ASBMT American Society for Blood and Marrow Transplantation

Total and Active Rabbit Antithymocyte Globulin (rATG;Thymoglobulin®) Pharmacokinetics in Pediatric Patients Undergoing Unrelated Donor Bone Marrow Transplantation

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Rabbit antithymocyte globulin (rATG; Thymoglobulin®) is currently used to prevent or treat graft-versus-host disease (GVHD) during hematopoietic stem cell transplantation (HSCT). The dose and schedule of rATG as part of the preparative regimen for unrelated donor (URD) bone marrow transplantation (BMT) have not been optimized in pediatric patients. We conducted a prospective study of 13 pediatric patients with hematologic malignancies undergoing URD BMT at St. Jude Children's Research Hospital from October 2003 to March 2005, to determine the pharmacokinetics and toxicities of active and total rATG. The conditioning regimen comprised total body irradiation (TBI), thiotepa, and cyclophosphamide (Cy); cyclosporine (CsA) and methotrexate (MTX) were administered as GVHD prophylaxis. Patients received a total dose of 10 mg/kg rATG, and serial blood samples were assayed for total rATG by enzyme linked immunosorbent assay (ELISA) and active rATG by florescein activated cell sorting (FACS). We found that our weight-based dosing regimen for rATG was effective and well tolerated by patients. The half-lives of total and active rATG were comparable to those from previous studies, and despite high doses our patients had low maximum concentrations of active and total rATG. There were no occurrences of grade iii-iv GVHD even in patients having low peak rATG levels, and the overall incidence of grade II GVHD was only 15%. None of the patients had serious infections following transplantation. These data support the use of a 10 mg/kg dose of rATG in children with hematologic malignancies because it can be administered without increasing the risk of graft rejection, or serious infection in pediatric patients with a low rate of GVHD. These conclusions may not apply to patients with nonmalignant disorders.

Biol Blood Marrow Transplant 15: 274-278 (2009)

KEY WORDS: Thymoglobulin, Hematopoietic stem cell transplantation, Bone marrow transplantation, Pediatrics, Pharmacokinetics, Rabbit antithymocyte globulin (rATG), Graft-versus-host disease (GVHD)

INTRODUCTION

Rabbit antithymocyte globulin (rATG), a polyclonal antibody that targets human T lymphocytes, is currently approved for treatment of acute rejection in renal transplant recipients. Because of its activity against T cells, it is frequently used for hematopoietic stem cell transplantation centers to prevent [1-7] or treat [8,9] graft-versus-host disease (GVHD) and to improve engraftment of donor stem cells during hematopoietic transplantation [1-7].

Currently, 2 polyclonal antibodies—Thymoglobulin® (rATG) and Atgam® (equine ATG, eATG)—are commercially available in the United States and differ in their biologic activities. Compared with eATG, rATG shows activity at lower doses (15 mg/kg versus 1.5 mg/kg, respectively). rATG also has a considerably longer half-life (5.7 versus 30 days, package insert) and higher specificity for human T lymphocytes than eATG, thereby favoring its use for hematopoietic stem cell transplant (HSCT). Our institution chose to use rATG because of its longer half-life and activity at lower doses.

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Financial disclosure: See Acknowledgments on page 278.

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Received October 6, 2008; accepted November 17, 2008
1083-8791/09/152-0001$36.00/0
doi:10.1016/j.bbmt.2008.11.027

There have been few pharmacokinetic studies of rATG in adults [2,10-12] and only 1 study [13] in children. The optimal dose and schedule of rATG as part of the preparative regimen for bone marrow transplantation (BMT) have not yet been defined. High or low doses of rATG can alter the risk of GVHD, graft rejection, or infectious complications [1,11,13]. At this time, low and high doses have not been firmly established.

To evaluate the pharmacokinetics of active and total rATG and toxicities associated with the dosing regimen, we performed a prospective clinical trial in children who underwent an unrelated donor (URD) BMT for hematologic malignancies at St. Jude Children's Research Hospital from October 2003 to March 2005. We assayed total and active rATG serum concentrations at multiple defined time points and assessed the effectivity and tolerance of the dose and schedule of rATG in this pediatric group.

