Basiliximab Combined with Low-Dose Rabbit Anti-Human Thymocyte Globulin: A Possible Further Step toward Effective and Minimally Toxic T Cell–Targeted Therapy in Kidney Transplantation

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In high-risk kidney transplant recipients, induction therapy with rabbit anti-human thymocyte globulin (RATG) reduces the risk for acute rejection but is associated with significant toxicity, opportunistic infections, and cancer. Using reduced doses of RATG combined with anti–IL-2 antibodies may achieve the same antirejection activity of standard-dose RATG but with a better safety profile. This randomized, open-label study compared the efficacy, tolerability, and costs of low-dose RATG (0.5 mg/kg per d) plus basiliximab (20 mg 4 d apart) *versus* standard-dose RATG (2 mg/kg per d) in 33 consecutive high-risk renal transplant recipients (living-related transplant recipients, sensitized patients or patients who received another transplant, and patients with delayed graft function) over 6 mo of follow-up. All patients received concomitant therapy with steroids, cyclosporin A, and azathioprine or mycophenolate mofetil. Seventeen patients received low-dose RATG plus basiliximab, and 16 received standard-dose RATG. Patient (100 *versus* 100%) and graft (94 *versus* 100%) survival were comparable in the two groups, but the incidence of fever (17.6 *versus* 56.5%; $P = 0.01$), leukopenia (23.5 *versus* 56.3%; $P < 0.05$), anemia (29.4 *versus* 62.5%; $P < 0.05$), cytomegalovirus reactivations (17.6 *versus* 56.5%; $P = 0.01$), the number of transfused units (0.5 ± 0.9 *versus* 2.0 ± 2.4; $P < 0.001$), and treatment costs (3652 ± 704 *versus* 5400 ± 1960 euro; $P = 0.001$) were lower with low-dose RATG plus basiliximab than with standard-dose RATG. There was one episode of biopsy-proven acute rejection on low-dose RATG plus basiliximab, and there were two on standard-dose RATG. In renal transplantation, induction therapy with basiliximab plus low-dose RATG effectively prevents acute rejection and is safer and more cost-effective than induction with standard-dose RATG.

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Events surrounding the renal transplantation procedure and initial hospitalization are important determinants of early and long-term allograft function (1). The initial immunosuppressive regimen may have a significant impact on these events, but the optimal immunosuppressive strategy for renal transplant recipients is not known. Induction therapy refers to the use of biologic agents, such as antilymphocyte antibodies, at the time of transplantation (2). It was conceived for the modification of the host immune system at the time of donor antigen presentation. Therefore, the purpose of the induction therapy is to produce profound peripheral blood lymphocyte depletion to block T cell activation and/or other immune cell function early posttransplantation (3). The additional advantage is to limit the use of calcineurin inhibitors in the immediate posttransplantation period in patients who have or at risk for delayed graft function (DGF), avoiding unsought renal vasoconstriction that negatively affects recovery of graft function (4). Both monoclonal and polyclonal agents have been used for induction therapy. However, because of the redundancy of the immune system, the broad specificity of the polyclonal agents produce more effective induction than monoclonal antilymphocyte therapy (5). Early 7- to 10-d induction course with polyclonal antilymphocyte globulin or horse-derived polyclonal anti-human thymocyte globulin has contributed to reduce the rate of acute rejection and improve early graft function in kidney transplant recipients (6,7). Even better results as far as frequency and severity of rejection episodes then were achieved with rabbit-derived polyclonal anti-human thymocyte globulin (RATG) (8), which thereafter became the standard biologic agent for induction therapy in kidney transplantation (9).

Nevertheless, the good efficacy profile of RATG induction therapy is burdened by significant toxicity (10). Symptoms of cytokine release syndrome such as fever, chills, leukopenia, and thrombocytopenia occur in 40 to 60% of patients during RATG

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infusion, which may require dose reduction or even treatment interruption (10–12). Moreover, induction therapy is associated with increased risk for cytomegalovirus (CMV) reactivation and posttransplantation lymphoproliferative disease, which may limit its risk-benefit and cost-effectiveness profile (13).

