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FEATURED CLINICAL TRIAL

Tacrolimus and cyclosporine have differential effects on the risk of development of bronchiolitis obliterans syndrome: Results of a prospective, randomized international trial in lung transplantation

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BACKGROUND: Chronic lung allograft dysfunction, which manifests as bronchiolitis obliterans syndrome (BOS), is recognized as the primary cause of morbidity and mortality after lung transplantation. In this study we assessed the efficacy and safety of two de novo immunosuppression protocols to prevent BOS.

METHODS: Our study approach was a multicenter, prospective, randomized (1:1) open-label superiority investigation of de novo tacrolimus vs cyclosporine, with both study arms given mycophenolate mofetil and prednisolone after lung transplantation. Cytolytic induction therapy was not employed. Patients were stratified at entry for cystic fibrosis. Primary outcome was incidence of BOS 3 years after transplant (intention-to-treat analysis). Secondary outcomes were survival and incidence of acute rejection, infection and other adverse events.

RESULTS: Group demographic data were well matched: 110 of 124 tacrolimus vs 74 of 125 cyclosporine patients were treated per protocol ($p < 0.01$ by chi-square test). Cumulative incidence of BOS Grade $\geq$1 at 3 years was 11.6% (tacrolimus) vs 21.3% (cyclosporine) (cumulative incidence curves, $p = 0.037$ by Gray's test, pooled over strata). Univariate proportional sub-distribution hazards regression confirmed cyclosporine as a risk for BOS (HR 1.97, 95% CI 1.04 to 3.77, $p = 0.039$). Three-year cumulative incidence of acute rejection was 67.4% (tacrolimus) vs 74.9% (cyclosporine) ($p = 0.118$ by Gray's test). One- and 3-year survival rates were 84.6% and 78.7% (tacrolimus) vs 88.6% and 82.8% (cyclosporine) ($p = 0.382$ by log-rank test). Cumulative infection rates were similar ($p = 0.91$), but there was a trend toward new-onset renal failure with tacrolimus ($p = 0.09$).

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chronic rejection

CONCLUSIONS: Compared with cyclosporine, de novo tacrolimus use was found to be associated with a significantly reduced risk for BOS Grade ≥1 at 3 years despite a similar rate of acute rejection. However, no survival advantage was detected.
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Lung transplantation has become a viable treatment option for selected patients with end-stage lung disease and leads to prolonged survival and improved quality of life. However, despite improvements in surgical techniques, immunosuppressive therapies and long-term care, survival rates reported by the registry of the International Society for Heart and Lung Transplantation (ISHLT) (79% at 1 year and 52% at 5 years) are lower than those reported for other solid-organ transplants.[1]1 The leading cause of death in long-term follow-up after lung transplantation is chronic allograft dysfunction due to obliterative bronchiolitis (OB) manifested by its physiologic correlate, bronchiolitis obliterans syndrome (BOS). OB is thought to result from chronic rejection leading to obliteration and scarring of the terminal bronchioles and causing a significant reduction in pulmonary function parameters, most specifically forced expiratory volume in 1 second (FEV_1).[2] In the absence of confounding variables, lung transplant recipients are considered to have BOS Grade ≥1 if they undergo a sustained (>3 weeks) ≥20% decline in FEV_1 from baseline of the average of the two best FEV_1 measurements obtained at least 3 weeks apart.[3]

Most immunosuppression regimens after lung transplantation are based on calcineurin inhibitors. The introduction of cyclosporine was responsible for the initial success of lung transplantation in the early 1980s as it allowed the use of less corticosteroids, and hence afforded superior wound-healing.[4] Its chief mechanism of action is the blockade of T-lymphocyte activation by inhibiting interleukin-2 (IL-2) synthesis. Tacrolimus is a macrolide lactone that was introduced in the 1990s and is now widely accepted as an alternative to cyclosporine.[1] Mechanisms of action and toxicities of tacrolimus and cyclosporine are similar, and tacrolimus has proven to be at least as effective as cyclosporine in solid-organ transplantation, including lung transplantation.[5–9] In vitro tacrolimus is 50-fold more potent than cyclosporine and has proven to be an effective rescue agent for patients with recurrent or refractory acute allograft rejection.[10,11] In one multicenter, retrospective, uncontrolled study, lung transplant recipients with BOS who converted from cyclosporine to tacrolimus had a reduced rate of decline in FEV_1.[10] Similar effects have also been demonstrated in a single-center analysis.[12]

