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Montelukast in chronic lung allograft dysfunction after lung transplantation

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KEYWORDS:
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outcome;
treatment

BACKGROUND: Chronic lung allograft dysfunction (CLAD) is a major cause of post–lung transplant mortality, with limited medical treatment options. In this study we assessed the association of montelukast treatment with pulmonary function and outcome in lung transplant recipients with progressive CLAD.

METHODS: We performed a retrospective study of all lung transplant recipients transplanted between July 1991 and December 2016 at our center and who were treated for at least 3 months with montelukast for progressive CLAD, despite at least 3 months of prior azithromycin therapy. Main outcome parameters included evolution of pulmonary function and progression-free and overall survival.

RESULTS: A total of 153 patients with CLAD (115 with bronchiolitis obliterans syndrome and 38 with restrictive allograft syndrome) were included, of whom 46% had a forced expiratory volume in 1 second (FEV_1) measure of between 66% and 80%, 31% an FEV_1 between 51% and 65%, and 23% an FEV_1 ≤50% of best post-operative FEV_1 at start of montelukast. Montelukast was associated with attenuation in rate of FEV_1 decline after 3 and 6 months, respectively (both $p < 0.0001$). Patients in whom FEV_1 improved or stabilized after 3 months of montelukast (81%) had significantly better progression-free ($p < 0.0001$) and overall ($p = 0.0002$) survival after CLAD onset, as compared to those with further decline of FEV_1 (hazard ratio [HR] 2.816, 95% confidence interval [CI] 1.450 to 5.467, $p = 0.0022$ for overall survival after CLAD onset in risk-adjusted multivariate analysis).

CONCLUSIONS: Montelukast was associated with a significant attenuation in rate of FEV_1 decline in a substantial proportion of patients with established CLAD, which correlated with better outcome. Further study is required regarding use of montelkast.

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Long-term survival after lung transplantation (LTx) is unsatisfyingly poor when compared with other types of solid-organ transplantation.[1] This lack of success is attributable to a higher prevalence of chronic lung allograft dysfunction (CLAD). At least 2 different clinical phenotypes of chronic rejection CLAD, namely a restrictive phenotype (restrictive allograft syndrome, RAS) and a more common obstructive phenotype (bronchiolitis obliterans syndrome, BOS), have been identified, of which RAS has the worst prognosis after onset (median survival 0.5 to 1.5 years vs 3 to 5 years in BOS).[2,3]

Treatment options for CLAD are limited and only redo transplantation may truly remediate CLAD in well-selected patients. However, a large proportion of patients will redevelop CLAD after redo transplantation, particularly in cases of previous RAS.[4] In BOS, current management guidelines recommend the following: avoiding sustained administration of high-dose corticosteroids, due harmful side effects and ineffectiveness; conversion of cyclosporine to tacrolimus; a trial of azithromycin for a least 3 months; fundoplication of the gastroesophageal junction in cases of documented gastroesophageal reflux; or retransplantation in selected cases.[5] For RAS, there are no formal guidelines and management is experimental; case reports have demonstrated some beneficial effects (i.e., improvement or stabilization of interstitial changes and lung function) with pirfenidone,[6] nintedanib,[7] or alemtuzumab.[8] Other therapeutic options for CLAD (particularly BOS) include total lymphoid irradiation (TLI)[9,10] or extracorporeal photopheresis (ECP).[11,12] TLI may reduce the rate of decline in graft function associated with BOS, particularly in "rapid decliners" (i.e., with forced expiratory volume in 1 second [FEV_1] decline >100 ml/month pre-TLI).[9,10] Similar effects have been seen with ECP, but mostly in BOS patients with a slowly progressive FEV_1 decline and increased airway lavage neutrophilia, whereas ECP is less likely to attenuate disease progression in rapid decliners (FEV_1 decline >100 ml/month pre-ECP), in BOS patients with low airway neutrophilia, and in patients with RAS, who, consequently, have the worst outcome after CLAD onset.[13]

In view of these findings, pharmacologic therapy for CLAD has been inadequate, and novel, preferably cheap and safe, treatment options to mitigate CLAD progression are urgently needed. Montelukast (MLK), a cysteinyl leukotriene receptor (CysLTR) antagonist, may be such a drug. (Mechanisms of action of cysteinyl leukotrienes and their antagonists are described in the Supplementary Material available online at www.jhltonline.org/.) MLK possesses a broad spectrum of anti-inflammatory properties, which target eosinophils, monocytes, and corticosteroid-insensitive neutrophils,[14] and may attenuate sub-epithelial and pulmonary fibrosis.[15,16] In pulmonary graft-vs-host disease (GvHD) after hematopoietic stem cell transplantation, characterized by small airways disease, obliteration, and sometimes concurrent parenchymal fibrosis, similar to CLAD after LTx, MLK was shown to be a safe, effective, and toxicity (steroid)-sparing supplement to standard therapy, resulting in improved FEV_1, reduced air trapping, improved exercise capacity, and/or reduced respiratory symptoms.[17–19] A recent consensus report on chronic pulmonary GvHD has stipulated MLK as first-line treatment (together with azithromycin and an inhaled corticosteroid) to prevent further FEV_1 decline.[20] After LTx, comparable beneficial effects with MLK were also suggested in a small, open-label pilot study, demonstrating attenuation of FEV_1 decline in BOS patients treated with MLK, compared with historic controls with BOS who never received MLK.[21] These positive findings were recently corroborated in a small, randomized "proof-of-concept" trial in late-onset BOS (>2 years after LTx) with slowly progressive FEV_1 decline despite prior azithromycin (<100 ml/month), again demonstrating attenuation of FEV_1 decline in patients with BOS Stage 1 with MLK ($n = 11$) compared with those patients taking placebo ($n = 8$), in whom lung function further declined.[22]

Given these findings, we hypothesized a possible beneficial effect of MLK in CLAD after LTx. The aim of this study was therefore to evaluate the association of long-term MLK treatment with evolution of pulmonary function (FEV_1) and progression-free and overall survival in a large cohort of LTx recipients with progressive CLAD despite at least 3 months of prior azithromycin therapy.

Methods

Study design and population

All patients who underwent lung or heart–lung transplantation at University Hospitals Leuven between July 1, 1991 and December 31, 2016 ($n = 965$) were retrospectively evaluated for the use of MLK for progressive CLAD, as per institutional protocol, since October 2008. CLAD status at initiation of MLK and duration of MLK treatment were assessed. Patients were excluded if they had received MLK for <3 months at the moment of analysis, if MLK was started prior to or within 3 months after initiation of azithromycin, if they in retrospect had no CLAD upon MLK initiation, or if CLAD status was not evaluable. The local ethics committee approved the study and all patients provided written informed consent to access their clinical and bio-banked data for research (S51577/ML5629).

Assessment of pulmonary function, CLAD status, and mortality

Pulmonary function was assessed (Master Screen, Jaeger, Hoechberg, Germany)[23] at baseline (best post-operative value), 6 months, and 3 months before start of MLK and at start of MLK, and then at 3 months, 6 months, and 1, 2, and 3 years after start of MLK (on the condition that patients were still alive without redo transplantation at that time). In case of redo transplantation, evolution over time after each transplant procedure was taken into account separately for outcome analyses.

CLAD was diagnosed as a persistent decline in FEV_1 of at least 20% compared with the 2 best post-operative values (baseline), without any other identifiable cause or confounding factors.[24] Sub-division was made between obstructive CLAD (BOS) and restrictive CLAD (RAS) using total lung capacity (TLC) (≥10% decrease vs baseline), forced vital capacity (FVC) (≥20% decline),

and/or FEV_1/FVC ratio (>0.70), in combination with persistent infiltrates on chest computed tomography (CT) scan, as described elsewhere.[24] CLAD severity at initiation of MLK was defined as FEV_1 66% to 80% (Stage 1), 51% to 65% (Stage 2), and ≤50% (Stage 3) of prior baseline FEV_1 (in liters), respectively.