In the past 10 yr, nondepleting mAb that specifically target activated T cells have been developed with the aim of providing effective immunosuppression without the adverse effects related to profound and aspecific T lymphocyte depletion with RATG (14–17). mAb against the CD25 subunit of the IL-2 receptor (IL-2R) on activated T cells, such as basiliximab (14–16) and the humanized antibody daclizumab (14,17), now are used widely because of their effectiveness with an excellent safety profile (14,15). A recent meta-analysis found that addition of an IL-2R antagonist over a standard immunosuppressive therapy significantly reduced the number of clinically diagnosed acute rejections by 34% and of steroid-resistant rejections by 49% (15). Of importance, the safety profile of IL-2R antagonists was similar to placebo, with an even lower incidence of CMV reactivation (15). Moreover, a recent randomized, multicenter study on renal transplant recipients at low immunologic risk found that the efficacy of IL-2R antagonists against acute allograft rejection was comparable to that of polyclonal antibodies (18). This however was not a uniform finding. Patients who were at high risk for rejection and DGF on cyclosporin A (CsA), mycophenolate mofetil (MMF), and steroids had two-fold excess acute rejection with anti–IL-2R antibodies as compared with polyclonal antibodies (19). Therefore, in sensitized patients or patients who received another transplant (19), IL-2R antibodies may not be as effective as polyclonal antibodies in preventing graft rejection.

Increasing efforts have been devoted to identifying novel induction protocols that can increase efficacy, particularly in high-risk patients, with the lowest possible toxicity. Some authors have proposed to use lower-than-standard doses of RATG to reduce severity of acute cytolysis syndromes and possible late complications of overimmunosuppression (20). In nonhuman primates that received a skin transplant, induction therapy with low-dose RATG was associated with a median survival time of the graft approximately one half shorter than that with standard-dose RATG (21). In humans who received a bone marrow transplant, low-dose RATG did not provide the same antirejection activity as standard dose and was associated with a 17% excess of graft-*versus*-host disease (22).

A possible explanation for the above findings is that, when used at low doses, RATG fails to achieve a complete and persisting lymphocyte depletion (21) and to block fully T cell activation, which may result in a less effective prevention of allograft acute rejection. If this hypothesis is correct, then add-on therapy with anti–IL-2R antibody in an induction protocol that is based on the use of low-dose RATG might be a rational strategy to inhibit fully T cell function even without achieving a complete T cell depletion. This is expected to prevent acute allograft rejection at least as effectively as standard-dose RATG but with less toxicity. Here we report the results of a pilot, explorative study that was designed to test the possibility that basiliximab that is given to patients with a clinical indication to induction therapy combined with low-dose RATG is at least as effective as RATG at standard dose, also with a favorable safety profile.

Materials and Methods

Patient Selection and Study Design

Our pilot, explorative study was aimed at comparing in a prospective, randomized, parallel-group design the risk-benefit profile of dual (basiliximab plus low-dose RATG) *versus* single (standard-dose RATG) T cell target therapy in a consecutive series of new incident renal transplant recipients who, per Renal Transplant Center policy, had a specific indication for induction therapy. We included all consecutive eligible patients who were selected for a kidney transplant according to the Nord Italia Transplant guidelines (23) and referred to our Renal Transplant Center from October 2000 to September 2003. Before randomization, eligible patients who provided written informed consent to study participation according to the Declaration of Helsinki were stratified into two groups. One group (group A) included recipients of a living-related transplant or patients who were at increased immunologic risk, such as sensitized (*i.e.*, with a panel reactive antibody titter >50%) or patients who received another transplant. The second group (group B) included patients with DGF, defined as need for dialysis therapy within 3 d posttransplantation without evidence of other causes of primary nonfunction of the graft, such as vascular thrombosis or urinary tract obstruction. Patients with a previous nonkidney transplant or recipients of multiple organ transplants or of kidneys from HLA-identical living donors were not included.

Within each group, patients were randomly assigned on a 1:1 basis to 7 d of treatment with low-dose RATG (0.5 mg/kg per d) plus basiliximab given in two 20-mg intravenous injections 4 d apart or standard-dose RATG (2 mg/kg per d). Group A patients were randomly assigned at the time of transplantation; group B patients were randomly assigned at the time of the first dialysis session. Patients' allocation to one of the two treatment regimens was centralized at the Unit of Biostatistics of the Clinical Research Center for Rare Diseases "Aldo e Cele Daccò" of the Mario Negri Institute for Pharmacologic Research under the responsibility of an independent investigator who was not involved in study design or conduct.

The study protocol was approved by the Ethical Committee of the "Azienda Ospedaliera O.R. di Bergamo." This study was investigator initiated and internally funded. There was no pharmaceutical company involvement in study design, conduct and monitoring, and data analysis and reporting.