It remains unclear whether de novo tacrolimus use can reduce the incidence of BOS when compared with cyclosporine after lung transplantation. To date, there are no published adequately powered, randomized, controlled trials in lung transplantation comparing efficacy and safety of cyclosporine and tacrolimus for primary immunosuppression. We therefore conducted a randomized, open-label, multicenter, investigator-driven trial comparing tacrolimus with cyclosporine—both arms in combination with mycophenolate mofetil (MMF) and prednisolone for the prevention of BOS in lung and heart–lung transplant recipients.

We partnered the calcineurin inhibitor with MMF instead of azathioprine. MMF is an ester pro-drug of mycophenolic acid (MPA), a potent and specific inhibitor of de novo purine synthesis blocking the proliferation of T and B lymphocytes. Superiority of MMF over its comparator, azathioprine, after lung transplantation has been suggested in small and non-randomized studies, but this was not confirmed in a larger, open-label, randomized study, which was unpowered by a high rate of treatment group switching from azathioprine to MMF.[13–15] However, large, randomized trials in renal and heart transplantation have demonstrated the greater efficacy of MMF for preventing acute allograft rejection when compared with azathioprine.[16–18]

Methods

Study design

This 3-year prospective, randomized (1:1), open-label, multicenter, investigator-driven superiority study in two parallel groups of adult lung transplant recipients was conducted in compliance with the provisions of the Declaration of Helsinki and good clinical practice guidelines. The study protocol was accepted by each local hospital research ethics committee. All patients provided written informed consent and were free to withdraw from the study at any time-point. The trial was proposed and designed by a steering committee consisting of members of the study group European and Australian Investigators in Lung Transplantation (EAILTx), representing experienced lung transplant centers from Australia, Austria, Belgium, Germany, Spain and Switzerland. The study was registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT01429844).

The investigation took place at 14 experienced lung transplantation centers in 5 European countries (Austria, Belgium, Germany, Spain and Switzerland) and Australia (see Appendix 2). Patients were screened for eligibility prior to transplantation. Inclusion and exclusion criteria are listed in Appendix 1. At time of transplantation, randomization was performed using a centralized, telephone-based computer randomization tool. Patients were assigned to receive tacrolimus or cyclosporine in combination with MMF and corticosteroids and were stratified for diagnosis of cystic fibrosis (CF). Stratification was performed because chronic airway infection, multiple-organ involvement and variable gastrointestinal absorption pose specific clinical problems in individuals with CF, which may have introduced an outcome bias if there were an imbalance of CF patients between groups.

Tacrolimus therapy was started immediately after transplantation with a continuous intravenous infusion of 0.01 to 0.03 mg/

kg/day. After extubation the mode of delivery was switched to oral administration twice daily (0.05 to 0.3 mg/kg/day). Doses were adjusted to trough levels. Target C_0 (trough) levels were 10 to 15 ng/ml for the first 3 months after transplantation and 8 to 12 ng/ml thereafter, with dose adjustments according to patient outcome.

Cyclosporine therapy was started immediately after transplantation with a continuous intravenous infusion of 1 to 3 mg/kg/day. After extubation, delivery was switched to oral administration (2 or 3 times daily at 4 to 18 mg/kg/day). Cyclosporine doses were adjusted to C_0 or C_2 levels according to local practice (C_0: trough level before drug intake; C_2: trough level 2 hours after drug intake). C_0 target trough levels were 200 to 300 ng/ml for the first 3 months after transplantation and 150 to 200 ng/ml thereafter. C_2 target levels have been reported previously.[19]

In both treatment groups, MMF therapy was also started immediately after surgery with fixed doses of 1 g MMF intravenously or via nasogastric tube twice per day for 2 or 3 days. After extubation, the mode of delivery was switched to oral administration (2 or 3 times daily at 2 to 3 g). Trough-level measurements for MPA were not mandated by the protocol, but, when performed, the doses were adjusted according to MPA trough levels targeting a level of 2 to 3 μg/ml (EMIT assay; Behring). Centers obtaining MPA levels were equally distributed between the two groups. The upper dose limit for MMF was 4 g/day. MMF dose adjustment required 3 consecutive out-of-range values or a clinical indication.

