HHS Public Access

Author manuscript

J Heart Lung Transplant. Author manuscript; available in PMC 2022 April 01.

Published in final edited form as:

J Heart Lung Transplant. 2021 April ; 40(4): 269–278. doi:10.1016/j.healun.2021.01.008.

Hyperbaric oxygen therapy to prevent central airway stenosis after lung transplantation

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Abstract

Background: Central airway stenosis (CAS) is a severe airway complication after lung transplantation associated with bronchial ischemia and necrosis. We sought to determine if hyperbaric oxygen therapy (HBOT), an established treatment for tissue ischemia, attenuates post-transplant bronchial injury.

Methods: We performed a randomized, controlled trial of usual care vs. HBOT (2 atmospheres absolute for 2 hours × 20 sessions) in subjects with extensive airway necrosis 4 weeks post-transplant. Endobronchial biopsies were collected at 4, 7, and 10 weeks post-transplant for quantitative PCR. Co-primary outcomes were incidence of airway stenting and acute cellular rejection (ACR) at one year.

Results: The trial was stopped after enrolling 20 subjects (n=10 per group) after a pre-planned interim analysis showed no difference between usual care and HBOT groups in stenting (both 40%), ACR (70% and 40%, respectively), or CAS (40% and 60%, respectively). Time-to-first-stent placement (median [IQR]) was significantly shorter in the HBOT group (150 [73–150] vs. 186 [167–206] days, P<0.05). Hypoxia-inducible gene expression was significantly increased in

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Authors contributions: B.D.K. and K.M. drafted the manuscript which was approved by all authors. All authors collected, analyzed, and/or interpreted the data. The overall research plan was conceived by S.L.S., and approved by all authors. All authors are accountable for the submitted work.

donor tissues at 4, 7, and 10 weeks post-transplant, but was not altered by HBOT. Subjects that developed CAS or required stenting had significantly higher *HMOX1* and *VEGFA* expression at 4 weeks (both P<0.05). Subjects that developed ACR had significantly *FLT1*, *TIE2*, and *KDR* expression at 4 weeks (all P<0.05).

Conclusions: Incidence of CAS is high after severe, established airway necrosis post-transplant. HBO therapy does not reduce CAS severity or stenting. Elevated *HMOX1* and *VEGFA* expression appears to associate with airway complications.

Keywords

lung transplantation; hyperbaric oxygenation; cell hypoxia/genetics; gene expression; postoperative complications

INTRODUCTION

Central airway stenosis (CAS) is a debilitating complication of lung transplantation that leads to loss of lung function, respiratory infections, hospitalizations, and possibly death[1–3]. While the pathophysiology is incompletely understood, accumulating evidence points to bronchial ischemia acquired intra-operatively due to sacrifice of the bronchial arteries[4–8]. Soon after transplant, donor bronchial tissue hypoxia is evident, with reduced tissue oxygen saturations and up-regulation of hypoxia-inducible factor-dependent (HIF) genes[6]. Severe bronchial ischemia is associated with post-transplant respiratory failure and prolonged hospitalization, and ultimately leads to mucosal necrosis, fibrosis, and central airway stenosis[6].

Strategies proposed to mitigate post-transplant bronchial ischemia include bronchial artery anastomosis[8,9], which prolongs operative time and may be technically challenging, topical application of HIF stabilizers[7], and hyperbaric oxygen therapy (HBOT)[10]. HBOT involves breathing pure oxygen in a chamber pressured to 1.4 atmospheres absolute (ATA) or higher[11], and is an established therapy for ischemic flaps and grafts[12,13]. In small case series HBOT has been reported to improve anastomotic healing after native tracheobronchial reconstruction[14–17]. Furthermore, HBOT can be safely administered to patients after lung transplantation[10,18] and may reduce the need for airway stent placement for CAS[10]. On this basis, we performed a randomized, controlled, phase 2 clinical trial evaluating the efficacy of HBOT to reduce airway complications following lung transplantation in patients with significant airway necrosis. We also serially measured bronchial mucosal gene expression of HIF-dependent genes to develop a timeline for host response, and validate our prior biomarker studies[6].

METHODS

Subject Selection

The study protocol was approved by the Duke Institutional Review Board (Pro00055849) and posted on www.clintrials.gov (NCT02363959). All subjects provided written informed consent prior to study procedures. Subjects were eligible for the study that had developed extensive (stage 3–4) (Table S1) post-transplantation airway necrosis[10] after lung

transplantation that did not spontaneously resolve after 2–3 weeks. Subjects were excluded that required mechanical ventilation with fraction of inspired oxygen (FiO_2) > 0.4, required extracorporeal membrane oxygenation at the time of screening, used inhaled nitric oxide at the time of screening, had a pneumothorax at the time of screening, were pregnant, or were unable to provide informed consent. Subjects that agreed to participate were randomized 1:1 to usual care or HBOT (total target enrollment of 40 subjects) using pre-prepared envelopes containing the group assignment.

Surgical Technique

Single or bilateral orthotopic lung transplantation (SOLT/BOLT) was performed as described[10,19]. Briefly, the membranous portions of the bronchi were sutured end-to-end using a running, 4–0 absorbable monofilament suture (e.g. PDS) with the smaller cartilaginous portion partially intussuscepted into the larger airway. When technically feasible, the donor bronchus is preferentially intussuscepted into the recipient bronchus. No tissue flap or additional coverage of the airway is performed. There were five surgeons during the time of the study that performed the airway anastomosis in a similar fashion. None of the enrolled subjects received lungs from donors after cardiac death (DCD) or after ex vivo lung perfusion (EVLP).

