REVIEW

HGT

Open camera or QR reader and scan code to access this article and other resources online.

# Gene Therapeutics for Surfactant Dysfunction Disorders: Targeting the Alveolar Type 2 Epithelial Cell

Sneha Sitaraman,[1] Konstantinos-Dionysios Alysandratos,[2,3] Jennifer A. Wambach,[4] and Maria P. Limberis[1,*]

[1]Spirovant Sciences, Inc., Philadelphia, Pennsylvania, USA; [2]Center for Regenerative Medicine, Boston University and Boston Medical Center, Boston, Massachusetts, USA; [3]The Pulmonary Center and Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA; [4]Division of Newborn Medicine, Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine and St. Louis Children's Hospital, St. Louis, Missouri, USA.

Genetic disorders of surfactant dysfunction result in significant morbidity and mortality, among infants, children, and adults. Available medical interventions are limited, nonspecific, and generally ineffective. As such, the need for effective therapies remains. Pathogenic variants in the *SFTPB, SFTPC*, and *ABCA3* genes, each of which encode proteins essential for proper pulmonary surfactant production and function, result in interstitial lung disease in infants, children, and adults, and lead to morbidity and early mortality. Expression of these genes is predominantly limited to the alveolar type 2 (AT2) epithelial cells present in the distal airspaces of the lungs, thus providing an unequivocal cellular origin of disease pathogenesis. While several treatment strategies are under development, a gene-based therapeutic holds great promise as a definitive therapy. Importantly for clinical translation, the genes associated with surfactant dysfunction are both well characterized and amenable to a gene-therapeutic-based strategy. This review focuses on the pathophysiology associated with these genetic disorders of surfactant dysfunction, and also provides an overview of the current state of gene-based therapeutics designed to target and transduce the AT2 cells.

**Keywords:** alveolar type 2 epithelial cell, AT2, pulmonary surfactant, gene therapy, viral vectors, *ABCA3*, *SFTPB*, *SFTPC*

## INTRODUCTION

The alveolar region of the human lungs is comprised of an extensively complex epithelial surface that mediates the exchange of $O_2$ and $CO_2$ required for respiration at birth. The alveolar surface is lined by alveolar type 1 (AT1) and type 2 (AT2) epithelial cells, which are in close apposition with endothelial cells.[1] In humans, the maintenance of pulmonary function is dependent on the proper function of AT2 cells, which synthesize and secrete surfactant, and serve as facultative progenitors of the distal lung. Pulmonary surfactant is composed of phospholipids (such as phosphatidylcholine and phosphatidylglycerol) and proteins (surfactant protein [SP]-A, SP-B, SP-C, and SP-D; produced intracellularly) that in combination reduce the surface tension and prevent alveolar collapse at end expiration (the composition and function of surfactant are reviewed in detail by Parra and Perez-Gil[2]).

The *SFTPB*, *SFTPC*, and *ABCA3* genes encode for key components required for proper production and function of pulmonary surfactant, and are predominantly expressed in the AT2 cells. Pathogenic variants in these genes result in diffuse parenchymal lung disease, which presents as severe neonatal respiratory distress syndrome (RDS), childhood interstitial lung disease (chILD), or adult pulmonary fibrosis, all of which are associated with substantial morbidity and mortality.[3–6]

*Correspondence: Dr. Maria P. Limberis, Spirovant Sciences, Inc., 3675 Market Street, Suite 900, Philadelphia, PA 19104, USA. E-mail: mlimberis@spirovant.com



DOI: 10.1089/hum.2022.130

Available medical therapies for genetic disorders of surfactant dysfunction are limited, not specific to the underlying genetic defect, and generally ineffective.[7,8] Lung transplantation, which is associated with substantial morbidity and mortality, has been performed for progressive respiratory failure associated with these disorders.[9] The genes central to the proper composition and function of pulmonary surfactant are well characterized, allowing for the development of a gene-based therapeutic strategy to target AT2 cells.

Herein, we provide an overview of the pathophysiology associated with each of these disorders that originate in the AT2 cells, the *in vivo* and *in vitro* model systems that recapitulate specific aspects of the disease phenotype and discuss the current state of gene therapeutics designed to target AT2 cells.

## GENETICS

RDS typically occurs in preterm infants due to a developmentally regulated deficiency of pulmonary surfactant production and presents shortly after birth with clinical symptoms that include tachypnea, subcostal and intercostal retractions, nasal flaring, grunting, hypoxemia, and impaired ventilation.[10] Reports of late preterm and full-term infants with severe, progressive RDS prompted the investigation of a genetically linked disruption of pulmonary surfactant production.[5] In 1993, Nogee et al. first reported on a full-term infant with congenital alveolar proteinosis and lethal neonatal RDS, who was later found to be homozygous for a loss-of-function variant in the *SFTPB*.[3] This report illustrated the importance of genetic testing full-term infants with severe and progressively worsening RDS.

In the United States, genetic testing for surfactant dysfunction disorders is now widely offered (https://www.ncbi.nlm.nih.gov/gtr/tests). The genetics and pulmonary pathophysiology associated with rare variants in the *SFTPB*, *SFTPC*, and *ABCA3* genes that cause surfactant dysfunction disorders are discussed in detail below. While not discussed here, other genes associated with surfactant dysfunction and diffuse lung diseases are reviewed in detail by Nogee[11] and Nogee and Ryan.[12]

## SP-B DEFICIENCY

The mature SP-B is a highly hydrophobic 79-amino acid (aa) protein derived from proteolytic processing of a 393-aa proprotein. SP-B is expressed primarily by AT2 cells, and is required for the maintenance of surface tension and prevention of alveolar collapse at end expiration[13,14] (https://gtexportal.org, accessed July 2022). SP-B deficiency is an autosomal recessive disorder that results from biallelic loss of function variants in *SFTPB*.[3] SP-B deficiency is considered to be the rarest genetic surfactant dysfunction disorder and affects $\sim$1 in 5 to 10 million births[15] (https://gnomad.broadinstitute.org, accessed July 2022).

The most common pathogenic *SFTPB* variant, c.397delCinsGAA; p.Pro133GlnfsTer95 (also noted as ''121ins2'' in earlier publications[16–18]) has a carrier frequency of $\sim$1 in 2,300 individuals, with an increased frequency of $\sim$1 in 1,200 among European, non-Finnish-descent individuals (Genome Aggregation Database, gnomAD; https://gnomad.broadinstitute.org/, v2.1.1, accessed July 2022), likely due to a founder effect. Further, this *SFTPB* variant has been identified in approximately two-thirds of infants with SP-B deficiency.[17]

These infants present with severe RDS shortly after birth and in the absence of lung transplantation will succumb to progressive respiratory failure within their first 3 months of life.[9] Rarely, SP-B deficiency is associated with delayed disease onset during infancy or survival past the first year of life owing to *SFTPB* variants that likely retain partial residual protein function.[19] Notably, parents of affected infants and adult carriers of *SFTPB* c.397delCinsGAA loss-of-function variant are typically healthy,[17] substantiating that $\sim$50% of SP-B levels are sufficient for normal lung function.

