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Brief Communication

A Randomized, Double-Blind, Placebo-Controlled, Multicenter Study of Rabbit ATG in the Prophylaxis of Acute Rejection in Lung Transplantation

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ATG-Fresenius S (ATG-F) is a polyclonal anti-human-T-lymphocyte immunoglobulin preparation that has been clinically developed to prevent episodes of acute cellular rejection. This study evaluated the efficacy and safety of ATG-F at doses of 5 and 9 mg/kg versus placebo in adult recipients of a primary lung allograft. The primary efficacy composite end point was defined as death, graft loss, acute rejection and/or loss to follow-up within 12 months of transplantation. The interim analysis showed the ATG-F 5 mg/kg treatment to be inefficacious, and it would be impossible to enroll enough patients to power the study to show a difference between the 9 mg/kg arm and the placebo arm. Therefore, the main focus of the study shifted to the safety end points and a descriptive analysis of the primary end point. At 12 months posttransplant, the efficacy failure rate was not significantly different between the ATG-F 9 mg/kg group and the placebo group (40.2% vs. 36.7%, respectively). This large study did not demonstrate a significant reduction in acute cellular rejection, graft loss or death with single-dose induction therapy with ATG-F within the first year after lung transplantation.

Keywords: Acute rejection, alloreactivity, induction immunosuppression, lung transplantation, rabbit ATG

Abbreviations: 6MWT, 6-min walk test; ATG, antithymocyte globulin; ATG-F, ATG-Fresenius S; AZA, azathioprine; BOS, bronchiolitis obliterans syndrome; CMV, cytomegalovirus; CRS, cytokine release syndrome; CSA, cyclosporine; DMSB, Data and Safety Monitoring Board; FEV1, forced expiratory volume in 1 s; ISHLT, International Society of Heart & Lung Transplantation; ITT, intent-to-treat; MMF, mycophenolate mofetil; TAC, tacrolimus

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Introduction

In lung transplantation, acute rejection and chronic rejection (in the form of the bronchiolitis obliterans syndrome [BOS]) are recognized risk factors for reduced survival (1,2). Induction therapy describes the peritransplant augmentation of immunosuppression using agents such as polyclonal antilymphocyte preparations, anti-CD52 antibodies and IL-2 receptor antagonists. The use of antithymocyte globulin (ATG) is well established in nonpulmonary solid organ transplantation for the prevention of acute rejection (3) and for the treatment of acute corticosteroid-resistant acute rejection (4).

The benefit of induction therapy in lung transplantation has not been established. Despite this, the Registry of the International Society of Heart & Lung Transplantation (ISHLT) suggests about one-half of all lung transplant recipients receive some form of induction therapy (5). A recently published study reported that approximately 70% of lung transplant recipients who were managed without induction therapy experienced acute rejection during the first year after transplantation, and up to 21% developed BOS within 3 years of transplantation (6). Furthermore, data from the Registry of the ISHLT indicate that antibody induction affords a small patient survival advantage (7). Consistent with the results from other solid organ transplant populations, a number of small studies have shown that induction therapy decreases the incidence of acute cellular rejection following lung transplantation (8–11). In the absence of large randomized studies, the role of depletional induction therapy in lung transplantation is unproven.

ATG-Fresenius S (ATG-F) is a concentrated, highly purified, polyclonal anti-human-T-lymphocyte immunoglobulin preparation derived from rabbits after immunization with a T-lymphoblast cell line (Jurkat) (12). ATG-F has been clinically developed to be used in combination with other immunosuppressants to enhance prophylaxis of acute rejection. While early studies in renal transplantation demonstrated that a single intraoperative induction dose of ATG-F improved kidney graft survival (13), this has not been confirmed in more recent studies (14). To date, the efficacy of ATG-F has not been rigorously studied in lung transplantation.

