Inhibiting CXCL12 blocks fibrocyte migration and differentiation and attenuates bronchiolitis obliterans in a murine heterotopic tracheal transplant model

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Objectives: Fibrocytes are integral in the development of fibroproliferative disease after lung transplantation. Undifferentiated fibrocytes (CD45+anti-collagen 1+CXCR4+) preferentially traffic by way of the CXCR4/CXCL12 axis and differentiate into smooth muscle actin-producing (CD45+CXCR4+ α -smooth muscle actin+) cells. We postulated that an antibody directed against CXCL12 would attenuate fibrocyte migration and fibro-obiteration of heterotopic tracheal transplant allografts.

Methods: A total alloantigenic mismatch murine heterotopic tracheal transplant model of obliterative bronchiolitis was used. The mice were treated with either goat-anti-human CXCL12 F(ab')₂ or goat IgG F(ab')₂. Buffy coat, bone marrow, and trachea allografts were collected and analyzed using flow cytometry. Tracheal luminal obliteration was assessed using hematoxylin-eosin and Direct Red 80 collagen stain.

Results: Compared with the controls, the anti-CXCL12-treated mice showed a significant decrease in tracheal allograft fibrocyte populations at 7 and 21 days after transplantation. Bone marrow and buffy coat aspirates showed the same trend at 7 days. In the anti-CXCL12-treated mice, there was a 35% decrease in luminal obliteration at 21 days (65% vs 100% obliterated; interquartile range, 38% vs 10%; $P = .010$) and decreased luminal collagen deposition at 21 and 28 days after transplantation ($P = .042$ and $P = .012$, respectively).

Conclusions: Understanding the role of fibrocytes in airway fibrosis after lung transplantation could lead to a paradigm shift in treatment strategy. Anti-CXCL12 antibody afforded protection against infiltrating fibrocytes and reduced the deterioration of the tracheal allografts. Thus, the CXCR4/CXCL12 axis is a novel target for the treatment of fibro-obiteration after lung transplantation, and the quantification of fibrocyte populations could provide clinicians with a biomarker of fibrosis, allowing individualized drug therapy. (J Thorac Cardiovasc Surg 2013;145:854-61)

Bronchiolitis obliterans syndrome (BOS) is a leading cause of morbidity and mortality after lung transplantation.¹ Lung fibroblasts and myofibroblasts are critical for the development of fibrosis and are thought to arise from 3 locations: the resident proliferation of fibroblasts, epithelial to mesenchyme transition, and bone marrow-derived mesenchymal progenitor cells, fibrocytes.²

Fibrocytes (CD45+anti-collagen 1 [Col1]+CXCR4+) are bone marrow-derived mesenchymal stem cells that are released into the circulation in response to numerous

inflammatory threats and microenvironmental cytokines. They travel to injured tissues by way of the CXCR4/CXCL12 chemokine axis. Fibrocytes differentiate into α -smooth muscle actin (α -SMA+) producing fibroblasts/myofibroblasts.^{2,3} Although fibrocytes have been shown to respond to several chemokines and express CCR3, CCR5, and CCR7 chemokines, the CXCR4/CXCL12 biologic axis is the predominate driving force for fibrocyte trafficking after airway injury.^{4,5}

Fibrocytes were first described in the context of normal wound healing; however, their importance in the progression of fibrotic and asthmatic lung disease has since been demonstrated.⁶ Patients with exacerbation of idiopathic pulmonary fibrosis (IPF) have been shown to have an elevated proportion of peripheral blood fibrocytes compared with patients with stable IPF. Furthermore, the total circulating fibrocyte population in those with IPF was found to be an independent predictor of death.⁷

In a prospective study aimed at quantifying circulating fibrocyte populations (CD45+Col1+) in patients after lung transplantation, we found a statistically significant increase in the circulating fibrocyte number in patients diagnosed with BOS (defined according to the forced expiratory

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Abbreviations and Acronyms

α -SMA	= α -smooth muscle actin
BOS	= bronchiolitis obliterans syndrome
Col1	= collagen 1
IPF	= idiopathic pulmonary fibrosis
mTOR	= mammalian target of rapamycin

volume in 1 second) compared with that in patients without BOS (8.9×10^5 cells/mL vs 2.96×10^5 cells/mL, respectively). Furthermore, we found significant incremental increases in circulating fibrocyte numbers with advancing BOS stage, suggesting a role for fibrocytes in BOS progression.⁸ This has been corroborated by another study, which found a statistically significant increase in the number of fibrocytes on staining for CXCR4/prolyl 4-hydroxylase in lung biopsy specimens from lung transplant patients with BOS compared with controls.⁹

