Narrative Review Article

Reversing AT2 Cell Senescence in Idiopathic Pulmonary Fibrosis via CRISPRi-Mediated Serpine1 (PAI-1) Modulation

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ABSTRACT

Idiopathic Pulmonary Fibrosis (IPF) is a progressive and fatal lung disease with a median survival of 2.5 to 3.5 years post-diagnosis. Current FDA-approved antifibrotic therapies, including pirfenidone and nintedanib, slow disease progression, yet fail to address the underlying cellular dysfunction driving fibrosis. A key factor driving IPF pathogenesis is the senescence of alveolar type 2 (AT2) cells, the progenitor cell population that maintains and repairs the alveolar epithelium. When AT2 cells undergo the Senescence-Associated Secretory Phenotype (SASP), AT2 cells lose their regenerative capacity and secrete pro-inflammatory and pro-fibrotic factors that contribute to scarring of the lung tissue, or fibrosis. SERPINE1, the gene encoding Plasminogen Activator Inhibitor-1 (PAI-1), which is significantly upregulated in AT2 cells of IPF patients, has been mechanistically linked to senescence through inhibition of p53 degradation, activating the p53-p21-Rb cell cycle arrest pathway. Conditional knockout studies in murine models suggest that AT2-specific PAI-1 regulation suppresses senescence and weakens IPF progression, establishing SERPINE1 as a causal driver rather than a passive biomarker. This proposal outlines a gene therapy that utilizes an adeno-associated virus (AAV) vector to deliver a CRISPR interference (CRISPRi) system composed of dCas9 and a KRAB repressor domain. This system would downregulate SERPINE1 transcription without the creation of double-strand DNA breaks, offering a safer alternative to conventional CRISPR-Cas9 therapies. By targeting the upstream driver of AT2 senescence, this approach has the potential to restore alveolar regenerative capacity, reverse disease progression, and meaningfully improve IPF patient outcomes.

Keywords: Idiopathic Pulmonary Fibrosis; IPF; CRISPRi; CRISPR Interference; SERPINE1; PAI-1; Senescence

INTRODUCTION

Idiopathic Pulmonary Fibrosis (IPF) is one of the most devastating diagnoses in modern pulmonary medicine. IPF is an incurable chronic, progressive interstitial lung disease characterized by irreversible scarring of lung parenchyma, leading to a gradual decline in pulmonary function by affecting alveolar walls and surrounding interstitial spaces (1). IPF gradually suffocates patients as their lungs lose the ability to transfer oxygen. The average age of onset for IPF is around 65 years, with patients surviving for about 2.5-3.5 years post-diagnosis (2–4). With a global incidence of approximately 17.7 per 100,000 individuals, IPF predominantly affects males, who account for around 70% of all IPF cases, and nearly 60-80% of IPF patients have had a history of smoking (5–10). The primary symptoms of IPF include non-productive cough, dyspnea, and fatigue (11) (Figure 1).

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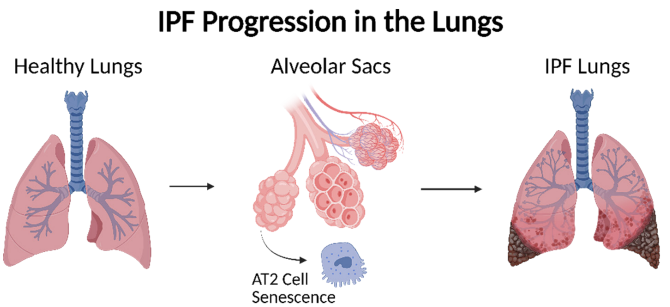

Figure 1. IPF Progression in the Lungs. *In healthy lungs, AT2 cells exhibit normal alveolar structure; however, in IPF, progressive dysfunction of AT2 cells contributes to AT2 cell senescence. This ultimately leads to widespread scarring (fibrosis) of the alveolar sacs, as seen in the IPF Lungs on the right. This figure demonstrates the progression from healthy alveolar function to fibrotic remodeling of the lungs, highlighting AT2 cells as an initiator for IPF and a key target for therapeutic intervention. Created in BioRender. Patel, V. (2026) https://BioRender.com/3mtmb82.*

Despite decades of research, the treatment options for IPF remain scarce. The two FDA-approved drugs, pirfenidone and nintedanib, are meaningful yet limited advances in IPF treatment. Both drugs have demonstrated the ability to limit declines in lung function and have decreased hospitalizations and mortality (12). Additionally, pirfenidone and nintedanib have been found to slow the annual decline in forced vital capacity (FVC) by around 44% to 57% (13). However, neither drug stops IPF progression or reverses the decline in lung function(14). These drugs' benefits come at the risk of gastrointestinal side effects such as diarrhea, nausea and vomiting, photosensitivity, and liver enzyme elevations, which reduce tolerability and patient adherence (15–18). These treatments mainly address downstream consequences of IPF, targeting myofibroblast activation and excessive extracellular matrix secretion that contribute to loss of alveolar function (19). Nintedanib and pirfenidone slow disease progression, but they do not address the underlying biological problem: the lung's inability to naturally repair and regenerate itself (20).

