EUROPEAN HEMATOLOGY ASSOCIATION
Ferrata Storti Foundation

Haematologica 2017
Volume 102(2):224-234

Anti-thymocyte globulin as graft-*versus*-host disease prevention in the setting of allogeneic peripheral blood stem cell transplantation: a review from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation is increasingly used as treatment for patients with life-threatening blood diseases. Its curative potential is largely based on immune-mediated graft-*versus*-leukemia effects caused by donor T cells contained in the graft. Unfortunately, donor T cells are also the cause of graft-*versus*-host disease. The vast majority of human leukocyte antigen-matched allogeneic hematopoietic stem cell transplants are nowadays carried out with peripheral blood stem cells as the stem cell source. In comparison with bone marrows, peripheral blood stem cells contain more hematopoietic stem/progenitor cells but also one log more T cells. Consequently, the use of peripheral blood stem cells instead of bone marrow has been associated with faster hematologic recovery and a lower risk of relapse in patients with advanced disease, but also with a higher incidence of chronic graft-*versus*-host disease. These observations have been the basis for several studies aimed at assessing the impact of immunoregulation with anti-thymocyte globulin on transplantation outcomes in patients given human leukocyte antigen-matched peripheral blood stem cells from related or unrelated donors. After a brief introduction on anti-thymocyte globulin, this article reviews recent studies assessing the impact of anti-thymocyte globulin on transplantation outcomes in patients given peripheral blood stem cells from human leukocyte antigen-matched related or unrelated donors as well as in recipients of grafts from human leukocyte antigen haploidentical donors.

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Received: April 29, 2016.
Accepted: August 24, 2016.
Pre-published: December 7, 2016.

doi:10.3324/haematol.2016.148510

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/102/2/224

Introduction

Allogeneic hematopoietic stem cell transplantation (HCT) is a potentially curative treatment for a wide range of hematologic malignancies.[1,2] Its anti-tumor activity relies in large part on immune-mediated graft-*versus*-leukemia (GvL) effects.[3,4] However, donor immune cells contained in the graft can also target healthy host

tissues causing graft-*versus*-host disease (GvHD).[5] GvHD can be divided into two syndromes, acute GvHD which is an inflammatory disease causing maculopapular erythematous rash, gastrointestinal symptoms, and/or cholestatic hepatitis, and chronic GvHD which generally occurs beyond day 100, is non-inflammatory, and shares many clinical features with autoimmune diseases.[6] Acute GvHD can be further divided into classical acute GvHD occurring within the first 100 days after transplantation, and late acute GvHD occurring later.[7,8] Chronic GvHD can also be subdivided, in this case between classical chronic GvHD in which only typical signs of chronic GvHD are present, and overlap syndrome in which typical manifestations of both acute and chronic GvHD coexist.[7,8] While chronic GvHD has been associated with GvL effects,[3,8-11] it is also the leading cause of mortality/morbidity in long-term transplant recipients[12,13] and impairs quality of life.

Although the pathogeneses of acute and chronic GvHD are distinct and involve several types of cells,[5,14] donor T cells play a pivotal role in both syndromes as demonstrated by the very low incidence of GvHD observed when profound *ex vivo* T-cell depletion is performed,[15] even in the HLA-mismatched setting.[16]

The vast majority of HLA-matched allogeneic HCT performed as treatment for acute leukemia in 2013 were carried out with peripheral blood stem cells (PBSC) as the stem cell source.[17] The use of PBSC instead of bone marrow (BM) in patients receiving grafts from HLA-matched donors has been associated with faster hematologic recovery and a lower risk of relapse in patients with advanced disease (due to greater GvL effects), but also with higher incidences of each of severe acute and extensive chronic GvHD.[18-20] These observations served as the basis for studies combining the use of PBSC with *in vivo* T-cell depletion of the graft with the aim of benefiting from the faster engraftment associated with the use of PBSC without exposing patients to high risks of severe GvHD. In this article, after briefly discussing mechanisms of action of the different brands of anti-thymocyte globulin (ATG), we review recent studies assessing the impact of immunoregulation with ATG on transplantation outcomes in patients given PBSC from HLA-matched donors as well as in those given grafts (PBSC plus granulocyte colony-stimulating factor-mobilized BM) from HLA-haploidentical donors, and propose indications for the use of ATG in those settings.

Anti-thymocyte globulin

Three preparations of ATG are currently available (Table 1).[21] ATGAM (ATG-h) consists of polyclonal IgG obtained from hyperimmune sera of horses immunized with human thymic cells. The two other brands of ATG consist of polyclonal IgG obtained from hyperimmune sera of rabbits immunized either with human thymocytes recovered from patients undergoing cardiac surgery (Thymoglobuline, ATG-T) or with the human Jurkat leukemic T-cell line (which was derived from the peripheral blood of a 14-year old boy suffering from acute T-cell leukemia[22]) [ATG Fresenius/Neovii (ATG-F)]. Although ATG-h is still currently used for *in vivo* T-cell depletion in the USA, two prospective randomized studies (including one performed almost 4 decades ago) failed to demonstrate its efficacy at preventing acute or chronic GvHD after HLA-matched BM transplantation (BMT).[23,24] Furthermore, a retrospective study from the Brazilian National Cancer Institute in a cohort of 40 patients with aplastic anemia receiving BMT from HLA-identical siblings observed higher incidences of grade II-IV acute GvHD (35% *versus* 0%, P=0.009) and moderate/severe chronic GvHD (3-year rate: 34% *versus* 0%, P=0.04) in the 20 patients conditioned with cyclophosphamide plus ATG-h (90 mg/kg total dose) than in those conditioned with cyclophosphamide plus ATG-T (8 mg/kg total dose).[25] These findings might be due to the fact that, in comparison to rabbit ATG, ATG-h induces less profound and less durable lymphopenia, even if it is administered at higher doses.[25,26]

Antigens targeted by ATG-T have been well described and include antigens expressed on T cells (such as CD2, CD3, CD4, CD7, or CD8), B cells, natural killer cells, macrophages and dendritic cells, as well as HLA class 1 and HLA-DR.[27] Recognition by ATG-T of B cells and dendritic-cell antigens can also be attributed to the presence of antigen-presenting cells, thymic stromal cells and B cells in thymus fractions, although they are composed mainly of T cells.[27] Furthermore, ATG-T also contains antibodies targeting antigens involved in cell adhesion and cell trafficking, as well as antigens involved in inflammation, apoptosis and cell proliferation.[27] Since ATG-F is produced by immunizing rabbits with a homogenous Jurkat cell line, and since during ATG-F (but not ATG-T) production rabbit IgG are adsorbed on human placental cells in addition to adsorption on human erythrocytes, the spectrum of antigens recognized by ATG-F is narrower than that recognized by ATG-T. For example, ATG-F does not contain or contains significantly fewer antibodies directed against CD3, CD4 or HLA-DR[28,29] (Figure 1). However, in contrast, compared to ATG-T, ATG-F contains more antibodies directed against CD107 (an antigen expressed on T cells during degranulation following antigenic stimulation).[29] Competitive binding experiments have demonstrated that

Table 1. Types of ATG.

Name	Type of antibodies	Lympho-depletion *in vivo*	GvHD prevention (total dose administered)
Antithymocyte globulin (ATG)			
ATGAM (ATG-h)	Polyclonal IgG from horses immunized with human thymocytes	+/-	–[24]*
ATG-Thymoglobuline (ATG-T)	Polyclonal IgG from rabbits immunized with human thymocytes	+	+ (2.5-10 mg/kg)
ATG-Fresenius / Neovii (ATG-F)	Polyclonal IgG from rabbits immunized with human Jurkat T leukemia cell line	+	+ (15-60 mg/kg)

*Champlin *et al.*[24]

ATG-T has stronger reactivity than ATG-F to activated peripheral blood mononuclear cells.[28] Furthermore, complement-mediated cytotoxic effects of ATG-T on peripheral blood mononuclear cells were stronger than those mediated by ATG-F[28] and ATG-T induced dendritic-cell apoptosis more effectively than similar doses of ATG-F.[30] Consequently, doses of ATG given for GvHD prohylaxis have been typically higher for ATG-F (15-60 mg/kg) than for ATG-T (2.5-10 mg/kg) (Table 1).

