ORIGINAL ARTICLE

Total lymphoid irradiation in progressive bronchiolitis obliterans syndrome after lung transplantation: a single-center experience and review of literature

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SUMMARY

Limited results about treatment with total lymphoid irradiation (TLI) in lung transplant (LTx) recipients suffering from progressive bronchiolitis obliterans syndrome (BOS) have been reported. We performed a retrospective analysis of all LTx recipients undergoing TLI for progressive BOS in our center, focusing on long-term outcomes regarding overall survival and lung allograft function. Treatment with TLI (2004–2017, $n = 20$, 1 BOS stage 1, 6 BOS stage 2, and 13 BOS stage 3) resulted in significant attenuation of the FEV_1-decline in the majority of patients, mainly in those with a rapid decline ($P = 0.0005$). This allowed bridging to redo-transplantation in five patients. However, three patients progressed from BOS to RAS following prior TLI. Overall patient survival was 44% at 2 years post-TLI and 38% after 17 years. Generally, TLI was well tolerated, with limited side effects and no serious adverse events. TLI may attenuate the decline in FEV_1 of LTx recipients with rapid progressive BOS and could thus help to bridge selected patients to redo-transplantation.

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Key words
bronchiolitis obliterans syndrome, chronic lung allograft dysfunction, lung transplantation, restrictive allograft syndrome, total lymphoid irradiation, outcome

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Introduction

Chronic lung allograft dysfunction (CLAD), affecting up to 50% of lung transplant (LTx) recipients after five years post-transplant, remains the leading cause of death beyond the first year post-transplant [1]. At least two distinct clinical phenotypes of CLAD have been identified: a less prevalent, but highly fatal, restrictive phenotype (restrictive allograft syndrome, RAS) and a more common obstructive phenotype (bronchiolitis obliterans syndrome, BOS) [2,3]. Currently, CLAD is defined as a persistent decline (≥20%) in measured FEV_1 value from the baseline value, computed as the mean of the best 2 postoperative FEV_1 measurements (taken >3 weeks apart), for at least 3 months and in the absence of other identifiable causes [2].

Since CLAD progression, either rapidly or more gradually, may result in respiratory insufficiency, accurate staging and clinical phenotyping should be performed as soon as possible during the disease course, in order to stratify for potential investigations and therapies, albeit truly effective treatments are limited. At best, disease progression can be attenuated, resulting in temporary stabilization or minor improvement of lung allograft function for a shorter (months) or longer (years) time, yet full reversal of CLAD with FEV_1 recovery to >80% of baseline is, per definition, never possible [2].

Total lymphoid irradiation (TLI) has been successfully used in the past as immunosuppressive salvage treatment in renal, cardiac, and lung allograft rejection [4]. Nevertheless, only limited experience of TLI in CLAD, exclusively for progressive BOS, has been reported to date. The current retrospective study evaluates efficacy and safety of TLI in LTx recipients at our center, and summarizes current literature on this subject. We focused on long-term outcomes regarding overall survival and lung allograft function, identifying patients with durable preservation of lung function, who were bridged to redo-transplantation or progressed to RAS after prior TLI.

Materials and methods

Study population

Data were retrospectively collected for all TLI-treated LTx recipients, transplanted between July 1991 and December 2017 at the University Hospitals Leuven, with follow-up until December 2018. All LTx recipients had lifelong, 3–4 monthly post-transplant follow-up at our institution. The local Ethics Committee approved the study, and all patients had provided written informed consent at the time of listing for LTx to access their clinical and biobanked data for research purposes (S51577). Demographic data, including CLAD status, patient and graft survival status, and time and cause of death, were obtained from the patients' electronic medical records. Lung function (FEV_1, FVC) before and after start of TLI was assessed until redo-transplantation, death, or end of follow-up. The rate of FEV_1-decline (milliliters per month), respectively, 6 and 3 months before versus after TLI was analyzed. "Slow" decline was defined as average monthly rate of FEV_1-decline <100 ml/month and "rapid" decline as FEV_1-decline ≥100 ml/month (measured over 3 months prior to TLI).

TLI protocol

All patients were similarly treated according to a standardized TLI protocol, consisting of a total dose of 8 Gray (Gy), administered in ten fractions of 0.8 Gy twice a week, for five weeks, delivered on all major supra- and infra-diaphragmatic perivascular lymph nodes, spleen, and thymus, as previously described [5,6]. Per protocol, patients stopped their cell-cycle inhibitor (azathioprine or mycophenolate mofetil) upon initiation of TLI, which was only restarted after completion of TLI, in the absence of concurrent leukopenia ($<4.0 \times 10^9$ cells/l). During and after TLI, all patients were seen at the outpatient clinic, during scheduled annual check-ups, or (any) unscheduled hospitalizations by their treating lung transplant physicians. Patients were monitored for signs of infection or bone marrow suppression, especially leukopenia. If infection occurred or white blood cell count decreased below 3.0×10^9 cells/l, administration of the next TLI-fraction was postponed until recovery. Standardized post-transplant management and follow-up were previously described [6,7].

Statistical analysis

All analyses were performed using GRAPHPAD PRISM 8.0.2 (San Diego, CA, USA). Results are expressed as mean (±standard deviation) or median (interquartile range), wherever appropriate. Groups were compared using *t*-test, Mann–Whitney test, and Wilcoxon signed rank test, respectively, depending on normality distribution and repeated measures. Fisher's exact or chi-square test was used to compare proportions, and Kaplan–Meier curves and log-rank test were used for survival analyses. All *P*-values are two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Study population

A total of 20 LTx recipients, all diagnosed with BOS, had been referred for TLI at the treating physician's discretion between 2004 and 2017 (Table 1). Six patients (30%) were included in an earlier report on TLI, with a maximum follow-up of 26 months at that time [6]. Median time of follow-up of the patients in the current study was 5.9 (4.2–15.2) years.

Median time from transplantation to BOS was 3.3 (1.5–5.4) years. Median time from BOS to start of TLI was 152.5 (74.5–276) days, with most patients being in BOS stage 3 at the time of TLI (13/20, 65%) and the remaining patients in BOS stage 2 (6/20, 30%) or BOS stage 1 (1/20, 5%). All patients (100%) received azithromycin prior to TLI, and median time from start of azithromycin to TLI was 213 (72.5–928.3) days; additionally, 13/20 patients (65%) were also treated with montelukast at the time of TLI, with median time between start of montelukast and TLI being 79 (34.5–168.5) days. Because of progressive BOS (i.e., further FEV_1-decline) despite treatment with azithromycin ± montelukast, all patients were referred for subsequent TLI. During later follow-up after TLI, 4/20 additional patients (20%) were initiated on montelukast for BOS progression, after a median of 6.2 (4.9–7.3) years following TLI.

