Transplant International

ORIGINAL ARTICLE

Outcomes with alemtuzumab induction therapy in lung transplantation: a comprehensive large-scale single-center analysis

Alberto Benazzo[1], Sophia Auner[1], Panja M. Boehm[1], Annika Morscher[1], Stefan Schwarz[1], Bogdan Vidmar[2], Fedja Dzubur[3], Thomas Schweiger[1], Ali M. Hoda[1], Bernhard Moser[1], Jose R. Matilla[1], Gabriella Muraközy[1], Georg Lang[1], Shahrokh Taghavi[1], Walter Klepetko[1], Konrad Hoetzenecker[1] & Peter Jaksch[1]

1 Department of Thoracic Surgery, Medical University of Vienna, Vienna, Austria
2 Division of Thoracic Surgery, Medical Center Ljubljana, Ljubljana, Slovenia
3 Clinical Center for Pulmonary Diseases, University Hospital Centre Zagreb, Zagreb, Croatia

Correspondence
Alberto Benazzo MD, Department of Thoracic Surgery, Medical University of Vienna, Währinger Guertel 18-20, A-1090 Vienna, Austria.
Tel.: +43 1 40400 56440;
fax: +43 1 40400 56420;
email:
alberto.benazzo@meduniwien.ac.at

The abstract of the herein presented study was presented at the 39th Annual Meeting of the International Society for Heart and Lung Transplantation.

SUMMARY

Alemtuzumab is a monoclonal antibody targeting CD52, increasingly used as induction therapy after transplantation. The aim of this study was to analyze the outcomes of alemtuzumab induction therapy followed by a low-dose maintenance immunosuppression in a large single-center cohort of lung transplant recipients. All patients, who received alemtuzumab induction followed by a low-dose maintenance immunosuppression were included in the analysis. Short- and long-term outcomes were analyzed. 721 lung transplant recipients, transplanted between January 2008 and June 2019, were included in this retrospective study. Freedom from higher-grade ACR at 1, 5, and 10 years was 98%, 96%, and 96%, respectively. Thirty-nine patients (5%) developed clinical AMR. Twenty-one percent of patients developed high-grade CKD. A total of 1488 infections were recorded. Sixteen percent were diagnosed within the first 3 months. Sixty-two patients (9%) developed a malignancy during follow-up. Freedom from CLAD at 1, 5, and 10 years was 94%, 72%, and 53%, respectively. Overall survival rates at 1, 5, and 10 years were 85%, 71%, and 61%, respectively. Alemtuzumab induction combined with a low-dose tacrolimus protocol is safe and associated with low rates of acute and chronic rejection, as well as an excellent long-term survival.

Transplant International 2021; 34: 2633–2643

Key words
alemtuzumab, immunosuppression, induction therapy, lung transplantation, rejection

Received: 6 June 2021; Revision requested: 15 October 2021; Accepted: 20 October 2021;
Published online: 16 November 2021

Background

According to the ISHLT, the most commonly used immunosuppression protocol in lung transplantation consists of an induction therapy, and a triple-drug maintenance immunosuppression including a calcineurin-inhibitor, an antiproliferative drug and steroids [1]. The rationale underlying induction immunosuppression is two-fold: to reduce the incidence of acute cellular rejection (ACR) and to reduce the dose of maintenance immunosuppression, thereby minimizing its adverse effects. Currently, IL-2 receptor antagonists are used by the majority of centers [1], followed by antithymocyte globulin (ATG) and alemtuzumab. Alemtuzumab is a

doi:10.1111/tri.14153

humanized monoclonal antibody targeting CD52, a surface molecule expressed on T and B lymphocytes, Natural Killer (NK) cells and macrophages. Its activation induces cell lysis leading to a long-lasting immune cell depletion between 6 to 24 months. [2,3] Induction therapy is associated with improved clinical outcomes in terms of acute and chronic rejection; however, there is still reluctance to use alemtuzumab as it might bear a higher risk for infections and malignancies. Alemtuzumab has been introduced in our clinical practice in 2008 and since 2013 has been broadly used as induction therapy. In 2014 our group published a prospective randomized controlled trial comparing alemtuzumab and ATG [4]. This study showed a reduced incidence of higher-grade ACR in the alemtuzumab group. Similarly, other groups observed superiority of alemtuzumab in terms of graft and patient survival. However, data on long-term results and safety are still missing.

The aim of this study was to comprehensively analyze short- and long-term outcomes of alemtuzumab induction therapy followed by a low-dose maintenance immunosuppression protocol in a large cohort of lung transplant recipients.

Methods

Study design

This is a retrospective descriptive study, analyzing a 10-years single-center experience with alemtuzumab induction combined with low-dose maintenance immunosuppression protocol. All patients receiving this protocol between January 2008 and June 2019 were included in the analysis. Primary endpoint was freedom from end-stage CLAD, defined as definite CLAD stage 3 to 4 [5], irrespective of phenotype. Secondary endpoints were patient survival, incidence of ACR, lymphocytic bronchiolitis (LB), antibody-mediated rejection (AMR), and donor-specific antibodies (DSA), rate of infections, malignancy and long-term kidney function. This study has been approved by the Institutional Ethical Committee of the Medical University of Vienna [ECS 1729/2020] and was conducted according the declaration of Helsinki.