## SP-C DYSFUNCTION

Pathogenic variants in *SFTPC* have been identified among individuals with diverse lung diseases, including neonatal RDS, chILD, and adult pulmonary fibrosis.[4,20,21] Lung disease resulting from SP-C dysfunction is rare, and its prevalence is difficult to estimate accurately. While information on the exact disease phenotype is not readily available, several pathogenic *SFTPC* variants are listed in gnomAD, suggesting a prevalence of $\sim$1 in 20,000 individuals. Pathogenic *SFTPC* variants are autosomal dominant with variable penetrance, even within families carrying the same *SFTPC* variant.[4,20–22] Approximately half of *SFTPC* variants are inherited, and half arise *de novo*.[23,24] The most common pathogenic *SFTPC* variant, c.218T>C (p.Ile73Thr, p.I73T), has been identified in multiple unrelated individuals of diverse ethnic backgrounds, and is present in $\sim$25% of individuals with SP-C–related lung disease.[21]

Individuals with pathogenic *SFTPC* variants typically present with chILD[4] or early-onset pulmonary fibrosis. Unlike surfactant proteins A, B, or D, SP-C is produced and expressed in the lungs exclusively by the AT2 cells.[25] Mature SP-C is a 35-aa, extremely hydrophobic protein that is derived from the proteolytic processing of an 191 or an 197 aa proprotein (proSP-C).[24] The terminal $\sim$100 aa of proSP-C have a high-level homology to the BRICHOS proteins that are misfolded in some familial (*i.e.*, British and Danish) dementias.[26] Proper folding of the BRICHOS domain is necessary for the processing and trafficking

of proSP-C to the lamellar bodies (LBs) in AT2 cells,[27] allowing for the incorporation of mature SP-C into pulmonary surfactant.

*SFTPC* variants result in a toxic gain of function, and histological examination of explanted lungs from symptomatic individuals revealed an abundant expression of proSP-C.[28] Most pathogenic *SFTPC* variants are located within the BRICHOS domain, with rare published reports of pathogenic variants within the mature SP-C.[29] Cell-based model systems[30–34] and mouse models[35–38] have been utilized to demonstrate that BRICHOS domain variants result in the accumulation of misfolded proteins leading to endoplasmic reticulum (ER) stress. This subsequently activates the unfolded protein response (UPR), and results in apoptosis, inflammation, and spontaneous lung fibrosis.

Non-BRICHOS *SFTPC* pathogenic variants, including the p.I73T variant, result in defective macroautophagy, inflammation, and spontaneous lung fibrosis.[28,39,40] It is difficult to predict the disease course of patients with pathogenic *SFTPC* variants, and to date a genotype–phenotype correlation has not been established.[7] While some patients with *SFTPC* variants have progressively worsening respiratory failure necessitating lung transplantation,[9] others stabilize with supportive care (*i.e.*, supplemental $O_2$, chronic mechanical ventilation, and/or anti-inflammatory medications[7,41]), and a subset remains relatively asymptomatic.[22]

## ATP-BINDING CASSETTE TRANSPORTER A3 DEFICIENCY

ATP-binding cassette transporter A3 (ABCA3) deficiency is an autosomal recessive disorder and is the most common genetic disorder of surfactant dysfunction.[5] The typical clinical presentation of ABCA3 deficiency includes neonatal RDS and chILD during infancy or early childhood.[42] While the incidence is rare, adults with biallelic *ABCA3* variants that present with early-onset pulmonary fibrosis have been reported.[8,42–45] ABCA3 is a 1,704 aa protein that shares considerable structural homology with another well-studied ATP-binding cassette transporter protein, the cystic fibrosis transmembrane conductance regulator (CFTR); similar to CFTR, ABCA3 has two membrane-spanning domains and two nucleotide-binding domains.[46] ABCA3 is developmentally regulated and is highly expressed in AT2 cells where it localizes to the limiting membranes of LBs. ABCA3 transports phospholipids, specifically phosphatidylcholine, from the cytoplasm to the LBs, where the phospholipids combine with SP-B and SP-C to produce pulmonary surfactant.[47,48] ABCA3 is also expressed at low levels in the brain, kidney, thyroid, and other tissues.[47]

To date, >300 disease-associated *ABCA3* variants have been reported, with the vast majority being either missense or private (*i.e.*, unique to individuals and families).[42] The most common pathogenic *ABCA3* variant is c.875A>T; p.Glu292Val (p.E292V), and is present in $\sim$1 in 220 individuals, with higher carrier frequencies reported among individuals of non-Finnish European descent (gnomAD). Although the p.E292V variant is commonly associated with chILD, there have been reports of individuals homozygous for this variant presenting with severe neonatal RDS or early-onset pulmonary fibrosis.[42,45,49]

Using the gnomAD frequencies of the *ABCA3* loss-of-function variants and the previously identified disease-associated variants (including p.E292V), we estimate the incidence of ABCA3 deficiency to be $\sim$1 in 90,000 live births. However, this incidence is more than likely an underestimate, as most pathogenic *ABCA3* variants are either missense variants without available functional data or are private,[8,42] and are therefore not always captured in the large databases containing exome and genome data from adults.

There are two mechanistic classes of *ABCA3* missense variants: those that disrupt intracellular trafficking of ABCA3 to the LBs (*i.e.*, p.L101P), and those that impair phospholipid transport into the LBs (*i.e.*, p.E292V).[50–52] Infants with biallelic loss-of-function *ABCA3* variants present with severe neonatal RDS and typically succumb to their disease within their first year of life.[8,42] The timing of disease onset, severity, and progression for infants and children with missense *ABCA3* variants are variable, and to date, survival outcomes are difficult to predict.[8,42]

## CURRENT TREATMENTS FOR GENETIC DISORDERS OF SURFACTANT DYSFUNCTION

Available medical therapies are limited, not gene specific, and generally ineffective.[7,8] The most common, noninvasive therapeutic strategy for infants with neonatal RDS, due to SP-B or ABCA3 deficiency, is exogenous surfactant replacement that leads to transient improvement in $O_2$ requirement lasting a few hours, although the response diminishes over time.[24] Anti-inflammatory medications, which include corticosteroids, hydroxychloroquine, and azithromycin, have also been used as treatments for both infants and children with ABCA3 deficiency, although with limited efficacy.[8,53,54] Children with pathogenic *SFTPC* variants may benefit from a regimen of steroid and hydroxychloroquine treatments in addition to chronic mechanical ventilation.[7,41]

Despite its obvious limitations, lung transplantation remains the definitive treatment option for progressive respiratory failure among infants and children but is only available at a few quaternary centers in the United States. The 5-year survival for infant and pediatric lung transplantation for genetic disorders of surfactant dysfunction is $\sim$60%, and is similar to that of other trans-

plant indications.[9] Transplantation-associated morbidities are frequent, and include hypertension, renal insufficiency, and post-transplant lymphoproliferative disorder.[9]

Furthermore, infants who undergo lung transplantation are at an elevated risk of speech and motor delays, hearing loss, and frequently require gastrostomy tube placement for nutritional support.[9] Bronchiolitis obliterans remains the most common cause of chronic lung transplant failure and develops in 30–50% of recipients within 5 years of transplantation.[9] While infant and pediatric lung transplantations for genetic disorders of surfactant dysfunction have been performed since 1993,[9,55] morbidity and mortality remain significant, thus highlighting the need for new and advanced therapies that include molecular therapeutics.