MATERIALS AND METHODS

Patients

Thirteen pediatric patients undergoing an URD BMT for hematologic malignancy were enrolled in a prospective clinical trial at St. Jude Children's Research Hospital from October 2003 to March 2005. All patients received non-T cell-depleted bone marrow grafts from unrelated donors. High-resolution HLA typing was performed for the loci A, B, C, DR, and DQ. Donors were considered acceptable if they matched the recipient at the allele level at 7 of 8 or 8 of 8 loci. The trial was approved by the St. Jude institutional review board, and all research participants gave informed consent and assent (as appropriate) for participation.

Conditioning Regimen

The conditioning regimen comprised 12 Gy total body irradiation (TBI; fractionated into 8 doses on days –8 to –5), thiotepa (5 mg/kg/dose for 2 doses on day –4), and cyclophosphamide (Cy; 60 mg/kg/dose on days –3 and –2). GVHD prophylaxis consisted of cyclosporine initiated on day −2 at 2.5 mg/kg/dose intravenously (i.v.) every 12 hours and adjusted to maintain trough concentrations between 170 and 250 ng/mL by the enzyme multiplied immunoassay technique (EMIT), and methotrexate i.v. (MTX; 15 mg/m^2/dose on day +1; 10 mg/m^2/dose on days +3, +6, and +11). rATG was administered intravenously via a central venous access device. All patients received 1 mg/kg rATG on day –4 over 1 hour and 3 mg/kg on days –3, –2, and –1 (total dose 10 mg/kg) over 6 hours each day. To minimize side effects, all patients received premedications (methylprednisolone, diphenhydramine, and acetaminophen) before each rATG dose and a continuous i.v. infusion of hydrocortisone (5 mg/kg) during rATG infusion.

Collection of Information on Adverse Events

Information on adverse events was collected prospectively and scored according to the common toxicity criteria (CTC) of the National Cancer Institute (NCI) Version 2 (http://ctep.cancer.gov/reporting/ctc_archive.html) and reported at least annually as appropriate to institutional committees and the Food and Drug Administration. Adverse events were assigned by the treating physician or principal investigator. Relationship to treatment and classification of event (expected or unexpected) for grade III and IV adverse events were detailed. Acute and chronic GVHD (aGVHD, cGVHD) were graded according to established criteria [14]. To assure patient safety and data validity and integrity, an independent Data Safety Monitoring Board (DSMB) at St. Jude conducted regular reviews of the study's clinical and safety-related data.

Measurement of Total and Active rATG

Blood samples (2-3 mL) were obtained at baseline (before administering the test dose of rATG), before each 3 mg/kg dose, and before reinfusion of stem cells. After the transplant, blood samples were obtained on days +1, +3, +5, and +7, and at weeks 1, 2, 4, 8, 16, and 24. Serum was separated within 2 hours of blood collection and stored at –70°C (Translational Trials Unit Core Pharmacology Laboratory, St. Jude Children's Research Hospital) until they were shipped on dry ice to Genzyme (Cambridge, MA) for processing. Total and active plasma rATG were determined by Genzyme by the method of Regan et al. [12]. Total plasma rATG was determined by enzyme-linked immunosorbent assay (ELISA) (limit of quantification 3.9 μg/mL) and active plasma rATG by FACS (limit of quantification 0.2 μg/mL).

Pharmacokinetic Methods

We used a 2-compartment pharmacokinetic model with first-order elimination (ADVAN 3 subroutine in NONMEM version V, double precision, level 1.1 using the first-order conditional estimation [FOCE] method with INTERACTION] [15]) to describe total and active rATG. The POSTHOC option was used to estimate individual parameters. Pharmacokinetic parameters estimated by the model were systemic clearance (CL), volume of the central compartment (V), and intercompartmental rate constants (k_{12}, k_{21}). Intersubject variability was modeled with an exponential model.

RESULTS

Of the 13 patients enrolled, 11 were male. The median age at BMT was 10 years (range: 2-16 years).