After *in vivo* infusion, all forms of ATG induce depletion of both T and antigen-presenting cells by complement-dependent lysis or antibody-dependent cellular cytotoxicity, apoptosis of activated T cells, and maintenance of dendritic cells in a tolerogeneic state.[27] Furthermore, rabbit (but not horse) ATG induces the generation of regulatory T cells (Treg), both *in vitro* and *in vivo*.[31-33] This is clinically relevant given accumulating evidence showing an important role for Treg in GvHD prevention after allogeneic HCT.[34-37]

The next paragraphs review the impact of both forms of rabbit ATG on transplantation outcomes in various transplantation settings. However since there has been no controlled head-to-head comparison between ATG-T and ATG-F reported thus far in the allogeneic HCT setting, one should be cautious when extrapolating data from medical literature referring to one of these two drugs.

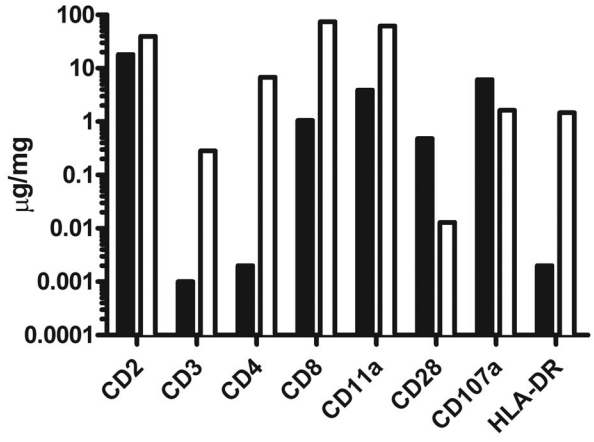

Figure 1. Quantification of ATG antibodies targeting specific human antigens. (adapted from Table 1 from Popow *et al.*[29]) Black bars represent ATG-F data and white bars ATG-T data.

Role of rabbit anti-thymocyte globulin in patients given HLA-matched peripheral blood stem cells after myeloablative conditioning

Pharmacokinetics of anti-thymocyte globulin and impact of post-transplant anti-thymocyte globulin serum levels on transplantation outcomes

Several studies have assessed ATG pharmacokinetics after PBSC transplantation. These studies demonstrated that residual total rabbit IgG levels or residual free T-cell-specific rabbit IgG levels were each influenced by the dose of ATG administered, although there was a considerable inter-patient variability. In detail, Forcina *et al.* assessed specific ATG-F pharmacokinetics in 22 patients who underwent allogeneic HCT after a myeloablative conditioning combining fludarabine and treosulfan.[38] ATG-F was administered at a dose of either 10 mg/kg/day (n=17) or 20 mg/kg/day (n=5) on days -4, -3 and -2 before transplantation. T-cell-specific rabbit IgG levels peaked at the end of the last dose of ATG-F administration and were four times higher in patients given the 20 mg/kg dose. These differences persisted on day 0. Furthermore, while patients given the 10 mg/kg dose reached sub-therapeutic specific rabbit IgG levels on day +10 after transplantation, those given the 20 mg/kg dose kept supra-therapeutic specific rabbit IgG levels beyond day +21 after the allogeneic transplant.

Waller *et al.* assessed ATG-T pharmacokinetics in 19 patients with high-risk hematologic malignancies who received $CD34^+$-selected, lymphocyte-depleted PBSC from partially HLA-matched related donors.[39] ATG-T was administered at a dose of 2.5 mg/kg/day (n=2, 10 mg/kg total dose) or 1.5 mg/kg/day (n=17, 6 mg/kg total dose) for 4 consecutive days (the last 4 days of the conditioning regimen). In comparison to patients given ATG-T at the 6 mg/kg total dose, those receiving a total dose of 10 mg of ATG-T had comparable total rabbit IgG levels (77±14 *versus* 62±22 µg/mL, P=0.4) but higher specific rabbit IgG levels on day 0 (25±8 *versus* 9±6 µg/mL, P=0.002). Furthermore, the number of days required to reach infra-therapeutic levels of specific rabbit IgG levels was 35±4 *versus* 17±9 days in patients given 10 or 6 mg/kg ATG-T total dose, respectively (P=0.01).

Remberger *et al.* prospectively assessed total ATG-T levels in 76 patients given PBSC (n=60) or BM (n=16) after myeloablative (n=37) or reduced-intensity (n=39) conditioning.[40] All patients received ATG-T at the dose of 2 mg/kg/day for 2-4 days (total dose 4 mg/kg to 8 mg/kg) with the last dose given on day -1. Day 0 and 7 total rabbit IgG levels were 49 and 26 µg/mL, respectively in patients receiving 6 mg/kg ATG-T total dose (n=46), *versus* 63 and 42 µg/mL respectively in patients receiving 8 mg/kg ATG-T total dose (n=26). The estimated half-life of total rabbit IgG was 9 days.

Analyses of the impact of ATG serum levels on transplantation outcomes were pioneered by the group of Jan Storek at the University of Calgary. This group correlated ATG serum levels on days 0 (immediately before graft infusion), 7 and 28 after allogeneic HCT with transplantation outcomes in a large cohort of patients conditioned with a myeloablative regimen combining fludarabine (250 mg/m^2 total dose), busulfan [12.8 mg/kg IV – pharmacokinetics adjusted - total dose], and ATG-T [given at the doses of 0.5 mg/kg on day –2, 2.0 mg/kg on day –1, and 2.0 mg/kg on day 0 (total, 4.5 mg/kg)]. Patients with leukemia also received 4 Gy total body irradiation. The authors demonstrated that high levels of serum ATG-T on days 7 and 28 each correlated with a low incidence of acute and chronic GvHD, but also with a high incidence of post-transplant lymphoproliferative disorder[41,42] In contrast, high ATG levels on day 0 were associated only with a low incidence of chronic GvHD.[42] Importantly, no associations were observed between ATG levels and relapse or non-relapse mortality. Subsequently, the same group demonstrated that high levels of ATG-T specificities capable of binding to Treg and invariant natural killer T cells on day 7 were associated with a low incidence of relapse.[43]

Impact of anti-thymocyte globulin on immune recovery

The first large study assessing the impact of ATG-T on immune recovery was reported by Bosch *et al.*[44] The authors compared immune recovery the first 2 years after transplantation in patients given PBSC either in Calgary after the fludarabine/busulfan/ATG-T regimen described above (n=125), or in Seattle after conditioning with cyclophosphamide 120 mg/kg and 12 Gy total body irradiation (and no ATG) (n=46). Post-grafting immunosuppression and flow-cytometry analyses were similar in the two cohorts of patients. Main observations were that, 1 month after allogeneic HCT, ATG-T patients had lower counts of B cells, as well as $CD4^+$ and $CD8^+$ T cells, while, for at least 1 year after transplantation, counts of naïve and memory $CD4^+$ T cells as well as of naïve $CD8^+$ T cells remained lower in patients given ATG-T than in those not given ATG. Comparable qualitative observations have been recently made in a cohort of 65 patients given PBSC after various myeloablative conditioning regimens at the University of Liège and who were given ATG-F (45 mg/kg total dose, n=37) or not (n=28).[45]

Non-randomized studies comparing anti-thymocyte globulin *versus* no anti-thymocyte globulin

A number of phase II studies have assessed the impact of rabbit ATG in patients given unmodified grafts after myeloablative conditioning[46-52] (Table 2). Collectively, these studies suggested that ATG decreased the incidence of grade III-IV acute and chronic GVHD without increasing non-relapse mortality (some studies even found lower non-relapse mortality with ATG), increased the incidence of post-transplantation lymphoproliferative disorders, and improved quality of life. A recent study from *La Societe Francophone de Greffe de Moelle et de Thérapie Cellulaire* (SFGM-TC) compared the impact of ATG according to stem cell source (PBSC *versus* BM) in patients given grafts from 10/10 HLA-matched unrelated donors following myeloablative conditioning regimens. Data from 356

Table 2. Selected non-randomized study comparing myeloablative allogeneic stem cell transplantation outcomes in patients given ATG or not.