"Response" to MLK, which was assessed at 3, 6, or 12 months after initiation of MLK, was defined as a >10% increase in FEV_1 (liters) vs FEV_1 (liters) at start of MLK; "progression" was defined as >10% decrease in FEV_1 (liters); and "stabilization" was defined as 10% decrease ≤ FEV_1 ≤ 10% increase after initiation of MLK. "Progression-free survival" was defined as freedom from CLAD progression (decline of FEV_1 >10% vs FEV_1 [liters] at start of MLK) at 12 months after initiation of MLK. Subjects not reaching 12-month follow-up were censored at last available FEV_1 after 3 or 6 months, respectively, at which time freedom from CLAD progression was taken into account. "Rapid decline" in FEV_1 prior to/after initiation of MLK was defined as an average FEV_1 decline of >100 ml/month.

Dates of CLAD, redo transplantation, death, or graft loss (defined as redo transplantation *or* death) and all other demographics and clinical parameters were obtained from the patients' individual electronic clinicopathologic medical files at University Hospitals Leuven, and then saved in a separate anonymized file at the University Lab of Respiratory Diseases (S51577).

Assessment of blood and BAL cellularity

Venous whole blood sampling was performed at start of MLK according to standard operating procedures. Total and differential leukocyte counts were performed by the University Hospital Clinical Lab.[25]

Broncho-alveolar lavage (BAL), together with transbronchial and central biopsies, was performed according to routine follow-up after 1, 3, 6, 12, 18, and 24 months, or upon suspected infection, acute rejection, or CLAD, as described elsewhere.[24,25] Historically, however, not all progressive CLAD patients underwent a new bronchoscopy before MLK initiation (especially if prior bronchoscopy was performed within the past 3 or 4 months), which was a decision made at the treating physician's discretion.

Immunosuppressive and prophylactic regimen

Standard outpatient follow-up, immunosuppressive regimen, infectious prophylaxis, and CLAD treatment were described in previous studies.[25,26] Briefly, all LTx recipients were in lifelong follow-up at 3- to 4-month intervals in our outpatient clinic. In suspected CLAD, immunosuppressive treatment was optimized if possible, and azithromycin (250 mg/day, 3 times a week) was initiated if patients were not already taking azithromycin, either because of prior lymphocytic bronchiolitis or as pre-emptive treatment, as per institutional protocol since 2014. As such, azithromycin-responsive allograft dysfunction (ARAD) was excluded upon CLAD diagnosis.[2] In established, progressive CLAD, treatment with MLK 10 mg/day was started. In cases of subsequent CLAD progression (i.e., further decline in FEV_1 after ≥3 to 6 months of MLK), rescue treatment with pulsed steroids, rabbit anti-thymocyte globulin (rATG), TLI, pirfenidone, antibody-directed therapy (pulsed methylprednisolone, followed by plasmapheresis, intravenous immunoglobulin [IVIg], and rituximab since 2013), or redo transplantation in selected cases was performed, based on the treating physician's discretion. ECP is not commonly available at our center for CLAD, although 2 included BOS patients did receive ECP rescue treatment: 1 abroad in compassionate use and 1 in a GvHD study protocol.

Statistical analysis

All analyses were performed using GraphPad Prism 5a software (GraphPad, Inc., San Diego, CA) or SAS version 9.3 (SAS Institute, Inc., Cary, NC). Results are expressed as mean ± standard deviation or as median with interquartile range, wherever appropriate. Groups were compared using *t*-test, Mann−Whitney test, Wilcoxon signed rank test, or one-way analysis of variance (ANOVA; either repeated-measures ANOVA with Bonferroni's post-test, or Kruskal−Wallis with Dunn's post-test), respectively, depending on normality distribution and repeated measures. Fisher's exact test or chi-square test was used to compare proportions and correlation analyses were performed using Spearman's rank test. Correlations between clinical variables and response/non-response to MLK were based on findings previously reported with the use of ECP in CLAD,[13] which demonstrated that patients with "RAS" or "rapid progressive BOS" were non-responsive to ECP treatment. Other variables taken into account for univariate correlation analysis were time between CLAD diagnosis and start of MLK, blood and BAL cell counts at CLAD diagnosis, and time to CLAD diagnosis. Kaplan−Meier survival curves with univariate log-rank analysis were used for time-to-event analysis for progression-free and overall survival. Multivariate Cox proportional hazards regression analysis was used to assess overall post-CLAD survival according to response to MLK, adjusting for underlying disease, type of LTx (S vs SS/HL), age, gender, time of CLAD onset, phenotype of CLAD (BOS vs RAS), and CLAD severity at start of MLK (Stage 1 vs 2 vs 3). For the end-point of CLAD, survival times were censored at death if this preceded CLAD, or else at end of current follow-up (March 1th 2017). All *p*-values are two-tailed and $p < 0.05$ was considered statistically significant.

Results

Study population

A total of 257 (27%) of all our LTx recipients were ever treated with MLK, of whom 207 (81%) received long-term (>3 months) MLK for CLAD, with the other 50 (19%) excluded from the current study (Figure 1).

To eliminate any bias on evolution of FEV_1 by initiation of azithromycin, we only report on the patients with established CLAD in whom MLK had been initiated >3 months after start of azithromycin (153 of 207; 74%). However, in some CLAD patients, MLK was initiated ≤3 months after start of azithromycin (54 of 207; 26%), as described in the Supplementary Material online (Figures S6 to S10) for completeness. Overall, comparable associations of MLK with pulmonary function evolution and survival were observed in this smaller CLAD cohort (Supplementary Material), as seen in the larger CLAD cohort, as described in what follows.

No severe adverse events attributable to MLK (i.e., allergic reaction, severe neuropsychiatric disorders with suicidality)[27] were reported by any of the patients or treating physicians since introduction of MLK for CLAD. Some patients mentioned vivid dreams after initiation of MLK, although MLK was not withdrawn in those cases.

