoietic Cell Transplantation

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Article history:
Received 20 September 2014
Accepted 14 November 2014

Key Words:
Antithymocyte globulin
Graft-versus-host disease
Hematopoietic cell transplantation

A B S T R A C T

Anti–T cell globulin (ATG) is polyclonal IgG from rabbits immunized with human thymocytes or a human T cell line. Prophylaxis using ATG infused with conditioning for adult marrow or blood stem cell transplantation reduces both acute and chronic graft-versus-host disease (GVHD). However, ATG is not or minimally efficacious in steroid refractory GVHD treatment. Regarding preemptive therapy, ATG is promising; however, further work is needed on establishing adequate biomarkers to be used as triggers for preemptive therapy before it can be used routinely. Relapse is not increased by ATG, except possibly in the setting of reduced-intensity conditioning. Infections are probably increased when using high but not low-dose ATG, except for Epstein-Barr virus–driven post-transplantation lymphoproliferative disorder, which may be increased even with low-dose ATG. Survival is not improved with ATG; however, survival free of immunosuppressive therapy is improved. Pharmacokinetics of ATG are highly variable, resulting in highly variable areas under the time-concentration curves. Optimized dosing of ATG might improve transplantation outcomes. In conclusion, ATG reduces GVHD and, thus, may improve quality of life, without compromising survival.

INTRODUCTION

Clinically significant graft-versus-host disease (GVHD), ie, grade 2 to 4 acute GVHD (aGVHD) or extensive or moderate/severe chronic GVHD (cGVHD), occurs in 40% to 90% of recipients of T cell–replete allogeneic hematopoietic cell transplantation (HCT) (for cGVHD, the up to 90% pertains to patients surviving 1 year). It leads to morbidity, mortality, and poor quality of life. Unfortunately, prophylaxis of GVHD with small molecule immunosuppressive drugs or with pure ex vivo T cell depletion (without in vivo T cell depletion) has been associated with increased relapse and infections [1-5]. Anti–T cell globulin (ATG) is promising as GVHD prophylaxis that may not result in increased relapse or fatal infections in adults undergoing bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT). This is less clear in the setting of pediatric BMT or PBSCT and adult and pediatric cord blood transplantation (CBT). Thus, here we review the use of ATG first in the setting of adult BMT/PBSCT and then in the setting of pediatric BMT/PBSCT and CBT. We also review the impact of ATG and ATG pharmacokinetics (PK) on immune reconstitution and its possible association with susceptibility to infections and relapse.

The name *anti–T cell globulin* is imprecise because ATG contains antibodies expressed not only on T cells but also other cells, and it does not contain total serum globulin but only IgG. A precise name would be ***A**nti–**T** cell and other cell Ig**G***.

ATG FORMULATIONS

As shown in Table 1, ATG is manufactured by immunizing animals with human thymocytes (ATGAM [Pfizer, New York, NY] and Thymoglobulin [Sanofi, Paris, France]) or Jurkat T lymphoblastoid cells (ATG-F [Neovii Biotech, Waltham, MA]) and subsequently extracting IgG from the sera of the immunized animals. Rabbits are used for the production of Thymoglobulin and ATG-F, whereas horses are used for the production of ATGAM. The rabbit products cause more profound and longer lymphocytopenia than the horse product, despite the horse product being given at a higher dose [6]. Interestingly, horse ATG appears to be more efficacious than rabbit ATG when treating aplastic anemia [6], though not all studies confirm this [7]. For prophylaxis of GVHD, rabbit ATG is efficacious whereas horse ATG is not efficacious [8-10]. In

Financial disclosure: See Acknowledgments on page 967.

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http://dx.doi.org/10.1016/j.bbmt.2014.11.676
1083-8791/

Table 1
ATG Formulations

	ATGAM (Pfizer)	Thymoglobulin* (Sanofi)	ATG-F* (Fresenius/Neovii)
Animal immunized	Horse	Rabbit	Rabbit
Human cells for the immunization of the animal	Thymocytes	Thymocytes	Jurkat cells (T lymphoblastoid cell line)
Lymphodepletion in vivo	±	+	+

* The immunized rabbits are pathogen free, the thymocytes (obtained from pediatric donors undergoing cardiac surgery in case of Thymoglobulin) are screened for known viruses, and the IgG from the immunized rabbits is pasteurized, ensuring safety. The rabbit IgG is exposed to human erythrocytes that adsorb antibodies against antigens on their surface. In case of ATG-F, the rabbit IgG is also adsorbed on human placental cells.

the randomized study of horse ATG prophylaxis in patients with aplastic anemia, the incidence of cGVHD was even higher in the horse ATG arm compared with the no ATG arm, though this was not statistically significant [8]. Thus, only rabbit ATG is reviewed further.

Thymoglobulin contains antibodies against multiple antigens, including CD1a, CD2, CD3/T cell receptor, CD4, CD5, CD6, CD7, CD8, CD11a/CD18 (LFA1), CD11b, CD16, CD19, CD20, CD25, CD28, CD29, CD30, CD32, CD38, CD40, CD44, CD45, CD49, CD50 (ICAM3), CD54 (ICAM1), CD56, CD58, CD61, CD81, CD82, CD95, CD98, CD99, CD102 (ICAM2), CD126, CD138, CD147, CD152 (CTLA4), CD184 (CXCR4),CD195 (CCR5), CD197 (CCR7), HLA class I, beta-2-microglobulin, and HLA class II [11,12]. Antigens targeted by ATG-F have not been studied as extensively as for Thymoglobulin; however, it is likely that the number of antigens targeted by ATG-F may be lower than that of Thymoglobulin (eg, CD4 and HLA-DR antibodies are lacking in ATG-F [12]). This may be because (1) Jurkat cells are relatively homogeneous whereas thymocytes are heterogenic (include T cell precursors, T cells, dendritic cells, B cells, plasma cells, macrophages, and stromal/epithelial cells), and (2) because during the production of ATG-F (but not Thymoblobulin), the rabbit IgG is adsorbed on human placental cells. Compared with Thymoglobulin, a higher concentration of ATG-F is needed to achieve the same degree of complement mediated lysis [13-15]. Perhaps this is the reason why a higher dose of ATG-F appears to be needed to achieve a similar degree of GVHD reduction. ATG-F for GVHD prophylaxis has been administered in recent studies at a dose of 15 to 60 mg/kg, whereas Thymoglobulin is administered at 2.5 to 10 mg/kg. The European Blood and Marrow Transplant Group recommends, based on consensus opinion, 30 mg/kg ATG-F or 7.5 mg/kg Thymoglobulin, divided into 3 doses administered on days -3, -2, and -1 (for 8/8 HLA allele-matched unrelated donor transplantation) [16]. Further work is needed to establish the optimal dosing. See the Pharmacokinetics section (below) for our opinion on the dosing.