The associations between fibrocyte numbers and BOS progression in human studies has suggested, but not yet proved, that fibrocytes have detrimental effects in pulmonary fibrosis. Given the correlation of fibrocyte number and clinical BOS stage and the importance of the CXCR4/CXCL12 axis in fibrocyte trafficking, we hypothesized that immunotherapy directed against CXCL12 would attenuate airway fibrosis and obliteration in a well-established murine heterotopic tracheal transplant model of obliterative bronchiolitis.^{10,11}

METHODS

Generation and Purification of Anti-CXCL12 IgG F(ab')₂ Fragments

Goat anti-human CXCL12 antibodies were purified using the Melon Gel IgG purification Kit (Thermo Scientific, Rockford, Ill). Normal goat IgG and purified goat anti-human CXCL12 IgG were digested with Immobilized Pepsin (Pierce, Rockford, Ill). Fragments were recovered and dialyzed against phosphate-buffered saline. F(ab')₂ were purified from Fc fragments using NUNC Protein G columns (Nunc, Rockford, Ill).

Mice

All mice (Jackson Laboratory, Bar Harbor, Maine) received humane care according to the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals." The Animal Care and Use Committee at the University of Virginia approved the study protocol.

Experimental Group Design

We used a heterotopic subcutaneous tracheal transplant model of bronchiolitis obliterans, as previously described.¹¹ A major histocompatibility complex class I and II mismatch was produced by transplanting 4 Balb/c (H-2^d) tracheas into 1 C57BL/6 (H-2^b) recipient. Four donor trachea allografts were used per recipient to ensure adequate tracheal tissue for fibrocyte determination and histologic examination. The mice were divided into 2 groups: the IgG control group [intraperitoneal injections of normal goat IgG F(ab')₂] and the anti-CXCL12 group [intraperitoneal injections of anti-CXCL12 F(ab')₂]. Each group included 6 recipients for a total of 120

donors and 30 recipients. Thus, there were a total of 240 donors and 60 recipients. All recipients were given intraperitoneal injections at days -1, 0, and 1, and then every other day until the endpoints. The mice were killed, and the allografts were collected on days 3, 7, 12, 21, or 28. Tracheal allografts from 5 of the 6 mice at each point were pooled for flow cytometry analysis, and the tracheal allografts from the sixth mouse were used for histologic examination and Sirius Red staining.

Fluorescence Activated Cell Sorter Analysis

Single cell suspensions isolated from bone marrow, peripheral blood, and trachea allografts were stained with peridinin-chlorophyll-protein complex-labeled CD45, phycoerythrin-labeled CXCR4 (BD Biosciences, San Diego, Calif), or isotype controls. Subsequently, the cells were permeabilized using cytofix/cytoperm for staining with DyLight-488 conjugated anti-Col1, phycoerythrin-labeled α -SMA, or isotype controls. Anti-Col1 and a rabbit IgG isotype control were conjugated using a DyLight-488 conjugation kit (Thermo Scientific, Rockford, Ill). Four-color analysis of the stained cells was performed on a FACSCanto II flow cytometer using FACS-*Diva*, version 6.0, software (BD Biosciences). All analyses were blinded.

Histologic Examination and Measurement of Fibrosis and Luminal Obliteration

The allograft tracheal tissues were fixed, embedded, sectioned, and stained with hematoxylin and eosin. Allograft trachea sections were photographed at 4 $\times$ magnification. Collagen deposition was quantified according to the percentage of luminal obliteration of the tracheal allografts using ImagePro Plus software. Eight allografts were measured in each group.

Collagen Staining and Densitometry

The tracheal sections were deparaffinized, rehydrated, and stained with Direct Red 80 (Sigma Aldrich, St. Louis, Mo). The images were captured for quantification using Image J software. The collagen signal in luminal fibro-obliteration tissue was semiquantified using the same software but with a different parameter setting.

Statistical Analysis

Fibrocytes from buffy coat, bone marrow, and tracheal single cell suspensions are presented as box and whisker plots. Each box and whisker plot at a given point represents 5 mice. Continuous data are reported as either the mean $\pm$ standard deviation or median and interquartile range. Independent sample comparisons were performed using either the Mann-Whitney *U* test or Kruskal-Wallis test. Statistical significance was set to $P < .05$. Group comparisons were unpaired, and the *P* values are 2-tailed. The analyses were performed using predictive analytics software (version 18.0.0; IBM Corp, Chicago, Ill).