Clinical protocol and definitions

Lung transplant recipients received alemtuzumab (Genzyme/Sanofi, Cambridge, USA) as a single intravenous dose of 30 mg after LTx at arrival at the intensive care unit (ICU). All patients received a dose-reduced maintenance immunosuppression according to Table 1. During follow-up, in case of deterioration of kidney function, infections requiring hospital admission or rejection, the target blood levels were adapted to the low or high end of the target range. PGD grades were prospectively recorded based on the most recent recommendation of the ISHLT working group [6]. Patients on prolonged postoperative ECMO with radiological signs of PGD were graded as PGD 3, those without pathologic chest X-ray were classified as "ungradable." Perioperative infectious prophylaxis was based on broad-spectrum antibiotics or adapted to resistance testing. All patients received a lifelong *Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole or atovaquon. Prophylactic inhalation therapy with amphotericin B and gentamycin or according to pretransplant airway colonization was provided for 1–3 months. CMV prophylaxis included CMV hyperimmunoglobulines (POD 1, 7, 14 and 21) together with valganciclovir for a minimum of 3 months. In high-risk patients (donor CMV IgG+, recipient CMV IgG-), a 12 months prophylaxis was performed. Surveillance bronchoscopy with transbronchial biopsy (TBB) and bronchoalveolar lavage (BAL) were performed 2 weeks and 1, 2, 3, 6, 12 months after transplantation and whenever clinically indicated. All patients received a chest computed tomography (CT) once a year. Biopsies were classified according to ISHLT criteria [7]. ACR grade A2 and LB grade B2 or higher were treated with a pulse of steroids for 3 days with consecutive dose tapering. In case of inadequate clinical response, ATG (2 mg/kg) was administered for 5 days. Until September 2016, DSAs was measured only in case of clinical deterioration. Afterward, DSAs screening was performed at each follow-up visit. DSAs were considered positive with a mean fluorescence intensity > 1000. AMR was defined

Table 1. Maintenance immunosuppression protocol.

Maintenance immunosuppression			
Time after LuTx	Tacrolimus trough blood level (ng/ml)	A prednisolone (mg/kg)	MMF
0–3 months	8–10	0.2	–
3–6 months	6–8	0.15	–
6–12 months	6–8	0.1	–
12–24 months	5–7	5 mg/d	1–1.5g twice daily
> 24 months	< 5	5 mg/d	1–1.5g twice daily

LuTx, lung transplantation; MMF, mycophenolate mofetil.

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according to the ISHLT consensus. [8] Diagnosis of CLAD was established by two independent physicians according to the consensus report of the ISHLT [5]. All patients with a decrease in lung function received azithromycin (250 mg three times a week) for at least three months, after exclusion of other treatable causes of lung allograft dysfunction. In case of further deterioration, recipients underwent six cycles of ECP within three months. In case of stabilization or improvement (defined as $\geq$ 10% in FEV_1 from start of ECP), recipients continued the therapy for 6 to 12 months, otherwise ECP was stopped and patients received best palliative care or were listed for retransplantation.

High-grade chronic kidney disease (CKD) was defined as eGFR <60 ml/min/1.73 m^2 for $\geq$ 3 months. With diagnosis of high-grade CKD, patients were switched to everolimus in combination with reduced dose of calcineurin inhibitors. Infectious complications, were defined as presence of pathogens with clinical signs and symptoms and need for specific treatment. CMV disease was defined as CMV DNAemia in combination with symptoms and signs of organ involvement together with histological or cytological verification of CMV infection [9].

Statistical analysis

Categorical variables were reported as absolute and relative frequencies (%), continuous variables as median (interquartile range, IQR) or mean ($\pm$ standard deviation). Relative frequencies were calculated based on the number of patients alive in follow-up at the respective timepoint. Chi-square tests, Fisher exact tests, Mann-Whitney *U*-tests, or ANOVA were used to compare variables as applicable. Survival curves were generated with the Kaplan-Meier method and compared by log-rank tests. Univariate and multivariable Cox regression were performed to find risk factors for CLAD or mortality. Variables were included in a multivariable Cox regression when they reached the level of significance in the univariate analysis. Data was analyzed using SPSS version 26.0 software or R 3.4.2 and graphics were designed with GraphPad Prism 6.

Results

Demographics

Seven-hundred twenty-one lung transplant recipients were included in the study (Table 2). Forty-seven percent of patients were female, median age was 51 years (IQR: 35–59). Most common underlying diagnosis was chronic obstructive pulmonary disease (COPD) (256, 36%). The majority of patients (395, 55%) received a bilateral lung transplantation. Eighty-eight percent of transplantations were performed on venoarterial ECMO and in 23% ECMO was prolonged into the postoperative period when the graft did not meet preset quality criteria [10]. At 72 h, the majority of patients were in PGD grade 0 while only 27 patients (4%) had a PGD grade 3. Median ICU time was 8 days (IQR: 5–19) and median in-hospital stay was 24 days (IQR: 18–37). Median length of follow-up was 1709 days (IQR: 754–2517).

Rejection and DSAs

A total of 4,132 surveillance biopsies have been performed in the study period. In 26 patients (4%), one or more episodes of higher-grade ACR was diagnosed during follow-up. Sixty-four patients (9%) had at least one episode of higher-grade LB. Freedom from ACR at 1, 5, and 10 years was 98%, 96%, and 96%, respectively (Fig. 1a). Freedom from LB at 1, 5, and 10 years was 95%, 90%, and 89%, respectively (Fig. 1b). No significantly worse patient and graft survival was observed in patients with higher-grade ACR or LB (Figures S1A,B and S2A,B).