While the underlying cause of IPF is unknown, hence "idiopathic", it is thought to arise from repeated lung damage and healing, which triggers chronic activation of fibroblasts and their differentiation into myofibroblasts (1, 21). These persistently activated myofibroblasts overproduce extracellular matrix, resulting in abnormal repair, scarring, and alveolar distortion (1). IPF is fundamentally driven by the dysfunction of alveolar type 2 pneumocytes (AT2 cells) (22). AT2 cells serve as the progenitor cells of the alveolar epithelium. They are capable of self-renewal and differentiation into alveolar type 1 pneumocytes (AT1 cells), which are essential for gas exchange and alveolar repair after injury. AT2 cells function as stem cells during both homeostasis and regeneration, rapidly proliferating to restore the lung epithelium following damage (23–26). In IPF, their regenerative capabilities drastically fail. Rather than properly responding to injury, AT2 cells get trapped in a state of cellular senescence. AT2 cells remain metabolically active and acquire a senescence-associated secretory phenotype (SASP), producing cytokines, chemokines, proteases, and growth modulators that contribute to fibroblast activation and impaired lung repair (27–30). Evidence from the lungs of IPF patients suggests that IPF AT2 cells accumulate in diseased tissue and express increased senescence markers, such as TP53, CDKN1A/p21, and CDKN2A/p16, compared to donor AT2 cells (28). It is AT2 cell senescence, not the loss of AT2 cells themselves, that specifically drives IPF progression (28).

Recent molecular analysis has confirmed that the SERPINE1 gene, which encodes plasminogen activator inhibitor-1, or PAI-1, is a key contributor to AT2 cell senescence in fibrotic lung diseases (26). PAI-1 is a major regulatory protein of the plasminogen activation system, which controls fibrinolysis, the physiological breakdown of clots, and extracellular matrix remodeling, maintaining the homeostatic balance in tissue repair processes(31) (Figure 2). However, in IPF patients, this tightly regulated process becomes heavily dysregulated. Studies of human IPF tissue show that IPF lungs express higher levels of PAI-1 and cell senescence markers p21 and p16, compared to healthy controls (26). At the molecular level, elevated PAI-1 binds to proteasome components and inhibits the degradation of p53 protein, a regulator of cellular stress responses (32). In healthy cells, p53 is normally degraded via the 26S and 20S proteasomes to maintain low levels(33). However, when PAI-1 inhibits the proteasome, p53 accumulates in the nucleus as it can no longer be degraded (32, 34). p53 subsequently activates the CDKN1A gene and thus upregulates expression of p21, a cyclin-dependent kinase inhibitor that blocks cell cycle progression by preventing the G1-to-S phase transition through inhibition of Retinoblastoma (Rb) protein phosphorylation, keeping Rb in its active, growth-suppressive form (35–37).

The activation of the p53-p21-Rb pathway results in irreversible growth arrest, or cellular senescence, where AT2 cells are metabolically active but lose their ability to proliferate and repair damaged alveolar tissue (38). These senescent AT2 cells develop the SASP phenotype, secrete pro-inflammatory cytokines and pro-fibrotic growth factors, and additional PAI-1, creating a self-amplifying loop to induce senescence in neighboring AT2 cells (35, 39, 40).

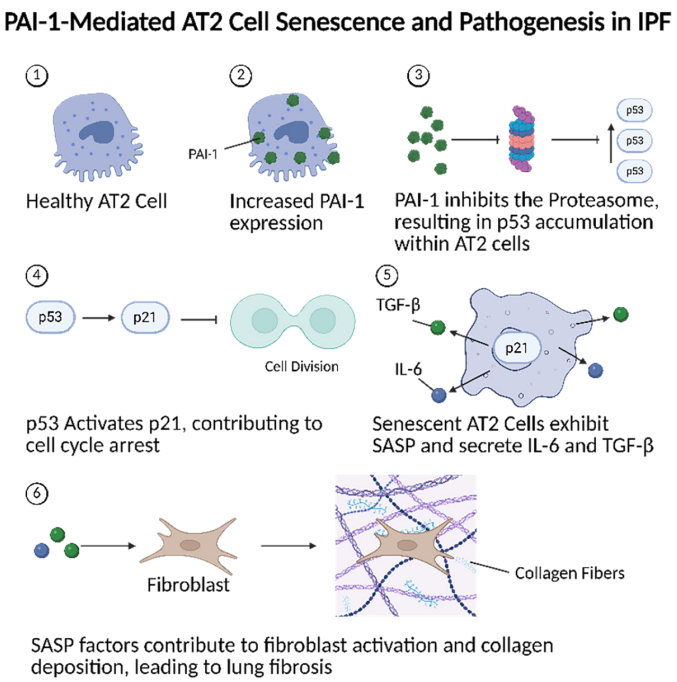

Figure 2. PAI-1-Mediated AT2 Cell Senescence and Pathogenesis in IPF. *In IPF, AT2 cells undergo a series of molecular changes that drive senescence and progressive lung fibrosis. (1) Healthy AT2 cells maintain normal function and alveolar regenerative capacity. (2) In IPF, PAI-1 expression is significantly increased in AT2 cells. (3) PAI-1 increasingly binds to and inhibits the proteasome, causing p53 accumulation within the cell. (4) Accumulated p53 activates p21, a cell cycle inhibitor, which results in cell cycle arrest. (5) Arrested AT2 cells adopt a Senescence-Associated Secretory Phenotype (SASP) and secrete cytokines like IL-6 and TGF-β. (6) SASP factors (IL-6 and TGF-β) activate resident fibroblasts, which deposit excessive collagen, leading to progressive scarring of the lung tissue in IPF. These steps illustrate the role of PAI-1 in AT2 cell senescence and IPF pathogenesis. Created in BioRender. Patel, V. (2026) https://BioRender.com/3giqn46.*