Bronchoscopy Protocol

Informed consent was obtained prior to each procedure. Bronchoscopies were performed at approximately 4 weeks, 7 weeks, and 10 weeks post-transplantation. Airway balloon dilation was performed for airway stenosis, defined as inability to traverse an airway that would otherwise permit passage of a 6.4 mm outer-diameter bronchoscope. Balloon dilation procedures were performed up to three times, in 2–3 week intervals. For subjects with stenosis refractory to three separate balloon dilation procedures, or who were at risk for complete airway obstruction, endobronchial stents were placed. Subjects with excessive granulation tissue underwent mechanical debridement as needed to open the airway lumen. Endobronchial biopsies (1–2 mm) of the airway epithelium were performed in triplicate at the main carina and the first subcarina of each donor bronchus. Subjects with prior SOLTs underwent biopsy of the main carina and the donor bronchus subcarina only. The endobronchial biopsy specimens were labeled numerically for blinding, placed immediately into RNA*later* RNA stabilization reagent (Qiagen), and stored at −80°C.

Hyperbaric Oxygen Therapy

Subjects randomized to HBOT breathed >99% medical-grade oxygen via head tent inside a multi-place hyperbaric chamber (Duke Center for Hyperbaric Medicine and Environmental Physiology, Durham, NC) pressured to 2 ATA for two hours daily for up to 20 sessions total. Subjects were evaluated after each treatment for signs or symptoms of barotrauma or oxygen toxicity.

Blinding

While the study was designed so the proceduralists were blinded to randomization, unblinding inevitably occurred during the study due to the clinical reporting of the HBOT

administration in the subjects' electronic medical record. Nevertheless, the proceduralists were blinded for as long as possible, until it was discoverable in the chart.

Crossover

Subjects randomized to usual care were eligible to crossover to HBOT between 8 – 12 weeks after initial study bronchoscopy if they: 1) developed CAS or another airway complication (e.g. dehiscence, malacia); or 2) failed to improve pseudomembrane severity based on a semi-quantitative scoring system (Table S1). For data analysis, subjects that crossed-over remained in their original group assignments per our intention-to-treat protocol.

Primary and Secondary Outcomes

The co-primary outcomes were need for airway stenting and development of acute cellular rejection (ACR) at one year. We chose ACR as a co-primary outcome for safety monitoring. The secondary outcomes were development of clinically significant central airway stenosis (CAS) due to fibrotic strictures, need for dilation/balloon bronchoplasty, development of clinically significant lung infection requiring antibiotics, development of airway dehiscence, chronic lung allograft dysfunction (CLAD)[20], development of other airway complications such as granulomatous strictures or bronchomalacia, and bronchial epithelial gene expression. Clinical outcomes were assessed at 12 months. An interim analysis was pre-planned after enrolling 20 subjects with the option to terminate the trial early for futility.

Polymerase Chain Reaction

Total RNA was extracted from endobronchial biopsy specimens using the RNeasy Midi Kit (Qiagen). RNA purity was confirmed on a 1.2% agarose gel and RNA was reverse transcribed into cDNA using the ImProm-II reverse transcription system (Promega). Quantitative real-time RT-PCR was performed on an ABI StepOnePlus using gene expression assays (Applied Biosystems). Gene expression assay primers were used to amplify *HMOX1*, *VEGFA*, *FLT1* (*VEGFR1*), *KDR* (*VEGFR2*), *TIE2* (*TEK*), and *TGFB1*. 18S rRNA was used as an endogenous control. Quantification of gene expression was determined by the comparative threshold cycle and relative quantification method. Each sample was assayed in triplicate. All mRNA work was performed in a blinded fashion.

Statistical Analysis

Assuming a CAS incidence of 60%, a reduction by HBOT to 10%[10], and alpha of 0.05, we calculated 14 subjects/group would achieve 80% power to detect statistical significance. We targeted 20 subjects/group (40 total) to compensate for subject withdrawal or drop-out.

Grouped data are shown as median (interquartile range) unless otherwise specified. Primary and secondary outcomes were analyzed by intention-to-treat analysis. Categorical data were analyzed using Fisher's exact test. PCR data were analyzed by one sample Wilcoxon signed-rank test against a hypothetical value of 1 with Bonferroni correction (Figure 2), or by 2-way ANOVA with Benjamin-Hochberg post-hoc test (to correct for multiple comparisons) for group differences within each time point (Figures 3–6) (Prism v8.1.1, GraphPad Software, San Diego, CA). $P<0.05$ was accepted as significant.

RESULTS

Twenty subjects (11 males, 9 females) that were 4.6 (4 – 5.6) weeks post-transplant were enrolled and randomized to either usual care (n=10) or hyperbaric oxygen therapy (n=10). Age and sex distributions were comparable between the groups: 54.5 (36 – 65) vs. 59.7 (41 – 62) years, and 50% vs. 40% female, respectively. Individual subject characteristics are shown in Table 1. Two subjects were screened but not randomized due to transplant-related complications developed prior to study activities (seizures, n=1; anastomotic dehiscence, n=1). The most common indications for transplant were interstitial lung disease (n=8) and cystic fibrosis (n=7). All but three subjects underwent BOLT (left SOLT, n=1; right SOLT, n=2). All patients had undergone evaluation for gastroesophageal reflux and esophageal dysmotility as part of their routine transplant evaluation. The groups were comparable with respect to gastroesophageal disorders (Table S2).

Additional post-transplant clinical factors are shown in Table S3. There were no significant differences between groups in ischemic times, primary graft dysfunction (PGD) severity[21], vasopressor-free days, usage of ECMO post-transplant, ventilator-free days, tracheostomy-free days, or time to readmission (all $p>0.05$). However, despite randomization, the number of oxygen-free days was significantly lower in the Usual Care group compared with the HBOT group ($p<0.05$).

For subjects randomized to HBOT, time to treatment was 8.5 (6 – 13) days after randomization. All subjects completed twenty HBOT sessions, except for one subject who discontinued HBOT after five sessions due to claustrophobia. Due to poorly resolving bronchial mucosal necrosis, two subjects that had randomized to usual care were offered to crossover to HBOT, one of which was agreeable and completed twenty HBO treatments.