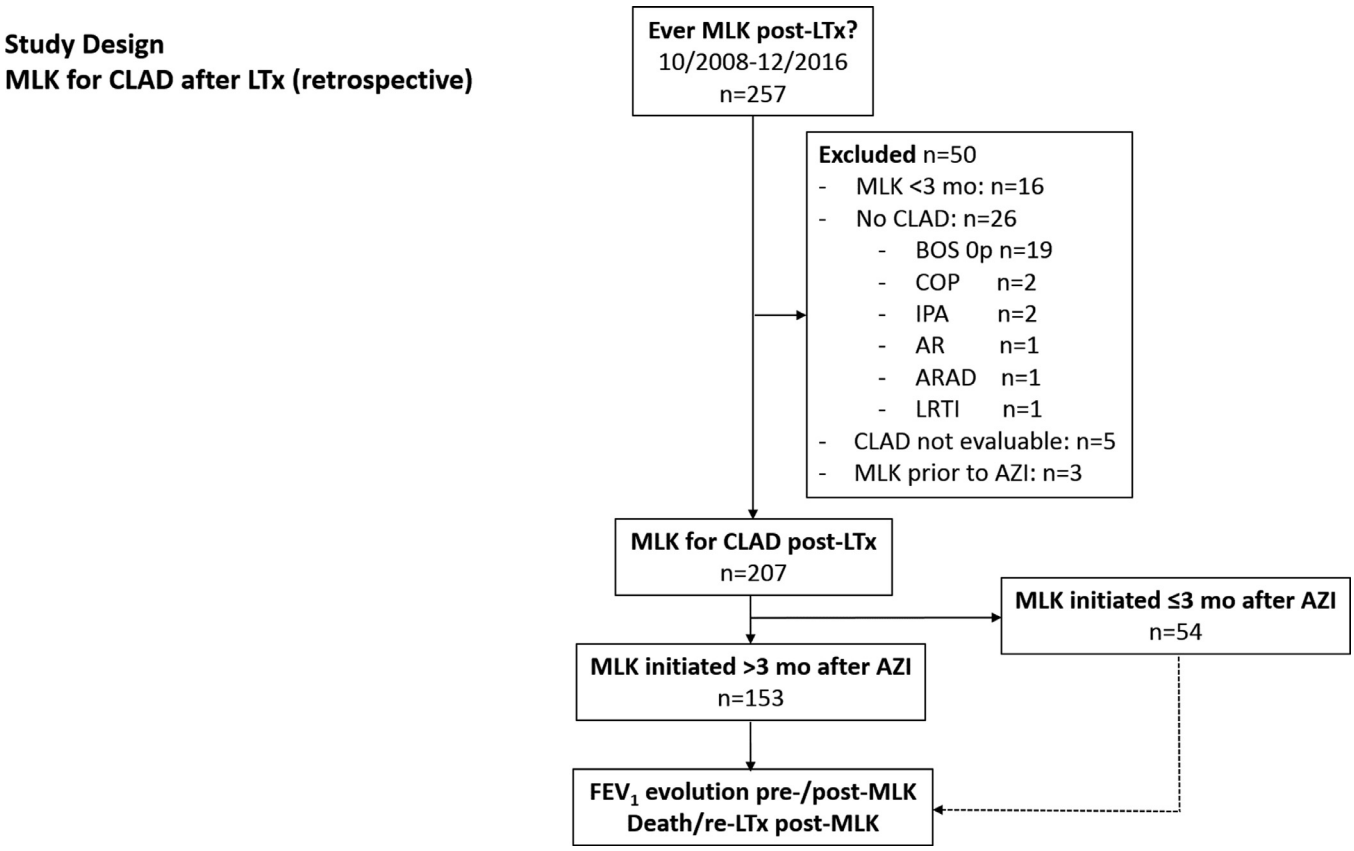

Figure 1 Flowchart of patient selection in our lung transplant cohort. A total of 257 (27%) LTx recipients in our cohort received MLK, of whom 207 (81%) received long-term (>3 months) MLK for CLAD, with the other 50 (19%) excluded from the study. Patients were further subdivided based on time of initiation of MLK after prior start of azithromycin (≤3 vs >3 months) for CLAD. Of the excluded patients, 16 were excluded because of MLK treatment for <3 months at current follow-up. Another 26 were excluded because, in retrospect, MLK had been initiated during BOS Stage 0-p ($n = 19$), after COP ($n = 2$), IPA ($n = 2$), acute rejection ($n = 1$), LRTI ($n = 1$) or azithromycin-responsive allograft dysfunction ($n = 1$) (all classified as non-CLAD). CLAD status was not evaluable in 5 patients, and another 3 were not included because MLK had been initiated before azithromycin. Abbreviations: AR, acute rejection; ARAD, azithromycin-responsive allograft dysfunction; AZI, azithromycin; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; COP, cryptogenic organizing pneumoniae; IPA, invasive pulmonary aspergillosis; mo, months; LTx, lung transplantation; LRTI, lower respiratory tract infection; MLK, montelukast.

Patients' characteristics

CLAD severity. CLAD severity at initiation of MLK was as follows: Stage 1 in 70 of 153 (46%); Stage 2 in 47 of 153 (31%); and Stage 3 in 36 of 153 (23%) patients. Patients' characteristics are summarized in Table 1. Most patients had been diagnosed with BOS (115 of 153, 75%), whereas 38 of 153 (25%) had RAS, among whom 6 (16%) initially had BOS that had progressed to RAS at the time of analysis (3 patients initially had BOS Stage 2, and 3 initially had BOS Stage 3).

Factors related to CLAD severity. Worse CLAD severity at start of MLK was significantly associated with higher blood leukocyte numbers ($r = 0.27$, $p = 0.0008$) and a higher blood neutrophil percentage ($r = 0.28$, $p = 0.0004$), thus with lower percentages of blood basophils ($r = -0.21$, $p = 0.009$), lymphocytes ($r = -0.23$, $p = 0.005$), and monocytes ($r = -0.18$, $p = 0.02$), but not blood eosinophils ($p =$ not statistically significant [NS]).

Despite all patients recieved azithromycin, worse CLAD severity also correlated with increased BAL total leukocytes ($r = 0.39$, $p = 0.002$) and higher BAL neutrophil percentage ($r = 0.54$, $p < 0.0001$), and thus lower BAL macrophages ($r = -0.52$, $p < 0.0001$) but not BAL lymphocyte or eosinophil percentages (both $p =$ NS). However, BAL was only performed in 61 of 153 (40%) patients at initiation of MLK (CLAD Stage 1: 40%; CLAD Stage 2: 57%; CLAD Stage 3: 25%), which may have biased BAL findings.

Rapid decliners during the 6 months pre-MLK displayed more severe CLAD ($r = 0.33$, $p < 0.0001$), and thus lower FEV_1 ($r = -0.16$, $p = 0.045$), but higher blood neutrophils ($r = 0.017$, $p = 0.036$) at the start of MLK. If FEV_1 decline during the 3-month interval pre-MLK only was considered, a rapid decline during this period was similarly associated with more severe CLAD stage ($r = 0.20$, $p = 0.013$) at start of MLK.

Evolution of pulmonary function with MLK

Overall changes in FEV_1. Figure 2 depicts the observed significant decrease in rate of FEV_1 decline before vs after initiation of MLK, respectively, for 3- and 6-month intervals. Figure 3 shows the evolution of FEV_1 over time before and

