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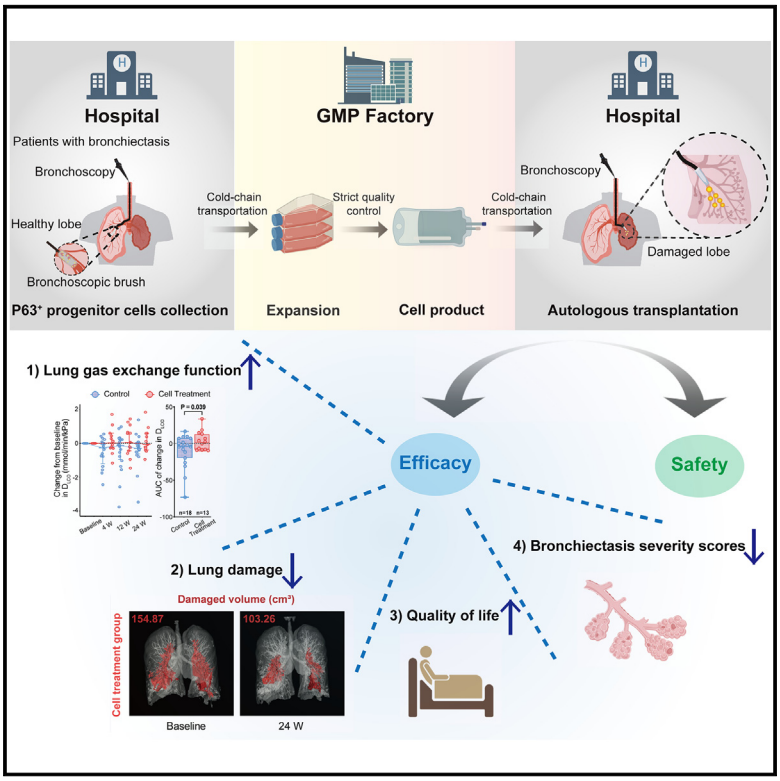
Autologous transplantation of P63^{+} lung progenitor cells in patients with bronchiectasis: A randomized, single-blind, controlled trial

Graphical abstract



Highlights

- Autologous P63^{+} progenitor cell transplantation is well tolerated
- Cell therapy improves lung function in patients with bronchiectasis
- Cell therapy shows reductions in lung damage and improvements in quality of life
- Higher P63 expression correlates with better therapeutic outcomes

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In brief

Yan et al. demonstrate that autologous P63^{+} progenitor cell transplantation enhances lung gas exchange function, quality of life, CT imaging outcomes, and bronchiectasis severity scores in patients with bronchiectasis. This study underscores the potential of regenerative cell therapy to promote lung repair, offering a promising strategy for the treatment of bronchiectasis.

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Autologous transplantation of P63$^+$ lung progenitor cells in patients with bronchiectasis: A randomized, single-blind, controlled trial

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SUMMARY

Non-cystic fibrosis bronchiectasis is a progressive respiratory disease with limited treatment options, prompting the exploration of regenerative therapies. This study investigates the safety and efficacy of autologous P63$^+$ progenitor cell transplantation in a randomized, single-blind, controlled, phase 1/2 trial. Thirty-seven patients receive bronchoscopic airway clearance (B-ACT) (n = 19) or B-ACT plus P63$^+$ progenitor cells (n = 18). Results show that compared to the control group, the change in D_{LCO} levels from baseline to 24 weeks post therapy is significantly higher in the cell treatment group (p value = 0.039). Furthermore, the patients in the cell treatment group demonstrate significantly reduced lung damaged area, improved SGRQ score, and ameliorated BSI and FACED scores within 4–12 weeks post therapy. Transcriptomic analysis reveals that progenitor cells with higher expression of P63 gene have better therapeutic efficacy. These findings suggest that P63$^+$ progenitor cells may offer a promising therapeutic approach for bronchiectasis. This study was registered at ClinicalTrials.gov (NCT03655808).

INTRODUCTION

Non-cystic fibrosis bronchiectasis (hereinafter referred to as "bronchiectasis") is a severe chronic respiratory disease characterized by permanent dilation of the airways, recurrent infection, persistent pulmonary epithelium damage, and inflammation.[1] The burden of bronchiectasis on patients is profound, with most suffering from daily symptoms of cough, sputum production, and intermittent exacerbations, ultimately leading to respiratory failure and diminished quality of life.[2] The global prevalence of bronchiectasis is increasing,[3,4] posing a significant health threat and economic burden to patients and society.[5,6] Despite various management strategies, including antibiotics, mucoactive agents, and bronchodilators, licensed treatments are lacking, and clinical interventions remain palliative, with limited evidence supporting their efficacy in repairing damaged lung tissue or restoring pulmonary function.[7–10] Consequently, there is an urgent need for regenerative therapies capable of repairing lung tissue damage and halting or reversing the progression of bronchiectasis.

The reparative processes of injured adult lung epithelium are mediated by the activation of various populations of lung-resident stem/progenitor cells, including P63$^+$ KRT5$^+$ basal progenitor cells located in the basal layer of the airway epithelium. However, the function of human P63$^+$ progenitor cells in lung regeneration remains controversial.[11–14] In recent decades, the function of human P63$^+$ progenitor cells in the lung regeneration process has remained a controversial issue. While some studies suggest their potential to regenerate bronchial and alveolar epithelium,[12–16] others indicate that P63$^+$ basal progenitors may contribute to persistent pathology, such as bronchiolization or dysplastic tissue formation.[17–19] Consequently, further investigation is warranted to determine the role of human P63$^+$ progenitor cells in lung repair, particularly in the context of bronchiectasis.

Previous studies showed that human P63$^+$ progenitor cells could be isolated from bronchoscopic brushed-off tissue from the patient's bronchi[20] and expanded in a feeder cell-based regenerative cloning culture (R-Clone) system. Transplantation

of human P63$^+$ progenitor cells into injured mouse lungs resulted in lung epithelial reconstitution and improved air exchange function.[21–23] More pre-clinical data in rodents and non-human primates also demonstrated the safety and feasibility of intrapulmonary P63$^+$ progenitor cell transplantation.[22,24] In an early pilot clinical trial performed in two patients with bronchiectasis, both patients have shown promising outcomes following autologous P63$^+$ progenitor cell transplantation, including significant improvements in pulmonary function and lung damage recovery.[21] In a recent phase 1 trial, autologous P63$^+$ progenitor cells were cloned and transplanted into patients with chronic obstructive pulmonary disease (COPD), resulting in statistically significant improvements in gas exchange function and walking distances.[24] These previous works demonstrated the feasibility of large-scale *in vitro* expansion and encouraged us to study the therapeutic potential of autologous P63$^+$ progenitor cells in patients with bronchiectasis.

RESULTS

Cloning P63$^+$ progenitor cells from patients with bronchiectasis

In healthy human lungs, the P63$^+$ KRT5$^+$ cells existed only in the airway epithelium. However, for those patients with severe lung diseases such as acute respiratory distress syndrome, idiopathic pulmonary fibrosis, and COPD, it is observed that the P63$^+$ KRT5$^+$ cells would appear in alveolar spaces, suggesting their possible participation of lung repair or regeneration process.[12,14,21,25] However, for patients with bronchiectasis with recurrent bacterial infection, it remains unclear whether the P63$^+$ progenitor cells mediated a similar process. In this study, we collected pulmonary tissues from 5 patients with bronchiectasis through surgical excision and performed immunostaining to examine the P63$^+$ KRT5$^+$ cell distribution in the lung. The result showed that in patient #1, all KRT5$^+$ cells were lined in the airway epithelium and none of them were found in alveolar spaces. In patient #2, the KRT5$^+$ cells were found in the alveolar spaces, forming a typical "bronchiolization" structure characterized by multi-layered cuboidal or columnar cells.[26,27] Interestingly, in patient #3, #4, and #5, we found that some of the KRT5$^+$ cells exhibited single-layered sphere morphology, forming air sac-like structures (Figure 1A). Interestingly, we noticed that all patient lungs except patient #1 were characterized by interstitial fibrosis. We also noticed that patient #3, #4, and #5 were all females with $\leq$2 years disease duration period and no bacterial growth in sputum culture, while patient #1 and #2 were males with $\geq$12 years disease duration period and detectable bacterial growth in sputum culture (Table S1). Immunostaining of consecutive pathological sections showed that the KRT5$^+$ air sac-like areas also expressed type I alveolar cell gene AQP5 and endothelial cell gene CD31 (Figure S1A). Altogether, these observations suggested that the P63$^+$ KRT5$^+$ progenitor cells might have alveolar repair function in the lungs of some patients with bronchiectasis, probably in those patients with recent disease onset and no active infection in the lung.

In order to further study the repair function of P63$^+$ progenitor cells in human, the cells were cloned from bronchiectasis patient airway and expanded similarly as previously reported.[24] Briefly, the process involved collecting tiny brush sample tissues from the 3rd–5th order bronchi of patients with bronchiectasis through bronchoscopy.[28] For tissue collection, the healthiest lung lobe was chosen based on high-resolution computed tomography (HRCT) imaging, and the relatively healthy airway inner surface was selected under bronchoscopic imaging. The collected tissues were then digested by recombinant enzyme and grown on the regenerative cell clone (R-Clone) culture system, which selectively promotes the growth and expansion of progenitor cells. To prevent microbial contamination, gentamicin sulfate at a concentration of 200 μg/mL was applied at the primary passage, but not in the following 2–5 passages. The cell products were subjected to standard quality assays to assess items including cell number, cell morphology, visible particles, pH value, osmotic pressure, sterility, mycoplasma, endotoxin, viral contamination, bovine serum albumin (BSA) level, feeder cell remains, and antibiotic remains (Figure 1B). The expression of representative progenitor cell markers, KRT5 and P63, was confirmed through immunofluorescent staining of the cell colonies (Figure 1C). The cells at the last passage were analyzed by flow cytometry, which showed >99% cells were KRT5$^+$/CD45$^-$/CD105$^-$/CD34$^-$ (Figures 1D and S1B). The non-tumorigenic potential of cells was confirmed by soft-agar colony formation assay (Figure S1C).

At the last passage, cells were cultured in feeder-free condition until they reached 85%–100% confluency. The cells were harvested using xeno-free TrypLE and suspended in 30 mL saline as the final product, which was sealed in a cell preservation bag and shipped as fresh cells to Ruijin Hospital by cold-chain transport (2°C–8°C) within 48 h. For transplantation, the cell suspension was warmed to room temperature and evenly distributed into the 6 pulmonary segments with the most severe lesions according to computed tomography (CT) results, using bronchoscopy with 5 mL for each segment.

Study population and baseline characteristics

In order to study whether the autologous P63$^+$ progenitor cells could repair the bronchiectasis lung, we conducted a single-blind, randomized, controlled clinical trial (NCT03655808) between June 2020 to May 2023, to investigate the effect of P63$^+$ progenitor cells on parenchyma repair in patients with bronchiectasis, which were diagnosed according to 2019 British Thoracic Society (BTS) guidelines.[29] Only patients with the diffusing capacity of the lungs for carbon monoxide (D_{LCO}) <80% of the predicted value were included in the study. Table S2 provides detailed patient inclusion and exclusion criteria.

Overall, we enrolled 37 patients with bronchiectasis in this study and randomly assigned them to the control or cell treatment group. Among them, 18 patients were assigned to the cell treatment group and 19 patients to the control group. Both the patients and the investigators, except for the bronchoscopy operators, remained masked to the group allocation for the duration of the study. Two patients, one in the cell treatment group and one in the control group, withdrew their previously written informed consent after randomization and did not receive treatment. Eventually, 18 and 17 patients were treated with bronchoscopic airway clearance treatment (B-ACT) or B-ACT plus

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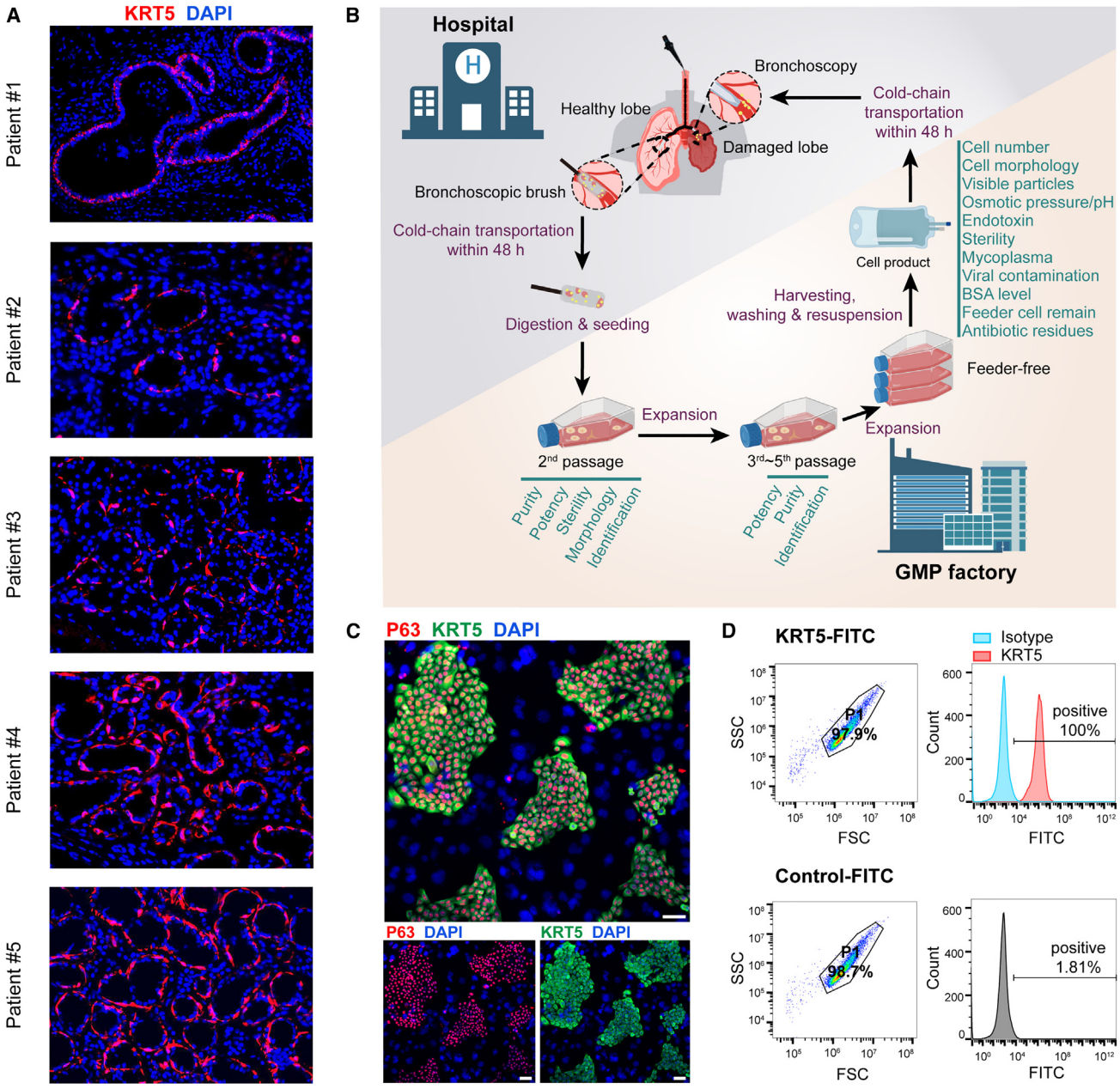

Figure 1. Characterization and cloning of P63$^+$ progenitor cells from patients with bronchiectasis

(A) Immunofluorescence staining showing KRT5$^+$ (red) cells in lung sections from different patients with bronchiectasis (n = 5). Nuclei were counterstained with DAPI (blue). Scale bar, 50 μm.

(B) A schematic diagram illustrating the manufacture, quality control, and clinical administration procedure of autologous P63$^+$ progenitor cell products.

(C) Cultured progenitor cell clones were immunostained with KRT5 and P63 markers. Scale bar, 100 μm.

(D) Fluorescence-activated cell sorting (FACS) gating strategy for cell identity and purity test. KRT5 was immunostained as a marker of progenitor cells. SSC, side-scatter; FSC, forward scatter.

autologous P63$^+$ progenitor cells transplantation, respectively (Figure S2). Other standard-of-care treatments were continued as well in both groups. The demographic and clinical characteristics of patients in both groups at baseline showed no statistically significant difference (Table 1). However, it is noted that the patients in the cell treatment group had non-significant lower diffusion capacity as measured by D_{LCO} level (p value = 0.191) and non-significant higher Bronchiectasis Severity Index (BSI) (p value = 0.242) at baseline. For B-ACT, 120–200 mL saline was instilled into the patient's lung followed by continuous suction to remove secretions in the respiratory tract. Patients in the cell treatment group were transplanted with 1–3 × 10^6

Table 1. Baseline demographic and clinical characteristics

Demographics	Cell treatment group (n = 17)	Control group (n = 18)	Total (n = 35)	p value
Age (years)[a]	53.7 ± 13.5	50.4 ± 14.1	52.0 ± 13.7	0.499
Female gender[b]	9 (52.9%)	11 (61.1%)	20 (57.1%)	0.738
Body mass index (kg/m^2)[a]	20.1 ± 3.5	19.8 ± 4.0	20.0 ± 3.7	0.832
Smokers[b]	3 (17.6%)	1 (5.6%)	4 (11.4%)	0.338
Bronchiectasis characteristics				
Duration of disease (years)[c]	16.0 (5.0, 31.0)	10.0 (6.5, 30.0)	12.0 (5.0, 30.0)	0.858
FEV1% predicted[a]	44.2 ± 18.3	49.1 ± 19.2	46.7 ± 18.7	0.442
D_{LCO}% predicted[a]	54.5 ± 18.6	62.4 ± 16.1	58.8 ± 17.6	0.191
BSI score[a]	10.9 ± 3.9	9.3 ± 4.1	10.1 ± 4.0	0.242
SGRQ score[a]	52.9 ± 20.3	44.1 ± 21.8	48.4 ± 21.2	0.225
Exacerbations in the past year[c]	1.0 (1.0, 1.5)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	0.386
Radiography involved lung lobes[c]	5.0 (4.0, 5.0)	4.0 (2.0, 5.0)	5.0 (3.0, 5.0)	0.207
Etiology				
Post TB infection[b]	2 (11.8%)	2 (11.1%)	4 (11.4%)	0.942
Post non-TB infection[b]	6 (35.3%)	8 (44.4%)	14 (40.0%)	
Idiopathic[b]	8 (47.1%)	7 (38.9%)	15 (42.9%)	
Other[b]	1 (5.9%)	1 (5.6%)	2 (5.7%)	
Comorbidities				
COPD[b]	4 (23.5%)	4 (22.2%)	8 (22.9%)	>0.999
Asthma[b]	1 (5.9%)	0 (0)	1 (2.9%)	0.486
Chronic rhinitis or sinusitis[b]	6 (35.3%)	4 (22.2%)	10 (28.6%)	0.471
Quality sputum culture				
Pseudomonas aeruginosa[b]	6 (35.3%)	4 (22.2%)	10 (28.6%)	0.471
Other[b]	2 (11.8%)	1 (5.6%)	3 (8.6%)	0.603
No bacterial growth[b]	9 (52.9%)	13 (72.2%)	22 (62.9%)	0.305
Medication for bronchiectasis				
Oral antibiotics[b]	11 (64.7%)	9 (50.0%)	20 (57.1%)	0.500
Oral corticosteroid[b]	2 (11.8%)	0 (0)	2 (5.7%)	0.229
Inhaled corticosteroid[b]	3 (17.6%)	3 (16.7%)	6 (17.1%)	>0.999
Inhaled bronchodilator[b]	10 (58.8%)	12 (66.7%)	22 (62.9%)	0.733
Mucolytics[b]	11 (64.7%)	16 (88.9%)	27 (77.1%)	0.121

[a]Data were presented as mean ± standard deviation (SD).
[b]Data were presented as patient number (percentage of patients).
[c]Data were presented as median (interquartile range, IQR).

autologous P63$^+$ progenitor cells per kilogram body weight (Table S3) through bronchoscopy. Patients were followed up at 4, 12, and 24 weeks post cell transplantation for safety and efficacy outcome analysis.

Safety analysis

Adverse events occurred in 82.4% of patients in the cell treatment group and 83.3% of those in the control group (p value >0.999) (Table 2). The most common adverse events were fever (37.1%), hemoptysis (i.e., coughing up bloody sputum; 25.7%), and increased sputum (20.0%). Grade 1 adverse events occurred in 9 (52.9%) patients in the cell treatment group and 14 (77.8%) patients in the control group. Grade 2 adverse events occurred in 8 (47.1%) patients in the cell treatment group and 8 (44.4%) patients in the control group. Two grade 3 serious adverse events (SAEs) occurred in 2 patients in the cell treatment group: one was pneumothorax and the other one was acute exacerbation of COPD with type 2 respiratory failure. Both patients were hospitalized and recovered after standard treatment. Among all these adverse events, 25 out of 66 events (37.88%) were considered related to bronchoscopic surgery, with 17 grade 1 events, 7 grade 2 events, and 1 grade 3 event (pneumothorax) (Table S4). Other 41 recorded adverse events (62.12%) were considered unlikely to be related to bronchoscopic procedure or cell transplantation, with 23 grade 1 events, 17 grade 2 events, and 1 grade 3 event, as judged by the investigators (Table S5). No grade 4 or 5 adverse events were recorded. There was no relationship between cell doses and the frequency of adverse events (correlation coefficient = −0.14; p value = 0.590). Additionally, key laboratory indexes, including white blood cells, neutrophil to lymphocyte ratio, alanine aminotransferase, aspartate transaminase, creatinine, and creatine kinase,

Table 2. Incidence of adverse events

Events	Cell treatment group (n = 17)	Control group (n = 18)	Total (n = 35)	p value
Any adverse event[a]	14 (82.4%)	15 (83.3%)	29 (82.9%)	>0.999
Fever[a]	8 (47.1%)	5 (27.8%)	13 (37.1%)	0.305
Hemoptysis[a,b]	3 (17.6%)	6 (33.3%)	9 (25.7%)	0.443
Sputum increased[a]	4 (23.5%)	3 (16.7%)	7 (20.0%)	0.691
Cough increased[a]	3 (17.6%)	3 (16.7%)	6 (17.1%)	>0.999
Fatigue[a]	3 (17.6%)	3 (16.7%)	6 (17.1%)	>0.999
COVID-19[a]	2 (11.8%)	4 (22.2%)	6 (17.1%)	0.658
Bronchiectasis exacerbation[a]	2 (11.8%)	2 (11.1%)	4 (11.4%)	>0.999
Pharyngeal discomfort[a]	1 (5.9%)	3 (16.7%)	4 (11.4%)	0.603
Other[a,c]	4 (23.5%)	5 (27.8%)	9 (25.7%)	>0.999
Serious adverse events[a,d]	2 (11.8%)	0 (0)	2 (5.7%)	0.229

[a]Data were presented as patient number (percentage of patients).
[b]The term "hemoptysis" included bloody sputum in this study.
[c]Other adverse events included chest discomfort, dizziness, dyspnea, nausea, influenza, and anxiety.
[d]Serious adverse events occurred in 2 patients, one for pneumothorax and another for acute exacerbation of COPD.

remained stable in both two groups after treatment (Figure S3). No death or tumor formation was reported in this trial and we are continuing life-long observation on the patients who received the cell therapy. Altogether, these data indicated that autologous $P63^+$ progenitor cell transplantation therapy had an acceptable safety profile among patients with bronchiectasis.

Primary efficacy outcomes

The primary efficacy outcome of the current study is the change of D_{LCO} after cell treatment. D_{LCO} is a measurement of the gas transfer capacity of lung. Unlike the typically analyzed forced expiratory volume in 1 s (FEV_1) parameter, which measures the airflow capacity, D_{LCO} represents the air exchange aspect of lung function that is quantitatively determined by the effective alveolar-capillary surface area. In chronic respiratory diseases including bronchiectasis, reduced gas transfer capacity was independently associated with higher mortality and lower quality of life.[30–33] In this clinical study, we planned to use the change of D_{LCO} as the primary efficacy outcome to evaluate the therapeutic effect. The data showed that the median change from baseline of D_{LCO} level in the cell treatment group was better than the control group at all follow-up time points. We then calculated the area under the curve (AUC) of D_{LCO} to quantify the overall change of D_{LCO} from baseline from 4 weeks to 24 weeks. It was observed that the patients in the cell treatment group exhibited significantly larger cumulative change compared to the control group (mean ± standard deviation [SD], 4.06 ± 13.14 vs. −9.84 ± 22.46; 95% confidence interval [CI], 0.73 to 27.06; p value = 0.039) (Figure 2A).

Similarly, we also analyzed the D_{LCO}% of predicted value. The data showed that the median change from baseline of D_{LCO}% of predicted value in the cell treatment group was also better than the control group at all follow-up time points. We then calculated the AUC of D_{LCO}% predicted to quantify the overall change of D_{LCO} from baseline from 4 to 24 weeks. It was observed that the patients in the cell treatment group exhibited significantly larger cumulative change compared to the control group in D_{LCO}% predicted (mean ± SD, 43.47 ± 153.16 vs. −118.37 ± 264.64; 95% CI, 7.23 to 316.44; p value = 0.041) (Figure 2B).

For chronic lung diseases, the minimum clinically important difference for D_{LCO} was 10%–11% of baseline D_{LCO}.[31,34] Therefore, in our analysis, we also calculated the number of patients with >10% D_{LCO} change. There were 30.8% of patients in the cell treatment group who had more than a 10% increase of baseline D_{LCO}% predicted at week 4, while none of the participants in the control group had >10% increase. There were only 15.4% of patients who had more than a 10% decrease of baseline D_{LCO}% predicted at week 4 in the cell treatment group, as compared with 50.0% of patients in the control group. The difference between groups was statistically significant (p value = 0.017) (Figure 2C and Table S6). A similar tendency was also observed at week 12 and week 24, although the difference was not statistically significant. Further subgroup analysis indicated that compared to the control group, the improvement of D_{LCO} and D_{LCO}% of predicted in the cell treatment group was consistent across most of the subgroups, except it was more pronounced in patients complicated with COPD (Figures S4 and S5). Altogether, the data indicated that in some of the patients with bronchiectasis, autologous $P63^+$ progenitor cell transplantation could significantly improve the gas exchange capacity of lung.

