Letter to the Editor

Evaluation of antithymocyte globulin pharmacokinetics and pharmacodynamics in children

To the Editor:

Since its introduction in the early 1970s, the use of antithymocyte globulin (ATG) has spread widely in the setting of hematopoietic stem cell transplantation (HSCT), mainly for preventing graft-versus-host disease (GVHD) and graft rejection.[1,2] ATG remains a double-edged sword because it is associated with a delayed immunologic recovery related to the depletion of mature T cells infused with the graft and impaired thymopoiesis.[3] This delayed immune reconstitution results in a higher risk of infections, especially viral reactivations.[3]

Thymoglobulin (Genzyme, Cambridge, Mass) is the most commonly used preparation of rabbit antithymocyte globulin (rATG) in pediatric HSCT. As a GVHD prophylaxis, children undergoing an allogeneic HSCT usually receive a Thymoglobulin dose ranging from 7.5 to 20 mg/kg administered for 3 to 4 consecutive days starting from day −9 or day −5 before graft infusion. As a salvage therapy, rATG is administrated early after the diagnosis of steroid-resistant acute GVHD with a great variability in dose and duration of treatment.[4,5] Thus determination of the optimal dose of ATG for prophylaxis and treatment of acute GVHD remains unclear and is based solely on experience.

To investigate this issue, we initiated a study in a pediatric population undergoing allogenic HSCT for primary immunodeficiency and receiving Thymoglobulin as part of the conditioning regimen. This study retrospectively analyzed all the medical records of children undergoing HSCT in the Department of Immuno-Hematology and Rheumatology of Necker Children's Hospital (Paris, France) between January 2013 and July 2014. All research participants or the participant's parents or legal guardian provided informed consent.

Two included patients received rATG for treatment of steroid-resistant grade IV acute GVHD during the posttransplantation period. Clinical and biological examinations (including lymphocyte counts) were performed before treatment and every day after dose administration. Active rATG concentrations were monitored and measured with a quantitative flow cytometric assay, as described previously with minor modifications.[6] Jurkat cells (50,000 in 50 μL) were incubated with 50 μL of standard and a 4-fold dilution of patients' sera. For the standard reference curve, rATG (5 mg/mL, Thymoglobulin, Genzyme) was diluted by making 2-fold dilutions in triplicate, establishing a final range from 0.01 to 5 μg/mL. Finally, the cells were washed, and flow cytometric analysis was performed immediately thereafter on a FACS scanner (Becton Dickinson Biosciences, Franklin Lakes, NJ). The reference curve was used to determine the concentration of active ATG in patients' samples.

A dynamic model linking active rATG concentrations and lymphocyte counts was considered (see Fig E1 in this article's Online Repository at www.jacionline.org). The lymphocyte time course was then obtained by means of addition of amounts in the "free lymph" and "rATG-lymph complex" compartments at a given time. Data were analyzed with Monolix software.[5] For goodness-of-fit evaluation, diagnostic plots were used, including normalized prediction distribution errors.[7]

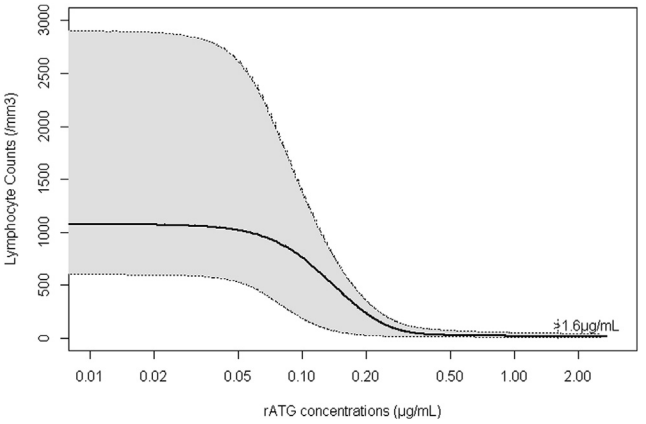


FIG 1. Concentration effect relationship of rATG.