Study	N. of ATG patients/ N. of control patients	Study design	Main observations. In comparison to control patients, ATG patients had:
Unrelated donors			
Zander *et al.*[46]	145 / 188	Retrospective analysis of CML patients given ATG-F (average dose 90 mg/kg) or not before unrelated BMT or PBSCT.	Faster leukocyte enraftment (P<0.001) Lower incidence of grade III-IV acute GvHD (P=0.03) Lower NRM (P=0.03) A trend for higher relapse (P=0.09) Better OS (P=0.03)
Schattenberg *et al.*[47]	34 / 22	Retrospective study in patients given ATG-T (8 mg or 16 mg/kg) or not before partially TCD unrelated BMT.	Lower NRM (P=0.004) Better DFS (P=0.005) Better OS (P=0.002)
Yu *et al.*[48]	54 / 42	Retrospective study in patients given ATG-F (16 mg/kg) or not before related or unrelated HCT.	Lower incidence of grade III-IV acute GvHD (P=0.03) Lower incidence of chronic GvHD (P=0.002) Higher incidence of infections (P=0.02) Better global quality of live (P<0.01) Less fatigue (P<0.01)
Ratanatharathorn *et al.*[50]*	76 / 121	Retrospective study in patients given ATG-T (4.5 mg/kg) or not before unrelated HCT (mainly PBSCT)	Lower incidence of grade III-IV acute GvHD (P=0.02) Lower incidence of chronic GvHD (P<0.001) Lower NRM (P=0.01) Better OS (P=0.01)
Binkert *et al.*[51]	120 / 145	Retrospective study in patients given ATG-F (35 or 60 mg/kg) or not before related (n=117) or unrelated (n=148) PBSCT	Slower neutrophil engraftment (P<0.001) Higher EBV infection Higher viral infection Lower incidence of chronic GvHD (P=0.03) Lower NRM and better OS with lower ATG doses
Ravinet *et al.*[53]	47 / 92	Retrospective study in patients given ATG-T or not before unrelated PBSCT	Lower incidence of grade III-IV acute GvHD (P=0.04) Lower incidence of chronic GvHD (P=0.03) Higher GvHD-free/relapse-free survival (P<0.01)
Ravinet *et al.*[53]	64 / 153	Retrospective study in patients given ATG-T or not before unrelated BMT	No statistically significant impact of ATG on outcomes
Related donors			
Bonifazi *et al.*[52]	47 / 146	Retrospective study of patients given ATG-F (15 or 30 mg/ kg) before PBSCT from HLA-identical siblings	Lower incidence of extensive chronic GvHD (P=0.03)
Wolschke *et al.*[49]*	79 / 159	Retrospective study in patients receiving PBSCT from sibling donors and given ATG-F (median dose of 30 mg/kg) or not.	Slower leukocyte enraftment (P=0.001) Higher incidence of PTLD (P=0.05) Lower incidence of grade II-IV acute GvHD (P=0.04) Lower incidence of chronic GvHD (P=0.002)

iTCD: *in vivo* T-cell depletion; ATG-T: ATG-Thymoglobuline; ATG-F: ATG-Fresenius/Neovii; Alem: alemtuzumab; BMT: bone marrow transplantation; HCT: hematopoietic cell transplantation; PBSCT: peripheral blood stem cell transplantation; CML: chronic myeloid leukemia; GvHD: graft-*versus*-host disease; OS: overall survival; DFS: disease-free survival; NRM: non-relapse mortality; *also includes patients given grafts after reduced intensity conditioning.

patients with acute myeloid leukemia or myelodysplastic syndrome were included in the analyses. Among patients given PBSC (n=139), those given ATG (n=47) had lower cumulative incidences of grade III-IV acute GvHD (HR 0.17, *P*=0.04) and chronic GvHD (HR 0.31, *P*=0.03) in comparison to those not given ATG.[53] Interestingly, the patients who received ATG had a significantly better GvHD-free/relapse-free survival (HR 0.48, *P*<0.01) than patients who did not. Notably, these correlations were not statistically significant in the group of patients who received BM as their source of stem cells (n=217).

Anti-thymocyte globulin dose-finding study

The best dose of ATG to use in patients who have been given PBSC after myeloablative conditioning has remained undefined. In an effort to address this issue, Deeg *et al.* performed a dose-finding study of ATG-T in patients with myeloid malignancies given PBSC after targeted busulfan plus cyclophosphamide conditioning.[54] The starting dose of ATG-T was 4.5 mg/kg total dose (0.5 mg/kg on day -3, and 2 mg/kg on each of days -2 and -1) and escalation was dependent on the occurrence of acute GvHD on the one hand, and Epstein–Barr virus (EBV) reactivation on the other hand. The authors identified a total dose of 6 mg/kg as the highest tolerable dose (in term of EBV reactivation). The incidences of grade II-IV acute and extensive chronic GvHD were comparable in patients given 4.5 or 6.0 mg/kg ATG-T, but were both lower in ATG-T patients (n=56) than in concurrent control patients (n=27) (50% *versus* 82% for grade II-IV acute GvHD, and 34% *versus* 82% for extensive chronic GvHD, respectively). This study suggests that administration of ATG-T at a total dose of 4.5 mg/kg to 6 mg/kg is safe and might successfully prevent GvHD in patients receiving PBSC from HLA-matched donors after myeloablative conditioning.

Randomized studies comparing anti-thymocyte globulin *versus* no anti-thymocyte globulin

Four randomized studies have assessed the impact of rabbit ATG on allogeneic HCT outcomes.[55-58] None of these studies was double-blinded and thus the possibility of a certain bias in the grading of GvHD cannot be ruled out.

- Unrelated donors

The first randomized study assessing the use of rabbit ATG was carried out by the *Gruppo Italiano Trapianti Midollo Osseo* (GITMO) in patients who underwent BMT from unrelated donors after myeloablative conditioning.[55] It is important to note that this study was performed before HLA high-resolution typing was available. In a first part of the study, 54 patients were randomized between no ATG or ATG-T 7.5 mg/kg total dose, while in the second part, 55 patients were randomized between no ATG or ATG-T 15 mg/kg total dose (Table 3). Patients not given ATG and those given 7.5 mg/kg ATG-T had similar incidences of grade III-IV acute GvHD, while patients given ATG-T 15 mg/kg had a lower incidence of grade III-IV acute GvHD but also a higher incidence of infections. Importantly, the incidence of extensive chronic GvHD was significantly lower among the patients given ATG-T 7.5 mg (38%) and ATG-T 15 mg (41%) than among the control patients (62%; *P*=0.04). However, ATG-T failed to improve overall survival, even with a long follow-up.[59]

Finke *et al.* and Socie *et al.* conducted a phase III randomized study comparing standard GvHD prophylaxis with cyclosporine and short-course methotrexate with or without added ATG-F.[56,60] The study included 201 patients who underwent unrelated BMT (n=37) or PBSC transplantation (n=164) after myeloablative conditioning. Patients were randomized between ATG-F (20 mg/kg on days -3, -2 and -1) or no ATG. The primary endpoint was grade III-IV acute GvHD or death within the first 100 days after the allogeneic HCT. Although the primary endpoint was not statistically different between the two groups (*P*=0.13), the study demonstrated that the patients given ATG had lower incidences of grade II-IV acute GvHD (33% *versus* 51%, *P*=0.01),[56] chronic GvHD (30% *versus* 60%, *P*<0.001), and extensive chronic GvHD (12% *versus* 45%, *P*<0.001).[60] However, ATG significantly delayed both neutrophil engraftment (26 *versus* 19 days to achieve $1x10^9$ neutrophils/L, *P*<0.001) and platelet engraftment (20 *versus* 37 days to achieve $50x10^9$ platelets/L, *P*<0.001), while five patients in the ATG arm but none in the control arm developed a post-transplant lymphoproliferative disorder.[56] Importantly, although there was only a statistically non-significant survival advantage for ATG patients (at 3 years 55% *versus* 43%, *P*=0.39), the 3-year probability of being alive and free of immunosuppressive drugs was three times higher among ATG patients than among control patients (53% *versus* 17%, *P*<0.001).[60]

Another randomized study in the unrelated allogeneic HCT setting was recently reported by Walker *et al.*[57] The main inclusion criteria were HLA-matched or 1/8 single HLA-mismatched unrelated donor, hematologic malignancy, myeloablative conditioning or reduced intensity conditioning (RIC), and PBSC or BM as the stem cell

Table 3. Randomized studies of rabbit ATG as GvHD prevention in patients given allogeneic hematopoietic cell transplantation.