Seventeen percent of recipients (n = 121) had pretransplant DSAs. Among them, 8% (n = 59) had DSA against HLA-Class I and 9% against HLA-Class II. Twenty-five percent (n = 177) developed de novo DSAs (dnDSAs): 10% were against both HLA-Class I and II, 5% against HLA-Class I and 10% against HLA-Class II. Freedom from AMR at 1, 5, and 10 years was 96%, 94%, and 93%, respectively (Fig. 1c). In 13 (33%) cases AMR was definite, in 17 (44%) probable and in 9 (23%) cases possible. Both patient and graft survival were significantly worse in AMR patients ($P < 0.001$ and $P < 0.001$, Figures S1C and S2C).

Twenty-eight percent (n = 189) of patients developed a CLAD during the study period. The majority (127, 67%) had a bronchiolitis obliterans syndrome (BOS), 10% (n = 19) had a restrictive allograft syndrome (RAS) and the remaining were classified as a mixed phenotype (43, 23%). At the time of this analysis, CLAD stage was distributed as follows: 26% (n = 49) were in stage 1, 17% (n = 33) in stage 2, 38% (n = 72) in stage 3 and 19% (n = 35) in stage 4. Freedom from CLAD at 1, 5, and 10 years was 94%, 72%, and 53%, respectively (Fig. 1d). Though not significant, patients with CLAD showed a trend toward a worse overall survival (P = 0.063, Figure S1D). As expected, graft

Table 2. Patients' characteristics.

Patients' characteristics		
Female (n, %)		337, 47%
Age at LuTx (median, IQR)		51 (35–59)
High-risk CMV mismatch (n, %)		153, 21%
Underlying diagnosis	COPD (*n*, %)	256, 36%
	Fibrosis (*n*, %)	181, 25%
	iPAH (*n*, %)	56, 8%
	CF (*n*, %)	156, 22%
	CLAD (*n*, %)	18, 2%
	Others (*n*, %)	54, 7%
Indication	LuTx (*n*, %)	703, 98%
	ReTx (*n*, %)	18, 2%
Type of Tx	DLuTX (*n*, %)	395, 55%
	SLuTX (*n*, %)	12, 2%
	Lobar (*n*, %)	47, 6%
	Size reduced (*n*, %)	267, 37%
ECLS Bridge (*n*, %)		69, 10%
Intraoperative VA-ECMO (*n*, %)		636, 88%
Prolonged postop ECMO (*n*, %)		164, 23%
PGD grade at 72 hrs	0 (*n*, %)	577, 80%
	1 (*n*, %)	44, 6%
	2 (*n*, %)	30, 4%
	3 (*n*, %)	27, 4%
	Ungradable (*n*, %)	43, 6%
ICU time (days) (median, IQR)		8 (5–19)
In-hospital stay (days) (median, IQR)		24 (18–37)
Best FEV1 post-Tx (L/sec) (median, IQR)		2.75 (2.3–3.4)
Best FEV1 post-Tx (%) (median, IQR)		95 (92–98)
Best TLC post-Tx (L) (median, IQR)		5.4 (4.6–6.3)
Best TLC post-Tx (%) (median, IQR)		82 (80–83)
Cumulative A score (mean ± SD)		0.05 ± 0.15
Cumulative B score (mean ± SD)		0.25 ± 0.27
Higher-grade ACR (*n*, %)		26, 4%
Higher-grade LB (*n*, %)		64, 9%
PreTx DSAs (*n*, %)		121, 17%
PreTx DSA Class I (*n*, %)		59, 8%
PreTx DSA Class II (*n*, %)		66, 9%
dnDSAs (*n*, %)		177, 25%
dnDSA DSA Class I (*n*, %)		106, 15%
dnDSA DSA Class II (*n*, %)		132, 20%
Antibody-mediated rejection (*n*, %)		39, 5%
AMR stage	Definite (*n*, %)	13, 33%
	Probable (*n*, %)	17, 44%
	Possible (*n*, %)	9, 23%
CLAD (*n*, %)		189, 28%
Time to CLAD stage 1 (days, IQR) (median, IQR)		710 (418–1526)
Time to CLAD stage 3 (days, IQR) (median, IQR)		1280 (657–2308)
CLAD phenotypes	BOS (*n*, %)	127, 67%
	RAS (*n*, %)	19, 10%
	Mixed (*n*, %)	43, 23%
Grade	1 (*n*, %)	49, 26%
	2 (*n*, %)	33, 17%
	3 (*n*, %)	72, 38%
	4 (*n*, %)	35, 19%

N, numbers; IQR, interquartile range; SD, standard deviation; LuTx, lung transplantation; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; iPAH, idiopathic pulmonary arterial hypertension; CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; ReTx, retransplantation; DLuTx, double lung transplantation; SLuTx, single lung transplantation; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; PGD, primary graft dysfunction; ICU, intensive care unit; ACR, acute cellular rejection; LB, lymphocytic bronchiolitis; DSA, donor-specific antibodies; AMR, antibody-mediated rejection; BOS, bronchiolitis obliterans syndrome; RAS, restrictive allograft syndrome.

survival was significantly worse in patients with CLAD ($P = 0.015$, Figure S2D).