Organ Procurement and Surgical Procedures

Donors were identified and selected through the Nord Italia Transplant network, and organs were procured by standard techniques and cold preserved. Cold ischemia times, defined as the interval from donor cross-clamp to organ removal from cold storage for anastomosis, were recorded. There were no changes in the surgical equipment and technical procedures throughout the whole study period. Briefly, the grafts were placed into the right or left iliac fossa, extraperitoneally, and verapamil was injected into the renal artery before vascular anastomosis. Saline, albumin, furosemide, and mannitol were administered before the renal artery unclamping according to a standard protocol that was applied to all patients who entered the study. Central venous pressure was monitored to guide fluid infusion during the surgery. After transplantation, all patients were transferred directly from the surgical room to our unit of nephrology, where they were followed up to discharge. After discharge, they were followed by serial visits in an outpatient clinic.

Posttransplantation Monitoring and Immunosuppression

All patients were on a standardized triple immunosuppressive regimen that included steroids, CsA, and MMF. They received 500 mg of methylprednisolone (MP) intravenously on the day of transplantation, 200 mg on days 1 and 2, 150 mg on day 3, and 100 mg on day 4 and then were shifted to oral prednisone 75 mg on day 5 and 50 mg on day 6 posttransplantation and methylprednisone 20 mg/d from day 7 to day 11, 16 mg/d from day 12 to day 60, 12 mg/d from day 61 to day 120, and 8 mg/d throughout the whole observation period. Intravenous CsA (3 to 5 mg/kg per d) was infused for 24 to 36 h posttransplantation. Then patients were shifted to oral CsA (8 to 10 mg/kg per d, progressively tapered to 4 mg/kg per d over the first month). Both intravenous and oral doses were adjusted to maintain blood trough levels within 250 to 440 ng/ml from days 0 to 7, 200 to 300 ng/ml from days 8 to 28, and 150 to 250 ng/ml up to study end. MMF (2 g/d) was started on posttransplantation day 1, and the dose was adjusted to avoid severe leukopenia (white blood cell [WBC]count $<2000/\mu l$) or thrombocytopenia (platelet count $<50{,}000/\mu l$). The indication for blood transfusions was *a priori* defined as an hemoglobin level <10 g/dl or a hematocrit $<30\%$.

Study Treatments

Group A. The first infusion of low-dose (0.5 mg/kg per d) or standard-dose (2 mg/kg per d) RATG (thymoglobulin; Sangstat, Milan, Italy) was started on the day of transplantation as soon as the intravenous injection of 500 mg of MP had been completed. Then, low-dose and standard-dose RATG were infused daily up to day 7 posttransplantation. RATG standard-dose and treatment duration were defined according to manufacturer guidelines, suggesting, for the prevention of acute rejection of a kidney graft, the daily infusion of 1.25 to 2.5 mg/kg for a period of 7 to 21 d (10), and reflected the standard induction protocol in use in our Renal Transplant Center. Daily doses were adjusted according to WBC count to avoid severe leukopenia (total WBC count <2000 cells/μl). In patients who were randomly assigned to the dual induction regimen, basiliximab (20 mg) was injected intravenously before transplantation and 4 d later.

Group B. At the time of the first dialysis session, CsA infusion was stopped and RATG infusion was started (same protocol as for group A patients) as soon as the first dialysis session was completed. In patients who were randomly assigned to the dual induction regimen, basiliximab (20 mg) was injected intravenously immediately before the first RATG infusion and 4 d later.

Patient Monitoring and Follow-Up

In all patients, routine clinical and laboratory parameters, including serum creatinine concentration, WBC and platelet counts, and leukocyte formula were monitored daily up to posttransplantation day 14 and thereafter every other day up to 1 mo, every week from month 2 to month 3 after transplantation, and then monthly up to the end of follow-up. Blood CsA concentrations were measured daily for the first 15 d posttransplantation and every month thereafter. The blood samples were collected just before ("trough level") and 2 hr after the morning dose of CsA. Additional evaluations were performed whenever deemed clinically appropriate.

Prophylaxis, Monitoring, and Treatment of CMV Reactivation

From randomization, all patients received per protocol a 14-d ganciclovir course, at doses adjusted according to serum creatinine levels. For the diagnosis of CMV reactivation, pp65 antigenemia was monitored at our Renal Transplant Center in all patients every 2 to 4 wk until 6 mo posttransplantation and whenever deemed clinically appropriate (24). A 14-d course of intravenous ganciclovir was started whenever a positive pp65 antigenemia was found and was continued for at least 1 wk after the CMV-positive cells had been cleared persistently from the circulation.

Diagnosis and Treatment of Rejection Episodes

The diagnosis of acute rejection episodes was established on the basis of clinical criteria, including a transient increase in serum creatinine concentration not explained by CsA trough levels above the target levels or by kidney hypoperfusion, vascular thrombosis, or urinary tract obstruction, and was confirmed by evidence of renal function recovery after a standard course of intravenous steroid pulses (MP 500 mg/d for 3 d, progressively tapered to the maintenance oral dose over 10 d). A kidney biopsy was performed per protocol to confirm or exclude the diagnosis of acute rejection in patients who had a clinical diagnosis and did not recover within 3 to 5 d of steroid therapy and in patients who had DGF and at 7 d posttransplantation still were dialysis dependent.