## PRECLINICAL MODELS OF GENETIC DISORDERS OF SURFACTANT DYSFUNCTION

To assess the translatability of any type of gene therapeutic, it is important to utilize sophisticated *in vivo* and *in vitro* model systems that mimic key features of the diseased alveoli. These model systems are discussed in detail below.

## MOUSE MODELS OF *SFTPB* DEFICIENCY

In humans, SP-B deficiency presents as RDS within hours of birth. This RDS phenotype was successfully recapitulated in a mouse model generated by the targeted disruption of the *Sftpb* locus in Swiss black mice, resulting in neonatal lethality in the affected offspring.[56] The *Sftpb* knockout (KO) mouse, a model of SP-B deficiency that develops lethal respiratory failure shortly after birth, is characterized by an absence of LBs in AT2 cells, a lack of tubular myelin in the airspaces, and an aberrant processing of SP-C proprotein.[56]

Although *Sftpb* KO mice closely phenocopied SP-B–deficient infants, it is interesting to note that upon histological examination of their lungs at E17.5, there were no signs of proteinosis or destruction of the respiratory epithelium, in stark contrast to the phenotypic changes observed in the lungs of SP-B–deficient infants.[56] This suggests that SP-B deficiency alone is not the cause for the complex repertoire of histological abnormalities observed in the lungs of SP-B–deficient infants.

Transgenic *SP-CrtTA:teto$_7$:SP-B* mice on the FVB/N background in which expression of *Sftpb* is doxycycline regulated also recapitulate the disease phenotype of SP-B deficiency.[57] In this model, when SP-B expression in AT2 cells was decreased by $\sim$75%, mice presented with respiratory failure, indicating that SP-B must be expressed at a minimum 25% of normal levels for proper surfactant homeostasis and respiratory function.[57] Lin et al. demonstrated that restoration of SP-B expression in AT2 cells, but not in club cells, was effective at rescuing the neonatal lethal phenotype of *Sftpb* KO mice.[58] The authors also showed that expression of SP-B in some, but not all, AT2 cells allowed for the postnatal survival of *Sftpb* KO mice.[58] In a subsequent study, Nesslein et al. demonstrated that in lung regions in which AT2 cells did not express SP-B, there was an obvious development of focal air space enlargement.[59]

## MOUSE MODELS OF *SFTPC* DYSFUNCTION

Several transgenic and knock-in (KI) mice with specific pathogenic *SFTPC* variants have been engineered to model the human pathophysiology associated with SP-C dysfunction.[35–38,40] Pathogenic variants in the BRICHOS domain result in a misfolded SP-C proprotein that is retained in the ER, activates ER stress, and subsequently undergoes proteasome-mediated degradation.[32] An animal model of SP-C dysfunction, the *Sftpc*$^{\Delta exon4}$ mouse, which expresses the BRICHOS domain variant Δexon4 under control of the 13 kb mouse *Sftpc* promoter, is characterized by severe defects in lung morphogenesis that lead to perinatal lethality as early as E18.[35] The severity of lung disease in the *Sftpc*$^{\Delta exon4}$ mouse model was reported to be directly proportional to the level of expression of the mutant SP-C proprotein.[35]

The mutant SP-C proprotein is unstable, and its transient expression in AT2 cells results in ER stress that induces apoptosis.[35] The p.L188Q *SFTPC* variant was identified in a large kindred and is inherited in an autosomal dominant manner with incomplete penetrance.[22] In the C57Bl6/J L188Q transgenic mouse model with intact WT *Sftpc* alleles, doxycycline-induced expression of the human p.L188Q BRICHOS domain variant[22] did not result in overt structural abnormalities in the lung, likely due to the low levels of p.L188Q expression.[36] Intratracheal challenge of C57Bl6/J L188Q transgenic mice with bleomycin resulted in lung fibrosis 3 weeks post-challenge, and was associated with activation of the ER stress response and an increased rate of apoptosis of AT2 cells.[36]

The p.L184Q KI mice on the C57Bl6/n background express the murine version of the human p.L188Q variant,[22] and present with oxidative and ER stress during postnatal AT2 cell expansion[38]; adult KI L184Q mice challenged with bleomycin, instilled intratracheally, developed persistent lung disease.[38] C57Bl6 mice harboring the human p.C121G BRICHOS variant[37] demonstrate disruptions in the disulfide bonds of the SP-C proprotein, as seen in human patients, and develop spontaneous lung fibrosis.[37] Induction of p.C121G allele expression in adult mutant mice activates the UPR and spontaneous fibrotic remodeling of the lung parenchyma after a phase of acute alveolitis.[37] Collectively, the findings of the studies noted above emphasize the role of ER

stress-mediated initiation or progression of fibrotic lung disease associated with the expression of *Sftpc* BRICHOS domain variants.

The SP-C linker domain variant p.I73T is the most commonly identified pathogenic variant in *SFTPC*, which results in the formation of a misfolded proprotein that escapes protein quality control and is misrouted to the plasma membrane.[39] Similar to the Δexon4 study described above, both heterozygous and homozygous p.I73T C57Bl6 mouse embryos present with arrested lung development as early as E18.5, which is associated with an acute increase in SP-C p.I73T mutant proprotein expression.[40] Tamoxifen-mediated induction of p.I73T expression in 8- to 12-week-old mice resulted in acute alveolar remodeling in the lungs, and tamoxifen-dose–dependent lethality as early as 5 days after induction; expression of p.I73T in adult mice also resulted in a phase of acute alveolitis followed by fibrotic lung remodeling.[40] Taken together, the *Sftpc* $^{I73T}$ and *Sftpc*$^{\Delta exon4}$ mouse models strongly suggest that the cytotoxic aggregation of mutant SP-C proprotein is associated with lethal lung disease.