PROPHYLAXIS VERSUS THERAPY OF GVHD WITH ATG

Prophylactic ATG is typically administered during conditioning. Because of its relatively long half-life (3 days to 6 weeks), ATG can persist in the HCT recipient for weeks to months, suppressing or killing T cells infused with the graft. This is thought to be the primary mechanism of reduced incidence of GVHD as reviewed below.

In contrast to the efficacy of ATG for GVHD prophylaxis, treatment of established GVHD with ATG has produced disappointing results [17,18]. However, this has been studied only for steroid refractory GVHD.

In the upcoming paragraphs, we will first review ATG use for GVHD prophylaxis (ATG in conditioning) and later ATG use for preemptive therapy (post-transplantation administration of ATG to patients at high risk of developing GVHD per early post-transplantation biomarkers).

GVHD Reduction by ATG in Conditioning for Adult BMT/PBSCT

The impact of ATG on GVHD has been studied in 5 randomized studies, multiple nonrandomized studies, and several studies comparing the GVHD incidence between patients with high versus low ATG serum levels (who were treated with a uniform dose of ATG) (Table 2). In all of the randomized studies and most of the nonrandomized studies, aGVHD and/or cGVHD incidence was reduced. Overall, the impact of ATG appears to be greater on cGVHD than aGVHD (Table 2). This is expected to lead to improved quality of life. This has been so far documented in 2 randomized studies and 1 nonrandomized study [19-21]. The anti-GVHD effect of ATG may be less pronounced in the setting of BMT compared with PBSCT [22].

The mechanism (how ATG reduces GVHD) is probably multifactorial, as ATG is polyclonal. ATG includes IgG specificities against antigens expressed on T cells, B cells, natural killer cells, granulocytes, monocyte/macrophages, dendritic cells, endothelial cells and nonhematolymphatic cells, all of which have been implicated in the pathogenesis of GVHD. Leading hypotheses are that ATG kills alloreactive T cells by inducing their apoptosis or complement lysis, interferes with alloreactive T cell traffic (eg, exit from blood to epithelial tissues) or function (eg, activation due to disruption of T cell antigen-presenting cell synapse, proliferation, cytokine production, cytotoxicity), or stimulates development of regulatory T cells [23-26]. Interestingly, a low ATG concentration may stimulate, whereas a high ATG concentration may inhibit, a mixed lymphocyte reaction [27]. Another hypothesis for the anti-GVHD effect is that ATG kills dendritic cells (that present alloantigens) via apoptosis or complement lysis [15,28,29], interferes with their maturation, or stimulates development of tolerogenic dendritic cells [30]. Among all immune cells, ATG has the highest affinity for naïve T cells [31], which are enriched for alloreactive T cells [32]. As ATG administration results in severe naïve T lymphocytopenia (Figure 1) [33-35], we hypothesize that many naïve T cells infused with the graft, including alloreactive T cells, are killed by ATG.

Relapse and ATG in Conditioning for Adult BMT/PBSCT

The impact of ATG on relapse appears to depend on the intensity of conditioning. In 19 of 19 studies on myeloablative conditioning transplantations or combined myeloablative and reduced-intensity conditioning (RIC) transplantations, including the 5 randomized studies, ATG prophylaxis was not associated with increased relapse. In contrast, in 4 of 6 studies on exclusively RIC transplantations, ATG prophylaxis was associated with increased relapse (Table 2). Use of ATG with very low intensity conditioning (eg, 2 Gy total body irradiation only) has not been reported.

The reason why ATG does not increase relapse (after myeloablative HCT) is not known. At least 2 hypotheses exist: (1) ATG selectively interferes with GVHD but not graft-versus-leukemia, and (2) ATG has a direct antileukemic effect,

Table 2
Impact of ATG on GVHD and Relapse after Adult BMT/PBSCT*

	Dose (mg/kg)	Controls	Acute GVHD	Chronic GVHD†	Relapse	Survival	Conditioning Intensity‡	Comment
Randomized studies:								
Bacigalupo [20] [64]	7.5-15 T	No ATG	↓§	↓	↔	↔	MA	↑ quality of life among ≥4 year survivors
Finke [49] [94]	60 F	No ATG	↓‖	↓	↔	↔	MA	cGVHD > aGVHD
Wang [46]	10 T	6 T	↓	↓?¶	↔	↔	MA	Haplo-identical donors
Bonifazi [60]	30 F	No ATG	↔	↓	↔	↔	MA	HLA matched sibs
Walker [21]	4.5 T	No ATG	↓	↓	↔	↔	MA	↑ quality of life
Nonrandomized studies:								
Zander [41]	≥40 F	No ATG	↓	↓	↔	↔	MA	
Shattenberg [42]	8-16 T	No ATG	↓	↔	↔	↔	MA	
Remberger [47]	6-10 T	4 T	↓	↔	↔	↔	MA	
Basara [95]	5-15 T, 45-60 F	No ATG	↔	↓	↔	↔	MA	
Russell [63]	4.5 T	No ATG	↔	↓	↑?#	↑	MA	
Mohty [96]	Varied	No ATG	↔	↓	↔	↔	MA	
Milano [52]	4.5-6 T	No ATG	↓	↓	Not given	↔	MA	
Soifer [61]	Varied	No ATG	↔	↓	↑	↓	RIC	
Yu [19]	16 F	No ATG	↓	↓	↔	↔	MA	↑ quality of life
Baron [97]	Varied	No ATG	↔	↓	↑	↔	RIC	
Bonifazi [98]	15-30 F	No ATG	↔	↓	↔	↔	MA	
Crocchiolo [43]	5 T	2.5 T	↓	↓	↔	↔	RIC	
Remberger [44]	8 T	6 T	↔	↔	↑	↔	RIC	
Wolschke [45]	Median 30 F	No ATG	↓	↓	↔	↔	MA, RIC	
Dulery [99]	Median 5 T	No ATG	↓	↓?**	↔	↔	MA, RIC	
Baron [100]	Varied	No ATG	↔	↓	↔	↔	RIC	
Devillier [62]	Median 7.5 T	Median 5 T	↔	↔	↑	↓	RIC	HLA-matched sibs
Studies of ATG levels:								
Remberger [101]	4-8 mg/kg T						MA, RIC	
	Day 0 level >70 mg/L	Day 0 level ≤70 mg/L	↓	↔	↔	Not given		
Podgorny [50]	4.5 mg/kg T						MA	
	Day 7 level >~1 mg/L	Day 7 level ≤~1 mg/L	↓	↓	↔	↔		
	Day 28 level >~0.04 mg/L	Day 28 level ≤~0.04 mg/L	↓	↓	↔	↔		
Chawla [102]	4.5 mg/kg T						MA	
	Day 0 level >~8 mg/L	Day 0 level ≤~8 mg/L	↔	↓	↔	↔		
	Day 7 level >~1.3 mg/L	Day 7 level ≤~1.3 mg/L	↓	↓	↔	↔		
	Day 28 level >~0.1 mg/L	Day 28 level ≤~0.1 mg/L	↓	↓	↔	↔		

T indicates Thymoglobulin; F, ATG-F; MA, myeloablative; RIC, reduced intensity conditioning.