	N. of patients	ATG brand / total dose (mg/kg)	Acute GvHD II-IV	Chronic GvHD	Non-relapse mortality	Relapse	Overall survival
			% ATG/ % no ATG (*P*)	% ATG/ % no ATG (*P*)	% ATG/ % no ATG (*P*)	% ATG/ % no ATG (*P*)	% ATG/ % no ATG (*P*)
Bacigalupo *et al.*[55]	54	T / 7.5	69 / 72 (0.6)	38 / 65 (0.08)	43 / 39 (0.7)[a]	10 / 12 (0.6)[a]	56 / 55 (0.8)[a]
Bacigalupo *et al.*[55]	55	T / 15	37 / 79 (0.001)	41 / 59 (0.3)	47 / 49 (0.9)[b]	36 / 18 (0.8)[b]	43 / 43 (0.8)[b]
Finke & Socie *et al.*[56,60]	201	F / 60	33 / 51 (0.01)	30 / 60 (<0.001)[a]	19 / 34 0.18)[a]	33 / 28 (0.5)[a]	55 / 43 (0.39)[a]
Kroger *et al.*[58]	155	F / 30	11 / 18 (0.13)	32 / 69 (<0.001)[c]	14 / 12 (0.6)[c]	32 / 26 (0.17)[c]	74 / 78 (0.5)[c]
Walker *et al.*[57]	196	T / 4.5	50 / 65 (0.01)[d]	22 / 33 (0.06)[b]	23 / 24 (NS)[b]	11 / 16 (NS)[b]	75 / 65 (0.24)[b]

[a]at 3 years; [b]at 1 year; [c]at 2 years; [d]grade I-IV at day 100. F: ATG-Fresenius; T: ATG-Thymoglobuline.

source. The primary endpoint was freedom from GvHD and systemic immunosuppressive treatment without resumption up to 12 months after transplantation. Of 203 randomized patients, 196 were eligible. These included 99 patients randomized to ATG-T (0.5 mg/kg on day -2 and 2 mg/kg on days -1 and +1), and 97 randomized to no ATG. Sixty-seven percent of patients received myeloablative conditioning (and 33% RIC), and in 88% of cases peripheral blood was the stem cell source. The main observations were that the primary endpoint was met in 37% of patients given ATG *versus* 16% of the patients not given ATG ($P<0.001$). Furthermore, ATG-treated patients had a significantly lower incidence of grade I-IV acute (50% *versus* 65% at 100 days, $P=0.01$) and chronic (22 *versus* 33, $P=0.06$) GVHD, without an increased risk of relapse (11% *versus* 16% at 1 year). However, there was a higher incidence of EBV reactivation among the patients given ATG (33% *versus* 3%). Finally, there was a non-statistically significant survival advantage for the ATG-treated patients (at 1 year: 75% *versus* 65%, $P=0.2$). Longer follow-up is needed to determine the impact of ATG on late relapses and on late non-relapse deaths.

The study by Walker *et al.* also prospectively assessed the impact of ATG-T on quality of life.[57] Patients assigned to the ATG-T arm had better Atkinson Life Happiness Scale Scores at 12 months and lower chronic GvHD symptom burden (assessed by the Lee scale) at 6 and 12 months after transplantation. These data suggest a beneficial impact of ATG on quality of life.

- HLA-identical sibling donors

The impact of ATG on transplantation outcomes has recently been tested in the setting of PBSC transplantation from HLA-identical sibling donors in patients suffering from acute leukemia in complete remission.[58] One hundred fifty-five eligible patients were randomized between ATG-F (n=83, administered at 10 mg/kg/day on days -3, -2 and -1) or no ATG (n=72). The 2-year cumulative incidence of chronic GvHD (primary endpoint of the study) was 32% in ATG patients *versus* 69% in controls ($P<0.001$). Other observations included slower leukocyte and platelet engraftment in ATG patients, while other transplantation outcomes (infections, acute GvHD, relapse incidence, non-relapse mortality, overall survival and progression-free survival) were not statistically significantly different between the two groups of patients (Table 3). Since this study only included patients in complete remission at the time of transplantation, further studies are needed to assess the impact of ATG in patients with advanced leukemia.

Role of rabbit anti-thymocyte globulin in patients given HLA-matched peripheral blood stem cells after reduced-intensity conditioning

RIC allogeneic HCT relies mainly on GvL effects for tumor eradication.[61-63] The biology of GvL effects remains poorly defined but has been thought to involve reactions to polymorphic minor histocompatibility antigens expressed either specifically on hematopoietic cells or more widely on a number of tissue cells.[64] As mentioned above, several studies have demonstrated a close relationships between GvHD and GvL responses after RIC or low-intensity immunosuppressive conditioning,[8-11,65] suggesting that the use of *in vivo* T-cell depletion might be harmful in that setting. However achievement/maintenance of complete remission has been observed in many patients given grafts after RIC/low-intensity conditioning who did not develop GvHD,[8-11,66] suggesting that clinical manifestations of GvHD are not universally required in order to achieve/maintain remissions.

Unfortunately, the randomized studies on the use or not of ATG were conducted mostly in patients undergoing myeloablative transplants. However, 33% of the patients included in the study by Walker *et al.* described above were given RIC or low intensity conditioning and, interestingly, the relapse incidence was similar between patients who were given ATG (n=33) and those who were not (n=31) also in this subgroup of patients.[57] Since it is hazardous to draw definitive conclusions based on data from a subgroup analysis of 64 patients, we also have to rely on phase II prospective and registry studies to estimate the impact of ATG specifically in the RIC allogeneic HCT setting.

Data from single center studies

The Marseille group conducted a number of phase II studies aimed at optimizing ATG-T dosing in patients given grafts after RIC conditioning.[67-70] They used the RIC regimen combining fludarabine and busulfan.[61] Post-grafting immunosuppression consisted of cyclosporine alone in the case of HLA-identical sibling donors, or cyclosporine plus mycophenolate mofetil in the case of unrelated donors. In a first study, they compared transplantation outcomes in patients given high (7.5-10 mg/kg total dose, n=46) or low (2.5 mg/kg total dose, n=55) dose ATG-T before transplantation from HLA-identical siblings. Incidences of grade II-IV acute GvHD ($P=0.001$) and chronic GvHD ($P=0.02$) were significantly lower in patients given the higher dose of ATG. However, this benefit was offset by a higher incidence of relapse in the group of patients given high-dose ATG.

In a second study, the authors retrospectively compared ATG-T 2.5 mg/kg total dose (n=124) to ATG-T 5 mg/kg total dose (n=105).[68] All patients received PBSC from either HLA-identical siblings (n=187) or unrelated donors (n=42). The main observations were that patients given 5 mg of ATG had lower incidences of grade II-IV acute GvHD (23% *versus* 42%, $P=0.002$) and chronic GvHD (35% *versus* 69%, $P<0.001$) than those given 2.5 mg/kg ATG. However, importantly, other transplantation outcomes (relapse, non-relapse mortality, overall survival and leukemia-free survival) were comparable between the two groups of patients. Similar observations were made when the analyses were restricted to patients transplanted as treatment for myeloid malignancies.[69]

Investigators from Karolinska University performed a retrospective study including data from 110 patients given unrelated PBSC (n=95) or BM (n=15) following chemotherapy-based RIC.[71] Transplantation outcomes were compared between patients given a total ATG-T total dose of 6 mg/kg (n=66) or 8 mg/kg (n=44). The authors observed a higher incidence of relapse ($P=0.04$) and lower leukemia-free survival rate ($P=0.04$) in patients given the higher dose of ATG.

More recently, Langston *et al.* reported the results of a retrospective study of 85 patients given PBSC (n=74) or BM (n=11) from unrelated donors at Emory University after fludarabine plus melphalan conditioning.[72] Patients with 10/10 HLA-matched unrelated donors were not

given ATG (n=54), while patients given grafts from HLA-mismatched donors were given ATG-T 6 mg/kg total dose. Remarkably, the authors observed comparable outcomes in the two cohorts of patients, suggesting that the addition of ATG was able to offset the negative impact of HLA-mismatch without increasing the incidence of relapse or infection.