## MOUSE MODELS OF *ABCA3* DEFICIENCY

Targeted disruption of the *Abca3* locus in mice results in neonatal lethality and is associated with a profound loss of LBs in AT2 cells.[60–63] Lungs of *Abca3*-deficient mice are characterized by a significant reduction in surfactant lipids, highlighting the essential role of ABCA3 in surfactant lipid metabolism and LB biogenesis.[60–63] In studies by Besnard et al., conditional deletion of *Abca3* from AT2 cells of the *SP-C-rtTA:TetO-Cre:Abca3*$^{f/f}$ mouse model (mixed strain background) resulted in surfactant deficiency and respiratory distress; 67% of the *Abca3*$^{\Delta/\Delta}$ pups died shortly after birth with surviving mice developing emphysema by 9 months of age.[64]

Notably, the surviving *Abca3*$^{\Delta/\Delta}$ mice present with two distinct AT2 cell populations: those that expressed ABCA3 and those that did not, suggesting that surviving ABCA3-expressing cells in the *Abca3*$^{\Delta/\Delta}$ mouse may compensate for ABCA3 deficiency and maintain adequate surfactant production. Consistent with this hypothesis, nontargeted ABCA3-expressing cells restored pulmonary homeostasis in the *Abca3*-conditional KO mouse model, in which *Abca3* expression was conditionally ablated through tamoxifen treatment for 4 days.[65]

Twelve weeks after tamoxifen treatment, expression of *Abca3* messenger RNA (mRNA) in AT2 cells was completely restored, concurrent with the proliferation of ABCA3-expressing AT2 cells that restored normal lung structure and pulmonary function.[65] The remarkable selective advantage of a small population of ABCA3-expressing cells (~23% of proSP-C$^+$ AT2 cells[65]) that was adequate for the restoration of alveolar homeostasis substantiates the therapeutic potential of a gene-based intervention for ABCA3 deficiency.

## *IN VITRO* MODELS OF GENETIC DISORDERS OF SURFACTANT DYSFUNCTION

In addition to animal models of genetic disorders of surfactant dysfunction, there is a need for *in vitro* model systems that recapitulate human disease pathobiology, which can be harnessed to assess variant pathogenicity, inform clinical decision making, and allow for the evaluation and development of effective therapeutic strategies. Overexpression of specific *SFTPC*[30–34,39,66–69] and *ABCA3*[50–52,61,70–72] variants by either plasmid transfection or adenovirus-based vector-mediated transduction of HEK293T and A549 cells has been utilized to study the downstream effects of mutant protein expression.

Recently, an ABCA3$^{-/-}$ A549 parent cell line with a single recombination target site or ''landing pad'' for genomic integration has been used for stable expression and functional characterization of individual *ABCA3* missense variants.[72] Others have attempted to ablate *SFTPB* in A549 and H441 cell lines through gene editing,[73] or have utilized patient-specific induced pluripotent stem cells (iPSCs) expressing mutant alleles from the endogenous locus that were differentiated to AT2-like cells.[18,28,74]

To model SP-B deficiency *in vitro*, Munis et al[73] used CRISPR/Cas9 gene editing of H441 cells grown at an air–liquid interface to generate *SFTPB* KO cells. In this model system, *SFTPB* KO cells had reduced transepithelial electrical resistance (TEER), which suggested a compromised epithelial barrier. Transduction of *SFTPB* KO cells with an F/HN-pseudotyped simian immunodeficiency virus (SIV)-based lentivirus (LV) vector encoding *SFTPB* resulted in measurable SP-B expression and restoration of functional epithelial barrier properties.[73]

In A549 or HEK293 cells transfected with various *SFTPC* BRICHOS variants (*SFTPC*$^{\Delta exon4}$, *SFTPC*$^{C121G}$, *SFTPC*$^{C186G}$, or *SFTPC*$^{L188Q}$), retention of proSP-C in the ER and subsequent intracellular aggregation of the misfolded proprotein has been reported, resulting in activation of the ER stress and UPR pathways leading to apoptotic cell death.[30,31,33,35,66,68] *In vitro* studies evaluating the consequences of overexpression of *SFTPC*$^{I73T}$ in A549 or HEK293 cells revealed a toxic gain of function due to the altered intracellular trafficking of proSP-C$^{I73T}$ to the plasma membrane.[34,39,67,69] Subsequent accumulation of misprocessed isoforms within the endosomes resulted in proteostasis perturbations characterized by a late block in autophagy and impaired mitophagy.[39]

While A549 and H441 (lung adenocarcinoma epithelial) or HEK293 (human embryonic kidney) cells are

typically used to study variants of surfactant genes, these lines do not possess the necessary AT2 machinery for surfactant production.[75,76] Three-dimensional (3D) model systems in combination with the development of methods for deriving human AT2-like cells from patient-derived iPSCs (iAT2s) provide a physiologically relevant disease model to study the biology of human distal lungs and aid in the development of novel molecular therapies for genetic disorders of surfactant dysfunction: iAT2s can produce surfactant, and are transcriptionally and ultrastructurally similar to primary AT2 cells.[18,77]

Specifically, iAT2s have been used to study the processing of SP-B and SP-C proproteins harboring pathogenic variants using patient-specific mutant iAT2s and their gene-edited progeny.[18,28] Jacob et al. generated iAT2s from an infant carrying the most common pathogenic *SFTPB* variant, p.Pro133GlnfsTer95, and reported that these iAT2s exhibited reduced *SFTPB* mRNA expression as a result of the nonsense-mediated decay of the mutant *SFTPB* transcript.[18]

Furthermore, no detectable SP-B protein or LBs were identified, as confirmed by transmission electron microscopy, recapitulating the patient disease phenotype. Intriguingly, ''footprint-free'' CRISPR/Cas9-based gene editing corrected the *SFTPB* variant, leading to increased *SFTPB* mRNA that restored LB ultrastructure and surfactant processing.[18] Another study evaluated the therapeutic benefit of a LV vector expressing *hSFTPB* in patient-derived iPSCs homozygous for the p.Pro133GlnfsTer95 *SFTPB* variant.[74] Differentiation of gene-corrected iPSCs into 3D cultures restored both LB formation and processing of surfactant proteins and phospholipids.[74]

Recently, patient-specific iPSCs heterozygous for the most common pathogenic *SFTPC* variant, $SFTPC^{I73T}$, were utilized to study the intrinsic epithelial dysfunction at the inception of lung disease.[28] When comparing the syngeneic mutant iAT2s to the corrected iAT2s, the mutant iAT2s misprocessed and mistrafficked proSP-C, in a manner similar to that observed in AT2 cells *in vivo*.[28] The accumulation of misprocessed proSP-C extended the observations made through the forced overexpression of mutant protein in heterologous cell lines,[34,39,67,78] patient bronchoalveolar lavage samples,[67] and the $Sftpc^{I73T}$ mouse model.[40]

Downstream consequences of mistrafficked proSP-C in the iAT2 model included proteostasis perturbations, mitochondrial dysfunction, metabolic reprogramming, and inflammatory activation.[28] Notably, gene editing to ablate the expression of mutant *SFTPC* restored normal AT2 cell-specific function in the patient-derived iAT2s *in vitro*.[79] Taken together, these studies suggest that patient-specific iAT2s can indeed be used as a reliable preclinical *in vitro* model.