Table 1 Patients' Characteristics

	MLK for CLAD ($n = 153$)	Response to MLK ($n = 124$)	No response to MLK ($n = 29$)	p-value
Age at LTx (years)	54.0 (42.5 to 59.0)	54.0 (42.5 to 59.0)	54.0 (44.5 to 58.0)	0.90
Sex, M/F (n)	67/86 (43.8/56.2)	56/68 (45.2/54.8)	11/18 (37.9/62.1)	0.54
Indication for LTx [n (%)]				0.95
Emphysema	80 (52.3)	63 (50.8)	17 (58.6)	
Pulmonary fibrosis	29 (19.0)	24 (19.4)	5 (17.2)	
CF or non-CF BRECT	16 (10.5)	14 (11.3)	2 (6.9)	
PAH (primary or Eisenmenger)	11 (7.2)	9 (7.3)	2 (6.9)	
OB	10 (6.5)	8 (6.5)	2 (6.9)	
LAM	3 (1.9)	3 (2.4)	0	
Other	4 (2.6)	3 (2.4)	1 (3.4)	
SSLTx or HLTx/SLTx [n (%)]	130/23 (85.0/15.0)	103/21 (83.1/16.9)	27/2 (93.1/6.9)	0.17
Year of transplantation (years)	2007 (2004–2011)	2007 (2003–2010)	2008 (2004–2011)	0.20
Time from LTx to CLAD (years)	3.9 (2.1 to 6.4)	4.0 (2.3 to 6.1)	3.2 (1.1 to 6.6)	0.17
Time from LTx to start AZI (years)	1.0 (0.06 to 3.5)	1.2 (0.1 to 3.8)	0.2 (0.0 to 2.7)	0.16
Time from LTx to start MLK (years)	5.2 (3.4 to 8.4)	5.3 (3.7 to 8.8)	3.8 (1.6 to 7.3)	0.023
Time between start AZI and MLK (years)	3.3 (1.5 to 5.7)	3.5 (1.9 to 5.8)	2.6 (4.0 to 1.1)	0.093
Time from CLAD to start MLK (years)	0.6 (0.08 to 2.2)	0.7 (0.1 to 2.8)	0.3 (0.1 to 0.7)	0.13
Total time of follow-up after LTx (years)	8.2 (5.3 to 11.0)	8.5 (5.7 to 11.9)	6.2 (3.8 to 9.7)	0.023[a]
Best FEV_1 after LTx (liters)	2.52 (2.13 to 3.26)	2.58 (2.13 to 3.32)	2.45 (2.15 to 3.16)	0.65
Best FEV_1 after LTx (% predicted)	88 (73 to 105)	89 (72.5 to 108)	88 (75.5 to 97)	0.41
FEV_1 at start MLK (liters)	1.53 (1.15 to 1.97)	1.53 (1.17 to 1.98)	1.53 (1.11 to 1.86)	0.55
FEV_1 at start MLK (% predicted)	54 (42 to 70)	55 (42.5 to 70.5)	48 (39 to 66.5)	0.29
CLAD severity at start MLK [n (%)]				0.64
CLAD Stage 1	70 (45.8)	59 (47.6)	11 (37.9)	
CLAD Stage 2	47 (30.7)	37 (29.8)	10 (34.5)	
CLAD Stage 3	36 (23.5)	28 (22.6)	8 (27.6)	
CLAD at end of follow-up [n (%)]				0.022[a]
BOS	115 (75.2)	98 (79)	17 (59)	
RAS	38 (24.8)	26 (21)	12 (41)	
Redo-LTx [n (%)]	12 (7.8)	7 (5.6)	5 (17.2)	0.036[a]
Time from LTx to redo LTx (years)	8.5 (4.8 to 12.2)	11.9 (8.5 to 15.1)	4.3 (2.9 to 7.5)	0.018[a]
Death at end of follow-up [n (%)]	54 (35.3)	37 (29.8)	17 (58.6)	0.0035[a]
Time from LTx to death (years)	7.7 (4.7 to 10.5)	7.9 (5.5 to 11.5)	6.4 (3.1 to 10.0)	0.19

Characteristics of patients treated for at least 3 months with montelukast for progressive CLAD despite previous use of azithromycin for at least 3 months, with subdivision according to response (increase/ stabilization of FEV_1) or no response (decrease of FEV_1) after 3 months of montelukast. Data are presented as mean ± standard deviation, median (interquartile range), or number (%). AZI, azithromycin; BOS, bronchiolitis obliterans syndrome; CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; F, female; FEV_1, forced expiratory volume in 1 second; HLTx, heart–lung transplantation; LTx, lung transplantation; LAM, lymphangioleiomyomatosis; M, male; MLK, montelukast; OB, obliterative bronchiolitis; PAH, pulmonary arterial hypertension; RAS, restrictive allograft syndrome; SLTx, single-lung transplantation; SSLTx, double (bilateral) lung transplantation.

[a]Statistically significant difference ($p < 0.05$).

after initiation of MLK in the entire CLAD cohort, demonstrating an attenuation in FEV_1 decline after MLK, as effect that was present throughout all stages of CLAD (see Figure S1 online). A similar effect was observed for FVC evolution after initiation of MLK (see Figures S2 and S3 online).

Individual changes in FEV_1. Figure 4 illustrates changes in FEV_1 in individual patients after 3 and 6 months of MLK. After 3 months of MLK, 16 of 151 (11%) patients were rapid decliners (FEV_1 decline >100 ml/month), most of whom (9 of 16, 56%) were already rapid decliners during the 3 months pre-MLK. If a 6-month interval was considered, 7 of 141 (5%) patients were rapid decliners after 6 months of MLK, of whom only 1 of 7 (14%) was a rapid decliner during the 6 months pre-MLK. This illustrates that most patients, either after 3 or 6 months of MLK, demonstrated an increase, stabilization, or slower decline in FEV_1, as detailed in what follows.

CLAD progression–free survival with MLK

Response to MLK. Figure 5 summarizes the proportion of patients demonstrating an increase, stabilization, or further decline in FEV_1 at 3, 6, or 12 months after initiation of MLK. The majority of patients after 3 (81%), 6 (66%), and 12 months of MLK (60% of evaluable patients) demonstrated increase/stabilization of FEV_1, an effect was seen in all stages of CLAD. Thus, a minority of patients demonstrated further CLAD progression after 3 months of MLK, which was seen in 11 of 70 (16%) of the patients who were started while in CLAD Stage 1, 10 of 46 (22%) evaluable patients started in CLAD Stage 2 (1 with no FEV_1 data at 3 months), and 8 of 35 (27%) evaluable patients started in CLAD Stage 3 (1 with no data) ($p = 0.58$). After 6 months, the proportions of non-responsive patients were: 21 of 66 (32%) evaluable patients in CLAD Stage 1 (4 with no

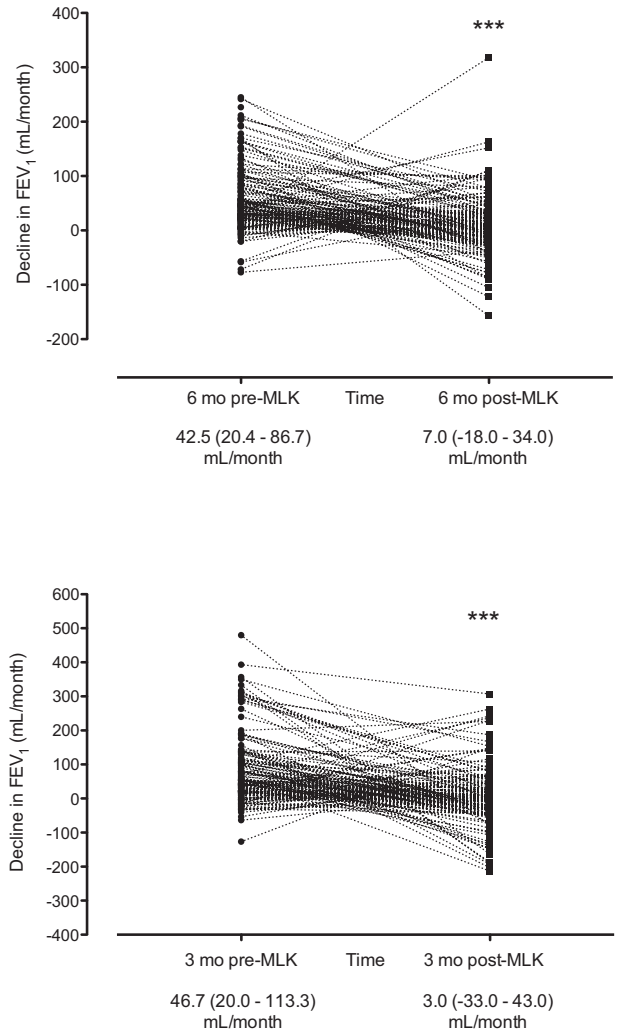

Figure 2 Monthly rate of decline in FEV_1 (ml/month) before and after initiation of montelukast (MLK) in patients with progressive chronic lung allograft dysfunction (CLAD) despite prior azithromycin for at least 3 months ($n = 153$). Upper graph: 6-month interval before/after initiation of MLK; lower graph: 3-month interval before/after initiation of MLK. $^{***}p < 0.001$.

FEV_1 data at 6 months); 14 of 44 (32%) in CLAD Stage 2 (3 with no data); and 13 of 31 (42%) in CLAD Stage 3 (5 with no data) ($p = 0.57$).