RESULTS

Attenuation of Fibrocyte Trafficking Using Anti-CXCL12 F(ab')₂ Antibody

We first assessed the ability of neutralizing anti-CXCL12 F(ab')₂ antibodies to attenuate fibrocyte trafficking and differentiation in the murine model of bronchiolitis obliterans. Bone marrow, buffy coat, and trachea allograft single-cell suspensions from Balb/C mice subcutaneously transplanted with BL6 trachea were analyzed for total undifferentiated (CD45+Col1+CXCR4+) and differentiated fibrocyte (CD45+Col1+ α -SMA+) cell populations.

The comparison of buffy coat isolates from mouse whole blood preparations revealed significant decrease in circulating undifferentiated fibrocytes in mice treated with

anti-CXCL12 at 7 days (Figure 1, A; $P = .028$) after tracheal transplantation. However, anti-CXCL12-treated mice at 12 days after transplantation (Figure 1, A; $P = .028$) had increased undifferentiated fibrocytes compared with controls. Figure 1, B shows a concomitant increase in total differentiated fibrocytes in buffy coat preparations from anti-CXCL12-treated mice at 3 days ($P = .047$), with a decrease in the differentiated fibrocyte population at 7 days ($P = .008$).

Bone marrow aspirates showed an increase in undifferentiated fibrocytes (Figure 1, C) in anti-CXCL12-treated mice at 12 days after transplantation ($P = .015$) and a decrease in undifferentiated fibrocytes at 7 days ($P = .008$) and 21 days ($P = .014$) after transplantation compared with the controls. Similarly, a significant increase was seen in the number of total differentiated fibrocytes (Figure 1, D) in the anti-CXCL12-treated mice at 12 days after transplantation ($P = .004$), with a decrease in the same population at 7 days after transplantation ($P = .008$).

As hypothesized, the single cell suspensions isolated from the tracheal allografts from anti-CXCL12-treated mice showed a significant decrease in both undifferentiated (Figure 1, E) and differentiated (Figure 1, F) fibrocytes at 7 days ($P = .009$ and $P = .009$, respectively) and 21 days ($P = .004$ and $P = .014$, respectively) after transplantation compared with the controls.

The mean ranks of undifferentiated and differentiated fibrocytes among buffy coat ($H = 18.95$, 7 df, $P = .008$ and $H = 21.92$, 7 df, $P = .003$, respectively), bone marrow ($H = 33.64$, 7 df, $P < .0001$ and $H = 34.01$, 7 df, $P < .0001$, respectively), and tracheal allograft single cell populations ($H = 30.29$, 7 df, $P < .0001$ and $H = 33.02$, 7 df, $P < .0001$, respectively) were significantly different among the control and anti-CXCL12-treated mice. Thus, a definite variation in fibrocyte number was found within each group.

Evaluation of Fibro-Obliteration on Tracheal Allografts

Representative hematoxylin and eosin images of tracheal allografts from the anti-CXCL12 and control mice at 3, 7, 12, and 21 days after transplantation are shown in Figure 2. Airway fibro-obliteration was scored using 2 methods. First, qualitative hematoxylin and eosin analysis was reviewed blindly by a lung pathologist who determined the percentage of luminal obliteration of tracheal allografts after tracheal transplantation (Figure 3). A significant decrease was seen in the median luminal obliteration at 21 days ($P = .010$) in the anti-CXCL12-treated mice (65% vs 100% obliterated in controls; interquartile range, 38% vs 10%). This trend was not seen at 28 days after transplantation ($P = .153$).

We next assessed the effect of anti-CXCL12 treatment on collagen deposition in trachea allograft lumens. Densometric

quantitative measurement of direct red 80 collagen staining was used to determine the total luminal collagen deposition during the same period. As expected, treatment with anti-CXCL12 resulted in a significant decrease in trachea allograft luminal collagen deposition at 21 days (1591.62 vs 6314.97 integrated density units; interquartile range, 3167.98 vs 10,725.91; $P = .042$) and 28 days (6871.09 vs 12,775.80 integrated density units; interquartile range, 5280.35 vs 8346.05; $P = .0012$) after transplantation (Figure 4). This is consistent with the hypothesis that inhibition of the CXCR4/CXCL12 biologic axis results in attenuated fibrocyte trafficking to, and differentiation in, tracheal allografts.