A total of 10 patients with 56 rATG and 136 lymphocyte measurements were available for analysis. Initial characteristics of the patients are displayed in Table E1 in this article's Online Repository at www.jacionline.org. Two patients had protein-losing enteropathy (PLE) in relation to grade IV acute GVHD during follow-up. No antibody to ATG was detected in these 2 patients.[6] rATG clearance increased as a linear function of weight ($P < 10^{-5}$, Wald test). After inclusion of body weight, a significant effect of PLE was found to dramatically increase both clearance of rATG and the elimination rate constant of rATG ($P < 10^{-5}$ and $P = .015$, respectively, Wald test). The effect of the infused lymphocyte dose was tested as a covariate influencing either clearance of rATG or the production rate constant of lymphocytes, or this dose was included in the dynamic model as an additional input into the "free lymph" compartment (see Fig E1). These 2 approaches did not show a significant effect of the injected lymphocytes on rATG clearance. All the model parameters were well estimated given their low relative standard error (see Table E2 in this article's Online Repository at www.jacionline.org). Goodness of fit shows that the prediction satisfactorily matches the observations (see Fig E2 in this article's Online Repository at www.jacionline.org).

According to Fig 1, an rATG threshold peak concentration of 1.6 μg/L appeared to be sufficient from 600 to 2900/mm^3 (first and third quartiles of the total lymphocyte count at baseline in our population) to ensure total lymphocyte depletion. Therefore the dosing scheme proposed should be used only in this range of baseline lymphocytes. According to simulations, a 12 mg/kg total dose achieved concentrations above the target level for at least 95% of patients. However, this dose should be split into 3 successive 4 mg/kg doses to maintain concentrations in the range of reported values. Accordingly, Fig 2 shows the predicted concentration time course of rATG after 3 successive daily doses of 4 mg/kg administered over 12 hours. This dosing scheme ensured concentrations of greater than the 1.6 μg/mL target for 95% of patients and achieved also a complete depletion of lymphocyte counts supporting its use at day −3 before HSCT. Because of the very short rATG half-life in children with PLE, multiple administrations of rATG might be needed.