Secondary efficacy outcomes

One secondary efficacy outcome of the current study is the change of the St George's Respiratory Questionnaire (SGRQ) score. SGRQ is used to assess the quality of life in patients with chronic respiratory diseases, and for the SGRQ score, a four-unit change has been proposed as clinically relevant.[35,36] At week 4, we observed that the proportion of patients with an improvement exceeding four units was 76.9% in the cell treatment group and 41.2% in the control group, which demonstrated a statistically significant difference (p value = 0.049) (Figure 2D and Table S6). A similar tendency was also observed at week 12 but not week 24. We also used two different multidimensional

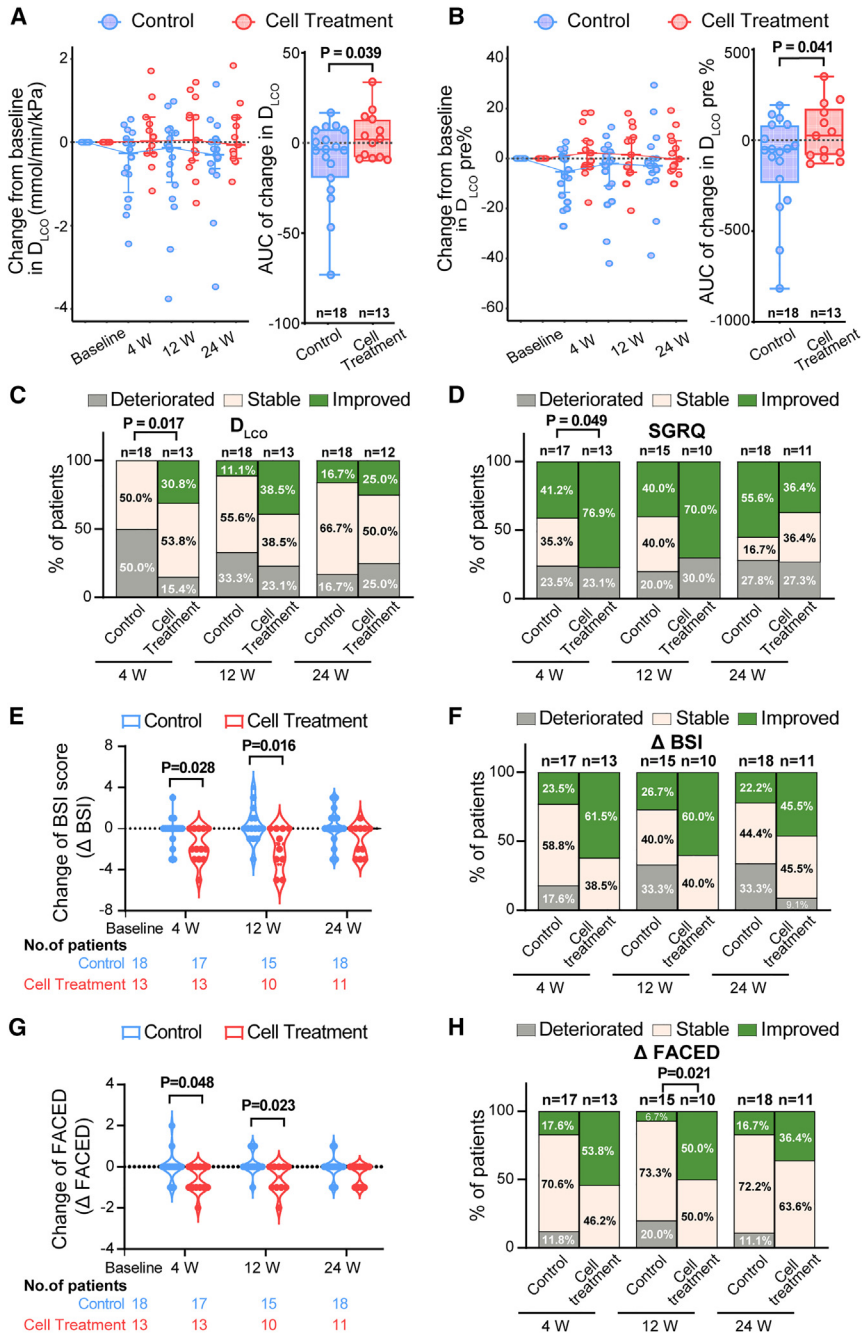

Figure 2. Changes of D_{LCO}, SGRQ, BSI score, and FACED score at different time points after cell treatment

(A) Left, changes of median D_{LCO} in both groups at week 4, 12, and 24. Data are represented as median (interquartile range, IQR). Right, boxplot showing the AUC of the D_{LCO} change from baseline to 24 weeks in both groups. Each dot indicated an individual patient.

(B) Left, changes of median D_{LCO}% of predicted in both groups at week 4, 12, and 24. Data are represented as median (IQR). Right, boxplot showing the AUC of the D_{LCO}% change from baseline to 24 weeks in both groups. Each dot indicated an individual patient.

(C) Column charts represent the proportion of patients who demonstrated >10% changes (improved) or < −10% changes (deteriorated) of D_{LCO} level at week 4, 12, and 24 after therapy.

(D) Column charts represent the proportion of patients who had >4 units changes (deteriorated) or < −4 units changes (improved) of SGRQ score at week 4, 12, and 24 after cell treatment.

(E) Violin plot showing changes in BSI score in both groups at week 4, 12, and 24.

(F) Column charts represent the proportion of patients whose BSI score improved or deteriorated for ≥1 unit at week 4, 12, and 24 after cell treatment.

(G) Violin plot showing changes in FACED score in both groups at week 4, 12, and 24.

(H) Column charts represent the proportion of patients whose FACED score improved or deteriorated ≥1 unit at week 4, 12, and 24 after cell treatment.

grading systems to assess the severity of bronchiectasis before and after cell treatment: the BSI and FACED scores.[37,38] Both scores could predict the exacerbation risk, hospitalization, and mortality of patients with bronchiectasis.[39] The data showed that the cell treatment group demonstrated a significant decline in both BSI score and FACED score compared to the control group (Figures 2E–2H and S6–S8, Tables S7 and S8). Altogether, our results indicated that the autologous P63^{+} progenitor cell transplantation could improve quality of life and decrease the severity of bronchiectasis during 4–12 weeks post cell transplantation, while at 24 weeks post cell transplantation, the beneficial effect was no longer obvious.

We also analyzed the HRCT data of patients before and after cell treatment. As the morphology of bronchiectasis and mucus plugs assessed by experts in a blind manner showed no obvious difference between the two groups, we used computational image processing software for in-depth analysis. Three-dimensional (3D) visualization of consecutive CT images by 3D Slicer could measure the damaged area, including bronchial dilation and inflammatory lesions. Figure 3A showed a representative 3D lung visualization image of patient #9003 from the control group, illustrating the increase in lung damage area (Figure 3B). In contrast, Figure 3C showed a representative 3D lung visualization image of patient #9018 from the cell therapy group, demonstrating that the lung damage area was largely decreased following P63^{+} progenitor cell transplantation (Figure 3D). Comparing to the control group, the patients in the cell treatment group demonstrated a statistically significant decrease in the damaged lung area over the course of 24 weeks (Figures 3B and 3D). Furthermore, we observed a significant

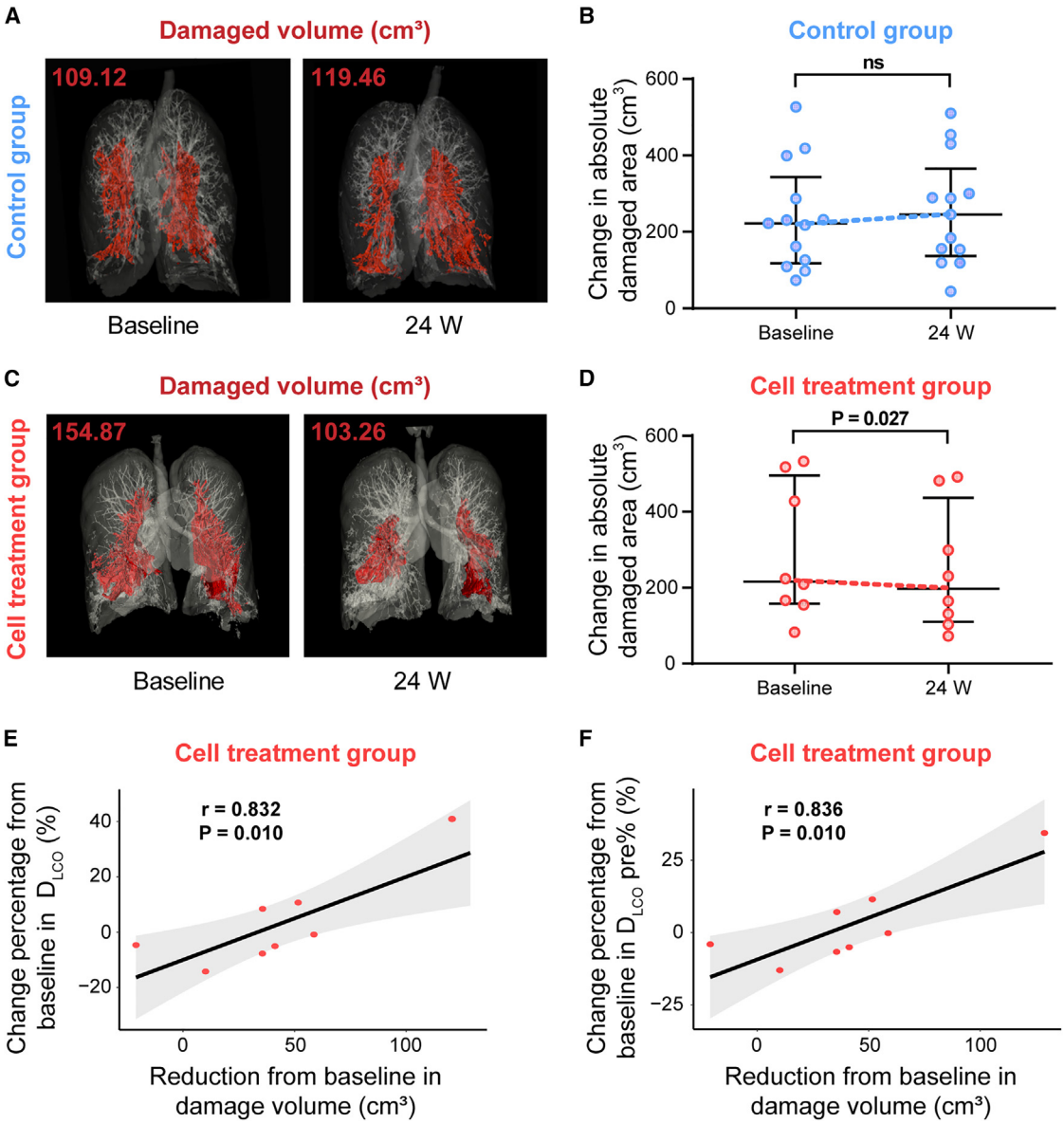

Figure 3. Changes in CT damaged areas at week 24 after cell treatment

(A) The lung of patient #9003 in the control group was scanned by HRCT and 3D visualized. The red zone indicated the lung damaged area.

(B) Changes in the absolute damaged area using HRCT 3D visualization and quantification analysis in the control group. Each dot represented an individual patient. Data are represented as mean ± standard error of the mean (SEM). The paired Student's t test was performed.

(C) The lung of patient #9018 in the cell treatment group was scanned by HRCT and 3D visualized. The red zone indicated the lung damaged area.

(D) Changes in the absolute damaged area using HRCT 3D visualization and quantification analysis in the cell treatment group. Each dot represented an individual patient. Data are represented as mean ± SEM. The paired Student's t test was performed.

(E) Pearson correlations between the change from baseline in the damaged volume and the D_{LCO} in the cell treatment group. Each dot represented an individual patient.

(F) Pearson correlations between the change from baseline in the damaged volume and the D_{LCO}% of predicted in the cell treatment group. Each dot represented an individual patient. Reduction from baseline = −1 × (change from baseline).

association between the change from baseline to 24 weeks in the damaged volume and the D_{LCO} in the cell treatment group (correlation coefficient = 0.832; *p* value = 0.010) (Figures 3E and S9A). Similarly, Pearson correlation analysis illustrated that the improvement of the damaged volume was also associated with the D_{LCO}% of predicted in the cell treatment group (correlation coefficient = 0.836; *p* value = 0.010) (Figures 3F and S9B). These results were consistent with our findings that autologous $P63^{+}$ progenitor cells transplantation could improve the gas exchange capacity in patients with bronchiectasis.

Furthermore, we observed significant improvement in alveolar ventilation and total lung capacity exclusively at week 4 (Table S7). In addition, we also observed that the increase of inspiratory capacity was larger in the cell treatment group than in the control group exclusively at week 24 (Table S7). Other efficacy outcomes including FEV_1, forced vital capacity (FVC), FEV_1/FVC, maximum mid expiratory flow, maximum voluntary ventilation, 6-min walking distance, and distance-saturation product were similar between two groups throughout the 24-week period after treatment (Table S7 and Figure S10).

Transcriptomic analysis of patient cells

We were particularly interested in understanding why some patients respond to the cell therapy better than others and hoping to identify factors that may determine treatment outcomes. Pearson correlation analysis illustrated that the improvement of the D_{LCO} level over the 24-week period was not associated with the dose of cells transplanted to patients in the cell treatment group (correlation coefficient = −0.18; p value = 0.616). Then we asked whether the difference in gene expression profiles of patients' progenitor cells might contribute to the difference in treatment efficacy. Therefore, we analyzed $P63^+$ progenitor cells isolated from 7 patients in the cell treatment group. Among them, 4 patients demonstrated a "complete response" to autologous cell transplant therapy, defined as patients with >10% D_{LCO} change from baseline level as well as improvement of SGRQ and mMRC (complete responsive [CR]-patient #9001, #9007, #9013, and #9018). The other 3 patients demonstrated "no response" to treatment (non-responsive [NR]-patient #9021, #9027, and #9035), whose D_{LCO} change is within ±10% of baseline level. We performed whole-genome RNA sequencing to analyze the transcriptome of progenitor cells from these 7 patients. Unsupervised principal component analysis of the whole-transcriptome data showed that four cell lines from CR patients showed a tendency to separate from three cell lines from NR patients (Figure 4A). These data suggested that the differences in overall gene expression profiles might be related to different treatment outcomes, as CR and NR patients were similar in terms of demographics, disease severity, comorbidities, lung function, and medication at baseline (Table S9).

Further studies showed that both groups expressed similar levels of the progenitor genes KRT5, SOX9, and SOX2 and cell proliferation markers KI67 and TOP2A (Figure 4B). However, progenitor cells from NR patients highly expressed 78 genes, and many of their functions were related to inflammation and virus, such as HLA-G and IRF9 (Figure 4C).[40] In contrast, progenitor cells from CR patients maintained higher expression level of the key transcriptional factor P63. Additionally, CR cells highly expressed other 67 genes, and many of their functions were related to lung development, such as FGF10, JAG1, and EREG (Figure 4D).[41–43] Gene ontology (GO) analysis indicated that the CR cells were enriched in genes related to wound healing, regeneration, and lung morphogenesis (Figures 4E and S11A). In contrast, GO analysis showed that NR cells highly expressed genes related to virus and interferon responses (Figures 4F and S11B). Protein-protein interaction network analysis showed that in CR patients, the proteins related to stem cell pathways frequently interacted with proteins involved in wound healing and responding to oxygen levels (Figure 4G). In contrast, in NR cells, proteins involved in type I interferon response interacted with proteins involved in innate immunity and virus resistance at high frequencies (Figure 4H), and we speculated that the NR cells might have been modified in patient lungs to fulfill the pathogen clearance function, instead of the normal tissue repair function. Overall, these data suggested that the P63-high normal progenitor cells might have a better therapeutic effect than the P63-low variant progenitor cells. These results were consistent with previous findings in patients with COPD[24] and indicated that the treatment of bronchiectasis needed to move toward an endophenotypic precision medicine approach.[44]

DISCUSSION

The clinical course of non-CF bronchiectasis is characterized by intermittent exacerbations and irreversible deterioration, which may progress to respiratory failure and even mortality. Current standard treatments, including antibiotics, mucoactive agents, bronchodilators, corticosteroids, and airway clearance therapy, offer only symptomatic relief and do not address the underlying structural lung damage. Thus, there is an urgent need for stem/progenitor cell treatment options aimed at lung regeneration. Our previous pilot clinical trial demonstrated the potential of autologous $P63^+$ progenitor cell transplantation to improve pulmonary function in two patients with bronchiectasis. Consecutive CT revealed regional bronchiectasis recovery in one of these patients.[21,45] Building upon this concept, the current randomized, controlled, single-blind clinical study aimed to investigate the safety and efficacy of intrapulmonary transplantation of $P63^+$ progenitor cells in a larger cohort.

The current study demonstrated the feasibility of isolating, expanding, and transplanting $P63^+$ progenitor cells in patients with bronchiectasis. The incidences of adverse events were similar between the two groups, with most events attributed to the bronchoscopy procedure or natural progression of bronchiectasis. SAEs occurred in 2 patients in the cell treatment group. One patient experienced a pneumothorax immediately after bronchoscopic surgery to collect $P63^+$ progenitor cells. Given the compromised nature of bronchial walls in patients with bronchiectasis,[46] this pneumothorax was likely due to the brush's manipulation during bronchoscopy. Therefore, future studies should ensure gentle bronchoscopic procedures performed by well-trained physicians. Another SAE involved a patient who developed a common cold and subsequently experienced an acute exacerbation of COPD 8 weeks after cell transplantation. This patient previously experienced 3–4 times of COPD acute exacerbations per year, and the cause of the exacerbation event this time was clear. Additionally, the occurrence time is far from transplantation surgery. Thus, both of the two SAEs were considered unrelated to cell transplantation therapy. These two patients recovered well following standard conservative treatment in the hospital. Importantly, among all patients, no tumor formation was observed during the entire follow-up period, indicating the overall safety of autologous $P63^+$ progenitor cell transplantation in patients with bronchiectasis.

In addition to safety evaluation, the data also revealed significant improvements in pulmonary gas transfer function (D_{LCO}),

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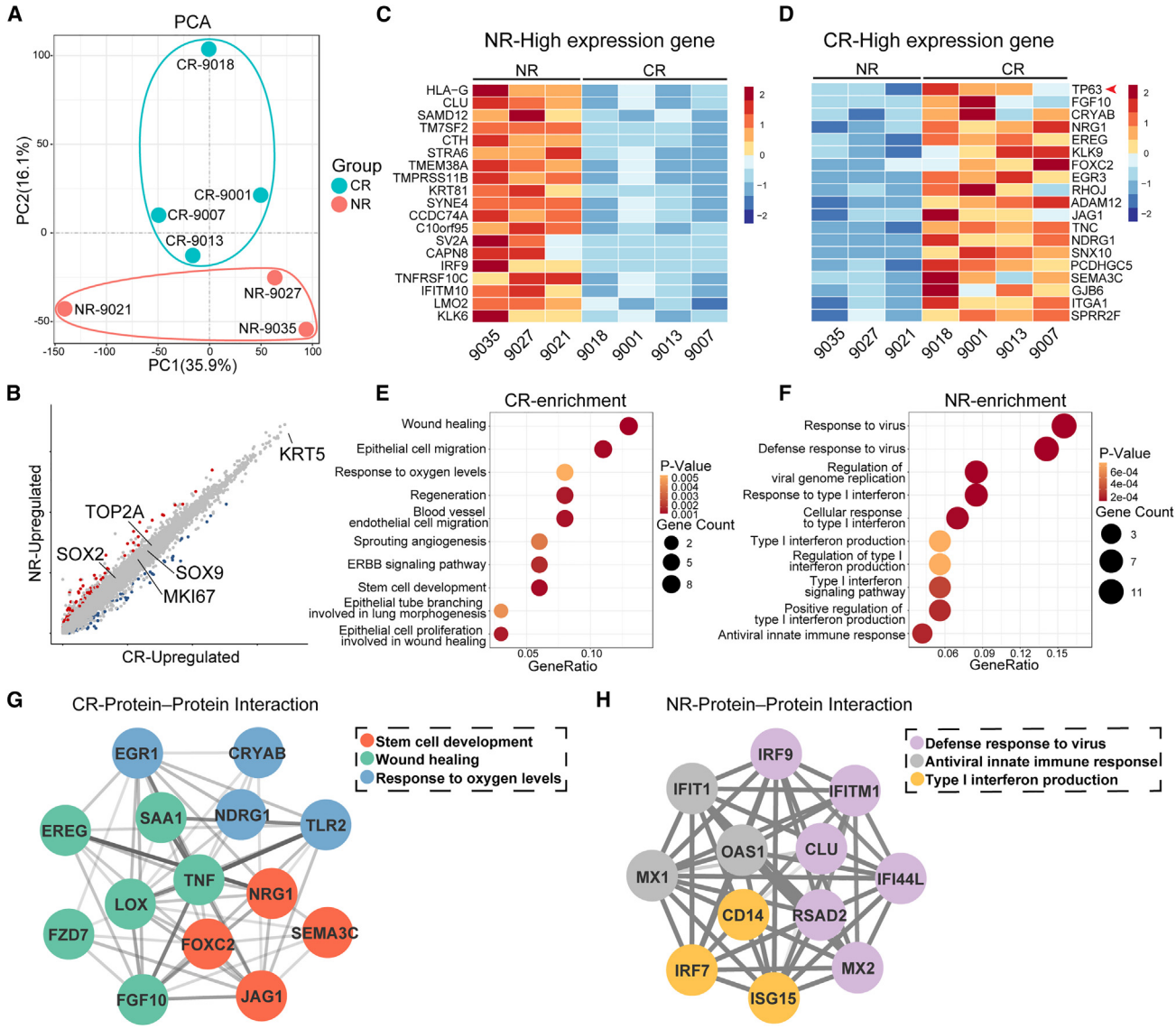

Figure 4. Transcriptomic analysis of progenitor cells derived from different patients

(A) Unsupervised principal component analysis of RNA-seq data progenitor cells derived from complete responsive (CR) and non-responsive (NR) patients (CR, $n = 4$; NR, $n = 3$).

(B) Scatterplot of gene expression of NR and CR cell lines. Gray dots represented genes showing no significantly different expression levels.

(C and D) Expression heatmap of gene sets differentially expressed in NR (C) and CR (D) progenitor cells (CR, $n = 4$; NR, $n = 3$).

(E and F) GO terms that were significantly enriched in the CR (E) and NR (F) groups (p value <0.05) (CR, $n = 4$; NR, $n = 3$).

(G and H) Protein interaction network analysis of the expression of proteins associated with specific GO terms and their interaction relationship in CR (G) and NR (H) groups (CR, $n = 4$; NR, $n = 3$).

quality of life, CT images, and bronchiectasis severity scores following cell transplantation, suggesting potential therapeutic benefits. Currently, pharmacotherapy for bronchiectasis primarily focuses on infection control using antibiotics and alleviating airflow restriction with short- or long-acting bronchodilators. However, these approaches fail to halt or reverse bronchiolar and alveolar destruction and do not positively impact gas transfer parameters (D_{LCO}). Previous studies have reported impaired D_{LCO} in 55.7% of patients with bronchiectasis, with a progressive decline of 2.9% per year.[44,47] D_{LCO} values below 85% of predicted values are significant predictors of all-cause mortality, even in the absence of apparent clinical respiratory disease.[48] In chronic respiratory conditions such as bronchiectasis and COPD, declining D_{LCO} is associated with higher mortality and lower quality of life,[30–33] independent of airflow obstruction severity and other clinical variables. In our study, a significant improvement in gas transfer function was observed within 12 weeks post therapy. The time-limited benefit of cell

transplantation could be due to that in the highly infectious microenvironment of bronchiectasis lungs, the transplanted cells cannot persistently engraft, in contrast to our previous finding of persistent beneficial effect in patients with COPD.[24] Consistently, CT imaging indicated partial lung injury repair in patients who underwent cell transplantation. Additionally, we observed improvements in BSI and FACED scores following cell treatment. These scoring systems are commonly used to assess the severity and prognosis of bronchiectasis and demonstrate high predictive power.[39]

The mechanism underlying $P63^+$ progenitor cell therapy's improvement of D_{LCO} and other health status in patients with bronchiectasis requires further investigation. As mentioned in the introduction, the exact function of $P63^+$ progenitor cells in the lungs of patients with various pulmonary diseases remains unclear. While these cells have demonstrated significant bronchiolar and alveolar repair potential, aberrant $P63^+$ basaloid cells have been found in the alveolar space of fibrotic lungs,[18] and $P63^+$ basal cell hyperplasia has been associated with persistent airway remodeling in COPD.[49,50] Regarding bronchiectasis, previous studies have indicated the expansion of $P63^+$ $KRT5^+$ lung basal progenitor cells in dilated bronchioles.[51] Additionally, we observed that these cells could form air sac-like structures in the alveolar spaces of some patients with bronchiectasis within two years of onset. This suggests that endogenous $P63^+$ cells may possess lung repair functions under certain conditions before becoming exhausted as the disease progresses. These repair processes likely involve multiple mechanisms, including the regeneration of damaged airway epithelium, re-epithelialization of injured alveolar spaces, and paracrine signaling of lung-repairing growth factors or anti-bacterial peptides. Interestingly, we found that the D_{LCO} improvement was associated with the higher expression level of P63 in progenitor cells in some patient samples. A similar observation was described in another phase 1 study using autologous $P63^+$ progenitor cells to treat COPD.[24] However, further investigations in animal models and human subjects are needed to fully elucidate the complex mechanisms underlying these observations. In the future, based on a better understanding of the repair mechanism, appropriate methods could be developed to select $P63^{high}$ cells for therapeutic purposes, or measures could be implemented to reprogram cells for improved therapeutic effects. Further research in this area holds the potential to enhance the efficacy of $P63^+$ progenitor cell therapy for bronchiectasis and other respiratory conditions.