Patient outcomes are shown in Table 2. After enrolling twenty patients, the trial was stopped after a pre-planned interim analysis showed no difference between groups in incidence of airway stenting, acute cellular rejection, CAS, airway balloon dilation, anastomotic dehiscence, airway infections, or CLAD (Table 2 and Table S4). Plaque scores were identical between both groups over time (Figure 1A). While the time to first dilation was also similar between usual care and HBOT groups (98 [93 – 128] vs. 97 [87 – 126] days, respectively) (Figure 1B), the time to stent placement was significantly shorter in the HBOT group (150 [73 – 150] vs. 186 [167 – 206] days, $P<0.05$) (Figure 1C). One subject (# 001) in the usual care group underwent stent placement for right mainstem bronchomalacia but did not require balloon dilation. A secondary analysis showed that oxygen-free days did not correlate with the need for stent placement, development of CAS, plaque score severity, or plaque score change over time.

Based on previous work that identified associations between hypoxia-inducible gene expression and post-transplant airway complications[6], we performed quantitative PCR on bronchial mucosal biopsies for select hypoxia-inducible genes. Donor/native tissue expression ratios were significantly >1 for *FLT1*, *HMOX1*, and *TGBF1* at 4 and 7 weeks, and for *VEGFA* at 4, 7, and 10 weeks (all $P<0.05$) (Figure 2). We also found elevated ratios for *KDR* at 7 and 10 weeks ($P<0.05$).

We next compared gene expression between the HBOT and usual care groups to determine if HBOT exposure altered hypoxic gene expression (Figure 3). Despite the randomization procedure, we initially found baseline differences in gene expression of *FLT1* and *TGFB1* at 4 weeks, prior to the initiation of HBOT. However, when accounting for oxygen-free days, these differences were no longer apparent. No other differences in mRNA levels of the measured genes (*FLT1*, *HMOX1*, *KDR*, *TIE2*, *VEGFA*, or *TGFB1*) were observed between the usual care and HBOT groups.

We then analyzed gene expression as a function of development of CAS and need for stent placement. We found significantly higher expression of *HMOX1* and *VEGFA* ($P<0.05$) at 4 weeks post-transplantation in subjects that would go on to develop CAS (Figure 4) and in subjects that required airway stenting (Figure 5). Furthermore, expression of *KDR*, the VEGFA receptor 2, was significantly lower at 10 weeks post-transplant in subjects that required airway stenting ($P<0.05$). An *HMOX1* donor/native gene expression ratio of > 40 was poorly sensitive for the development of CAS or need for stenting (36% and 38%, respectively) but 100% specific with a positive predictive value of 100%. Similarly, VEGFA donor/native gene expression ratio > 5 was poorly sensitive for development of CAS or need for stenting (43% and 46%, respectively), but 100% specific with a positive predictive value of 100%. Finally, we analyzed gene expression as a function of development of acute cellular rejection. There were significantly higher mRNA levels of *FLT1*, *KDR*, and *TEK* (all $P<0.05$) at 4 weeks post-transplant in subjects that would develop rejection compared with subjects that would not (Figure 6). However, there were no differences noted at 7 or 10 weeks post-transplant.

DISCUSSION

We report the first randomized, controlled trial of hyperbaric oxygen therapy compared with usual care to treat established airway necrosis after lung transplantation. While many clinical factors are known to contribute to ischemic airway complications, such as hypotension, prolonged mechanical ventilation, infection, PGD, and ACR[1,2,6,22–24], our hypothesis was that HBOT might improve donor bronchial mucosal healing and reduce the incidence of CAS and need for stenting. However, the study was terminated early for futility, as there was no difference in incidence of airway complications or acute cellular rejection. However, we did find a significantly shorter time to stent placement in the HBOT group. We also validated two mRNA biomarkers (*HMOX1* and *VEGFA*) associated with development of airway complications, and discovered three novel RNA biomarkers (*FLT1*, *KDR*, and *TIE2*) associated with development of acute cellular rejection.

Our study rationale was that HBOT is safe[10], has been reported to promote healing in native airways following tracheobronchial resections[14–17], and therefore may be useful in post-transplant donor airway ischemia. Lung transplantation is unlike other solid organ transplant procedures as there is no anastomosis of the arterial (bronchial) blood supply. This renders the transplanted lung dependent on low flow, retrograde perfusion from the poorly oxygenated pulmonary circulation[8], which predisposes to donor airway ischemia, a leading risk factor for airway complications[4–8]. This system mimics ischemic grafts and flaps, where HBOT has been shown to improve oxygen delivery, recruit endothelial stem cells, promote

neovascularization and fibroblast proliferation, and reduce inflammation[12,13,25,26]. Therefore, we performed a randomized, controlled trial of HBOT vs. usual care in lung transplant patients with established airway necrosis. However, we found no difference between groups in incidence of airway complications, such as CAS, need for balloon dilation, need for airway stenting, infection, or dehiscence, and no difference in development of ACR or CLAD. We chose ACR as a co-primary outcome to monitor for any effect of HBOT on ACR in either direction: HBOT has immunosuppressive effects and has been studied as a treatment for ACR in other solid organ allografts[27]; however, T-cell metabolism is largely oxidative[28] and we could not rule out that lung T-cells could become activated after hyperoxic exposure. Because we only enrolled subjects with severe, established airway necrosis that persisted at 4 weeks post-transplant, it is possible that the donor airway injury was too advanced for HBOT to have had any effects. Future studies could administer HBOT as soon as post-transplant airway necrosis is recognized, or even preemptively to all patients post-operatively[16].

Our study did identify several notable clinical findings. First, the incidence of CAS in our patient population that was enriched for airway necrosis was 40–60%, much higher than the generally reported incidence of 10–15%[2,3], further strengthening the relationship between airway ischemia, necrosis, and stenosis. Second, the incidence of airway stenting was the same between groups, but occurred significantly sooner in the HBOT group. The reason for this is not entirely clear, but was seen in our earlier case-control study as well[10], and is suggestive of an accelerated fibrotic healing response in ischemic airway tissues by HBOT similar to that seen in other tissues[12,26,29].

We have previously shown that mRNA levels of HIF-dependent genes, such as *VEGFA*, *FLT1*, *HMOX1*, were upregulated in ischemic donor airways at 4 weeks and associated with post-transplant respiratory complications[6]. In this study, we again found mRNA levels of these genes were higher at 4 week. We also found they remained elevated through 10 weeks post-transplant, longer than was previously known. We further hypothesized that by augmenting oxygen delivery, HBOT might down-regulate donor bronchial mucosal HIF-dependent gene expression, although we found no such effect on the measured genes. However, we did find that *HMOX1* and *VEGFA* mRNA levels were significantly associated with the development of CAS and need for stent placement. Taken together, these findings validate our earlier observations[6], and suggest the HIF pathway may be a viable target for pharmacologic intervention[7].