Despite the advantages of mouse models,[37,38,40] there are significant differences in the degree and type of surfactant dysfunction-associated lung disease that develops in these models, which limit their effective use for human disease modeling and evaluation of therapeutics. The ideal human AT2 cell dysfunction model should allow for the study of disease pathogenesis by utilizing patient-derived cells that would reveal the cascade of mechanistic events associated with the inception, progression, and resolution of lung disease. However, the study of pathobiology in primary human AT2 cells is hindered by the difficulty of accessing patient-derived samples, especially from patients at the early stages of the disease. Although iPSC-based models provide a unique opportunity for disease modeling using human cells, they also have several limitations.

The main limitation is that most of the established protocols generate cells that are less mature when compared with their *in vivo* counterparts, due in part to their more proliferative state compared with the typically quiescent AT2 cells.[80] Importantly, primary AT2 cells in culture are more proliferative and lose some degree of maturation compared with their quiescent *in vivo* parent cells.[80] Further, primary AT2 cells derived from patients with pathogenic variants in the surfactant protein genes have yet to be stably maintained *in vitro*, limiting comparative studies between patient-specific iAT2s and primary AT2 cells cultured from these patients.

## GENE THERAPY FOR GENETIC DISORDERS OF SURFACTANT DYSFUNCTION

Gene therapy remains the most promising therapeutic strategy for genetic disorders of surfactant dysfunction. In addition to the availability of several candidate gene therapeutics, the associated genes, *SFTPB*, *SFTPC,* and *ABCA3*, are extensively characterized,[81,82] and several animal models and sophisticated cell culture systems are available for preclinical testing. The two most important challenges of gene therapy for these disorders are delivery to target cell and the cell-specific expression of the therapeutic transgene.

Several strategies can now be employed for AT2 cell-restricted transgene expression, including, but not limited to, cell-specific promoters and/or enhancer elements. Various virus and nonvirus-based vector systems have been utilized for effective gene transfer to the respiratory epithelium.[83] Furthermore, there are technologies now available and also under development that allow for precise deposition of gene therapeutics to specific regions of the neonatal lungs. These technologies are reviewed by Katz et al. and McCarron et al.[84,85] In the next section, we review the viral-based and nonviral-based gene therapeutics that have a demonstrated transduction of primary AT2 cells (also summarized in Table 1).

## ADENOVIRAL VECTORS

Recombinant adenoviral (rAdV) vectors have broad tropism in various species *in vivo,* and rAdV-mediated

**Table 1.** *Viral-based vectors used for transduction of cells of the alveolar epithelium* in vivo *and alveolar type 2 cells* in vitro

| *Vector* | *Serotype or Envelope* | *Transgene* | *AT2 Cell-Specific* Ex Vivo *or* In Vivo *Model System(s)* | *Vector Dose* | *References* |
|---|---|---|---|---|---|
| AdV-based vector | Ad5 | β-Galactosidase (LacZ) | Human fetal lung AT2 cells and fibroblast cocultures | $1.0\times10^{3}$ p/cell | 86 |
| | | | Human fetal lung explants (22 weeks gestation) | $1.0\times10^{11}$ p/explant | |
| | | | Mouse lungs | $2.5\times10^{11}$ p/mouse, IT | |
| | | SP-C$^{1-197}$, SP-C$^{\Delta exon4}$ | Mouse AT2 cells | 50 MOI | 35 |
| | | GFP, Bcl-xL, Bcl-2 | Mouse lungs | $2.0\times10^{10}$ optical particle unit/mouse, IT | 87 |
| | | | Mouse AT2 cells | $2.0\times10^{7}$–$2.0\times10^{9}$ PFU/well | |
| | | FoxM1B, FoxM1B-TA, LacZ | Mouse AT2 cells | 20 MOI | 88 |
| | | | Mouse lungs | $5.0\times10^{8}$ PFU, IT | |
| | | ABCA3 variants | Isolated human precursor lung epithelial cells | $3.0\times10^{3}$ VP/cell | 72 |
| AAV-based vector | AAV1 | hALP | Mouse lungs | $6.0\times10^{10}$–$3.0\times10^{11}$ GC/mouse, IN | 98 |
| | AAV2 | LacZ | Mouse lungs | $1.0\times10^{10}$ p/mouse, IN | 97 |
| | | GFP | Human embryonic stem cell-derived lung bud organoids | $3.5\times10^{8}$–$1.0\times10^{9}$ GC | 103 |
| | AAV4 | GFP | Pig lungs | $1.0\times10^{12}$ VG/pig (bronchoscope-guided delivery to left lobe) | 100 |
| | AAV5 | hALP | Mouse lungs | $6.0\times10^{10}$–$3.0\times10^{11}$ GC/mouse, IN | 98 |
| | | LacZ | Mouse lungs | $1.0\times10^{10}$ p/mouse, IN | 97 |
| | AAV6 | ffLuc, hALP | Mouse lungs | $1.0\times10^{11}$ VG/mouse, IN | 99 |
| | | GFP | Human embryonic stem cell-derived lung bud organoid | $3.5\times10^{8}$–$1.0\times10^{9}$ GC | 103 |
| | AAV6.2 | GFP | Human embryonic stem cell-derived lung bud organoid | $3.5\times10^{8}$–$1.0\times10^{9}$ GC | 103 |
| | AAV6.2FF | ffLuc, hALP | Mouse lungs | $1.0\times10^{11}$ VG/mouse, IN | 99 |
| | | GFP | Human embryonic stem cell-derived lung bud organoid | $3.5\times10^{8}$–$1.0\times10^{9}$ GC | 103 |
| | | m*Sftpb*, h*SFTPB,* GFP, mCherry, ffLuc | Mouse lungs | $1.0\times10^{11}$ VG/mouse, IT | 104 |
| | | | Precision cut lung slices (human) | $1.0\times10^{8}$–$1.0\times10^{10}$ VG | |
| | | mCherry, Cre, *Sftpc*-miR | Mouse lungs | $5.0\times10^{11}$–$4.0\times10^{12}$ VG/mouse, IN | 105 |
| | AAV8 | ACE2, GFP | Mouse lungs | $4.0\times10^{11}$ VP/mouse, IT | 101 |
| | AAV9 | LacZ, α1-antitrypsin | Mouse lungs | $1.0\times10^{11}$ GC/mouse, IN | 102 |
| LV-based vector | VSV-G | EGFP | Rat AT2 cells | 0.1–50 MOI | 108 |
| | | EGFP | Mouse lungs | $1.0\times10^{8}$ international units, IT | 110 |
| | | CDK4, hTERT, SV40 | Human AT2 cells | Not available | 109 |
| | | GFP | iPSC-derived AT2 cells | 50 MOI | 111 |
| | | GFP | Mouse lungs | $0.5$–$3.0\times10^{8}$ TU/mouse, IT | 116 |
| | SARS-CoV2 Spike | GFP | iPSC-derived AT2 cells | 30 MOI | 111 |
| | Sendai Virus—F and HN proteins | ffLuc | Mouse lungs | $5.0\times10^{8}$ TU/mouse, IN | 117 |
| | Mokola virus glycoprotein | GFP | Mouse lungs | $0.5$–$3.0\times10^{8}$ TU/mouse, IT | 116 |
| | Lymphocytic choriomeningitis virus glycoprotein | GFP | Mouse lungs | $0.5$–$3.0\times10^{8}$ TU/mouse, IT | 116 |
| | Ebola virus glycoprotein | GFP | Mouse lungs | $0.5$–$3.0\times10^{8}$ TU/mouse, IT | 116 |