Cost Analysis

Cost analysis took the perspective of healthy services providers, and the costing method determined the direct health care costs that were associated with each treatment. All resource use was valued at prices in euro in 2003. The costs of basiliximab, RATG, and CsA administered during the anuria period were estimated on the basis of respective prices multiplied by the days on therapy and by dose. Costs for ganciclovir prophylaxis and treatment of CMV reactivation episodes during the follow-up also were quantified by a similar approach. Costs for days of hospitalization, dialysis sessions, and transfused red blood cell units were obtained from the Economical Department of our hospital.

Statistical Analyses

The analysis was intention to treat. Comparisons between the two treatment groups were done by unpaired t test, Wilcoxon rank-sums test, χ^2 test, or Fisher exact test as appropriate. Comparison analyses considered the two treatment groups as a whole and within the subgroup of patients with DGF (including group A patients who had been randomly assigned at the time of transplantation). Data are expressed as mean $\pm$ SD or median (interquartile range) if not otherwise indicated. $P < 0.05$ was considered as statistically significant.

Sample Size

This was a pilot, explorative study, and the sample size was not calculated *a priori* on the basis of an expected treatment effect. Therefore, we planned to include into the study all eligible patients who were identified throughout a predefined recruitment period of 3 yr. Because approximately 12 patients who satisfied the selection criteria were expected to be referred to the center every year, a total of 36 patients were expected to enter the study during the predefined recruitment period. On the basis of previous experience, we predicted a 70% incidence of leukopenia or thrombocytopenia in patients who were receiving standard RATG doses (25). In those who were receiving low-dose RATG, we assumed an incidence of events (25%) similar to that previously observed in patients who had similar characteristics and were not given RATG induction therapy (24). Therefore, we calculated that, with the expected number of patients and incidence of events, the study had a 70% power to detect as statistically significant ($\alpha = 0.05$, two-tailed test) the expected difference in leukopenia or thrombocytopenia occurrence (70 *versus* 25%) between the two treatment groups. Because of the explorative nature of the study, this was considered a reasonable sam-

ple size to compare the tolerability profile (at least in terms of bone marrow toxicity) of the two study treatments.

Results

A total of 33 eligible patients were identified during the recruitment period, and all of them consented to enter the study. Seventeen were randomly assigned to low-dose RATG plus basiliximab, and 16 were randomly assigned to standard-dose RATG treatment. Characteristics of patients at randomization and of corresponding donors were very well comparable (Table 1). Seventeen patients (nine on basiliximab plus low-dose RATG, eight on standard-dose RATG) were randomly assigned at the time of transplantation (group A), and 16 patients (eight in each treatment group) were randomly assigned at the time when dialysis therapy was started because of DGF (group B). Six patients of group A (three in each treatment regimen) developed DGF after randomization, and their outcome data were considered together with those of the other 16 patients with DGF.

Efficacy

Patient and graft survival at 6 mo posttransplantation were comparable in the two treatment arms (94 and 100% *versus* 100 and 100% in the low-dose RATG plus basiliximab *versus* standard-dose RATG, respectively). There was only one graft loss in the standard-dose RATG that was due to a refractory, biopsy-proven vascular rejection. The graft was explanted 2 mo posttransplantation, after a second biopsy showed a scarred kidney with global glomerulosclerosis and diffuse tubulointerstitial fibrosis.

Overall, patients who were receiving low-dose RATG plus basiliximab showed a trend to a faster graft function recovery (Figure 1A) as compared with those who were receiving standard-dose RATG dose. In the subgroup with DGF, the number of dialysis sessions (1.7 ± 0.7 *versus* 1.3 ± 0.6) and of days to last dialysis (3.4 ± 3.2 *versus* 2.2 ± 2.8) and to first spontaneous, non–dialysis-induced reduction in serum creatinine (5.0 ± 3.6 *versus* 4.1 ± 2.9) were comparable in the low-dose RATG plus basiliximab *versus* standard-dose RATG group, respectively. Length of initial (8.4 ± 3.2 *versus* 9.5 ± 4.8 d) and subsequent hospitalizations (8.5 ± 13.7 *versus* 7.4 ± 13.8 d) was comparable in low-dose RATG plus basiliximab *versus* the standard-dose RATG group as well.