Prior studies have shown chronic rejection (CLAD) is associated with small airway ischemia[30–33], and activation of the HIF-1α-VEGFA-VEGFR2 (KDR) pathway[34]. Moreover, experimental CLAD is attenuated by HIF-1α transfection[33], suggesting this pathway is not merely a biomarker for disease but is involved in pathogenesis. While we did not show a significant difference between the HBOT and usual care groups in development of CLAD (0% vs. 10%, respectively), our study only followed subjects for one year, where the incidence of CLAD is still low. Therefore, we are limited in assessing whether HBOT affected CLAD incidence.

Prior studies have correlated gene expression in various lung tissues (e.g. bronchoalveolar lavage cells, transbronchial biopsy, lung explant) with ACR[35] and CLAD[36–38]. This work has focused primarily on pro-fibrotic and immunologic genes. Our study focused on hypoxic gene expression and is the first to show associations between ACR and angiogenic markers, such as the VEGFA pathway (*KDR* and *FLT1*), and *TIE2* expression. TIE2 is an angiogenic cell surface marker previously associated with chronic renal allograft rejection[39] and CLAD[33,40], but is a novel finding for ACR. Our study is limited due to measuring gene expression in large airways rather than small ones where acute cellular rejection occurs. However, we hypothesize that these small airways display hypoxic gene expression too, especially 1) those with wall thickness > 1 mm, which is the maximum distance that oxygen diffuses[41]; and 2) because they are perfused (down to respiratory bronchioles) by the bronchial arteries[42] which are sacrificed at transplant. However, these findings are hypothesis generating and require confirmation in future lung ACR studies.

Our study has several limitations. First, it was not possible to use a blinded study design, which may have biased the results, particularly the use of stents. However, the randomization procedure itself was blinded and our stent insertion followed a strict protocol (see Methods) which would limit this. Second, despite the randomization step, there were still imbalances between the groups with respect to oxygen-free days. However, as the Usual Care group had fewer oxygen-free days, this would have biased the study in favor of the HBOT intervention, which we did not see.

In conclusion, the study provided no support for the use of HBOT to reduce airway complications in lung transplant patients with established airways ischemia. Higher levels of *HMOX1* and *VEGFA* RNA expression at 4 weeks appeared to be associated with increasing airways complications, such as CAS and need for stenting, irrespective of whether receiving HBOT or not, and may be pharmacologic targets for airway-directed therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors thank the chamber operators, nurses, and physicians at the Duke Center for Hyperbaric Medicine and Environmental Physiology for assistance with this study, and thank the Duke Lung Transplant clinical coordinators and patient care providers.

Funding: Duke Pulmonary and Duke Hyperbaric Center Divisional funds, and NHLBI (K08HL130557, PI: Kraft).

FINANCIAL CONFLICTS OF INTEREST:

The study was funded by Duke Pulmonary Divisional funds and Duke Hyperbaric Center funds. Dr. Kraft reports additional funding from the NIH/NHLBI (K08HL130557). None of the funding sources had any role in data collection, analysis, or interpretation, or in approving or disapproving of the manuscript. The authors have no other conflicts of interest pertinent to the submitted work.

GLOSSARY

A1AT	Alpha-1-anti-trypsin

ACR	Acute cellular rejection
ANOVA	Analysis of Variance
ATA	Atmospheres absolute
BM	Bronchomalacia
BOLT	Bilateral orthotopic lung transplant
CAS	Central Airway Stenosis
cDNA	Complimentary DNA
CF	Cystic fibrosis
CLAD	Chronic lung allograft dysfunction
COPD	Chronic obstructive pulmonary disease
DCD	Donors after cardiac death
ECMO	Extracorporeal membrane oxygenation
EVLP	Ex vivo lung perfusion
FiO_2	Fraction of inspired oxygen
FLT1	Vascular Endothelial Growth Factor A Receptor 1
GS	Granulomatous stricture
GVHD	Graft-versus-host disease
HBOT	Hyperbaric oxygen therapy
HIF	Hypoxia-inducible factor
HMOX1	Heme oxygenase-1
HP	Hypersensitivity pneumonitis
IPF	Idiopathic pulmonary fibrosis
IQR	Interquartile range
KDR	Vascular Endothelial Growth Factor A Receptor 2
mRNA	Messenger ribonucleic acid
PCR	Polymerase chain reaction
PDS	Polydioxanone suture
PGD	Primary graft dysfunction
RNA	Ribonucleic acid

rRNA	Ribosomal ribonucleic acid
SOLT	Single orthotopic lung transplant
TGFB1	Transforming growth factor beta-1
TIE2	Angiopoietin Receptor 1
VEGFA	Vascular Endothelial Growth Factor A