The identification of PAI-1 as a causal mediator of AT2 cell senescence leads to a unique therapeutic opportunity (26, 32). A novel yet effective treatment option for AT2 cell senescence could involve incorporating gene therapies, namely CRISPR interference (CRISPRi), to downregulate the SERPINE1 gene in AT2 cells to decrease AT2 cell senescence (41). A modified form of CRISPR-Cas9, CRISPRi operates by precisely targeting and repressing gene expression at the transcriptional level (42). Rather than cutting out subsections of DNA using a Cas9 protein and inducing double-strand breaks (DSBs), which can lead to mutagenesis, CRISPRi uses a deactivated Cas9 (dCas9) to prevent DNA cleavage (43, 44). The dCas9 is guided to specific DNA sequences through a single guide RNA (sgRNA), which binds to the promoter region of the target sequence (42, 43). Once bound, the dCas9-sgRNA system obstructs transcriptional elements, including RNA polymerase, to block mRNA synthesis, ultimately silencing SERPINE1 expression (45).

This proposal suggests a novel therapeutic strategy that utilizes CRISPRi to selectively downregulate SERPINE1 expression in AT2 of IPF patients to prevent these critical progenitor cells from entering senescence and restore their regenerative abilities. This approach shifts from managing the consequences of IPF to addressing its root cause: dysfunction of the lung's regenerative capabilities (46). By reversing, or merely delaying, AT2 cells from becoming senescent, this strategy preserves the regenerative cell pool while decreasing the pro-inflammatory and pro-fibrotic signals that drive IPF. The goal of this proposal is to establish an effective treatment that can restore the lung's capacity for self-repair to not only potentially offer IPF patients a slower decline, but also functional recovery.

REMOVAL OF SERPINE1 (PAI-1) REDUCES AT2 CELL SENESCENCE AND FIBROSIS

Before discussing the treatment plan outlined in this proposal, it is essential to understand and establish that downregulation of SERPINE1 (PAI-1) can reduce AT2 cell senescence and fibrosis. A 2017 study by Jiang and colleagues investigated the mechanistic role of SERPINE1 (PAI-1) in promoting AT2 cell senescence and the effect of its removal (26). The researchers aimed to uncover if increased PAI-1 expression results in AT2 senescence and whether PAI-1 leads to senescence by increasing p53 levels. AT2 cells from adult rat lungs (L2) were treated with bleomycin, mimicking features of

lung fibrosis; subsequently, levels of PAI-1, p53, and p21 proteins, as well as the activity of senescence-associated β-galactosidase (SA-β-gal), were found to increase (26, 47). The researchers then silenced PAI-1 with PAI-1 siRNA, resulting in the reduction of basal levels of p53 and p21 in L2 cells. These findings suggest that PAI-1 (and thus SERPINE1) positively regulates p53 and p21 expression and mediates AT2 senescence by activating the p53-p21 pathway (26).

In a subsequent experiment, Jiang and colleagues subjected L2 cells to PAI-1 knockdown via shRNA and then exposed them to bleomycin-induced DNA damage (26). Compared to NT shRNA controls, PAI-1 shRNA L2 cells showed significantly decreased bleomycin-induced activation of the p53 pathway due to reductions in serine-18 phosphorylated p53, total p53 protein levels, and downstream p21 expression (all calculated as a ratio over β-actin levels): 1.60 (NT shRNA controls) vs. 0.30 (PAI-1 shRNA L2 cells), 0.80 vs. 0.25, and 0.55 vs. 0.10, respectively (26). The decrease in serine-18 phosphorylated p53 suggests lower p53 activation and reduced stability, while reductions in p53 and p21 reflect a decrease in cell-cycle arrest signals. Additionally, 60% of PAI-1 siRNA cells were positive for proliferating cell nuclear antigen (PCNA), a reliable indicator for cell proliferation and DNA repair, compared to 20% of NT siRNA controls (26, 48). Similar results were observed when PAI-1 was inhibited via a small molecular inhibitor, TM5275 (26). In addition to bleomycin, the researchers used another anticancer drug, doxorubicin, to trigger senescence (26, 49). Many studies have demonstrated that doxorubicin can give rise to interstitial pneumonia and fibrosis (26, 50–52). L2 cells were transfected with PAI-1 shRNA or NT shRNA and were treated with saline or doxorubicin. Results indicate that basal levels of p53 protein in L2 cells, expression of doxorubicin-induced p53, and p21 expression decreased in PAI-1 shRNA-transfected cells (26).