A 500-mg to 1-g intravenous dose of methylprednisolone was given during the transplant surgical procedure before the start of reperfusion, followed by 3 doses of 125 mg every 8 hours in the intensive care unit. Prednisolone was started on Day 1 post-operatively at 0.5 to 1 mg/kg/day (twice daily), then tapered to 0.1 to 0.2 mg/kg/day within the first 3 months. Other steroids were given in prednisolone equivalent doses.

Patients were followed for 3 years (regular visits at 1 and 2 weeks, then at 1, 2, 3, 6, 9 and 12 months, and then every 6 months thereafter). Data were entered into an electronic case report form (eCRF) and regularly monitored and checked for inconsistencies by an independent monitor who was also responsible for query management. After completion of the follow-up period source data verification was performed by independent data management specialists who visited the centers and checked patient records for completeness of data. Reporting follows the CONSORT Statement.[20]

Outcome measures

The primary outcome measure was cumulative incidence of BOS at 3 years post-transplantation. BOS was defined according to ISHLT criteria applied by each center to their local data and reviewed for accuracy by an independent data-monitoring organization. Secondary outcome measures included 1- and 3-year rates of: (1) acute allograft rejection (determined by clinical criteria or transbronchial lung biopsy); (2) patient and graft survival; (3) infections; (4) adverse events; and (5) treatment failure, defined as drug discontinuation (e.g., conversion to a different immunosuppression regimen).

Statistical analysis

Sample size calculations were performed assuming a BOS incidence at 3 years of 36% in the cyclosporine group and a reduction of at least 15% in the tacrolimus group.[21] We calculated that 140 patients per treatment group were needed to achieve an 80% power of detecting the stated difference in BOS incidence between groups with statistical significance (2-sided $\alpha = 0.05$).

Patient survival was assessed by Kaplan–Meier analysis and compared by log-rank test. To reflect the study design all conducted analyses were stratified by cystic fibrosis (CF). Assumption of proportional hazards was assessed using Schoenfeld residuals, and, when found to be valid, a pooled analysis with CF as an additional risk factor was performed. To assess the influence on BOS we analyzed death as a competing event and calculated cumulative incidence curves, which were compared using Gray's test stratified for CF status.

To calculate sub-distributional hazard rates, the methods of Fine and Gray were used. To adjust for potential confounders, proportional sub-distribution hazards models were applied. Relevant confounders were identified using a backward selection procedure. To assess the impact of acute rejection (AR), we implemented an extended Cox proportional hazards model for BOS using time-dependent covariates for AR. The primary analysis population of the study was determined according to the intention-to-treat principle (all patients randomized were analyzed according to their original treatment group assigned), whereas the safety population referred to all patients who underwent transplantation and received at least one dose of study drug. A 2-sided $p < 0.05$ was considered statistically significant. All statistical analyses were done with SPSS, version 15 (SPSS, Inc.), and R 2.9 (R Development Core Team) software.

Results

Patients

From January 2001 until June 2003, a total of 274 patients from 14 centers in 6 countries were assessed for eligibility, of whom 265 were randomized and transplanted (Figure 1). Fifteen patients did not receive study drug and 1 patient was retransplanted within 3 days, leaving 249 patients in the intent-to-treat population.

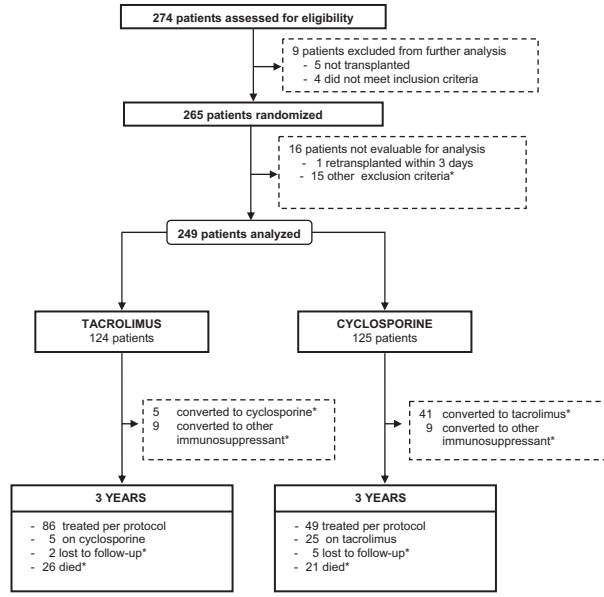

Figure 1 Trial profile. *See text for detailed reasons for exclusion from BOS analysis, conversion from allocated immunosuppressant, cause of death and loss to follow-up.