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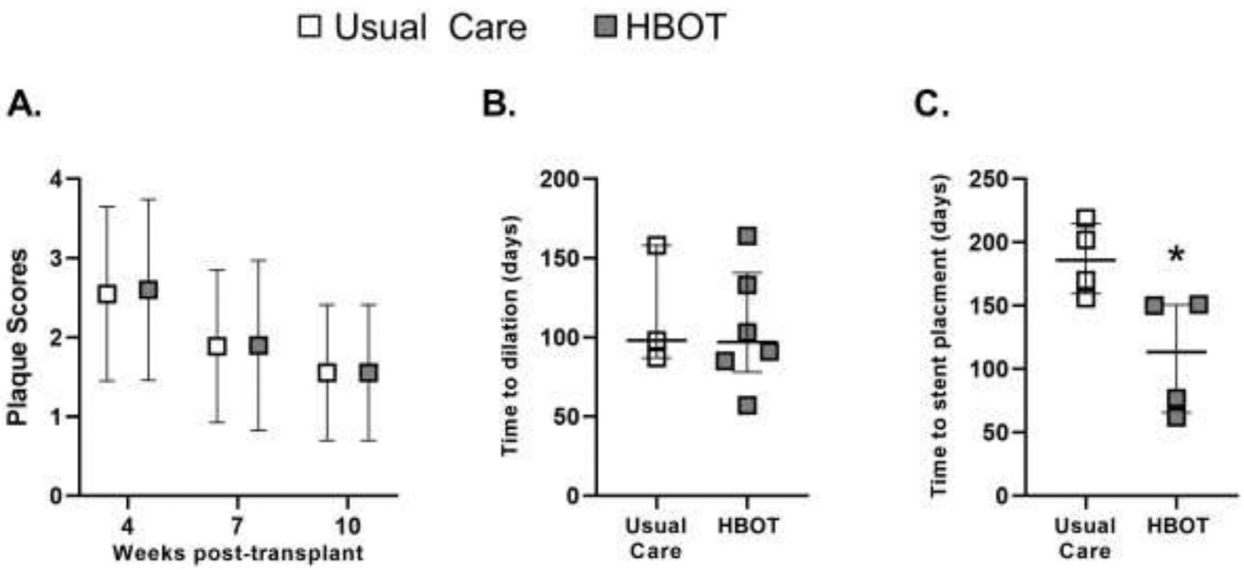

Figure 1: Clinical outcomes.

(A) Plaque scores (mean ± SD) over time for Usual care (open boxes) and HBOT (grey boxes) groups. (B) Time to first balloon airway dilation (median and IQR). (C) Time to first stent placement (median and IQR). *P<0.05 by Mann Whitney test.

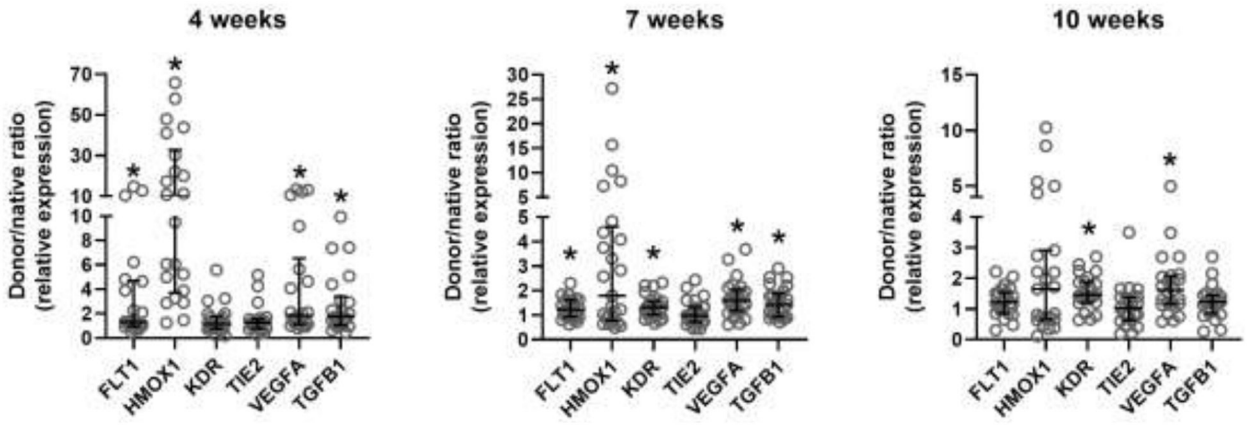

Figure 2: Donor/native tissue gene expression ratios.

Bronchial mucosal gene expression shown as donor/native ratio (relative to 18S mRNA) at 4, 7, and 10 weeks post-transplant. *P<0.05 by one sample Wilcoxon signed-rank test compared to hypothetical value of 1.