Overall, CLAD progression during the 12-month period after initiation of MLK occurred in 59 of 153 (39%) patients, with the other 94 (61%) free from CLAD progression during the first year after MLK. Obviously, patients with further CLAD progression after 3 months of MLK subsequently demonstrated lower FEV_1 at 6 ($r = -0.30$, $p = 0.0030$) and 12 ($r = -0.26$, $p = 0.0068$) months after start of MLK, and were thus more likely to show CLAD progression during the 12 months after initiation of MLK ($r = 0.51$, $p < 0.0001$).

Factors related to (non-)response to MLK after 3 months. FEV_1 was less likely to increase/stabilize after 3 months of MLK in patients with a rapid decline during the 6-month interval preceding MLK ($r = 0.27$, $p = 0.0008$), despite the fact that MLK was initiated sooner after CLAD diagnosis ($r = 0.23$, $p = 0.0040$) in these rapid decliners compared to patients with a slower FEV_1 decline. Therefore, these rapid decliners more often demonstrated further CLAD progression during the 12 months after start of MLK ($r = 0.20$, $p = 0.012$) and subsequently had a shorter time to graft loss after CLAD diagnosis ($r = -0.17$, $p = 0.037$). If rapid decline during the 3-month interval preceding MLK only was considered, again these rapid decliners were less likely to respond after 3 months of MLK ($r = 0.24$, $p = 0.0024$), despite earlier initiation of MLK after CLAD onset ($r = 0.32$, $p < 0.0001$), which similarly was associated with a shorter time to graft loss after CLAD diagnosis ($r = -0.28$, $p = 0.0006$). Next to rapid decliners, RAS patients were also more likely to demonstrate further progression after 3 months of MLK ($r = 0.19$, $p = 0.019$).

Patients demonstrating an increase/stabilization of FEV_1 after 3 months of MLK had lower blood leukocyte ($r = 0.18$, $p = 0.028$) and higher blood eosinophil ($r = -0.16$, $p = 0.045$) and basophil ($r = -0.18$, $p = 0.031$) levels compared to patients with further CLAD progression (Table 2), with the latter showing higher BAL lymphocyte ($r = 0.31$, $p = 0.014$) and BAL eosinophil ($r = 0.26$, $p = 0.041$) levels. Again, BAL data at start of MLK were not available for all patients (Table 2).

Management of CLAD progression. Generally, CLAD progression during later follow-up was managed by redo transplantation (11 of 153, 7%) or by medical treatment (38 of 153, 25%)—either plasma exchange + IVIg with/without rituximab alone ($n = 7$) or followed by subsequent redo transplantation ($n = 1$), off-label pirfenidone as monotherapy ($n = 8$), or pirfenidone in combination with plasma exchange + IVIg with/without rituximab ($n = 4$), TLI ($n = 4$), rATG ($n = 1$), or ECP ($n = 2$); or by best-supportive care only (38 of 153, 25%).

Overall survival and graft loss with MLK

Overall survival. Median overall post-LTx survival was 13.4 years, and comparable for patients in CLAD Stages 1, 2, and 3 ($p = 0.47$). Median overall survival after CLAD diagnosis was 7.3 years and was again comparable for different CLAD stages ($p = 0.73$), as was graft loss after CLAD diagnosis, with a median of 6.5 years ($p = 0.69$) (see Figure S4 online). BOS patients demonstrated significantly better outcomes compared with RAS patients (see Figure S5 online).

Stratified post-CLAD survival according to response to MLK. Compared to patients with an increase/stabilization of FEV_1 after 3 months of MLK ($n = 124$ of 153, 81%), non-responders/progressing patients ($n = 29$ of 153, 19%) demonstrated significantly worse post-CLAD survival ($p = 0.0002$). Importantly, median survival after CLAD diagnosis in patients with increase/stabilization of FEV_1 was more than double that of non-responders: 7.3 years vs 3.4 years ($p = 0.0002$) (Figure 6).

Multivariate Cox proportional hazards regression analysis (adjusted for underlying disease, type of LTx, age, gender, time of CLAD onset, phenotype of CLAD, and CLAD stage at start of MLK) demonstrated that non-response after 3 months of MLK (vs increase/stabilization of FEV_1) was an independent risk factor for mortality after CLAD

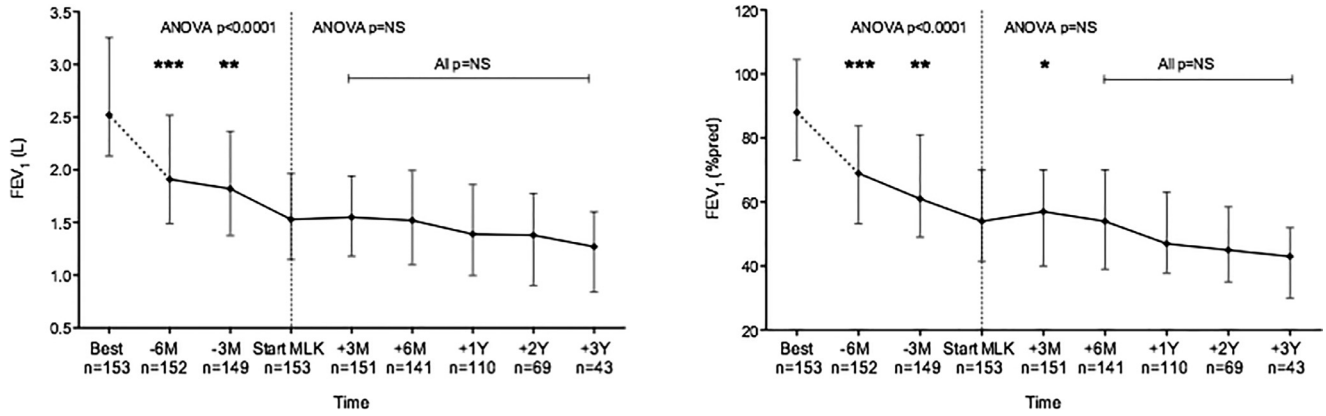

Figure 3 Evolution of FEV_1 (L, liters; %pred, percent predicted) over time after initiation of montelukast in patients with progressive chronic lung allograft dysfunction (CLAD) despite prior azithromycin for at least 3 months ($n = 153$). Data presented as median (interquartile range). Dotted line represents start of montelukast. ANOVA was used to assess evolution of FEV_1 before initiation of montelukast (comparing time-points "−6M" and "−3M" vs "start MLK") and after initiation of montelukast (comparing time-points "start MLK" vs "+3M" and "+6M"). All individual time-points (except "Best") were compared with "start MLK," (NS: $p > 0.05$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Abbreviations: Best, best post-operative FEV_1 value; −6M, 6 months before start of montelukast; −3M, 3 months before start of montelukast; start MLK, at start of montelukast; +3M, 3 months after start of montelukast; +6M, 6 months after start of montelukast; +1Y, 1 year after start of montelukast; +2Y, 2 years after start of montelukast; +3Y, 3 years after start of montelukast.