Table 1. Main donor and recipient characteristics in the two treatment regimens

	Standard-Dose RATG (n = 16)	Low-Dose RATG (n = 17)
Gender (M/F)	4/12	7/10
Sensitized recipients	2	2
Second transplantation	4	4
Delayed graft function	11	11
Comorbid conditions	10	10
hypertension	7	10
diabetes	1	0
other	2	1
Cadaveric donor	14	14
Living donor	2	3
Mean cold ischemia time (h)	17.6	15.4

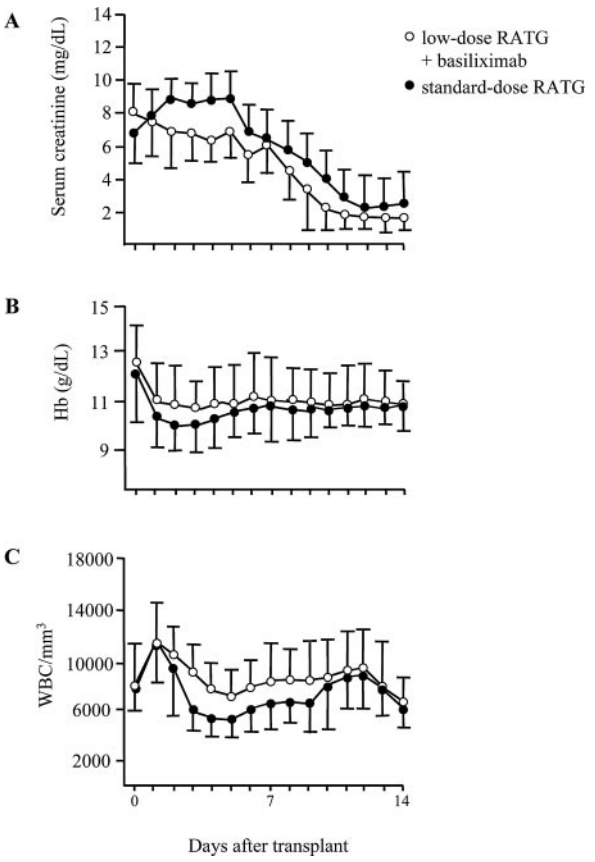

Figure 1. Time course of serum creatinine concentration (A), hemoglobin (Hb) concentration (B), and white blood cell (WBC) count (C) in the low-dose rabbit anti-human thymocyte globulin (RATG) plus basiliximab group (○) and in the RATG standard-dose group (●) during the first 2 wk after transplantation.

Overall, there were three biopsy-proven acute rejection episodes (one on low-dose RATG plus basiliximab and two on standard-dose RATG). All of them occurred in patients with DGF, during the anuria period. At study end, serum creatinine levels were comparable on RATG low-dose plus basiliximab and on RATG standard-dose in the study group as a whole (1.6 ± 0.2 *versus* 1.7 ± 0.2 mg/dl) as well as in the subgroup of patients with DGF (1.4 ± 0.3 *versus* 1.4 ± 0.5 mg/dl).

Safety Analyses

Both regimens were well tolerated; no patient had severe cytokine release syndromes, such as serum sickness–like syndrome or acute respiratory distress syndrome, during RATG infusion; WBC and hemoglobin levels tended to decrease in both treatment groups during RATG infusion; but changes in both parameters were lower in patients who were receiving low-dose RATG plus basiliximab than in those who were receiving standard-dose RATG (Figure 1, B and C). Episodes of

leukopenia (defined as WBC counts <3500/μl) and anemia (hemoglobin reduction to <10 g/dl) that required red blood cells transfusions were significantly less frequent on low-dose RATG plus basiliximab than on standard-dose RATG therapy, in particular among patients with DGF (Table 2). Fever was significantly less frequent during low-dose RATG plus basiliximab than during standard-dose RATG treatment (Table 2). A significantly lower number of patients who were receiving low-dose RATG plus basiliximab required reduction in RATG dose schedule throughout the treatment period because of WBC reduction (Table 2). Of note, at the end of the RATG therapy course (day 7 after transplantation), the number and the percentage of circulating lymphocytes were significantly higher in those who were receiving low-dose RATG plus basiliximab than in those who were receiving standard-dose RATG treatment (1.7 ± 1.0 *versus* 3.4 ± 3.9%; Figure 2). On the contrary, the number of circulating WBC, neutrophils, and monocytes did not differ between the two treatment arms (Figure 2).