Table 1. Patient Demographics

Patient	Diagnosis	Age(years)	Weight(kg)	Disease Statusat Transplant	Days toANC >500/mm^3	Days toPlatelets >20× 10^3/mm^3 without Transfusion x7 days
001	AML	12	40.8	CR2	17	49
002	JCML	2	13.4	PD	22	32
003	AML	16	60.4	CR1	23	48
004	AML	10	38.7	CR1	23	38
005	ALL	3	14.5	CR3	16	28
006	CML	10	36.7	CP1	19	27
007	CML	16	86.6	CP1	19	26
008	MDS	12	40	SD	19	33
009	CML	8	29.3	CP2	16	18
010	JCML	3	14.1	CR1	21	29
011	ALL	10	34.9	CR3	17	25
012	AML	2	11.1	CR1	17	34
013	ALL	14	48.5	CR2	15	25

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; JCML, juvenile chronic myelogenous leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; CR, complete remission; CP, chronic phase; PD, progressive disease; SD, stable disease; ANC, absolute neutrophil count.

The median weight was 36.7 kg (range: 11.1-86.6 kg). Three patients had chronic myelogenous leukemia (CML; 2 in first chronic phase and 1 in the second chronic phase), 3 had acute lymphoblastic leukemia (ALL; 1 CR1 and 2 CR3), 4 had acute myelogenous leukemia (AML; 3 CR1 and 1 CR2), 2 had juvenile CML (JCML; 1 CR1 and 1 progressive disease [PD]), and 1 had myelodysplastic syndrome (MDS; stable disease) (Table 1). Sex, diagnosis, and age did not affect the results (data not shown).

We observed a biphasic elimination of total and active rATG in our patients (Figure 1). Mean CL for the population for total and active rATG was 198 and 4530 mL/day, respectively. The intersubject variability (coefficient of variation [CV%]) in the CL for total and active rATG were 47% and 61%, respectively. When accounting for the positive relation between CL and weight (kg) (Figure 2), intersubject variability (CV%) significantly decreased ($P < .0001$) to 37% and 50%, respectively. On the basis of post hoc estimates, the median beta half-lives for total and active rATG were 27.3 days (range: 25.7-30.4 days) and 12.5 days (range: 5.8-22.4 days), respectively. In addition, the median actual C_{max} values were 57.7 (range: 23.7-80.7) and 4.0 (range: 1.6-8.0) μg/mL, respectively.

Total rATG was measurable in all patients until week 4 and in 11 of 13 patients until week 8. Mean total rATG on day 0 was 53 μg/mL (range: 23.7-80.7 μg/mL) and on day 28 was 15.9 μg/mL (range: 5.1-20.6 μg/mL). Active rATG was measurable in all patients through week 2, 10 patients at week 4, and undetectable (<0.2 μg/mL) in 7 patients at week 8. Mean active rATG on day 0 was 4 μg/mL (range: 1.6-8.0) and on day 28 was 1.13 μg/mL (range <0.2-4.64). Corresponding median concentrations for total and active rATG showed similar elimination curves (data not shown).

All patients achieved neutrophil engraftment at a median of day +19 (range: 15-23 days) and platelet engraftment at a median of day +32 (range: 15-34 days). There were no episodes of grade iii or iv GVHD; 2 patients developed grade 2 GVHD. There were no grade iii or iv nonhematologic serious adverse events, no grade iii or iv serious adverse events related to rATG administration, and no serious infections. One of the patients developed cGVHD (overall incidence of 7.6%). Patients were tested weekly by polymerase chain reaction (PCR) in peripheral blood for cytomegalovirus (CMV), adenovirus (ADV), and Epstein Barr Virus (EBV). All detectable copy numbers for CMV were indications for treatment with ganciclovir or foscarnet. EBV was treated with rituximab if copy numbers exceeded 2000 copies/μg total cellular DNA. Three patients reactivated CMV in the first 45 days posttransplant. One patient developed a positive PCR for ADV, and there were 6 episodes of positive PCR for EBV all with copy numbers less than our threshold for treatment.

DISCUSSION

Our weight-based dosing regimen (total dose 10 mg/kg) of rATG was effective and well tolerated by pediatric patients undergoing an URD BMT for hematologic malignancy. There was no grade 4 nonhematologic toxicity, the overall incidence of aGVHD (maximum grade II) was 15%, and there were no occurrences of grade iii-iv GVHD, despite 11 of 13 patients having peak total rATG levels <70 μg/mL on day 0. Also, there were no serious infections in any patient after transplant. Only 1 of 13 patients relapsed: his day 0 concentration of rATG was <70 μg/mL, and he had an active rATG of >1 μg/mL for 2 weeks (average follow-up 3 years) [2,11].