Comorbidities

A detailed description of comorbidities is presented in Table 3. Serum creatinine as well as eGFR slightly deteriorated within the first year after transplantation but stabilized thereafter (Fig. 2). Twenty-one percent ($n = 148$) of patients developed high-grade CKD. At 5 years, 6% ($n = 21$) of patients were in CKD stage 3a, 10% ($n = 35$) in CKD stage 3b, 4% ($n = 14$) in CKD stage 4, and less than 1% in CKD stage 5. At 10 years, 20% ($n = 6$) of patients had a CKD stage 3a, 10% ($n = 3$) a CKD stage 3b, and 7% ($n = 2$) a CKD stage 4. Two patients (0.2%) required chronic dialysis in the follow-up.

Differential count of leukocyte subpopulation within the first year after transplantation showed a significant cell depletion involving mainly $CD4^+$ and $CD8^+$ T cells, as well as B cells (Tables 4 and S1). In the study cohort, a total of 1488 infections were recorded during follow-up. The vast majority (1036, 69%) could be treated on an outpatient basis, 408 (27%) required hospitalization in the normal ward and only in 64 (4%) cases an ICU admission was necessary. The majority (824, 54%) of infections involved the lower respiratory tract, followed by upper respiratory tract infections (270, 18%), gastrointestinal infections (252, 17%), and urinary tract infections (162, 11%). Within the first year after transplantation, 406 infections were diagnosed in our

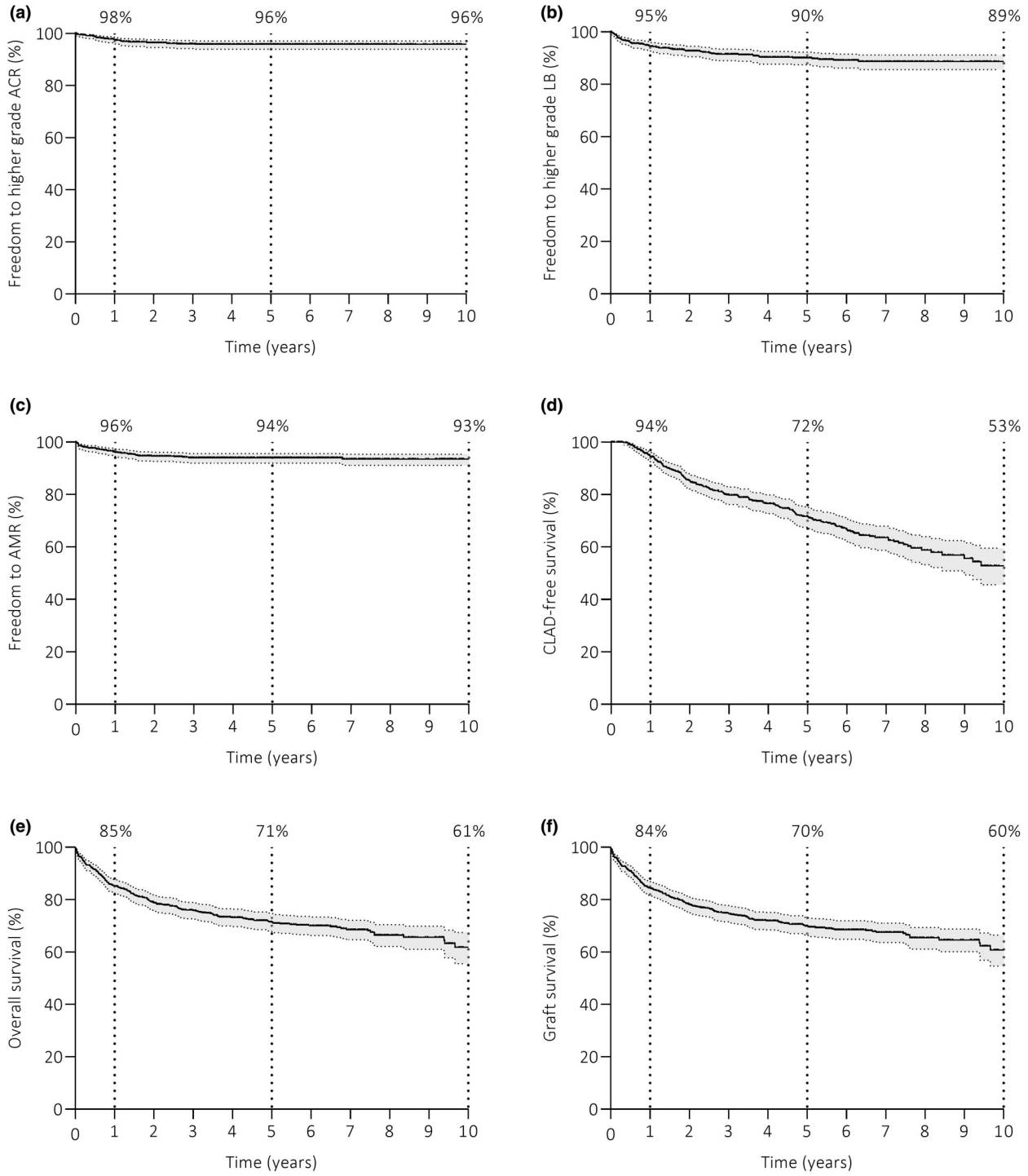

Figure 1 Kaplan–Maier curves showing (a) freedom to higher-grade ACR, (b) freedom to higher-grade LB, (c) freedom from clinical AMR, (d) CLAD-free survival, (e) overall survival, (f) graft survival. Abbreviations. ACR: acute cellular rejection, LB: lymphocytic bronchiolitis, AMR: antibody-mediated rejection, CLAD: chronic lung allograft dysfunction.

cohort. Sixteen percent (n = 67) were diagnosed within the first 3 months, 48% (n = 194) between month 3 and 6 after transplantation and the remaining (145, 36%) between month 6 and 12. Sixty-one patients (8%) were diagnosed with a pulmonary Pseudomonas Aeruginosa infection, 20 (3%) suffered from a CMV disease, among them 11 (1.5%) presented with CMV pneumonia.