Table 1 Patient Demographics, Failure to Maintain per Protocol Immunosuppression, Primary End-point and Selected Secondary End-points

	Tacrolimus group	Cyclosporine group	p[a]
N	124	125	
Age (years) (mean ± SD)	46.4 ± 13.4	44.4 ± 13.7	0.82
Male gender (%)	69 (56)	73 (58)	0.70
Transplant indication (%)			
Idiopathic pulmonary fibrosis	22 (18)	33 (26)	0.09
Emphysema	39 (31)	28 (22)	0.15
Cystic fibrosis	32 (26)	30 (24)	0.77
Idiopathic pulmonary hypertension	6 (5)	9 (7)	0.60
α_1-anti-trypsin deficiency	7 (6)	5 (4)	0.57
Other	18 (15)	20 (16)	0.86
Type of transplant (%)			
Double lung	86 (69)	99 (79)	0.08
Single lung	32 (26)	21 (17)	0.09
Heart—lung	6 (5)	5 (4)	0.65
Reasons for failure to maintain per protocol immunosuppression			
BOS	2	17	<0.01
Refractory/recurrent acute rejection	1	14	<0.01
Cytopenia	3	3	1.00
Nephrotoxicity	0	4	0.12
Neurotoxicity	3	0	0.12
Side effects	0	4	0.12
Other	5	8	0.38
Totals	14	50	<0.01
Primary end-point			
BOS Grade ≥1 at 3 years (%)[b]	11.6	21.3	0.037
Secondary end-points			
1-year survival (%)[c]	84.6	88.6	0.82
3-year survival (%)[c]	78.7	82.8	0.38
≥1 acute rejection at 1 year (%)[b]	65.7	73.2	0.12
≥1 acute rejection at 3 years (%)[b]	67.4	74.9	0.11
New-onset renal dysfunction (%)	22.8	16.8	0.09
Infections per 100 patient-days (%)	0.336	0.343	0.91
Bacterial (%)	68.2	70.1	0.38
Fungal (%)	3.4	3.9	0.47
Viral (%)	28.5	26.0	0.21

[a]Calculated by Student's *t*-test for age, all other comparisons by chi-square test.
[b]Event rate at time-point estimated by cumulative incidence functions (Gray's test).
[c]Event rate at time-point estimated by Kaplan—Meier analysis (log-rank test).

The groups were well-balanced with respect to patient demographics, etiology of end-stage lung disease and type of transplantation (Table 1). Of note, 26% patients in the tacrolimus group and 24% patients in the cyclosporine grouphad CF. The last patient completed follow-up in June 2006. Source data verification was performed in 2007. Missing data were tracked, retrieved and confirmed until 2009. The statistical analysis was performed in 2010.

A change from the assigned treatment protocol was significantly more frequent in the cyclosporine group (50 of 125 patients [40%]) than in the tacrolimus group (14 of 124 patients [11.3%], $p < 0.01$ by chi-square test). Forty-one of 50 cyclosporine patients were converted to tacrolimus and 9 to other regimens, whereas 5 of 14 tacrolimus patients were converted to cyclosporine and 9 to other regimens. In the cyclosporine group, treatment failure due to BOS (17 of 50 patients, 34%) or refractory or recurrent acute rejection (14 of 50 patients, 28%) were the main reasons for failure to maintain the per-protocol immunosuppression, whereas in the tacrolimus group non-BOS or rejection-associated effects (11 of 14 patients, 78.6%) were the main reason (Table 1).