Limitations of the study

The current work has several limitations that warrant attention in future research. Firstly, considering the diverse etiologies of bronchiectasis, the relatively small number of participants and the single-center nature of the study may limit the generalizability of the findings, especially the beneficial effect in patients without severe deficiency of gas exchange function. Therefore, additional verification in larger, multicenter cohorts is necessary to establish the safety and clinical efficacy of $P63^+$ progenitor cell transplantation. Secondly, the trial was not specifically designed to elucidate the etiology of enrolled patients, leading to potential variability in responses to cell therapy due to the heterogeneous nature of non-CF bronchiectasis. Additionally, the genetic and epigenetic background of progenitor cells cloned from individual patients may contribute to distinct responses to therapy. Future studies should consider stratifying patients based on etiological factors and explore the impact of genetic and epigenetic variations on treatment outcomes. Thirdly, the 24-week follow-up duration may be insufficient to assess long-term safety and efficacy, particularly regarding exacerbation frequency and overall mortality. Longer-term follow-up periods are necessary to evaluate the durability of treatment effects and potential late-onset adverse events. Finally, we noted a higher drop-out rate in the cell treatment group. This was mainly due to an uneven geographic distribution of patients after randomization. A larger proportion of patients in the control group (47.06% vs. 27.78%) resided in the Yangtze River Delta (YRD) region of China, which is closer to the Shanghai Ruijin Hospital where the study took place. Due to the stringent COVID-19 lockdown policy enforced in Shanghai in 2022,[52] several patients in the cell treatment group who resided outside of the YRD region were lost to follow-up. We hope that future studies will be able to address the limitations of the current study and provide more reliable evidence regarding the safety and efficacy of the treatment.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jieming Qu (jmqu0906@163.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

RNA-seq data generated during this study have been deposited at GEO and the accession number is listed in the key resources table.

Qualified researchers may request access to individual patient-level clinical data for eligible studies. However, due to proprietary considerations, the datasets generated and/or analyzed during the current study are not publicly available. All data will be shared in aggregate form and can be requested once the article has been published, if there is not a reasonable likelihood of participant reidentification. To request access to patient-level data, please contact the lead contact, who will decide whether or not to provide the data.

No custom code was generated.

Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

J.Q. and W. Zuo were responsible for the study design and coordination of all study-related activities and contributed to the evaluation and interpretation of study data and manuscript writing. M.Z., Y.F., L.Z., Y.G., T.Y., and Y.H. performed the bronchoscopy. M.Z. and Y.F. generated the random allocation sequence. C.D., Q.Z., X.W., J.Z., R.D., L. Ni, and Z.B. recruited the patients and assigned participants to groups. J.Y., W. Zhang, X.L., L. Niu, M.S., P.W., and T.Z. collected and assembled the data. J.Y. and W. Zhang did the statistical analysis. All authors were involved in data interpretation and the writing, revision, and critical review of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. J.Q., W. Zuo, and M.Z. accessed and verified the underlying data.

DECLARATION OF INTERESTS

W. Zuo and T.Z. have an equity interest in Regend Therapeutics, holding the patent for human lung progenitor cell isolation and expansion technique.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Cytokeratin 5 Monoclonal Antibody	Thermo Fisher	Cat#MA5-14473; RRID: AB_10979451
Rabbit CD31 Polyclonal Antibody	Proteintech	Cat#28083-1-AP; RRID: AB_2881055
Rabbit Recombinant Anti-Aquaporin 5 antibody	Abcam	Cat#ab92320; RRID: AB_2049171
Mouse Anti-p63 antibody	Abcam	Cat#ab735; RRID: AB_305870
Rabbit Recombinant Anti-Cytokeratin 5 antibody	Abcam	Cat#ab52635; RRID: AB_869890
Alexa Fluor-conjugated Donkey 488	Thermo Fisher	Cat#A21206; RRID: AB_2535792
PE Mouse Anti-Human CD45 Antibody	BD Pharmingen	Cat#560975; RRID: AB_560975
PE Mouse Anti-Human CD34 Antibody	BD Pharmingen	Cat#560941; RRID: AB_10522562
FITC Mouse Anti-Human CD105	BD Pharmingen	Cat#561443; RRID: AB_10714629
FITC Mouse Anti-Human IgG Antibody	BD Pharmingen	Cat#560952; RRID: AB_2648727
PE Mouse IgG1 κ Isotype Ctrl Antibody	BD Pharmingen	Cat#557646; RRID: AB_10519360
Alexa-conjugated Donkey 594 secondary antibodies	Life Technologies	Cat#A-21207; RRID: AB_141637
Alexa-conjugated Donkey 488 secondary antibodies	Life Technologies	Cat#A-21206; RRID: AB_2535792
Chemicals, peptides, and recombinant proteins		
Citrate Buffer (pH = 6)	Sigma-Aldrich	Cat#c9999
DAPI	Roche	Cat#10236276001
PBS	Multicell	Cat#311-425-CL
Vectashield	Vector Labs	Cat#H-1000-10
TRIzol Reagent	Invitrogen	Cat#15596026CN
Deposited data		
RNA-Seq data	This paper	GEO: GSE261102
Software and algorithms		
Statistical Package for the Social Science (version 25.0)	IBM Corp	https://www.ibm.com/cn-zh/products/spss-statistics
GraphPad Prism (version 9.0)	GraphPad Software, Inc.	https://www.graphpad.com
R package using ggplot2 (version 3.4.2)	R Project	https://cran.r-project.org/web/packages/ggplot2/index.html
R package using DESeq2 (version 1.38.3)	R Project	https://bioconductor.org/packages/release/bioc/html/DESeq2.html
R package using ClusterProfiler (version 4.6.2)	R Project	https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html
R (version 4.2.3)	R Project	https://www.r-project.org
Cytoscape (version3.10.0)	Cytoscape Consortium	https://cytoscape.org
R package using pheatmap (version 1.0.12)	R Project	https://cran.r-project.org/web/packages/pheatmap/index.html
3D Slicer (version 5.2.2)	Fedorov et al., 2012	https://www.slicer.org
FlowJo	N/A	https://www.flowjo.com

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Trial design

A randomized, single-blind, controlled trial was conducted at Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. Patients enrollment began on June 21, 2020, and the last patient follow-up visit was on May 17, 2023. All the eligible patients met the criteria by the day of enrollment. Written informed consent was obtained after discussion with the patient or an appropriate surrogate. This clinical trial was approved by the Ethics Commission of Ruijin Hospital (2018-10-5) and registered with ClinicalTrials.gov, number

NCT03655808. Detailed clinical trial protocol was shown in Data S1. The cell dose range (1–3 × 10^6 kg/body weight) was chosen based on previous studies.[21,24] Within the given range, for each patient, the exact cell dose was determined by the cell number harvested at last.

Participants

Patients aged 18 to 75 years, with a diagnosis of bronchiectasis confirmed by chest HRCT and remaining clinically stable for at least 2 weeks, were recruited from the outpatient clinics of Ruijin Hospital. In addition, all enrolled patients had a D_{LCO} of less than 80% of the predicted value, were suitable for bronchoscopy, and were willing to receive autologous $P63^+$ progenitor cells transplantation therapy. The key exclusion criteria included active pulmonary tuberculosis, uncontrolled asthma, extremely severe COPD, respiratory failure, major hepatic or renal dysfunction, pregnancy or breast-feeding. A complete list of inclusion and exclusion criteria was shown in Table S2.

Randomization and blinding

As the number of participants was relatively small and the trial was single center, eligibility patients were assigned according to a random number table, with sequentially numbered in a 1:1 ratio generated by computer, to receive either B-ACT plus autologous $P63^+$ progenitor cells transplantation (cell treatment group) or B-ACT therapy only (control group). The opaque sealed envelope method was used to conceal the allocation sequence. Both patients and investigators, except for the bronchoscopy operators, remained masked to the treatment assignment for the duration of the study. That is, only the investigators who performed the bronchoscopy were unblinded. The non-blinded investigators should not disclose any blind information to other investigators, participants, or clinic staff.

Interventions

Firstly, a comprehensive assessment of patients was conducted to check whether the patients were able to tolerate the bronchoscopy. Mainly through the following examinations: blood test, infectious diseases related indicators detections, electrocardiogram, chest HRCT, pulmonary function examination, and arterial blood gas analysis if necessary. Preoperative analysis and discussion were conducted according to the requirement of bronchoscopy. Patients and their families were fully informed before the bronchoscope, and the informed consents were signed. The bronchoscopy was performed by board-certified respiratory physicians of Ruijin Hospital using a flexible fiberoptic bronchoscope. Before the bronchoscopy, oropharyngeal and laryngeal anesthesia was obtained by administration of 2 mL of nebulized 4% lidocaine, followed by 1 mL of 2% topical lidocaine sprayed into the patient's oral and nasal cavities. Operators checked whether the patients had active denture and removed it in time to prevent aspiration. Oxygen was given to one side of the nasal tract and oxygen saturation and pulse were monitored. For patients in the control group, they were only given B-ACT therapy. B-ACT therapy was performed on all patients in both groups according to the protocol described in our previous study.[53] In brief, continuous suction was performed with the sputum aspirator from the trachea to the subsegmental bronchi during the entering of the bronchoscope to remove the visible secretions from the entire respiratory tract, and then operators used 120–200 mL normal saline to collect lavage fluid (the volume various depending on the operator's judgment). For patients in the cell treatment group, on the basis of B-ACT therapy, airway tissues were collected from patients in the cell treatment group by a disposable 2-mm brush. Operators gently glided the brush back and forth 1 or 2 times in 3∼5^{th} order bronchi within the relatively healthy area of the lung, which showed no obvious sign of lesions based on chest HRCT scans and bronchoscopic scope.

The obtained tissues were washed and enzymatically digested to form a single-cell suspension, which was then cultured under the R-Clone system, a patented technique of Regend Therapeutics, Ltd. Before releasing for therapeutic purpose, the expanded $P63^+$ progenitor cells underwent a series of tests, including cell identity, cell purity, viable cell count, viability, sterility, mycoplasma detection, biological efficacy, endotoxin, viral contamination, BSA remain content, antibiotic remain content, tumorigenicity pH, osmolality, product appearance, and visible particles.

The $P63^+$ progenitor cells product contained 1 to 3 × 10^6 cells/kg body weight suspended in 30 mL sterile 0.9% normal saline and was shipped to Ruijin Hospital in an ice box with a real-time monitoring and alarm device for temperature and location to ensure the required storage conditions (2–8°C). Shipping of cell products by car from the Regend cell factory to the hospital generally took less than 3 h. Upon receipt, the cellular product was inspected and 0.5 mL was kept as the retained sample in a liquid nitrogen storage tank in Ruijin Hospital. The rest was immediately sent to the bronchoscope room.

Patients underwent examinations 1–3 days before the second bronchoscopy and physicians assessed the patients again to determine whether they were suitable for bronchoscopy. For patients in the control group, they were given B-ACT therapy again. And for patients in the cell treatment group, after B-ACT therapy, they were also given autologous $P63^+$ progenitor cells transplantation. Cell suspension was pre-warmed to approximately 37°C 15 min before use and kept in a syringe for later use.

During the cell transplantation process, all the patients received standard monitoring systems, including electrocardiogram, heart rate, non-invasive arterial blood pressure, and peripheral oxygen saturation (SpO_2) in the operating room. The patients were asked to open the mouth as wide as possible and then the oral cavity and hypopharynx mucosa were sprayed with 2% lidocaine 3 times within 20 min before the procedure. A bite block was placed between the teeth of patients, and the operator advanced the fiberoptic bronchoscope downward along the oropharyngeal curve until the epiglottis and glottis were visible. The fiberoptic bronchoscope was inserted into the trachea after the front of the bronchoscope had passed through the vocal cords.

Six lung segments with the most severe lesions were selected by the team of doctors before bronchoscopy according to CT results. After the bronchoalveolar lavage was completed, the lavage fluid in the affected area was required to aspirate as much as possible. When the oxygen saturation of patients maintained above 92%, 5 mL of the cell suspension was slowly and gently pushed into each lung segment via the working channel of the bronchoscope with a 20 mL syringe in around 30 s, and the severely damaged lung segment could be injected more than once.

After bronchoscopy, patients were told to fast, avoid coughing violently, and keep a supine position without pillow for at least 2 h. Physicians closely monitored the physical signs of patients including body temperature, pulse, respiration, blood pressure, oxygen saturation, and any signs of adverse reaction. Patients was discharged from the hospital 2–3 days after bronchoscopy.

Outcomes

Patients were followed up by clinical physicians at Week 4, Week 12, and Week 24 in Ruijin Hospital after the second bronchoscopy operation. In order to evaluate the safety and tolerability of autologous $P63^+$ progenitor cells transplantation, we monitored adverse events from enrollment through 24 weeks after treatment. Meanwhile, we recorded the clinical information and laboratory tests of all the participants on baseline, and Week 4, 12, and 24. The data included the following: (1) demographic data, principal symptoms, medical history related to bronchiectasis, comorbidity, and medicine treatment; (2) vital signs and physical examination results; (3) laboratory tests, including blood and urine routine examinations, chemistry panels assessing liver and kidney function, lactate dehydrogenase (LDH), CK, blood glucose, and arterial blood gas analysis; (4) electrocardiogram results.

The primary efficacy endpoints were the changes from baseline in D_{LCO} after treatment. Efficacy was also evaluated with respect to the following secondary endpoint measures: the changes from baseline in other pulmonary function parameters including FEV_1, FVC, FEV_1/FVC, MMEF, and MVV, at Week 4, 12, and 24; the changes from baseline in 6MWD and DSP at Week 4, 12, and 24; the changes from baseline in SGRQ at Week 4, 12, and 24; the changes from baseline in BSI and FACED scores at Week 4, 12, and 24. These endpoints were compared between the cell treatment group and the control group. Data collections were performed according to standardized protocol by clinical physicians involved in this research.

Serial pulmonary function tests were all performed according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines.[54–59] This included measurement of the flow-volume curve and spirometry, lung volume by single breath dilution and plethysmography, airway resistance during panting at functional residual capacity (FRC), and D_{LCO}. Predicted values were selected using a reference model by Zheng and associates.[60] Short-acting bronchodilators, if any, were withdrawn for at least 4 h, and long-acting bronchodilators for 12 h prior to the examinations.[57]

BSI score and FACED score were applied to determine the severity and prognosis of bronchiectasis. The BSI score was a combination of clinical parameters, including the age, body mass index, prior exacerbations and prior hospitalization in the preceding year, mMRC grading, FEV_1% of predicted, *Pseudomonas aeruginosa* infection, colonization with other potentially pathogenic microorganisms (PPMs, including *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Staphylococcus aureus*, *Klebsiella* spp., and other clinically significant bacteria) and the number of bronchiectatic lobes. BSI score of $\leq$4, 5–8, and $\geq$9 denoted mild, moderate, and severe bronchiectasis, respectively.[38] FACED score incorporated variables including FEV_1% of predicted, age, colonization of *Pseudomonas aeruginosa*, radiological extension, and mMRC grading. FACED score of $\leq$2, 3–4, and $\geq$5 denoted mild, moderate, and severe bronchiectasis, respectively.[37]

A standardized data collection spreadsheet was designed to obtain data of patients from electronic medical records. Two attending physicians independently reviewed the data collection forms to double check the data validity.

CT image analysis

All CT examinations were performed adhered to the common chest protocol: the patient was installed in a supine position with arms raised and held the breath at full inspiration during acquisition. Chest CT images were acquired using a GE Revolution APEX CT (GE Healthcare, Milwaukee, USA). The scan parameters are summarized as follows: helical, 100KVp, 80mm collimation, 0.5 s rotation time, 0.992 pitch, 1$\sim$1.25mm slice thickness, B70f very sharp kernel. HRCT is critical to establish the diagnosis of bronchiectasis according to 2019 BTS guidelines.[29] The direct signs of CT to establish a diagnosis of bronchiectasis include: (1) bronchial dilatation (internal lumen diameter greater than accompanying pulmonary artery, bronchoarterial ratio >1); (2) lack of airway tapering >2 cm distal to point of bifurcation; (3) airway visibility within 1 cm of the costal pleura of fissures. And the indirect signs include: (1) bronchial wall thickening; (2) mucoid impaction/fluid-filled airways (tubular or Y-shaped structures; branching or rounded opacities in cross section $\pm$ air-fluid levels); (3) bronchiolitis (clustered ill-defined centrilobular nodules with a tree-in-bud configuration); (4) mosaic attenuation caused by air trapping; (5) mosaic perfusion of the pulmonary identified on contrast-enhanced dual energy CT of the pulmonary parenchyma; (6) bronchial artery hyperplasia.[61]

CT image quantification and 3D visualization were performed with the open-source 3D Slicer, version 5.2.2 (https://www.slicer.org). Damaged lung areas with attenuation values of between $-$600 and 2500 Hounsfield units on CT images were automatically selected, with minor manual adjustment by experts. The percentage of damaged areas of the lungs is defined as the ratio to the total volume of both lungs (except trachea and bronchi) and is calculated by using the "Segment Statistics" function in the software.

METHOD DETAILS

Immunostaining

For immunostaining, section slides underwent antigen retrieval in citrate buffer (pH = 6, Sigma-Aldrich, USA) heated in a microwave oven for 20 min. The following antibodies were utilized for immunostaining: KRT5 (1:500, MA5-14473, Thermo Fisher), P63 (1:200, ab735, Abcam), CD31 (1:200, Proteintech, 28083-1-AP), AQP5 (1:300, Abcam, ab92320). For immunofluorescence staining, Alexa-conjugated Donkey 488/594 secondary antibodies (1:200, Life Technologies, USA) along with DAPI (Roche, USA, 10236276001) were used. The tissue slides underwent auto-fluorescence removal and were mounted using mounting media (Vectashield, Vector Labs, H-1000-10). Slides were observed under a fluorescent microscope (Olympus).

Flow cytometry

Cells were digested into single cell suspensions, washed with PBS (Multicell, 311-425-CL), and then resuspended in PBS at a concentration of 1×10^6 cells/mL. Flow cytometry staining was conducted in a standard protocol. Following staining, cells were transferred into FACS tubes and each tube was analyzed on a Beckman CytoFLEX within 1 h. The gate was defined to remove debris and doublet cells using FSC and SSC. Positive and negative cells were identified by the isotype control group. Antibodies used include: Anti-KRT5 (Abcam, Ab52635, 1:500), Alexa Fluor-conjugated Donkey 488 (Thermo Fisher, A21206, 1:200), PE Mouse Anti-Human CD45 Antibody (BD Pharmingen, 560975, 1:200), PE Mouse Anti-Human CD34 Antibody (BD Pharmingen, 560941, 1:200), FITC Mouse Anti-Human CD105 (BD Pharmingen, 561443, 1:200), FITC Mouse Anti-Human IgG Antibody (BD Pharmingen, 560952, 1:200), 488 Mouse IgG1 κ Isotype Ctrl Antibody (BD Pharmingen, 557782,1:200) and PE Mouse IgG1 κ Isotype Ctrl Antibody (BD Pharmingen, 557646,1:200).

Bulk RNA-Sequencing and bioinformatics

Total RNA was extracted from progenitor cells using TRIzol Reagent (Invitrogen, Life Technologies, 15596026CN) following the manufacturer's instructions. Subsequently, the extracted RNA was treated with DNase I (Invitrogen, Life Technologies, USA) to remove any contaminating DNA. The cDNA library was then constructed and sequenced, and the BGI-NSG platform was utilized. The sequencing data obtained was then subjected to filtering using SOAP nuke.[62] The filtering process involved the following steps: (1) removing reads containing sequencing adapters, (2) removing reads with a low-quality base ratio (base quality less than or equal to 15) higher than 20%, and (3) removing reads with an unknown base ('N' base) ratio higher than 5%. Following the filtering steps, clean reads were obtained and stored in FASTQ format for further analysis. The clean data were mapped to the reference genome (hGRC38) by HISAT (v2.1.0).[63] The expression level of genes was calculated by RSEM (v1.2.8) and FPKM (Fragments Per Kilobase per Million) of each gene was calculated based on the length of the gene and read counts mapped to this gene.

Analysis of RNA-Seq data was performed by R (version 4.2.3). PCA and differential expression analysis were performed using the DESeq2 R package (1.38.3). A P-value of 0.01 and an absolute fold change of 2 were set as the threshold for significant differential expression. Visualization of heatmap was generated through R packet pheatmap (1.0.12). GO enrichment analysis of differentially expressed genes was performed by the ClusterProfiler R package. GO terms with a P-value <0.05 were considered significantly enriched by differentially expressed genes and the results were visualized by the enrichplot R package using dot plots. Protein-protein interaction (PPI) network was constructed to map the differentially expressed genes (DEGs) to the protein by using Cytoscape (3.10.0).

QUANTIFICATION AND STATISTICAL ANALYSIS

As the trial was an early phase study, the sample size was based on clinical consideration, rather than statistical consideration, to provide safety and efficacy information with the need to minimize exposure to subjects. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard error of the mean (SEM)/standard deviation (SD) or median ($25\sim75^{th}$ interquartile range [IQR]). The Shapiro-Wilk test was applied to assess the data normality. The unpaired Student's t test was used for normally distributed variables and the Mann-Whitney U test was used for non-normally distributed variables unless otherwise noted. Categorical variables were compared by the Chi-square test or Fisher's exact test. Pearson correlation test was computed for correlation analysis. For the primary endpoint analysis, the AUC of the change from baseline to 4–24 weeks in D_{LCO} was calculated and Welch's t-test was used to examine the difference between the cell treatment and control groups. For secondary endpoints, the difference between the cell treatment and control groups was tested using the Mann-Whitney U test, and the median differences were calculated using the Hodges-Lehmann estimation. If patients missed pulmonary function tests, the last results of the D_{LCO} test after cell treatment were carried forward to the missing visits for primary endpoint analysis. Other missing values for secondary endpoints and safety analyses were not imputed. Analyses were presented with two-sided P-values, with the level of significance set at 0.05. All statistical analysis and diagramming were performed by SPSS (version 25.0), GraphPad (version 9.0), and R package using ggplot2 (version 3.4.2).

ADDITIONAL RESOURCES

This study has been registered with ClinicalTrials.gov, NCT03655808.

Cell Reports Medicine, Volume *5*

Supplemental information

Autologous transplantation of P63^{+} lung progenitor cells in patients with bronchiectasis: A randomized, single-blind, controlled trial

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Supplementary information

Table S1. Demographic and clinical characteristics of bronchiectasis patients who provided pulmonary tissues through surgical excision. Related to Figure 1.

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5
Age (years)	33	61	67	58	50
Gender	Male	Male	Female	Female	Female
Body mass index (kg/m^2)	25.95	22.99	27.25	24.56	24.63
Smoking history (pack years)	None	35	None	None	None
Principal symptoms	Cough, sputum, hemoptysis	Cough, sputum, hemoptysis	Cough, hemoptysis	Cough, chest pain	Cough, sputum, hemoptysis
Duration of disease (years)	17	12	1	1	2
FEV_1 % predicted	103.6	65.4	74.3	96.1	83.4
Infection status	*klebsiella pneumoniae*	*Streptococcus intermadius*	No bacterial growth in the sputum culture	No bacterial growth in the sputum culture	No bacterial growth in the sputum culture
BSI score	9	10	4	2	7
Comorbidities	None	Tuberculosis, gout	None	None	Tuberculosis
Medication for bronchiectasis	Inhaled bronchodilator	Mucolytics	Oral antibiotics, mucolytics	None	Oral antibiotics, mucolytics

Site of surgical tissues	Inferior lobe of left lung	Inferior lobe of left lung	Inferior lobe of left lung	Inferior lobe of right lung	Inferior lobe of right lung
Airway destruction condition[a]	Expanded alveolar spaces, alveolar histiocytosis, metaplasia	Expanded alveolar spaces, alveolar hemorrhage, focal abscess	Edema, alveolar hemorrhage and disruption, metaplasia	Alveolar histiocytosis, necrotic material in the bronchial lumen	Expanded alveolar spaces, alveolar hemorrhage
Immune infiltration condition[a]	Lymphocytes, plasma cells, and eosinophils infiltration	Lymphocytes infiltration, lymphoid follicular formation	Lymphocytes, plasma cells, and eosinophils infiltration, lymphoid follicular formation	Lymphocytes and plasma cells infiltration, lymphoid follicular formation	Lymphocytes, plasma cells, and eosinophils infiltration
Fibrosis condition[a]	Not observed	Interstitial fibrosis	Interstitial fibrosis	Interstitial fibrosis	Slight interstitial fibrosis

[a]These were assessed according to histopathological examination of specimens.

Table S2. List of key inclusion and exclusion criteria. Related to STAR Methods.

Inclusion criteria:
Aged between 18 to 75;
Remaining clinically stable;
Diagnosed with bronchiectasis according to the guidelines;
D_{LCO} < 80% of the predicted value;
Being capable of doing pulmonary function tests;
Tolerant to bronchofiberscopy;
Written informed consent signed.
Exclusion criteria:
Pregnant or lactating;
Patients positive for syphilis or HIV;
Patients with malignant tumors;
Patients with serious comorbidities;
Patients with serious systemic diseases;
Patients with serious kidney dysfunction;
Patients with serious liver dysfunction;
Patients with serious heart disease (NYHA class III~IV);
Patients with a history of abusing alcohol and illicit drugs;
Patients participated in other clinical trials in the past 3 months;
Patients could not understand the test procedures and use the test equipment;
Patients assessed as inappropriate to participate in this clinical trial by the investigator.

Table S3. Dose information in study patients. Related to STAR Methods.

Patient #	The date of $P63^+$ progenitor cells collection	Days from collection to transplantation (days)	Group	Dose ($\times 10^6$ cells/kg)[a]
9001	2020.8.12	28	Cell treatment	2.37
9002	2021.7.2	78	Cell treatment	1.78
9007	2021.5.7	46	Cell treatment	1.13
9008	2021.7.2	78	Cell treatment	2.49
9010	2021.9.16	132	Cell treatment	2.13
9013	2021.8.27	45	Cell treatment	2.12
9015	2021.10.19	42	Cell treatment	1.49
9017	2021.10.21	34	Cell treatment	1.70
9018	2021.11.11	48	Cell treatment	2.00
9021	2022.9.7	292	Cell treatment	2.40
9024	2022.3.7	74	Cell treatment	2.83
9027	2022.10.17	42	Cell treatment	2.28
9028	2022.8.15	31	Cell treatment	2.11
9029	2022.9.9	35	Cell treatment	2.34
9032	2022.10.13	49	Cell treatment	2.00
9034	2022.10.21	42	Cell treatment	2.41
9035	2022.10.27	48	Cell treatment	2.27
		67.29 ± 63.20[b]		2.11 ± 0.41[b]

[a]Cells suspended Perfedex preservation solution for long-term shipment.

[b]Data were presented as mean ± standard deviation (SD).