Table 3. Comorbidities.

Comorbidities		
Higher-grade CKD in FU (*n*, %)		148, 21%
Time to CKD (days) (median, IQR)		398 (365–674)
SCr > 2.5 mg/dl at 3 years (*n*, %)		8, 2%
SCr > 2.5 mg/dl at 5 years (*n*, %)		7, 2%
SCr > 2.5 mg/dl at 10 years (*n*, %)		2, 7%
CKD stage ≥ 3 at 3 years	3a (*n*, %)	40, 8%
	3b (*n*, %)	47, 10%
	4 (*n*, %)	18, 4%
	5 (*n*, %)	1, 0.2%
CKD stage ≥ 3 at 5 years	3a (*n*, %)	21, 6%
	3b (*n*, %)	35, 10%
	4 (*n*, %)	14, 4%
	5 (*n*, %)	1, 0.3%
CKD stage ≥ 3 at 10 years	3a (*n*, %)	6, 20%
	3b (*n*, %)	3, 10%
	4 (*n*, %)	2, 7%
	5 (*n*, %)	0
Post-transplant diabetes (*n*, %)		79, 11%
Total outpatient infections (*n*, %)		1036, 69%
Total inpatient infections (*n*, %)		408, 27%
Total ICU infections (*n*, %)		64, 4%
Number of infections within 1st year	within 3 months, (*n*, %)	67, 16%
	3 to 6 months, (*n*, %)	194, 48%
	6 to 12 months, (*n*, %)	145, 36%
Total URTI (*n*, %)		270, 18%
Total LRTI (*n*, %)		824, 54%
Total GI (*n*, %)		252, 17%
Total UTI (*n*, %)		162, 11%
Colonization Pseudomonas Aeruginosa	Pretransplant (*n*, %)	50, 7%
	within 1st year (*n*, %)	111, 15%
	after 1st year (*n*, %)	32, 4%
Infection with P. Aeruginosa (*n*, %)		61, 8%
Colonization with aspergillus (*n*, %)		126, 17%
CMV	Reactivation (*n*, %)	270, 37%
	Disease (*n*, %)	20, 3%
Malignancies	PTLD (*n*, %)	13, 2%
	non-PTLD (*n*, %)	49, 7%
Time to malignancy (median, IQR)		869 (339–1778)
Non-PTLD primary organ	Lung (*n*, %)	7, 1%
	Colorectal (*n*, %)	3, 0.4%
	Dermatological (*n*, %)	20, 2.8%
	Hepatobiliary (*n*, %)	1, 0.1%
	Gynecological (*n*, %)	7, 1%
	Breast (*n*, %)	2, 0.3%
	Head and neck (*n*, %)	1, 0.1%
	Esophageal (*n*, %)	1, 0.1%
	Gastric (*n*, %)	1, 0.1%
	Urological (*n*, %)	5, 0.7%
	Hematological (*n*, %)	1, 0.1%

N, numbers; IQR, interquartile range; SD, standard deviation; CKD, chronic kidney disease; FU, follow-up; SCr, serum creatinine; ICU, intensive care unit; URTI, upper respiratory tract infections; LRTI, lower respiratory tract infections; GI, gastrointestinal infections; UTI, urinary tract infections; CMV, cytomegalovirus; PTLD, post-transplant lymphoproliferative disorder.

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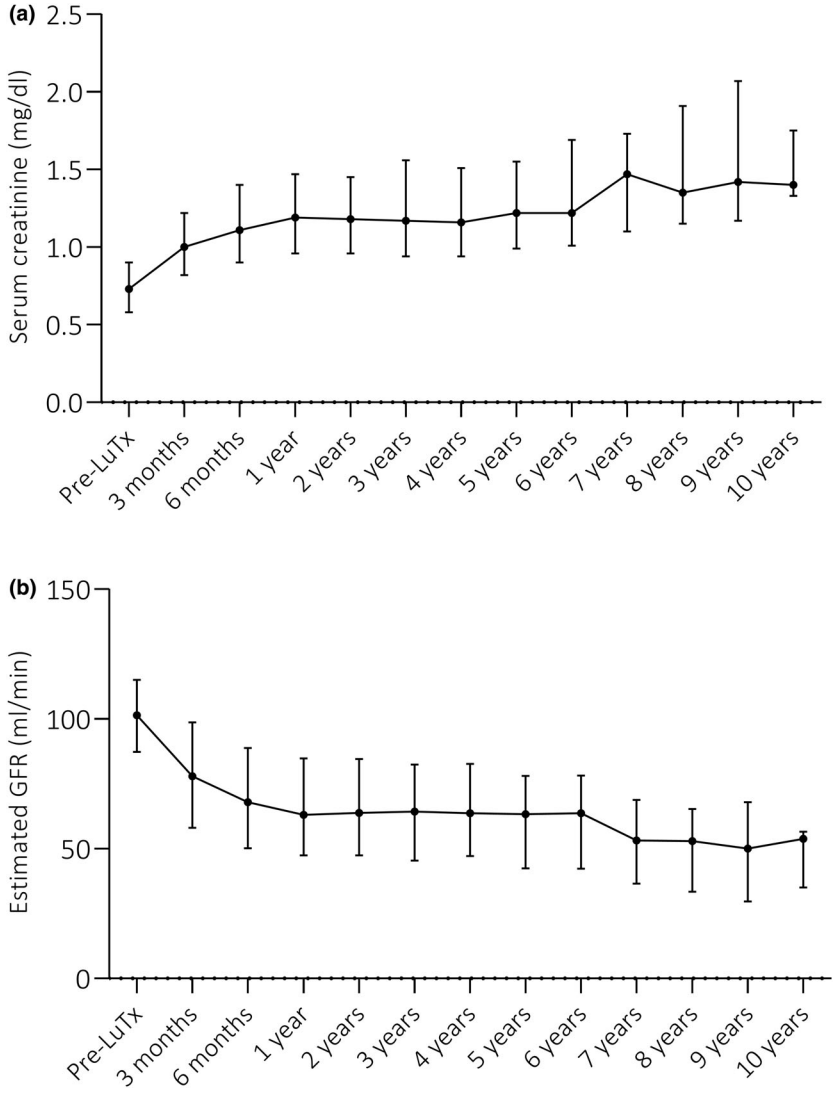