We observed 2 phases of clearance for both total and active rATG in our patients, which is consistent with the results of Waller et al. [11] and Regan et al. [12]. Although the dose and schedule given to our patients were different from those of previously published studies (Waller et al. [11], 6 mg/kg; Guttmann et al. [10], 7 mg/kg; Seidel et al. [13], dose range

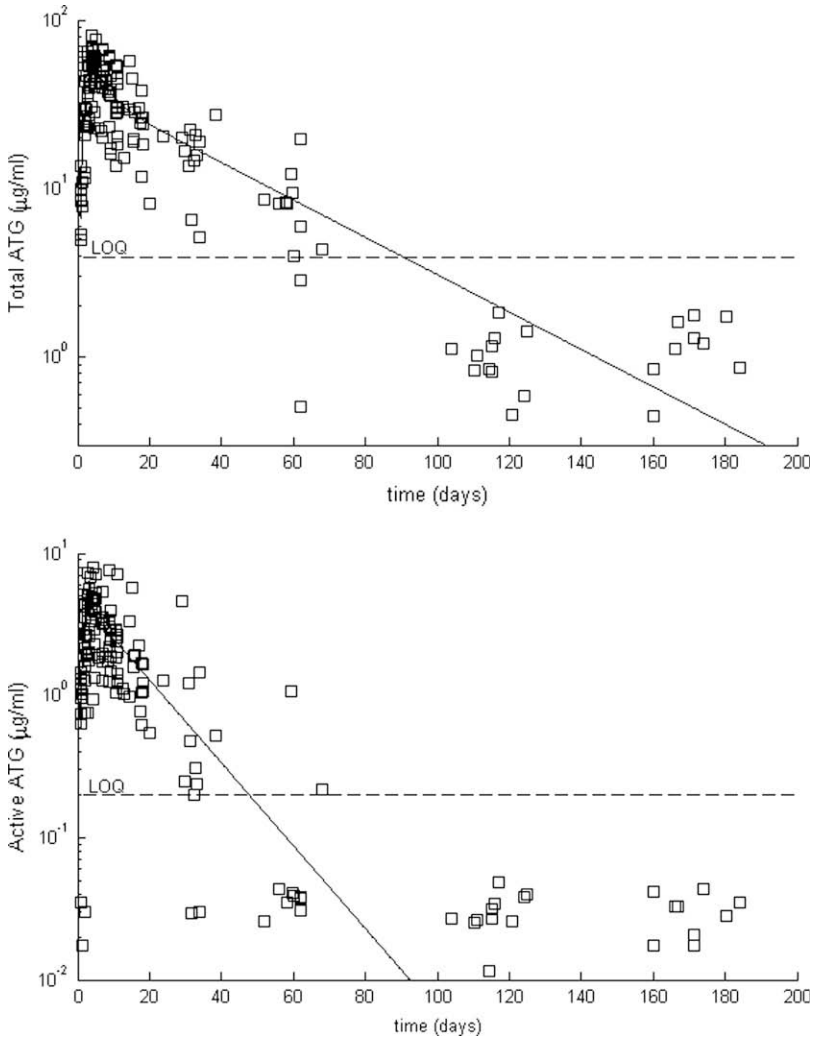

Figure 1. Total rATG (top) and active rATG (bottom) versus time. Squares represent data and the solid curve represents the population average model fitted curve. LOQ: limit of quantification (3.9 mg/mL for total ATG and 0.2 mg/mL for active ATG). Because the assay only indicated that a level was below the LOQ rather than assigning a specific value to it, we randomly assigned it a level below the LOQ to improve the visual interpretation of the results. Time line represents time from first rATG dose. No patient had measurable rATG concentrations prior to the first dose.