Outcome measures

Primary outcome

At 3 years, the cumulative incidence of BOS was lower in the tacrolimus group than in the cyclosporine group (11.6% vs 21.3%) ($p = 0.037$ by Gray's test, pooled over strata) (Tables 1, 2 and 3). Cox proportional hazards assumption for treatment was fulfilled ($p = 0.68$) and the treatment

Table 2 Cause-specific Hazard Ratios from Competing Risks Regression Model

	HR	95% CI	*p*-value
BOS (cyclosporine vs tacrolimus)	1.98	(1.04–3.77)	0.039
Patients with cystic fibrosis	0.86	(0.41–1.79)	0.690
Death without BOS (cyclosporine vs tacrolimus)	0.73	(0.39–1.37)	0.330
Patients with cystic fibrosis	0.53	(0.22–1.25)	0.150

CI, confidence interval; HR, hazard ratio.

effect was not stratum-dependent ($p = 0.61$). In the overall population, a significant difference in BOS-free survival between the tacrolimus and cyclosporine groups was found ($p = 0.037$). Proportional sub-distribution regression analysis revealed an unadjusted hazard ratio of 1.97 (95% confidence interval [CI] = 1.04 to 3.76, $p = 0.039$) for cyclosporine patients to develop BOS in comparison to tacrolimus patients.

Secondary outcomes

Survival. Intention-to-treat analysis revealed no difference between the tacrolimus and cyclosporine groups in the proportion of patients surviving at 1 and 3 years (84.6% vs 88.6% and 78.7% vs 82.8%, respectively). Kaplan–Meier analysis did not reveal a significant treatment effect on survival ($p = 0.382$ by log rank test) (Figure 2). Overall, there were 26 deaths in the tacrolimus group and 21 deaths in the cyclosporine group. The proportional hazards (PH) assumption for CF was found to be valid ($p = 0.260$) and CF was not found to be an independent prognostic factor for survival using a Cox PH model (hazard ratio [HR] = 0.58, confidence interval [CI] 0.27 to 1.25, $p = 0.167$). Only 1 patient underwent retransplantation, so the rate of patient and graft survival was virtually identical.

The dominant causes of death in the cyclosporine vs tacrolimus groups, respectively, were infection (7 vs 8 patients), cerebrovascular events (3 vs 3 patients), multiple-organ failure (4 vs 1 patient), graft failure (2 vs 2 patients) and anastomotic dehiscence (2 vs 1 patient). Other reasons for death were cardiovascular events ($n = 6$), secondary hemorrhage ($n = 1$), cytomegalovirus infection ($n = 3$), reperfusion edema ($n = 1$) and pancreatic carcinoma ($n = 1$). There was no significant difference between groups in the cumulative incidence of deaths that were not related to

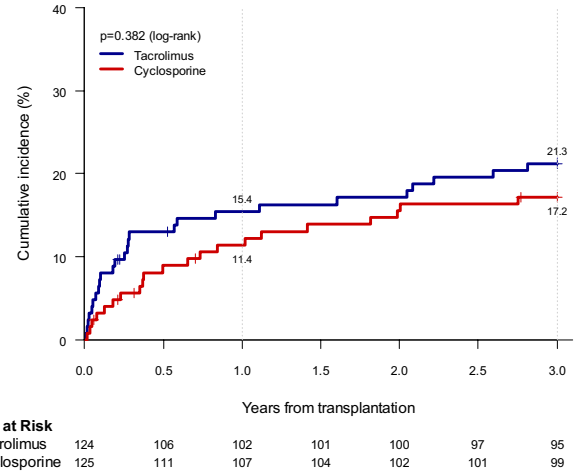

Figure 2 Cumulative mortality for the tacrolimus and cyclosporine groups ($p = 0.331$ by log-rank test).

BOS using Gray's test on cumulative incidence curves ($p = 0.331$) or sub-distributional hazards modeling ($p = 0.330$) (Figure 3 and Table 2).

Acute rejection. There was no significant difference in the cumulative rate of AR at 3 years between treatment groups ($p = 0.43$) using a 2-sample test for equality of proportions (Table 3). Incidence functions for AR treating BOS or death as competing events did not reveal a difference between treatment groups ($p = 0.118$) (Figure 4).

Patients with 2 or more acute rejection episodes had a significantly higher risk of death than patients with only one or no acute rejection episode (HR 3.75, 95% CI 1.74 to 8.06, $p < 0.01$).