AAV, adeno-associated virus; ABCA3, ATP-binding cassette transporter A3; AdV, adenovirus; AT2, alveolar type 2; ffLuc, firefly luciferase; GC, genome copies; hALP, human placental alkaline phosphatase; IN, intranasal; iPSC, induced pluripotent stem cell; IT, intratracheal; LV, lentivirus; MOI, multiplicity of infection; p, particles; PFU, plaque-forming unit; SP-C, surfactant protein C; VG, vector genomes; VP, viral particles; VSV-G, vesicular stomatitis virus glycoprotein.

gene transfer to AT2 cells both *in vivo* and *ex vivo* has been studied extensively.[35,72,86–89] In a study by Strayer et al,[86] a replication-deficient AdV vector expressing LacZ was evaluated in adult mice, *ex vivo* in normal human lung explants, and *in vitro* in A549 and also H441 cells. As evidenced by β-galactosidase, the rAdV-mediated SP-B–driven LacZ expression was restricted to AT2 cells and club cells.[86] Another AdV vector, AdV5F35++, a chimeric rAdV5 vector with the AdV35 fiber with two mutations (D207G, T245A), resulted in extensive transduction of AT2 cells in the lungs of nonhuman primates.[89]

In studies by Alapati et al., *in utero* rAdV-mediated gene editing of the p.I73T *Sftpc* variant in heterozygous mouse fetuses at E16 increased the survival of newborn mice beyond 24 h, concurrent with a marked improvement in alveolarization at postnatal day 7,[79] thus providing important proof of concept for therapeutic virus vector-based gene editing for toxic gain of function associated with *SFTPC* variants.

While the large packaging capacity of AdV vectors can certainly accommodate the coding sequence of all the surfactant-associated genes (*SFTPB* [1.2 kb], *SFTPC* [0.6 kb], and *ABCA3* [5.1 kb]), AdV vectors trigger host cellular immune responses,[90,91] which may lead to detrimental cytopathic effects when delivered into the distal lungs. Despite approaches to engineer AdV vectors for improved targeting and to prevent *in vivo* immunological responses,[92] AdV vectors result in poor and transient (*i.e.*, <14 days[93]) expression *in vivo*, which necessitates AdV vector readministration to maintain the therapeutic benefit conferred by the expression of the transgene. To date, effective readministration of AdV vectors in lungs *in vivo* is hampered by pre-existing and/or acquired vector-specific antibodies.

## ADENO-ASSOCIATED VIRAL VECTORS

Compared with rAdV vectors, recombinant adeno-associated viral (AAV) vectors have a favorable safety profile,[94–96] and lead to sustained expression for the lifetime of the transduced cell. The small ($\sim$4.7 kb) packaging capacity of rAAV limits their use to the *SFTPB* and *SFTPC* genes. Several AAV capsids (*i.e.*, AAV5, AAV6, AAV8, AAV9) were shown to transduce AT2 cells *in vivo*[97–102] and in *ex vivo* organoid models.[103] rAAV6.2FF, containing the p.F129L, p.Y445F, and p.Y731F mutations in the AAV6 capsid, was used by Kang et al[104] to express the *SFTPB* complementary DNA (cDNA) in the *SP-CrtTA:(teto)$_7$:SP-B*–conditional KO mouse model. Restoration of SP-B proprotein expression led to the survival of neonatal and adult mice coupled with phenotypic changes in the diseased alveoli after gene therapy; LB structure was maintained but most importantly, respiratory failure was rescued.

Notably, the authors showed that intratracheal delivery of rAAV6.2FF did not elicit any local or systemic immune responses.[104] In a subsequent study, Rindler et al[105] used the rAAV6.2FF vector to deliver microRNAs (miRNAs) in the lungs of C57Bl6 mice to target AT2 cells and inhibit *Sftpc* expression, resulting in a >90% reduction in expression, thus providing a rationale for a gene silencing strategy for pathogenic *SFTPC* variants. Since AAV vectors remain episomal and do not integrate, vector readministration is necessary for maintenance of therapeutic transgene expression.

The effectiveness of readministration can be impacted by the level of serum circulating AAV vector-specific neutralizing antibodies. Although readministration of either AAV2/9[102] or AAV2/6[106] in mouse lungs was shown to be feasible, the rapid increase in AAV vector-specific neutralizing antibodies precluded the successful intranasal or intratracheal readministration and subsequent transduction with AAV2,[106] AAV2/5,[102] or AAV5/5.[107] Collectively, these studies suggest the use of an immunosuppressive regimen or alternate AAV serotypes for effective readministration.

## LV VECTORS

LV vectors have a packaging capacity of $\sim$7.5 kb and can thus accommodate large transgenes, rendering them a highly suitable vector platform for the *in vivo* delivery of *ABCA3*. In addition, LV vectors can be pseudotyped with various envelope glycoproteins to either enhance or confer tissue- or cell-specific tropism. Vesicular stomatitis virus glycoprotein (VSV-G)–pseudotyped LV vectors have been previously shown to transduce AT2 cells and iAT2s.[108–111] In combination with the SP-C promoter (3.7 kb, 5′ flanking sequence), transgene expression was restricted to the AT2 cells *in vivo*.[110]

Notably, the 3.7 kb SP-C promoter was shown to regulate expression in the bronchiolar epithelium,[112,113] whereas the shorter 2 kb SP-C promoter improved targeting to the AT2 cells.[114] It should be noted that while the 2 kb promoter drove relatively weak transgene expression in AT2-like cell lines (*i.e.*, MLE12, MLE15, and A549), vector-mediated gene expression was improved by the addition of a CMV enhancer element.[115] VSV-G–pseudotyped LV vector–mediated delivery of *SFTPB* to iPSC-derived lung organoids harboring the p.Pro133GlnfsTer95 *SFTPB* variant highlights the potential of the therapeutic application of LV vectors for the treatment of genetic disorders of surfactant dysfunction. HIV-based LV vectors pseudotyped with glycoproteins derived from the Ebola Zaire virus, the lymphocytic choriomeningitis virus, the Mokola virus,[116] and SIV-based vector pseudotyped with glycoproteins derived from the Sendai virus have been reported to transduce AT2 cells in the distal lungs *in vivo*.[117]