Figure 2 Long-term kidney function. This figure shows that kidney function worsens rapidly within the first 6 months after transplantation and then stabilize along the follow-up. Abbreviations. GFR: glomerular filtration rate.

Sixty-two patients (9%) developed a malignancy during follow-up. Median time to diagnosis was 869 days (IQR: 339–1778). Thirteen (2%) patients developed a post-transplant lymphoproliferative disorder (PTLD), the majority of them (7, 54%) within the first year after transplantation. Of the remaining 49 (7%) cases, 20 (3%) patients had a skin tumor, followed by 7 (1%) lung tumors, 7 (1%) gynecological tumors, and 5 (1%) urological tumors. Patients with PTLD showed a worse survival compared to the remaining cohort (at 1 year 61% vs. 85%, at 5 years 35% vs. 71%, $P = 0.005$). Patients with non-PTLD malignancy had comparable overall survival to the remaining cohort ($P = 0.404$). The low incidence of severe infections and malignancy with preserved kidney function and low rates of rejections resulted in improved quality of life in our cohort.

Survival and retransplantation

Mean patient survival time was 3331 days (CI: 3167–3495). Overall survival rates at 1, 5, and 10 years were 85%, 71%, and 61%, respectively (Fig. 1e, Table S4). Mean graft survival time was 3283 days (CI: 3118–3447). Rates of graft survival at 1, 5, and 10 years were 84%, 70%, and 60%, respectively (Fig. 1f). Nineteen recipients underwent retransplantation. In the majority (13, 70%) the indication was CLAD, followed by infectious complications in 4 cases, primary organ failure in 1 case and vanishing bronchus intermedius syndrome in

Table 4. Leukocyte subpopulation counts over time.

Counts of leukocyte subpopulations						
	1st month		6th month		12th month	
Time points	Median	IQR	Median	IQR	Median	IQR
$CD4^+$ T cells (abs/μl)	2	1–5	73	43–104	140	99–201
$CD8^+$ T cells (abs/μl)	5	2–21	116	61–258	245	109–338
B cells (abs/μl)	1	1–5	13	7–27	48	24–95
NK cells (abs/μl)	55	37–86	204	147–317	207	147–310

a further case. Time to retransplantation was 630 days (IQR: 156–1052).

Risk factor analysis

Univariate and multivariable Cox regression analysis (Fig. 3, Tables S2 and S3) were performed to identify risk factors for overall survival and occurrence of CLAD. The following independent risk factors for survival were found: higher-grade LB (HR: 1.98, CI: 1.05–3.75, $P = 0.036$), AMR (HR: 3.82, CI: 1.86–7.82, $P < 0.001$), RAS phenotype (HR: 2.86, CI: 1.26–6.44, $P = 0.011$), mixed phenotype (HR: 1.89, CI: 1.04–3.47, $P = 0.038$).

The following risk factors for CLAD could be identified: higher-grade ACR (HR: 2.45, CI: 1.33–4.54, $P = 0.004$), dnDSAs (HR: 1.75, CI: 1.22–2.52, $P = 0.003$), AMR (HR: 3.81, CI: 2.21–6.58, $P < 0.001$), total number of infections per patients (HR: 1.08, CI: 1.03–1.14, $P = 0.001$), and post-transplant lymphoproliferative disease (HR: 2.65, CI: 1.06–6.66, $P = 0.038$). Female gender (HR: 0.67, CI: 0.48–0.93, $P = 0.017$) and cystic