TLI safety and toxicity

Temporary discontinuation of TLI occurred in 5/20 patients (25%) due to leukopenia, 4/20 patients (20%) experienced mild to moderate (toxicity grade 1–2) nausea, anorexia or dysphagia during TLI. No hospital admissions because of infection occurred during TLI. Two patients (10%) died during TLI (after, respectively, 5 and 7 out of 10 planned fractions) due to further rapid decline of pulmonary function leading to fatal respiratory insufficiency, for which palliative care treatment was given.

Lung allograft function

Pulmonary function study population

Pulmonary function was available in all patients prior to TLI, whereas after TLI, this was the case in 16/20 patients (80%): two patients died during TLI (both with rapid FEV_1-decline) and two patients (one rapid and one slow FEV_1-decline) were unable to perform spirometry between cessation of TLI and subsequent death, which occurred for both 4 months later due to progressive respiratory insufficiency.

FEV_1 at start of TLI in these 4 patients without post-TLI spirometry (3 in BOS stage 3 and 1 in BOS stage 2) was significantly lower compared to FEV_1 of the 16 patients with consecutive post-TLI spirometries: 0.94 ± 0.22 l vs. 1.44 ± 0.68 l ($P = 0.025$). Mean monthly rate of FEV_1-decline during the 3 months before TLI, however, was somewhat lower in these four patients compared to the 16 other patients: 86.5 ± 94.2 ml/month vs. 245.6 ± 170.1 ml/month, ($P = 0.096$).

Pre-TLI versus post-TLI spirometry

In the 16 patients with consecutive spirometric data, significant attenuation of the monthly rate of FEV_1-decline occurred after TLI (Fig. 1): 245.6 ± 170.9 ml/month during the 3-month interval pre-TLI vs. 64.4 ± 118.4/month during the 3 months post-TLI ($P = 0.0008$); and 196.8 ± 116 ml/month during the 6-month interval pre-TLI vs. 59.0 ± 91.3 ml/month during the 6 months post-TLI ($P = 0.0020$; Fig. 2).

Overall, the monthly rate of FEV_1-decline decreased in all, but one, patients (15/16, 93.7%) at 3 months post-TLI. The latter patient (BOS 3) without attenuation of the FEV_1-decline following TLI subsequently died 124 days after TLI due to respiratory failure, secondary to pulmonary infection with multiple organ failure.

Remarkably, an absolute *increase* in FEV_1 (respectively, with +480 and +440 ml) was seen in 2/16 patients (12.5%) at 6 months post-TLI compared to start of TLI (both BOS 3).

In contrast to the observed attenuation of the FEV_1-decline, no effect was seen regarding the monthly rate of FVC-decline: 120 ± 101 ml/month during 3 months pre-TLI vs. 90 ± 132 ml/month during 3 months post-TLI ($P = 0.63$); and 81.5 ± 52.7 ml/month during 6 months pre-TLI vs. 71.7 ± 59.1 ml/month during 6 months post-TLI ($P = 0.63$), respectively.

Slow versus rapid FEV_1-decline and response to TLI

Four patients (25%) were slow decliners and 12/16 (75%) were rapid decliners pre-TLI. Notably, attenuation of the monthly rate of FEV_1-decline at 3 months after TLI occurred exclusively in rapid decliners (309.9 ± 146.9 ml/month pre-TLI vs. 65.5 ± 132.8 ml/month post-TLI, $P = 0.0005$), whereas this effect was absent in slow decliners (52.5 ± 27.2 ml/month pre-TLI vs. 61.0 ± 73.2 ml/month post-TLI, $P = 0.87$; Fig. 3).

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Table 1. Patient demographics.

	TLI for BOS n = 20 (2004–2017)
LTx indication, *n*	
COPD	9 (45%)
Pulmonary vascular disease/ Eisenmenger's syndrome	5 (25%)
Obliterative bronchiolitis	3 (15%)
Cystic fibrosis	2 (10%)
Interstitial lung disease	1 (5%)
Sex m/f, *n*	6/14
Type of LTx, *n*	
Bilateral lung	16 (80%)
Heart–lung	4 (20%)
Total time of follow-up after LTx, year	5.9 (4.2–15.2)
Time from LTx to BOS diagnosis, year	3.3 (1.5–5.4)
Time from start of azithromycin (100%) to start of TLI, day	213 (72.5–928.3)
Time from start of montelukast (65%) to start of TLI, day	79 (34.5–168.5)
Time from BOS diagnosis to start of TLI, day	152.5 (74.5–276)
FEV_1 at start of TLI, l	1.14 (0.94–1.69)
BOS stage at start of TLI, *n*	
BOS 1	1 (5%)
BOS 2	6 (30%)
BOS 3	13 (65%)
Blood leukocyte count at start of TLI (n = 20)	
Total cell number, ×10*9/l	7.7 (5.6–12.1)
Neutrophils, %	66.0 (59.4–84.1)
Lymphocytes, %	20.7 (8.2–26.4)
Monocytes, %	7.6 (5.2–9.4)
Eosinophils, %	1.2 (1.0–4.1)
Basophils, %	0.3 (0.1–0.6)
Anti-HLA antibodies at start of TLI, *n*	
Unknown	10 (50%)
Negative	7 (35%)
Positive	3 (15%)
Bronchoalveolar lavage cells at start of TLI (n = 15/20)	
Total cell number, ×10*3/ml	92 (56–181)
Macrophages, %	69.5 (26–85)
Lymphocytes, %	8.6 (1.5–22.2)
Neutrophils, %	5.5 (2–70)
Eosinophils, %	1.5 (0–4.6)
Transbronchial biopsies at start of TLI (n = 14/20), *n*	
A0B0	10 (71%)
A0B1	2 (14%)
AxB3	1 (7%)
A2B0	1 (7%)
Current status, *n*	
Alive	7 (35%)
Dead	13 (65%)
Retransplanted after TLI	5 (25%)
Time from start of TLI to death, day	365 (134.5–437.0)
Time from start of TLI to redo-transplantation, day	586 (147.5–2372)

BOS, bronchiolitis obliterans syndrome; HLA, human leukocyte antigen; TLI, total lymphoid irradiation.

Summary of patients' demographics of the lung transplant (LTx) recipients treated with TLI for progressive BOS in our center. Results are presented as absolute values (percentage) or median (interquartile range), where appropriate.

In the 12 rapid decliners, the greatest reduction in monthly rate of FEV_1-decline (3 months pre-TLI vs. 3 months post-TLI) was seen in those patients with the shortest interval between start of azithromycin and TLI [$r = -0.63$ (-0.89 to -0.071), $P = 0.031$]. No associations whatsoever were seen between either the magnitude of change in monthly rate of FEV_1-decline and post-transplant time of TLI initiation, nor with time to start of montelukast, time interval between montelukast and TLI, time to CLAD onset, CLAD stage at TLI, FEV_1 (Liters) at TLI, time interval between CLAD onset and TLI, nor with total leukocyte numbers or differential cell numbers in blood or bronchoalveolar lavage at start of TLI.