* Table contains only studies in which the difference in the incidence of GVHD or relapse was unequivocally attributed to ATG (and not due to confounding factors).

† In studies in which the incidence of both any cGVHD and extensive cGVHD were compared between the ATG-treated and no (or low dose) ATG-treated, only the results of the comparison of extensive cGVHD is indicated here.

‡ Conditioning intensity is categorized as MA, RIC, or nonmyeloablative (NMA) according to Bacigalupo et al. [103]. The category listed here is that pertaining to the majority of patients in the study.

§ aGVHD reduced with 15 but not 7.5 mg/kg.

‖ cGVHD > aGVHD denotes greater reduction of cGVHD than aGVHD. In the randomized study of Finke/Socie et al. [49,94], aGVHD grade 2 to 4 was reduced 1.5-fold, aGVHD grade 3 to 4 was reduced 2.1-fold, and extensive cGVHD was reduced 3.7-fold.

¶ Difference between patients receiving 10 versus 6 mg ATG was significant only for any cGVHD, not for moderate to severe cGVHD.

P value for difference in relapse incidence was .05.

** *P* value for difference in extensive cGHVD incidence was .057.

because it contains antibodies against antigens commonly expressed by both thymocytes/Jurkat cells and leukemic cells. We favor the latter hypothesis because, although there are no experimental data supporting the former hypothesis, ATG has been shown to induce apoptosis and complement lysis of leukemic cell lines and primary leukemic cells (acute leukemia blasts, chronic lymphocytic leukemia cells) in vitro [36-40].

Infections and ATG in Conditioning for Adult BMT/PBSCT

Total infections

Studies evaluating the impact of ATG on infections are listed in Table 3. In most of them, the authors compared the percent of patients who died of an infection among ATG versus no/low-dose–ATG treated patients. In 6 of 8 such studies, no difference was found, suggesting that ATG does not lead to increased infectious mortality [41-46]. However, in the study of Bacigalupo, higher mortality because of infections (primarily bacterial) was found when using 15 mg/kg but not 7.5 mg/kg Thymoglobulin (compared with no ATG) [20], suggesting that ATG can lead to infectious mortality if used at a high dose. Consistent with that, Remberger found a trend toward increased infectious mortality with 10 mg/kg compared with 4 to 8 mg/kg Thymoglobulin [47]. This is also consistent with Hamadani's study evaluating the percentage of patients with ≥1 infection (fatal and nonfatal) among those treated with 7.5 mg/kg versus 6.0 mg/kg Thymoglobulin—the percentage was significantly higher in the 7.5 mg/kg group [48]. Apart from the study of Hamadani, there are 2 studies evaluating whether ATG leads to increased infections in general (fatal and nonfatal) (Finke