The kinetics of total nonluminal tracheal collagen deposition showed a slightly different pattern from that of luminal deposition (data not shown). A significant decrease in tracheal fibro-obliteration in the anti-CXCL12-treated mice at 12 ($P < .0001$) and 21 ($P < .0001$) days after transplantation compared with controls, with no significant difference seen at 3, 7, and 28 days ($P > .05$).

DISCUSSION

The importance of fibrocyte biology has been established in multiple pancorporeal fibrotic processes, including normal wound repair, nephrogenic fibrosing dermatomyopathy, scleroderma, chronic pancreatitis, cystitis, liver fibrosis, and multiple lung pathologies marked by recurring inflammation and repair.⁶ We have previously shown a correlation between increased circulating fibrocyte levels and the development and progression of BOS in patients after lung transplantation.⁸ These studies have suggested that fibrocytes might contribute to the pathology of pulmonary fibrosis.

Given the potential clinical importance of fibrocytes in the progression of fibrotic pathology, we sought to identify the effect of an anti-CXCL12 F(ab')₂ antibody in mitigating tracheal fibro-obliteration. Despite its shortcomings, we used a murine tracheal transplant model of bronchiolitis obliterans, because it is well described and less technically demanding and time consuming compared with other models and reproducible in its production of obliterative airway disease. The critics of this model have noted that this is a large airway representation of small airway disease and that it fails to use a vascularized or aerated allograft. Other models of bronchiolitis obliterans have been developed, including the orthotopic tracheal model¹²; however, just as in the heterotopic model, this is a large airway model and is not vascularized. Furthermore, it is technically challenging and fails to uniformly develop histopathologic bronchiolitis obliterans. Similarly, single lung transplantation has been successfully performed in mice. However, these transplanted lungs allografts have not been shown to reliably develop histopathologic lesions of bronchiolitis obliterans.¹³

In the present study, we have demonstrated that an anti-CXCL12 antibody effectively attenuates fibrocyte

FIGURE 1. Box and whisker plots depicting total fibrocyte populations in both anti-CXCL12 F(ab')₂-treated and control goat IgG F(ab')₂-treated mice after heterotopic transplant of allograft tracheas. *Box and whiskers* represent 25th to 75th and 10th to 90th percentiles, respectively; *transverse lines* represent the median. Each box and whisker plot at a given point represents 5 mice. A, C, E, Undifferentiated, CD45+collagen 1 (Col1)+CXCR4+ fibrocyte populations over time in buffy coat (BC), bone marrow (BM), and tracheal allograft isolations (Trach), respectively. B, D, F, Differentiated, CD45+Col1+smooth muscle actin (SMA)+ fibrocyte populations in BC, bone marrow, and trachea allograft isolations, respectively. **P* < .05, Mann-Whitney *U* test.

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FIGURE 2. Representative images of hematoxylin and eosin–stained histopathologic sections of (left), goat IgG F(ab')₂–treated or (right), anti-CXCL12 F(ab')₂–treated mice at 3, 7, 12, 21, and 28 days after heterotopic transplantation of allograft trachea.

trafficking to, and differentiation in, heterotopic trachea allografts. This antibody simultaneously mitigated graft disease progression and fibro-obliteration. Our findings have been corroborated by evidence from other studies that found that blocking the CXCR4/CXCL12 axis with an anti-CXCL12 antibody¹⁴ or a direct CXCR4 antagonist, AMD3100,¹⁵ protected against pulmonary fibrosis in bleomycin models of IPF—a process defined by cryptogenic fibro-obliteration of the lung parenchyma.

Recent evidence has pointed toward a duality in fibrocyte biology as a cell capable of both immune modulation and extracellular matrix repair.¹⁶ The differentiation of fibrocytes into cells capable of fulfilling these roles is dependent on the cytokine milieu and progresses chronologically during the disease course from an early role in an inflammatory phenotype to late differentiation into collagen-producing reparative cells.¹⁷ The results of the present study have shown a temporal fibrocyte trafficking pattern consistent with this paradigm in which fibrocytes respond to tracheal tissue injury by CXCL12 chemotaxis and propagate a temporal fibrotic process. In the acute stages after transplantation, there is an increase in bone marrow production of both undifferentiated (CD45+Col1+CXCR4+) and differentiated (CD45+Col1+ α -SMA+) fibrocytes in anti-CXCL12–treated and control mice. As expected, there was a lag in the appearance of both cell populations in buffy coat preparations.