Blood parameters

Blood leukocyte numbers and differential cell counts at start of TLI are summarized in Table 1. Three months post-TLI (results available in 18/20 patients), a significant decrease in total blood leukocyte number [5.7 (3.6–8.8) $\times$ 10^9 cells/l, $P = 0.0058$] and increase in % monocytes [10.3 (8.0–14.7), $P = 0.0046$] were seen, whereas % neutrophils, lymphocytes, eosinophils, and basophils were similar (all $P > 0.05$).

The occurrence of anti-HLA antibodies at start of TLI and during later follow-up is summarized in Fig. 4. Anti-HLA antibodies were overall identified in 6/20 (30%) patients and were donor-specific (DSA) in all these cases. None of the 3 patients with DSA at start of TLI cleared their DSA after TLI, yet follow-up in these patients was short (respectively, 127, 541, and 384 days after TLI). Of the six patients who developed DSA, 3 (50%) have died, while three patients are currently alive (one after redo-transplantation).

Bronchoalveolar parameters

Bronchoalveolar lavage (BAL) was performed in 15/20 (75%) patients within 3 months (mean 45 ± 23 days) prior to TLI (rapid FEV_1-decline $n = 12$ and slow FEV_1-decline $n = 3$); lavage cell numbers are summarized in Table 1. BAL cultures revealed *Pseudomonas aeruginosa* in two patients (both with rapid FEV_1-decline), which was *lege ar*tis treated, on average 27 days prior to TLI; BAL cultures were negative for microbes in the other patients. No BAL was performed shortly after TLI in any of the TLI-treated patients, making it impossible to assess evolution of BAL cellularity with TLI.

No significant differences were seen regarding BAL total cell numbers, % macrophages, lymphocytes, neutrophils or eosinophils between patients with a rapid FEV_1-decline pre-TLI ($n = 12$) and those with a slow decline ($n = 3$) (all $P > 0.05$).

Of the 15 patients in whom BAL was performed, 14 had concurrent transbronchial biopsies taken (Table 1): The majority demonstrated no rejection ($n = 10$, A0B0), two showed A0B1 grade rejection, one disclosed AxB3, and 1 had A2B0 (which was the only biopsy treated with pulse steroids, despite which FEV_1 further decreased, leading to initiation of TLI).

Biopsies of patients with a slow FEV_1-decline pre-TLI ($n = 3$) demonstrated A0B0 ($n = 2$) or A0B1 ($n = 1$), while those with a rapid FEV_1-decline pre-TLI ($n = 11$) mostly demonstrated A0B0 ($n = 8$) and A0B1, AxB3 or A2B0 in the other three patients, respectively. Histologic rejection grade as a binary parameter was neither associated with the rate of FEV1-decline, nor with response to TLI. Finding on biopsies are therefore unlike to explain the observed difference in FEV_1 evolution in rapid versus slow decliners prior to TLI, nor response to TLI.

Outcome

BOS to RAS progression

Interestingly, 3/16 (18%) patients with consecutive spirometries after TLI progressed from BOS to RAS, after a median of 407 (292–3008) days following BOS onset and 65 (36–2468) days after TLI (respectively, two patients with BOS stage 2 and 1 with BOS stage 3 at TLI; two with rapid FEV_1-decline and 1 with slow FEV_1-decline pre-TLI).

Ultimately, two RAS patients died of respiratory insufficiency (respectively, 40 and 373 days post-TLI) and one RAS patient later underwent redo-LTx

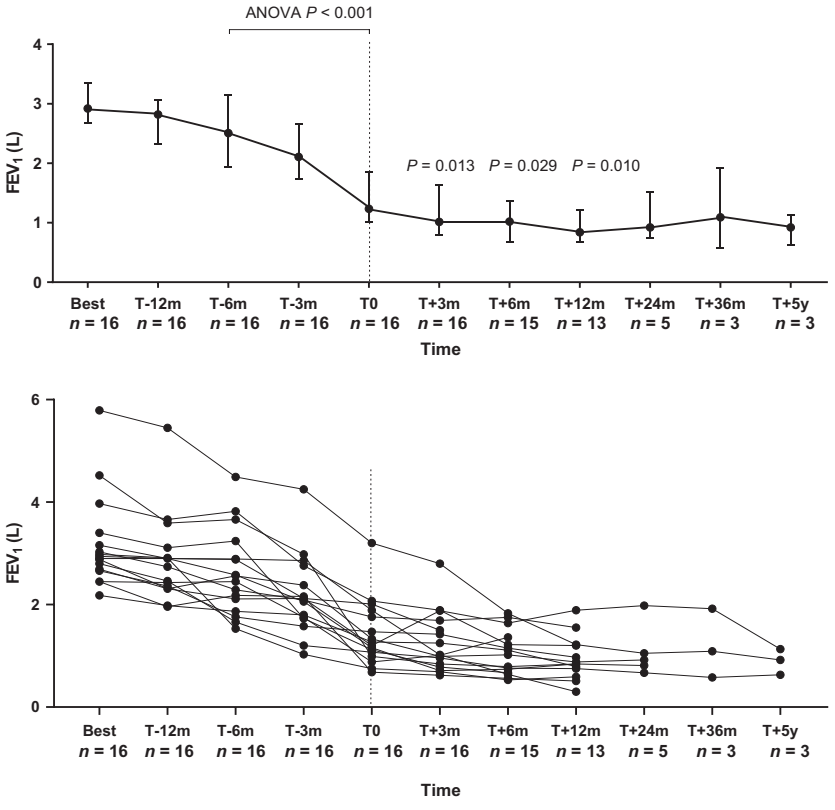

Figure 1 Evolution of FEV_1 in lung transplant recipients treated with TLI for progressive BOS. Evolution of FEV_1 over time before and after start of TLI in lung transplant (LTx) recipients with progressive BOS (upper panel). ANOVA *P*-value indicates significant decline from 6 months pre-TLI to start of TLI (T0). Later, attenuation of the FEV_1-decline is seen, although FEV_1 at 3, 6, and 12 months after start of TLI overall remained significantly lower ($P < 0.5$) compared to FEV_1 at start of TLI (T0). Lower panel depicts the individual trajectories of each included patients' FEV_1 over time before and after TLI. BOS, bronchiolitis obliterans syndrome; TLI, total lymphoid irradiation.