7.5-40 mg/kg), the half-lives for total and active rATG were similar to those found in previous adult studies [2,10-12]. Conversely, despite receiving higher doses, the peak total concentrations of rATG for our patients were 67% and 28% lower than in studies by Waller et al. [11] and Guttmann et al. [10], respectively. In the only other pediatric study Seidel and colleagues [13] reported concentrations of active rATG approximately 60% higher than our median peak concentration of 4 μg/mL at a dose 25% lower than the dose used in our study. These differences might be attributed to differences in sampling times between their study and ours. Remberger et al. [2] have suggested that peak concentrations of total rATG <70 μg/mL before and 1 week after BMT increases patient's risk of developing grade II–IV aGVHD. In our study, only 2 patients had total rATG concentrations of >70 μg/mL at day 0 and neither developed GVHD. On the other hand, only 2 of 11 patients who had day 0 concentrations <70 μg/mL developed grade II GvHD (overall incidence 15%).

Waller et al. [11] have suggested that <1 μg/mL of active rATG is subtherapeutic. The 2 patients who developed aGVHD had <1 μg/mL active rATG concentrations by week 2. The concentrations of active rATG were measurable to day 28 in 10 patients who had low incidence of GVHD. This low incidence in our patients might have been because active rATG persisted in the serum; however, inhibition of T cell stimulation by phytohemagglutinin (PHA) in vitro by rATG (as described by Remberger et al. [5]) was not performed as samples were not assayed in real time and thus T cell function could not be assessed.

The primary limitation of our study is the low patient numbers, yet we were able to describe the pharmacokinetics of rATG in pediatric patients in a prospective study. These data support a hypothesis

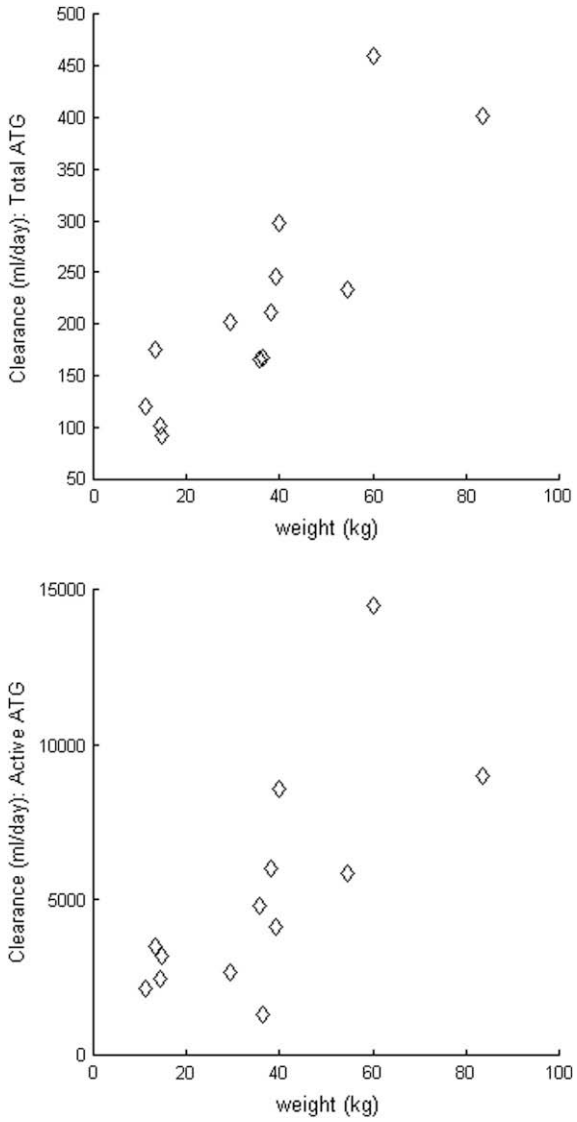

Figure 2. Total rATG clearance (top) and active rATG clearance (bottom) versus weight.

that a total rATG dose of 10 mg/kg can be safely administered without increasing the risk of GVHD, graft rejection, or serious infection in pediatric patients with heamtologic malignancies. The definition of low- or high-dose rATG remains to be determined in the BMT population, although an attempt has been made by Remberger and colleagues [16] to delineate differences in outcome in patients who received different doses of rATG. Using retrospective data from 1989-2003, they evaluated BMT outcomes from 4 different doses of rATG (4 mg/kg, 6 mg/kg, 8 mg/kg, and 10 mg/kg). They found a higher incidence of infections in the 10 mg/kg patients, who received transplants in the 1990s. Our data did not show an increased risk of infections in our patients; this may be because of differences in infection prophylaxis over the past 18 years. Whether total thymoglobulin or active thymoglobulin concentrations are more predictive of GVHD, engraftment or risk of relapse also remain to be answered.