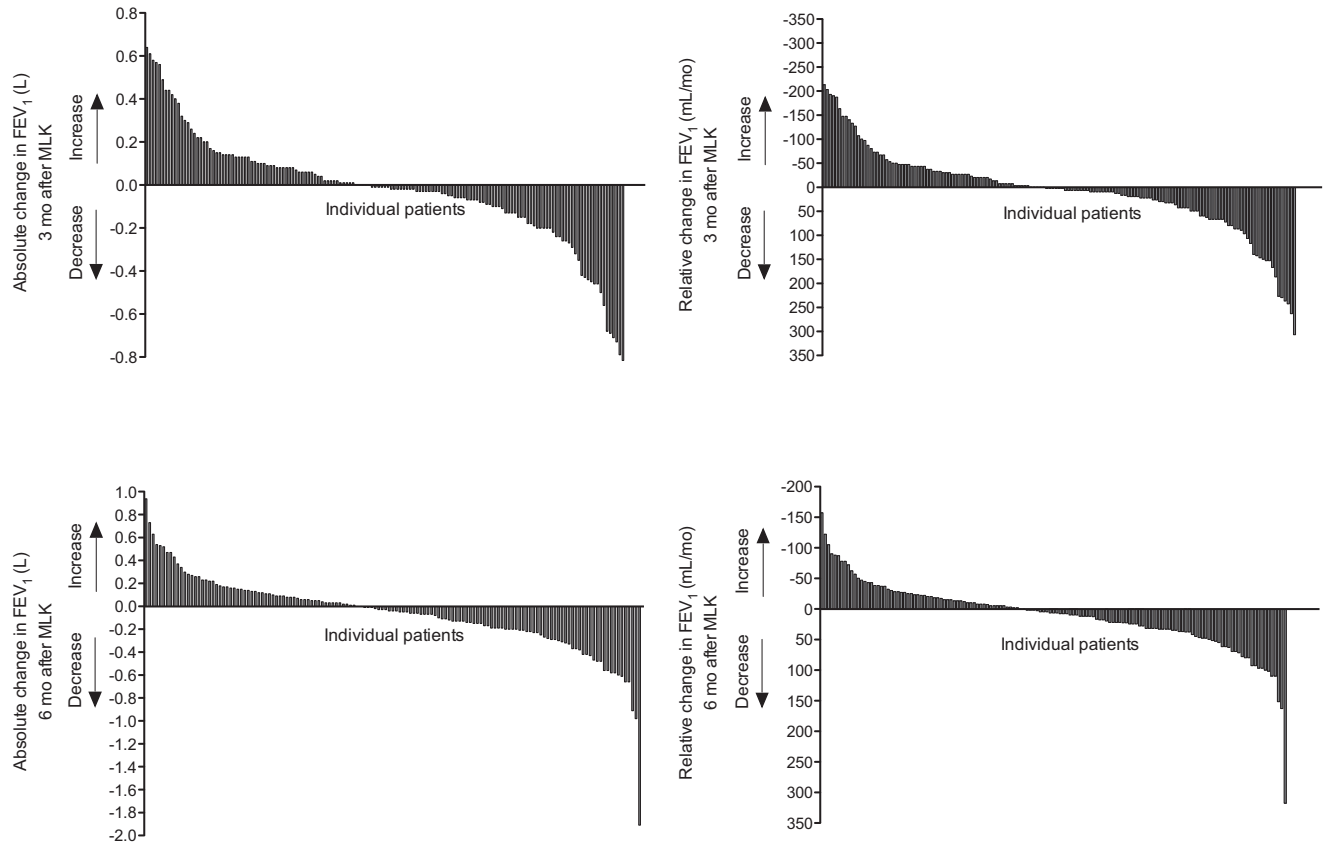

Figure 4 Waterfall plots depicting change in FEV_1 in individual patients after start of montelukast. Individual distribution of each patient's change in FEV_1 (increase, stable, decrease) after initiation of montelukast, expressed both in absolute values (L, liters) and relative values (ml/month), in a cohort of LTx recipients with progressive chronic lung allograft dysfunction (CLAD) despite prior azithromycin for at least 3 months ($n = 153$). Upper panels: change in FEV_1 3 months after start of montelukast; lower panels: change in FEV_1 6 months after start of montelukast.

diagnosis (hazard ratio [HR] 2.816, 95% confidence interval [CI] 1.450 to 5.467, $p = 0.0022$).

Other factors related to graft loss. Graft loss after LTx was associated with an RAS phenotype ($r = 0.22$, $p = 0.0050$), more severe CLAD stage ($r = 0.29$, $p = 0.0003$), and lower FEV_1 at start of MLK ($r = -0.40$, $p < 0.0001$), as well as with non-response after 3 ($r = 0.25$, $p = 0.0022$), 6 ($r = 0.35$, $p < 0.0001$), or 12 ($r = 0.22$, $p = 0.022$) months of MLK,

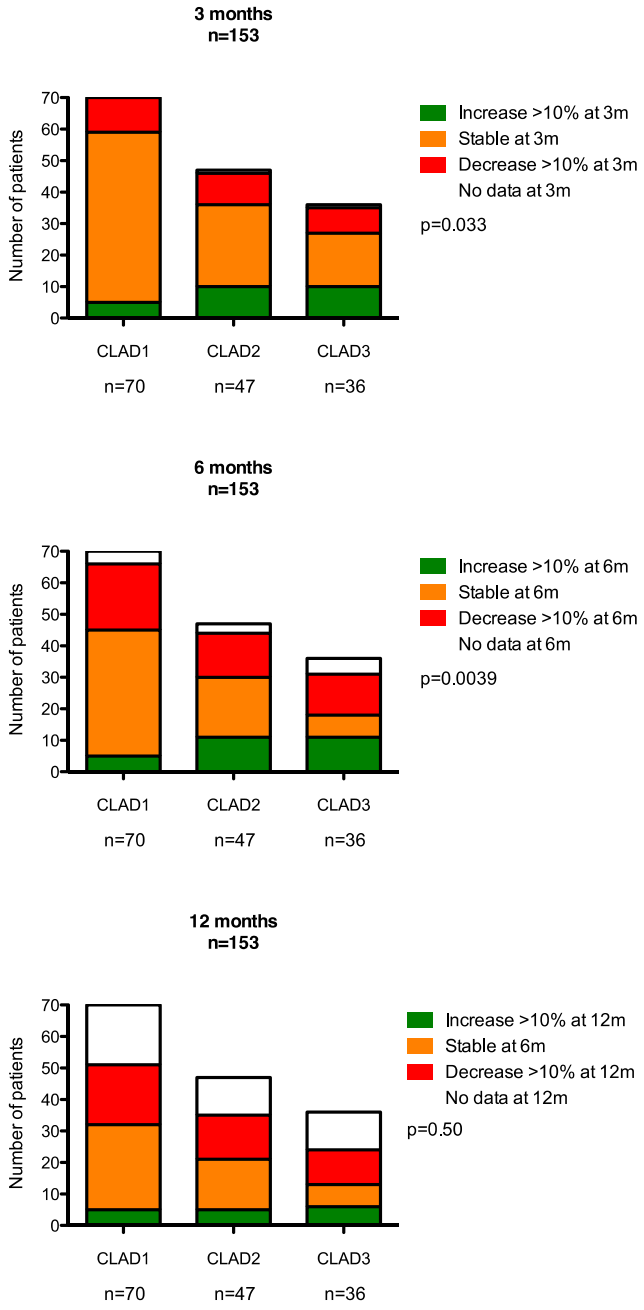

Figure 5 Proportional distribution of patients according to chronic lung allograft dysfunction (CLAD) status, response to montelukast (increase, stabilization, or decline of FEV_1), and time after initiation of montelukast (3, 6, or 12 months) in patients with progressive CLAD despite prior azithromycin for at least 3 months ($n = 153$).

respectively, and thus overall with CLAD progression during the 12 months after initiation of MLK ($r = 0.40$, $p < 0.0001$).

Finally, most likely due to its association with CLAD severity and/or progression, higher blood leukocyte ($r = 0.16$, $p = 0.050$), higher BAL leukocyte ($r = 0.33$, $p = 0.0077$), and higher BAL neutrophil ($r = 0.35$, $p = 0.0082$) numbers at start of MLK were associated with graft loss after CLAD diagnosis. On the other hand, higher blood eosinophils ($r = -0.25$, $p = 0.0023$), basophils ($r = -0.20$, $p = 0.015$), and monocytes ($r = -0.19$, $p = 0.017$) at start of MLK were associated with better outcome with regard to future graft loss.