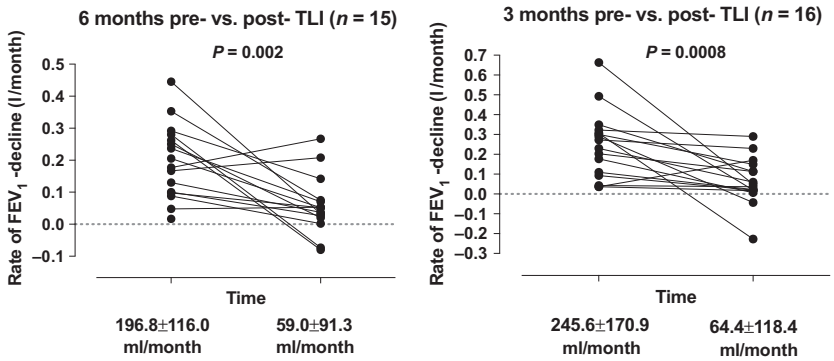

Figure 2 Rate of FEV_1-decline over time pre- and post-TLI in all lung transplant recipients treated with TLI for progressive BOS. Significant reduction in the monthly rate of FEV_1-decline over 6 and 3 months interval before/after start of TLI was seen in lung transplant (LTx) recipients treated with TLI for progressive BOS (P = 0.002 and P = 0.008, respectively). BOS, bronchiolitis obliterans syndrome, TLI, total lymphoid irradiation.

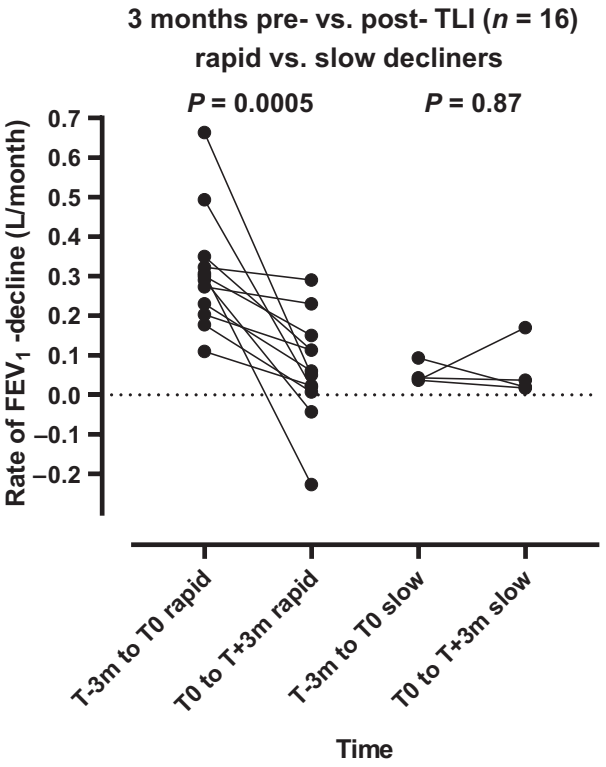

Figure 3 Rate of FEV_1-decline over time pre- and post-TLI in rapid versus slow decliners. Significant reduction in the rate of FEV_1-decline over 3-month interval before/after start of TLI was seen in lung transplant (LTx) recipients with rapid FEV_1-decline (≥100 ml/month) pre-TLI (P = 0.0005), but not in patients with slow FEV_1-decline (P = 0.87). BOS, bronchiolitis obliterans syndrome; TLI, total lymphoid irradiation.

10.8 years post-TLI and is currently still alive 2.4 years after redo-LTx.

In these three patients evolving from BOS to RAS, anti-HLA antibodies/DSA were unknown both at TLI and during later follow-up in one patient, unknown at TLI but positive during later follow-up in one patient (DR + DQ); and negative at TLI but positive during later follow-up in 1 patient (DQ), which may suggest that the occurrence of DSA plays a role in the pathogenesis of transition from BOS to RAS.

Allograft and patient survival

A total of 5/20 patients (25%) underwent redo-transplantation following TLI, after a median of 586 (147.5–2372) days post-TLI. Currently, 4/5 (80%) redo-transplant patients are alive, with a median time of follow-up after redo-transplantation of 4.8 (1.7–11.2) years, whereas one patient died 10.5 years after redo-transplantation due to recurrence of BOS.

Overall, freedom from graft loss (i.e., death or redo-transplantation) at 2 years post-TLI was 27% and overall patient survival (not censored at redo-transplantation) was 44% at 2 years post-TLI; and 38% after 17 years (Fig. 5).

Discussion

In this retrospective case series, we demonstrated favorable effects of salvage TLI treatment for progressive BOS, resulting in attenuation of the FEV_1-decline in some patients, mainly in rapid decliners. This helped bridging to redo-transplantation in a substantial proportion of patients, with acceptable long-term outcomes. Some patients, however, later progressed from BOS to RAS, in which DSA may be involved.

The rate of FEV_1-decline in BOS is variable, yet patients presenting with a FEV_1 (or FVC)-decline of ≥10% within the first 6 months after CLAD onset or with a FEV_1-decline of ≥100 ml/month during follow-up (so-called "rapid" decliners) appear to have a particularly poor survival [7–9]. Similar findings are reported

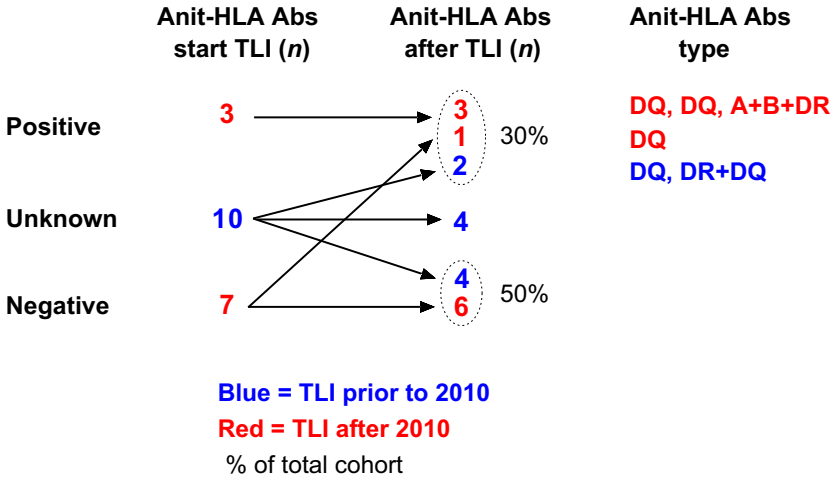

Figure 4 Anti-HLA antibodies in the cohort of lung transplant recipients treated with TLI for progressive BOS. The occurrence of anti-HLA antibodies (including type of anti-HLA antibodies) in the cohort of TLI-treated patients at start of TLI and during follow-up after TLI. Blue numbers indicate patients treated before 2010, red numbers patients treated after 2010, at which moment systematic anti-HLA assessment using Luminex was introduced in our center. All anti-HLA antibodies were donor-specific.

in BOS after hematopoietic stem cell transplantation (HSCT) [10]. Treatment options for BOS are limited [11]. However, given the supposed role of immune-mediated allograft damage in BOS onset and progression, salvage therapies targeting the immune system are often considered, such as extracorporeal photopheresis (ECP) or TLI.