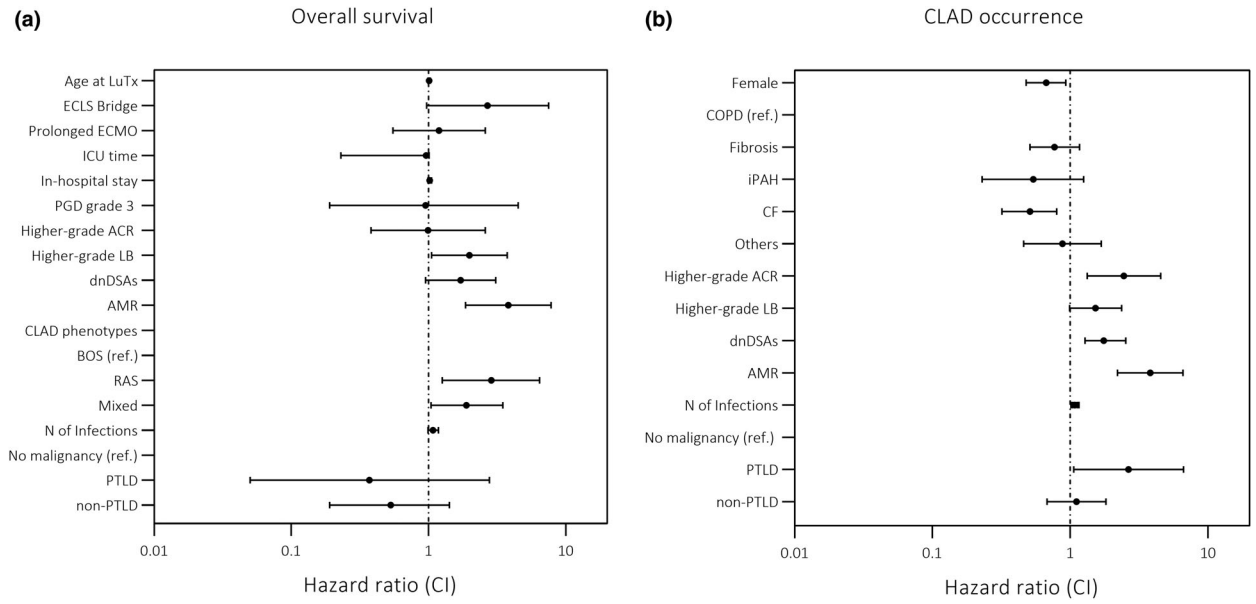

Figure 3 Forest plots showing results of multivariable Cox regression for overall survival and graft survival. In (a) higher-grade LB (HR: 1.98, CI: 1.05–3.75, $P = 0.036$), AMR (HR: 3.82, CI: 1.86–7.82, $P < 0.001$), RAS phenotype (HR: 2.86, CI: 1.26–6.44, $P = 0.011$) and mixed phenotype (HR: 1.89, CI: 1.04–3.47, $P = 0.038$) were identified as risk factors for survival. In (b) higher-grade ACR (HR: 2.45, CI: 1.33–4.54, $P = 0.004$), dnDSAs (HR: 1.75, CI: 1.22–2.52, $P = 0.003$), AMR (HR: 3.81, CI: 2.21–6.58, $P < 0.001$), total number of infections per patients (HR: 1.08, CI: 1.03–1.14, $P = 0.001$) and post-transplant lymphoproliferative disease (HR: 2.65, CI: 1.06–6.66, $P = 0.038$) have been identified as risk factors for CLAD occurrence. Female gender (HR: 0.67, CI: 0.48–0.93, $P = 0.017$) and cystic fibrosis (HR: 0.51, CI: 0.32–0.80, $P = 0.004$) were protective against CLAD. Abbreviations., LuTx: lung transplantation, ECLS: extracorporeal life support, ECMO: extracorporeal membrane oxygenation, ICU: intensive care unit, PGD: primary graft dysfunction, ACR: acute cellular rejection, LB: lymphocytic bronchiolitis, DSA: donor-specific antibodies, AMR: antibody-mediated rejection, BOS: bronchiolitis obliterans syndrome, RAS: restrictive allograft syndrome, PTLD: post-transplant lymphoproliferative disorder, COPD: chronic obstructive pulmonary disease, iPAH: idiopathic pulmonary arterial hypertension, CF: cystic fibrosis, CLAD: chronic lung allograft dysfunction.

fibrosis (HR: 0.51, CI: 0.32–0.80, $P = 0.004$) were protective factors against CLAD.

Discussion

To the best of our knowledge, this study represents the first large-scale analysis of long-term of an immunosuppression strategy based on alemtuzumab induction therapy and followed by a low-dose dual drug maintenance immunosuppression. The herein presented results show that dreaded side effects of induction therapy, namely severe infections and malignancies, are uncommon but episode of ACR, LB, and AMR have become rare events. Consequently, CLAD rates were low and long-term survival was excellent.

Alemtuzumab is a humanized monoclonal antibody targeting CD52, which is expressed on several immune cells and its activation induces cell lysis leading to immune cell depletion [2,3]. B-cell counts usually recover 3 to 6 months and T-cell counts 12 to 24 months after the treatment [2]. Moreover, clinical data support the expansion of regulatory T (Tregs) and B (Bregs) cells. [6,11]

To date, published data on the use of alemtuzumab in lung transplantation are scarce. In 2011 Shyu and colleagues presented their single-center experience with different immunosuppression strategies, including alemtuzumab induction. In their 5-year experience, the alemtuzumab group showed improved patient and graft survival compared to the noninduction and daclizumab group. Moreover, freedom from ACR, LB, and bronchiolitis obliterans (BO) was superior in the alemtuzumab group [12]. Furuya and colleagues reviewed the UNOS database and identified 738 patients receiving alemtuzumab induction therapy [13]. These patients had an improved survival and a lower risk for CLAD. Finally, our center conducted the only prospective randomized controlled trial published to date comparing alemtuzumab and ATG [4]. In this study, a significant reduction of higher-grade acute cellular rejection rates was observed in the alemtuzumab group. These findings could be confirmed in the current analysis of over 700 patients. The herein presented low incidence of ACR and LB, demonstrated by both the low cumulative biopsies scores and an excellent freedom from higher-grade episodes, significantly differs from the ACR rates published by the ISHLT. According to the registry data, at least 27–29% of recipients experienced a higher-grade ACR episode within the first postoperative year, irrespective of induction strategies [1]. Higher-grade ACR and LB are well-known risk factors for the development of both BOS and RAS. [14] Also, in the current study, ACR was independently associated with an increased risk of CLAD in the multivariable analysis. The low incidence of ACR in our patient cohort reflected in the longer freedom from CLAD as compared to the international standards [1,15].