Table S4. Adverse events likely related to bronchoscopy. Related to Table 2.

Events	Any Grade[c]		Grade 1[c]		Grade 2[c]		Grade 3[c]		Grade 4~5[c]	
	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)
Fever[a]	7(41.18%)	2 (11.11%)	4 (23.53%)	1 (5.56%)	3 (17.65%)	1 (5.56%)	0 (0)	0 (0)	0 (0)	0 (0)
Hemoptysis[ab]	3 (17.65%)	3 (16.67%)	3 (17.65%)	3 (16.67%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sputum increased[a]	1 (5.88%)	0 (0)	1 (5.88%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pharyngeal discomfort[a]	1 (5.88%)	3 (16.67%)	1 (5.88%)	3 (16.67%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chest discomfort[a]	1 (5.88%)	1 (5.56%)	0 (0)	1 (5.56%)	1 (5.88%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dyspnea[a]	1 (5.88%)	0 (0)	0 (0)	0 (0)	1 (5.88%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea[a]	1 (5.88%)	0 (0)	0 (0)	0 (0)	1 (5.88%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pneumothorax[a]	1 (5.88%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.88%)	0 (0)	0 (0)	0 (0)

[a]Data were presented as patient number (percentage of patients).

[b]The term “hemoptysis” included bloody sputum in this study.

[c]The severity grade of adverse events was defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 5.0.

Table S5. Adverse events likely unrelated to bronchoscopy. Related to Table 2.

Events	Any Grade[c]		Grade 1[c]		Grade 2[c]		Grade 3[c]		Grade 4~5[c]	
	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)	Cell treatment (*N* = 17)	Control (*N* = 18)
Fever[a]	1 (5.88%)	3 (16.67%)	1 (5.88%)	0 (0)	1 (5.88%)	2 (11.11%)	0 (0)	0 (0)	0 (0)	0 (0)
Hemoptysis[ab]	0 (0)	3 (16.67%)	0 (0)	3 (16.67%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sputum increased[a]	3 (17.65%)	3 (16.67%)	3 (17.65%)	3 (16.67%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cough increased[a]	3 (17.65%)	3 (16.67%)	3 (17.65%)	3 (16.67%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fatigue[a]	3 (17.65%)	3 (16.67%)	2 (11.76%)	2 (11.11%)	1 (5.88%)	1 (5.56%)	0 (0)	0 (0)	0 (0)	0 (0)
COVID-19[a]	2 (11.76%)	4 (22.22%)	0 (0)	0 (0)	2 (11.76%)	4 (22.22%)	0 (0)	0 (0)	0 (0)	0 (0)
Bronchiectasis exacerbation[a]	2 (11.76%)	2 (11.11%)	0 (0)	0 (0)	2 (11.76%)	2 (11.11%)	0 (0)	0 (0)	0 (0)	0 (0)
Dizziness[a]	1 (5.88%)	1 (5.56%)	1 (5.88%)	1 (5.56%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dyspnea[a]	0 (0)	1 (5.56%)	0 (0)	1 (5.56%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Influenza[a]	0 (0)	1 (5.56%)	0 (0)	0 (0)	0 (0)	1 (5.56%)	0 (0)	0 (0)	0 (0)	0 (0)
Anxiety[a]	0 (0)	1 (5.56%)	0 (0)	0 (0)	0 (0)	1 (5.56%)	0 (0)	0 (0)	0 (0)	0 (0)
Acute exacerbation of COPD[a]	1 (5.88%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.88%)	0 (0)	0 (0)	0 (0)

[a]Data were presented as patient number (percentage of patients).

[b]The term “hemoptysis” included bloody sputum in this study.

[c]Severity grade of adverse events were defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 5.0.

Table S6. Number and percentage of patients who improved or deteriorated more than the minimal clinically important difference in two groups for D_{LCO}, FEV_1, 6MWD, SGRQ score, and mMRC grading at Week 4, 12, and 24. Related to Figure 2.

	Cell treatment group	Control group	P-value
D_{LCO} % predicted change percentage from baseline			
Week 4	*N* = 13	*N* = 18	
> 10%[a]	4 (30.8%)	0 (0)	
-10% to 10%[a]	7 (53.8%)	9 (50.0%)	**0.017**
< -10%[a]	2 (15.4%)	9 (50.0%)	
Week 12	*N* =13	*N* =18	
> 10%[a]	5 (38.5%)	2 (11.1%)	
-10% to 10%[a]	5 (38.5%)	10 (55.6%)	0.237
< -10%[a]	3 (23.1%)	6 (33.3%)	
Week 24	*N* = 12	*N* = 18	
> 10%[a]	3 (25.0%)	3 (16.7%)	
-10% to 10%[a]	6 (50.0%)	12 (66.7%)	0.665
< -10%[a]	3 (25.0%)	3 (16.7%)	
FEV_1 change from baseline			
Week 4	*N* = 13	*N* = 17	
> 100 mL[a]	2 (15.4%)	1 (5.9%)	
-100 to 100 mL[a]	9 (69.2%)	11 (64.7%)	0.535
<100 mL[a]	2 (15.4%)	5 (29.4%)	
Week 12	*N* = 10	*N* = 15	
> 100 mL[a]	0 (0)	3 (20.0%)	
-100 to 100 mL[a]	9 (90.0%)	7 (46.7%)	0.120
<100 mL[a]	1 (10.0%)	5 (33.3%)	
Week 24	*N* = 11	*N* = 18	
> 100 mL[a]	0 (0)	4 (22.2%)	
-100 to 100 mL[a]	8 (72.7%)	9 (50.0%)	0.285
<100 mL[a]	3 (27.3%)	5 (27.8%)	
6MWD change from baseline			
Week 4	*N* = 12	*N* = 17	
> 30 m[a]	5 (41.7%)	5 (29.4%)	
-30 to 30 m[a]	6 (50.0%)	8 (47.1%)	0.621
<30 m[a]	1 (8.3%)	4 (23.5%)	
Week 12	*N* = 10	*N* = 15	
> 30 m[a]	5 (50.0%)	6 (40.0%)	0.512

-30 to 30 m[a]	4 (40.0%)	4 (26.7%)	
<30 m[a]	1 (10.0%)	5 (33.3%)	
Week 24	*N* = 11	*N* = 18	
> 30 m[a]	5 (45.5%)	5 (27.8%)	
-30 to 30 m[a]	4 (36.4%)	6 (33.3%)	0.563
<30 m[a]	2 (18.2%)	7 (38.9%)	
SGRQ score change from baseline			
Week 4	*N* = 13	*N* = 17	
> 4 units[a]	10 (76.9%)	7 (41.2%)	
-4 to 4 units[a]	0 (0)	6 (35.3%)	**0.049**
< -4 units[a]	3 (23.1%)	4 (23.5%)	
Week 12	*N* = 10	*N* = 15	
> 4 units[a]	7 (70.0%)	6 (40.0%)	
-4 to 4 units[a]	0 (0)	6 (40.0%)	0.102
< -4 units[a]	3 (30.0%)	3 (20.0%)	
Week 24	*N* = 11	*N* = 18	
> 4 units[a]	4 (36.4%)	10 (55.6%)	
-4 to 4 units[a]	4 (36.4%)	3 (16.7%)	0.485
< -4 units[a]	3 (27.3%)	5 (27.8%)	
mMRC grading change from baseline			
Week 4	*N* = 13	*N* = 17	
> 1 unit[a]	6 (46.2%)	4 (23.5%)	
-1 to 1 unit[a]	6 (46.2%)	11 (64.7%)	0.423
< -1 unit[a]	1 (7.7%)	2 (11.8%)	
Week 12	*N* = 10	*N* = 15	
> 1 unit[a]	7 (70.0%)	0 (0)	
-1 to 1 unit[a]	1 (10.0%)	10 (66.7%)	**<0.0001**
< -1 unit[a]	2 (20.0%)	5 (33.3%)	
Week 24	*N* = 11	*N* = 18	
> 1 unit[a]	3 (27.3%)	5 (27.8%)	
-1 to 1 unit[a]	6 (54.5%)	11 (61.1%)	0.874
< -1 unit[a]	2 (18.3%)	2 (11.1%)	

[a]Data were presented as patient number (percentage of patients).

Table S7. Key secondary endpoint results at Week 4, 12, and 24. Related to Figure 2 and Figure S10.

Endpoint	Cell treatment group		Control group		Difference in change, Cell treatment vs Control group (95% CI) [c]	P-value
	N subjects [a]	Change from baseline[b]	N subjects[a]	Change from baseline[b]		
Week 4						
VA (L)	13	0.08 (-0.18, 0.40)	15	-0.08 (-0.42, 0.07)	0.26 (0.01, 0.89)	**0.037**
VA % predicted	13	2.60 (-3.00, 7.55)	15	-1.70 (-8.80, 1.50)	4.90 (0.20, 15.00)	**0.033**
TLC % predicted	13	2.90 (-3.25, 11.40)	17	-1.80 (-10.05, -0.05)	5.80 (0.10, 18.30)	**0.043**
FEV_1 (L)	13	0.01 (-0.07, 0.06)	17	0 (-0.14, 0.04)	0.03 (-0.04, 0.12)	0.245
FEV_1 % predicted	13	1.90 (-0.70, 3.00)	17	0.20 (-4.85, 1.10)	2.10 (-0.50, 5.70)	0.079
FVC (L)	13	-0.02 (-0.12, 0.15)	17	-0.05 (-0.13, 0.04)	0.04 (-0.06, 0.18)	0.385
FVC % predicted	13	0.70 (-3.30, 4.55)	17	-1.20 (-5.45, 0.95)	2.40 (-1.40, 6.20)	0.213
FEV_1/FVC	13	0.01 (-5.48, 2.66)	17	-2.31 (-4.32, 2.02)	0.38 (-4.05, 4.13)	0.805
MMEF (L/s)	13	0 (-0.03, 0.05)	17	-0.07 (-0.28, 0.06)	0.07 (-0.05, 0.26)	0. 363
MVV (L/min)	12	0.83 (-3.19, 7.26)	16	3.95 (0, 6.31)	-1.61 (-6.20, 8.02)	0.599
6MWD (m)	12	20.50 (-1.00, 85.91)	17	2.73 (-32.85, 46.35)	29.84 (-18.00, 79.00)	0.263
DSP	12	35.40 (0.21, 81.00)	16	17.51 (-10.86, 30.74)	25.45 (-16.03,72.77)	0.302
SGRQ	13	-16.65 (-21.05, 0.73)	17	-1.27 (-8.60, 4.42)	-11.84 (-19.60, 1.69)	0.103
BSI score	13	-2.00	17	0 (-0.50, 0)	-2.00 (-3.00, 0)	**0.028**

		(-3.00, 0)				
FACED score	13	-1.00 (-1.00, 0)	17	0 (0, 0)	-1.00 (-1.00, 0)	**0.048**
mMRC grading	13	0 (-1.00, 0)	17	0 (-0.50, 0)	0 (-1.00, 0)	0.263
Week 12						
FEV_1 (L)	10	0 (-0.08, 0.03)	15	-0.03 (-0.12, 0.08)	0.03 (-0.08, 0.11)	0.495
FEV_1 % predicted	10	-0.05 (-3.60, 1.30)	15	-1.90 (-6.30, 3.31)	1.00 (-3.70, 5.40)	0.765
FVC (L)	10	0.06 (-0.05, 0.08)	15	-0.06 (-0.29, 0.12)	0.12 (-0.06, 0.28)	0.129
FVC % predicted	10	1.00 (-2.38, 2.78)	15	-4.00 (-11.30, 3.60)	3.50 (-2.10, 10.40)	0.196
FEV_1/FVC	10	-2.97 (-4.76, 2.27)	15	0.83 (-0.29, 3.09)	-3.49 (-6.34, 0.69)	0.091
MMEF (L/s)	9	-0.06 (-0.17, -0.01)	15	0 (-0.17, 0.07)	-0.05 (-0.21, 0.07)	0.263
MVV (L/min)	10	2.37 (-1.95, 12.50)	15	-0.15 (-4.74, 12.00)	3.18 (-6.52, 14.32)	0.531
6MWD (m)	10	40.96 (-12.75, 105.50)	15	24.00 (-51.00, 57.00)	38.34 (-36.00, 107.00)	0.261
DSP	9	62.56 (26.85, 85.18)	13	24.09 (-15.86, 42.15)	39.74 (-8.47, 76.08)	0.110
SGRQ	10	-7.29 (-16.18, 10.16)	15	-1.68 (-16.92, 3.16)	-4.09 (-12.93, 10.89)	0.807
BSI score	10	-1.50 (-3.50, 0)	15	0 (-1.00, 1.00)	-2.00 (-4.00, 0)	**0.016**
FACED score	10	-0.50 (-1.00, 0)	15	0 (0, 0)	-1.00 (-1.00, 0)	**0.023**
mMRC grading	10	-1.00 (-1.00, 0.25)	15	0 (0, 1.00)	-1.00 (-1.00, -1.00)	**0.012**
Week 24						
IC (L)	10	0.12	13	-0.12	0.22 (0.04, 0.48)	**0.018**

		(-0.10, 0.28)		(-0.26, 0.02)		
FEV_1 (L)	11	0 (-0.10, 0.02)	18	-0.01 (-0.11, 0.08)	0.01 (-0.10, 0.08)	0.774
FEV_1 % predicted	11	-0.30 (-4.40, 1.10)	18	-0.90 (-5.40, 4.40)	-0.25 (-5.20, 3.70)	0.912
FVC (L)	11	0.03 (-0.13, 0.09)	18	0.01 (-0.25, 0.11)	0.04 (-0.15, 0.18)	0.642
FVC % predicted	11	-0.40 (-2.10, 1.70)	18	-1.65 (-9.53, 2.93)	1.00 (-3.90, 4.60)	0.642
FEV_1/FVC	11	0.99 (-3.46, 0.85)	17	-0.01 (4.43, 3.05)	-0.97 (-4.48, 2.39)	0.547
MMEF (L/s)	11	-0.01 (-0.08, 0.04)	17	0 (-0.18, 0.06)	-0.01 (-0.15, 0.09)	0.890
MVV (L/min)	10	0.83 (-4.30, 8.45)	15	0.96 (-6.21, 7.87)	0.24 (-7.20, 8.07)	0.935
6MWD (m)	11	9.00 (-27.00, 100.80)	18	-0.57 (-59.88, 34.25)	42.12 (-28.46, 106.36)	0.276
DSP	11	44.83 (-33.64, 77.49)	15	0.20 (-53.73, 38.54)	29.06 (-21.95, 88.83)	0.198
SGRQ	11	-2.22 (-20.03, 10.06)	18	-6.13 (-11.17, 7.12)	-0.06 (-12.47, 12.56)	> 0.999
BSI score	11	0 (-3.00, 0)	18	0 (-0.25,1.00)	-1.00 (-3.00, 0)	0.092
FACED score	11	0 (-1.00, 0)	18	0 (0, 0)	0 (-1.00, 0)	0.238
mMRC grading	11	0 (-1.00, 0)	18	0 (-1.00, 0)	0 (-1.00, 1.00)	0.808

CI, confidence interval; VA, alveolar ventilation; TLC, total lung capacity; MMEF, maximum mid expiratory flow; MVV, maximum voluntary ventilation; DSP, distance-saturation product; IC, inspiratory capacity.

[a]Patients No. at Week 4/12/24.

[b]Data are shown as median (interquartile range, IQR).

[c]Differences are expressed as Hodges-Lehmann estimator and 95% CI.

Table S8. Number and percentage of patients who improved or deteriorated in the individual components of BSI score and FACED score at Week 4, 12, and 24. Related to Figure 2 and Figure S6, S7, S8.

	Cell treatment group	Control group	P-value
BSI-the BMI parameter change from baseline			
Week 4	*N* = 13	*N* = 17	
Improvement[a]	1 (7.7%)	0 (0)	
No change[a]	12 (92.3%)	17 (100%)	0.433
Worsening[a]	0 (0)	0 (0)	
Week 12	*N* =10	*N* =15	
Improvement[a]	1 (10.0%)	0 (0)	
No change[a]	9 (90.0%)	15 (100%)	0.400
Worsening[a]	0 (0)	0 (0)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	1 (9.1%)	0 (0)	
No change[a]	10 (90.9%)	18 (100%)	0.379
Worsening[a]	0 (0)	0 (0)	
BSI-the FEV_1 % of predicted parameter change from baseline			
Week 4	*N* = 13	*N* = 17	
Improvement[a]	0 (0)	2 (11.8%)	
No change[a]	13 (100%)	11 (64.7%)	0.068
Worsening[a]	0 (0)	4 (23.5%)	
Week 12	*N* =10	*N* =15	
Improvement[a]	1 (10.0%)	2 (13.3%)	
No change[a]	9 (90.0%)	11 (73.3%)	0.769
Worsening[a]	0 (0)	2 (13.3%)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	0 (0)	2 (11.1%)	
No change[a]	10 (100%)	13 (72.2%)	0.499
Worsening[a]	1 (9.1%)	3 (16.7%)	
BSI-the acute exacerbation parameter change from baseline			
Week 12	*N* =10	*N* =15	
Improvement[a]	0 (0)	0 (0)	
No change[a]	10 (100%)	14 (93.3%)	> 0.999
Worsening[a]	0 (0)	1 (6.7%)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	0 (0)	0 (0)	> 0.999

No change[a]	11 (100%)	17(94.4%)	
Worsening[a]	0 (0)	1 (5.6%)	
BSI-the mMRC grading parameter change from baseline			
Week 4	*N* = 13	*N* = 17	
Improvement[a]	4 (30.8%)	1 (5.9%)	
No change[a]	9 (69.2%)	15 (88.2%)	0.138
Worsening[a]	0 (0)	1 (5.9%)	
Week 12	*N* =10	*N* =15	
Improvement[a]	2 (20.0%)	0 (0)	
No change[a]	8 (80.0%)	13 (86.7%)	0.141
Worsening[a]	0 (0)	2 (13.3%)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	1 (9.1%)	1 (5.6%)	
No change[a]	8 (72.7%)	17 (94.4%)	0.257
Worsening[a]	2 (18.2%)	0 (0)	
BSI-the *Pseudomonas aeruginosa* infection parameter change from baseline			
Week 4	*N* = 13	*N* = 17	
Improvement[a]	4 (30.8%)	2 (11.8%)	
No change[a]	9 (69.2%)	15 (88.2%)	0.360
Worsening[a]	0 (0)	0 (0)	
Week 12	*N* =10	*N* =15	
Improvement[a]	4 (40.0%)	1 (6.7%)	
No change[a]	6 (60.0%)	13 (86.7%)	0.121
Worsening[a]	0 (0)	1 (6.7%)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	4 (36.4%)	3 (16.7%)	
No change[a]	7 (63.6%)	12 (66.7%)	0.264
Worsening[a]	0 (0)	3 (16.7%)	
BSI-the other microorganisms parameter change from baseline[b]			
Week 12	*N* =10	*N* =15	
Improvement[a]	0 (0)	1 (6.7%)	
No change[a]	10 (100%)	14 (93.3%)	> 0.999
Worsening[a]	0 (0)	0 (0)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	0 (0)	0 (0)	
No change[a]	11 (100%)	17(94.4%)	> 0.999
Worsening[a]	0 (0)	1 (5.6%)	
FACED-the *Pseudomonas aeruginosa* infection parameter change from baseline			

Week 4	*N* = 13	*N* = 17	
Improvement[a]	4 (30.8%)	2 (11.8%)	
No change[a]	9 (69.2%)	15 (88.2%)	0.360
Worsening[a]	0 (0)	0 (0)	
Week 12	*N* =10	*N* =15	
Improvement[a]	4 (40.0%)	1 (6.7%)	
No change[a]	6 (60.0%)	13 (86.7%)	0.121
Worsening[a]	0 (0)	1 (6.7%)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	4 (36.4%)	3 (16.7%)	
No change[a]	7 (63.6%)	12 (66.7%)	0.264
Worsening[a]	0 (0)	3 (16.7%)	
FACED-the mMRC grading parameter change from baseline			
Week 4	*N* = 13	*N* = 17	
Improvement[a]	4 (30.8%)	1 (5.9%)	
No change[a]	9 (69.2%)	15 (88.2%)	0.138
Worsening[a]	0 (0)	1 (5.9%)	
Week 12	*N* =10	*N* =15	
Improvement[a]	2 (20.0%)	0 (0)	
No change[a]	8 (80.0%)	13 (86.7%)	0.141
Worsening[a]	0 (0)	2 (13.3%)	
Week 24	*N* = 11	*N* = 18	
Improvement[a]	1 (9.1%)	1 (5.6%)	
No change[a]	9 (81.8%)	17 (94.4%)	0.470
Worsening[a]	1 (9.1%)	0 (0)	
FACED-the FEV_1 % of predicted parameter change from baseline[c]			
Week 4	*N* = 13	*N* = 17	
Improvement[a]	0 (0)	0 (0)	
No change[a]	13 (100%)	16 (94.1%)	> 0.999
Worsening[a]	0 (0)	1 (5.9%)	

[a]Data were presented as patient number (percentage of patients).

[b]Data of the age, hospitalization, and radiological severity parameters of BSI score at Week 4, 12, and 24, and the other microorganisms parameter at Week 4 were not shown as all patients had no change.

[c]Data of the age and radiological severity parameters of FACED score at Week 4, 12, and 24, and the FEV_1 % of predicted parameter at Week 12 and 24 were not shown as all patients had no change.

Table S9. Baseline characteristics of complete responsive (CR) and non-responsive (NR) patients in the cell treatment group. Related to Figure 4.

Demographics	CR patients (*N* = 4)	NR patients (*N* = 3)	P-value
Age (years)[a]	50.5 ± 14.8	49.0 ± 10.5	0.888
Female gender[b]	3 (75.0%)	2 (66.7%)	> 0.999
Body mass index (kg/m^2)[a]	23.3 ± 1.7	22.3 ± 4.2	0.669
Smokers[b]	0 (0)	1 (33.3%)	0.429
Bronchiectasis characteristics			
Duration of disease (years)[a]	22.0 ± 18.7	14.3 ± 6.7	0.535
FEV_1 (L)[a]	1.0 ± 0.4	1.6 ± 0.7	0.174
D_{LCO} (mmol/min/kPa)[a]	4.6 ± 0.6	6.1 ± 1.3	0.085
6MWD (m)[a]	443.5 ± 123.5	411.0 ± 92.7	0.720
SGRQ score[a]	45.9 ± 13.7	51.1 ± 22.4	0.718
BSI score[a]	8.3 ± 4.0	10.8 ± 1.5	0.311
FACED score[a]	3.5 ± 1.3	2.3 ± 1.5	0.322
mMRC grading[c]	1.5 (1.0, 2.5)	2.0 (2.0, 2.5)	0.400
Etiology			
Post infection[b]	1 (25.0%)	1 (33.3%)	> 0.999
Idiopathic[b]	3 (75.1%)	2 (66.7%)	
Comorbidities			
COPD[b]	1 (25.0%)	1 (33.3%)	> 0.999
Chronic rhinitis or sinusitis[b]	0 (0)	1 (33.3%)	0.429
Hypertension[b]	1 (25.0%)	0 (0)	> 0.999
Diabetes[b]	0 (0)	1 (33.3%)	0.429
Quality BALF culture			
Pseudomonas aeruginosa[b]	3 (75.0%)	1 (33.3%)	0.486
No bacterial growth[b]	1 (25.0%)	2 (66.7%)	
Medication for bronchiectasis			
Oral antibiotics[b]	3 (75.0%)	2 (66.7%)	> 0.999
Inhaled bronchodilator[b]	3 (75.0%)	1 (33.3%)	0.486
Mucolytics[b]	2 (50.0%)	2 (66.7%)	> 0.999

[a]Data were presented as mean ± standard deviation (SD).

[b]Data were presented as patient number (percentage of patients).

[c]Data were presented as median (interquartile range, IQR).

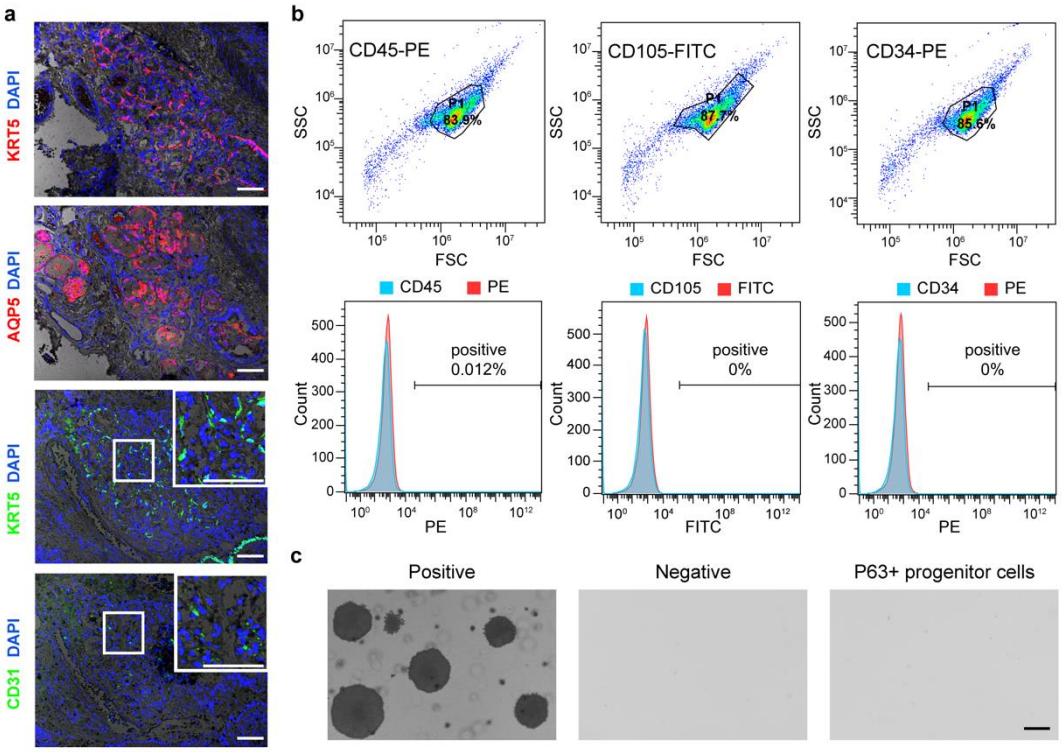


Fig. S1 Immunofluorescence images and quality control of cultured lung progenitor cells. Related to Figure 1. a, Immunofluorescence staining showing the expression of KRT5, AQP5, and CD31 with nuclear staining using DAPI (blue). KRT5 expression (red) and AQP5 expression (red) are shown in the same field for Patient #5. KRT5 expression (green) and CD31 expression (green) are shown in the same field for Patient #3. Scale bar, 50 μm. **b,** FACS gating strategy for purity test by immunostaining with anti-CD45, CD105, and CD34 antibodies. **c,** Soft agar assay, also known as a tumorigenicity test, showing the lack of tumor formation by $P63^{+}$ lung progenitor cells. Human melanoma cells served as a positive control, while growth-arrested 3T3 cells served as a negative control. Scale bar, 200 μm.