The overall incidence of infectious episodes was significantly lower on low-dose RATG plus basiliximab than on standard-dose RATG therapy (29.4 *versus* 66.6%; $P < 0.05$), this excess being largely explained by a lower incidence of CMV reactivations in patients who were receiving low-dose RATG plus basiliximab than in those who were receiving standard-dose RATG (Table 2). All events were observed within the first 3 mo after transplantation (Figure 3). In all cases, patients who showed signs of CMV reactivation (including pp65 antigenemia) fully recovered with ganciclovir therapy. No patient developed lymphoproliferative disorders or other malignancies during the follow-up period.

Costs

The average cumulative, daily doses of RATG were 18.8 and 92.5 mg (equivalent to 0.5 and 1.6 mg/kg per d) in the low-dose plus basiliximab and standard-dose groups, respectively. Per-patient costs of low-dose RATG and standard-dose RATG were 1642 and 5400 euro, respectively. Cost of basiliximab treatment was 2010 euro per patient. Therefore, per-patient costs of treatment with low-dose RATG plus basiliximab were significantly lower than those for standard-dose RATG (Table 3). In addition, costs for blood transfusions and ganciclovir therapy were significantly lower for patients who were receiving low-dose RATG plus basiliximab than for those who were receiving standard-dose RATG. As a result, per-patient treatment costs were approximately 3000 euro less on low-dose RATG plus basiliximab than on standard-dose RATG (Table 3).

Discussion

In this study, we found that in renal transplant recipients who were receiving lymphocyte-depleting therapy, combined treatment with basiliximab plus low-dose RATG was as effective as standard-dose RATG in limiting acute rejection episodes but was safer and less expensive. Only three patients had rejection throughout the observation period. These all were patients with DGF, which is not surprising given evidence already available on the negative impact of DGF on the risk for allograft rejection (4,26,27). Among the 11 patients who received the dual induction protocol, only one had acute rejection, which can be taken to indicate even high-risk group benefits of the novel regimen. Clinical implications of such findings are major as early rejection represents an important risk factor for long-term graft loss, particularly in recipients who had a delayed recovery of renal function after transplantation (28,29). In the context of DGF, effective prevention of acute rejection is crucial to allow postponing calcineurin-inhibitor therapy, which can adversely influence recovery from ischemia-reperfusion injury (4,29).

Patients who were receiving low, as compared with standard, RATG doses had a lower incidence of anemia that required red blood cell transfusions, of leukopenia that required RATG dose reduction, and of CMV reactivation that required ganciclovir therapy. Therefore, on average, patients who received combined therapy received significantly fewer units of packed red cell units and lower cumulative doses of ganciclovir. The costs saved for these medications, combined with the costs saved with the reduced RATG dose requirements, ex-

Table 2. Patients with side effects in the two treatment regimens throughout the whole study period[a]

	Overall		DGF Patients	
	Standard-Dose RATG ($n = 16$)	Low-Dose RATG ($n = 17$)	Standard-Dose RATG ($n = 11$)	Low-Dose RATG ($n = 11$)
Fever	9	3[b]	7	3
WBC < 3500/mm^3	9	4	7	3
Hb reduction > 2 g/dl	1	5	8	4
need for blood transfusions	1	5[c]	1	3[d]
no. of per-patient transfused units	2.1 ± 0.7	0.5 ± 0.4[e]	2.9 ± 0.7	0.6 ± 0.6[e]
Infections	1	5	8	5
CMV reactivations	9	3[b]	7	3

[a]WBC, white blood cell; Hb, hemoglobin; CMV, cytomegalovirus.
[b]$P < 0.05$, [c]$P = 0.01$, [d]$P < 0.001$, [e]$P < 0.001$ *versus* standard-dose RATG.