Data from registry studies

A first registry study assessing the impact of *in vivo* T-cell depletion on outcomes in the RIC setting was reported by Soiffer *et al.* on behalf of the Center for International Blood and Marrow Transplant Research (CIBMTR).[73] The study included data from 1676 adult patients given BM (n=203) or PBSC (n=1473) after various RIC regimens as treatment for a heterogeneous group of hematologic malignancies. Patients were divided into three groups: one group was not given either ATG or alemtuzumab (controls, n=879), one group was given ATG (n=584; including 160 patients who received horse ATG and 405 patients who received ATG-T at a median total dose of 7 mg/kg), and one group was given alemtuzumab (n=213). In multivariate analyses, in comparison to control patients, those given ATG had a similar incidence of grade III-IV acute GvHD (HR 0.9, P=0.2), a lower incidence of chronic GvHD (HR 0.7, P<0.001), a higher incidence of non-relapse mortality (HR 1.3, P=0.01), and a higher incidence of relapse (HR 1.5, P<0.001). This translated into significantly worse overall survival (HR 1.3, P=0.002) and disease-free survival (HR 1.5, P<0.001) in patients given ATG.

The Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT) revisited this issue in a more homogeneous cohort of 1,250 patients with acute myeloid leukemia in first complete remission given PBSC from HLA-identical siblings.[74] A total of 554 patients did not receive any form of *in vivo* T-cell depletion (control group), whereas ATG and alemtuzumab were given in 444 and 252 patients, respectively. In multivariate analyses, the use of ATG was associated with a lower risk of chronic GvHD (HR=0.6, P<0.001) and a lower risk of extensive chronic GvHD (HR=0.5, P<0.001). Furthermore, in contrast to what was observed in the CIBMTR study, ATG patients had a similar risks of relapse (HR=1.1, P=0.40) and non-relapse mortality (HR=0.9, P=0.6), and similar overall survival (HR=0.9, P=0.6) and leukemia-free survival (HR=1.0, P=0.8) in comparison to control patients (Figure 2). The impact of ATG on the risk of relapse and on leukemia-free

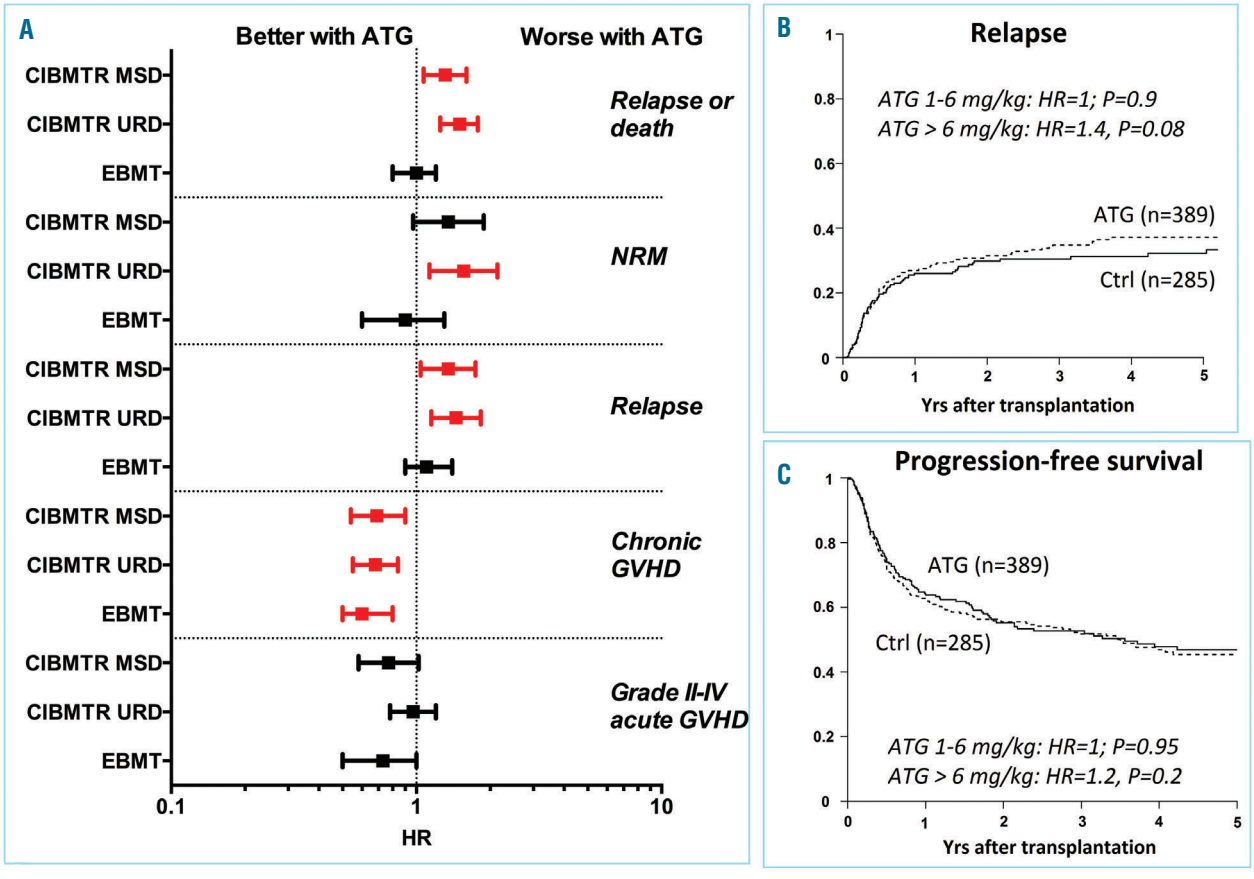

Figure 2. Impact of ATG on transplantation outcomes in patients given grafts after reduced-intensity conditioning. (A) Forest plots showing the results of multivariate analyses from two large registry studies assessing the impact of ATG on transplantation outcomes either in patients with various hematologic malignancies [study from the Center for International Blood and Marrow Transplant Research (CIBMTR)[73]] or in patients with acute myeloid leukemia in first complete remission [study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT)[74]]. (B) Relapse incidence and (C) progression-free survival in the subroup of patients from the EBMT study given peripheral blood stem cells (PBSC) after busulfan-based RIC (n=674, including 389 patients given ATG) [74].

survival in the subgroup of patients transplanted following a busulfan-based RIC is shown in Figure 2B,C. Within this subgroup of patients, the relapse incidence was similar in those given <6 mg/kg ATG and in those not given ATG (HR=1.1, *P*=0.9), while there was a suggestion of a higher incidence of relapses in patients given ATG at a dose ≥6 mg/kg (HR 1.4, *P*=0.08).

Taken together, these phase II and registry study data suggest that a total dose of ATG-T around 5 mg/kg might be optimal in the RIC setting. This assumption should be tested in an appropriate phase III trial with a uniform conditioning regimen and post-grafting immunosuppression.[75]

Role of rabbit anti-thymocyte globulin in patients given HLA-matched peripheral blood stem cells after low-intensity immunosuppressive conditioning

The impact of ATG has also been investigated in the setting of PBSC transplantation following low-intensity immunosuppressive conditioning. The Stanford group developed a low-intensity conditioning regimen combining ATG-T (7.5 mg total dose given at the dose of 1.5 mg/kg from day -11 to day -7) and 8 Gy total lymphoid irradiation in ten fractions followed by PBSC transplantation (TLI-ATG regimen).[76,77] This conditioning allowed sustained engraftment and GvL effects (mainly in patients who achieved full donor T-cell chimerism) with a very low incidence of GvHD.[76,77] In murine models of transplantation, this was achieved through Th2 polarization of donor T cells by recipient invariant natural killer/T cells (still present at transplantation given their relative resistance to ionizing radiation),[78] and through expansion of donor Treg by recipient invariant natural killer/T cells.[78]

The Belgian Society of Hematology conducted a phase II randomized study comparing low-intensity transplantation following a fludarabine plus 2 Gy total body irradiation regimen (n=49) developed by the Seattle group or following TLI-ATG (n=45).[79] The main observations were that overall survival rates were comparable in the two study arms (53% in patients treated with fludarabine and total body irradiation *versus* 54% in TLI-ATG patients) although the TLI-ATG patients had a significantly lower incidence of moderate/severe chronic GvHD (18% *versus* 41%, *P*=0.02) but a higher risk of relapse/progression at 4 years (50% *versus* 22%, *P*=0.02). The low incidence of chronic GvHD in TLI-ATG recipients could be attributed in part to higher Treg /naïve CD4 T-cell ratios early after transplantation.[80]

Role of rabbit anti-thymocyte globulin in patients given HLA-haploidentical peripheral blood stem cells