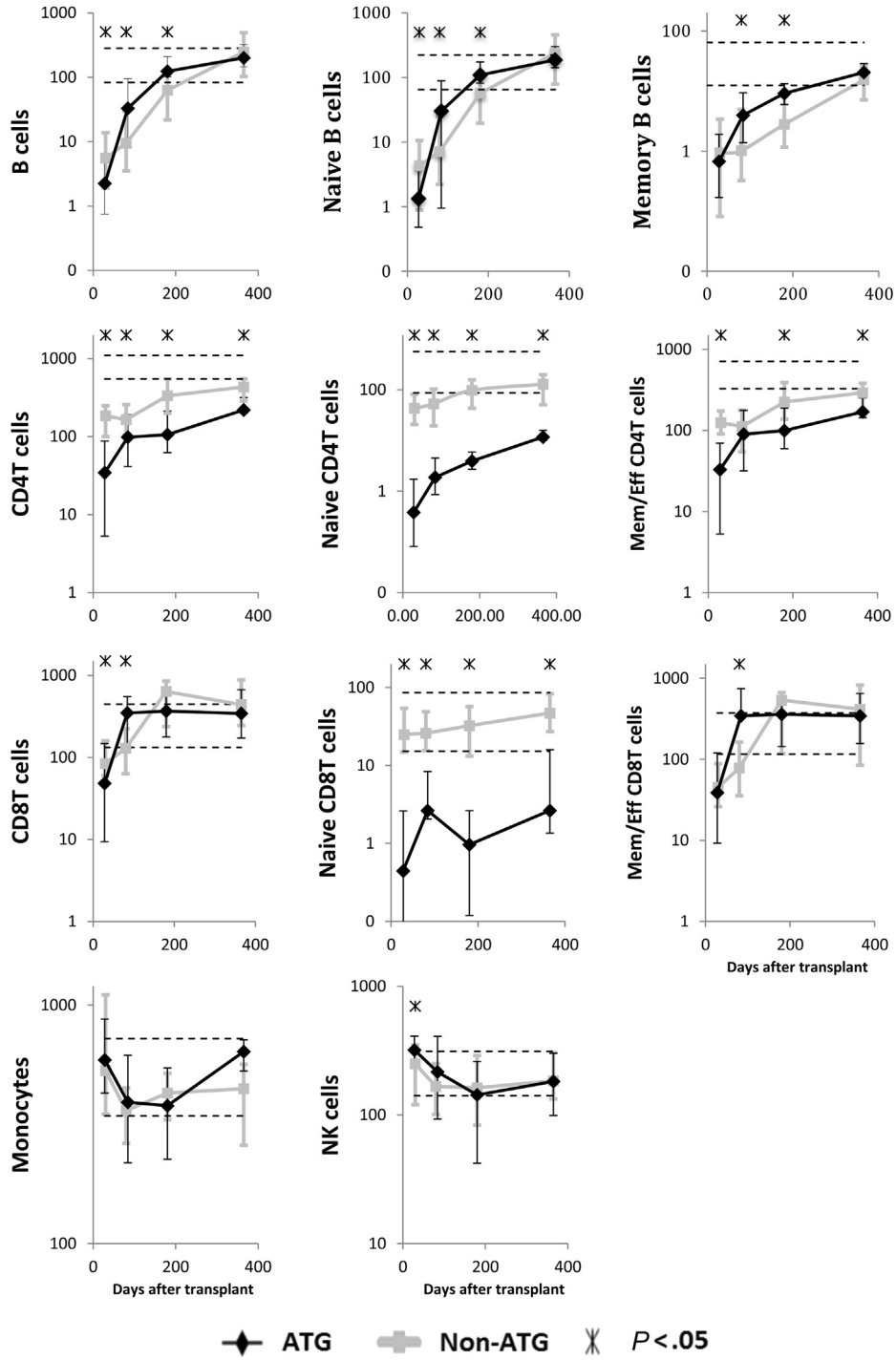

Figure 1. Median immune cell subset counts in recipients of blood stem cells conditioned with ATG (black diamonds) versus without ATG (gray squares). The time points displayed are 1, 3, 6 and 12 months after transplantation. Error bars indicate the 25th to 75th percentiles. Stars indicate a significant difference ($P < .05$) between ATG-conditioned and non–ATG-conditioned patients. Normal values are shown as horizontal dashed lines (10th and 90th percentiles). Days after transplantation are shown on all x-axes. On all y-axes, values are cells per microliter blood. Mem/Eff indicates memory/effector. From Bosch et al. [33], with permission.

et al. [49], and Podgorny et al. [50]/Hoegh-Petersen et al. [51]). The strength of these 2 studies is that they evaluated the impact of ATG on not only the percentage of patients with ≥1 infection, but also on the infection rate (density over a time period), which is more sensitive and more clinically relevant. In Finke's study, no difference in either the percentage of patients with ≥1 infection or the infection rate was found between ATG versus non–ATG-treated patients. This was true for any infection (due to any microorganism) as well as viral, bacterial, and fungal infections. Similarly, in Podgorny/Hoegh-Petersen's study, no difference between patients with high versus low ATG levels on day 7 was found in any, bacterial, and fungal infections when using both the percentage of patients with ≥1 infection method and the infection rate method. However, a small but statistically significant difference was found in viral infections when

Table 3
Impact of ATG on Infections after Adult BMT/PBSCT

	Dose (mg/kg)	Controls	What Was Compared	ATG Impact on Infections	Comment
Randomized studies:					
Bacigalupo [20]	7.5-15 T	No ATG	% Patients with a fatal infection	↔ (7.5), ↑ (15)	
Finke [49] [94]	60 F	No ATG	% Patients with ≥1 any, viral, bacterial, or fungal infection	↔ any, ↔ viral, ↔ bacterial, ↔ fungal	Trend toward ↑ % patients with PTLD
Wang [46]	10 T	6 T	% Patients with a fatal any infection, or a fatal viral infection	↔ any, ↑ viral	↑ % patients with PTLD
Nonrandomized studies:					
Zander [41]	≥40 F	No ATG	% Patients with a fatal infection	↔	
Shattenberg [42]	8-16 T	No ATG	% Patients with a fatal infection	↔	
Remberger [47]	10 T	4-8 T	% Patients with a fatal infection	↑? ($P = .09$)	Trend toward ↑ % patients with ≥1 HSV, VZV and CMV disease
Hamadani [48]	7.5 T	6 T	% Patients with ≥1 any, viral or bacterial infection, % patients with ≥1 CMV reactivation (>4,000 copies/mL)	↑ any, ↑? viral, ↑ bacterial, ↑ CMV reactivation	
Soifer [61]	Varied	No ATG	% Patients with PTLD	↑	
Yu [19]	16 F	No ATG	% Patients with ≥1 opportunistic infection	↑	
Crocchiolo [43]	5 T	2.5 T	% Patients with a fatal infection	↔	
Remberger [44]	8 T	6 T	% Patients with a fatal infection	↔	
Wolschke [45]	median 30 F	No ATG	% Patients with a fatal infection	↔	Trend toward ↑ % patients with PTLD
Studies of ATG levels:					
Podgorny [50] and Hoegh-Petersen [51].*	Day 7 level > ~1.4 mg/L	Day 7 level ≤ ~1.4 mg/L	1. % Patients with ≥1 any, viral, bacterial or fungal infection;	1. ↔ any, ↔ viral, ↔ bacterial, ↔ fungal	
			2. Rates of any, viral, bacterial or fungal infection;	2. ↔ any, ↑ viral, ↔ bacterial, ↔ fungal	
			3. % Patients with PTLD	3. ↑ PTLD	
	Day 28 level > ~0.08 mg/L	Day 28 level ≤ ~0.08 mg/L		1. ↔ any, ↔ viral, ↔ bacterial, ↔ fungal 2. ↔ any, ↔ viral, ↔ bacterial, ↔ fungal 3. ↑ PTLD	
Chawla [102].*	Day 0 level > ~8 mg/L Day 7 level > ~1.9 mg/L Day 28 level > ~0.1 mg/L	Day 0 level ≤ ~8 mg/L Day 7 level ≤ ~1.9 mg/L Day 28 level ≤ ~0.1 mg/L	% Patients with CMV reactivation (>25,000 IU/mL plasma), % Patients with PTLD	↔ CMV, ↔ PTLD ↔ CMV, ↑ PTLD ↔ CMV, ↑ PTLD	

* All patients received 4.5 mg/kg Thymoglobulin.

using the rate method but not when using the percentage of patients with ≥1 infection method.

Viral infections

Consistent with the increase in viral infection rate in Hoegh-Petersen's study [51], there was a trend toward increased percentage of patients with ≥1 herpes simplex virus, varicella zoster virus, or cytomegalovirus (CMV) infection among patients treated with 10 versus 4 to 8 mg/kg Thymoglobulin in Remberger's study [47], an increased percentage of patients who died because of a viral infection among those treated with 10 versus 6 mg/kg Thymoglobulin in Wang's study (despite death due to any infection not being different) [46], and an increased percentage of patients with lower respiratory tract viral infection (that did not increase transplantation-related mortality) in patients treated with 4.5 to 6.0 mg/kg versus no Thymoglobulin in Milano's study [52]. The cumulative incidence of Epstein-Barr virus (EBV)–induced post-transplantation lymphoproliferative disorder (PTLD) has been conspicuously increased with ATG in some studies (Table 3). Fortunately, fatal PTLD is rare when using rituximab or EBV-specific T cells prophylactically, preemptively (when EBV DNAemia has exceeded a threshold), or promptly (with early signs of PTLD) [53,54].

Collectively, at low doses (<8 mg/kg), ATG appears not to increase total infections or fatal infections. Viral infections, particularly PTLD, appear to be increased. With higher ATG doses, mortality due to infections may be substantially increased.