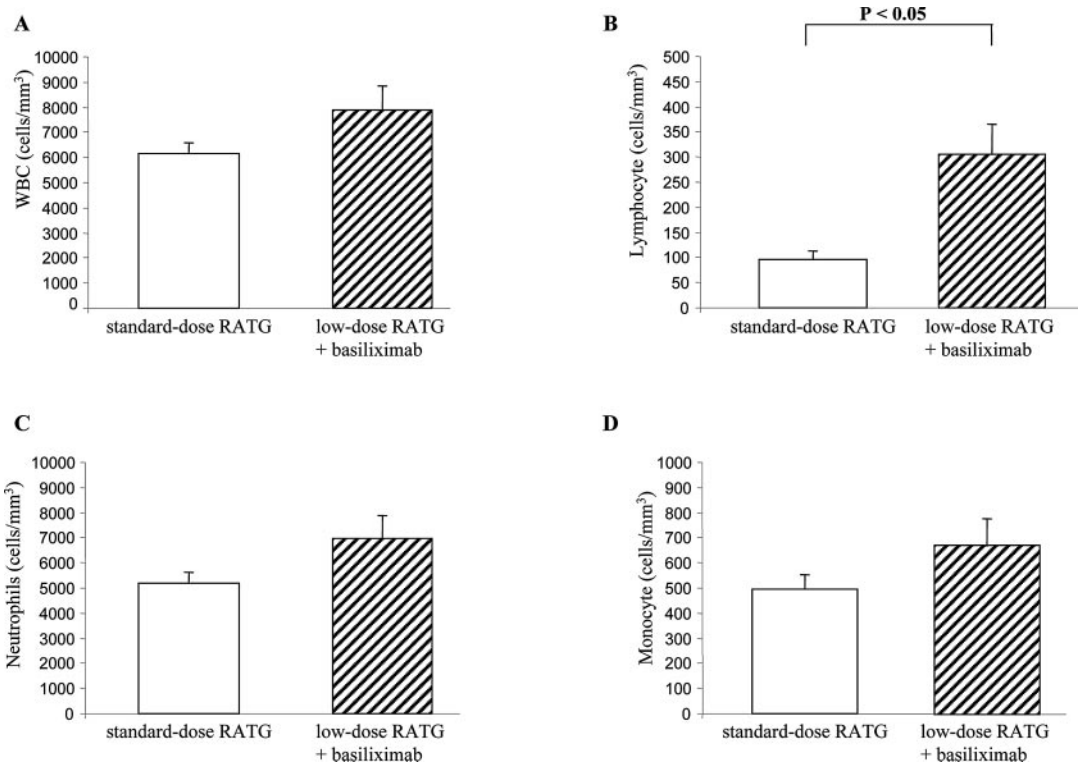

Figure 2. Absolute number of circulating WBC (A), lymphocytes (B), neutrophils (C), and monocytes (D) in the low-dose RATG plus basiliximab group (▨) and in the standard-dose RATG group (□) on day 7 after transplantation.

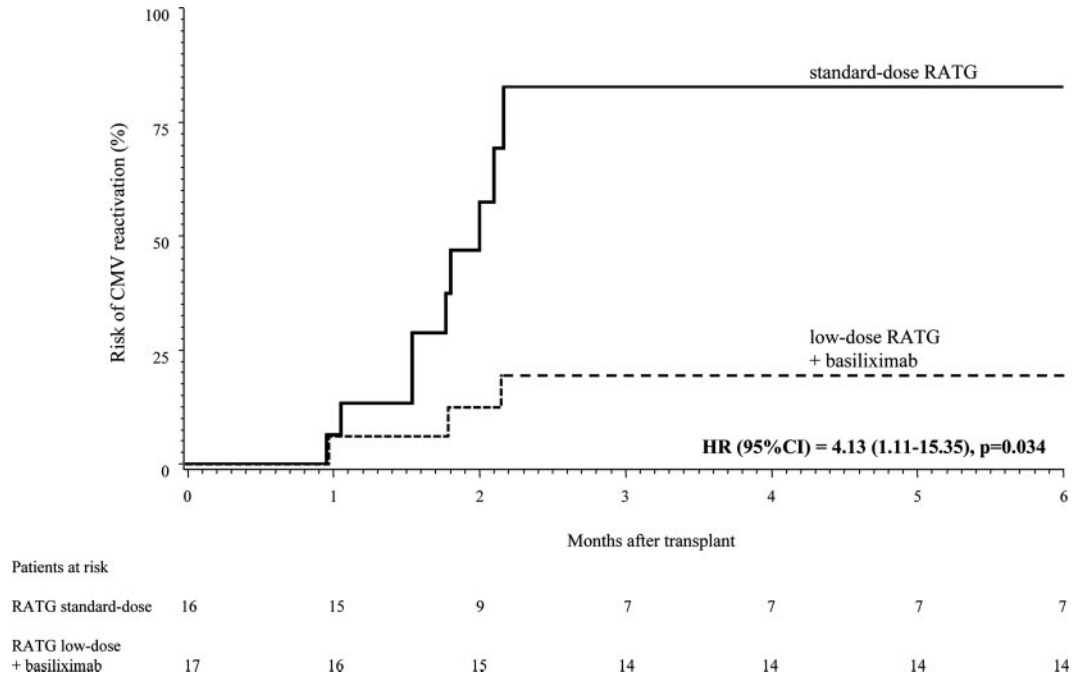

Figure 3. Kaplan-Meier curves of the percentage of cytomegalovirus (CMV) reactivation in the two treatment arms during the whole follow-up period. HR, hazard ratio; CI, confidence interval.

ceeded the extra costs for basiliximab therapy and allowed for an overall cost saving of approximately 3000 euro per patient in the combined treatment group.