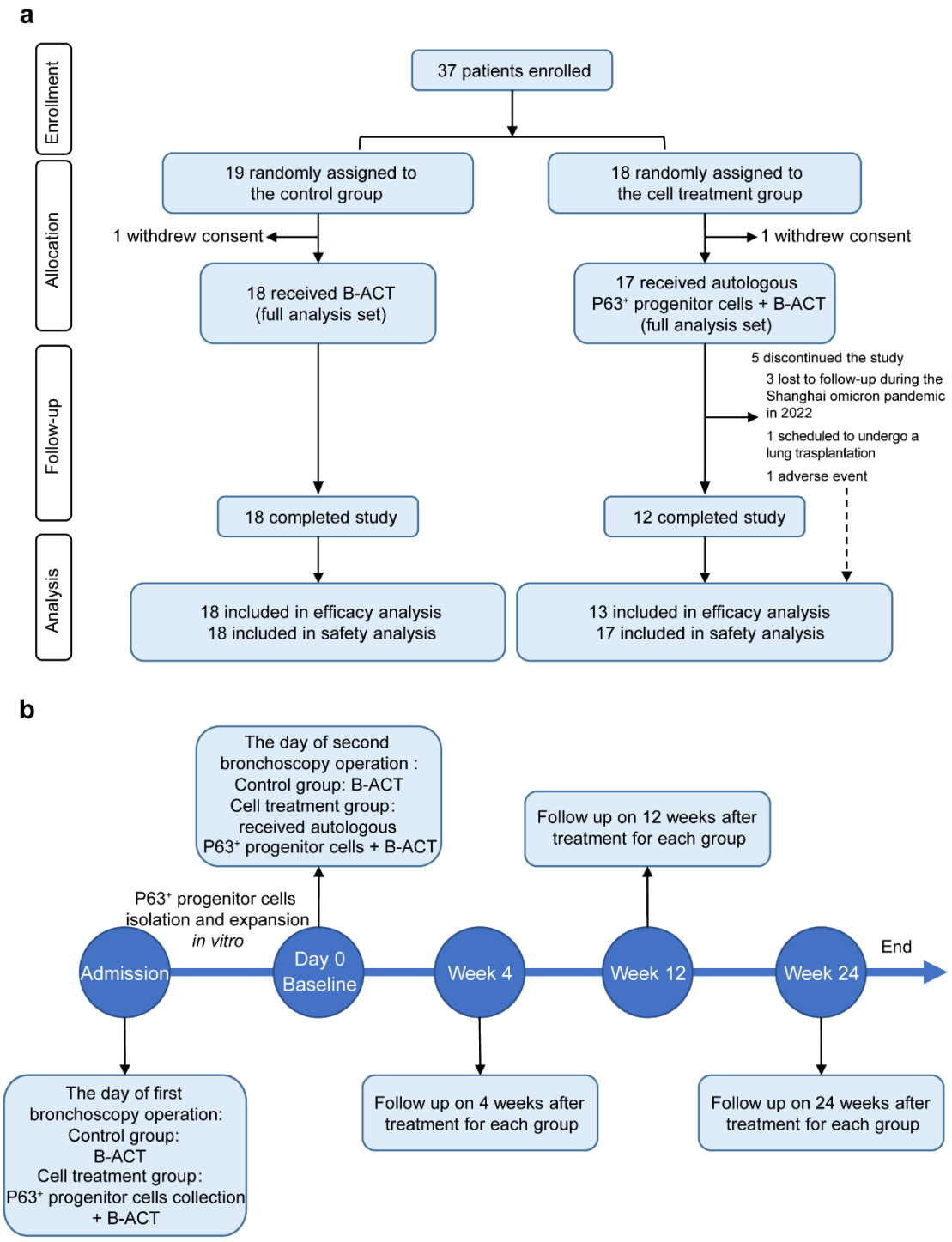


Fig. S2 Flow diagram and timeline for the study design. Related to STAR Methods. a, The detailed procedure of the study. **b**, The timeline of the study. B-ACT, bronchoscopic airway clearance therapy.

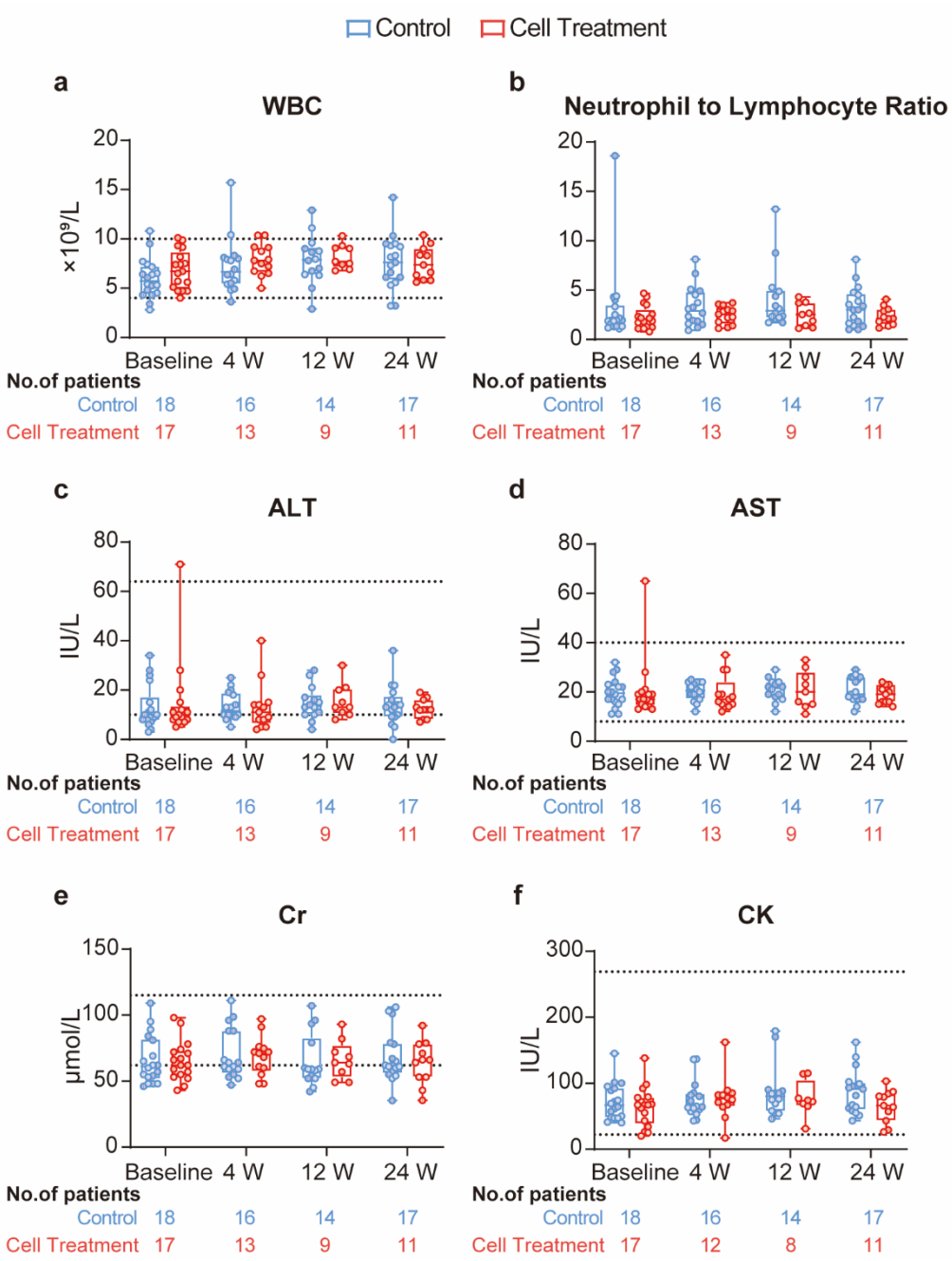


Fig. S3 Boxplot showing changes in clinical laboratory evaluations following cell therapy. Related to Table 2. The horizontal line within each box represents the median value; the bottom and top lines of the box represent the 25th and 75th percentiles, respectively; and the horizontal lines below and above the box represent the lowest and highest values, respectively.

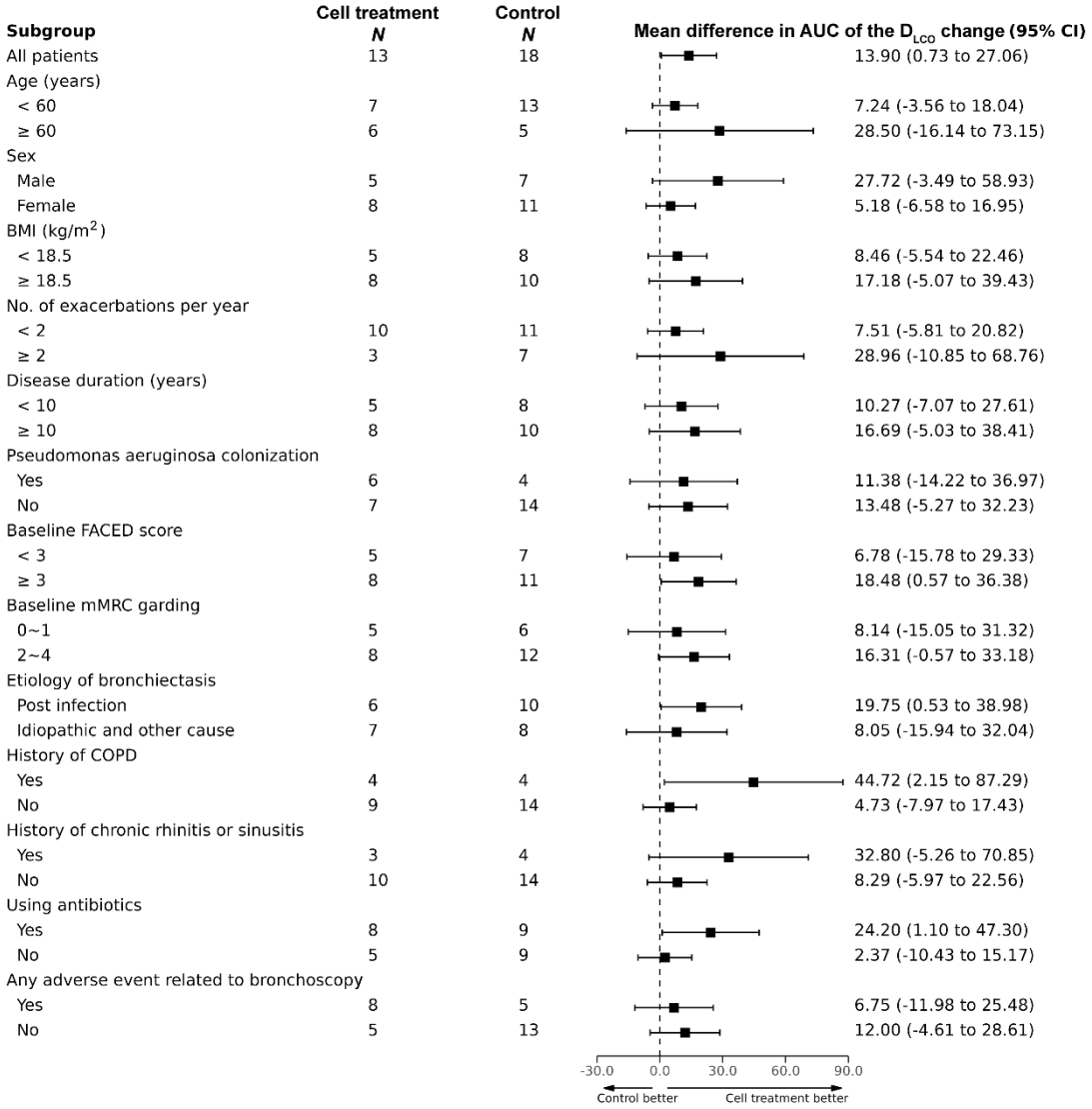


Fig. S4 Forrest plots of the AUC of the D_{LCO} change from baseline to 24 weeks in subgroup analysis. Related to Figure 2. AUC, the area under the curve; BMI, body mass index; mMRC, modified British Medical Research Council; COPD, chronic obstructive pulmonary disease.

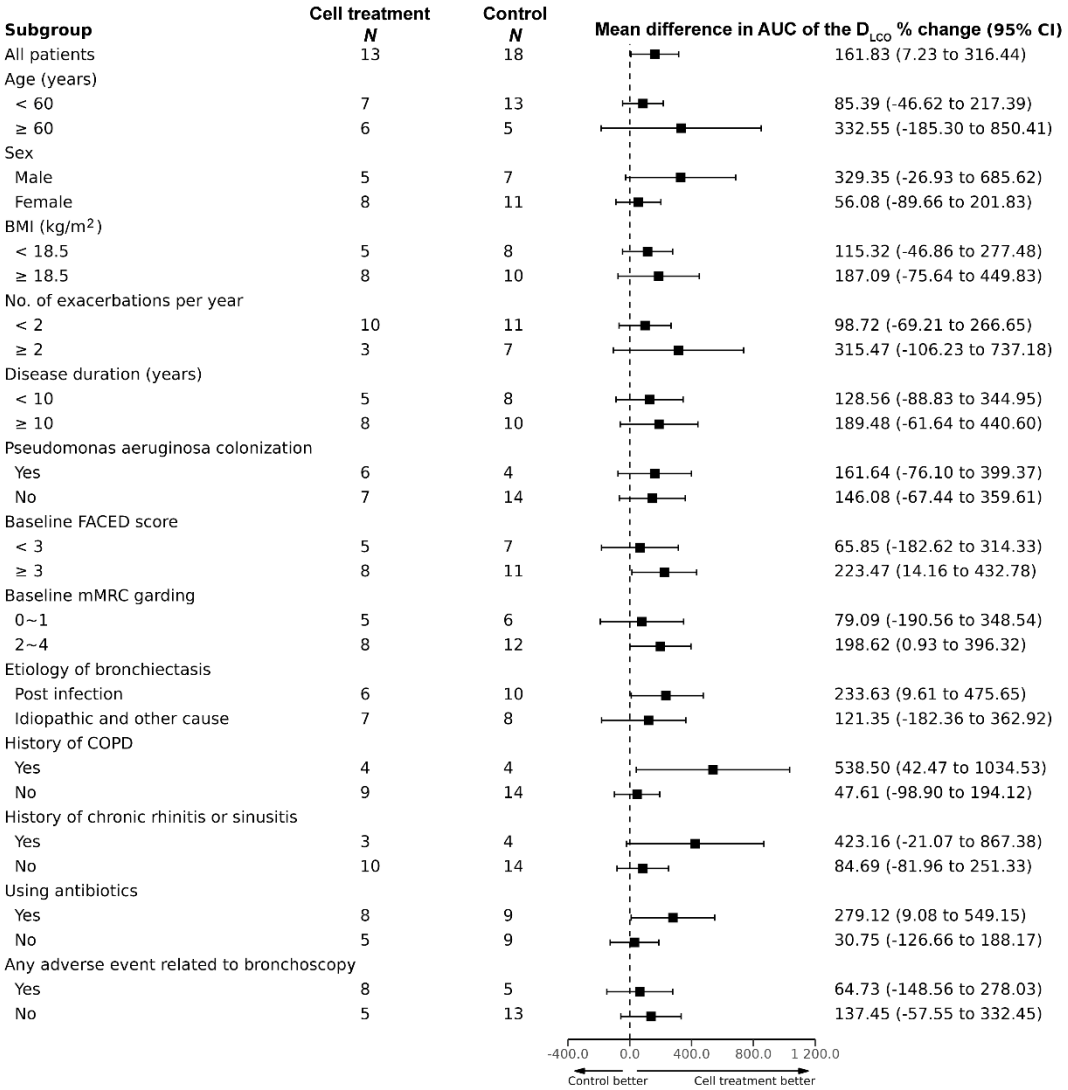


Fig. S5 Forrest plots of the AUC of the D_{LCO} % of predicted value change from baseline to 24 weeks in subgroup analysis. Related to Figure 2. AUC, the area under the curve; BMI, body mass index; mMRC, modified British Medical Research Council; COPD, chronic obstructive pulmonary disease.

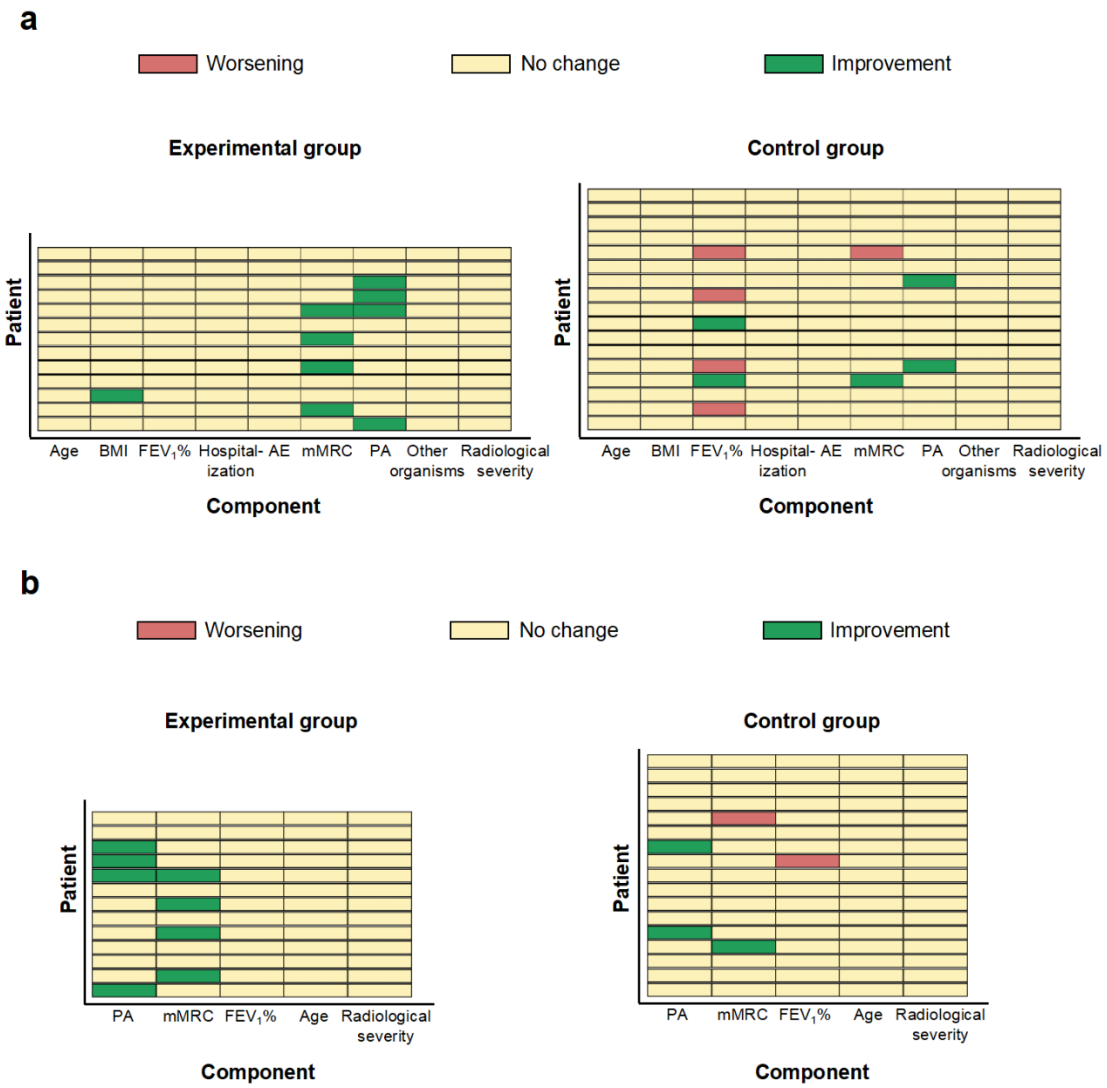


Fig. S6 Components of BSI and FACED scores at Week 4 for patients in two groups. Related to Figure 2 and Table S8. Components of BSI score (**a**) and FACED score (**b**) at Week 4 for patients in the experimental group (left) (n = 13) and the control group (right) (n = 17). Each row represents an individual patient. Green represents improvement, yellow no change, and red worsening, as compared with baseline values. BMI, body mass index; FEV_1, forced expiratory volume in 1 second; AE, acute exacerbation; mMRC, modified British Medical Research Council; PA, *Pseudomonas aeruginosa*.

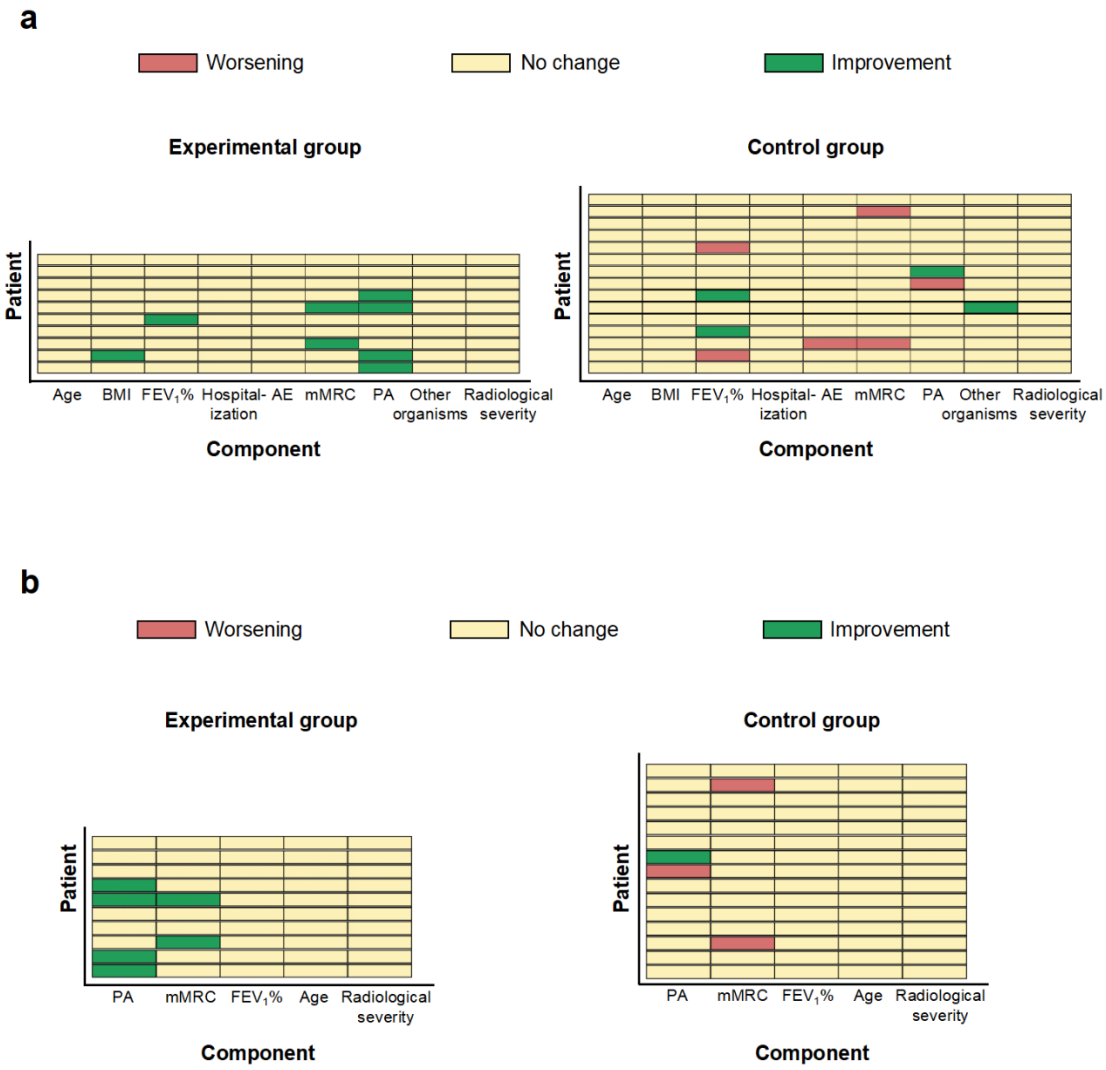


Fig. S7 Components of BSI and FACED scores at Week 12 for patients in two groups. Related to Figure 2 and Table S8. Components of BSI score (**a**) and FACED score (**b**) at Week 12 for patients in the experimental group (left) (n = 10) and the control group (right) (n = 15). Each row represents an individual patient. Green represents improvement, yellow no change, and red worsening, as compared with baseline values. BMI, body mass index; FEV_1, forced expiratory volume in 1 second; AE, acute exacerbation; mMRC, modified British Medical Research Council; PA, *Pseudomonas aeruginosa*.