ACKNOWLEGMENTS

Financial disclosure: This work was supported in part by NIH Cancer Center Support Grant P 30CA 21765, and American Lebanese Syrian Associated Charities (ALSAC).

REFERENCES

1. Bacigalupo A, Lamparelli T, Bruzzi P, et al, for Gruppo Italiono Trapianti Midollo Osseo (GITMO). 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;98:2942-2947.
2. Remberger M, Sundberg B. Rabbit-immunoglobulin G levels in patients receiving thymoglobulin as part of conditioning before unrelated donor stem cell transplantation. *Haematologica*. 2005; 90:931-938.
3. Russell JA, Savoie ML, Blogh A, et al. Allogeneic transplantation for adult acute leukemia in first and second remission with a novel regimen incorporating daily intravenous busulfan, fludarabine, 400 cGY total-body irradiation, and thymoglobulin. *Biol Blood Marrow Transplant*. 2007;13:814-821.
4. Remberger M, Storer B, Ringden O, Anasetti C. Association between pretransplant thymoglobulin and reduced non-relapse mortality rate after marrow transplantation from unrelated donors. *Bone Marrow Transplant*. 2002;29:391-397.
5. Remberger M, Mattsson J, Ringden O. Polyclonal anti-T-cell globulin as part of the preparative regimen for pediatric allogeneic stem-cell transplantation. *Pediatr Transplant*. 2001;5:285-292.
6. Basara M, Baurmann H, Kolbe K, et al. Antithymocyte globulin for the prevention of graft-versus-host disease after unrelated hematopoietic stem cell transplantation for acute myeloid leukemia: results from the multicenter German cooperative study group. *Bone Marrow Transplant*. 2005;35:1011-1018.
7. 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;12:573-584.
8. Simpson D. New developments in the prophylaxis and treatment of graft versus host disease. *Expert Opin Pharmacother*. 2001;2: 1109-1117.
9. McCaul KG, Nevill TJ, Barnett MJ, et al. Treatment of steroid-resistant acute graft-versus-host disease with rabbit antithymocyte globulin. *J Hematother Stem Cell Res*. 2000;9:367-374.
10. Guttmann RD, Caudrelier P, Alberici G, Touraine JL. Pharmacokinetics, foreign protein immune response, cytokine release, and lymphocyte subsets in patients receiving thymoglobuline and immunosupression. *Transplant Proc*. 1997;29(Suppl7A):24S-26S.
11. Waller EK, Langston AA, Lonial S, et al. Pharmacokinetics and pharmacokynamics of anti-thymocyte globulin in recipients of partially HLA-matched blood hematopoietic progenitor cell transplantation. *Biol Blood Marrow Transplant*. 2003;9:460-471.
12. Regan JF, Lyonnais C, Campbell K, Van Smith L, Buelow R. for the US Thymoglobulin® Multi-Center Study Group. Total and active thymoglobulin levels: effects of dose and sensitization on serum concentrations. *Transpl Immunol*. 2001;9:29-36.
13. Seidel MD, Fritsch G, Matthew-Martin S, et al. Antithymocyte globulin pharmacokinetics in pediatric patients after hematopoietic stem cell transplantation. *J Pediatr Hematol Oncol*. 2005;27: 532-536.
14. Glucksbert H, Storb R, Feder A, et al. Clinical manifestations of graft-versus host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation*. 1974;18:295-304.
15. *NONMEM Users' Guide Parts I-VIII*. San Francisco, CA: NONMEM Project Group, University of California at San Francisco; 1998.
16. Remberger J, Svahn B, Mattsson J, Ringden O. Dose study of thymoglobulin during conditioning for unrelated donor allogeneic stem-cell transplantation. *Transplantation*. 2004;78(1):122-127.