Discussion

In this study we have retrospectively assessed the association of long-term MLK treatment with pulmonary function and survival in a large cohort of LTx recipients with progressive CLAD despite prior azithromycin treatment. We found that MLK was associated with significant attenuation in the rate of FEV_1 decline in a substantial proportion of patients with established CLAD, which was independent of CLAD stage and associated with significantly better CLAD progression–free and overall survival during later follow-up. Multivariate risk-adjusted analysis demonstrated non-response/progression after 3 months of MLK to be an independent risk factor for mortality after CLAD diagnosis. Slowly progressing BOS patients had a better response to MLK, whereas rapid decliners and patients with an RAS phenotype were less likely to respond to MLK. Blood and/or BAL cellularity may be able identify patients who are more likely to respond to MLK (i.e., those with higher blood eosinophils) vs patients who are less likely to respond (i.e., those with higher blood/BAL leukocytes and neutrophils). Although further proof is required, the current findings are important, given the current unmet medical need of pharmacologic therapy for CLAD.

CysLTs (LTC4, LTD4, and LTE4) display important bronchoconstrictive and pro-inflammatory effects through interactions with Type 1 and 2 cysteinyl leukotriene receptors ($CysLT_1R$ and $CysLT_2R$).[28,29] MLK is a selective antagonist of $CysLT_1R$ and possesses a broad range of anti-inflammatory and anti-fibrotic activities (refer to Supplementary Material online).[30–33] There is increasing evidence of leukotriene involvement in the pathogenesis of lung allograft rejection. A potential role for leukotriene B_4 (LTB4), a derived form of the unstable leukotriene A_4 (LTA_4) by LTA_4 hydrolase more upstream from the CysLTs, was demonstrated in a murine trachea transplant model.[34] LTB_4 is a potent neutrophil chemoattractant and induces airway inflammation by inhibiting neutrophil apoptosis and inducing directional neutrophil migration, regulating differentiation of immune cells and expression of cytokines, such as interleukin-1, tumor necrosis factor-α, and matrix metalloproteinases. In this murine model, LTB_4 was also shown to have potent chemotactic activity for $CD8^+$ effector T lymphocytes, in which recruitment into the airways and associated inflammation could be blocked by a (pre-clinical) LTB_4 receptor antagonist. Also, reduced inflammation and fibroproliferation was seen in the small airways of LTB_4 receptor–deficient mice compared with wild-type mice, which was again attributable to reduction of $CD8^+$ T-cell–mediated lung injury. These findings were corroborated by increased expression of LTB_4 receptors on graft-specific T cells isolated from the airways of LTx recipients with obliterative bronchiolitis (OB), when compared with healthy controls. Recently, these findings were further substantiated by demonstrating that MLK reduced plasma LTB_4 levels and attenuated subsequent tracheal fibrosis in a rat heterotopic trachea transplant model, despite the fact that LTB_4 exerts its primary function through another receptor (BLT1), which is not a direct target for MLK.[35] Altogether,

Table 2 Cellular Characteristics According to Response to Montelukast in Study Cohort

	Improved/stable FEV_1 after 3 months MLK	Decline in FEV_1 after 3 months MLK	*p*-value
Blood	(*n* = 122)	(*n* = 29)	
Total leukocytes	7.05 (5.7 to 9.3)	8.3 (6.4 to 12.3)	0.028[a]
Neutrophils (%)	65.7 (54.5 to 73.5)	68.6 (57.8 to 82.9)	0.17
Eosinophils (%)	2.1 (1.1 to 3.8)	1.6 (0.65 to 3.1)	0.045[a]
Basophils (%)	0.3 (0.2 to 0.6)	0.2 (0.1 to 0.5)	0.031[a]
Lymphocytes (%)	21.5 (13.6 to 30.9)	18.3 (8.5 to 27.8)	0.20
Monocytes (%)	9.9 (8.3 to 11.6)	10.1 (6.5 to 11.2)	0.43
BAL	(*n* = 41)	(*n* = 20)	
Total leukocytes	145.0 (48.5 to 612.5)	199.0 (46.5 to 593.8)	0.73
Macrophages (%)	75.2 (13.1 to 90.5)	55.0 (18.6 to 79.7)	0.31
Lymphocytes (%)	4.5 (1.0 to 9.1)	7.5 (4.9 to 15.8)	0.016[a]
Neutrophils (%)	12.0 (2.0 to 84.0)	18.9 (7.9 to 70.7)	0.57
Eosinophils (%)	0.0 (0.0 to 1.0)	0.8 (0.0 to 5.0)	0.043[a]

Blood and bronchoalveolar lavage (BAL) total and differential (%) cell counts according to evolution of pulmonary function (based on FEV_1) after initiation of MLK in patients with progressive chronic lung allograft dysfunction despite prior azithromycin for at least 3 months. Increase, stabilization, or decline in FEV_1 was defined as described in the Methods. Data after 3 months of MLK were unavailable for 2 patients (thus, 151 of 153 patients included). Data are presented as median (interquartile range). BAL, brochoalveolar lavage; FEV_1, forced expiratory volume in 1 second; MLK, montelukast

[a]Statistically significant difference ($p < 0.05$).

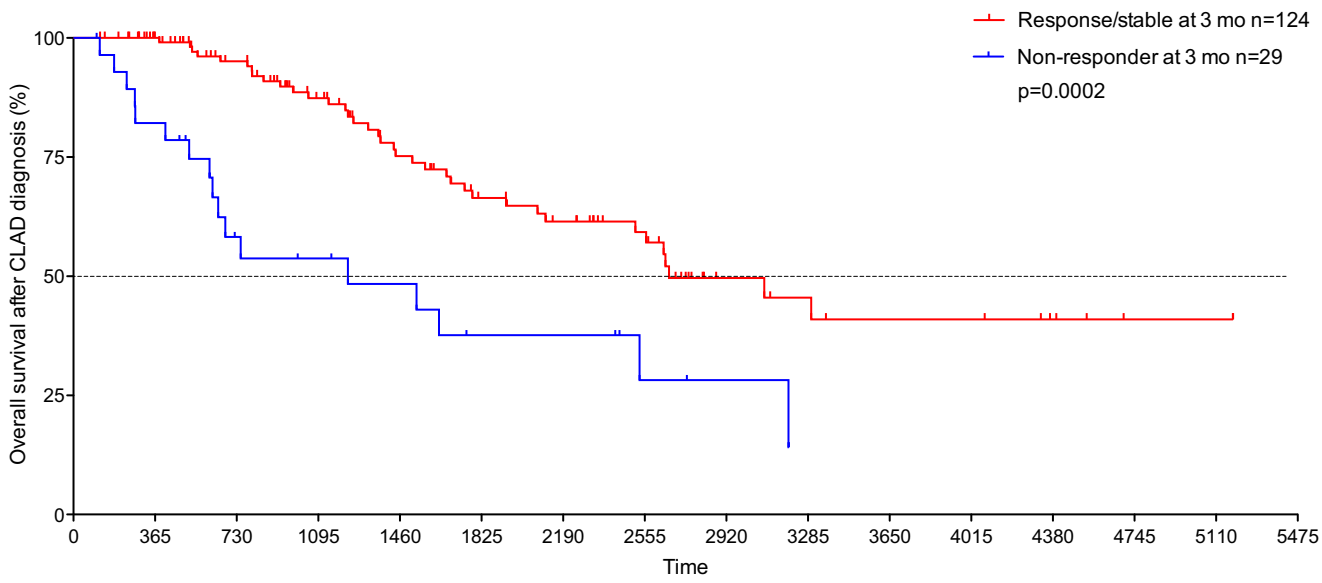

Figure 6 Survival according to response to montelukast. Kaplan–Meier survival curve with log-rank *p*-values depicts overall survival after diagnosis of chronic lung allograft dysfunction (CLAD) according to response to montelukast in patients with progressive CLAD, despite prior azithromycin for at least 3 months (*n* = 153). Time is expressed in days. Dotted line represents 50% survival.