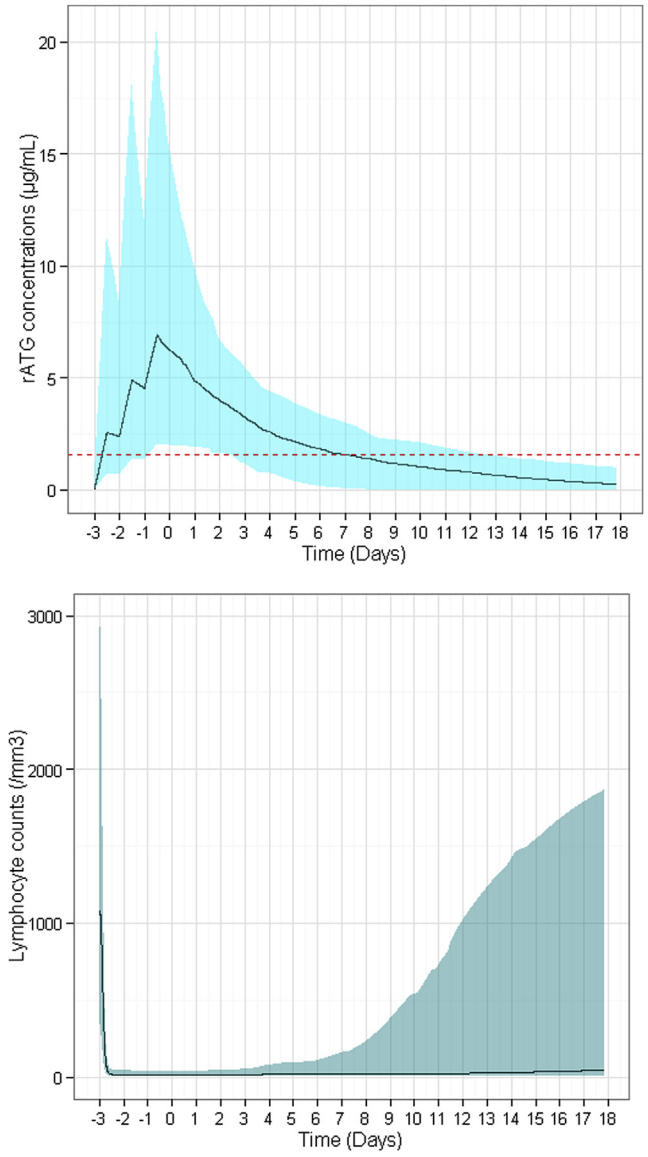


FIG 2. Predicted concentration time course of rATG after 3 successive doses of 4 mg/kg in patients without PLE. The *shaded areas* stand for the fifth and ninety-fifth percentiles of predictions. The *dashed line* corresponds to the threshold 1.6 µg/mL concentration.

This study described rATG pharmacokinetics and pharmacodynamics in 10 children. Body weight was the most significant parameter on clearance and explained a substantial part of the observed variability. Clearance appears to increase with body weight linearly from age 1 to 10 years (ie, the first and third quartiles of our population), which is in agreement with the results of Call et al,[8] who reported a linear correlation between rATG clearance and body weight, supporting a unique milligram per kilogram dose for rATG in this age range.

Beyond body weight, PLE explained also a part of the remaining variability in rATG clearance. These patients had a 3-fold increase in clearance (2.22 vs 6.8 L/y). The PLE effect was satisfactorily estimated; however, the effect size of PLE on rATG clearance was evaluated only in 2 patients and should be cautiously taken. Nevertheless, the kinetics of both lymphocytes and rATG in these patients are clearly atypical compared with values in others, and the active rATG half-life appeared to be dramatically decreased (4.5 days vs 18 hours in patients with PLE).

The elimination clearance found in this study for rATG was consistent with the value reported in a recent population pharmacokinetic study published by Admiraal et al[9] performed in a large group of children (2.22 vs 2.20 L/d). However, in our study we went further and combined both rATG pharmacokinetics and immune reconstitution. The simulations performed with this model allowed us to derive the rATG target concentration (1.6 µg/mL) to achieve *in vivo* total depletion of lymphocyte counts. This value is consistent with the findings reported by Genestier et al,[10] who observed *in vitro* that the ATG effect was concentration dependent, with maximal cytotoxicity at 1 µg/mL.

In conclusion, this study reports on rATG pharmacokinetics in children. Therapeutic targets were successfully derived and simulations were performed to provide hypotheses regarding a pediatric dosing strategy. These assumptions should be prospectively confirmed.

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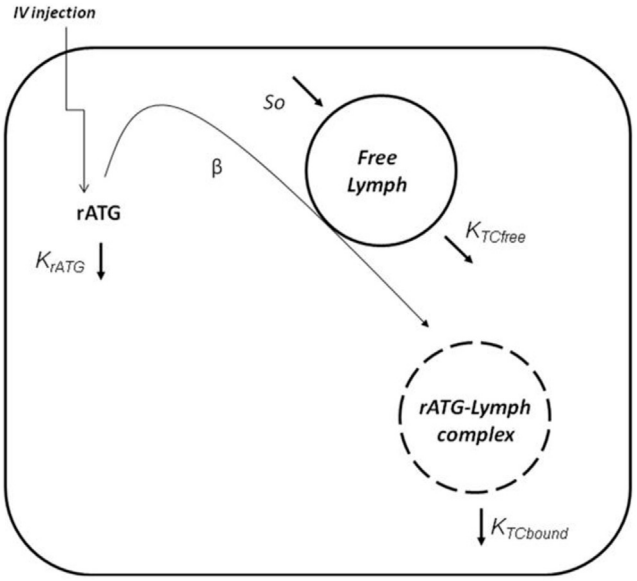


FIG E1. Schema of the mechanistically based pharmacokinetic and pharmacodynamic model used for rATG concentration and lymphocyte count time course determinations. Lymphocytes are produced at a rate labeled *So*. These cells die at a rate labeled K_{TCfree} and bind rATG according to the rate constant β. Antibody-lymphocyte complexes are cleared at the rate $K_{TCbound}$. Antibodies are administered according to the individual dose and rate of infusion and are cleared with the elimination rate constant of rATG (K_{rATG}). If we assume a system at steady state before initiating rATG treatment, the initial conditions of the system are as follows: rATG(0) = 0; FreeLymph(0) = So/K_{TCfree}; and rATG-Lymph complex(0) = 0.