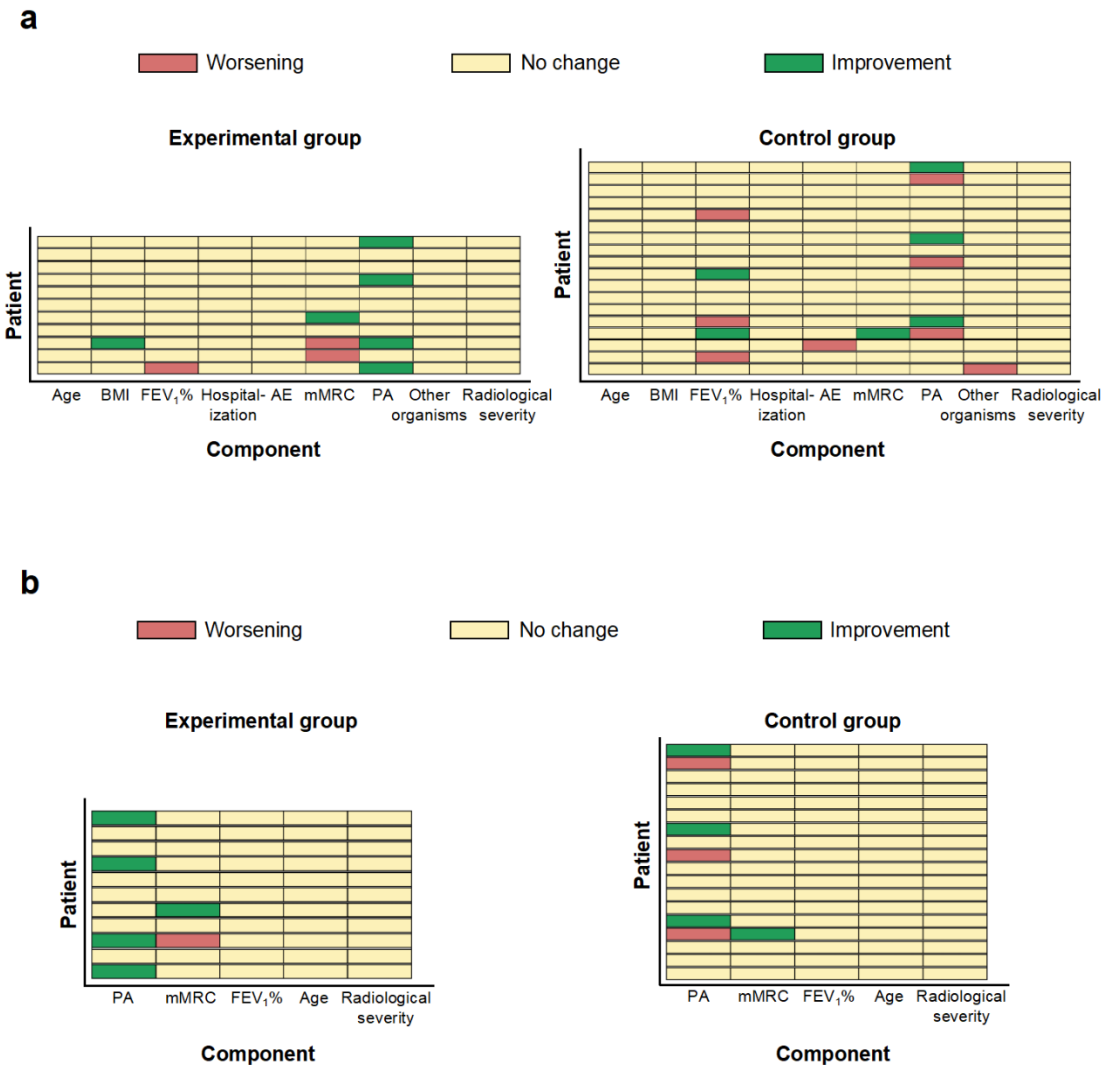


Fig. S8 Components of BSI and FACED scores at Week 24 for patients in two groups. Related to Figure 2 and Table S8. Components of BSI score (**a**) and FACED score (**b**) at Week 24 for patients in the experimental group (left) (n = 11) and the control group (right) (n = 18). Each row represents an individual patient. Green represents improvement, yellow no change, and red worsening, as compared with baseline values. BMI, body mass index; FEV_1, forced expiratory volume in 1 second; AE, acute exacerbation; mMRC, modified British Medical Research Council; PA, *Pseudomonas aeruginosa*.

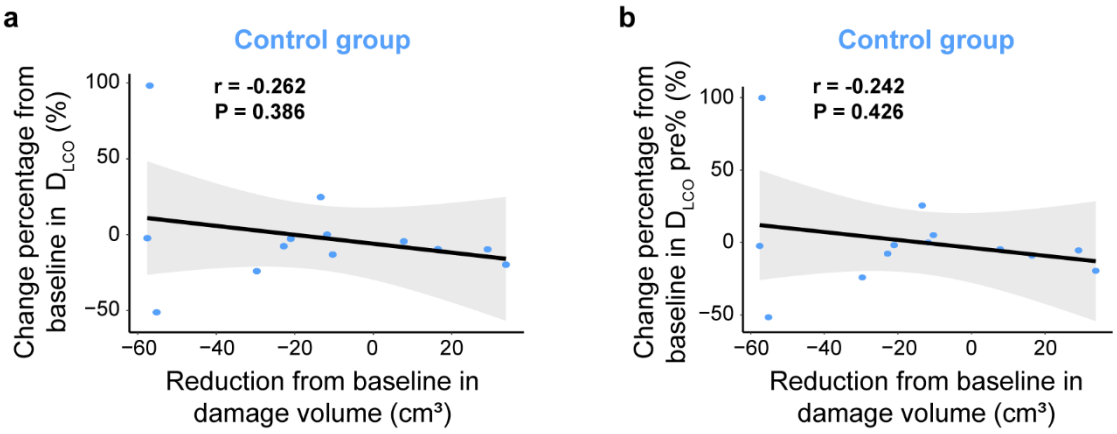


Fig. S9 Pearson correlations between the change from baseline to 24 weeks in the damaged volume and diffusing capacity in the control group. Related to Figure 3. Each dot represented an individual patient. Reduction from baseline = -1 × (change from baseline).

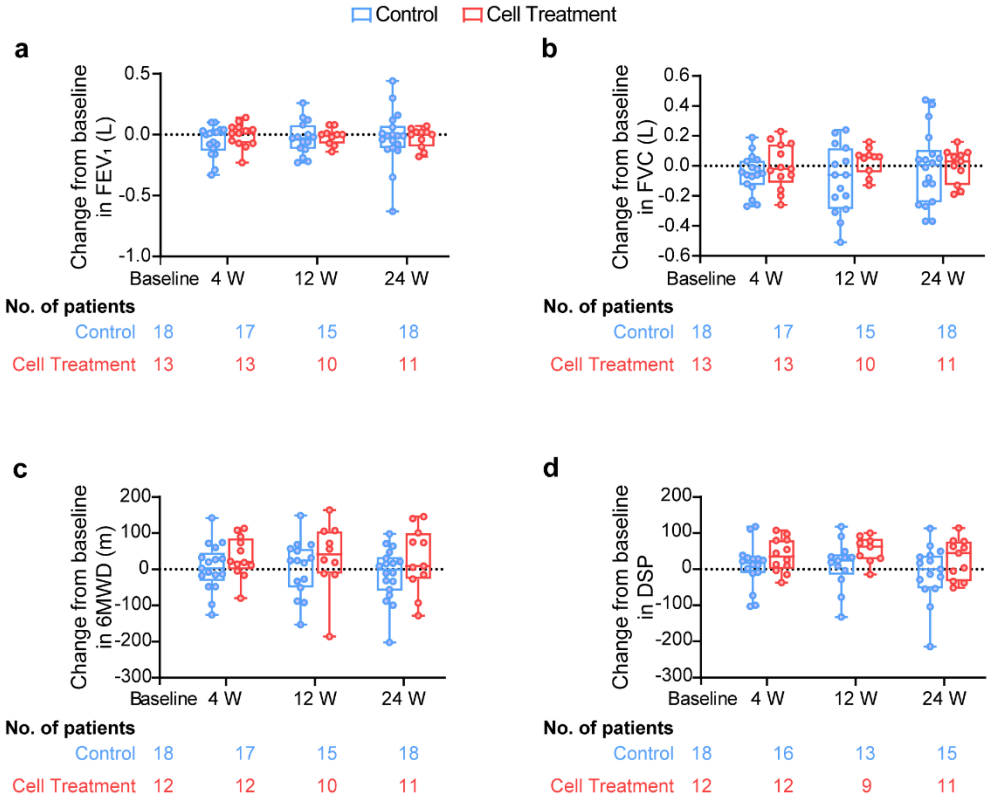


Fig. S10 Boxplot showing changes in FEV_1, FVC, 6MWD, and DSP at different time points. Related to Table S7. a, Changes in FEV_1 at Week 4, 12, and 24. **b**, Changes in FVC at Week 4, 12, and 24. **c**, Changes in 6MWD at Week 4, 12, and 24. **d**, Changes in DSP at Week 4, 12, and 24.

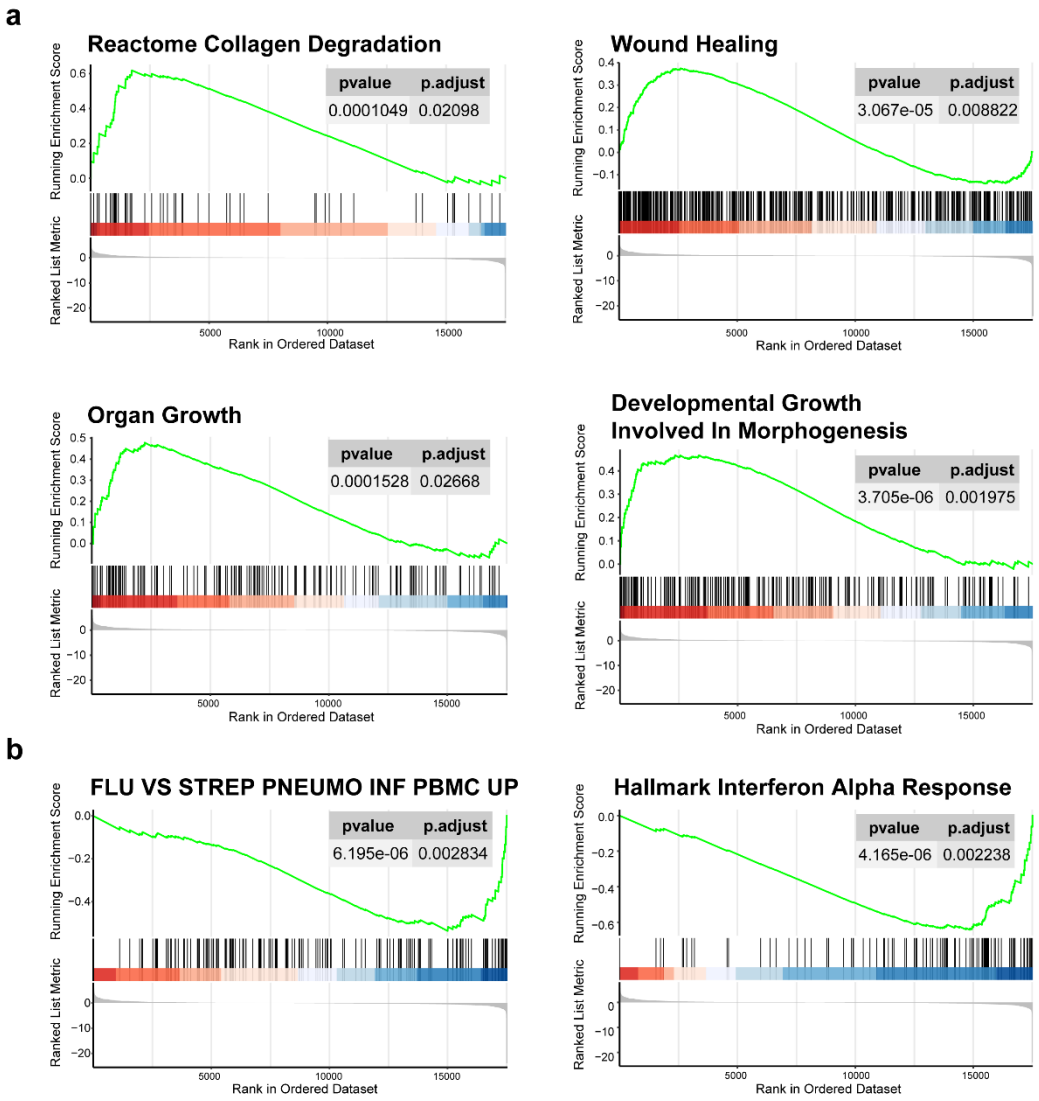


Fig. S11 Gene set enrichment analysis (GSEA) of RNA-Seq data from complete responsive (CR) and non-responsive (NR) progenitor cells. Related to Figure 4. a, b, GSEA showed that the CR (**a**) and NR (**b**) group was enriched in MSigDB gene sets compared to the NR and CR group (CR, n = 4; NR, n = 3).

Data S1: Clinical trial protocol of the study, related to the STAR Methods.

An Exploratory Study on Autologous Bronchial Basal Cell Transplantation for the Treatment of Bronchiectasis

Version 4.0 – March 2, 2020

Sponsor: Ruijin Hospital, Shanghai Jiao Tong University School of Medicine

Principal investigator: Jie-Ming Qu

Ethics Reference Number: 2018-10-5

ClinicalTrials. gov: NCT03655808

Confidentiality Statement:

The information contained in this research proposal is provided solely for the review of the researchers involved in this project, the ethics committee, and relevant institutions. It is strictly prohibited to disclose any information to third parties not associated with this research without the approval of the Principal Investigator (PI).

TABLE OF CONTENTS

1 INTRODUCTION

Bronchiectasis is a chronic respiratory disease characterized by permanent and irreversible bronchial wall dilation and thickening. There has been a remarkable increase in its incidence and prevalence during the past 20 years. The latest statistics estimated that over 1.5% of women and 1.1% of men in the general population have physician-diagnosed bronchiectasis in China. Patients with bronchiectasis usually present with chronic cough and sputum production, and their clinical course is characterized by intermittent exacerbations, which can eventually develop into respiratory failure, causing loss of work ability and self-care ability, and even death. The condition worsens progressively and irreversibly, and the socioeconomic burden of the disease has also been increasing.

Current commonly used treatment methods in clinical practice include antibiotics, mucoactive agents, bronchodilators and corticosteroids, and airway clearance therapy. However, traditional treatments only provide symptomatic relief and fail to fundamentally solve the problem of lung structural damage. Lobes resection surgery is one of the treatments for bronchiectasis with poor prognosis, but this technology has relatively short development time, unclear indications and efficacy, and a high incidence of complications.

1.1 STUDY RATIONALE

Transplant therapy, which includes mature organ, tissue, and emerging cell transplant, is the main option for treating end-stage organ failure diseases so far. However, organ and tissue transplants come with significant disadvantages, such as the severe shortage of sources and obvious immune rejection. Allogeneic transplants, which are the most common type, often lead to varying degrees of immune rejection reactions. The use of immunosuppressants to treat this increases the risk of various opportunistic infections. Additionally, organ and tissue transplants require long surgical time, high technical requirements, and complex procedures. In the field of lung disease, the number of lung transplant surgeries performed in China in 2016 was less than 300, which was far from meeting the huge demand of patients.

Cell transplantation technology, particularly hematopoietic cell transplantation (transfusion and bone marrow transplantation), skin cell transplantation, and corneal cell transplantation, have been successfully used in clinical treatment and are increasingly valued by the medical community. These technologies provide a promising alternative to organ and tissue transplants and could help mitigate the current shortage of sources. Cell therapy has been utilized for the treatment of blood-related diseases, such as blood transfusion and bone

marrow transplantation since the 1970s. Over the years, it has been increasingly applied to other tissue and organ diseases, such as skin cell transplantation for burns, mesenchymal stem cell transplantation for liver failure and metabolic diseases, and limbal stem cell transplantation for corneal injuries. In cell therapy, different types of cells were cultured and used for distinct purposes, such as to enhance immune function, eliminate pathogens and tumor cells, promote tissue and organ regeneration, and aid in disease treatment.

Autologous stem/progenitor cell therapy has several advantages: 1) since the cells are sourced from the patient's own body, there is no risk of immune rejection; 2) there is no risk of tumorigenesis as the cells come from adult tissues and organs that are already part of the patient's body; 3) cells have plasticity and can actively divide and migrate to supplement apoptotic or necrotic cells of the same tissue or type in an appropriate environment; 4) the administrative procedure is simple and no complicated surgery is needed; and 5) cell transplantation can help tissue regeneration by multiple mechanisms: migrating to damaged tissue sites and differentiating into normal tissue cells, playing a role in repairing the damaged tissue, while at the same time, activating paracrine mechanisms by secreting various anti-inflammatory factors and inhibiting pro-inflammatory factor secretion. In conclusion, cell transplantation, with its advantages of good therapeutic effect, minimal side effects, personalized

and precision therapy, is currently being applied to the treatment of various diseases, including blood system diseases, tumors, diabetes, cardiovascular diseases, and nervous system diseases.

1.2 BACKGROUND

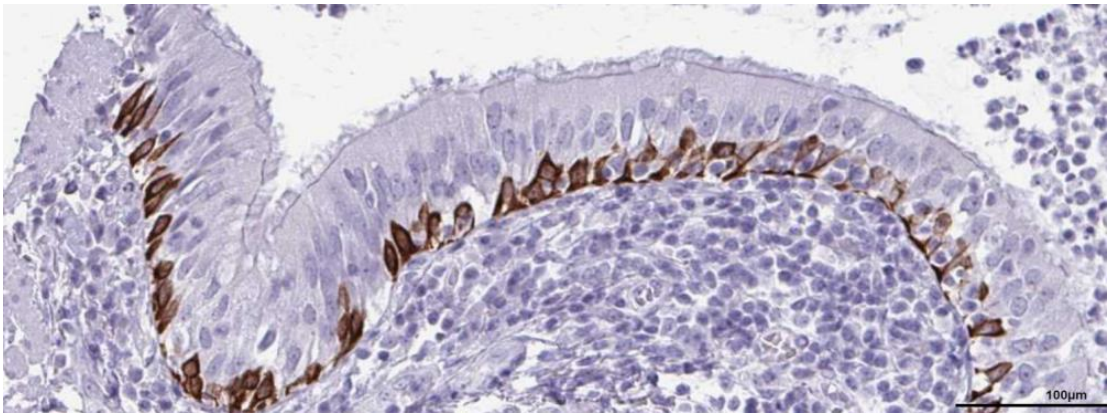
Recent high-level international scientific research has shown that a special population of **bronchial basal cells (also called KRT5^{+}/P63^{+} distal airway stem cells or lung basal progenitor cells in some literature)** in the lungs can regenerate and repair damaged tissue (Hong, *et.al.*, *AJP*, 2004; Kumar, *et.al.*, *Cell*, 2011; Zuo, *et.al.*, *Nature*, 2015; Vaughan, *et.al.*, *Nature*, 2015), making them a promising type of "seed" cells for the treatment of lung tissue damage that cannot be naturally repaired by the body. These cells are located in the basal layer of the airway epithelium and specifically express the KRT5 and P63 antigens. They function as adult tissue stem/progenitor cells in lung, and are relatively active in cell division and migration, could produce cells to replace other types of epithelial cells that have been dismissed. They have plasticity and can directly repair the structure of bronchi and alveoli epithelium.

Such bronchial basal cells can be obtained by bronchoscopic brushing, and isolated, purified, and extensively

expanded by appropriate methods. It has been proven that bronchial basal cell transplantation can directly repair damaged lungs in experimental animal models. Since bronchial basal cells are derived from autologous tissue, there is no immune rejection issue. Also, because bronchial basal cells are derived from adult tissue organs and are themselves part of the body, there is no risk of tumor formation. In the animal experiment, even when 100 times the human dose of bronchial basal cells was administered *via* the trachea in animals, there was no tumor formation observed.

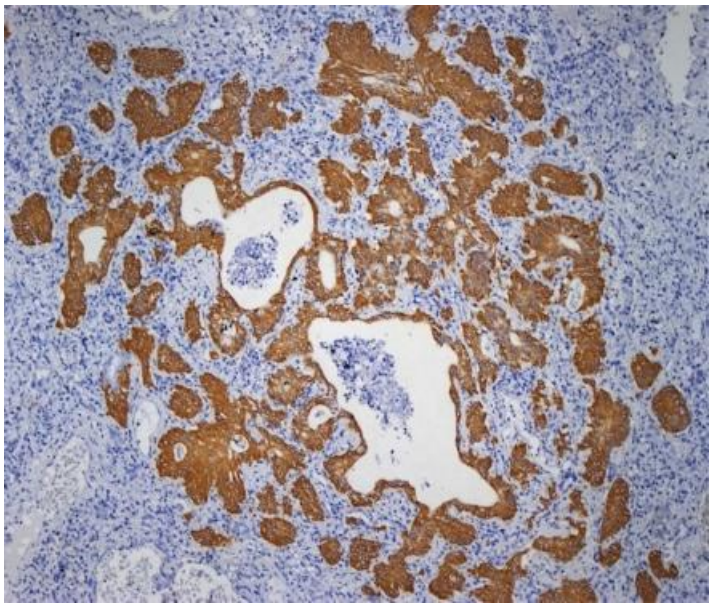
1.2.1 IDENTIFICATION AND CHARACTERIZATION OF BRONCHIAL BASAL CELL

Recent studies have revealed the existence of bronchial basal cells located in the basal layer of the bronchi in the lungs. These cells express the KRT5 marker gene, as well as the P63 gene. Although they are relatively rare in mouse lungs, they are widespread in human lungs. Please refer to the image below for more details:

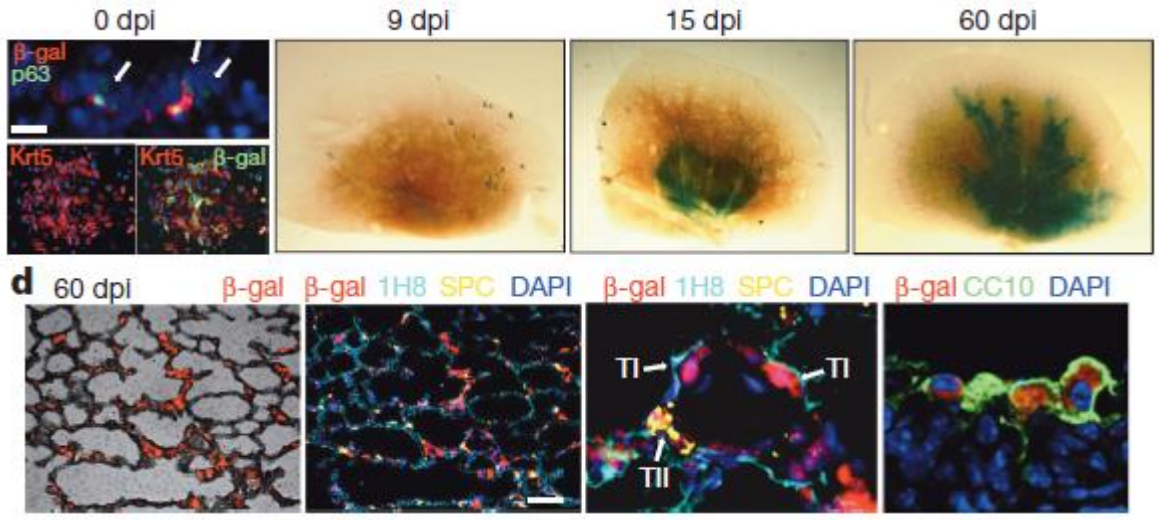


Human bronchial basal cells (KRT5 IHC staining)

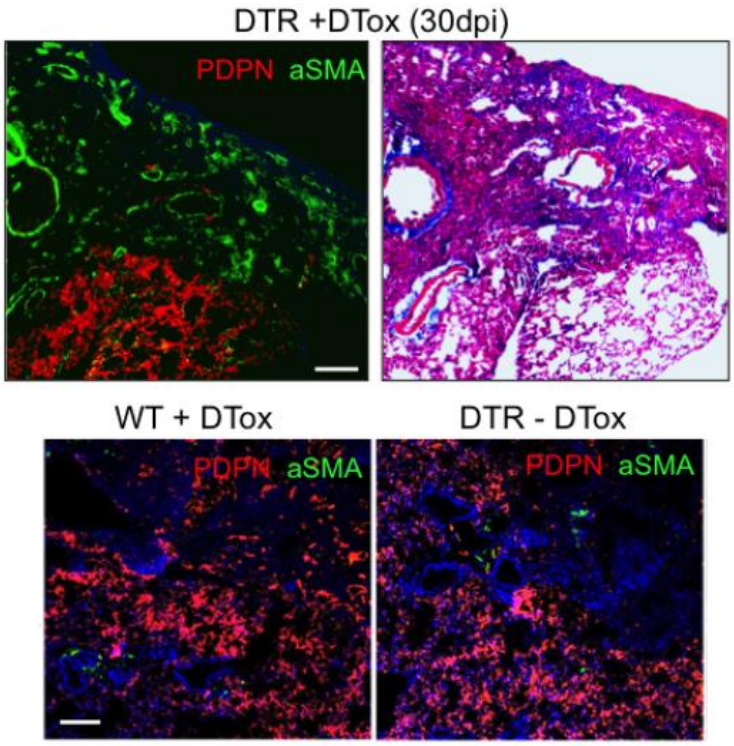
Importantly, we have observed a significant proliferation of $KRT5^{+}$ bronchial basal cells in the bronchial basal layer in the injury area of ARDS patient lungs, which has begun to form bronchiole and air sac-like structures as shown in the figure below. This suggests the potential role of bronchial basal cells in lung repair.



To prove the potential of bronchial basal cells to differentiate into mature alveolar and bronchial epithelial cells, Professor Wei Zuo's team utilized genetic lineage tracing to track the proliferation, migration, and differentiation of bronchial basal cells in mice following influenza virus infection. This demonstrated that these cells are indeed capable of repairing lung tissue. The results are shown in the figure below (Zuo, *et.al.*, *Nature*, 2015).