Although results of ECP in BOS vary greatly between studies, overall around two-thirds of patients may demonstrate either slowing or cessation of disease progression, and increase in FEV_1 was observed in 12–25% of subjects receiving ECP [12]. However, ECP is less likely to attenuate BOS progression in rapid decliners (FEV_1-decline $\geq$100 ml/month pre-ECP), in BOS patients with low airway neutrophilia, and in patients with RAS [8]. Obvious reasons for ECP being less efficacious in rapid decliners may be that ECP: (i) requires some time (usually some weeks) to be logistically organized (i.e., need for central intravenous line and treatment-slots); and (ii) needs some time (again several weeks) before its indirect immunomodulatory effects (supposedly through T-cell apoptosis, antigen-presenting cell, and regulatory T-cell activation) may become evident. As such, precious time—and thus lung function—is lost. Although significant adverse events are rare, ECP remains a technically complicated and costly procedure, therefore not available in every center (including ours). A much less costly rescue treatment for progressive BOS may be montelukast [7], yet rapid decliners also seemed to benefit less from this therapy, most likely because it again takes some time to attain the anti-inflammatory and anti-fibrotic effects of montelukast.

Another salvage therapy for progressive BOS is TLI, directly delivering targeted irradiation to lymphoid tissue, which can be rapidly initiated in most centers (i.e., <2 weeks following treatment decision in our center). TLI was first used to treat Hodgkin's disease and is nowadays still used in the context of nonmyeloablative conditioning prior to allogeneic HSCT [13]. In the 80s and 90s, TLI emerged as treatment for cardiac [4,14–17] and renal allograft rejection [18,19]. Yet, because of concerns about long-term radiation-related side effects, such as myelodysplasia and leukemia, and because newer immunosuppressive treatments became available, TLI was reserved as ultimate salvage therapy in this setting. More recently, regained interest for TLI in BOS arose, given the devastating disease progression leading to respiratory insufficiency and death in most BOS patients (Table 2).

The effects of TLI in BOS have been reported in a limited number of case series, summarized in Table 2. In what follows, we will describe the main findings of these series, focusing on evolution of pulmonary function and—for the first time—specifically on responders versus nonresponders to TLI. Already in 1998, Diamond *et al.* reported on 11 BOS patients (all double lung transplant), of which 4/11 patients (36%) demonstrated a beneficial response with TLI (i.e., 40% FEV_1-decline during 3 months pre-TLI vs. 1% FEV_1 improvement during 3 months post-TLI), which was durably sustained after 24–72 weeks. The other seven patients failed

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to respond within 8 weeks post-TLI. These nonresponders had either lower FEV_1 at start of TLI (i.e., five cases with mean FEV_1 of 1.0 vs. 1.53 l in the four responders), or active pulmonary infection with *Nocardia* or *Aspergillus*, worsening during TLI (two cases) [20].

In 2005, Fisher *et al.* reported on 37 BOS patients (single and double lung transplant). A total of 27/37 patients (73%) completed at least 8 out of 10 planned TLI-fractions (vs. 90% in our series because of two deaths during TLI). Bone marrow suppression was the major side effect responsible for not completing scheduled treatment [5]. In almost all cases, however, this was a numerical, asymptomatic toxicity. Severe, but nonlethal, infection occurred in two patients (5%). In the patients who completed ≥8/10 fractions, significant attenuation of the monthly rate of FEV_1-decline was seen [122.7 ml/month pre-TLI to 25.1 ml/month post-TLI ($P = 0.004$)], with a *mean* (95% CI) net change in rate of FEV_1-decline of 97.5 (48.2–146.7) ml/month. Remarkably, FEV_1 *improved* in about 50% of patients after TLI. Interestingly, the most pronounced effect of TLI on attenuation of the FEV_1-decline appeared to occur in both patients with the most rapid BOS progression prior to TLI (i.e., FEV_1-decline >600 and >400 ml/month, respectively). Moreover, all 12 patients with a FEV_1-decline ≥100 ml/month pre-TLI demonstrated a reduction to <100 ml/month post-TLI, similar to our results.

These findings corroborated those of a prior report by the same group on 12 BOS patients (most of which were therefore likely also included in the study of Fisher) [21]. In this study by Charon *et al.* in 2000 (including mostly single lung recipients), the FEV_1-decline was significantly ($P = 0.05$) reduced at a median time of 497 (range 70–869) days following TLI (21). In retrospect, 4/12 (33%) patients in this study demonstrated a sustained *increase* in FEV_1 post-TLI.

More recently, at least four other series of TLI in BOS (mostly including patients treated with azithromycin) reported similar findings, demonstrating reduction of the rate of FEV_1-decline or even stabilization of FEV_1 following TLI in the majority of patients (Table 2) [5,22–24]. In the largest series to date, including 45 BOS patients [24], median FEV_1-decline decreased from

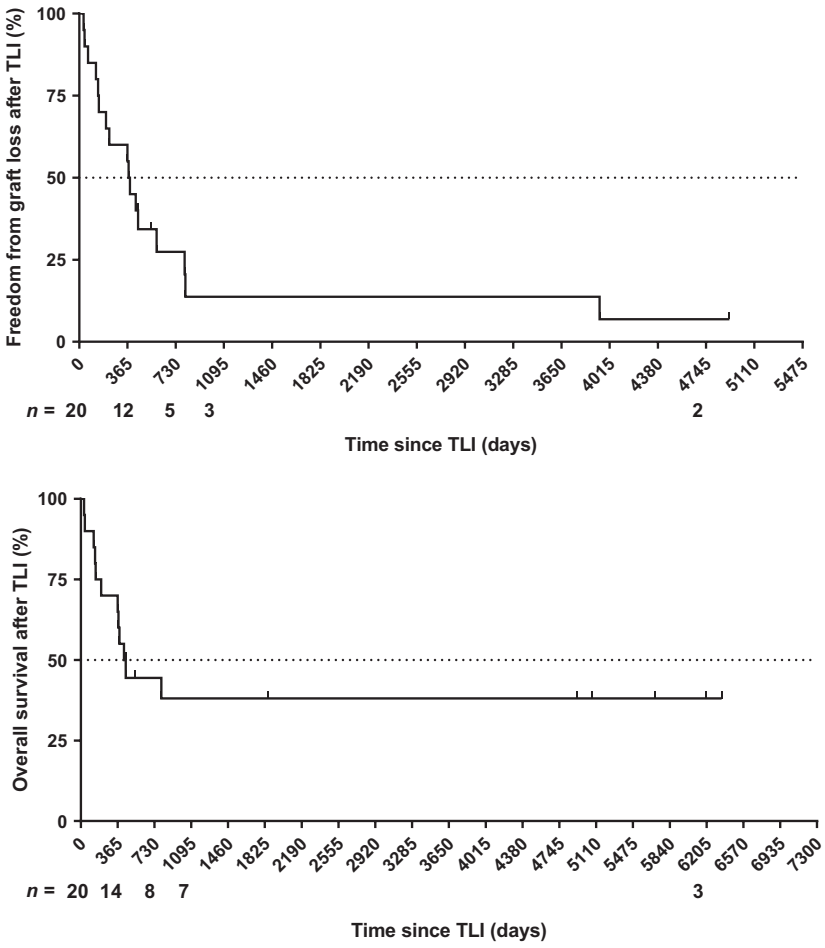

Figure 5 Graft loss and overall survival in lung transplant recipients treated with TLI for progressive BOS. Kaplan–Meier survival curves depicting freedom from graft loss (death or redo-transplantation), which was 27% at 2 years after TLI (upper panel), and overall patient survival (not censored for redo-transplantation), which was 44% at 2 years post-TLI and 38% after 17 years (lower panel). A total of five patients (25%) underwent redo-transplantation following TLI (median time between TLI and redo-transplantation was 586 (147.5–2372) days). Currently, four of these five (80%) redo-transplant patients are still alive, with a median follow-up time after redo-transplantation of 4.8 (1.7–11.2) years.