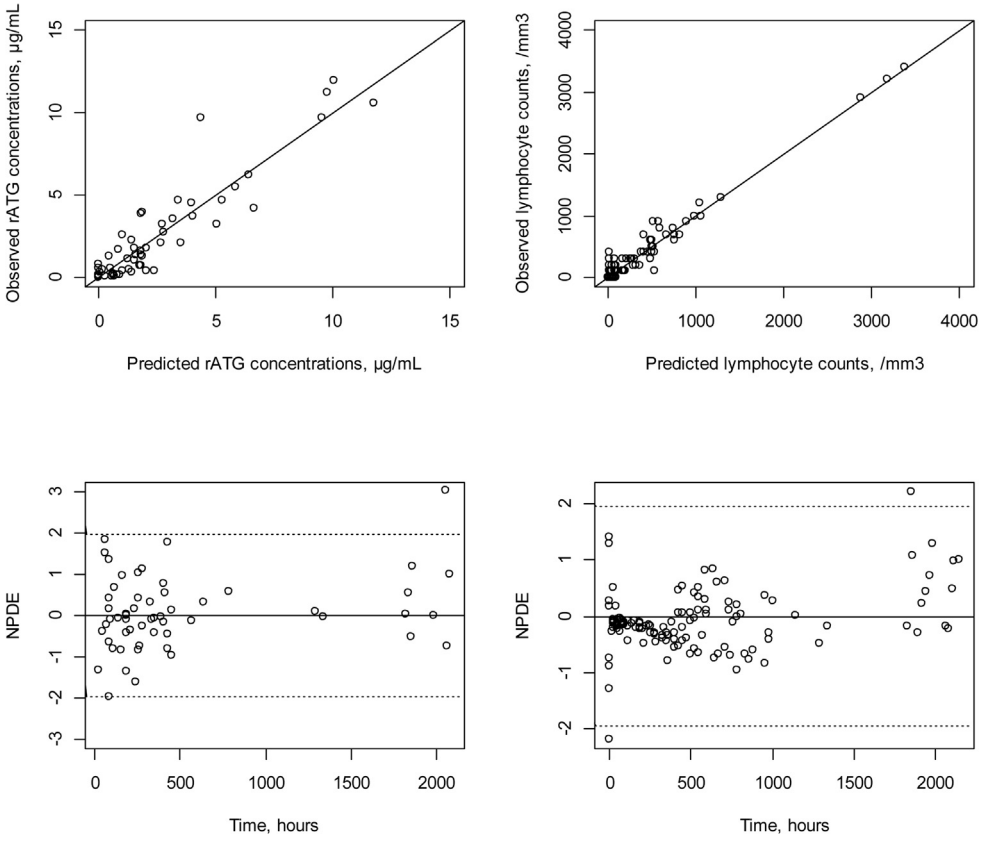


FIG E2. Goodness-of-fit plots: observed versus population-predicted rATG concentrations and lymphocyte counts and normalized prediction distribution errors versus time for both rATG concentrations and lymphocyte counts. *NPDE,* Normalized prediction distribution error.

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TABLE E1. Patients' demographics