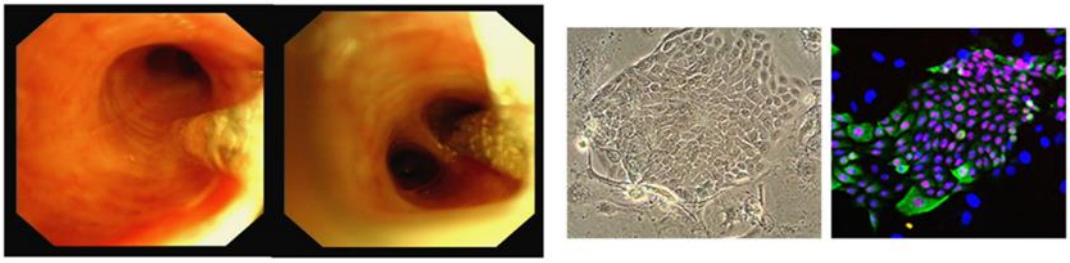
Further animal experiments have also demonstrated that if the activated bronchial basal cells were genetically eliminated using a diphtheria toxin receptor (DTR) in mice (DTR + DTox group), not only could lung function not be repaired, but tissue fibrosis could also occur. It can be inferred that maintaining a sufficient number of bronchial basal cells in the body is essential for repairing the lungs and inhibiting the occurrence of lung fibrosis, as shown in the figure below:



1.2.2 COLLECTION, ISOLATION, AND CULTURE OF HUMAN BRONCHIAL BASAL CELLS

Our team obtained small amounts of bronchial basal cells through bronchoscopic brushing. The obtained tissue was washed and digested into single-cell suspension, which was then cultured using a patented technology that simulates the basal layer environment *in vitro* using a combination of growth factors. This system selectively amplified bronchial basal cells, while other types of mature epithelial cells and fibroblasts could not grow and would naturally undergo apoptosis. After a period of expansion, bronchial basal cells could be stored in liquid nitrogen cell banks for long-term preservation. Before being used

in subjects, bronchial basal cells underwent a series of strict tests, including microbial contamination detection, cell morphology, cell viability, genetic characteristics, and KRT5 marker gene detection, *etc*. The bronchial basal cells obtained from brushing and P_0 culture are shown in the figure below:



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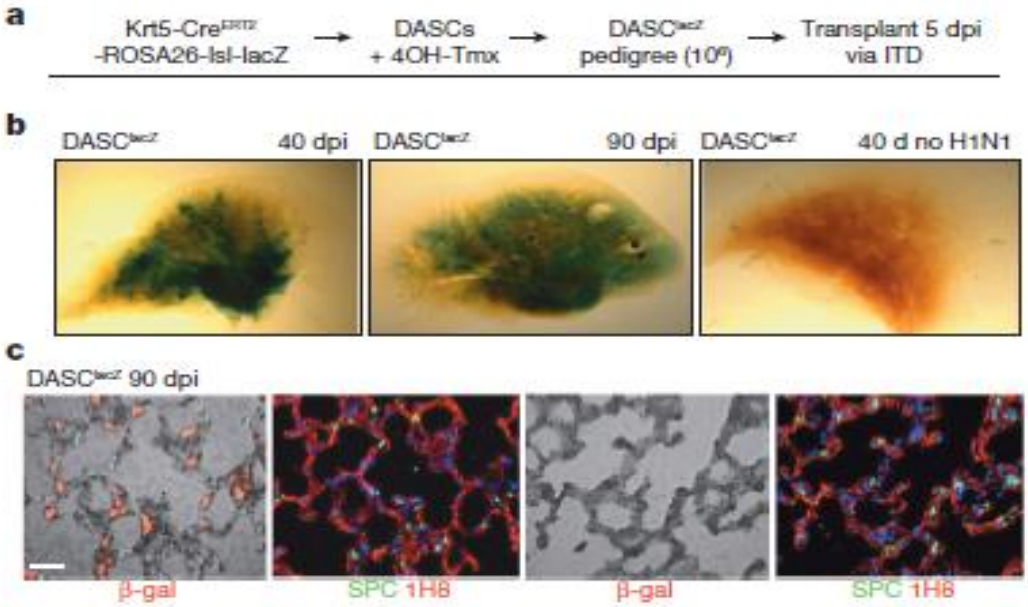
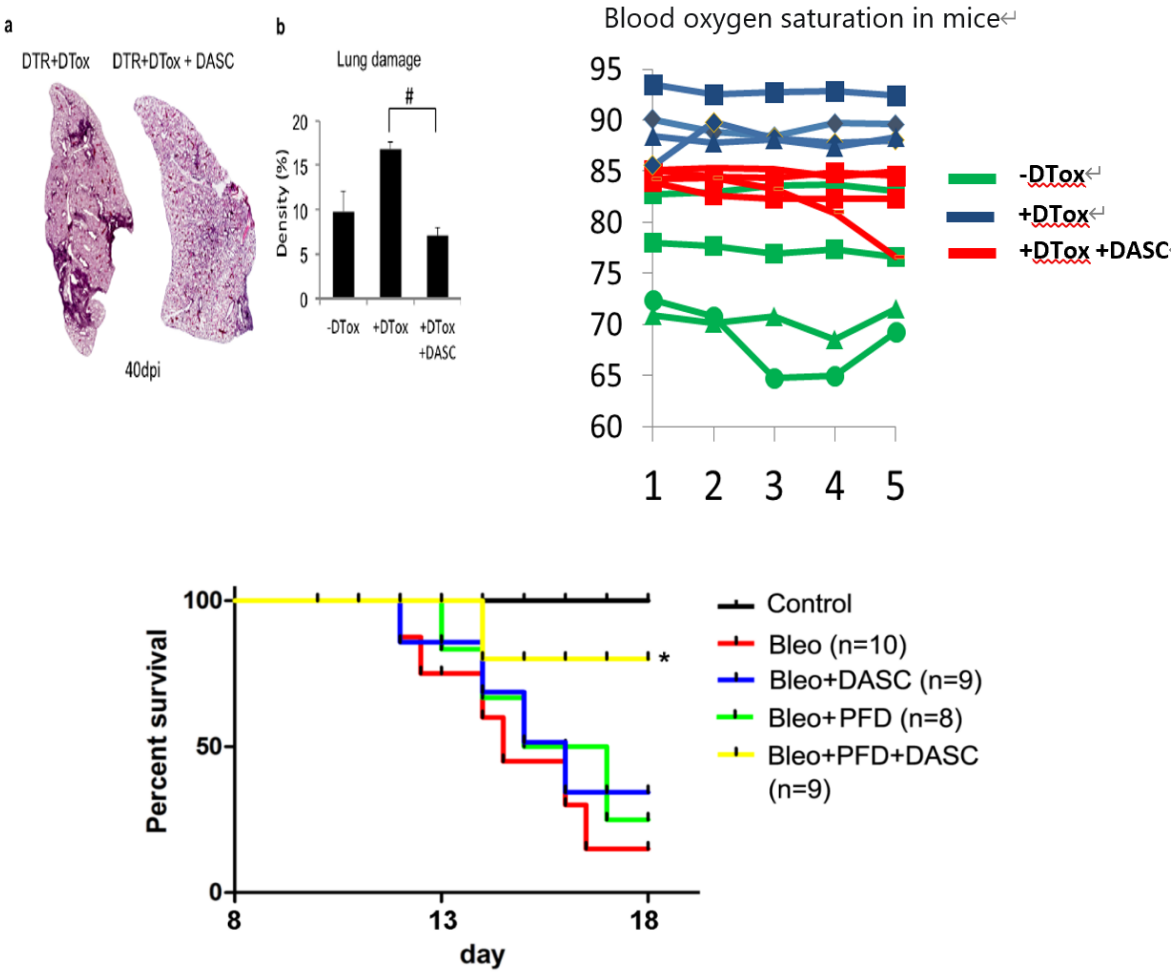
1.2.3 MOUSE BRONCHIAL BASAL CELL TRANSPLANTATION (ARDS MODEL BASED ON INFLUENZA VIRUS)

In the mouse model of ARDS induced by the H_1N_1 influenza virus, we genetically labeled bronchial basal cells with blue LacZ and then transplanted them into the mouse lungs *via* the trachea. We observed that the donor cells integrated extensively into the recipient's lungs and differentiated into mature alveoli and bronchial structures. It is worth noting that the transplantation of bronchial basal cells into the lungs of healthy mice without injury was completely unsuccessful, which to some extent guarantees the relative safety of transplantation. The figure below shows the data (Zuo, *et.al.*, *Nature*, 2015):



For ARDS mice whose lung injuries could not be naturally repaired due to the lack of bronchial basal cells, the basal cell

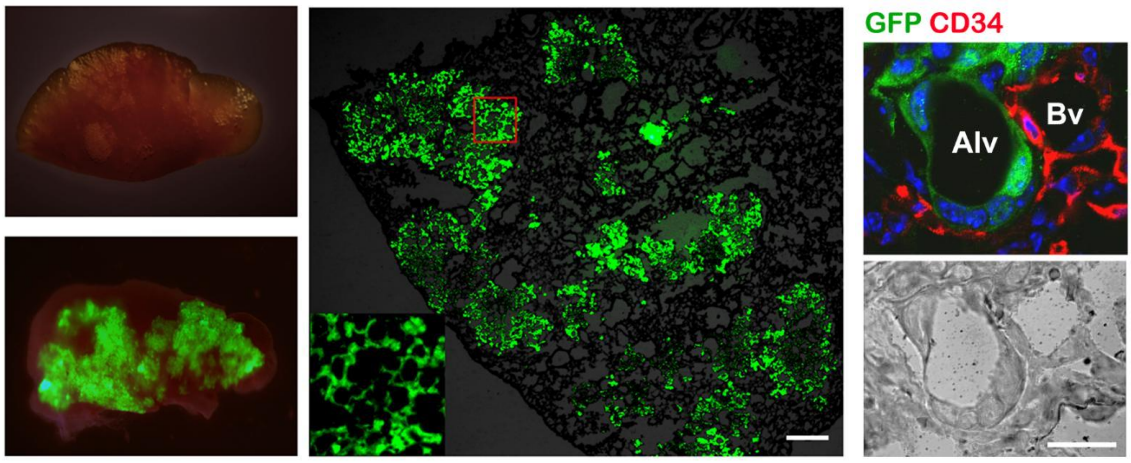
transplantation helped restore their morphological structure and function (blood oxygen saturation). At the same time, transplanting bronchial basal cells in lung-injured mice treated with bleomycin significantly increased their survival time. The figure below shows this:



1.2.4 HUMAN BRONCHIAL BASAL CELL TRANSPLANTATION (BASED ON BLEOMYCIN-INDUCED PULMONARY FIBROSIS MODEL)

In the mouse model of lung injury induced by bleomycin, human bronchial basal cells (marked with GFP fluorescence)

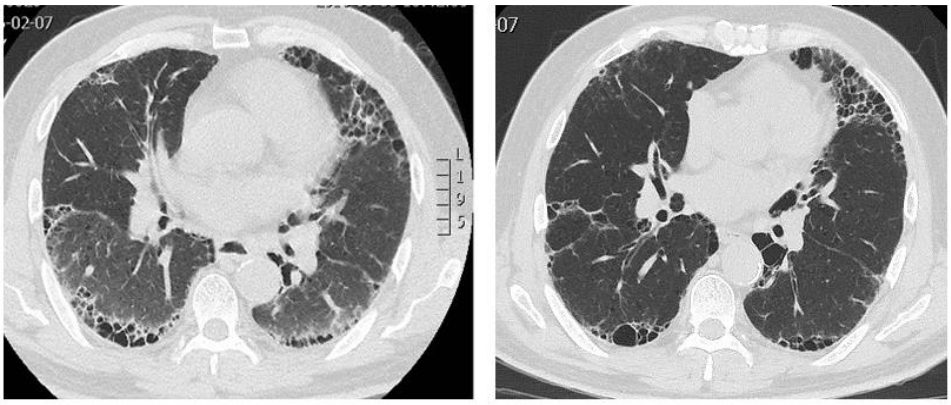
transplanted into the injured area of the recipient's lungs were observed to integrate extensively three weeks after transplantation. They differentiated into mature human alveolar epithelium and bronchial structures. The newly generated human bronchiole-alveolar epithelium recruited surrounding capillaries, reconstructing functional units with gas exchange ability. Importantly, by rebuilding the epithelial tissue structure, the transplantation of bronchial basal cells effectively reduced the proliferation and activation of fibroblasts (α-SMA positive) in the lungs. The transplanted bronchial basal cells and their differentiated offspring in the body survived almost indefinitely. The process is shown in the figure below:



1.2.5 PILOT CLINICAL TRIAL OF AUTOLOGOUS BRONCHIAL BASAL CELL TRANSPLANTATION FOR THE TREATMENT OF INTERSTITIAL LUNG DISEASE

In 2016, Regend Therapeutics collaborated with Shanghai East Hospital to investigate the potential of autologous bronchial basal cell transplantation therapy. The treatment was administered to a 59-year-old male who was diagnosed with interstitial lung disease at the First Affiliated Hospital of Guangzhou Medical University in 2015. In April 2016, this subject underwent autologous bronchial basal cell transplantation therapy at Shanghai East Hospital. Three weeks post-transplantation, CT imaging revealed significant improvement in interstitial lesions (as depicted in the figure below). Various lung function indicators, such as FVC (forced vital capacity), FEV_1 (forced expiratory volume in one second), and D_{LCO} (diffusing capacity of the lungs for carbon monoxide), demonstrated varying degrees of enhancement. Additionally, the 6-minute walk test significantly improved, and symptoms of wheezing exhibited significant improvement.

Before transplantation　　3 weeks post transplantation



As of December 2016, a total of five cases for autologous bronchial basal cell transplantation therapy for interstitial lung disease had been completed at Shanghai East Hospital. The results showed exceptionally high levels of safety and promising preliminary efficacy.

1.2.6 PILOT CLINICAL TRIAL OF AUTOLOGOUS BRONCHIAL BASAL CELL TRANSPLANTATION FOR THE TREATMENT OF BRONCHIECTASIS

After obtaining approval from the ethics committee of the Southwest Hospital of the Third Military Medical University of PLA, two patients with bronchiectasis (one of whom had comorbid COPD) received autologous bronchial basal cell transplantation therapy in our department in 2016. Three months after treatment, we observed significant improvements in various pulmonary function indicators, including FVC, VC MAX, FEV_1, and D_{LCO}, as well as a decrease in C-reactive protein levels and a significant increase in the 6-minute walk test. Furthermore, according to the subjects' self-reports, their symptoms were significantly relieved, as shown in the table below:

	FVC (%Pred)			FEV1(%Pred)			VC MAX (%Pred)			TLC-SB (%Pred)		
Days post transplantation	-1	30	90	-1	30	90	-1	30	90	-1	30	90
Patient 1	69.4	58.3	79.5	36.6	31.9	47.5	74.1	62.1	81.5	75.9	120.6	88.2
Pateint 2	24.3	61.2	62.4	20.8	34.6	33.3	24.8	62.2	60.4	22.2	67.6	71.9

	RV-SB (%Pred)			FRC-SB (%Pred)			DLCOc/VA (%Pred)			6MWT (m)		
Days post transplantation	-1	30	90	-1	30	90	-1	30	90	-1	30	90
Patient 1	94.5	245.0	116.0	71.9	194.3	89.7	128.2	136.3	120.2	453	504	558
Pateint 2	15.7	73.1	101.0	40.7	112.0	120.3	14.9	103.9	97.3	399	453	468

1.2.7 OTHER RELEVANT STUDIES

Currently, there are several clinical studies registered on ClinicalTrials.gov investigating the use of autologous and allogeneic mesenchymal stem cell transplantation therapy, including adipose-derived stem cells and bone marrow-derived mesenchymal stem cells, for the treatment of COPD. Nine clinical trials have been registered so far (NCT02645305, NCT02216630, NCT00683722, NCT01110252, NCT02161744, NCT02348060, NCT02041000, NCT02412332, NCT01559051), with six being conducted in the United States, two in Brazil, and one in Vietnam. Two small-sample studies using allogeneic mesenchymal stem cell transplantation therapy for COPD in the United States and Brazil have been completed and reported high safety levels with almost no adverse reactions, but they did not show significant improvement in lung function or other therapeutic endpoints. In contrast, our preliminary attempts at autologous bronchial basal cell transplantation therapy in our hospital have demonstrated significant improvements in lung function indicators such as FEV_1, FVC, MMEF, MVV, 6-minute

walk test (6MWT), and St. George's Respiratory Questionnaire (SGRQ) score. These results suggest that by fundamentally repairing the damaged lung structure of COPD patients, autologous bronchial basal cell transplantation therapy may help to restore lung function.

2 RESEARCH OBJECTIVES

The objective of this study is to evaluate the safety and efficacy of autologous bronchial basal cell transplantation in the treatment of bronchiectasis.

3 STUDY DESIGN

3.1 OVERALL DESIGN

This trial is a randomized, single-blind, controlled pilot study.

3.2 NUMBER OF PARTICIPANTS

A total of 76 participants are expected to be enrolled and randomly assigned to the control or cell treatment group at a 1:1 ratio.

3.3 RANDOMIZATION AND MASKING

Eligibility patients will be assigned according to a random number table, with sequentially numbered in a 1:1 ratio generated by computer, to receive either B-ACT + autologous bronchial basal cell transplantation therapy (cell treatment group) or B-ACT therapy (control group). The opaque sealed envelope method will be used to conceal the allocation sequence. Both patients and investigators, except for the bronchoscopy

operators, remain masked to the treatment assignment for the duration of the study. That is, only the investigators who perform the bronchoscopy are unblinded. The non-blinded investigators should not disclose any blind information to other investigators, participants, or clinic staff.

4 STUDY POPULATION

4.1 DIAGNOSTIC CRITERIA

The diagnosis will be based on the 2019 British Thoracic Society (BTS) guidelines for bronchiectasis.

4.2 INCLUSION CRITERIA

- 18~75 years, outpatients with chronic cough, sputum production, and a clinical diagnosis of bronchiectasis (confirmed by chest high-resolution computed tomography [HRCT] scan);
- Remaining clinically stable (respiratory symptoms not significantly exceeding the daily variations, and in the absence of acute exacerbation of bronchiectasis or acute upper respiratory tract infection within the previous 2 weeks);
- D_{LCO} % predicted < 80%;

- Being capable of doing pulmonary function tests;
- Being eligible for bronchoscopy and willing to receive autologous bronchial basal cell transplantation therapy;
- Patients or their family members voluntarily participated in the study and signed the informed consent.

4.3 EXCLUSION CRITERIA

- Pregnant or lactating women or women of childbearing age who were planning to conceive;
- Positive serological tests for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), or syphilis (HBV carriers and patients with stable chronic hepatitis B could be accepted if titers of HBV DNA < 500 IU/mL or copies < 1000 copies/mL; patients with curative hepatitis C were eligible if HCV RNA tests were negative)
- Malignant tumors;
- Co-existing active pulmonary tuberculosis, pulmonary embolism, pneumothorax, multiple huge bullae, uncontrolled asthma, acute exacerbation of chronic bronchitis, or extremely severe chronic obstructive pulmonary disease (COPD);
- A history of severe systemic diseases (*i.e.*, poorly controlled diabetes, myocardial infarction, unstable angina pectoris, liver cirrhosis, acute glomerulonephritis)

- Leukopenia (WBCs < 4 × 10^9 /L) or agranulocytosis (WBCs < 1.5 × 10^9 /L or neutrophils < 0.5 × 10^9 /L) for any cause;
- Significant kidney dysfunction (Cr being 1.5 times higher than the upper limit of normal);
- Significant liver dysfunction (ALT, AST, total bilirubin > 2 times of the upper limit of normal);
- A history of mental disorders, or suicide risk, or epilepsy, or other central nervous system disease;
- Clinically significant arrhythmia (*i.e.*, ventricular tachycardia, frequent supraventricular tachycardias, atrial fibrillation, atrial flutter, second- or third-degree atrioventricular block);
- A history of alcohol or drug abuse;
- Participated in other clinical trials within 3 months before screening;
- Subjects with poor compliance, difficult completing the study;
- Any other conditions that might increase the risk of subjects or interfere with the clinical trial.

4.4 REMOVING CRITERIA

- Participants who do not meet the inclusion criteria or meet the exclusion criteria.

- Participants who are unwilling or unable to continue participating in this trial.
- Participants who are unable to evaluate efficacy due to treatment interruption.
- Participants who interrupt treatment due to pregnancy.

4.5 WITHDRAWAL CRITERIA

Subject-Requested Withdrawal:

According to the provisions of the informed consent form, participants have the right to withdraw from the clinical study at any time, or if a participant, without explicitly withdrawing from the clinical study, no longer undergoes medication and testing and becomes lost to follow-up (also considered withdrawal or dropout). Participants who meet the inclusion criteria for entry into the clinical study, regardless of when or for what reason they withdraw before completing the specified observation and follow-up periods, are considered dropout cases.

For participants who withdraw, the researcher should clearly state the reasons for withdrawal and the withdrawal time, and should (or as much as possible) conduct appropriate observation and evaluation of the withdrawn participants, complete the specified withdrawal assessment, and fill out the corresponding records. For those who withdraw due to adverse reactions or

abnormal laboratory findings, tracking should continue until the adverse reactions disappear or the laboratory findings return to normal/baseline levels. Properly preserving trial data related to dropout cases is crucial, and a comprehensive analysis set should be compiled for them.

Researcher-Requested Subject Withdrawal Conditions:

- The Medical Ethics Committee deems it necessary to stop.
- Inability to collect a sufficient number of bronchial basal cells according to the planned protocol, or failure to successfully amplify autologous basal cells due to reasons such as drug-resistant bacterial infection, or the quality of amplified autologous basal cells is unsuitable for clinical researchers.
- Occurrence of a severe adverse event making the participant unsuitable to continue the trial.
- Occurrence of severe other concurrent diseases during the clinical study.
- Use of any anticancer drugs during the clinical study.
- Poor compliance of the participant, who no longer undergoes cell transplantation therapy or testing before completing the entire clinical study, cannot adhere to the planned clinical study, including not adhering to prescribed

medications, or any other factor that may affect efficacy observation.

- Participation in other clinical trials during the clinical study.
- Acute worsening of relevant symptoms in the participant.
- Other situations deemed necessary by the researcher to withdraw from the study.

5 STUDY INTERVENTIONS

5.1 INVESTIGATIONAL DRUG

Autologous bronchial basal cells

Dosage: 1~3 × 10^6 cells/kg body weight, which can be adjusted according to the severity of bronchiectasis.

5.2 ADMINISTRATION

5.2.1 COLLECTION, SEPARATION, AND CULTURE OF BRONCHIAL BASAL CELLS BEFORE TREATMENT

Using the bronchoscopic brush method, a small amount of tissue is collected and bronchial basal cells are isolated from the 3~5th level bronchi of the human lung after B-ACT completed. The obtained tissue is washed and enzymatically digested to form a single-cell suspension, which is then cultured. The basal cell culture system is a patented technology of the sponsor

(Regend Therapeutics Co., Ltd.), which uses a combination of special growth factors and composite materials *in vitro* to simulate the basal environment and selectively expand bronchial basal cells, while other types of terminally differentiated epithelial cells and fibroblasts cannot survive and naturally die. After a period of expansion, bronchial basal cells can be stored in a liquid nitrogen cell bank for long-term preservation. Before being used for subjects, bronchial basal cells need to undergo a series of rigorous tests, including identity, purity, sterility, endotoxin, viral contamination, bovine serum albumin (BSA) remaining and antibiotic remaining, *etc*.

Strict quality control of the transplanted bronchial basal cells, including relevant testing to ensure that the cells meet the quality standards:

- Cell viability ≥ 90%;
- Expression of marker KRT5;
- Purity greater than 90%;
- No bacterial or fungal contamination;
- No mycoplasma contamination;
- No bovine virus contamination;
- Endotoxin content lower than 0.1 EU/mL;
- Cells have a karyotype of 46XX or 46XY;

- Potential for differentiation into alveolar-like structures or HOPX-positive cells.
- Gentamicin residues in cell suspension are less than 5.4 ppb.

5.2.2 BRONCHIAL BASAL CELL TREATMENT

After locating the lesion through chest CT, the subject will be placed in the supine position and receive local anesthesia. Cell suspension is pre-warmed to approximately 37 ^{o}C 15 minutes before use, and then kept in a syringe for later use. A fiberoptic bronchoscope will be used to perform B-ACT. After the bronchoalveolar lavage is completed, the lavage fluid in the affected area of the bronchial lobe or segment will be aspirated as much as possible. Six lung segments with the most severe lesions are selected by the team of doctors before bronchoscopy according to CT results. After lavage and when the oxygen saturation of patients reaches > 92%, 5 mL of the cell suspension will be slowly and gently pushed into each lung segment *via* the working channel of the bronchoscope with a 20 mL syringe in around 30 seconds, and the severely damaged lung segment may be injected multiple times. After the successful injection of bronchial basal cells, the feeding tube will be removed first, followed by the fiberoptic bronchoscope. Non-invasive positive pressure ventilation treatment may be added if necessary. Then

allow the subject to maintain a flat position for 2 hours after transplantation. The subject should not drink water for 2 hours after surgery and is advised to minimize coughing. If necessary, oral codeine may be given.

5.2.3 TRANSPLANTATION TREATMENT DURATION AND COURSE FOR BRONCHIAL BASAL CELL THERAPY

The duration of bronchial basal cell transplantation treatment is determined by the completion time of cell expansion. Following the completion of bronchial basal cell tissue sampling, the subsequent processes include cell isolation, cryopreservation, culture, and expansion. Generally, these procedures take 4~8 weeks. Upon completion of the expansion, various quality tests are conducted in the production workshop. The product is then released, transported to the cell therapy unit, and the cell therapy is completed.

Currently, the course of bronchial basal cell transplantation treatment consists of a single session, meaning the treatment is administered only once.

5.3 PRIOR AND CONCOMITANT MEDICATIONS

In principle, subjects can continue to use medications they were taking before the start of this treatment to exclude any interference from sudden discontinuation of medication on the

study results. Conventional bronchiectasis medications can be used for treatment. For subjects who were already receiving conventional bronchiectasis medication before the screening, the dosage should remain stable from the start of the screening period throughout the entire study. For subjects who are recently diagnosed and have not received any treatment, they may begin using conventional bronchiectasis medications from the start of the screening period and maintain a stable dosage throughout the entire study. For all concomitant medications, the researcher should record the details on the Concomitant Drug Use page of the Case Report Form (CRF), including the reason for medication/treatment, administration/treatment methods, and start and end dates.

5.4 HANDLING OF CASES INELIGIBLE FOR INCLUSION DURING THE CELL THERAPY PREPARATION PROCESS

If a participant, originally meeting the inclusion and exclusion criteria, becomes ineligible for cell therapy between the completion of cell collection and the initiation of cell therapy (usually a period of 4~8 weeks), the participant will be considered disqualified, and the preparation of cell therapy will be temporarily halted. The cells that have been cultured, either completed or partially completed, will be cryopreserved

according to the relevant regulations of the production workshop. After being disqualified, the participant may undergo observation and intervention for a period. When their physical condition permits, they can participate in screening again. If they meet the inclusion criteria, there is no need to recollect cells; they can directly enter the trial, reschedule the treatment time, and use the cells after resuscitation, cultivation, and testing. Participants can undergo multiple screenings until the end of the study. If, until the end of the study, a participant has not met the inclusion criteria, they will be excluded, and the cells will be either destroyed or cryopreserved for an extended period at the participant's request.