Recently, the development of dnDSAs has increasingly gained recognition within the lung transplant community [8]. According to the literature, dnDSAs can be found in 25–55% of recipients and are associated with increased mortality and higher risk for CLAD [16–18]. Of note, the incidence of dnDSAs has never been specifically investigated in lung transplant recipients receiving alemtuzumab before. Twenty-five percent of recipients in our cohort developed de novo DSAs and the majority was directed against HLA-Class II. This rate is comparable to the rates reported by other centers [16–18]. Thirty-nine patients, equivalent to 5% of the whole cohort, were diagnosed with a clinical AMR. The incidence was lower compared to other series [19]; however, survival in these patients was poor with less than 50% at 5 years.

According to the literature, the incidence of chronic kidney disease after lung transplantation ranges between 20% and 70% [1,20], it is associated with increased long-term mortality [21]. The ISHLT data reports that 13–15% of lung transplant recipients have a serum creatinine higher than 2.5 mg/dl in the long-term follow-up and 25% developed with a severe renal dysfunction [1]. In our cohort, 2–7% of patients showed a serum creatinine higher than 2.5 mg/dl within 10 years and approximately 20% of the patients were diagnosed with a severe chronic kidney disease in the long-term follow-up. The introduction of alemtuzumab in our clinical practice allowed for a significant reduction of CNI, with a consequent preservation of kidney function. This was possible due to the profound immunodepletion driven by this agent.

One of the main concerns behind the reluctance of using alemtuzumab as induction therapy is the long-lasting immunodepletion of T- and B cells, which is thought to increase the risk for severe infection and malignancies. However, to date, no evidence can be found in the literature supporting these concerns in solid organ recipients treated with alemtuzumab [12,13,22–24]. Van Delden *et al.* recently published a large analysis of infectious diseases in a transplant cohort, including 3541 solid organ transplant recipients, among them 286 lung recipients [25]. The authors observed a total of 3520 infectious events and a cumulative incidence of 62% at 12 months after lung

transplantation. In addition, a multi-center prospective study including 236 lung transplant recipients reported a 36% incidence of pneumonia within the first 180 days [26]. In our cohort, observed infection were similar to these international standards or even lower. Over the whole study period, only 4% of recipients required ICU admission for infectious complications and approximately 70% were treated on an outpatient basis.

We acknowledge that this study is not free of limitations. First, its retrospective nature comes with the possibility of miscoded data. Then, with a timeframe of ten years a temporal bias cannot be excluded. During the study period, however, protocols and treatment regimens have not changed. Then, mTOR-inhibitors are routinely used in the long-term follow-up in recipients with an eGFR < 60ml/min for more than 3 months. This results in reduced trough levels of CNI and in a possible further bias interpreting the results. Finally, a further limitation is the lack of a matched control group, since the described immunosuppression protocol has been exclusively used for the last 10 years in our institution. This does not allow any inference on superiority of the proposed immunosuppression protocol over other induction agents. Still, the reported experience may improve the general clinical knowledge in our field, thereby consistently improving outcomes after lung transplantation.

In conclusion, this single-center analysis represents one of the largest and most comprehensive study on immunosuppression in the modern era. The findings presented herein show that alemtuzumab induction led to low rates of acute and chronic rejection as well as excellent long-term survival. Moreover, the low infection rate and incidence of malignancy confirmed the safety of the presented protocol.

Authorship

AB and PJ: initiated and designed the project and directed the research. AB, SA, PMB, AM, SS: participated in data collection. AB, KH, PJ: wrote the manuscript. AB, SA, PMB, AM, SS, BV, FD, TS, AMH, BM, JRM, GM, GL, ST, WK, KH, PJ: performed revision and correction of the manuscript. AB, KH, PJ: participated in data analysis and explanation.

Funding

The authors have declared no funding.

Conflicts of interest

The authors have declared no conflicts of interest.

Acknowledgements

None.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Kaplan–Maier curves for overall survival comparing in A) patients with ACR, in B) patients with LB, in C) patients with AMR and in D) patients with CLAD versus non-CLAD patients. Abbreviations. ACR: acute cellular rejection, LB: lymphocytic bronchiolitis, AMR: antibody-mediated rejection, CLAD: chronic lung allograft dysfunction.

Figure S2 Kaplan–Maier curves for graft survival comparing in A) patients with ACR, in B) patients with LB, in C) patients with AMR and in D) patients with CLAD versus non-CLAD patients. Abbreviations. ACR: acute cellular rejection, LB: lymphocytic bronchiolitis, AMR: antibody-mediated rejection, CLAD: chronic lung allograft dysfunction.

Table S1 Tacrolimus trough blood level (ng/ml).

Table S2 Cox regression for overall survival.

Table S3 Cox regression for occurrence of CLAD.

Table S4 Causes of death.

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