Adverse events

Cumulative incidence of post-operative onset of renal dysfunction, defined as a persistent increase in serum creatinine of >2 mg/dl or dialysis dependency, was lower in the cyclosporine group than in the tacrolimus group (16.8% vs 22.8%), but the difference did not reach statistical significance ($p = 0.09$). Cumulative incidence of infection was similar in both treatment groups with overall infection rates of 0.34 infection per 100 patient-days in the tacrolimus group vs 0.34 infections per 100 patient-days in the cyclosporine group ($p = 0.91$). Bacterial, fungal and viral infections were evenly distributed between groups (Table 1).

Table 3 Incidence of Acute Rejection by Treatment and Cystic Fibrosis Status

	Tacrolimus			Cyclosporine		
	Not CF	CF	Total	Not CF	CF	Total
AR episodes	98	41	139	110	41	151
Patient-days	76,514	28,641	105,155	77,497	26,590	104,087
AR episodes per 100 patient-days	0.128	0.143	0.132	0.142	0.154	0.145

AR, acute rejection; CF, cystic fibrosis.

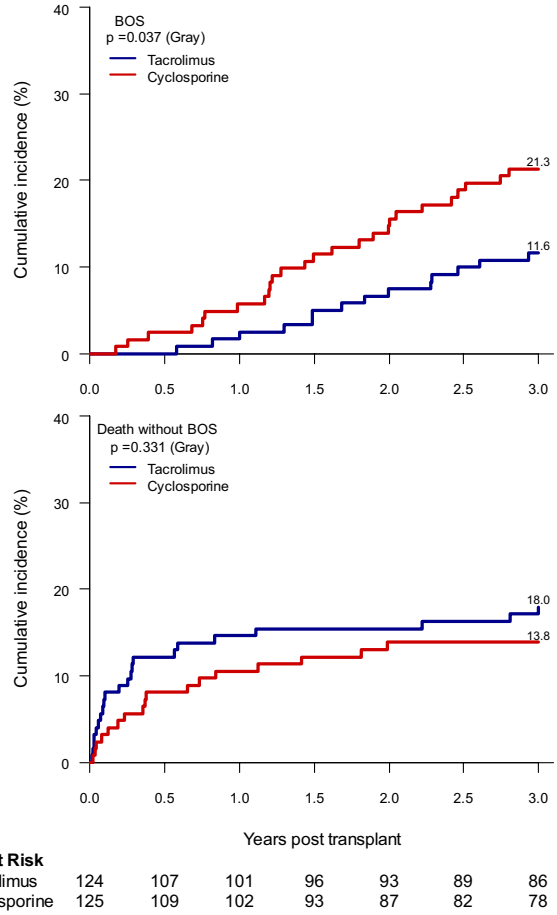

Figure 3 Cumulative incidence of BOS ($p = 0.037$ by Gray's test) and death without BOS ($p = 0.331$ by Gray's test) for the tacrolimus and cyclosporine groups.

Discussion

Synopsis of the key findings

Calcineurin inhibitors are commonly used in combination with anti-proliferative agents and steroids for the prevention of acute and chronic rejection in patients undergoing lung transplantation. This is the first prospective, randomized, controlled, multicenter trial to demonstrate superiority of tacrolimus over cyclosporine in combination with MMF and steroids for the development of BOS, the surrogate for chronic rejection and the main reason for death in long-term follow-up after lung transplantation. Competing events analysis and Gray's test revealed a significantly lower burden of BOS for tacrolimus-treated patients at 3 years, which was confirmed by Cox regression analysis showing a 1.97-fold higher risk of developing BOS within 3 years for cyclosporine-treated patients compared with tacrolimus-treated patients.

Despite these findings, the overall incidence of BOS in the cyclosporine group was only 21.3% at 3 years, which was 14.7% lower than the estimated 36% incidence based on ISHLT registry data, and the difference in BOS incidence between groups was 9.7%, which was 5.3% lower than the powering assumption on which the study was based.[21] Patient numbers in both treatment groups were also lower than in the pre-study sample size calculations (125 vs 140 patients [cyclosporine] and 124 vs 140 patients [tacrolimus], respectively). Therefore, the final overall power of the study was lower than calculated (57% vs 80%), which may alter the conclusiveness of the results. Nevertheless, the treatment effect on the primary end-point was significant ($p = 0.037$ by Gray's test, pooled over strata) and the value of post-ex power calculations remains controversial.