These findings warrant further research into enhanced AT2 cell targeting through the use of alternate glycoproteins to pseudotype LV-based gene therapeutics, particularly given that VSV-G–pseudotyped LV vectors have relatively poor lung epithelial cell tropism *in vivo*.[118] To sustain long-lived expression in the airways, LV vectors should be able to be readministered. Sinn et al. demonstrated in mice successful intranasal readministration of GP64-pseudotyped feline immunodeficiency virus-based vector.[119]

Further, Griesenbach et al. demonstrated the feasibility of daily or monthly readministration of the F/HN-pseudotyped SIV vector into mouse airways.[117] Collectively, these studies provide important proof of concept for sustaining and/or enhancing transgene expression in the lungs. While marginal, the theoretical risk for insertional mutagenesis induced by the integration of LV vectors remains. Current LV vectors appear to target terminally differentiated cells, adding an additional layer of safety should this adverse event occur.

## NONVIRAL VECTORS

Compared with viral-based gene therapeutics, nonviral-based gene therapeutics have a favorable *in vivo*

immunogenicity profile.[120,121] Although nonviral vectors (such as lipid nanoparticles) have been used to target AT2 cells *ex vivo*,[122] although with moderate success, there is an apparent lack of reported *in vivo* alveoli-directed gene therapeutic applications, suggesting the limitation of this specific platform technology.

## CONCLUSION

A gene therapeutic for the genetic disorders of surfactant dysfunction holds great promise. Current gene therapy strategies require improvement of AT2 cell-specific expression and the incorporation of silencing strategies to control off-target effects, particularly in cells of the bronchiolar epithelium. Molecular studies conducted in mouse models of surfactant dysfunction disorders suggest that targeting 20–30% of AT2 cells is sufficient to reverse the phenotypic changes associated with specific surfactant gene variants (*i.e.*, *SFTPB* and *ABCA3* variants). The translation, however, of these studies to the target patient population, particularly the neonates with actively developing lungs, is unknown.

Furthermore, the application of a gene therapeutic in these patients is complicated by the limitations of timely identification of candidate patients—infants are often identified at different centers, and very few centers in the United States offer lung transplantation. For these disorders that are characterized by progressively worsening lung disease, gene therapy may provide a bridge to lung transplantation by prolonging the viability of the diseased lungs.

Gene therapy for ultrarare diseases is actively being pursued, and its importance is emphasized by the newly formed Bespoke Gene Therapy Consortium, a partnership between the National Institutes of Health, the U.S. Food and Drug Administration, and multiple public and private organizations that aim to develop customized gene therapeutics for these diseases. In conclusion, the application of gene therapy in neonates, infants, and children with genetic disorders of surfactant dysfunction is feasible, and warrants further investigation for future clinical development.

## ACKNOWLEDGMENTS

We thank Nicholas W. Keiser and Stephen Tottey (Spirovant Sciences, Inc.) for their critical review of the article.

## AUTHORS' CONTRIBUTIONS

S.S. contributed to conceptualization, writing—original draft, review and editing. K.-D.A. and J.A.W. performed writing—original draft, review and editing. M.P.L. provided conceptualization, supervision, writing—review and editing.

## AUTHOR DISCLOSURE

S.S. and M.P.L. are employees of Spirovant Sciences, Inc. All other authors have no competing financial interests.

## FUNDING INFORMATION

This work was supported in part by an I.M. Rosenzweig Junior Investigator Award from The Pulmonary Fibrosis Foundation and an Integrated Pilot Grant Award through Boston University Clinical & Translational Science Institute (1UL1TR001430) to K.-D.A., NIH NHLBI (R01HL149853 and U01HL13475) and Children's Discovery Institute grants to J.A.W.

## REFERENCES

1. Knudsen L, Ochs M. The micromechanics of lung alveoli: Structure and function of surfactant and tissue components. Histochem Cell Biol 2018; 150(6):661–676.
2. Parra E, Perez-Gil J. Composition, structure and mechanical properties define performance of pulmonary surfactant membranes and films. Chem Phys Lipids 2015;185:153–175.
3. Nogee LM, de Mello DE, Dehner LP, et al. Brief report: Deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. N Engl J Med 1993;328(6):406–410.
4. Nogee LM, Dunbar AE, 3rd, Wert SE, et al. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. N Engl J Med 2001;344(8):573–579.
5. Shulenin S, Nogee LM, Annilo T, et al. ABCA3 gene mutations in newborns with fatal surfactant deficiency. N Engl J Med 2004;350(13): 1296–1303.
6. Hamvas A, Deterding RR, Wert SE, et al. Heterogeneous pulmonary phenotypes associated with mutations in the thyroid transcription factor gene $NKX_{2-1}$. Chest 2013;144(3):794–804.
7. Kröner C, Reu S, Teusch V, et al. Genotype alone does not predict the clinical course of SFTPC deficiency in paediatric patients. Eur Respir J 2015;46(1):197–206.
8. Kröner C, Wittmann T, Reu S, et al. Lung disease caused by ABCA3 mutations. Thorax 2017;72(3): 213–220.
9. Eldridge WB, Zhang Q, Faro A, et al. Outcomes of lung transplantation for infants and children with genetic disorders of surfactant metabolism. J Pediatr 2017;184:157.e2–164.e2.
10. Lagoski MHA, Wambach JA. Pathophysiology and Management of Respiratory Distress Syndrome. In: Fanaroff and Martin's Neonatal Perinatal Medicine: Diseases of the Fetus and Infant, 11th ed. (Martin R, Fanaroff A, Walsh M, eds.): Elsevier, Philadelphia, p. 1159–1173, 2019.