Jiang and colleagues also tested whether increased PAI-1 causes AT2 cell senescence in fibrotic lung diseases, such as IPF, through an *in vivo* tamoxifen-inducible, AT2 cell-specific PAI-1 conditional knockout (CKO) mouse model with exons 4 and 5 of PAI-1 floxed (flanked by loxP sites for Cre-mediated excision) (26). Results from polymerase chain reaction (PCR), immunofluorescence and western analysis depict that tamoxifen injection resulted in a deletion of exons 4 and 5 of PAI-1 specifically in CKO mice. Testing if PAI-1 deletion in AT2 cells protects AT2 cells from bleomycin-induced senescence, Jiang and colleagues intraperitoneally injected CKO and wild-type (PAI-1 fl/fl; floxed but intact gene) mice with tamoxifen and then with 2U/kg bleomycin (26). 14 days later, mice were euthanized, lung tissue was collected, and AT2 cells were isolated. Double-immunofluorescence staining and X-gal staining results demonstrate that PAI-1 deletion in CKO mice reduces the percentage of AT2 cells expressing PAI-1/p53, p21, and SA-β-gal, compared to PAI-1 fl/fl mice: 60% (PAI-1 fl/fl) vs. 30% (PAI-1 deletion), 55% vs. 20%, and 17.5% vs. 7.5% (26). Similar to results from cultured L2 cells, PAI-1 deletion increases Rb phosphorylation within AT2 cells of mice *in vivo*, suggesting that loss of PAI-1 promotes cell-cycle progression by relieving Rb-mediated cell cycle arrest and possibly reducing senescence (26).

The authors also tested whether deletion of PAI-1 in AT2 cells can protect mice from bleomycin-induced fibrosis (26). Eight-to-ten-week-old CKO and PAI-1 fl/fl mice were injected with tamoxifen and then challenged with 2U/kg bleomycin and euthanized 14 days later. It was found that deleting PAI-1 (SERPINE1 gene) in AT2 cells in CKO mice resulted in a decrease in weight (from 6 grams to 4 grams) and a reduction in the accumulation of bronchoalveolar lavage fluid (BALF) (from 5.0 ng/mL to 3.5 ng/mL), compared to fl/fl controls. Additionally, PAI-1 deletion also resulted in decreases in collagen deposits, hydroxyproline accumulation (150% to 120% of saline), and expression of procollagen 1α2 (150% for PAI-1 fl/fl to 75% of saline for CKO), procollagen 1α1(175% to 75%), and alpha-smooth muscle actin (500% to 250%) in CKO cells compared to PAI-1 fl/fl cells (26). Excessive extracellular matrix accumulation, including increased collagen deposition and activated myofibroblast markers like αSMA, is a hallmark of fibrotic lung diseases, such as IPF, and can distort tissue architecture and impair lung function (1, 53). Decreases in collagen and smooth muscle actin suggest that the deletion of PAI-1 in AT2 cells *in vivo* can mitigate or reverse the fibrotic remodeling process that occurs in fibrotic lung diseases, such as IPF.

The results of this study convey that genetic deletion of SERPINE1 in AT2 cells reduced bleomycin and doxorubicin-induced AT2 cell senescence and reduced lung fibrosis severity, establishing causality rather than mere association (26). This study suggests that PAI-1 causes AT2 senescence by upregulating p53 expression and thereby activating the p53-p21-Rb pathways, resulting in cell-cycle arrest (26). Using siRNA and shRNA to suppress SERPINE1 to prevent AT2 cell senescence, with no detectable off-target effects, demonstrates that

partial, non-permanent gene repression is adequate for therapeutic benefit, directly supporting CRISPRi as a rational strategy to achieve stable transcriptional silencing without permanent genomic alterations. Collectively, this study establishes that AT2 cell PAI-1 plays an important role in the development of lung fibrosis through AT2 cell senescence, and interventions targeting SERPINE1 expression in AT2 cells can break this cycle found in many fibrotic lung diseases, including IPF (26).

A 2020 study by Rana et al. found that TGF-β1, a potent profibrotic cytokine, induces AT2 cell senescence rather than apoptosis, and AT2 cells express large amounts of PAI-1 (SERPINE1) in fibrotic diseases (54). Given these findings, the researchers sought to investigate whether removing PAI-1 (SERPINE1) could prevent AT2 cell senescence and its downstream effects. To understand whether PAI-1 plays a role in TGF-β1-induced AT2 cell senescence, Rana and colleagues isolated mouse lung AT2 cells from PAI-1–deficient ($PAI\text{-}1^{-/-}$) mice, where SERPINE1 had been removed from all cells via germline knockout, and wild-type ($PAI\text{-}1^{+/+}$) mice and treated them with TGF-β1 (54). Genetic deletion of PAI-1 in AT2 cells provided near-complete protection against TGF-β1-induced senescence: while TGF-β1 increased SA-β-gal-positive senescent cells from 2% to 30% in wild-type AT2 cells, PAI-1 knockout cells maintained baseline senescence levels at approximately 2% even after TGF-β1 treatment. PAI-1 deletion also decreased basal caspase 3/7 activity (a marker of apoptotic cell death) from approximately 25,000 relative luminescence units (RLU) to 15,000 RLU (54). PAI-1 abolished TGF- β1-stimulated secretion of multiple profibrotic SASP components: IL-6 was reduced from approximately 130 pg/mL in TGF-β1-treated wild-type cells to around 60 pg/mL in PAI-1−/− cells, with IL-4, IL-13 and PDGF being reduced from approximately 14 pg/mL to 7 pg/mL, 25 pg/mL to 15 pg/mL, and 65 pg/mL to 35 pg/mL, respectively.