The current study was designed to assess the efficacy and safety of ATG-F at doses of 5 and 9 mg/kg versus placebo for prophylaxis against acute cellular rejection in adult recipients of a primary lung allograft. Doses of ATG-F were based upon the approved dose of 5 mg/kg in solid organ transplant recipients (12) and a single intraoperative dose of 9 mg/kg supported by previous studies in renal transplantation (8,15). Inclusion of the higher dose in this Phase III study aimed to assess the best balance between efficacy and safety in the setting of lung transplantation. The study drug was given in the early postoperative period after an assessment of clinical stability had been completed.

Methods

Study design, randomization and conduct

An international, multicenter, double-blind, randomized parallel group design was employed (ClinicalTrials.gov identifier: NCT00105183). Initially, 240 patients were to be enrolled, randomized in a 1:1:1 ratio to ATG-F 5 mg/kg, ATG-F 9 mg/kg or placebo. Inclusion and exclusion criteria were applied immediately after the transplant operation. The main postoperative exclusion criteria included evidence of clinical instability (full details are available in the Supplementary text).

Block randomization was completed at the study center within 6–24 h after release of the surgical cross-clamps. The demonstration of postoperative stability was undertaken to ensure reserve against any cytokine side effects, while focusing on those who were now likely to survive to benefit from long-term efficacy. Investigators were able to assign each eligible patient a unique number through an Interactive Voice Response System that also facilitated randomization. Unblinding was planned after all patients completed 12-month follow-up. Independent Human Studies Committee approval was obtained from all 18 participating centers, and all patients provided written informed consent. The first patient entered on December 14, 2004, and the last patient out was on January 25, 2011.

Patients

Adult patients ($\geq$18 years) receiving a primary single or double lung allograft were eligible for the study.

Prophylaxis, study drug administration and immunosuppressive drug regimens

Premedication for ATG-associated cytokine release syndrome (CRS) (16) included diphenhydramine (50 mg enterally), acetaminophen (1000 mg enterally) and methylprednisolone (125 mg intravenously) administered 1 h prior to the infusion of the study drug. Standard infection prophylaxis consisting of antiviral, antifungal and antibiotic drugs was given to all patients according to local practices. Following randomization, the study drug was administered as a 500-mL infusion over 4–24 h in a double-blinded fashion. Placebo consisted of 0.9% sodium chloride. In addition, patients were required to receive a protocol-defined immunosuppressive regimen consisting of one of the following: tacrolimus (TAC) and azathioprine (AZA) with corticosteroids, cyclosporine (CSA) and mycophenolate mofetil (MMF) with corticosteroids, or TAC and MMF with corticosteroids. Each center was stratified to evenly include all three arms.

Efficacy and safety assessments

The primary efficacy end point was a composite of death, graft loss, acute rejection and/or loss to follow-up within 12 months of transplantation. Assuming rejection rates of 25% for patients on ATG-F and 50% for patients on placebo with a power of 80% and a sequential analysis design (10), sample size calculations estimated that 80 patients would be needed in each arm of the study. Secondary efficacy end points included: subset analysis of the primary end point for different calcineurin inhibitor regimens; death or graft loss at 6 and 12 months; incidence, frequency, severity and time to acute rejection; incidence of BOS (4) and distance covered in the 6-min walk test (6MWT). Safety end points included the incidence and severity of adverse events and the incidence of infections and malignancies.

Assessment of acute rejection

Transbronchial lung biopsies were performed for surveillance at 1, 2, 3, 6 and 12 months after transplantation and for clinical indications at the transplant center's discretion. Acute cellular rejection was graded by local histopathological interpretation of transbronchial biopsies using the ISHLT criteria. The efficacy end point for acute rejection in this study was defined as either a single episode of Grade $\geq$A2, or three or more episodes of Grade A1 (13). Recurrent acute rejection was defined as the reappearance of a Grade A2 event following histological resolution of the previous episode. Steroid-resistant acute rejection was defined as Grade $\geq$A2 rejection that failed to improve to Grade A1 or resolve within 4 weeks of a 3-day course of high-dose intravenous methylprednisolone therapy. The specifics of the management of acute rejection were according to each center's own protocol. BOS was defined as per ISHLT guidelines (1).