Patient ID	Weight (kg)	Age (y)	Disease	PLE	Conditioning regimen	rATG	Donor	GVHD prophylaxis	CD34 dose ($\times 10^6$/kg)	Engraftment	GVHD
001	50	17	GATA2	No	Busulfan* Fludarabine, 160 mg/m^2	10 mg/kg day −5 to day −2	URD 9/10	*In vitro* T depletion	6.35	No	No
002	14	3	CGD	No	Busulfan* Fludarabine, 160 mg/m^2 Thiotepa 10 mg/kg	10 mg/kg day −5 to day −2	Haploidentical	*In vitro* T depletion	18.03	Yes (day + 18)	No
003	9.1	13	CID	No	Busulfan* Fludarabine, 160 mg/m^2	10 mg/kg day −5 to day −2	URD 10/10	Cyclosporine/MMF	19.89	Yes (day + 16)	Grade III (day + 17)
004	8.5	1	SCID-X1	No	Busulfan* Fludarabine, 160 mg/m^2	10 mg/kg day −5 to day −2	Haploidentical	*In vitro* T depletion	14.80	Yes (day + 34)	No
005	6.3	0.29	FLH	No	Busulfan* Fludarabine, 180 mg/m^2 Thiotepa, 5 mg/kg	20 mg/kg day −14 to day −11	Haploidentical	*In vitro* T depletion	14.82	Yes (day + 15)	No
006	32	11	Fanconi	No	Fludarabine, 100 mg/m^2 Cyclophosphamide, 60 mg/kg	10 mg/kg day −5 to day −2	URD 10/10	Cyclosporine/MMF	5.00	Yes (day + 12)	Grade I (day + 60)
007	9.7	0.8	MHCII	No	Busulfan* Fludarabine, 160 mg/m^2	10 mg/kg day −5 to day −2	URD 10/10	Cyclosporine/MMF	18.33	Yes (day + 15)	Grade III (day + 9)
008†	16	6	GM-CSFR	Yes	Busulfan* Fludarabine, 160 mg/m^2	10 mg/kg day −5 to day −2	URD 10/10	Cyclosporine/MMF	7.57	Yes (day + 15)	Grade IV (day + 22)
009	9.7	1.17	SCID	No	Busulfan* Fludarabine, 160 mg/m^2	10 mg/kg day −5 to day −2	Haploidentical	*In vitro* T depletion	20.08	Yes (day + 16)	No
010†	10	1.58	CD40L	Yes	Busulfan* Fludarabine, 160 mg/m^2	10 mg/kg day −5 to day −2	URD 10/10	Cyclosporine/MMF	3.87	Yes (day + 22)	Grade IV (day + 48)

CD40L, CD40 ligand deficiency; *CGD*, chronic granulomatous disease; *CID*, combined immunodeficiency; *FLH*, familial lymphohistiocytosis; *GATA2*, GATA2 deficiency; *GM-CSFR*, GM-CSF receptor deficiency; *MHCII*, MHC class II deficiency; *MMF*, mycophenolate mofetil; *SCID*, severe combined immunodeficiency; *SCID-X1*, X-linked severe combined immunodeficiency; *URD*, unrelated donor.

*Busulfan dosage was adapted according to daily drug monitoring to reach a total area under the curve of 90 ± 5 mg/mL·h.

†ATG was administrated as salvage therapy for grade IV acute GVHD.

TABLE E2. Population pharmacokinetic and pharmacodynamic parameters of rATG

Parameters	Median	RSE (%)	*P* value
CL (mL/h)	92.5	18	
θWT_{CL}	1.22	17	<10−4
θPLE_{CL}	1.13	25	<10−4
K_{rATG}	0.0062	30	
θPLE_{KrATG}	1.79	41	.015
β	$9.82 \times 10-5$	1	
So	9.73	23	
K_{TCfree}	0.00905	0	
$K_{TCbound}$	0.762	0	
ωCL	0.282	48	
ωK_{rATG}	0.891	22	
ωSo	0.744	23	
σrATG (μg/mL)	1.28	10	
σLymph (/mm^3)	149	6	

β, Rate constant of complex lymphocyte-antibody formation; *CL*, clearance of rATG; K_{rATG}, elimination rate constant of rATG; $K_{TCbound}$ elimination rate constant of lymphocytes bound to antibodies; K_{TCfree}, elimination rate constant of unbound lymphocytes; $\sigma Lymph$, residual variability for lymphocyte counts; θPLE_{CL}, influential factor of PLE on CL; θPLE_{KrATG}, influential factor of body weight on K_{rATG}; $\sigma rATG$, residual variability for rATG concentrations; *RSE*, Relative standard error (Standard error of estimate/Estimate × 100); *So*, production rate constant of lymphocytes; θWT_{CL}, influential factor of bodyweight on CL.