Next, the researchers investigated whether factors secreted by SASP AT2 cells influence alveolar macrophage (AM) gene expression and if PAI-1 plays a role in this (54). Rana and colleagues collected conditioned medium (CM) from TGF-β1–treated, senescent AT2 cells (with and without deletion of the SERPINE1 gene) and subsequently incubated AMs with the CM. They found that CM from senescent AT2 cells strongly induced pro-fibrotic and alternative action markers such as Fizz1, Ym1, STAT6, iNOS, TNF-α, IL-4, IL-6, IL-13, and TGF-β1 (54). However, this stimulatory effect was partially or completely lost when AMs were cultured in CM from PAI-1–deficient AT2 cells. These findings highlight that PAI-1-driven AT2 cell senescence influences AMs to adopt a pro-fibrotic phenotype that possibly contributes to the progression of IPF (54).

Rana et al. then investigated if the presence or absence of TM5275, an inhibitor of PAI-1, could protect AT2 cells from TGF-β1–induced senescence and the associated secretion of profibrotic mediators. They treated rat lung AT2 (L2) cells with 2ng/mL TGF-β1 in the presence or absence of TM5275. TGF-β1 treatment increased AT2 cell senescence, as indicated by higher senescence-associated-β-gal (SA-β-gal) activity, upregulation of p16 and PAI-1, and reduced Rb phosphorylation (54). However, TM5275 treatment virtually blocked these effects on SA-β-gal activity, p16, and Rb phosphorylation. In L2 cells treated with only TGF-β1, approximately 40% were positive for SA-β-gal, the level of p16 relative to GAPDH was 1.5, and the level of phosphorylated Rb relative to GAPDH was 0.5. By contrast, L2 cells treated with TGF-β1 plus TM5275 had a reduction in SA-β-gal–positive cells to 10%, p16 levels decreased to 0.75, and phosphorylated Rb increased to 1.0, suggesting that pharmacological inhibition of PAI-1 via TM5275 can reduce TGF-β1–induced AT2 cell senescence and restore cell cycle regulatory signaling(54)n. Although TGF-β1 resulted in the secretion of two senescence markers, IGFBP3 and IL-6, by AT2 cells, TM5275 almost completely blocked these markers along with IL-4, IL-13, and PDGF, suggesting that PAI-1 removal is sufficient to block TGF-β1-induced AT2 cell senescence and downstream profibrotic signaling. Similar to their earlier CM experiments with cultured AMs, the researchers cultured NR8383 rat macrophages with CM from TGF-β1–treated L2 cells and found that TM5275 treatment drastically diminished expression of profibrotic macrophage activation markers, conveying that PAI-1 downregulation decreases profibrotic macrophage activation from senescent AT2 cells through paracrine signaling (54).

This study demonstrates that genetic deletion via germline knockout and pharmacologic inhibition of PAI-1 can reduce AT2 cell senescence, suppress SASP, and disrupt paracrine activation of profibrotic AMs(54)8mzB1. The results suggest that PAI-1 (and thus SERPINE1) plays an important role in AT2 senescence, and its removal results in many therapeutic benefits that can reduce and reverse fibrosis.

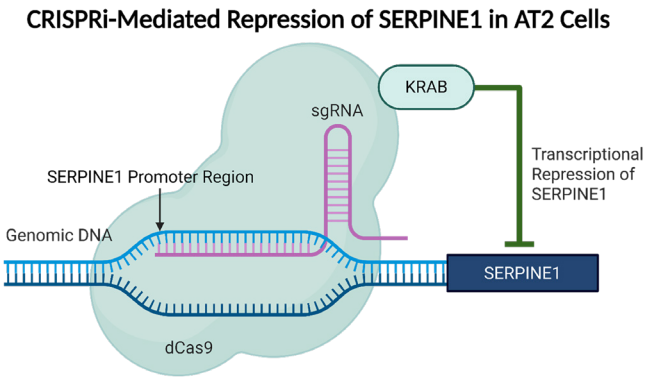

Figure 3. CRISPRi-Mediated Repression of SERPINE1 in AT2 Cells. *CRISPRi utilizes a dCas9 fused to a KRAB repressor domain, guided to the SERPINE1 promoter region by a sequence-specific sgRNA. After binding, the dCas9-KRAB complex blocks transcriptional factors from accessing the SERPINE1 gene, decreasing SERPINE1 mRNA transcription and thus PAI-1 protein levels within AT2 cells. By suppressing SERPINE1 expression via CRISPRi, this approach aims to interrupt the PAI-1-driven senescence described in Figure 2 to restore the regenerative capacity of AT2 cells and decrease fibrosis. Created in BioRender. Patel, V. (2026) https://BioRender.com/3mtmb82.*