Statistical analysis

Time-to-event data were analyzed using Kaplan–Meier methodology. All descriptive statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC). No adjustments for multiplicity or for covariate effects were made. All efficacy parameters were assessed for the intent-to-treat (ITT) population including all patients randomized to study treatment by study arm. A per protocol analysis for all ITT patients without major protocol deviations was planned as a secondary analysis set.

Interim analysis

The study protocol mandated an interim analysis to re-estimate the necessary sample size when 50% of patients had completed 12 months of follow-up. The ATG-F 9 mg/kg treatment arm was compared to placebo for the primary end point to test for inefficacy or overwhelming drug effectiveness (17). If the p-value was $>$0.49, the Data and Safety Monitoring Board (DSMB) could recommend stopping the study for inefficacy (null hypothesis of treatment equality would be accepted). Conversely, if the 1-sided p-value was $<$0.000025, the independent DSMB could recommend stopping the study for overwhelming efficacy (ATG-F 9 mg/kg more efficacious than placebo). The same tests could also be applied to the ATG-F 5 mg/kg treatment arm. If the study was continued, the number of patients to maintain a conditional power of 80% was recalculated.

Results

Changes to study conduct

The interim analysis showed the ATG-F 5 mg/kg treatment to be inefficacious, and the DMSB recommended stopping enrollment for this patient group. Results of the interim analysis also revealed that it would be impossible to enroll enough patients to power the study to show a significant difference between the ATG-F 9 mg/kg and the placebo arm. Therefore, the main focus of the study shifted to the safety end points. The target enrollment was reduced from 240 to 220, and randomization continued in a 1:1 ratio between the ATG-F 9 mg/kg and placebo treatments until 80 patients were enrolled in each of these two arms.

Patient disposition

Overall, 223 patients were enrolled; however, 2 patients (1 ATG-F 9 mg/kg; 1 placebo) were considered too unwell to receive the study drug, and this resulted in a safety population of 221 patients. Recipient and donor demographics for the safety population are shown in Table 1. Most parameters were matched between the groups, although there was a higher number of cytomegalovirus (CMV) mismatched patients (donor CMV seropositive/recipient CMV seronegative) in the ATG-F 5 mg/kg treatment group.

Immunosuppressive medication

The full dose was not administered to a few patients because of clinical concerns about side effects during infusion: 5 patients (6.1%) in the ATG-F 9 mg/kg group (3 CRS, 1 anaphylaxis, 1 raised pulmonary artery pressure), 2 patients (3.2%) in the ATG-F 5 mg/kg group (1 anaphylaxis, 1 hyperventilating), and 1 placebo patient (detail not recorded). All patients received corticosteroids, and the proportions of patients in each treatment group receiving TAC/CSA or MMF/AZA was balanced among the groups. Overall, 133 patients (60.1%) were initially started on TAC. During the course of the study, switching CSA to TAC was reported in 4/31 (12.9%), 9/29 (31.0%), and 14/28 (50.0%) patients treated with ATG-F 9 mg/kg, ATG-F 5 mg/kg, and placebo, respectively, whereas only 5 patients (ATG-F 9 mg/kg, n = 4; ATG-F 5 mg/kg, n = 1) were switched from TAC to CSA. Exposure to TAC (mean trough levels 8–12 ng/mL) and CSA (mean trough levels 200–350 ng/mL) was similar across all three treatment groups (data not shown).