The mechanism (why low-dose ATG has no impact on the incidence of infections other than viral infections/PTLD) is not known. We offer the following 3 hypothetical

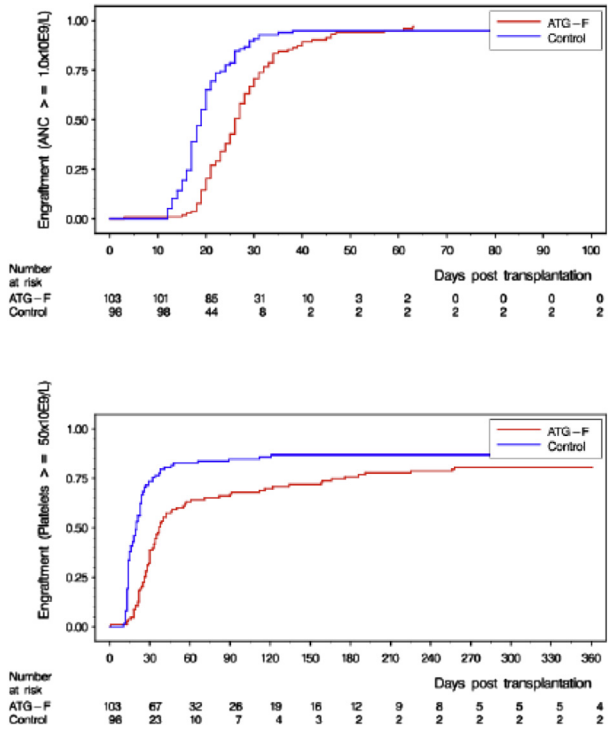

Figure 2. Engraftment kinetics in patients randomized to treatment with ATG-F (60 mg/kg) versus no ATG (control). ANC indicates absolute neutrophil count. From Finke et al. [49], with permission.

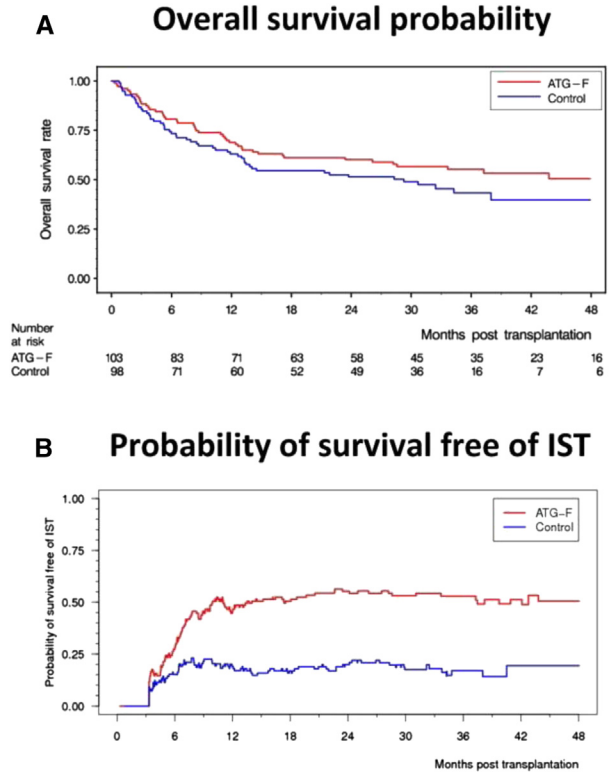

Figure 3. Survival and survival free of immunosuppressive therapy (IST) in patients randomized to ATG-F in conditioning versus no ATG. Overall survival (A) was not significantly different between the 2 groups ($P = .39$). For survival free of IST (B), significance of difference was not given but was probably high as the hazard ratio for receiving IST was .31 ($P < .0001$). From Socie et al. [94], with permission.

explanations: (1) GVHD (or its treatment) is a major risk factor for infections, so with lower incidence of GVHD due to ATG, fewer GVHD-associated infections are expected; (2) ATG kills primarily naïve T cells, whereas memory/effector T cells, enriched for T cells against common pathogens, are relatively spared [31,33-35]; and (3) ATG improves the reconstitution of natural killer cells, B cells, and CD8 T cells (Figure 1) [33].

Engraftment and ATG in Conditioning for Adult BMT/PBSCT

Transient neutropenia and thrombocytopenia have been reported after administering ATG to recipients of solid organ grafts [55-59]. For HCT recipients, data on whether ATG delays engraftment or increases the likelihood of graft failure has been inconsistent. In the randomized studies of ATG-F versus no ATG [49,60], median time to neutrophil engraftment was delayed by 3 to 7 days and median time to platelet engraftment was delayed by 7 to 14 days, and there was a trend toward increased incidence of platelet (but not neutrophil) nonengraftment (Figure 2). In the published articles on the randomized studies of Thymoglobulin [20,46], there was no impact on the median time to neutrophil engraftment or the incidence of neutrophil nonengraftment. However, delayed platelet engraftment was noted in the Bacigalupo study (significant with the dose of 15 but not 7.5 mg/kg) [20] and a trend toward increased incidence of platelet nonengraftment by day 100 was noted in the Wang study [46]. In the nonrandomized studies listed in Table 2, ATG was reported to have no effect on the speed of engraftment or the incidence of nonengraftment in all but 2 studies. The 2 exceptions arrived at different conclusions: Soiffer's study found decreased day 60 platelet engraftment from 92% in the non-ATG group to 88% in the Thymoglobulin group [61], whereas Zander's study found faster engraftment of leukocytes (neutrophils not reported) in the ATG-F group [41]. Our (Albertan) unpublished data on 295 adult BMT/PBSCT recipients who received Thymoglobulin 4.5 mg/kg with conditioning and in whom we correlated day 7 ATG levels [50] with time to neutrophil engraftment suggest no negative impact of Thymoglobulin on neutrophil engraftment. On the contrary, we observed a trend toward earlier neutrophil engraftment with higher Thymoglobulin levels (Spearman rank correlation coefficient r = -.10, $P = .10$). Collectively, in spite of some controversy, based on the randomized studies, it is likely that ATG-F has a negative effect on both neutrophil and platelet engraftment and Thymoglobulin on platelet engraftment.

Survival and ATG in Conditioning for Adult BMT/PBSCT

No impact of ATG on overall survival has been demonstrated in the 5 randomized studies and all but 3 of the nonrandomized studies listed in Table 2. The 3 exception studies arrived at different conclusions: In Soiffer's and Devillier's studies, which used RIC, the survival after ATG versus no or lower dose ATG was decreased, which was attributed to increased incidence of relapse [61,62]. In Russell's study, which used myeloablative conditioning, survival was increased, which was attributed to decreased incidence of nonrelapse death [63]. Whereas ATG appears not to improve overall survival, it appears to improve survival free of immunosuppressive therapy for cGVHD (Figure 3) and, by extrapolation, survival with good quality of life.