Table 2. Summary of published data on TLI in lung transplantation.

Author, reference	Year of TLI	CLAD	N	BOS severity at TLI	Post-Tx time of TLI	Effect on rate of FEV_1-decline	% Response
Diamond *et al.* [20] (St. Louis, USA)	1995–1996	BOS	11	BOS 2/3	1.5 year	"stabilization" in 4/11 ptsFEV_1 − 40%/3 month vs. +1%/3 monthTLI bridge to re-transplant in $n = 1$	36
Chacon *et al.* [21] (Newcastle, UK)	1990–1995	BOS	12	BOS 2/3	>1.5 year	"reduction"monthly rate of FEV_1-decline not reported ($P = 0.05$)	50
Fisher *et al.* [5] (Newcastle)	1989–2001	BOS	37	BOS 1 19%BOS 2/3 81%	>1.5 year	"reduction," 27/37 pt completed TLIFEV_1 − 122.7 ml/month vs. −25.1 ml/month ($P = 0.004$)	81
Verleden *et al.* [6] (Leuven, BE)	2000–2009	BOS	6	BOS 2/3	3.5 year	"reduction"FEV_1 − 221 ml/month vs. −79 ml/month ($P = 0.041$)TLI bridge to re-transplant in $n = 2$	100
Saez *et al.* [22] (Barcelona, ESP)	2011–2013	BOS	12	BOS 1 25%BOS 2 33%BOS 3 42%	?	"reduction," 11/12 pt completed TLIFEV_1 − 0.71 l/year vs. 0.13 l/year	83
Soresi *et al.* [23] (Harefield, UK)	2012–2014	BOS	27	BOS 1 22%BOS 2 48%BOS 3 30%	3.0 year	"stabilization"FEV_1 1.86 ± 0.59 l baseline vs. 1.73 ± 0.83 l ($P = 0.097$) at 1 year post-TLITLI bridge to re-transplant in $n = 1$Survival 58.6% at 2 years post-TLI	73.9
Miller *et al.* [24] (Newcastle, UK)	2004–2014	BOS	45	?	2.75 year	"reduction," 37/45 pt completed TLIFEV_1 − 174.7 ml/month vs. −105.3 ml/month ($P = 0.018$)	?
Total			150				70.6%

?, data not available; BOS, bronchiolitis obliterans syndrome; TLI, total lymphoid irradiation.

Summary of published data on TLI in lung transplantation, full explanation is given in the main manuscript. Overall, beneficial effects of TLI on BOS progression, as demonstrated by stabilization or reduction in the rate of FEV_1-decline, were reported in the majority of patients.

174.7 ± 132.9 ml/month pre-TLI to 105.3 ± 146.7 ml/month post-TLI, resulting in a net *median* change in rate of FEV_1-decline of 69.4 ml/month ($P = 0.018$). Again FEV_1 improved in several patients post-TLI, and rapid decliners pre-TLI appeared to demonstrate a more pronounced attenuation of the FEV_1-decline with TLI [24].

Finally, in two patients with refractory BOS following allogeneic HSCT, who received TLI, respectively, 30 and 26 months after HSCT, FEV_1 either stabilized at 6 months post-TLI or significantly improved in the other patient, which effect was sustained at 10 months post-TLI [25]. In all published series, including ours, TLI was generally well tolerated, with main cause of preemptive cessation of radiotherapy being bone marrow suppression, mostly asymptomatic, rather than respiratory infections [22–24].

In most studies to date, including ours, there are thus patients with rapid progressive BOS demonstrating a spectacular response to TLI. For instance, in our study, two patients with a FEV_1-decline >450 ml/month pre-TLI (i.e., 663 and 493 ml/month, respectively) were among the top responders, with their monthly rate of FEV_1-decline plunging to 50 and 20 ml/month at 3 months post-TLI, respectively. It is unclear why TLI has such an important effect in these patients, but they may represent a subtype of BOS wherein there is a very active immune response directed toward the allograft, which is abruptly tempered by lymphoid tissue irradiation. The exact mechanism of action of TLI in these rapid progressive BOS patients is unclear; however, it may be similar to that in refractory acute cellular allograft rejection, in whom TLI may reverse intractable cellular rejection, as previously demonstrated in a small series of heart–lung and lung transplant recipients [26].

Importantly, TLI may buy these rapid decliners precious time and hence help to bridge them to possible redo-transplantation, which up to now had only been reported in four cases, including two of our own previous study [6]. Our current data show that patients undergoing redo-transplantation following TLI ($n = 5$ in our present series) may have a very good long-term outcome, with survival up to at least 10 years after redo-transplantation [i.e., median follow-up time 4.8 (1.7–11.2) years in our series].

Data on long-term outcomes after TLI, including graft and patient survival, have unfortunately not been consistently reported to date, hampering comparison of outcomes between series. While most studies mainly focused on FEV_1 as outcome parameter, there is only a single study reporting 58.6% survival after 2 years [23]. In our series, overall patient survival at 2 years post-TLI was 44%, yet with one patient surviving up to 13 years after TLI without redo-transplantation. However, given the low number of cases and lack of general guidelines or recommendations on redo-transplantation, we cannot make a firm statement regarding the timing and selection criteria of eligible candidates for redo-transplantation following TLI, nor on the appropriate timing of surgery, yet it seems wise to postpone redo-transplantation until after recovery of TLI-induced leukopenia.

In future years, immunophenotyping may perhaps provide further insights into the involved mechanisms and thus help to identify patients more likely to respond to TLI. In a prospective study of 26 progressive BOS patients (despite at least 6-week azithromycin) treated with TLI between 2012 and 2014, a total number of circulating B cells, naïve B cells, plasmablasts, switched memory B cells, and naïve $CD8^+$ T cells were inversely associated with patient survival after TLI (i.e., better survival in patients with lower pre-TLI cell numbers, which is contra-intuitive to what would be expected from the immunosuppressive effects of TLI), while no relation with DSA was seen [27]. In our series, we found no association between DSA, circulating, or local leukocyte numbers (including lymphocytes) on the one hand, and the attenuation of the rate of FEV_1-decline with TLI on the other hand, yet lymphocyte subset analysis was not performed. We noticed, however, a significant decrease in total blood leukocyte numbers and increase in % blood monocytes after TLI, but the exact importance of these finding remains to be elucidated.