As demonstrated in Figure 5, there is an early accumulation of undifferentiated fibrocytes in tracheal allografts that might result from the trafficking of fibrocytes already in circulation. However, there is a lack of early concomitant fibrosis, suggesting that these fibrocytes are responding to an inflammatory milieu that exists acutely after transplantation by differentiating along antigen-presenting or immunomodulatory cell lines instead of along a reparative cell lineage. A comparison of control and anti-CXCL12 mice demonstrated a more rapid and, ultimately, more significant decrease in fibrocyte numbers in anti-CXCL12–treated mice and a more rapid and, ultimately, more significant increase in tracheal fibrosis in the control mice. A Kruskal-Wallis analysis demonstrated that both control and anti-CXCL12 fibrocyte populations ($P < .001$) and tracheal collagen deposition ($P < .001$) are significantly different.

On closer analysis, the difference between the control and anti-CXCL12–treated mice was most pronounced at day 7, when the anti-CXCL12–treated mice have a strikingly decreased number of both fibrocyte populations in buffy coat and trachea allograft isolations. Although the control mice continued to experience an increase in fibrocyte trafficking and differentiation into grafted airways, both fibrocyte populations in the anti-CXCL12–treated mice greatly decreased. Therefore, day 7 might be significant in that it represents when the pressure to differentiate toward an inflammatory lineage subsides and the pressure to differentiate toward a reparative lineage increases.

FIGURE 3. Box and whisker plots depicting percentage of luminal fibrosis in tracheal allograft sections in both anti-CXCL12 F(ab')₂-treated and control goat IgG F(ab')₂-treated mice as assessed by a lung pathologist in a blinded fashion. *Box and whiskers* represent 25th to 75th and 10th to 90th percentiles, respectively; *transverse lines* represent the median. **P* < .05, Mann-Whitney *U* test.

Thus, the larger fibrocyte populations on day 7 in the control mice are able to respond to this pressure and, as a result, fibro-obliteration occurs more rapidly than in the anti-CXCL12-treated mice.

Our data have shown that inhibiting the CXCL12/CXCR4 axis had little effect on the acute bone marrow production of fibrocytes but instead appeared more vital in preventing the trafficking of fibrocytes from the bone marrow into the circulation and from the circulation into the extravascular compartment. Furthermore, the difference in kinetics of collagen deposition between tracheal tissue and

lumen implicates the CXCL12/CXCR4 axis in the trafficking of fibrocytes through the tracheal tissue and into the tracheal lumen. At day 28, no difference was seen in tracheal tissue collagen deposition between the anti-CXCL12-treated mice and controls; however, the treated mice showed a significant decrease in total luminal collagen deposition, indicating that although the anti-CXCL12 antibody did not completely prevent the fibrotic disease from progressing in the tracheal allograft tissue, it mitigated total airway obliteration.

The inhibition of CXCL12 could be responsible for the decreased population of differentiated fibrocytes in the trachea allografts and buffy coat isolations and the decrease in luminal fibrosis in the anti-CXCL12-treated mice. However, it is likely that these changes were, in large part, a consequence of diminished trafficking and extravasation of undifferentiated fibrocytes into the proper milieu for differentiation. Although a drastic decrease was seen in both buffy coat and tracheal undifferentiated and differentiated fibrocyte populations in the anti-CXCL12-treated mice, there was a substantial increase in the same populations in the control mice. This might suggest that in the control mice, undifferentiated fibrocytes were more readily able to traffic to the tracheal allografts and differentiate into fibroblastic, collagen-producing cells. Although the difference in fibrocytes populations might not account for the entire difference in fibro-obliteration between the anti-CXCL12-treated and control mice, it does seem to be a major contributor to disease progression.

However, we still saw an increasing accumulation of collagen deposition in the tracheal allografts from the anti-CXCL12-treated mice. This might have been due, in part, to other less important chemokines (CCR7/CCR2) that are able to recruit fibrocytes into the allografts. Mouse fibrocytes have been shown to express CCR7, CXCR4, and CCR2 and respond to a number of different ligands.¹⁸ The CXCR4/CXCL12 axis is known to be the predominant receptor/ligand interaction for trafficking to a grafted airway and subsequent extravasation of fibrocytes into tissue.^{5,18,19} These other receptor/ligand interactions might help to explain the differences in kinetics between the anti-CXCL12-treated and control groups. Furthermore, local differentiation of fibroblasts and epithelial to mesenchyme transition play a role in fibro-obliteration.² The stimuli for fibrocyte production in the bone marrow and subsequent movement to blood is not clear, but it appears that other cytokine/receptor interactions might predominate. Thus, blocking the CXCR4/CXCL12 axis, although instrumental for inhibiting the progression of fibro-obliteration, does not, by itself, eliminate collagen deposition.