CRISPRI DELIVERY MECHANISMS

To date, no study has used CRISPRi to target SERPINE1 in AT2 cells specifically. Although CRISPRi-mediated downregulation of SERPINE1 in AT2 cells has not yet been reported, research by Werder et al. provides strong proof of concept that CRISPRi can effectively modulate SERPINE1 expression in human AT2 cells (55). In this study, the researchers used induced pluripotent stem cells, which were human cells that can be directed to develop into any type of cell, and differentiated them into iPSC-derived AT2 cells (iAT2s) (55) (Figure 3). They modified them with an inducible CRISPRi mechanism made of two components. The first component was a dCas9-KRAB fusion protein, which is a deactivated version of the Cas9 enzyme that is fused to a KRAB repressor domain that silences gene expression when brought to a specific genomic location. It was integrated into the cells' safe harbor locus, AAVS1, under a doxycycline-inducible TRE promoter (55). The second component was the targeting system, which was a set of sgRNAs delivered via a lentiviral vector. After the addition of doxycycline, the dCas9-KRAB protein was expressed and directed by the sgRNAs to the target gene, where the KRAB domain blocks transcription through silencing factors. The researchers were able to knock down nine genes linked to Chronic Obstructive Pulmonary Disease (COPD) risk in iAT2s and discovered that silencing these genes resulted in changes in AT2 differentiation, maturation, proliferation, and injury response (55). As further proof of concept, the researchers demonstrate that CRISPRi knockdown of SFTPC, a gene encoding surface protein C (SPC), which is exclusively expressed in AT2 cells, was efficient and continuous over a long period throughout the differentiation from iPSC to iAT2 (55–58).

Although Werder and colleagues did not directly target SERPINE1, their findings are highly relevant to the therapeutic interventions put forth in this paper (55). Werder et al. establish that CRISPRi can modulate processes in human AT2 cells, such as proliferation, maturation, and injury response, many of which are disrupted by the senescence phenotype in IPF (35, 39, 40, 55). If this same CRISPRi delivery mechanism were used to silence SERPINE1 instead of the COPD genes, it would be highly likely to see suppression of PAI-1, diminishing the senescence pathway and restoring AT2 regenerative function, in theory. The delivery system used by Werder et al. is an *in vitro* approach and not suitable for use in a living organism (55). In a patient, it would not be feasible to alter the AAVS1 locus in existing AT2 cells or to use lentiviral vectors, due to their risk of insertional mutagenesis (59). For the *in vivo* strategy proposed in this paper, adeno-associated virus (AAV) delivery offers a safer and more clinically translatable alternative for delivering the CRISPRi system. AAV vectors can be administered into the lungs through intratracheal instillation and can carry the dCas9-KRAB and sgRNA components, with AT2-specific expression further ensured by driving dCas9-KRAB under an SPC promoter, which is selectively active in AT2 cells. Collectively, the successful use of CRISPRi to induce gene knockout by Werder et al., combined with the established safety and lung-targeting capacity of AAV, supports the biological feasibility of the therapeutic strategy in this proposal: AAV-delivered CRISPRi-mediated downregulation of SERPINE1 in AT2 cells to reduce senescence and restore regenerative capacity in IPF patients.

THERAPEUTIC IMPLICATIONS, LIMITATIONS, AND FUTURE DIRECTIONS

Why SERPINE1 Is the Optimal Target

In the context of IPF and cellular senescence, it's important to understand the reason for targeting

SERPINE1 in place of other senescence-related genes. Some of the more intuitive genes of interest may be p53 or p21 due to their well-established role in coordinated cell cycle arrest among senescent cells (60). However, directly silencing these genes poses many risks, as they both function as tumor suppressors, as p53 guards against uncontrolled cell growth by activating p21, which acts as a cyclin-dependent kinase (CDK) inhibitor (61, 62). Trying to downregulate either or both genes could facilitate tumor progression in the lung epithelium. SERPINE1 is the stronger gene of interest as it sits upstream of the p53-p21-Rb pathway instead of within it, minimizing the risk of altering genes that could facilitate tumor growth. The p53 protein would still be present and would perform its tumor suppressor function of responding to cancer signals, but it would be maintained below the threshold at which its accumulation drives AT2 senescence.

SERPINE1 may have some involvement in cancer biology and tumor progression as PAI-1 has been recognized as a biomarker in breast, colorectal, ovarian, bladder, and non-small cell lung cancers (63–72). It has also been found that the SERPINE1 gene is an extremely reliable biomarker for breast cancer, ovarian cancer, renal cell carcinoma, head and neck squamous cell carcinoma, bladder cancer, colorectal cancer, gastric cancer, and non-small cell cancers (73). These findings may indicate that PAI-1 can function as a tumor-promoting factor in certain biological contexts. Tumor-promoting effects are often associated with elevated PAI-1 levels in cancer literature. However, many cancers actively upregulate PAI-1 levels, and it is the resulting overexpression that correlates with worse prognosis among cancer patients, not the presence of PAI-1 at baseline levels (72, 74). This is relevant because PAI-1 is also overexpressed in IPF, as senescent AT2 cells produce abnormally high levels of PAI-1, which results in further senescence and fibrosis within the lung (26, 54). Additionally, unlike p53 or p21, SERPINE1 is not classified as a tumor suppressor, and individuals with PAI-1 deficiency do not exhibit increased cancer risk, as would individuals with p53 or p21 deficiency, but rather only a mild bleeding tendency, confirming that diminished levels of PAI-1 do not create conditions favorable for tumor formation (75, 76). In IPF, PAI-1 is overexpressed and creates a microenvironment that drives progressive fibrosis, mirroring the overexpression state that cancer literature often identifies as pro-tumorigenic. The proposed CRISPRi strategy aims to reduce PAI-1 from pathologically elevated levels back to baseline levels, not eliminate it entirely. In theory, this strategy would be expected to alleviate the pro-senescence and pro-fibrotic effects of PAI-1 upregulation in AT2 cells without creating a PAI-1-deficient state. Thus, by targeting SERPINE1, a gene upstream of the p53-p21-Rb pathway, CRISPRi-mediated PAI-1 downregulation offers the arguably most favorable strategy for reversing AT2 cell senescence.