Efficacy failure

The efficacy failure rates are presented in Table 2. At 12 months posttransplant, the efficacy failure rate among patients in the ATG-F 9 mg/kg group and in the placebo group was comparable (40.2% vs. 36.7%, respectively). However, in the 5 mg/kg group the efficacy failure rate was higher than in the placebo group (48.4% vs. 36.7%, respectively). Among the components of efficacy failure, there was a trend toward a higher death rate in the 9 mg/kg group (15.9%) as compared to the 5 mg/kg group (11.3%) and the placebo group (8.9%). In addition, the acute rejection rate was higher in the 5 mg/kg group (35.5%) than in either the 9 mg/kg group (26.8%) or the placebo group (25.3%; Table S1). The overall number of episodes of acute

Table 1: Key demographics (safety population)

Parameter	ATG-F 9 mg/kg (n = 82)	ATG-F 5 mg/kg (n = 61)	Placebo (n = 78)	p-Value placebo vs. pooled ATF-G
Recipient				
Age years, mean (SD)	55.4 (8.7)	55.0 (10.3)	52.8 (11.5)	ns
Gender male, n (%)	46 (56.1)	35 (57.4)	42 (53.8)	ns
Race, n (%)				
White	74 (90.2)	52 (85.2)	74 (94.9)	ns
Black	6 (7.3)	8 (13.1)	2 (2.6)	ns
Smoking history, n (%)	70 (85.4)	45 (73.8)	59 (75.6)	ns
Body mass index kg/m^2, mean (SD)	23.7 (3.9)	23.3 (3.9)	23.8 (4.7)	ns
Mean panel reactive antibody level, % (SD)	5.9 (15.8)	5.3 (17.5)	6.6 (21.0)	ns
Primary disease, %				
Chronic obstructive pulmonary	48.8	54.1	48.7	ns
Disease				ns
Idiopathic pulmonary fibrosis	14.6	16.1	21.8	ns
Alpha-1 antitrypsin deficiency	13.4	8.2	6.4	ns
Cystic fibrosis	2.4	8.2	1.3	
Bilateral lung transplant, %	73.2	80.3	78.2	ns
Donor				
Age years, mean (SD)	37.4 (14.7)	36.4 (13.9)	31.8 (13.0)	ns
Gender male, n (%)	49 (59.8)	37 (60.7)	58 (71.8)	ns
Donor–recipient compatibility				
CMV IgG donor positive/recipient negative, n (%)	12 (14.6)	18 (29.5)	8 (10.3)	ns

ATG-F, ATG-Fresenius S; CMV, cytomegalovirus.

Table 2: Efficacy failure rates and death or graft loss (ITT population)

End point	ATG-F 9 mg/kg (n = 82)	ATG-F 5 mg/kg (n = 62)	Placebo (n = 79)
Efficacy failure at month 12, n (%)[1]	33 (40.2)[2]	30 (48.4)[3]	29 (36.7)
Death	13 (15.9)	7 (11.3)	7 (8.9)
Graft loss	0	0	1 (1.3)
Biopsy-proven acute rejection	22 (26.8)	22 (35.5)	20 (25.3)
Loss to follow-up	1 (1.2)	5 (8.1)	4 (5.1)
Death or graft loss, n (%)[4]			
Month 6	9 (11.0)	2 (3.2)	5 (6.3)
Month 12	13 (15.9)	7 (11.3)	8 (10.1)

ATG-F, ATG-Fresenius S; ITT, intent-to-treat.
[1]No significant differences between ATG-F dose groups and placebo for all components, chi-square test.
[2]Not significantly different to placebo but numerically similar, chi-square test p = 0.65.
[3]Not significantly different to placebo but numerically similar, chi-square test p = 0.16.
[4]No significant differences between ATG-F dose groups and placebo for all time points, chi-square test.

rejection and its severity and clinical relevance were also not statistically different between the groups (Tables S2 and S3).

The efficacy failure rate in each treatment group was similar regardless of the initial baseline therapy (CSA vs. TAC, MMF vs. AZA; Table S4). Kaplan–Meier estimates for time to each individual end point were not statistically different between treatment groups, except at 3 months posttransplant for the cumulative proportion of deaths between the ATG-F 9 mg/kg group (7/82; 8.5%) and the placebo group (1/79; 1.3%; p = 0.03; Table S1). Recipient survival and freedom from acute rejection are shown in Figures 1 and 2, respectively.