Interestingly, immunomodulation and induction of immune tolerance with TLI, most likely via apoptotic lymphocyte depletion and upregulation of regulatory natural killer T cells [28], has been described in several preclinical animal models [29–35]. Similar findings in human heart transplantation were described, with profound depression of $CD4^+$ and to a lesser extent $CD8^+$ lymphocyte numbers after TLI, allowing for prolonged (up to 8 years) immunosuppressant-*free* survival [36]. Using a tolerance-induction regimen containing TLI, immunosuppressive drug withdrawal and subsequent long-term (up to 14 years) graft survival was also achieved in renal allograft recipients [37–40]. Finally, a combination regimen including TLI could desensitize kidney recipients pre-transplant (i.e., reduce anti-HLA antibody levels) and reverse antibody-mediated rejection post-transplant, most likely through inhibition of B-cell proliferation [19]. Besides this, several studies have reported on the beneficial effects of TLI in therapy-resistant chronic graft-versus-host disease following HSCT [41].

Long-term complications of TLI in adults are mostly limited in severity and asymptomatic [4,42]. Nevertheless, delayed TLI treatment because of abnormal blood count (65%) and infections (20%) was reported in a significant proportion of BOS patients [24]. Importantly, to our knowledge, there have been no cases of post-transplant lymphoproliferative disorder reported in patients who received TLI for acute allograft rejection or BOS, including our current series.

Inevitable limitations of our study are inherent to its single-center, retrospective design, such as low patient numbers, absence of controls, and large study-period spanning 13 years, during which different prophylactic and therapeutic strategies have been applied. Indeed, only a limited number of BOS patients were referred for TLI at the treating physician's discretion in our center between 2004 and 2017 (20 patients, or on average 1.5 patient/year). Some other BOS patients refused/did not consent for TLI, because they were afraid of the possible side effects and opted for symptomatic treatment. Others were considered not to be eligible candidates because of recurrent respiratory infections or because of developing other comorbidities during the course of their follow-up (i.e., malignancy and cardiovascular problems) precluding them from TLI treatment. In retrospect, it is however impossible to identify these individuals in our current database, because these reasons for not starting TLI were not recorded at that time. Also, interaction of TLI with other drugs, such as azithromycin or montelukast, cannot be fully excluded. Ideally, a randomized, preferably multicenter, trial should be performed to assess efficacy and safety of TLI in BOS. Following initial stabilization after TLI, some patients may later progress to RAS, the reason for which remains unclear and needs further investigation in a larger cohort.

In conclusion, we report on our experience of TLI for progressive BOS, demonstrating that TLI may be an acceptable salvage treatment attenuating disease progression in some patients, particularly in rapid decliners, in whom it may be worthwhile considering using this therapeutic strategy to help to bridge to redo-transplantation.

Authorship

Description of each author's contribution or role: ML: performed research/study, collected data, analyzed data, wrote the paper, and critically assessed the paper. JK: collected data, analyzed data, wrote the paper, and critically assessed the paper. ML, AV, HB, GPLA, BMV, SEV, EKV, APN, LJC, DEVR, and GMV: collected data and critically assessed the paper. RV: designed research/study, collected data, analyzed data, wrote the paper, and critically assessed the paper.

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Conflicts of interest

The authors of this manuscript have no conflicts of interest to disclose. The authors confirm that that the work described has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form in English or in any other language, without the written consent of the copyright holder. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. All authors contributed in an important manner to the study design, data collection and analysis, or writing of the paper according to the guidelines of the International Committee of Medical Journal Editors (ICMJE). All authors have read and approved the manuscript, all authors take responsibility for the manuscript, and the submitting author has permission from all authors to submit the manuscript on their behalf.

REFERENCES

1. Kulkarni HS, Cherikh WS, Chambers DC, *et al.* Bronchiolitis obliterans syndrome-free survival after lung transplantation: an International Society for Heart and Lung Transplantation Thoracic Transplant Registry analysis. *J Heart Lung Transplant* 2019; **38**: 5.