The reason overall survival may not be improved (despite the reduction of GVHD without significantly impacting relapse or fatal infections) is not known. As ATG appears to reduce primarily cGVHD and deaths due to cGVHD and/or its treatment can occur many years after transplantation, perhaps improved survival will have become apparent with longer follow-up. In all but 2 of the studies listed in Table 2, the median follow-up was <5 years or not given. The 2 studies with >5-year median follow-up were the Russell study, which showed a marginal improvement of survival with 4.5 mg/kg Thymoglobulin versus no ATG [63] and the Bacigalupo study, which showed no difference in survival [20,64]. In the latter study, there was a trend toward improved survival among ATG versus non–ATG-treated patients who survived 1 year after transplantation ($P = .09$) [64], consistent with the notion that survival benefit might become apparent only after many years of follow-up, as cGvHD is associated with late mortality.

Anomalies of ATG-conditioned Adult BMT/PBSCT

What the transplantation community has learned since the 1970s using patients conditioned without ATG may not always apply to ATG-conditioned patients. One example is the apparently high incidence of PTLD with ATG (Table 3). Here, we provide additional 2 examples of differences between transplantations with versus without ATG: (1) impact of donor CMV serostatus on survival of CMV-seropositive recipients and (2) risk factors for cGVHD.

D-R+ (donor CMV seronegative and recipient CMV seropositive) patients had similar survival compared with D+R+ patients in multiple studies in which most or all patients were conditioned without ATG [65]. Among patients conditioned with ATG, D-R+ patients in our study had lower survival compared to D+R+ patients (42% versus 56% at ~2 years), due to difference in nonrelapse mortality [66]. This is consistent with 3 other studies on ATG-conditioned patients [67-69]. The reason for the discrepancy between non-ATG and ATG-conditioned patients is not known. Perhaps the T cell–replete grafts from seronegative donors contain enough naïve CMV-specific T cells that, in the absence of ATG, can differentiate after transplantation into memory/effector cells and protect the recipient against CMV complications, whereas in the presence of ATG, the naïve CMV-specific T cells from the graft are killed or inhibited from differentiating into the memory/effector cells. Also, ATG could kill or inhibit recipient CMV-specific T cells surviving the conditioning chemo/radiotherapy, which in the absence of ATG, prevent CMV complications [70]. These 2 hypotheses are consistent with the fact that after ATG conditioning, D-R+ patients have fewer CMV-specific T cells and more CMV reactivations and CMV diseases than D+R+ patients [66].

Recognized risk factors for cGVHD after T cell–replete transplantation include HLA-mismatched or unrelated donor, older patient, older donor, female donor for male recipient, and blood stem cell graft [71]. In an Australian and Albertan study of 356 ATG-conditioned BMT/PBCST recipients, none of the above risk factors applied [72]. Instead, surprisingly, younger patient and absence of total body irradiation in conditioning were identified as risk factors for developing cGVHD.

We hypothesize that the lack of knowledge of the "anomalies" of ATG-conditioned transplantations may have contributed to why survival with ATG was not superior to survival without ATG in most studies listed in Tables 2 and 3, which showed decreased GVHD and no impact on relapse or fatal infections. Perhaps, survival of ATG-conditioned patients would surpass that of non-ATG patients if, for example, only CMV-seropositive donors were chosen for seropositive patients. Moreover, most patients used in the studies listed in Tables 2 and 3 underwent transplantation before the era of routine EBV DNAemia monitoring and preemptive or prompt therapy of PTLD; thus, a higher incidence of fatal PTLD among ATG versus non–ATG-conditioned patients may have also contributed to the "no survival difference" finding in most of the studies.

PREEMPTIVE THERAPY OF GVHD AFTER ADULT BMT/PBSCT

Given that low-dose ATG does not worsen survival after myeloablative BMT/PBSCT and improves quality of life by reducing GVHD, it is our bias that ATG prophylaxis (ATG given with conditioning) should be routinely used with adult myeloablative BMT/PBSCT. However, in our (Albertan) experience, approximately 40% adult PBSCT recipients still develop clinically significant GVHD despite prophylaxis with 4.5 mg/kg Thymoglobulin. Increasing the dose of ATG should further lower the incidence of GVHD [46,47] but may be associated with an unacceptable increase in fatal infections. Even more prolonged infusion-related side effects of higher dose ATG (eg, more fever or rigors), despite being typically easily manageable, may be unwelcome by patients. Thus, a higher dose of ATG or an extra dose (on top of the 4.5 mg/kg) might be justified only for patients at high risk of developing significant GVHD. Given that pretransplantation risk factors, such as HLA-mismatched or unrelated donor, patient age, donor age, or female donor for male recipient, discriminate poorly or not at all between patients at high versus low risk of developing GVHD after low-dose ATG prophylaxis [73], early post-transplantation biomarkers may be needed to guide the preemptive therapy. Bacigalupo et al. pioneered the use of early post-transplantation biomarkers, specifically serum cholinesterase, gamma glutamyl transferase, urea, and total protein. Based on these biomarkers on day 7, patients at high risk of GVHD were randomized to receive 2 or 3 extra doses of ATG, 1.25 mg/kg each, between day 7 and 11. This resulted in 2- to 3-fold decrease in the incidence of both grade 3 and 4 aGVHD and extensive cGVHD [74]. As expected from the studies of ATG prophylaxis, relapse or fatal infections were not increased, and survival was virtually identical. Thus, despite quality of life not reported in Bacigalupo's study, it is likely that preemptive ATG on top of low-dose prophylactic ATG further improves quality of life (due to reduction of cGVHD) and should theoretically be recommended, although it does not improve survival.

However, in practice, preemptive ATG cannot be presently recommended to be used at multiple centers because biomarkers that are valid for stratifying patients into high versus low risk of GVHD at 1 center may not be valid at another center. We evaluated the performance of biomarkers used in the Bacigalupo's study [74] using Albertan patients and failed to validate the biomarkers (Table 4). It is not clear whether the difference between Bacigalupo's and our results is due to different treatment/supportive care practices or different patient ethnicities. In summary, preemptive use of ATG is promising, but centers wishing to apply it should first identify which 1 or few of currently existing biomarker candidates stratify their patients into high versus low risk of GVHD. Ferrara, Paczesny, Levine, and others work on discovering new biomarkers with high positive and negative predictive values for development of clinically significant

Table 4
Lack of Validation of Genoa Biomarkers for Prediction of GVHD in Alberta

Biomarker (on Day 7)	Genoa* Association with Transplantation-Related Mortality (Surrogate for Acute and/or Chronic GVHD) is Shown as Arrow‡ and Univariate *P* value is Given in Parentheses.	Alberta† Associations with Acute GVHD/Chronic GVHD are Shown as Arrow‡ and Univariate *P* Values are Given in Parentheses
GGT	↑ (.004)	↔ (.17)/↔ (.45)
Cholinesterase	↑ (.0007)	↔ (.59)/↑ (.04)
Total protein	↓ (.0003)	Not done
Albumin	↓ (.008)	↔ (.21)/↑ (.01)
Blood urea nitrogen	↑ (<.0001)	↔ (.86)/↔ (.55)

GGT indicates Gamma glutamyl transferase.