Secondary efficacy end points

The higher incidence of death or graft loss in the ATG-F 9 mg/kg group (Table 2) reflects a larger number of deaths occurring in the first 3 months after transplantation in this group (Figure 1). A total of five patients died within the first 30 days of the study, and all of these were in the 9 mg/kg treatment group. The causes of these deaths included acute rejection (n = 2), disseminated intravascular coagulation (n = 1) and hemorrhage (n = 1), graft loss (n = 1), and multisystem organ failure (n = 1). By 3 months after transplantation, a total of 7 (8.5%) patients had died in the 9 mg/kg group, and 1 patient in each of the 5 mg/kg and placebo groups (1.6% and 1.3%, respectively) had died. This difference in the death rate among the groups was still evident at 12 months; however, there was not a statistically significant difference in the death rate between either the ATG-F 9 or 5 mg/kg group compared to the placebo group during the first year after transplantation. The 6MWT distance, while not completed on all patients, was shorter for both ATG-F groups compared with placebo at 30 days but thereafter improved and was similar among the groups by 1 year posttransplantation. The mean FEV1 increased slightly during the study for the 9 and 5 mg/kg groups, but not for the placebo group. From Days 30 to 365, the mean FEV1 forced expiratory volume in 1 s (FEV1) increased from 2.0 to 2.4 L in the 9 mg/kg group and from 2.3 to 2.6 L in the 5 mg/kg group, but remained at 2.3 L in the placebo group (Table S5). At 1 year, BOS was diagnosed in 1/82 (1.2%), 7/62 (11.3%) and 4/79 (5.1%) of the ATG-F 9 mg/kg, ATG-F 5 mg/kg and placebo dose groups, respectively (Figure 3).

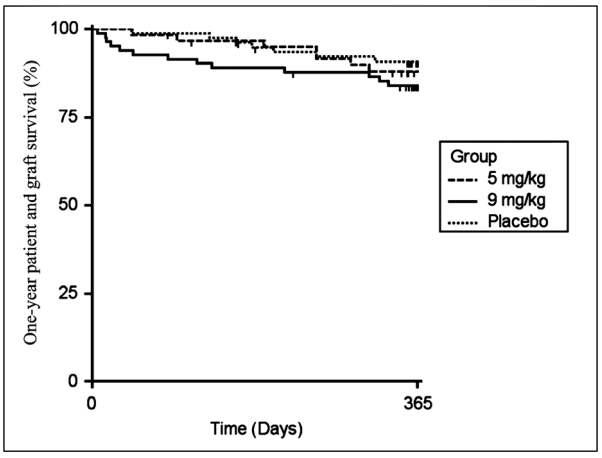

Figure 1: One-year patient and graft survival.

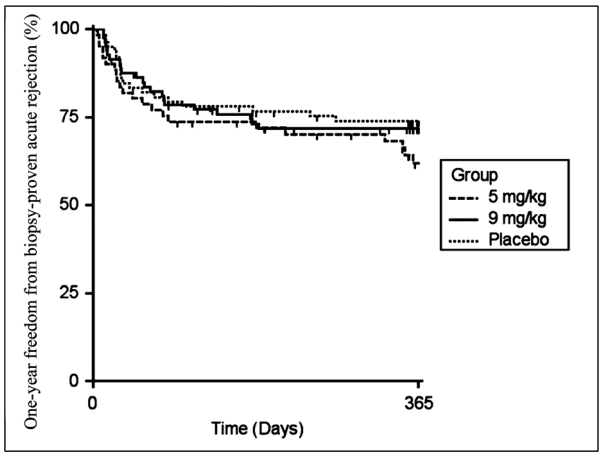

Figure 2: One-year freedom from biopsy-proven acute rejection.