We found a trend toward a lower rate of acute rejection in the tacrolimus group at 1 and 3 years. In this regard it is noted that 50 patients in the cyclosporine group failed to maintain their assigned immunosuppressive group due to refractory and recurrent acute rejection episodes. Most were switched to tacrolimus. Only 14 of 124 (11.3%) tacrolimus-treated patients were withdrawn from the assigned immunosuppressive protocol, in contrast to 50 of 125 (40%) cyclosporine-treated patients ($p < 0.01$ by chi-square test).

Despite concerns regarding the higher immunosuppressive potency of tacrolimus, we did not detect an increase in infections or side effects. Bacterial, fungal and viral infections were similarly distributed between study groups. Cyclosporine-treated patients showed a lower rate of new-onset renal dysfunction, but the difference did not reach statistical significance ($p = 0.09$). Overall patient survival was excellent in both groups, demonstrating the efficacy and safety of both immunosuppressive regimens, but also underscoring the great experience of the participating centers. Overall survival was significantly higher than that seen in ISHLT registry results. Stratification for CF was performed, but CF was not determined to have an impact on study outcomes in any of the statistical analyses examined.

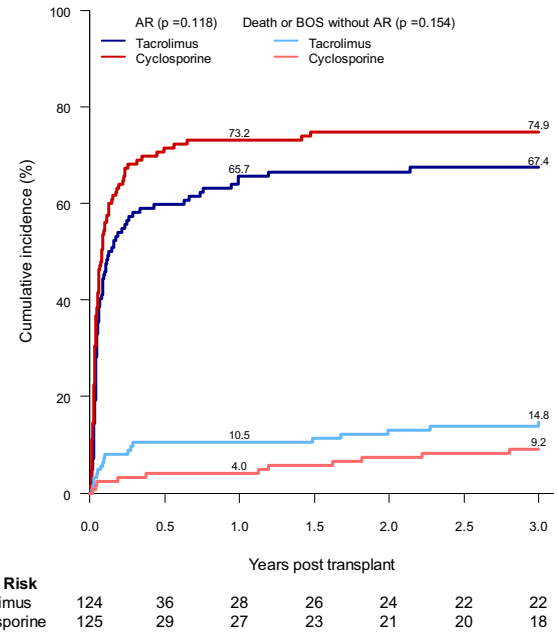

Figure 4 Cumulative incidence of AR ($p = 0.118$ by Gray's test) and death or BOS without previous AR ($p = 0.154$ by Gray's test) for the tacrolimus and cyclosporine groups.

Possible mechanisms and explanations

Both calcineurin inhibitors share very similar mechanisms of action. They permeate the cell membrane freely and bind to immunophilins: cyclosporine to cyclophilin and tacrolimus to FK 506–binding protein (FKBP). The complexes formed inhibit gene transcription for the expression of molecules such as interleukin-2 and CD154, thereby inhibiting T-lymphocyte activation. Tacrolimus shows an increased binding affinity to FKBP, rendering tacrolimus 50-fold more potent than cyclosporine in vitro and 10 times more potent in vivo. Different effects of tacrolimus and cyclosporine on IL-10 synthesis and transforming growth factor (TGF) synthesis have also been described.[22,23] These differences may relate to the increased immunosuppressive efficacy of tacrolimus compared with cyclosporine.

Comparison with findings from other published studies

There are few published randomized, controlled trials comparing tacrolimus and cyclosporine immunosuppression after lung transplantation. Most trials have compared combinations of tacrolimus and cyclosporine with azathioprine. In 1995, Keenan et al reported the 2-year results of a Pittsburgh single-center study showing a significant reduction in BOS and AR incidence in 133 patients treated with tacrolimus with azathioprine vs cyclosporine with azathioprine.[7] This effect was not appreciable when long-term follow-up data were made available in abstract form.[24]