* Based on Sormani et al. [104] and Bacigalupo et al. [74]. Transplantation-related mortality was used as surrogate for GVHD, as per the authors the transplantation-related mortality was mostly due to GVHD.

† Based on Pratt et al. [105] and unpublished data of Pratt and Storek (May 2014). Acute GVHD refers to grade 2 to 4 acute GVHD; chronic GVHD refers to chronic GVHD treated with systemic immunosuppressive therapy.

‡ ↑ indicates that higher serum level of biomarker is associated with a higher likelihood of GVHD, ↓ indicates that lower serum level of biomarker is associated with a higher likelihood of GVHD, and ↔ indicates no significant association of biomarker with GVHD.

GVHD, but so far only in the setting of no ATG [75,76]. This work, if conducted also on ATG-conditioned patients, might lead to discovering 1 or a few universally applicable biomarkers (valid in any center). This would, hopefully, lead to universal applicability of preemptive therapy with ATG.

IMPACT OF ATG IN CONDITIONING ON GVHD, RELAPSE, INFECTIONS, AND SURVIVAL IN PEDIATRIC BMT/PBSCT

Whatever has been learned about ATG in adults cannot be assumed to apply to children. The main reason is that the incidence of GVHD (without ATG) is lower in children compared with adults. In adults, the small increase in viral infections due to ATG is far outweighed by the significant reduction of GVHD; however, the risk to benefit ratio may not be as favorable in children. Furthermore, dosing of ATG is less clear in pediatric patients than it is in adult patients. Most of the studies that have analyzed the dosing were combined studies, including adults and pediatrics [44,47]. A small pediatric study by Call et al. suggested that 10 mg/kg Thymoglobulin is a safe dose [77]. Another pediatric study compared 7.5 to 10 mg/kg with 15 to 40 mg/kg Thymoglobulin [78]. There was no added benefit of the high dose (aGVHD incidence was <10% and cGVHD incidence was 0% in both the low- and the high-dose groups); however, the high dose resulted in substantially increased incidence of PTLD [78]. Consistent with that, we (Boelens et al.) recently showed in a study including bone marrow and cord blood donors that higher post-transplantation ATG exposures are not associated with lower incidence of GVHD but are associated with worse T cell reconstitution, and the worse T cell reconstitution is associated with higher nonrelapse mortality, presumably due to infections [79]. Also consistent with that, a recent randomized trial comparing 30 mg ATG-F versus 15 mg ATG-F showed that nonrelapse mortality was lower in the 15 mg/kg group, which resulted in higher leukemia-free survival [80]. Neither GVHD nor relapse incidence were different between the 2 doses. Collectively, high-dose ATG (>10 mg/kg Thymoglobulin or ≥ 30 mg/kg ATG-F) has an unfavorable risk to benefit ratio in children. Studies are needed to determine the risk to benefit ratios of various low doses.

IMPACT OF ATG IN CONDITIONING ON GVHD, RELAPSE, INFECTIONS AND SURVIVAL IN ADULT AND PEDIATRIC CBT

GVHD incidence is lower after CBT than it is after BMT/PBSCT, so the benefit of ATG may be lower in the setting of CBT. Infections occur more frequently after CBT than after BMT/PBSCT (in part because cord blood graft contains fewer T cells and virtually no memory/effector T cells), so the risk of ATG may be higher in the setting of CBT. Even though cord blood T cells are mostly naive, in the absence of ATG, the naïve T cells can differentiate early after CBT into memory/effector cells and, thus, protect the recipient against viral infections and relapse [81,82]. With ATG, the naïve T cells may be eliminated or made unable to differentiate into the memory/effector cells, which appears to lead to frequent viral reactivations/diseases [83,84] and relapse [85]. Thus, the risk to benefit ratio of ATG after CBT is expected to be less favorable than it is after BMT/PBSCT. However, this has not been evaluated in a prospective study. In the largest retrospective study, survival was lower with ATG (~10 mg/kg between day -5 and 0) than without ATG (61% versus 71%), however, this was not statistically significant [83]. In the second largest retrospective study, survival was significantly lower with ATG (median 5 mg/kg between approximately day -6 and -5) than without ATG (38% versus 57%; $P = .02$) [86].

PHARMACOKINETICS AND TIMING OF PROPHYLACTIC ATG IN RELATION TO GRAFT INFUSION

The PK of ATG are likely influenced by its binding to a diversity of target antigens and Fc receptors. This is likely to cause variability and nonlinearity in both distribution and elimination of ATG. Furthermore, a minority of patients may have pre-existing neutralizing anti-ATG antibodies or develop these antibodies after transplantation, which may cancel the anti-GVHD effect [87].

Only a few studies have described the PK of ATG, using noncompartmental analysis or applying linear 1- or 2-compartment models [77,78,88-90]. Levels of total ATG (total rabbit IgG) or active ATG (rabbit IgG capable of binding to human lymphocytes or a T cell line) were measured. The half-life after HCT is longer for the total than the active ATG [87]. The active ATG appears more associated with pharmacodynamic (PD) effects; however, this has not been studied rigorously. In all studies, substantial interpatient variability was observed (Figure 4). Estimates of the half-life of ATG have varied from 2 days to 6 weeks. In 1 study, nonlinear PK were observed as a more than proportional increase of maximal concentration and half-life with increasing dose [78]. The high variability is possibly related to the size and age of the patient (baby, child, or adult) or to HCT-related factors, such as timing of ATG administration and cell counts present in the recipient before conditioning or in the infused graft. The relative and absolute size of the leukocyte subpopulations vary with age [91] and are influenced by the treatment of the disease before HCT. We (Boelens et al.) have embarked on a study of ATG PK and PD in a large pediatric cohort (n > 250, combined CBT and BMT/PBSCT). So far we have found that PK is influenced by patient weight and lymphocyte count before conditioning [92]. Regarding PD, higher post-transplantation exposure (area under the time-concentration curve) was associated with poorer T cell