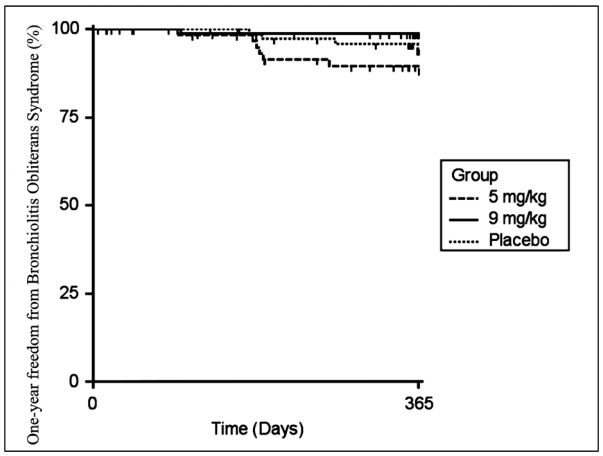

Figure 3: One-year freedom from bronchiolitis obliterans syndrome.

Safety

The incidence of treatment-related adverse events and serious adverse events was higher in the ATG-F groups and increased in a dose-related manner compared with placebo (Table 3). Although there were exceptions, infections and malignancies generally occurred with higher frequencies in the ATG-F treatment groups. Malignancies were diagnosed infrequently in the ATG-F 5 mg/kg group (two events) but occurred more frequently in the ATG-F 9 mg/kg group (six events; Table 3). However, five of the eight total malignancies in the two ATG groups were skin cancers. Mean serum creatinine concentrations tended to increase after transplantation, but were similar in all three treatment groups at 1 year posttransplantation. Thrombocytopenia was the most frequent treatment-related adverse event and it was ATG-F dose-related (Table 4). CRS and pyrexia occurred in approximately 10% of patients in each ATG-F group.

Discussion

This study was designed to test the effectiveness of ATG-F as an induction agent in lung transplantation in a large randomized, prospective, multicenter trial. Compared to placebo, induction therapy with a single dose of ATG-F at

Table 3: Adverse events over 1 year (safety population)

Adverse event parameter, n (%)	ATG-F 9 mg/kg (n = 82)	ATG-F 5 mg/kg (n = 61)	Placebo (n = 78)	p-Value placebo vs. pooled ATG-F
Summary				
Treatment-emergent	82 (100.0)	61 (100.0)	78 (100.0)	ns
Treatment-related	58 (70.7)	42 (68.9)	38 (48.7)	0.002
Serious	65 (79.3)	44 (72.1)	52 (66.7)	0.13
Serious treatment-related	21 (25.6)	14 (23.0)	10 (12.8)	0.04
Leading to study drug discontinuation	5 (6.1)	2 (3.3)	1 (1.3)	0.17
Leading to study withdrawal	0	0	1 (1.3)	0.17
Infection treatment-emergent >10% patients				
Candidal lung infection	20 (24.4)	20 (32.8)	21 (26.9)	0.87
Aspergillus infection	13 (15.9)	19 (31.1)	21 (26.9)	0.45
Pseudomonal lung infection	15 (18.3)	19 (31.1)	22 (28.2)	0.69
Staphylococcal infection	11 (13.4)	11 (18.0)	19 (24.4)	0.10
Pneumonia	9 (11.0)	9 (14.8)	12 (15.4)	0.56
Cytomegalovirus viremia	10 (12.2)	9 (14.8)	7 (9.0)	0.34
Cytomegalovirus infection	12 (14.6)	8 (13.1)	8 (10.3)	0.43
Enterococcal infection	10 (12.2)	5 (8.2)	4 (5.1)	0.17
Stenotrophomonas infection	6 (7.30	7 (11.5)	7 (9.0)	0.98
Malignant neoplasms treatment-emergent				
Basal cell carcinoma	3 (3.7)	1 (1.6)	0	0.14
Squamous cell carcinoma	1 (1.2)	0	1 (1.3)	0.66
Breast cancer *in situ*	0	1 (1.6)[1]	0	0.46
Adenocarcinoma	1 (1.2)[1]	0	0	0.46
B cell lymphoma	1 (1.2)[1]	0	0	0.46
Benign neoplasm treatment-emergent				
Lung neoplasm	1 (1.2)	0	2 (2.6)	0.25
Skin papilloma	2 (2.4)	0	0	0.29
Dysplastic naevus syndrome	1 (1.2)	0	0	0.46
Melanocytic naevus	1 (1.2)	0	0	0.46
Seborrheic keratosis	1 (1.2)	0	0	0.46
Uterine leiomyoma	1 (1.2)	0	0	0.46