Although only 20-30% of patients have an HLA-identical sibling donor, virtually all patients have a potential HLA-haploidentical donor. Historically, HLA-haploidentical transplants were performed by transplanting a "megadose" of *in vitro*-selected CD34$^+$ cells.[81-83] This technique resulted in extensive *in vitro* T-cell depletion that prevented severe GvHD but also affected immune reconstitution leading to high incidences of both infections and disease relapse/progression.[84]

Fortunately, several novel approaches have been developed in the last decades and have resulted in more favorable outcomes.[85] These approaches include co-infusion of megadoses of CD34$^+$ cells with donor Treg and conventional T cells at a 2:1 ratio,[36] and T-cell-repleted HLA-haploidentical transplantation with either high-dose post-transplantation cyclophosphamide,[86] or intensive pharmacological immunosuppression including ATG. Using the latter strategy, the Bejing group developed a protocol combining granulocyte-colony-stimulating factor-mobilized BM and PBSC, as well as administration of ATG-T to prevent both graft rejection and GvHD.[87] ATG-T was administered from days -5 to -2 at the dose of either 2.5 mg/kg/day or 1.5 mg/kg/day. The conditioning regimen consisted of cytarabine (4 g/m^2/day on days -10 to -9), busulfan (4 mg/kg/day orally or 3.2 mg/kg i.v. on days –8 to –6), cyclophosphamide (1.8 g/ m^2/day on days -5 to -4), or semustine (250 mg/m^2 on day -3), while post-grafting immunosuppression was obtained with cyclosporine, mycophenolate mofetil, and short-course methotrexate. The outcomes of 1,210 consecutive patients offered HLA-haploidentical transplantation following this strategy were reported recently. The incidence of grade III-IV acute GvHD was 12%, while the 3-year cumulative incidence of chronic GvHD was 50%. At 3 years, progression-free and overall survival rates were 67% and 70%, respectively.

The same group of authors recently reported the results of a phase III non-inferiority trial investigating two doses of ATG-T (6 mg/kg total dose *versus* 10 mg/kg total dose) before infusion of granulocyte-colony-stimulating factor-mobilized BM and PBSC from HLA-haploidentical donors.[88] The primary endpoint was the incidence of grade III-IV acute GvHD. In comparison to patients given ATG-T 10 mg/kg total dose, those given 6 mg/kg total dose had a higher incidence of grade III-IV acute GvHD (16% *versus* 4%, *P*=0.005). Furthermore, the 1.5-year cumulative incidence of chronic GvHD was 65% *versus* 45% in the ATG-T 6 mg/kg and ATG-T 10 mg/kg groups, respectively (*P*=0.01). However, the incidences of septicemia (5% *versus* 12%, *P*=NS), EBV reactivation (10% *versus* 25% at 1 year, *P*<0.001) and post-transplant lymphoproliferative disorder (2% *versus* 8%, *P*=0.03) were each lower in the ATG-T 6 mg/kg group than in the ATG-T 10 mg/kg group. This could be attributed to slower immune recovery in patients given ATG-T 10 mg/kg total dose. Relapse, progression-free and overall survival rates were similar in the two arms.

The Milan group developed another protocol of HLA-haploidentical transplantation. The study included 121 patients, most with advanced disease. The conditioning regimen combined treosulfan (14 g/m^2/day on days -6 to -4) and fludarabine (30 mg/m^2/day on days -6 to -2), and post-grafting immunosuppression combining ATG-F 10 mg/kg on days -4 to -2, sirolimus and mycophenolate mofetil.[89] The incidence of grade III-IV acute GvHD was 22%, while the 2-year cumulative incidence of chronic GvHD was 47%. At 3 years, the cumulative incidences of non-relapse mortality, progression-free survival and overall survival were 31%, 20% and 25%, respectively.

Summary: possible indications for anti-thymocyte globulin in patients transplanted with peripheral blood stem cells

In summary, three prospective randomized studies have demonstrated that ATG decreases the incidence of

Table 4. Proposed indications for immunoregulation with ATG in patients given PBSC from allogeneic donors.

	Recommendation for ATG	Dose and timing of ATG
Myeloablative PBSCT from matched sibling donors[58]	standard of care	ATG-F 10 mg/kg/day on days -3, -2 and -1.
Myeloablative PBSCT from HLA-matchedunrelated donors[56,60,57]	standard of care	ATG-F 20 mg/kg/day on days -3, -2 and -1*. ATG-T 0.5 mg/kg on day -2 and 2 mg/kg on days -1 and +1.
RIC-PBSCT fludarabine-busulfan[68]	recommended	ATG-T 2.5 mg/kg/day on days -2 and -1.
Non-myeloablative PBSCT	developmental	/
HLA-haplo-identical stem cell transplantation (Bejing approach)[88]	standard of care	ATG-T 2.5 mg/kg/day from days -5 to -2.

* some centers use smaller doses such as 15 mg/kg total dose.

chronic GvHD without increasing the risk of relapse or non-relapse mortality in patients given HLA-matched related[90] or unrelated PBSC after myeloablative conditioning.[60] This suggests that ATG might become a standard of care in that setting (Table 4) although ATG has also been associated with infusion reactions, delayed hematopoietic and immune recovery, and increased risks of cytomegalovirus and EBV infections. Potential limitations of these studies are that they allowed different conditioning regimens (based on total body irradiation or chemotherapy), that they included patients with various risks of disease relapse, and that they lacked statistical power to assess the impact of ATG on disease relapse in high-risk patients. Future phase III studies should deal with these limitations and also, ideally, compare ATG administration with new methods of GvHD prophylaxis such as post-transplant cyclophosphamide.[91] Another important comparison that should be addressed in a phase III study is that between a combination of PBSC with ATG *versus* BM without ATG.

In the RIC setting, a retrospective study from the CIBMTR observed that *in vivo* T-cell depletion with ATG increased the risk of relapse and decreased disease-free survival in a cohort of patients transplanted for various hematologic malignancies.[73] However, several phase II studies[68,69] as well as a retrospective study by the ALWP of the EBMT[74] suggested that low doses of ATG efficiently prevented chronic GvHD without impairing leukemia-free or overall survival, while the use higher doses of ATG (>6 mg/kg ATG-T) was associated with a higher risk of relapse. Prospective randomized studies are thus needed to confirm the impact of *in vivo* T-cell depletion on outcomes specifically in the RIC setting.

Conclusions

Three brands of ATG are currently commercialized. ATG-h (ATGAM) is currently available almost only in the USA. ATG-h induces less lymphopenia than the two brands of rabbit ATG.[26] However, its impact on GvHD prevention has remained uncertain and is not supported by data from phase III studies. Although the two brands of rabbit ATG (ATG-T and ATG-F) share some similarities (such as inducing a profound and more durable lymphopenia than ATG-h), they diverge by the nature and intensity of antigens recognized.[29] Large studies comparing the impact of ATG-T *versus* ATG-F on immune recovery and post-transplant lymphoproliferative disorder are lacking. Consequently, data observed with one rabbit ATG product cannot be automatically extended to the other rabbit ATG formulation. Proposed indications/doses for immunoregulation with ATG in patients given PBSC from allogeneic donors are summarized in Table 4.

Acknowledgments

FB is senior research associate of the national fund for scientific research (F.R.S., FNRS), Belgium.