2. Verleden GM, Glanville AR, Lease ED, *et al.* Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment –

a consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant* 2019; **38**: 493.
3. Glanville AR, Verleden GM, Todd JL, *et al*. Chronic lung allograft dysfunction: definition and update of restrictive allograft syndrome – a consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant* 2019; **38**: 483.
4. McKay C, Knight KA, Wright C. Beyond cancer treatment – a review of total lymphoid irradiation for heart and lung transplant recipients. *J Med Radiat Sci* 2014; **61**: 202.
5. Fisher AJ, Rutherford RM, Bozzino J, Parry G, Dark JH, Corris PA. The safety and efficacy of total lymphoid irradiation in progressive bronchiolitis obliterans syndrome after lung transplantation. *Am J Transplant* 2005; **5**: 537.
6. Verleden GM, Lievens Y, Dupont LJ, *et al*. Efficacy of total lymphoid irradiation in azithromycin nonresponsive chronic allograft rejection after lung transplantation. *Transplant Proc* 2009; **41**: 1816.
7. Vos R, Van den Eynde R, Ruttens D, *et al*. Montelukast in chronic lung allograft dysfunction after lung transplantation. *J Heart Lung Transplant* 2019; **38**: 516.
8. Greer M, Dierich M, De Wall C, *et al*. Phenotyping established chronic lung allograft dysfunction predicts extracorporeal photopheresis response in lung transplant patients. *Am J Transplant* 2013; **13**: 911.
9. Todd JL, Neely ML, Finlen Copeland CA, Frankel CW, Reynolds JM, Palmer SM. Prognostic significance of early pulmonary function changes after onset of chronic lung allograft dysfunction. *J Heart Lung Transplant* 2019; **38**: 184.
10. Kwok WC, Liang BM, Lui MMS, *et al*. Rapid versus gradual lung function decline in bronchiolitis obliterans syndrome after haematopoietic stem cell transplantation is associated with survival outcome. *Respirology* 2019; **24**: 459.
11. Meyer KC, Raghu G, Verleden GM, *et al*. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. *Eur Respir J* 2014; **44**: 1479.
12. Benden C, Haughton M, Leonard S, Huber LC. Therapy options for chronic lung allograft dysfunction-bronchiolitis obliterans syndrome following first-line immunosuppressive strategies: a systematic review. *J Heart Lung Transplant* 2017; **36**: 921.
13. Kohrt H, Lowsky R. Total lymphoid irradiation for graft-versus-host disease protection. *Curr Opin Oncol* 2009; **21** (Suppl. 1): S23.
14. Tallaj JA, Pamboukian SV, George JF, *et al*. Total lymphoid irradiation in heart transplantation: long-term efficacy and survival – an 18-year experience. *Transplantation* 2011; **92**: 1159.
15. Lim TS, O'Driscoll G, Freund J, Peterson V, Hayes H, Heywood J. Short-course total lymphoid irradiation for refractory cardiac transplantation rejection. *J Heart Lung Transplant* 2007; **26**: 1249.
16. Asano M, Gundry SR, Razzouk AJ, *et al*. Total lymphoid irradiation for refractory rejection in pediatric heart transplantation. *Ann Thorac Surg* 2002; **74**: 1979.
17. Chin C, Hunt S, Robbins R, Hoppe R, Reitz B, Bernstein D. Long-term follow-up after total lymphoid irradiation in pediatric heart transplant recipients. *J Heart Lung Transplant* 2002; **21**: 667.
18. Myburgh JA, Meyers AM, Margolius L, *et al*. Total lymphoid irradiation in clinical renal transplantation–results in 73 patients. *Transplant Proc* 1991; **23**: 2033.
19. Zhu D, Qi G, Tang Q, *et al*. Combination of total lymphoid irradiation, low-dose IVIG and ATG as rescue therapy for highly sensitized and antibody-mediated rejection renal transplant recipients. *Clin Transpl* 2014; 215.
20. Diamond DA, Michalski JM, Lynch JP, Trulock EP 3rd. Efficacy of total lymphoid irradiation for chronic allograft rejection following bilateral lung transplantation. *Int J Radiat Oncol Biol Phys* 1998; **41**: 795.
21. Chacon RA, Corris PA, Dark JH, Gibson GJ. Tests of airway function in detecting and monitoring treatment of obliterative bronchiolitis after lung transplantation. *J Heart Lung Transplant* 2000; **19**: 263.
22. Saez B, Bravo C, Loor K, *et al*. Total lymphoid irradiation in progressive bronchiolitis obliterans syndrome after lung transplantation [abstract]. *Eur Respir J* 2014; **44**: P3323.
23. Soresi S, Sabashnikov A, Popov AF, *et al*. Early aggressive total lymphoid irradiation after lung transplantation: a tough battle against chronic lung allograft dysfunction [abstract]. *Transpl Int* 2015; **28**(S4): 78.
24. Miller R, Hartog B, Frewet J, *et al*. Total lymphoid irradiation (TLI) for the management of bronchiolitis obliterans syndrome (BOS) post lung transplant: a single centre experience [abstract]. *J Heart Lung Transplant* 2016; **35**(S4): 70.
25. Lewis JA, Jenrette JM, Watkins JM, *et al*. Total lymphoid irradiation for the treatment of refractory bronchiolitis obliterans following allogeneic hematopoietic stem cell transplantation [abstract]. *Biol Blood Marrow Transplant* 2010; **16**: S246.
26. Valentine VG, Robbins RC, Wehner JH, Patel HR, Berry GJ, Theodore J. Total lymphoid irradiation for refractory acute rejection in heart-lung and lung allografts. *Chest* 1996; **109**: 1184.
27. Schmack B, Cano Romero F, Hester J, *et al*. Immunophenotyping pre total lymphoid irradiation for BOS after lung transplantation may predict response to therapy [abstract]. *J Heart Lung Transplant* 2017; **36**(S4): 35.
28. Weiss L, Slavin S. Effect of total lymphoid irradiation on natural killer cells and cytotoxic and primed T lymphocytes. *Isr J Med Sci* 1994; **30**: 253.
29. Jansson K, Dreckmann K, Sommer W, *et al*. Splenocyte infusion and whole-body irradiation for induction of peripheral tolerance in porcine lung transplantation: modifications of the preconditioning regime for improved clinical feasibility. *Transplant Direct* 2017; **3**: e170.
30. Nagasaki K, Obara H, Xiong A, *et al*. Liver allografts are toleragenic in rats conditioned with posttransplant total lymphoid irradiation. *Transplantation* 2007; **84**: 619.
31. Bakonyi A, Berho M, Ruiz P, *et al*. Donor and recipient pretransplant conditioning with nonlethal radiation and antilymphocyte serum improves the graft survival in a rat small bowel transplant model. *Transplantation* 2001; **72**: 983.
32. Hayamizu K, Zeng D, Strober S. Donor cells that facilitate tolerance to rat heart allografts after posttransplant total lymphoid irradiation and rabbit anti-thymocyte globulin. *Transplant Proc* 1999; **31**(3B Suppl.): 25S.
33. Horton PJ, Hawthorne WJ, Walters S, *et al*. Tolerance induction in a large-animal model using total lymphoid irradiation and intrathymic bone marrow. *Transplantation* 2008; **86**: 1830.
34. Myburgh JA, Smit JA, Pieterz GA, Ling S, Sandrin MS, McKenzie IF. Tolerance in baboon kidney transplantation with total lymphoid irradiation (TLI) and anti-CD3/CD4-idarubicin conjugates. *Transplantation* 2001; **72**: 1150.

35. Halperin EC. Non-human to human organ transplantation: its biologic basis and a potential role for radiation therapy. *Int J Cancer* 2001; **96**: 76.
36. Comerci GD Jr, Williams TM, Kellie S. Immune tolerance after total lymphoid irradiation for heart transplantation: immunosuppressant-free survival for 8 years. *J Heart Lung Transplant* 2009; **28**: 743.
37. Trivedi HL, Kaneku H, Terasaki PI, *et al.* Clonal deletion using total lymphoid irradiation with no maintenance immunosuppression in renal allograft recipients. *Clin Transpl* 2009; 265.
38. Scandling JD, Busque S, Shizuru JA, *et al.* Chimerism, graft survival, and withdrawal of immunosuppressive drugs in HLA matched and mismatched patients after living donor kidney and hematopoietic cell transplantation. *Am J Transplant* 2015; **15**: 695.
39. Scandling JD, Busque S, Dejbakhsh-Jones S, *et al.* Tolerance and withdrawal of immunosuppressive drugs in patients given kidney and hematopoietic cell transplants. *Am J Transplant* 2012; **12**: 1133.
40. Kuruvila CK, McCalmon RT, Schreiber DP, Kortz WJ. Total lymphoid irradiation for pretransplant immunosuppression: nine year follow-up. *Kidney Int Suppl* 1997; **61**: S89.
41. Hautmann AH, Wolff D, Hilgendorf I, *et al.* Total nodal irradiation in patients with severe treatment-refractory chronic graft-versus-host disease after allogeneic stem cell transplantation: response rates and immunomodulatory effects. *Radiother Oncol* 2015; **116**: 287.
42. Thomas O, Mahé M, Campion L, *et al.* Long-term complications of total body irradiation in adults. *Int J Radiat Oncol Biol Phys* 2001; **49**(1): 125.