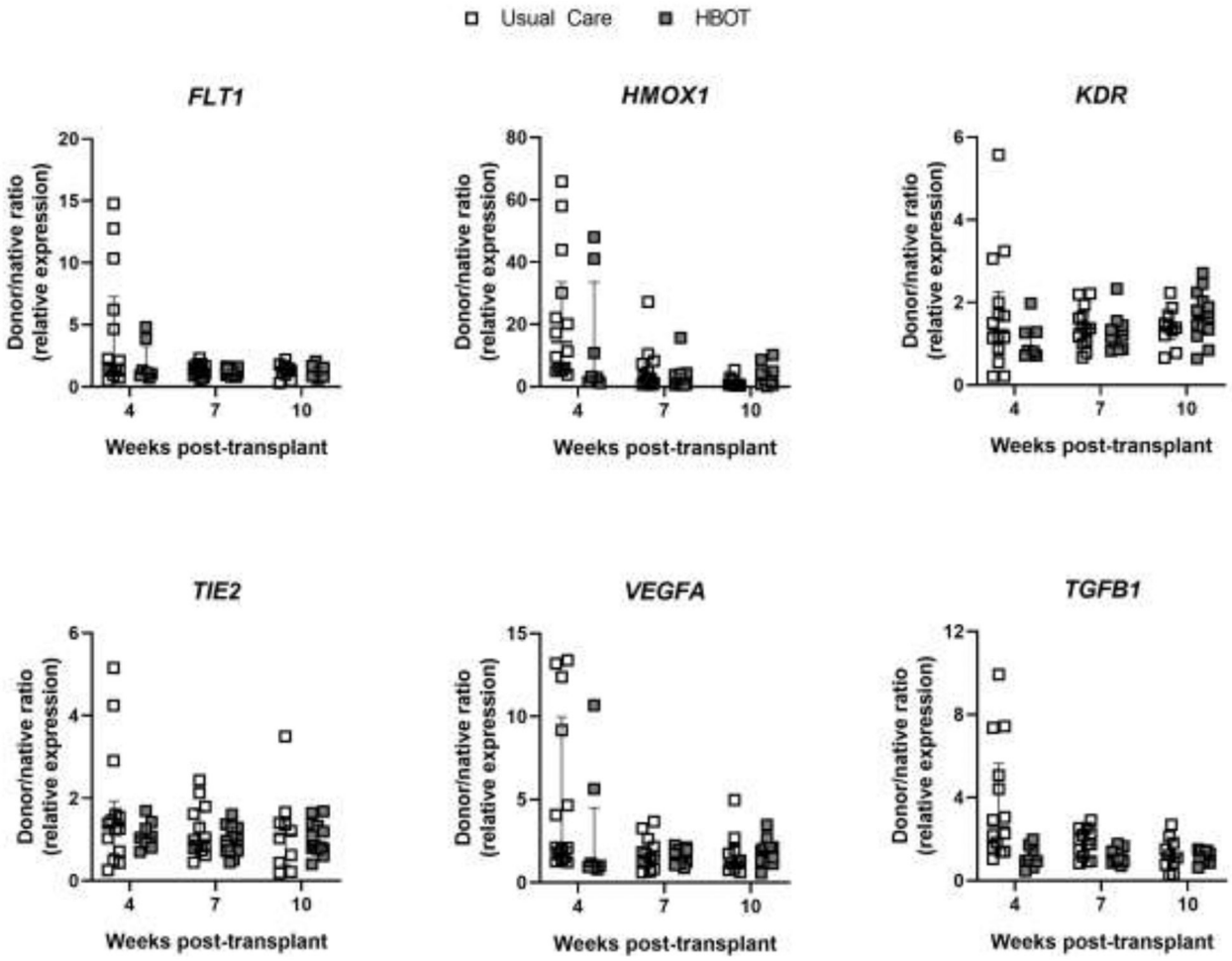

Figure 3: Gene expression of subjects by group assignment (Usual Care vs. HBOT).

Bronchial mucosal gene expression shown as donor/native ratio (relative to 18S mRNA) in Usual Care (open boxes) and HBOT (grey boxes) groups for *FLT1*, *HMOX1*, *KDR*, *TIE2*, *VEGFA*, and *TGFB1* at 4, 7, and 10 weeks post-transplant. Error bars are median with IQR.

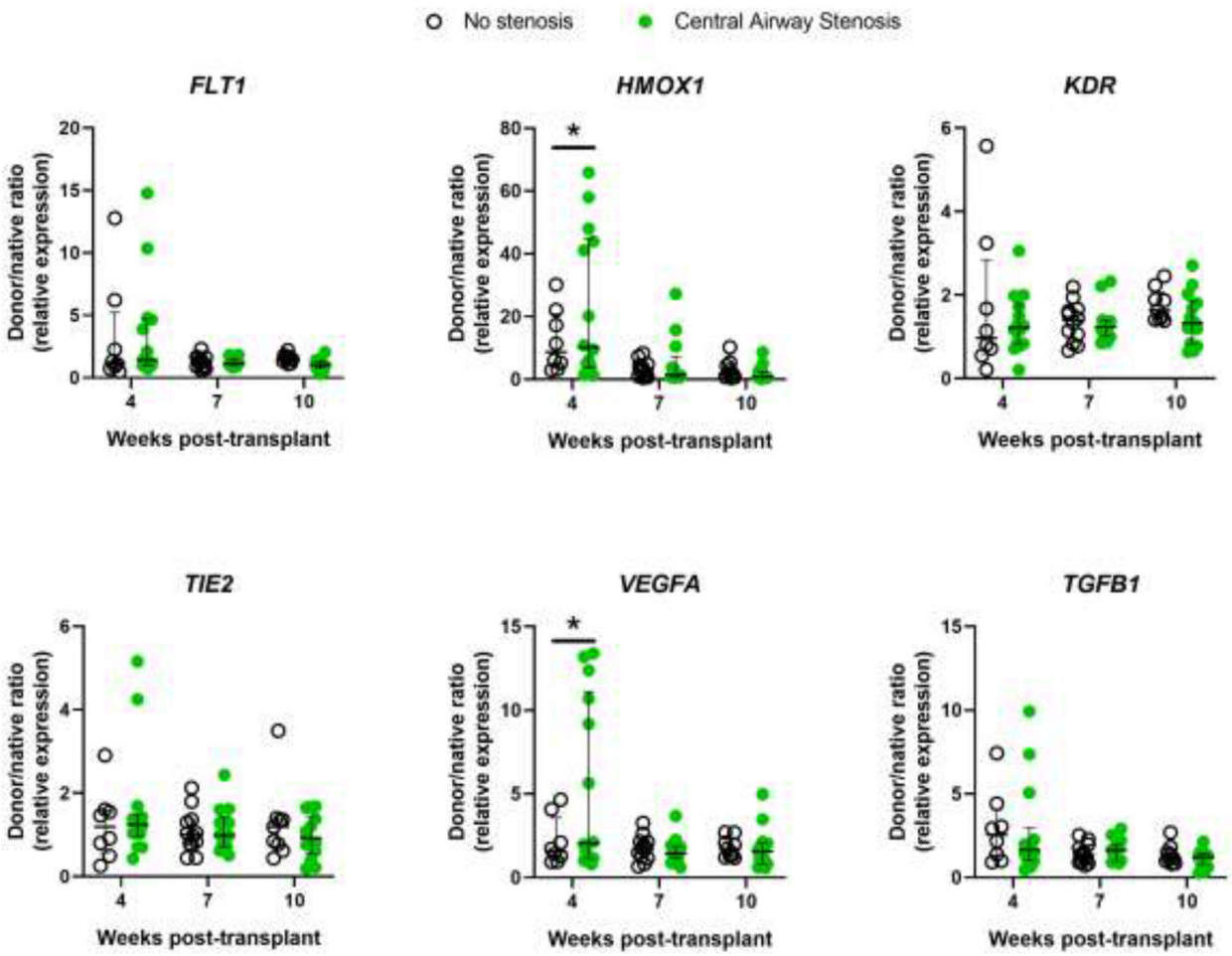

Figure 4: Gene expression of subjects by Central Airway Stenosis status.

Bronchial mucosal gene expression shown as donor/native ratio (relative to 18S mRNA) in subjects without central airway stenosis (open circles) and with central airway stenosis (green circles) for *FLT1*, *HMOX1*, *KDR*, *TIE2*, *VEGFA*, and *TGFB1* at 4, 7, and 10 weeks post-transplant. Error bars are median with IQR. *P<0.05 for group differences within each time point by 2-way ANOVA with the Benjamin-Hochberg post-hoc test.