References

1. Baron F, Storb R. Allogeneic hematopoietic cell transplantation as treatment for hematological malignancies: a review. Springer Semin Immunopathol. 2004;26(1-2):71-94.
2. Sureda A, Bader P, Cesaro S, et al. Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. Bone Marrow Transplant. 2015;50(8):1037-1056.
3. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med. 1981;304(25):1529-1533.
4. Kolb HJ, Schmidt C, Barrett AJ, Schendel DJ. Graft-versus-leukemia reactions in allogeneic chimeras. Blood. 2004;103(3):767-776.
5. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol. 2012;12(6):443-458.
6. Duarte RF, Greinix H, Rabin B, et al. Uptake and use of recommendations for the diagnosis, severity scoring and management of chronic GVHD: an international survey of the EBMT-NCI Chronic GVHD Task Force. Bone Marrow Transplant. 2014;49(1):49-54.
7. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2005;11(12):945-956.
8. Thepot S, Zhou J, Perrot A, et al. The graft-versus-leukemia effect is mainly restricted to NIH-defined chronic graft-versus-host disease after reduced intensity conditioning before allogeneic stem cell transplantation. Leukemia. 2010;24(11):1852-1858.
9. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol. 2005;23(9):1993-2003.
10. Baron F, Labopin M, Niederwieser D, et al. Impact of graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation for acute myeloid leukemia: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Leukemia. 2012;26(12):2462-2468.
11. Storb R, Gyurkocza B, Storer BE, et al. Graft-versus-host disease and graft-versus-tumor effects after allogeneic hematopoietic cell transplantation. J Clin Oncol. 2013;31(12):1530-1538.
12. Pidala J, Kurland B, Chai X, et al. Patient-reported quality of life is associated with severity of chronic graft-versus-host disease as measured by NIH criteria: report on base-

line data from the Chronic GVHD Consortium. Blood. 2011;117(17):4651-4657.
13. Wingard JR, Majhail NS, Brazauskas R, et al. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. J Clin Oncol. 2011;29(16):2230-2239.
14. Socie G, Ritz J. Current issues in chronic graft-versus-host disease. Blood. 2014;124 (3):374-384.
15. Ho VT, Soiffer RJ. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood. 2001;98 (12):3192-3204.
16. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. Blood. 2014;124(5):822-826.
17. Passweg JR, Baldomero H, Bader P, et al. Hematopoietic SCT in Europe 2013: recent trends in the use of alternative donors showing more haploidentical donors but fewer cord blood transplants. Bone Marrow Transplant. 2015;50(4):476-482.
18. Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. J Clin Oncol. 2005;23(22):5074-5087.
19. Anasetti C, Logan BR, Lee SJ, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med. 2012;367(16):1487-1496.
20. Savani BN, Labopin M, Blaise D, et al. Peripheral blood stem cell graft compared to bone marrow after reduced intensity conditioning regimens for acute leukemia: a report from the ALWP of the EBMT. Haematologica. 2016;101(2):256-262.
21. Storek J, Mohty M, Boelens JJ. Rabbit anti-T cell globulin in allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2015;21(6):959-970.
22. Schneider U, Schwenk HU, Bornkamm G. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. Int J Cancer. 1977;19(5):621-626.
23. Weiden PL, Doney K, Storb R, Thomas ED. Antihuman thymocyte globulin for prophylaxis of graft-versus-host disease. A randomized trial in patients with leukemia treated with HLA-identical sibling marrow grafts. Transplantation. 1979;27(4):227-230.
24. 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(10):4582-4585.
25. Atta EH, de Sousa AM, Schirmer MR, Bouzas LF, Nucci M, Abdelhay E. Different outcomes between cyclophosphamide plus horse or rabbit antithymocyte globulin for HLA-identical sibling bone marrow transplant in severe aplastic anemia. Biol Blood Marrow Transplant. 2012;18(12):1876-1882.
26. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. N Engl Med. 2011;365 (5):430-438.
27. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. Leukemia. 2007;21(7):1387-1394.
28. Popow I, Leitner J, Majdic O, et al. Assessment of batch to batch variation in polyclonal antithymocyte globulin preparations. Transplantation. 2012;93(1):32-40.
29. Popow I, Leitner J, Grabmeier-Pfistershammer K, et al. A comprehensive and quantitative analysis of the major specificities in rabbit antithymocyte globulin preparations. Am J Transplant. 2013;13(12): 3103-3113.
30. Naujokat C, Berges C, Fuchs D, Sadeghi M, Opelz G, Daniel V. Antithymocyte globulins suppress dendritic cell function by multiple mechanisms. Transplantation. 2007;83(4): 485-497.
31. Feng X, Kajigaya S, Solomou EE, et al. Rabbit ATG but not horse ATG promotes expansion of functional CD4+CD25highFOXP3+ regulatory T cells in vitro. Blood. 2008;111(7):3675-3683.
32. Shimony O, Nagler A, Gellman YN, et al. Anti-T lymphocyte globulin (ATG) induces generation of regulatory T cells, at least part of them express activated CD44. J Clin Immunol. 2012;32(1):173-188.
33. Ehx G, Hannon M, Beguin Y, Humblet-Baron S, Baron F. Validation of a multicolor staining to monitor phosphoSTAT5 levels in regulatory T-cell subsets. Oncotarget. 2015;6(41):43255-43266.
34. Cohen JL, Trenado A, Vasey D, Klatzmann D, Salomon BL. CD4(+)CD25(+) immunoregulatory T cells: new therapeutics for graft-versus-host disease. J Exp Med. 2002;196(3):401-406.
35. Hannon M, Lechanteur C, Lucas S, et al. Infusion of clinical-grade enriched regulatory T cells delays experimental xenogeneic graft-versus-host disease. Transfusion. 2014;54(2):353-363.
36. Martelli MF, Di Ianni M, Ruggeri L, et al. HLA-haploidentical transplantation with regulatory and conventional T cell adoptive immunotherapy prevents acute leukemia relapse. Blood. 2014; 124(4):638-644.
37. Edinger M, Hoffmann P, Ermann J, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat Med. 2003;9(9):1144-1150.
38. Forcina A, Seitz I, Martinius H, et al. Pharmacokinetics (PK) study of antithymocyte globulins fresenius (ATG-F) prior to allogeneic stem cell transplantation: implications for timing of graft and early adoptive immunotherapy infusions. Blood. 2013;122 (21):4536.
39. Waller EK, Langston AA, Lonial S, et al. Pharmacokinetics and pharmacodynamics of anti-thymocyte globulin in recipients of partially HLA-matched blood hematopoietic progenitor cell transplantation. Biol Blood Marrow Transplant. 2003;9(7):460-471.
40. Remberger M, Sundberg B. Low serum levels of total rabbit-IgG is associated with acute graft-versus-host disease after unrelated donor hematopoietic stem cell transplantation: results from a prospective study. Biol Blood Marrow Transplant. 2009;15(8):996-999.
41. Podgorny PJ, Ugarte-Torres A, Liu Y, Williamson TS, Russell JA, Storek J. High rabbit-antihuman thymocyte globulin levels are associated with low likelihood of graft-vs-host disease and high likelihood of post-transplant lymphoproliferative disorder. Biol Blood Marrow Transplant. 2010;16(7):915-926.
42. Chawla S, Dharmani-Khan P, Liu Y, et al. High serum level of antithymocyte globulin immediately before graft infusion is associated with a low likelihood of chronic, but not acute, graft-versus-host disease. Biol Blood Marrow Transplant. 2014;20(8):1156-1162.
43. Hoegh-Petersen M, Amin MA, Liu Y, et al. Anti-thymocyte globulins capable of binding to T and B cells reduce graft-vs-host disease without increasing relapse. Bone Marrow Transplant. 2013;48(1):105-114.
44. Bosch M, Dhadda M, Hoegh-Petersen M, et al. Immune reconstitution after anti-thymocyte globulin-conditioned hematopoietic cell transplantation. Cytotherapy. 2012;14 (10):1258-1275.
45. Servais S, Menten-Dedoyart C, Beguin Y, et al. Impact of pre-transplant anti-T cell globulin (ATG) on immune recovery after myeloablative allogeneic peripheral blood stem cell transplantation. PloS One. 2015;10(6): e0130026.
46. Zander AR, Kroger N, Schleuning M, et al. ATG as part of the conditioning regimen reduces transplant-related mortality (TRM) and improves overall survival after unrelated stem cell transplantation in patients with chronic myelogenous leukemia (CML). Bone Marrow Transplant. 2003;32(4):355-361.
47. Schattenberg A, van der Meer A, Preijers F, et al. Addition of ATG to the conditioning regimen is a major determinant for outcome after transplantation with partially lymphocyte-depleted grafts from voluntary unrelated donors. Bone Marrow Transplant. 2004;33(11):1115-1121.
48. Yu ZP, Ding JH, Wu F, et al. Quality of life of patients after allogeneic hematopoietic stem cell transplantation with antihuman thymocyte globulin. Biol Blood Marrow Transplant. 2012;18(4):593-599.
49. Wolschke C, Zabelina T, Ayuk F, et al. Effective prevention of GVHD using in vivo T-cell depletion with anti-lymphocyte globulin in HLA-identical or -mismatched sibling peripheral blood stem cell transplantation. Bone Marrow Transplant. 2014;49(1):126-130.
50. Ratanatharathorn V, Deol A, Ayash L, et al. Low-dose antithymocyte globulin enhanced the efficacy of tacrolimus and mycophenolate for GVHD prophylaxis in recipients of unrelated SCT. Bone Marrow Transplant. 2015;50(1):106-112.
51. Binkert L, Medinger M, Halter JP, et al. Lower dose anti-thymocyte globulin for GvHD prophylaxis results in improved survival after allogeneic stem cell transplantation. Bone Marrow Transplant. 2015;50 (10):1331-1336.
52. Bonifazi F, Bandini G, Arpinati M, et al. Intensification of GVHD prophylaxis with low-dose ATG-F before allogeneic PBSC transplantation from HLA-identical siblings in adult patients with hematological malignancies: results from a retrospective analysis. Bone Marrow Transplant. 2012;47(8): 1105-1111.
53. Ravinet A, Cabrespine A, Socie G, et al. Impact of thymoglobulin by stem cell source (peripheral blood stem cell or bone marrow) after myeloablative stem cell transplantation from HLA 10/10-matched unrelated donors. A report from the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. Transplantation. 2015; 100(8):1732-1739.
54. Deeg HJ, Storer BE, Boeckh M, et al. Reduced incidence of acute and chronic graft-versus-host disease with the addition of thymoglobulin to a targeted busulfan/cyclophosphamide regimen. Biol Blood Marrow Transplant. 2006;12(5):573-584.
55. Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host

disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). Blood. 2001;98(10):2942-2947.
56. Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009;10(9):855-864.
57. Walker I, Panzarella T, Couban S, et al. Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. Lancet Oncol. 2016; 17(2):164-173.
58. Kroger N, Solano C, Wolschke C, et al. Antilymphocyte globulin for prevention of chronic graft-versus-host disease. New Engl J Med. 2016;374(1):43-53.
59. Bacigalupo A, Lamparelli T, Barisione G, et al. Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. Biol Blood Marrow Transplant. 2006;12(5):560-565.
60. Socie G, Schmoor C, Bethge WA, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. Blood. 2011;117(23):6375-6382.
61. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91(3):756-763.
62. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood. 1997;89(12):4531-4536.
63. Gill S, Porter DL. Reduced-intensity hematopoietic stem cell transplants for malignancies: harnessing the graft-versus-tumor effect. Annu Rev Med. 2013;64(101-117.
64. Warren EH, Deeg HJ. Dissecting graft-versus-leukemia from graft-versus-host-disease using novel strategies. Tissue Antigens. 2013;81(4):183-193.
65. Valcarcel D, Martino R, Caballero D, et al. Sustained remissions of high-risk acute myeloid leukemia and myelodysplastic syndrome after reduced-intensity conditioning allogeneic hematopoietic transplantation: chronic graft-versus-host disease is the strongest factor improving survival. J Clin Oncol. 2008;26(4):577-584.
66. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97(11):3390-3400.
67. Mohty M, Faucher C, Blaise D. Graft-versus-host-disease and granulocyte colony-stimulating factor administration after allogeneic stem cell transplantation. Leukemia. 2005;19(4):500-503.
68. Crocchiolo R, Esterni B, Castagna L, et al. Two days of antithymocyte globulin are associated with a reduced incidence of acute and chronic graft-versus-host disease in reduced-intensity conditioning transplantation for hematologic diseases. Cancer. 2013;119(5):986-992.
69. Devillier R, Crocchiolo R, Castagna L, et al. The increase from 2.5 to 5 mg/kg of rabbit anti-thymocyte-globulin dose in reduced intensity conditioning reduces acute and chronic GVHD for patients with myeloid malignancies undergoing allo-SCT. Bone Marrow Transplant. 2012;47(5):639-645.
70. Crocchiolo R, Esterni B, Castagna L, et al. Two days of antithymocyte globulin are associated with a reduced incidence of acute and chronic graft-versus-host disease in reduced-intensity conditioning transplantation for hematologic diseases. Cancer. 2013;119(5):986-992.
71. Remberger M, Ringden O, Hagglund H, et al. A high antithymocyte globulin dose increases the risk of relapse after reduced intensity conditioning HSCT with unrelated donors. Clin Transplant. 2013;27(4):E368-374.
72. Langston AA, Prichard JM, Muppidi S, et al. Favorable impact of pre-transplant ATG on outcomes of reduced-intensity hematopoietic cell transplants from partially mismatched unrelated donors. Bone Marrow Transplant. 2014;49(2):185-189.
73. Soiffer RJ, Lerademacher J, Ho V, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Blood. 2011;117(25):6963-6970.
74. Baron F, Labopin M, Blaise D, et al. Impact of in vivo T-cell depletion on outcome of AML patients in first CR given peripheral blood stem cells and reduced-intensity conditioning allo-SCT from a HLA-identical sibling donor: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 2014;49(3):389-396.
75. Rubio MT, Labopin M, Blaise D, et al. The impact of graft-versus-host disease prophylaxis in reduced-intensity conditioning allogeneic stem cell transplant in acute myeloid leukemia: a study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Haematologica. 2015;100(5):683-689.
76. Lowsky R, Takahashi T, Liu YP, et al. Protective conditioning for acute graft-versus-host disease. N Engl J Med. 2005;353(13):1321-1331.
77. Kohrt HE, Turnbull BB, Heydari K, et al. TLI and ATG conditioning with low risk of graft-versus-host disease retains antitumor reactions after allogeneic hematopoietic cell transplantation from related and unrelated donors. Blood. 2009;114(5):1099-1109.
78. Pillai AB, George TI, Dutt S, Strober S. Host natural killer T cells induce an interleukin-4-dependent expansion of donor CD4+CD25+Foxp3+ T regulatory cells that protects against graft-versus-host disease. Blood. 2009;113(18):4458-4467.
79. Baron F, Zachee P, Maertens J, et al. Nonmyeloablative allogeneic hematopoietic cell transplantation following fludarabine plus 2 Gy TBI or ATG plus 8 Gy TLI: a phase II randomized study from the Belgian Hematological Society. J Hematol Oncol. 2015;8(1):4.
80. Hannon M, Beguin Y, Ehx G, et al. Immune recovery after allogeneic hematopoietic stem cell transplantation following Flu-TBI versus TLI-ATG conditioning. Clin Cancer Res. 2015;21(14):3131-3139.
81. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. N Engl J Med. 1998;339(17):1186-1193.
82. Kanakry CG, Fuchs EJ, Luznik L. Modern approaches to HLA-haploidentical blood or marrow transplantation. Nat Rev Clin Oncol. 2016;13(1):10-24.
83. Apperley J, Niederwieser D, Huang XJ, et al. Haploidentical hematopoietic stem cell transplantation: a global overview comparing Asia, the European Union, and the United States. Biol Blood Marrow Transplant. 2016;22(1):23-26.
84. Ciceri F, Labopin M, Aversa F, et al. A survey of fully haploidentical hematopoietic stem cell transplantation in adults with high-risk acute leukemia: a risk factor analysis of outcomes for patients in remission at transplantation. Blood. 2008;112(9):3574-3581.
85. Rubio MT, Savani BN, Labopin M, et al. Impact of conditioning intensity in T-replete haplo-identical stem cell transplantation for acute leukemia: a report from the acute leukemia working party of the EBMT. J Hematol Oncol. 2016;9(1):25.
86. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2002;8(7):377-386.
87. Wang Y, Chang YJ, Xu LP, et al. Who is the best donor for a related HLA haplotype-mismatched transplant? Blood. 2014;124(6):843-850.
88. Wang Y, Fu HX, Liu DH, et al. Influence of two different doses of antithymocyte globulin in patients with standard-risk disease following haploidentical transplantation: a randomized trial. Bone Marrow Transplant. 2014;49(3):426-433.
89. Peccatori J, Forcina A, Clerici D, et al. Sirolimus-based graft-versus-host disease prophylaxis promotes the in vivo expansion of regulatory T cells and permits peripheral blood stem cell transplantation from haploidentical donors. Leukemia. 2015;29(2):396-405.
90. Bonifazi F, Solano C, Wolschke C, et al. Prevention of chronic GvHD after HLA-identical sibling peripheral hematopietic stem cell transplantation with or without anti-lymphocyte globulin (ATG). Results from a prospective, multicenter randomized phase III trial (ATGfamilystudy). Blood. 2014;124(21):37.
91. Kanakry CG, Tsai HL, Bolanos-Meade J, et al. Single-agent GVHD prophylaxis with posttransplantation cyclophosphamide after myeloablative, HLA-matched BMT for AML, ALL, and MDS. Blood. 2014;124(25):3817-3827.