these findings suggest a major role for leukotrienes in development of airway fibrosis/OB and/or pulmonary fibrosis in CLAD after LTx. Nevertheless, the presence of leukotrienes in airways of LTx recipients remains to be confirmed. However, increased numbers of tissue eosinophils and mast cells in explanted (end-stage) CLAD lungs,[36] along with increased levels of immunoglobulin E (IgE) in BAL fluid of CLAD patients upon diagnosis,[37] may serve as indirect evidence of leukotriene involvement in CLAD, as eosinophils, mast cells, and IgE, together with leukotrienes, are well-known cooperative components for initiation and propagation of inflammatory responses. As such, blood eosinophilia (reflecting tissue eosinophilia) may be a useful biomarker for possible response to MLK in CLAD patients.

Not all CLAD patients seem to benefit equally from MLK, and this requires further investigation. Responders were more likely patients with a slower decline in FEV_1 (i.e., <100 ml/month), especially if these patients demonstrated increased blood eosinophilia. Non-responders were more likely patients with a rapid FEV_1 decline or with an RAS phenotype. An MLK dose of 10 mg/day may be too low to attenuate aggressive disease progression in the latter cases. MLK 10 mg/day is the standard dose used for adults

with asthma (its main indication), and is the dose used in previous studies in GvHD patients and in our pilot studies in CLAD.[21,22] Meanwhile, since the initial submission of our manuscript, we have empirically initiated high-dose MLK (10 mg, 3 times daily) in 2 new BOS patients with rapid FEV_1 decline and 1 new RAS patient, but without significant attenuation in rate of decline after 4 to 6 weeks in either case. The effects of higher MLK doses in these "non-responders" (including possible increase in side effects), however, should be assessed in a larger group of patients for a longer duration before conclusions can be drawn on its efficacy in this setting.

Whether MLK may be used as preventive therapy for CLAD, similar to and perhaps synergistic with azithromycin,[24,26] is currently unknown, but may be a topic for future research. Other limitations of our study include its retrospective, cross-sectional design and lack of "controls" not receiving MLK, which would evidently be a historic group of CLAD patients not treated with MLK. Even so, such a cohort would most likely be biased by different patient characteristics (e.g., proportionally more single-sided LTx, which negatively affects outcome) and diagnostic, therapeutic, or prophylactic management. On the other hand, in our study, every patient served as his/her own control over time, and previous studies have already demonstrated a progressive FEV_1 decline in historic controls or placebo-treated patients with CLAD,[21,22] which may represent the "natural course" of CLAD. This was demonstrated by Lama et al, who described a progressive FEV_1 decline after onset of BOS, with the steepest slope of decline in the initial 6 months and a more gradual slope of FEV_1 decline over the next 12 months.[39] However, their BOS cohort included mainly single lung recipients with native lung emphysema, so their findings may need confirmation in bilateral lung recipients with CLAD, even though CLAD progression over time (especially in rapid decliners and RAS patients) is now commonly reported in most LTx centers.[3,11,13]

In most of the patients in our study the rate of FEV_1 (and FVC) decline was already significantly attenuated after 3 months of MLK, with improved FEV_1 in a significant number of CLAD patients. These findings were corroborated in the smaller cohort of patients for whom MLK was initiated for CLAD ≤3 months after previous start of azithromycin ($n = 54$; compare Figure S1B vs Figure S6B online). Drawing firm conclusions from the findings in this smaller cohort, however, is difficult because a time-dependent (late) effect of azithromycin may have still played a role in the observed attenuation of FEV1 decline around the time of start of MLK. Indeed, rarely patients may show a somewhat delayed beneficial effect on FEV1 after initiation of azithromycin (i.e., only stabilization after 4 to 8 weeks instead of <4 weeks). However, if a CLAD patient does not respond to azithromycin after 12 weeks, a later response to azithromycin is very unlikely.[2,5] Altogether, however, the observed increase/stabilization of FEV_1 and later CLAD progression–free evolution in most of our >200 CLAD patients after initiation of MLK seemingly contradicts the "natural decline" in CLAD over time. Moreover, stratified survival analysis according to response to MLK after 3 months as a binary parameter (i.e., increase/stabilization after MLK vs non-response/further decline) clearly demonstrates that non-response after 3 months of MLK was an independent risk factor for later mortality, which is an important finding for early identification of CLAD patients at risk, which perhaps may need intensified follow-up or rescue-treatment to avoid further FEV_1 decline. Thus, together with its good tolerance and low cost, the magnitude of effect size of montelukast may be such that the observed association promotes further consideration, while falling short of causality. Nonetheless, our findings would preferably require further validation in an independent LTx cohort.

Another limitation of our study is that information regarding a possible "CLAD-triggering" event(s) was historically not captured in our transplant CLAD database. This information may have clarified whether responders were more likely to have a preceding infectious CLAD trigger(s). However, MLK was initiated, on average, 0.6 year after CLAD onset (so not immediately after a possible triggering event), and in most patients no specific CLAD trigger could be found, as we described previously in a small RAS cohort ($n = 38$, in which 55% of patients had no clear identifiable trigger preceding RAS diagnosis).[3] Another limitation is that data on anti–human leukocyte antigen (DSA) antibodies were not included, because these data were only available in 37% (59 of 153) of the study patients. The reason is that DSA measurement by Luminex technology has only been performed on a routine basis since 2010 at our center. In patients with available DSA data, however, presence/absence of DSA did not correlate with response/no response after 3 months of MLK ($p = 0.34$), nor with CLAD progression during the 12 months after start of MLK ($p = 0.18$). Moreover, it is unlikely that MLK would directly affect B lymphocytes/DSA production, despite the fact that that MLK does have some direct biologic effects, including induction of apoptosis, on T lymphocytes through CysLTR blockade.[38]

CLAD patients who have previously never received azithromycin or MLK for CLAD, and perhaps may have died sooner after CLAD diagnosis, were not included in this study, which may have skewed our survival analysis. On the other hand, as both of these drugs were only introduced in the past decade, we believe the current CLAD cohort is a good representation of what can currently be expected for patients diagnosed with CLAD, both with regard to overall survival and subsequent evolution of pulmonary function after CLAD diagnosis. These contemporary data could therefore be used to design future studies assessing the effects of novel therapies and/or more targeted or personalized treatment in CLAD. Importantly, median survival after CLAD onset in patients with FEV_1 increase/stabilization after MLK was 7.3 years, suggesting that "progression-free survival" instead of "mortality" should be used as primary end-point in future outcome studies—except for non-responders, in whom there is an urgent need for life-saving therapeutic interventions, given their average survival of only 3.4 years after CLAD onset. Sub-analysis of "rescue" treatment options for the latter patients were outside the scope of this study, but probably should also be targeted

(i.e., anti-fibrotic drugs and/or antibody-mediated therapy for RAS,[6,7] TLI for BOS with rapid FEV_1 decline,[9,10] etc.).

In conclusion, we have demonstrated that montelukast was associated with significant attenuation in the rate of FEV_1 decline in a substantial proportion of LTx recipients with established CLAD, which correlated with better outcome. These findings require further validation in other LTx cohorts, given that there are currently few effective therapeutic options for CLAD.

Disclosure statement

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Supplementary data

Supplementary data associated with this article can be found in the online version at www.jhltonline.org/.

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