Broader Biological Effects of SERPINE1 Downregulation

Much of this paper has focused on the role of PAI-1 in AT2 cell senescence through the p53-p21-Rb cell cycle arrest pathway. However, the impact of PAI-1 overexpression in IPF may extend beyond this single transduction pathway. Rana et al. found that TGF-β1-induced senescence in mouse AT2 and rat L2 cells led to SASP, in which AT2 cells secrete pro-inflammatory and pro-fibrotic signals, such as IL-4 and IL-13 (54). These SASP-associated signals acted on alveolar macrophages through paracrine signaling, stimulating the expression of genes that create increased fibrosis and scarring in the lung (54). These findings confirm that senescent AT2 cells not only fail to regenerate but also alter existing immune function by reprogramming nearby macrophages toward a phenotype that increases fibroblast activation, myofibroblast differentiation, and collagen deposition (35, 77, 78). Yet, when Rana et al. inhibited PAI-1 expression with TM5275 or deleted PAI-1 genetically, SASP was blocked in AT2 cells, and alveolar macrophages were no longer in a pro-fibrotic state (54). This research suggests that PAI-1 is not only involved in AT2 dysfunction but also in a broader fibrotic cascade (78). Thus, the proposed CRISPRi strategy is significant due to its role in decreasing pulmonary fibrosis. It would not be limited to restoring AT2 cell regeneration but also reducing the secretion of pro-fibrotic SASP factors. Targeting SERPINE1 accomplishes two important goals: reversing regenerative defects in AT2 cells while limiting the paracrine signaling that sustains fibrotic progression in the lungs of IPF patients.

Limitations and Experimental Gaps

Despite the mechanistic framework established in this proposal, several key limitations must be acknowledged. Importantly, no current study to date has directly applied CRISPRi to downregulate SERPINE1 in AT2 cells in human IPF patients. The mechanism proposed in this study integrates three validated lines of evidence: first, that PAI-1 drives AT2 cell senescence through the p53-p21-Rb pathway (26, 28, 54), second, that AAV vectors

may efficiently transduce AT2 cells *in vivo* through intratracheal instillation (58), and third, that CRISPRi can effectively silence genes in iPSC-derived AT2 cells and produce meaningful biological changes (55). Each of these points has been confirmed independently, yet each of these components has not been combined into a single mechanism that has been experimentally tested. Thus, experimental validation is necessary to confirm the effectiveness of the CRISPRi mechanism outlined in this proposal.

Another important factor is the way AT2 senescence was induced. The relationship between PAI-1 and AT2 senescence was established using multiple chemical inducers, including bleomycin, which causes DNA strand breaks; doxorubicin, which intercalates DNA and causes oxidative stress; and TGF- β1, a pro-fibrotic cytokine (26). While both bleomycin and doxorubicin are highly accepted models of inducing senescence, they represent only two of the many pathways in which AT2 cells can become senescent in human IPF patients. In actual IPF patients, AT2 senescence is driven by a more nuanced and diverse combination of factors, including chronic aging, telomere shortening, and mitochondrial dysfunction (28, 35, 79). Hence, it remains unclear whether CRISPRi-mediated SERPINE1 suppression would be equally effective against other forms of AT2 senescence driven by multiple factors. However, this proposal specifically focuses on reversing PAI-1-mediated senescence achieved through the p53-p21-Rb pathway, and broader applicability will need to be evaluated through future research.

There exists a significant gap between preclinical studies and clinical application. The AAV delivery research supporting this proposal came from studies in mice and hamsters, where intratracheal instillation achieved efficient transduction of AT2 cells (58). However, no CRISPRi therapy has been specifically delivered to AT2 cells in human patients. Transitioning from rodents to humans may introduce challenges, including differences in lung size, transduction pathways, and structural complexity, that could affect the effectiveness of the CRISPRi mechanism. Additionally, there are some safety considerations related to PAI-1 suppression. PAI-1 deficiency may cause bleeding, characterized by delayed surgical bleeding, menstrual bleeding, and excessive bruising (75). These effects may be present among patients who undergo CRISPRi delivery, resulting in PAI-1 reduction. However, the proposed CRISPRi strategy differs from a complete deficiency in that it targets only AT2 cells and aims for partial reduction of PAI-1. Together, these factors highlight the importance of selectively altering SERPINE1 overexpression while preserving the IPF lung microenvironment.

Future Directions

Currently, the most important next step is direct experimental confirmation of the efficacy of the proposed strategy. This would begin with *in vitro* testing of CRISPRi targeting the SERPINE1 gene in human IPF AT2 cells or iPSC-derived AT2 cells, specifically measuring PAI-1 reduction, decreases in senescence markers, and restoring AT2 cell proliferation. Successful *in vitro* validation would be followed by testing *in vivo* in IPF mouse models through AAV-delivered CRISPRi. The sgRNA should follow the mechanism established by the Jiang et al. conditional mouse model, which solely targeted exons 4 and 5 of SERPINE1 and still resulted in reductions in fibrosis, highlighting that knocking out these specific regions may be sufficient for biologically meaningful PAI-1 suppression (26).