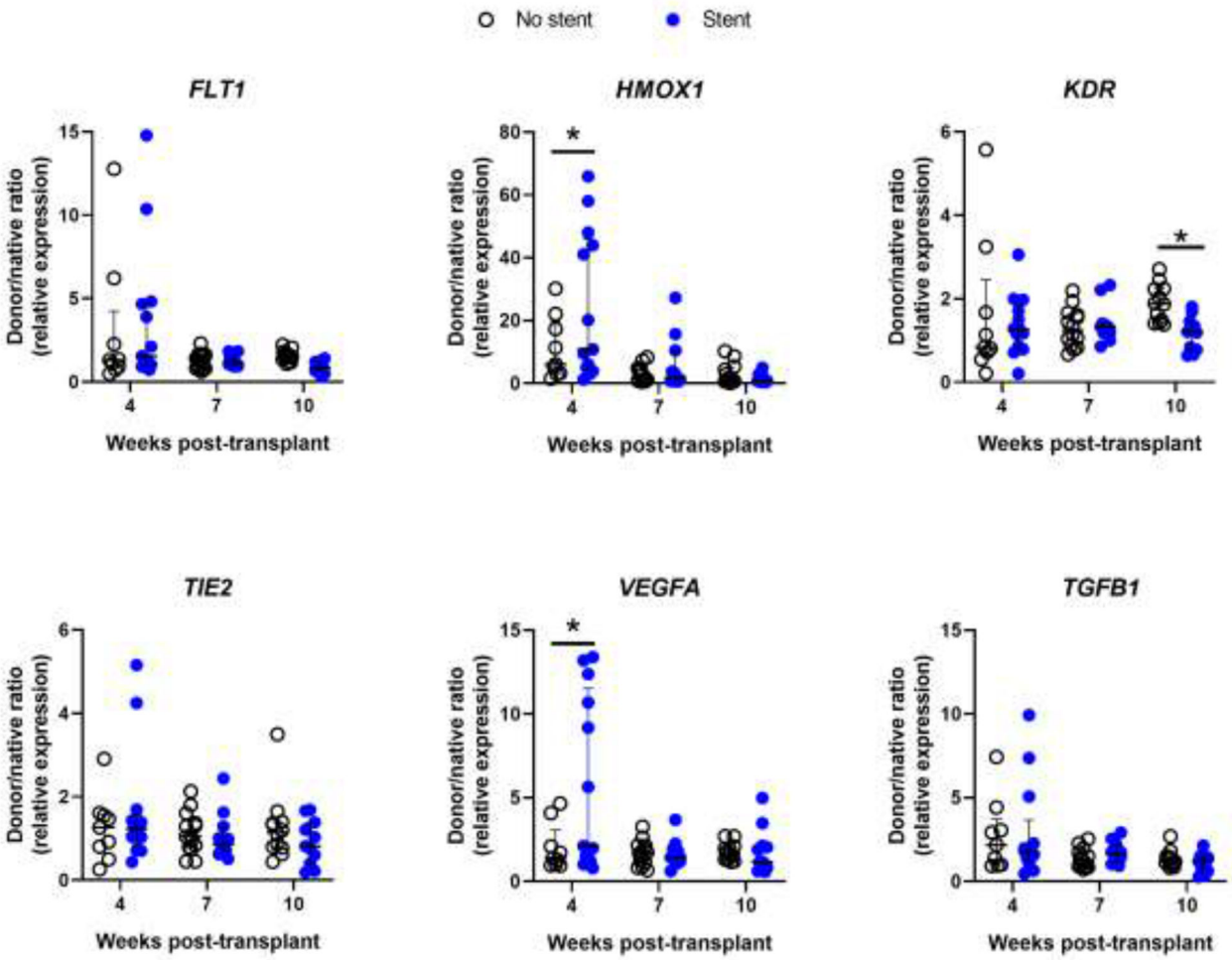

Figure 5: Gene expression of subjects by Airway Stent status.

Bronchial mucosal gene expression shown as donor/native ratio (relative to 18S mRNA) in subjects without airway stents (open circles) and with airway stents (blue circles) for *FLT1*, *HMOX1*, *KDR*, *TIE2*, *VEGFA*, and *TGFB1* at 4, 7, and 10 weeks post-transplant. Error bars are median with IQR. *P<0.05 for group differences within each time point by 2-way ANOVA with the Benjamin-Hochberg post-hoc test.