ATG-F, ATG-Fresenius S.
[1]Possibly related to study drug.

Table 4: Treatment-related adverse events ≥5% patients (safety population)

Parameter	ATG-F 9 mg/kg (n = 82)	ATG-F 5 mg/kg (n = 61)	Placebo (n = 78)	p-Value placebo vs. pooled ATG-F
Hematological	36 (43.9)	13 (21.3)	8 (10.3)	>0.001
Thrombocytopenia	27 (32.9)	9 (14.8)	2 (2.6)	>0.001
Anemia	8 (9.8)	4 (6.6)	3 (3.8)	0.20
Leukopenia	8 (9.8)	2 (3.3)	0	0.017
Infections	27 (32.9)	20 (32.8)	23 (29.5)	0.61
Respiratory *Candida* infection	7 (8.5)	7 (11.5)	8 (10.3)	0.92
Pulmonary *Aspergillus* infection	3 (3.7)	6 (9.8)	5 (6.4)	0.99
Pulmonary Pseudomonal infection	1 (1.2)	8 (13.1)	4 (5.1)	0.72
Cytomegalovirus viremia	4 (4.9)	6 (9.8)	2 (2.6)	0.16
Staphylococcal infection	3 (3.7)	1 (1.6)	5 (6.4)	0.19
Systemic disorders	18 (22.0)	13 (21.3)	2 (2.6)	0.05
Pyrexia	9 (11.0)	7 (11.5)	2 (2.6)	0.03
Cytokine release syndrome	9 (11.0)	6 (9.8)	0	0.003
Thoracic disorders	16 (19.5)	11 (18.0)	9 (11.5)	0.16
Dyspnea	5 (6.1)	3 (4.9)	1 (1.3)	0.12
Pleural effusion	6 (7.3)	1 (1.6)	1 (1.3)	0.17
Lung infiltration	1 (1.2)	4 (6.6)	0	0.09
Vascular disorders	15 (18.3)	6 (9.8)	8 (10.3)	0.35
Hypotension	10 (12.2)	3 (4.9)	4 (5.1)	0.29
Cardiac disorders	11 (13.4)	8 (13.1)	3 (3.8)	0.02
Atrial fibrillation	5 (6.1)	3 (4.9)	2 (2.6)	0.30
Gastrointestinal disorders	8 (9.8)	7 (11.5)	4 (5.1)	0.17
Nausea	5 (6.1)	5 (8.2)	1 (1.3)	0.06

ATG-F, ATG-Fresenius S.

either 9 or 5 mg/kg had no significant impact on the primary composite end point of acute rejection, graft loss or death during the first year after transplantation. The results from this study do not support the efficacy of single-dose induction with ATG-F in lung transplant recipients.

The prevalence of acute cellular rejection during the first year after transplantation was similar between the placebo group (25.3%) and the ATG-F treatment groups (35.5% at 5 mg/kg; 26.8% at 9 mg/kg) in this study, and these rates are equivalent to the 30% rate in the ISHLT Registry data. The concomitant immunosuppression employed in this study was based on CSA or TAC with MMF or AZA and steroids. These drug combinations are well established and effective in lung transplantation, and in the subset analysis there was no evidence that the choice of maintenance immunosuppressive regimen significantly affected the primary outcome.