Beyond CRISPRi, future research can explore combining therapeutic strategies to mitigate IPF progression. One promising approach would be to combine CRISPRi with existing antifibrotic drugs such as pirfenidone or nintedanib (3, 11, 19). These drugs slow the progression of fibrosis by targeting downstream fibroblast activation, but they do not address the root cause of AT2 senescence: suppressed proliferative capacity in AT2 cells. CRISPRi would target the root cause, while pirfenidone or nintedanib would mitigate fibrotic damage. This combinatorial approach yields a synergistic therapeutic outcome that no single therapy could achieve alone. Another promising approach is the use of monoclonal antibodies to reduce circulating PAI-1 levels or key SASP mediators. Anti-PAI-1 monoclonal antibodies could provide systemic reduction of PAI-1 by binding to secreted PAI-1 protein in the extracellular space, limiting its ability to inhibit plasminogen activators and promote senescence, though this approach may not fully address the intracellular PAI-1-driven senescence pathway in AT2 cells (80, 81). Because AAV-delivered CRISPRi may require days or even weeks to achieve its full therapeutic effect, the administration of monoclonal antibodies could help mitigate the spread of IPF during the transition period between CRISPRi introduction and when it achieves full effectiveness. This multi-targeted approach could prevent new senescence (CRISPRi) while decreasing the existing level of senescence proteins (anti-PAI-1 monoclonal antibodies), attacking IPF pathogenesis at multiple points.

Looking ahead, if patient safety is established, the pathway toward human trials would begin with *ex vivo* testing of CRISPR in AT2 cells from human IPF lungs, followed by a Phase I clinical trial with a small cohort of IPF patients. Ultimately, careful patient consideration will be essential for clinical translation of this mechanism. This approach will most benefit patients with early or moderate IPF, where AT2 cells are still present but senescent, rather than patients in late-stage IPF, where excessive fibrosis may have already replaced alveolar tissue. These future directions represent a clear and achievable path from theoretical proposal to clinical reality, and if validated, CRISPRi-mediated SERPINE1 downregulation could be the first regenerative treatment that targets IPF at its origin rather than treating downstream consequences.

Bioethical Considerations and Translational Barriers

The therapeutic mechanism presented in this proposal raises some important bioethical considerations for using gene therapies in clinical settings. An important concern is the risk of unintended off-target effects. Although CRISPRi is safer than traditional CRISPR-Cas9 because it doesn't create DSBs, the dCas9-KRAB complex could still bind to unintended genomic regions other than SERPINE1, which could disrupt AT2 cell function in unpredictable ways (82). Because AT2 cells are the primary cells responsible for alveolar repair, even minimal off-target suppression could impair genes involved in proliferation, differentiation, or surfactant production (55). Moreover, introducing dCas9-KRAB, a protein derived from bacteria, may trigger an immune response against transduced AT2 cells, possibly worsening AT2 cell depletion through an unintended immune response (83–85).

Given the lack of studies on applying CRISPRi to complex diseases such as IPF, regulatory oversight will be essential for monitoring patient health and ensuring the efficacy of the therapy. Future applications will require approval by regulatory agencies such as the FDA, with rigorous preclinical safety protocols and long-term follow-up plans (86). Finally, equitable access to this therapy must remain a priority (87). Approved gene therapies currently cost millions of dollars per patient, with Casgevy, the world's first FDA-approved CRISPR cell therapy, costing $2.2 million per patient (88). If the price of CRISPRi-mediated SERPINE suppression is similar, it would be financially inaccessible to many IPF patients, many of whom are most likely older adults on fixed incomes. If this therapy is to achieve its goal of serving as a meaningful treatment for IPF, the path to clinical reality must not only include scientific confirmation but also managing off-target effects, minimizing immune responses, ensuring the therapy is effective and priced in a manner that makes it accessible to a majority of IPF patients, regardless of socioeconomic status.

CONCLUSION

This proposal highlights the various factors that support the use of CRISPRi gene editing in AT2 cells of IPF patients, focusing on downregulating the SERPINE1 gene encoding the PAI-1 protein, while also acknowledging limitations and future directions. Downregulation of SERPINE1, a senescence-associated gene, offers a promising avenue to reverse AT2 dysfunction and restore the regenerative capacity of AT2 cells in IPF patients. Moving forward, the use of CRISPRi with other therapies, such as monoclonal antibodies, could be an emerging possibility that would require rigorous testing. Preclinical studies will be needed to assess the long-term safety of AAV-CRISPRi delivery mechanisms and their efficacy as a clinical intervention to hinder IPF progression and contribute to disease decline. This manuscript advances the field of CRISPR-based regenerative therapies for pulmonary medicine by elucidating the rationale for AAV-delivered, AT2-specific CRISPRi targeting of SERPINE1, rather than targeting downstream fibrotic pathways, offering a mechanism to preserve progenitor cell function and restore alveolar regenerative capacity. In summary, this proposal puts forward a gene therapy in which an AAV vector delivers a CRISPRi system to downregulate SERPINE1 in AT2 cells to ultimately restore AT2 progenitor function and reactivate alveolar repair in IPF lungs. This therapeutic approach could transform IPF treatment by actively reversing disease progression, extending survival, and meaningfully improving patient outcomes.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this work.

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