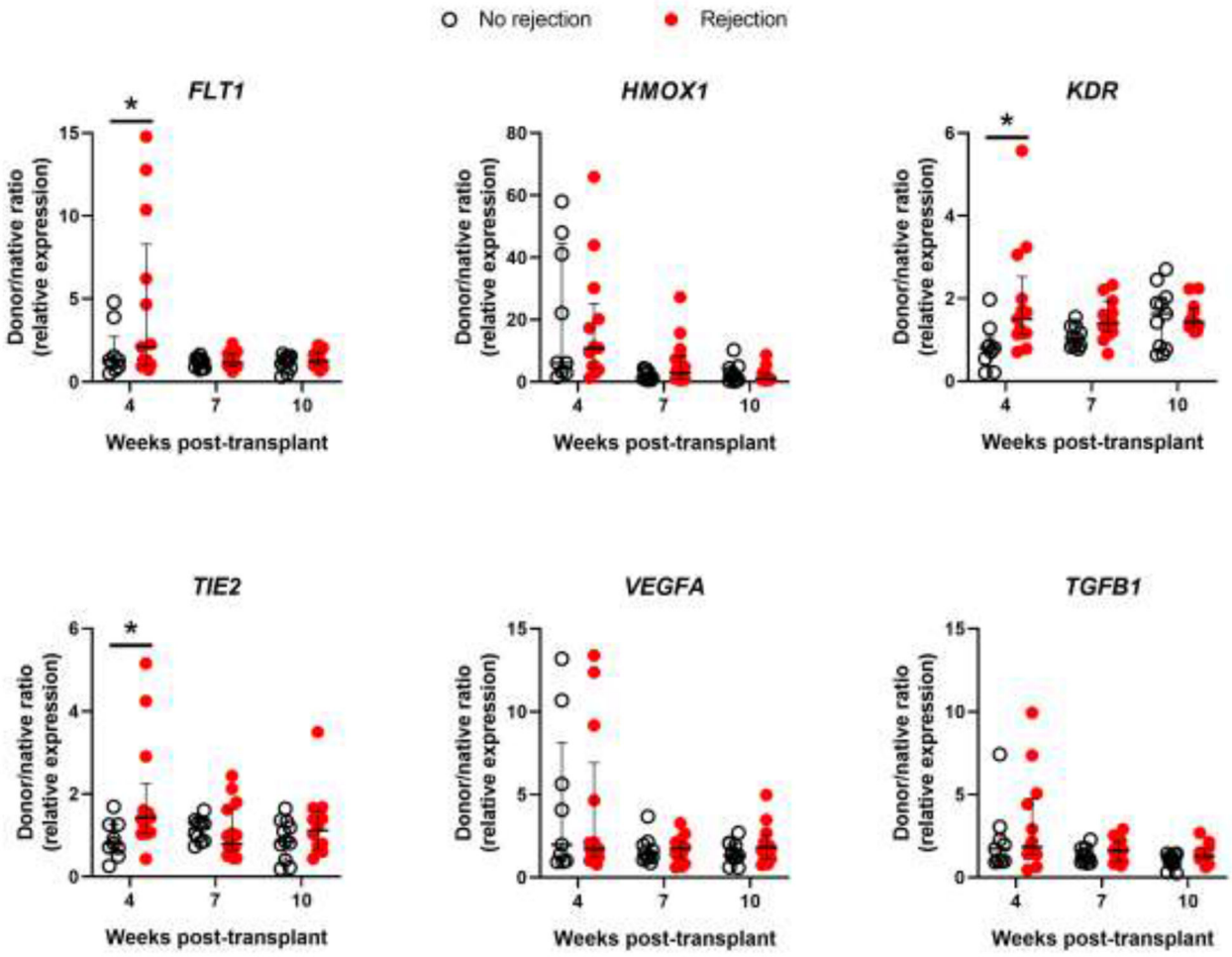

Figure 6: Gene expression of subjects by Acute Cellular Rejection status.

Bronchial mucosal gene expression shown as donor/native ratio (relative to 18S mRNA) in subjects without ACR (open circles) and with ACR (red circles) for *FLT1*, *HMOX1*, *KDR*, *TIE2*, *VEGFA*, and *TGFB1* at 4, 7, and 10 weeks post-transplant. Error bars are median with IQR. *P<0.05 for group differences within each time point by 2-way ANOVA with the Benjamin-Hochberg post-hoc test.

Table 1.

Patient Characteristics

Subject ID	Age	Sex	Lung Disease	Donor Lung	Weeks post-transplant	Group Assignment	Necrosis Score (Right/Left)
001	58	M	IPF	Bilateral	3.9	Usual care	3/2
002	67	M	IPF	Right	4.6	Hyperbaric oxygen	3/0
003	78	M	IPF	Right	2.6	Usual care	4/0
004	50	F	CF	Bilateral	4.0	Usual care	3/3
005	20	F	CF	Bilateral	3.3	*Screened, not enrolled*	2/1
006	31	M	CF	Bilateral	4.1	Usual care	2/1
007	19	M	CF	Bilateral	2.3	Usual care	3/3
008	60	M	A1AT deficiency	Bilateral	8.3	Hyperbaric oxygen	3/1
009	63	F	COPD	Bilateral	7.7	Usual care	3/3
010	66	F	IPF	Left	3.6	Usual care	0/4
011	37	F	CF	Bilateral	6.9	Hyperbaric oxygen	3/1
012	29	F	CF	Bilateral	5.1	Usual care	3/3
013	58	M	Chronic HP	Bilateral	4.7	Hyperbaric oxygen	4/3
014	73	M	IPF	Bilateral	4.3	Usual care	3/3
015	51	F	IPF	Bilateral	5.6	Hyperbaric oxygen	1/3
016	31	F	CF	Bilateral	7.3	Hyperbaric oxygen	3/1
017	49	F	Kartagener’s syndrome	Bilateral	4.9	Usual care	2/3
018	62	F	COPD	Bilateral	3.9	Hyperbaric oxygen	4/3
019	76	M	COPD	Bilateral	5.6	Hyperbaric oxygen	3/3
020	30	M	CF	Bilateral	4.6	Hyperbaric oxygen	4/3
021	30	M	GVHD/drug toxicity	Bilateral	3.9	*Screened, not enrolled*	4/4
022	60	M	IPF	Bilateral	4.1	Hyperbaric oxygen	3/3

Abbreviations: A1AT, Alpha-1-antitrypsin; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; F, female; GVHD, graft-versus-host-disease; HP, hypersensitivity pneumonitis; IPF, idiopathic pulmonary fibrosis; M, male.

Table 2.

Primary and Secondary Endpoints

	Co-Primary Endpoints		Secondary Endpoints						
Group Assignment	**Stent**	**ACR**	**GS**	**Dehiscence**[a]	**Infections**[b]	**BM**	**CAS**	**Dilation**	**CLAD**
Usual care, n (%)	4 (40)	7 (70)	1 (10)	1 (10)	3 (30%)	2 (20)	4 (40)	3 (30)	1 (10)
Hyperbaric oxygen, n (%)	4 (40)	4 (40)	0 (0)	1 (10)	5 (50%)	1 (10)	6 (60)	6 (60)	0 (0)

Abbreviations: ACR, acute cellular rejection; CAS, central airway stenosis due to fibrotic strictures; CLAD, chronic lung allograft dysfunction; GS, granulomatous strictures; BM, bronchomalacia.

[a]Includes full thickness mucosal breakdown.

[b]Airway infections at 10 weeks based on bronchoalveolar lavage fluid culture. See Table S3 for additional details on microbiology at all time points.