The most frequently reported adverse events during induction therapy with polyclonal lymphocyte-depleting antibodies include signs of the cytokine release syndrome (10). These signs

Table 3. Per-patient costs (euro) throughout the whole study period[a]

	Standard-Dose RATG ($n = 16$)	Low-Dose RATG ($n = 17$)
Study drug	5,344 ± 490	3,737 ± 171[b]
Initial hospitalization	11,325 ± 1,650	10,658 ± 991
Subsequent hospitalizations	2,081 ± 1,009	1,941 ± 907
Dialysis therapy	174 ± 41	135 ± 32
Red blood cell transfusions	190 ± 55	49 ± 21[c]
CMV prophylaxis and treatment of infectious episodes	1,061 ± 166	480 ± 109[b]
Total	18,094 ± 1,625	15,059 ± 1,029

[a]Data are expressed as mean ± SE.
[b]$P = 0.01$, [c]$P < 0.01$ *versus* standard-dose RATG.

are dose dependent, and our findings demonstrated that they can be prevented fully by reduction of the dose of RATG to approximately one fourth the standard dose. Of note, leukopenia and thrombocytopenia were prevented by use of lower-than-standard doses of RATG not only in the study group as a whole but also in renal transplant recipients who were dialysis dependent because of DGF. This is consistent with the possibility that RATG preparations contain cross-reactive antibodies that entail risk for leukopenia and neutropenia in a dose-dependent way (30). This also could explain the excess of symptomatic anemia, in particular in patients who had DGF and were receiving standard-dose RATG. No obvious confounding factor such as surgical bleeding or blood occult losses or iron deficiency seemed to explain the remarkably different transfusion need between the low- and standard-dose RATG groups. Anemia, associated with leukopenia and thrombocytopenia, reflects also a generalized bone marrow suppression that has been documented in patients who were given polyclonal lymphocyte-depleting antibody preparations (31), which may include toxicity on $CD34^+$ stem cells, that is dose dependent (32). Erythroid cell function is impaired further by fewer monocyte- and lymphocyte-released growth factors, as it occurs with leucopenia and lymphocyte depletion *via* RATG (33). Among these, granulocyte-macrophage colony-stimulating factor and IL-3, produced predominantly by monocytes and T cells (34–36), have most of the effect on erythroid growth (33,37). In patients with DGF, erythropoiesis is already impaired by insufficient renal synthesis of erythropoietin (38), and the uremic milieu may create additional bone marrow toxicity (39). This likely explains severe anemia in patients who had DGF and received standard-dose RATG that was limited on low-dose RATG. In this setting, partial lymphocyte depletion can be offset by anti IL-2R antagonists.

The significantly increased incidence of CMV reactivations in patients who received standard-dose RATG—but not low-dose RATG—likely reflected a state of overimmunosuppression, consistent with the profound and persistent lymphocyte depletion. Patients who received low-dose RATG plus basiliximab had a rate of CMV reactivations comparable to that of other no-induction regimens (40). Timing of CMV reactivation was comparable in both study arms, and no event occurred after 3 mo posttransplantation. This can be taken to suggest that the lower incidence of CMV in patients who were given combined therapy did not reflect simply a delay in viral reactivation. These findings suggest that at lower-than-standard doses, RATG induction does not result in excess immunosuppression, even in combination with anti–IL-2R mAb. Our study was not powered to assess whether the low-dose regimen also could avoid the excess long-term risk for opportunistic infections, lymphoproliferative disorders, or malignancies associated with standard thymoglobulin-based induction regimens (41–43). On the basis of previous findings, however, patients who were exposed to less RATG were at lower risk (20). Other studies found that IL-2R antagonists do not increase the risk for lymphomas (15,44) and solid tumors (15,44), suggesting that add-on therapy with IL-2R antagonists can be relatively safe.

The finding that the CsA dose that was required to maintain comparable trough levels was not different in the two arms of this study indicates that the theoretical possibility of interference between basiliximab and CsA metabolism (45) is not an issue here. That renal function recovery tended to be faster in those who were receiving the dual induction regimen as compared with standard RATG permits exclusion also of any nephrotoxic effect.

Conclusion

The results of our pilot, explorative study suggest that basiliximab plus low-dose RATG therapy may be a valuable option to prevent safely allograft rejection in renal transplant recipients who are at increased immunologic risk or have DGF. This dual regimen also may save costs as compared with standard RATG induction therapy. Larger trials are needed to evaluate the risk-benefit profile of dual T cell target treatment as compared with standard RATG in patients at different immunologic risk or combined with maintenance regimens that may include tacrolimus and mammalian target of rapamycin inhibitors. The long-term effectiveness of such regimens also will be worth evaluating.

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