Our studies have provided novel insight into the kinetics of fibrocyte trafficking during the progression of airway fibro-obliteration and demonstrate the potential that inhibiting this axis has for slowing the disease process.

FIGURE 4. Box and whisker plots depicting percentage of tracheal luminal fibrosis as measured using Direct Red 80 collagen stain at 21 and 28 days after transplantation. *Box and whiskers* represent 25th to 75th and 10th to 90th percentiles, respectively; *transverse lines* represent the median. **P* < .05, Mann-Whitney *U* test.

FIGURE 5. Combined kinetics of both undifferentiated, CD45+collagen 1 (*Col1*)+CXCR4+ fibrocytes in tracheal allografts, as determined by flow cytometry, and tracheal tissue collagen deposition, as determined by Direct Red 80 densitometry of tracheal allograft sections. A, Kinetics of control, goat IgG F(ab')₂-treated mice showed more gradual and less complete decrease in tracheal allograft undifferentiated fibrocyte number with a concomitant more rapid and greater increase in tracheal collagen deposition compared with (B) anti-CXCL12 F(ab')₂-treated mice. Kruskal-Wallis analysis demonstrated that both control and anti-CXCL12 fibrocyte populations ($P < .001$) and tracheal collagen deposition ($P < .001$) were significantly different.

Anti-CXCL12 F(ab')₂ afforded protection against infiltrating fibrocytes and deterioration of allograft tracheal epithelium and collagen deposition. Thus, the CXCL12/CXCR4 axis provides a novel therapeutic target for the treatment of fibroproliferative disease. Given the clinical effect that fibrotic diseases have on morbidity and mortality, additional study is warranted in both animal models and humans to identify the role of this axis in molding fibrocyte differentiation in inflammatory and reparative milieus.

Newer immunomodulatory drugs directed toward inhibition of the mammalian target of rapamycin (mTOR), such as sirolimus and everolimus, offer significant hope in the prevention of allograft rejection.²⁰ Previous studies have

shown that therapy with rapamycin attenuates fibrocyte trafficking from bone marrow and prevents fibrocyte and collagen deposition in lungs challenged with bleomycin by altering CXCR4 expression.⁵ Others have shown everolimus to be a potent inhibitor of proliferation of ex vivo lung fibroblasts cultured from transbronchial biopsy samples harvested from patients after lung transplantation.²¹ Also, in 2 separate randomized control trials of lung transplant recipients, those patients receiving maintenance everolimus were less likely to have clinically suspected and biopsy proven acute graft rejection compared with the controls.^{22,23} Because these drugs are currently approved by the Food and Drug Administration for the prevention of

solid organ rejection and their potency for immune modulation, they might prove to be superior alternatives to anti-CXCL12 F(ab')₂. Despite this potential, many therapeutic challenges remain to the proper administration of these drugs. In lung transplant recipients receiving sirolimus, an increased risk of severe venous thromboembolism was present,²⁰ and in separate trials, patients treated with sirolimus experienced airway anastomosis dehiscence and generalized poor wound healing.^{24,25} Thus, many clinicians have decided to administer sirolimus after completion of bronchiolar wound healing,²⁴ and, as such, the use of sirolimus across all centers was 8% at 1 year and 18% at 5 years after transplantation.¹

CONCLUSIONS

An optimal outcome of mTOR inhibition would allow mitigation of fibroproliferative disease and, at the same time, normal wound repair. We suggest that given the integral nature of mTOR and the CXCR4 biologic axis to the pathogenesis of fibrosis, monitoring fibrocyte populations could offer a new biomarker for optimizing mTOR inhibitor therapy. Quantification of the circulating and lung allograft fibrocyte populations might provide the clinician with an individualized view of fibrotic stage/progression in lung transplant patients. Using these populations as biomarkers, it might be possible to tailor mTOR inhibitor drug therapy to a dose that would both maximize gain and minimize unwanted side effects on an individual basis, effectively opening the door to a more personalized medical approach for these patients.

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