The leading cause of death beyond the first year is the development of chronic lung allograft syndrome/BOS. Indeed, BOS is present in up to 70% patients at 10 years after transplantation (7). In 2008, Hartwig et al (18) described a cohort where rabbit ATG induction delayed the development of BOS. However, like the present study, the overall incidence of acute cellular rejection and overall survival was no different between the induction and conventional treatment arms. While some previous studies have suggested that TAC, rather than CSA, confers some protection against BOS (6,19,20), a *post hoc* sub-set analysis according to drug combination did not show that ATG-F induction with TAC maintenance preferentially protected against acute rejection or efficacy failure.

The interim analysis showed that the ATG-F 5 mg/kg dose was not efficacious. Recruitment to this treatment arm was then discontinued. The higher dosage arm also lacked statistical power to achieve superiority over placebo. Nevertheless, the study was completed with fewer patients and assessments, and results between treatment groups were compared in a descriptive sense. ATG-F failed to confer any advantages in the efficacy parameters.

The safety profile of ATG-F in this study was consistent with the mechanism of action and the well-known side effects of this preparation (12). An increased liability for infection and thrombocytopenia versus placebo therapy was observed, but pseudomonas and *Aspergillus* lung infections were actually lower in the high ATG-F dose group. Malignant neoplasms were restricted to two events with the low dose of ATG-F, but increased to six separate events with ATG-F 9 mg/kg (cutaneous basal cell carcinoma, n = 3; cutaneous squamous cell carcinoma, n = 1; adenocarcinoma, n = 1; B cell lymphoma, n = 1). Similarly, drug-specific complications such as pyrexia and CRS occurred more frequently with ATG-F, especially at the higher dose. These adverse events, study drug discontinuations and deaths showed the low dose of ATG-F to be better tolerated. Of concern, the high ATG-F dose was associated with an increased number of deaths in the first 3 months after transplantation.

The strengths of this study derive from the prospective, double-blind design and the inclusion of a large number of patients recruited from many centers, indeed one of the largest blinded lung transplant studies ever performed. However, the inclusion criteria, which permitted a number of different drug maintenance regimens, resulted in several sub-groups by drug regimen that confounded comparisons of ATG-F with placebo therapy. Furthermore, the switch from CSA to TAC in a considerable number of patients blurred the efficacy assessment within the ITT treatment comparison. The protocol also stipulated that ATG-F should be given no sooner than 4 h and no later than 24 h after transplantation. The delayed administration of ATG-F might have contributed to the apparent inefficacy in the current setting. Finally, the selected doses of ATG-F might not have been optimal for highly immunogenic organs even though previous studies had suggested safety and efficacy of the 9 mg/kg single dose in kidney transplant recipients (11,13).

In conclusion, the present study design did not demonstrate a positive benefit-to-risk profile for single-dose ATG-F induction in lung transplant recipients.

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Participating Centers (Enrolled Patients)

Australia: The Alfred Hospital (36 patients), Melbourne

Austria: University Hospital of Vienna (50)

Canada: Toronto General Hospital (15)

USA: Temple University (2), Philadelphia; Mayo Clinic—Transplant Center (2); Emory University (3), Atlanta; University of Kentucky (9); University of Pennsylvania (9), Philadelphia; University of Texas (4), San Antonio; Stanford University (3); University of Iowa (4); Cleveland Clinic (7); Cedars-Sinai Medical Center (4); Baptist Medical Oklahoma City (5); Vanderbilt University (10), Nashville; The Methodist Hospital (8), Houston; Washington University (36), St. Louis; University of California (16), San Francisco.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary Methods

Table S1: Cumulative proportion of biopsy-proven acute rejection.

Table S2: Episodes of biopsy-proven acute rejection (ITT population, per recipient).

Table S3: Episodes of treated acute rejection (ITT population, number of episodes, includes biopsy proven and clinician defined).

Table S4: Efficacy failure according to baseline immunosuppression.

Table S5: Pulmonary function tests (ITT population).