In a 2-center, prospective, randomized trial, the Munich and Vienna groups compared tacrolimus and cyclosporine in combination with MMF and reported a high immunosuppressive potency of both regimens, but no significant difference was detected in freedom from acute rejection or survival after 1 year. The lack of difference was probably due to the small patient numbers or inhomogeneously distributed confounding variables.[9]

More recently, Hachem et al published the results of a randomized, controlled, single-center trial of tacrolimus vs cyclosporine in combination with azathioprine in 90 patients after lung transplantation.[6] The primary end-point was a composite of a cumulative acute rejection (A score) of $\geq$3, a cumulative lymphocytic bronchiolitis (B score) of $\geq$4, or the onset of BOS Stage 0-p. Tacrolimus was associated with a lower burden of AR and lymphocytic bronchiolitis and a trend toward greater freedom from BOS Stage 0-p than cyclosporine. Incidence of BOS Stage 1 was not significantly different between groups, although a trend toward less BOS was seen in tacrolimus-treated patients.

In contrast to previously published randomized trials our study was sufficiently powered to detect a difference in BOS incidence 3 years after lung transplantation. For the first time it has been shown that a de novo immunosuppressive protocol using tacrolimus in combination with MMF leads to a reduced rate of BOS after lung transplantation when compared with a de novo immunosuppressive protocol using cyclosporine and MMF. Cyclosporine treatment carried a 2-fold higher risk for BOS at 3 years when compared with tacrolimus treatment. The magnitude of this observation may have been greater if not for the high number of patients in the cyclosporine group who were converted to tacrolimus due to refractory or recurrent AR. Tacrolimus reduced the occurrence of BOS but was not associated with a survival advantage; in fact, survival in the cyclosporine-treated group was marginally greater, albeit not significantly. Given the fact that BOS is the most important long-term prognostic factor for survival, a survival benefit for tacrolimus-treated patients remains a realistic possibility in longer term follow-up.[25]

Limitations of the study

This study was mainly limited by the large number of patients converted to immunosuppressive protocols other than initially assigned, which was more frequent in the cyclosporine group than in the tacrolimus group. Failure to complete the trial per protocol may have influenced the incidence of primary or secondary parameters despite the use of an intent-to-treat analysis. Allowing individual center determination of BOS grade could theoretically bias results, but each center was highly experienced in the application of the ISHLT criteria and would have been aware of confounding variables. Similarly, a clinical diagnosis of rejection is not as specific as a histopathologic diagnosis.

In conclusion, this prospective, randomized, controlled, multicenter investigator-driven trial strongly suggests that a de novo protocol of tacrolimus, in combination with MMF, significantly reduces the risk for development of BOS in lung transplant patients when compared with a protocol of cyclosporine and MMF. Survival rates were excellent in both study groups and, despite the higher immunosuppressive potency of tacrolimus, we did not detect an increase in infections or other side effects with this drug, although there was a trend for worse renal function. The study was not powered to detect a survival advantage at 3 years, but the lower rate of BOS in the tacrolimus group may translate to improved survival in longer term follow-up.

Disclosure statement

The authors have no conflicts of interest to disclose. The study was funded by Astellas Pharma (formerly Fujisawa Pharmaceuticals) and Roche. The funding companies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix 1

Inclusion criteria:

- Male or female recipients of a first heart–lung, bilateral or single-lung allograft suitable to receive triple immunosuppressive therapy with tacrolimus or cyclosporine, MMF and corticosteroids per standard guidelines.[27,28]

- Age range = 18 to 66 years.
- Able to understand the purposes and risks of the study.
- Female patients of child-bearing age agreeing to maintain effective birth control practice during the follow-up period.

Exclusion criteria:

- Need for immunosuppressive regimen other than study medication or received additional organ transplantations.
- Pregnant women, nursing mothers or women unwilling to use adequate contraception.
- Serologic evidence of human immunodeficiency virus, hepatitis B surface antigen or hepatitis C virus antibodies.
- Pan-resistant infections with *Burkholderia cepacia* or mycobacteria during the last 12 months preceding lung transplant.
- Renal insufficiency (creatinine clearance <40 ml/min).
- Patients in need of invasive ventilator devices or extracorporeal membrane oxygenation.

Appendix 2
List of participating centers:

Australia: St. Vincent's Hospital, Sydney.
Austria: Vienna General Hospital, Vienna.
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