Considerations regarding the standards or range of various pre-cell therapy examinations for participants mainly involve the researcher further verifying the inclusion and exclusion criteria before the participant undergoes cell therapy. If the participant still meets the inclusion criteria, does not meet the exclusion criteria, and there are no special circumstances, the researcher, after comprehensive assessments, deems the participant suitable for cell therapy, and the treatment proceeds as normal. If the participant does not meet the inclusion criteria or meets the exclusion criteria, the treatment will be temporarily halted (cells that have been cultured will be cryopreserved, and treatment will be given at a later date). For instance, if there is significant progression of the participant's condition, worsening of

pulmonary inflammation, higher risk during bronchoscopy, follow-up CT (as required or treatment interval exceeding 8 weeks) reveals concurrent lung cancer with a life expectancy of less than 1 year, or the anticipated frequent risk of acute exacerbation after cell therapy is high, the researcher, after comprehensive assessment, deems the participant unsuitable for cell therapy. These situations can be considered as criteria or a range where treatment is not acceptable.

5.5 HANDLING OF CASES WITH CELL CULTURE FAILURES

If, during the cell culture process before treatment, it is discovered that the cells cannot be cultured, or there is abnormal cell proliferation, or quality inspection reveals that the cells do not meet quality requirements, the reasons need to be investigated. The technical department should design a response plan, arrange for the participant to undergo resampling, and conduct cell culture again. Cells that have been cultured (if found to be non-compliant) should be destroyed according to relevant regulations.

If the re-culturing is successful, the participant will proceed with normal enrollment in the clinical trial. If re-culturing still fails, the participant will be excluded from the clinical trial.

Note: Preliminary research results from the applicant indicate that the success rate of the first cell culture is higher than 90%, and the success rate of two consecutive cultures is higher than 95%.

6 PACKAGING AND LABELING

6.1 PACKAGING SPECIFICATIONS

30 mL per package.

6.2 PACKAGING REQUIREMENTS

The product will be packaged in sealed sterile bags.

6.3 LABELS

Autologous Bronchial Basal Cells
(For clinical research use by the subject only)

Batch: _____ ID: _____
Indication: Bronchiectasis
Specification: 30 mL
Color: White or transparent
Cell count:
Dosage and administration: After suspension in saline, the cells will be locally introduced into the lungs at a dose of 1-3 × 10^6/Kg/person.
Storage: 2-8 °C
Shelf life: 12 hours
Date of manufacture: As indicated in the package insert
Notes: These cells are sterile products and should be used strictly under the guidance of a physician. If you have any questions, please consult your physician promptly. Unused cells and packaging need to be collected.
Regend Therapeutics Co., Ltd.

6.4 CELL ALLOCATION

The cells will be strictly used for autologous treatment. Once the cells are prepared, they will be immediately transported to the clinical facility by a designated individual and received by responsible personnel. The cells will be used by the managing physician of the subject.

6.5 PRODUCT STORAGE AND APPLICATION

Storage conditions: 2~8 ^{o}C

The cells will be shipped to Ruijin Hospital in an ice box with a real-time monitoring and alarm device for temperature, and delivered to the airway using a bronchoscope.

6.6 DRUG MANAGEMENT

A designated individual will be responsible for managing the cells. The cells will be counted after each use, and any unused cells and packaging will be collected and returned.

6.7 CODE ESTABLISHMENT

The patient identification number will be consistent with the cell preparation number. The numbering will start from the patient's admission to the hospital, and all related documents will use the same numbering system.

6.8 TRANSPORT QUALITY ASSURANCE MEASURES

1) Firstly, ensure that the real-time temperature recorder is in good condition (with sufficient battery and calibrated within the last year). Set the temperature alarm's upper limit to 8.1 ^{o}C and the lower limit to 1.9 ^{o}C.

2) The packaged cellular preparations (including internal and external packaging) are placed in a temperature-controlled box balanced at 2-8 ^{o}C for transportation. Simultaneously, an opened real-time temperature recorder is placed in the temperature-controlled box to monitor the temperature throughout the transportation. Set the recording interval of the temperature recorder to 15 minutes, meaning it will automatically record the temperature inside the temperature-controlled box every 15 minutes. Ensure uninterrupted temperature detection, continuous recording, data storage, and alerts for exceeding limits throughout the transportation.

3) Upon arrival of the cellular preparations at the clinical institution, personnel should inspect the packaging integrity of the cellular preparations. Confirm that the temperature displayed on the temperature recorder is within the range of 2-8 ^{o}C. Additionally, the transportation personnel should promptly retrieve the temperature recorder to verify the temperature conditions inside the temperature-controlled box throughout the transportation and complete relevant records.

4) Within 3 hours of cellular transportation to the clinical institution, institution personnel conduct a release inspection of the cells to exclude the influence of the transportation process on the quality of the stem cells.

7 SAFETY AND EFFICACY ASSESSMENT INDICATORS

7.1 SAFETY ASSESSMENTS

The main safety endpoint is the incidence of adverse events. The following assessments will be conducted during the follow-up:

7.1.1 PHYSICAL AND VITAL SIGN EXAMINATION

- Physical and vital sign examination include height, weight, medical history, *etc*.

7.1.2 LABORATORY EXAMINATIONS

- Complete blood count: red blood cell count, white blood cell count, hemoglobin, platelets, white blood cell differential count, mean corpuscular volume, hematocrit, and mean corpuscular hemoglobin concentration.
- Urinalysis: urine pH, specific gravity, protein, glucose, ketones, occult blood, white blood cells, and urobilinogen.

- Biochemical analysis: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), uric acid (URIC), total protein (TP), albumin (ALB), creatine kinase (CK), lactate dehydrogenase (LDH), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (Cr), blood glucose (Glu), sodium ion (Na^+), potassium ion (K^+), chloride ion (Cl^-).

7.1.3 12-LEAD ELECTROCARDIOGRAM (ECG) EXAMINATION

- The 12-lead ECG examination should record heart rate, rhythm, PQ or PR interval, QRS interval, QT interval (uncorrected), and QTc (QT/RR1/2), and provide an overall evaluation (normal, clinically insignificant abnormalities, clinically significant abnormalities, requiring further explanation). The signed original ECG will be archived at the study center, and the ECG examination results will be recorded on the CRF.

7.1.4 FIBER-OPTIC BRONCHOSCOPY EXAMINATION

1) Preoperative Preparation: Thoroughly understand the participant's medical history, conduct a physical examination, perform CT scans, and conduct laboratory tests. Explain the

purpose and significance of the examination, addressing concerns. Ensure a fasting period of at least 4 hours. Administer intramuscular atropine 0.5 mg 30 minutes before, with sedatives for anxious individuals.

2) Local Anesthesia: Combine 2% lidocaine throat spray with inhalation anesthesia until a sensation of obstruction in the throat.

3) Procedure Steps: Position the participant supine, inserting the flexible bronchoscope through the nasal passages. Observe tracheal mucosal folds, cartilaginous rings, epiglottis, bronchi openings, mucosal smoothness, color, presence of abnormalities, and record details.

4) Specimen Collection: If abnormalities like new growths are identified, perform a bronchoscopic biopsy for specimen collection.

5) Postoperative Care: Restrict oral intake for 2 hours, allowing eating or drinking only after anesthesia effects dissipate. Explain potential blood-tinged sputum. Administer antibiotics for postoperative fever and provide oxygen therapy for breathlessness and hypoxemia.

7.1.5 ARTERIAL BLOOD GAS ANALYSIS

- pH, P_aCO_2, P_aO_2, the concentration of HCO_3^-.

7.2 EFFICACY ASSESSMENTS

7.2.1 PRIMARY EFFICACY ENDPOINTS

- The change from baseline in D_{LCO} at Week 4, 12, and 24 (after treatment).

7.2.2 SECONDARY EFFICACY ENDPOINTS

- The changes from baseline in other pulmonary function parameters except D_{LCO}, including FEV_1, FVC, FEV_1/FVC, MMEF, and MVV, at Week 4, 12, and 24
- The changes from baseline in the 6-minute walking distance (6MWD) and the composite index (distance-saturation product, DSP) at Week 4, 12, and 24
- The changes from baseline in the modified medical research council (mMRC) chronic dyspnea scale at Week 4, 12, and 24
- The changes from baseline in the SGRQ score at Week 4, 12, and 24
- The changes from baseline in bronchiectasis severity index (BSI) score and FACED score at Week 4, 12, and 24
- Imaging of lung by HRCT at Week 4, 12, and 24

8 FOLLOW-UP PLAN

All subjects must sign an informed consent form before screening.

The study physician will provide the subjects with complete and truthful information about the background, objectives, trial design, direct and indirect benefits that the subjects can obtain, potential risks, and any other relevant information related to this clinical trial, in a quiet and private environment. The subjects will be allowed to think independently and discuss with the physician, ask any questions they have, and receive help to fully understand all the information. After the subjects make a voluntary decision to participate, they and the study physician will sign the informed consent form simultaneously.

Please refer to the table below for details on the specific study procedures:

Project	Screening (collection)	Baseline	4 weeks	12 weeks	24 weeks
Sign of informed consent	X				
Inclusion / exclusion criteria screen	X	X			
Medical history inquiry	X	X			
Symptom inquiry	X	X	X	X	X
Physical examination	X	X	X	X	X

Vital signs examination	X	X	X	X	X
Blood routine test	X	X	X	X	X
Blood pregnancy test[1]		X			
Urine routine test		X	X	X	X
Blood biochemical test		X	X	X	X
Blood coagulation test	X	X			
Creatine kinase test		X	X	X	X
Serological test of syphilis, HBV, HCV, HIV	X				
Electrocardiogram	X	X	X	X	X
Chest HRCT	X		X	X	X
Bronchoscopic brushing	X				
Cell transplantation		X			
Arterial blood gas analysis		X	X	X	X
Pulmonary function testing	X	X	X	X	X
6MWT and DSP		X	X	X	X
SGRQ		X	X	X	X
BSI score		X	X	X	X
FACED score		X	X	X	X
Combined medicine	X	X	X	X	X
Adverse event evaluation	X	X	X	X	X

Note: The day of cell transplantation is considered day 0 of the study; all follow-up procedures for baseline will be completed

before bronchial basal cell infusion except for adverse events, and bronchoscopy will only be performed during the non-acute phase. The follow-up time may vary by approximately 7 days before and after the scheduled time.

[1]Female subjects (postmenopausal women who have completed one year of menopause are not required to participate).

9 STUDY INTERVENTION DISCONTINUATION AND TERMINATION

9.1 CRITERIA FOR DISCONTINUATION

The clinical trial must be discontinuation if the following situations occur:

- If a subject experiences intolerable adverse events or serious adverse events, and the investigator determines that the risk of continued participation in this trial is greater than the benefit to the subject, the trial must be terminated. Appropriate treatment measures should be taken, and the subject should be included in the full analysis set for safety analysis.
- If the subject's condition worsens after treatment, the subject should be included in the efficacy and safety analysis for the protocol set.

- If the investigator or sponsor terminates the study for any reason, the reason for termination should be submitted to the ethics committee.
- Once a subject withdraws from this trial, they cannot re-enter the study.

9.2 CRITERIA FOR TERMINATION

The clinical trial will be ended when the following situations occur:

- Enough subjects have been recruited;
- Follow-up has been completed for the last subject recruited;
- Statistical analysis has been completed for all safety and efficacy indicators for all subjects.

10 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

10.1 DEFINITION OF ADVERSE EVENTS (AE)

Any unexpected and unfavorable medical occurrence related to any medical intervention in the study, regardless of its association with bronchial basal cell therapy, is considered an adverse event (AE). AE includes clinically significant laboratory abnormalities that indicate damage to a disease and/or organ.

All observed or subject-reported adverse events should be recorded on the adverse events page of the CRF.

For each adverse event, the time of occurrence, severity, duration, management measures, and outcome of the event should be described, and analysis should be conducted to determine the causal relationship with the study drug and whether it meets the criteria for a serious adverse event.

The analysis of the association between adverse events and bronchial basal cell therapy should take into account the following factors:

- Whether there is a reasonable temporal sequence between the adverse event and the time of bronchial basal cell therapy;
- Whether the clinical or pathological manifestations of the adverse event are consistent with known knowledge of bronchial basal cell therapy;
- Whether the adverse event can be explained by the original disease, subject's factors, or environmental factors.

10.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event (SAE) is defined as an adverse event that results in any of the following:

- Death;
- Life-threatening;
- Requires hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect;
- Cancer.

If a subject experiences a serious adverse event during the trial, regardless of whether it is related to bronchial basal cell therapy, the investigator should take appropriate treatment measures immediately to ensure the subject's safety.

10.3 METHOD AND FREQUENCY OF AE DETECTION

While observing the efficacy, closely monitor for adverse events or unforeseen toxic side effects. Safety indicators are set in the observation index, as follows:

10.3.1 PHYSICAL AND VITAL SIGNS EXAMINATION

Physical and vital signs examination including height, weight, medical history, *etc*.

10.3.2 LABORATORY TESTS

- Blood routine: red blood cell count, white blood cell count, hemoglobin, platelet, white blood cell differential count, mean red cell volume, hematocrit, and mean corpuscular hemoglobin concentration.
- Urine routine: urine pH, specific gravity, protein, urine sugar, ketone bodies, occult blood, white blood cells, and urinary bilirubin.
- Blood biochemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), uric acid (URIC), total protein (TP), albumin (ALB), creatine kinase (CK), lactate dehydrogenase (LDH), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (Cr), blood glucose (Glu), sodium ion (Na^+), potassium ion (K^+), and chloride ion (Cl^-).

10.3.3 12-LEAD ELECTROCARDIOGRAM (ECG) EXAMINATION

The 12-lead ECG examination should record heart rate, rhythm, PQ or PR interval, QRS interval, QT interval (uncorrected), and QTc (QT/RR1/2) and give an overall evaluation (normal, clinically insignificant abnormality, clinically

significant abnormality, need further explanation). The signed original ECG will be archived at the study center, and the ECG examination result will be recorded on the CRF.

10.3.4 ARTERIAL BLOOD GAS ANALYSIS

Fiber-optic bronchoscopy examination:

1) Preoperative Preparation: Thoroughly understand the participant's medical history, conduct a physical examination, perform CT scans, and conduct laboratory tests. Explain the purpose and significance of the examination, addressing concerns. Ensure a fasting period of at least 4 hours. Administer intramuscular atropine 0.5 mg 30 minutes before, with sedatives for anxious individuals.

2) Local Anesthesia: Combine 2% lidocaine throat spray with inhalation anesthesia until a sensation of obstruction in the throat.

3) Procedure Steps: Position the participant supine, inserting the flexible bronchoscope through the nasal passages. Observe tracheal mucosal folds, cartilaginous rings, epiglottis, bronchi openings, mucosal smoothness, color, presence of abnormalities, and record details.

4) Specimen Collection: If abnormalities like new growths are identified, perform a bronchoscopic biopsy for specimen collection.

5) Postoperative Care: Restrict oral intake for 2 hours, allowing eating or drinking only after anesthesia effects dissipate. Explain potential blood-tinged sputum. Administer antibiotics for postoperative fever and provide oxygen therapy for breathlessness and hypoxemia.

10.3.5 ARTERIAL BLOOD GAS ANALYSIS

- pH, P_aCO_2, P_aO_2, the concentration of HCO_3^-.

10.4 RECORDING OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

For any adverse events and serious adverse events that occur during the study period, their symptoms, severity, onset time, duration, treatment measures, and outcomes should be recorded in the CRF. The relationship between the event and the study cells should be evaluated, and the researcher should record, sign, and date the information in detail.

10.5 ASSESSMENT OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

10.5.1 SEVERITY DETERMINATION

- Mild adverse event: The subject has symptoms, but the reaction is slight and tolerable, does not affect normal

activities, and the symptoms are transient and self-resolving.

- Moderate adverse event: The symptoms affect the subject's normal daily life and persist for a longer duration.
- Severe adverse event: The subject's body function is impaired, leading to loss of normal work and life ability, and the symptoms persist for an even longer duration.

10.5.2 CAUSALITY DETERMINATION

The relationship between adverse events and study cells can be classified into the following categories:

- Definitely related: There is evidence of using the study drug, the occurrence of adverse events is reasonably related to cell therapy, and it is more reasonable to explain the adverse events with cell therapy than other reasons.
- Probably related: There is a reasonable correlation between the occurrence of adverse events and cell therapy in time. Adverse events can be explained by other reasons.
- Possibly unrelated: There is evidence of using cell therapy, and the adverse events may be better explained by other reasons.

- Unrelated: No cell therapy was conducted, or there is no correlation between cell therapy and the occurrence of adverse events, or there are other clear causes of adverse events.
- Unable to determine.

10.6 FOLLOW-UP OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Subjects experiencing adverse events will be followed up *via* telephone interview once per quarter, and the recovery of the subjects will be recorded in detail.

10.7 REPORTING OF SERIOUS ADVERSE EVENTS

In the event of a serious adverse event, the clinical research unit must take immediate measures to protect the safety of the subjects and report to the Drug Supervision Administration, sponsor, and ethics committee within 24 hours. The investigator should sign and date the report. The sponsor will ensure compliance with all legal and regulatory reporting procedures.

10.8 ADVERSE REACTIONS AND TREATMENT METHODS

Adverse reactions should be anticipated before the operation, and timely treatment should be provided if adverse reactions occur. The patients should receive corresponding symptomatic treatment.

When an adverse event occurs, the observing physician may decide whether to discontinue the observation according to the patient's condition. For cases where treatment is stopped due to adverse reactions, a follow-up investigation should be conducted, and the management process and results should be recorded in detail.

10.9 POTENTIAL RISKS AND MANAGEMENT STRATEGIES

- Risks associated with bronchoscopy: The common complications of bronchoscopy include anesthesia accidents, bleeding, pneumothorax, laryngospasm, hypoxemia, infection, postoperative fever, and other unexpected events. Currently, clinical practices in bronchoscopy are highly mature and exhibit good safety. This study involves only sputum suction and injection in specific lung segments, and the occurrence of complications is very low.
- Risk Monitoring for Anesthesia-Related Events: 1) Anesthesia physicians must conduct thorough

preoperative visits, explaining procedures and formulating suitable plans. 2) Verify equipment functionality before anesthesia and ensure preparedness for any patient receiving anesthesia. 3) Monitor vital signs closely, adhere to a double-check system for medications, and stay with the patient throughout anesthesia.4) Prepare for intravenous anesthesia, securing loose teeth and ensuring gentle tracheal intubation. 5) Conduct postoperative follow-ups within 48 hours, promptly reporting any issues to senior physicians for intervention.

- Other possible risks during bronchial basal cell therapy: Regend Therapeutics follows GMP to establish bronchial basal cell production and quality systems. Trained personnel, especially the manager, ensure compliance, preventing contamination risks with clear roles, regular training, and effective reporting mechanisms.
- Multiple organ dysfunction: Due to the rich capillary network in the lungs, bronchial basal cells, if excessively absorbed into the bloodstream, may lead to multi-organ dysfunction. Our method, delivered through the trachea, minimizes the risk compared to intravenous administration, reducing the likelihood of organ dysfunction to less than 0.01%.

- Emergency management of massive hemoptysis: Massive hemoptysis is a life-threatening complication of bronchiectasis, defined as a single episode exceeding 200 ml or a total of 500 mL within 24 hours. It can lead to suffocation. Preventing asphyxiation is the primary focus. Ensure airway patency, improve oxygenation, and stabilize hemodynamics. For lesser amounts of hemoptysis, reassure the patient and have them rest on the affected side. In cases of asphyxiation, place the patient in a 45° head-down position, clear blood clots from the mouth, and gently tap the healthy side of the back to facilitate blood drainage. If these measures are ineffective, prompt endotracheal intubation or, if necessary, a tracheostomy should be performed.

11 DATA MANAGEMENT AND STATISTICAL ANALYSIS

11.1 DATA MANAGEMENT

11.1.1 COMPLETION AND SUBMISSION OF CRF

The investigator is responsible for completing CRF for each eligible case. Completed CRFs will be reviewed by the trial sponsor and archived.

11.1.2 DATA ENTRY AND MODIFICATION

A designated person, appointed by the trial sponsor, will enter CRF data into the database. Any modifications to the data should follow the CRF modification requirements.

11.1.3 DATA REVIEW

Following the completion of data entry and verification, a designated person appointed by the trial sponsor will review the data and make the final determination of the analysis population.

11.1.4 DATA LOCK

Data can be locked once the following conditions are met:

- All data has been entered into the database;
- All queries have been resolved;
- The analysis population has been defined and determined.

11.2 STATISTICAL ANALYSIS

11.2.1 SAMPLE SIZE DETERMINATION

A sample size of 76 subjects is not based on any statistical considerations. The sample size is based on the clinical consideration to provide safety and efficacy information with the need to minimize exposure to subjects in a pilot study.

11.3 STATISTICAL METHODS

11.3.1 PROPOSED STATISTICAL METHODS

- Descriptive Statistics:

Outliers: Statistical and professional analysis is used to determine whether to include outliers.

Missing data: For individual subjects with missing primary efficacy data, the method of imputation is determined based on statistical and professional judgment.

Descriptive statistics: n, mean, standard deviation (SD), median, Q1, Q3, and range (minimum and maximum), *etc*.

- Inferential Statistics:

Continuous data: Unpaired Student's t-test and Mann-Whitney U test.

Count data: Chi-square test and Fisher's exact test.

11.3.2 PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint in the study is the change of D_{LCO} after therapy. D_{LCO} data will be expressed as both absolute value (mmol/min/kPa) or % of predicted (%). The difference between the cell treatment and control groups of the changes in D_{LCO} from baseline to Week 4, 12, and 24, is tested using the Mann-Whitney U test, and the median differences are calculated using the Hodges-Lehmann estimation.

11.3.3 STATISTICAL EXPRESSION

- Tables are mainly used to present the results and should be self-explanatory, with a title, caption, and number of cases.
- Results of repeated measures data are presented in tables, with statistical graphs attached to increase readability.
- Analyses are presented with two-sided P values, with the level of significance set at 0.05.

11.4 STATISTICAL SOFTWARE AND GENERAL REQUIREMENTS

All statistical analysis and diagramming are performed by SPSS (version 25.0) and GraphPad (version 9.0).

12 TRIAL MANAGEMENT

12.1 COMPLIANCE WITH GCP REQUIREMENTS

This clinical trial fully complies with the Good Clinical Practice (GCP) guidelines and is strictly conducted according to the specified requirements.

12.2 PROTECTION OF SUBJECT PRIVACY

This clinical trial respects the privacy of subjects, strictly protects subject privacy, and prevents leakage of subject information.

12.3 QUALITY CONTROL AND ASSURANCE

12.3.1 QUALITY CONTROL

- Determination of the study protocol: The clinical research protocol is discussed and negotiated by all researchers participating in this clinical trial. After reaching a consensus, it is submitted to the ethics committee for approval.
- Quality control measures for the laboratory: the sponsor has standardized testing indicators, standard operating procedures, and quality control procedures.
- Qualifications of researchers: Researchers participating in clinical trials must have professional expertise, qualifications, and capabilities in clinical research. After qualification review, personnel requirements are relatively fixed.
- Pre-trial training: Pre-trial training for researchers is conducted to ensure full understanding and awareness of the clinical research protocol and its specific indicators.

The entire clinical trial process should strictly follow the relevant operating norms.

- Measures to ensure subject compliance: Detailed information on the huge benefits of successful cell therapy is provided to subjects, which may help prevent the pain of future loss of lung function, and subject compliance is monitored.
- The abnormal judgment criteria for laboratory examinations are based on the normal reference range of the inspection unit.
- All observation results and findings in the clinical study should be verified to ensure the reliability of the data and ensure that all conclusions in the clinical study are derived from original data. Corresponding data management measures are taken in the clinical study and data processing stages.
- Based on the original observation records of the subjects, researchers ensure that the data are entered correctly (consistent with the actual situation of the subjects), complete (without omissions), clear (neat handwriting and easy to identify), and timely on the CRF.

12.3.2 QUALITY ASSURANCE

- The sponsor appoints a monitor to ensure the protection of the rights and interests of subjects in the clinical study, the accuracy and completeness of research records and report data, and ensure that the study follows the approved protocol and relevant regulations.
- All observation results and findings in the trial should be verified to ensure the reliability of the data and ensure that all conclusions in the clinical trial are derived from original data. Quality control is used at every stage of data processing to ensure that all data is reliable and processed correctly.
- The sponsor performs strict quality testing of cell preparation, provides a cell out-of-factory testing report, and ensures the quality of the cells.

12.4 SUBJECT CODING, RANDOM NUMBER TABLE, AND CRF PRESERVATION

The researcher should keep all study materials, including confirmation of all subjects participation (effective cross-check of different recording data, such as original records in hospitals), all original informed consent forms signed by the subjects, all case observation forms, and detailed records of cell distribution and

use. The researcher should keep the clinical study materials for at least 5 years after the end of the study.

13 ETHICAL CONSIDERATIONS

This clinical study will adhere to the Helsinki Declaration (2008 version) and relevant regulations and guidelines for the management of cellular clinical research in China. Before the commencement of the clinical trial, the research center ethics committee must approve the study protocol.

Before each participant is enrolled in the study, the investigating physician is responsible for providing complete and comprehensive written information about the purpose, procedures, and potential risks of the study to the participant or their designated representative. Participants should be informed that they have the right to withdraw from the study at any time. Each participant must be provided with an informed consent form before being enrolled in the study. The investigating physician is responsible for ensuring that each participant signs the informed consent form before entering the clinical trial and keeping it in the study records.

14 EXPECTED PROGRESS AND COMPLETION DATES OF CLINICAL TRIALS

1) Research Start Date: The implementation of this protocol will commence after obtaining approval from the Ethics Committee.

2) Midterm Clinical Coordination Meeting: The timing for convening the midterm clinical coordination meeting will be determined based on the progress and completion status of the clinical study.

3) Clinical Study Completion Date: The clinical study is expected to be completed within 36 months from the initiation of the research.

4) Data Collection, Statistical Analysis, and Summary of Clinical Study: The collection, statistical analysis, and summarization of clinical study data will be completed within 6 months after the completion of the study and receipt of the statistical analysis report.

15 MAIN REFERENCES

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Vaughan A, Brumwell, *et al*. Lineage-negative progenitor mobilize to regenerate lung epithelium after major injury. *Nature* 2015 517, 621-625.