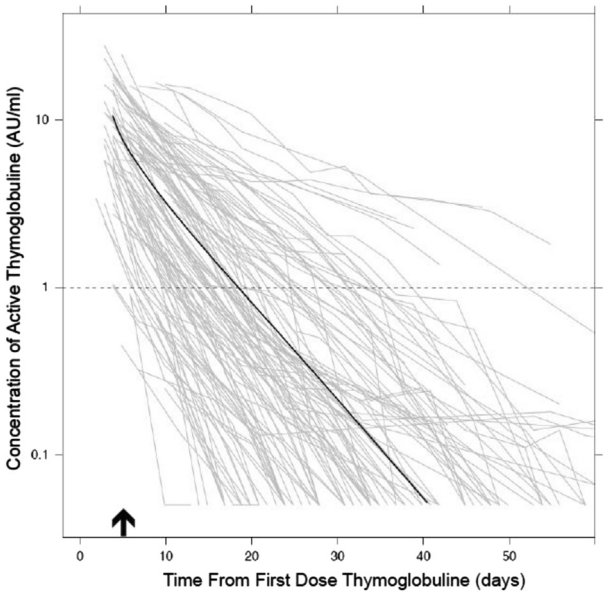

Figure 4. Pharmacokinetics of ATG. Thymoglobulin (approximately 10 mg/kg) was infused between transplantation day -5 (here denoted as day 0, ie, the day of the first Thymoglobulin infusion) and transplantation day 0 (here denoted as day 5 after the first Thymoglobulin infusion; transplantation day 0 is indicated by the arrow). Thereafter, serum levels of active ATG (rabbit IgG capable of binding to a human T cell line [HUT] [87]) were measured in 121 patients undergoing transplantation within the pediatric blood and marrow transplant program in Utrecht (66% cord blood, 29% bone marrow, 5% peripheral blood stem cells). Regarding the Thymoglobulin dose, 94% patients received between 9 and 11 mg/kg, 4% <9 mg/kg and 2% >11 mg/kg. The dotted line denotes the assumed lowest pharmacodynamically relevant serum level of Thymoglobulin. AU indicates arbitrary units.

reconstitution [79], which was associated with lower overall survival due to higher incidence of relapse and higher non-relapse mortality, presumably from infections. Further and more detailed analyses are currently being performed using both the above pediatric cohort as well as an adult cohort ($n > 250$) to get better and more detailed information on the association between ATG exposure before or after HCT and T cell reconstitution and HCT outcomes (eg, GVHD, relapse, infections).

The timing of ATG administration is also important. Late ATG administration (close to day 0) likely kills/inhibits donor T cells (infused with the graft) to a greater degree than early administration (eg, before day -5), whereas killing/inhibiting host T cells, host antigen presenting cells, and leukemic cells may be similar. Thus, compared with the early administration, the late administration is expected to result in less GVHD [93] and more viral infections [83,84]. It is also theoretically conceivable that the late administration is more likely to delay engraftment, whereas the early administration might facilitate engraftment.

It is impossible to make a firm recommendation on the dose and timing of ATG, as insufficient data are available, and transplantation settings may vary, especially the type of GVHD prophylaxis used in addition to ATG. For pediatric dose, see discussion in the section "Impact of ATG in Conditioning on GVHD, Relapse, Infections, and Survival in Pediatric BMT/PBSCT" (above). For adult BMT/PBSCT dose, we are of the opinion that the European Blood and Marrow Transplant Group consensus-recommended dose (7.5 mg/kg Thymoglobulin or 30 mg/kg ATG-F, divided into 3 doses administered on days -3, -2, and -1, in combination with methotrexate and a calcineurin inhibitor) [16] is adequate for HLA-matched and mismatched unrelated donor transplantation, though a lower dose (as for matched siblings, see next sentence) could be considered for matched unrelated donor transplantation. In the HLA-matched sibling donor setting, 4.5 to 6.0 mg/kg Thymoglobulin or 16 mg/kg ATG-F (dose associated with improved quality of life without affecting survival [19]) might suffice, particularly if the last infusion is given on day -1 or day 0 (before graft infusion). Based on our (Storek's and Mohty's) experience, for matched sibling transplantation, we would recommend the 4.5 to 6.0 mg/kg Thymoglobulin dose, with the last infusion on day -1 (for 5 to 6 mg/kg total dose) or day 0 (for 4.5 mg/kg total dose).

FUTURE PERSPECTIVES

Adequate immune reconstitution is important as all limitations of HCT (relapse, infections, GVHD) are associated with either immune deficiency or immune dysregulation. Given the high PK variability, an individualized ATG dosing, resulting in a predictable immune reconstitution and predictable likelihood of GVHD, infections, and relapse, may further improve the outcomes of HCT. Future detailed immune reconstitution studies, including multiple immune cell subsets not only in blood but also in tissues, and other biomarkers of infections/GVHD/relapse in association with ATG PK may provide improved insight into the biology of the desired effect (graft-versus-leukemia) and the complications (GVHD, infections) of HCT.

CONCLUSION

In the setting of adult myeloablative PBSCT, ATG prophylaxis definitely reduces GVHD, primarily cGVHD; thus, ATG probably improves quality of life. This may not be associated with increased relapse or fatal infections. ATG may not improve survival. Hopefully, the impact of ATG on quality of life, relapse, infections, and survival will be definitely resolved in 2 randomized trials that have so far been reported only in an abstract form [21,60] and in an ongoing trial by Soiffer et al. comparing ATG-F to no ATG (ClinicalTrials.gov identifier NCT01295710). It remains to be determined whether survival will be improved after we have learned more about the anomalies of ATG-conditioned HCT (eg, high mortality of CMV-seropositive recipients of grafts from CMV-seronegative donors, or high incidence of PTLD) and adjusting our donor selection and supportive care accordingly. It also remains to be determined whether the survival or quality of life will be improved after we have found biomarkers predicting GVHD with high sensitivity and specificity and implemented preemptive therapy accordingly. Regarding CBT and pediatric BMT/PBSCT, the benefit (reduction of GVHD) may not be as pronounced as after adult PBSCT and the risks (particularly viral diseases after CBT) may be higher. More studies are needed to determine whether the risk to benefit ratio warrants use of ATG in the setting of CBT and pediatric BMT/PBSCT. PK and PD studies will hopefully provide more insight into variables influencing the ATG exposure before and after HCT and their effects. This may lead to optimized dosing and timing of ATG administration.

ACKNOWLEDGMENTS

The authors thank the patients for participating in ATG-related research. We also thank the physicians, data managers, research nurses, technicians and other staff of the Alberta Blood and Marrow Transplant Program, the Utrecht

and Leiden pediatric blood and marrow transplantation programs, and the Leiden Academic Center for Drug Research for helping to generate unpublished data presented in this review, as well as all research and clinical personnel from around the world who generated or helped to generate the published data reviewed here.

Financial disclosure: Alberta Innovates-Health Solutions, Alberta Cancer Foundation, Buckley Family Cancer Research Excel Award, and ZonMW Priority Medicine Children Program (The Netherlands) provided grant support.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: J.S. and J.J.B. wrote the paper. M.M. critically reviewed the manuscript.

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