11. Nogee LM. Genetic causes of surfactant protein abnormalities. Curr Opin Pediatr 2019;31(3):330–339.
12. Nogee LM, Ryan RM. Genetic testing for neonatal respiratory disease. Children 2021;8(3):216.
13. Nogee LM. Genetics of the hydrophobic surfactant proteins. Biochim Biophys Acta 1998; 1408(2–3):323–333.
14. Weaver TE. Synthesis, processing and secretion of surfactant proteins B and C. Biochim Biophys Acta 1998;1408(2–3):173–179.
15. Garmany TH, Wambach JA, Heins HB, et al. Population and disease-based prevalence of the common mutations associated with surfactant deficiency. Pediatr Res 2008;63(6):645–649.
16. Nogee LM, Garnier G, Dietz HC, et al. A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. J Clin Invest 1994;93(4):1860–1863.
17. Nogee LM, Wert SE, Proffit SA, et al. Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. Am J Respir Crit Care Med 2000;161(3 Pt 1):973–981.
18. Jacob A, Morley M, Hawkins F, et al. Differentiation of human pluripotent stem cells into functional lung alveolar epithelial cells. Cell Stem Cell 2017;21(4):472.e10–488.e10.
19. Lopez-Andreu JA, Hidalgo-Santos AD, Fuentes-Castello MA, et al. Delayed presentation and prolonged survival of a child with surfactant protein B deficiency. J Pediatr 2017;190:268.e1–270.e1.
20. Lawson WE, Grant SW, Ambrosini V, et al. Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. Thorax 2004; 59(11):977–980.
21. Cameron HS, Somaschini M, Carrera P, et al. A common mutation in the surfactant protein C gene associated with lung disease. J Pediatr 2005;146(3):370–375.
22. Thomas AQ, Lane K, John Phillips I, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med 2002;165(9):1322–1328.
23. Nogee LM, Dunbar AE, 3rd, Wert S, et al. Mutations in the surfactant protein C gene associated with interstitial lung disease. Chest 2002; 121(3 Suppl):20s–21s.
24. Nogee LM. Alterations in SP-B and SP-C expression in neonatal lung disease. Annu Rev Physiol 2004;66:601–623.
25. Weaver TE, Conkright JJ. Function of surfactant proteins B and C. Annu Rev Physiol 2001;63: 555–578.
26. Johansson H, Nordling K, Weaver TE, et al. The Brichos domain-containing C-terminal part of pro-surfactant protein C binds to an unfolded poly-val transmembrane segment. J Biol Chem 2006;281(30):21032–21039.
27. Willander H, Askarieh G, Landreh M, et al. High-resolution structure of a BRICHOS domain and its implications for anti-amyloid chaperone activity on lung surfactant protein C. Proc Natl Acad Sci U S A 2012;109(7):2325–2329.
28. Alysandratos KD, Russo SJ, Petcherski A, et al. Patient-specific iPSCs carrying an SFTPC mutation reveal the intrinsic alveolar epithelial dysfunction at the inception of interstitial lung disease. Cell Rep 2021;36(9):109636.
29. Beers MF, Mulugeta S. Surfactant protein C biosynthesis and its emerging role in conformational lung disease. Annu Rev Physiol 2005; 67:663–696.
30. Wang WJ, Mulugeta S, Russo SJ, et al. Deletion of exon 4 from human surfactant protein C results in aggresome formation and generation of a dominant negative. J Cell Sci 2003;116(Pt 4): 683–692.
31. Mulugeta S, Nguyen V, Russo SJ, et al. A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation. Am J Respir Cell Mol Biol 2005;32(6): 521–530.
32. Bridges JP, Xu Y, Na CL, et al. Adaptation and increased susceptibility to infection associated with constitutive expression of misfolded SP-C. J Cell Biol 2006;172(3):395–407.
33. Mulugeta S, Maguire JA, Newitt JL, et al. Misfolded BRICHOS SP-C mutant proteins induce apoptosis via caspase-4- and cytochrome c-related mechanisms. Am J Physiol Lung Cell Mol Physiol 2007;293(3):L720–L729.
34. Stewart GA, Ridsdale R, Martin EP, et al. 4-Phenylbutyric acid treatment rescues trafficking and processing of a mutant surfactant protein-C. Am J Respir Cell Mol Biol 2012;47(3): 324–331.
35. Bridges JP, Wert SE, Nogee LM, et al. Expression of a human surfactant protein C mutation associated with interstitial lung disease disrupts lung development in transgenic mice. J Biol Chem 2003;278(52):52739–52746.
36. Lawson WE, Cheng DS, Degryse AL, et al. Endoplasmic reticulum stress enhances fibrotic remodeling in the lungs. Proc Natl Acad Sci U S A 2011;108(26):10562–10567.
37. Katzen J, Wagner BD, Venosa A, et al. An SFTPC BRICHOS mutant links epithelial ER stress and spontaneous lung fibrosis. JCI Insight 2019;4(6): e126125.
38. Sitaraman S, Martin EP, Na CL, et al. Surfactant protein C mutation links postnatal type 2 cell dysfunction to adult disease. JCI Insight 2021; 6(14):e142501.
39. Hawkins A, Guttentag SH, Deterding R, et al. A non-BRICHOS SFTPC mutant (SP-CI73T) linked to interstitial lung disease promotes a late block in macroautophagy disrupting cellular proteostasis and mitophagy. Am J Physiol Lung Cell Mol Physiol 2015;308(1):L33–L47.
40. Nureki SI, Tomer Y, Venosa A, et al. Expression of mutant Sftpc in murine alveolar epithelia drives spontaneous lung fibrosis. J Clin Invest 2018;128(9):4008–4024.
41. Liptzin DR, Patel T, Deterding RR. Chronic ventilation in infants with surfactant protein C mutations: An alternative to lung transplantation. Am J Respir Crit Care Med 2015;191(11): 1338–1340.
42. Wambach JA, Casey AM, Fishman MP, et al. Genotype-phenotype correlations for infants and children with ABCA3 deficiency. Am J Respir Crit Care Med 2014;189(12):1538–1543.
43. Doan ML, Guillerman RP, Dishop MK, et al. Clinical, radiological and pathological features of ABCA3 mutations in children. Thorax 2008;63(4): 366–373.
44. Klay D, Platenburg M, van Rijswijk R, et al. ABCA3 mutations in adult pulmonary fibrosis patients: A case series and review of literature. Curr Opin Pulm Med 2020;26(3):293–301.
45. Tomer Y, Wambach J, Knudsen L, et al. The common ABCA3(E292V) variant disrupts AT2 cell quality control and increases susceptibility to lung injury and aberrant remodeling. Am J Physiol Lung Cell Mol Physiol 2021;321(2):L291–L307.
46. Denman L, Yonker LM, Kinane TB. The classification of ATP-binding cassette subfamily A member 3 mutations using the cystic fibrosis transmembrane conductance regulator classification system. Pediatr Investig 2018;2(1):17–24.
47. Mulugeta S, Gray JM, Notarfrancesco KL, et al. Identification of LBM180, a lamellar body limiting membrane protein of alveolar type II cells, as the ABC transporter protein ABCA3. J Biol Chem 2002;277(25):22147–22155.
48. Whitsett JA, Weaver TE. Alveolar development and disease. Am J Respir Cell Mol Biol 2015; 53(1):1–7.
49. Turcu S, Ashton E, Jenkins L, et al. Genetic testing in children with surfactant dysfunction. Arch Dis Child 2013;98(7):490–495.
50. Matsumura Y, Ban N, Ueda K, et al. Characterization and classification of ATP-binding cassette transporter ABCA3 mutants in fatal surfactant deficiency. J Biol Chem 2006;281(45):34503–34514.
51. Matsumura Y, Ban N, Inagaki N. Aberrant catalytic cycle and impaired lipid transport into intracellular vesicles in ABCA3 mutants associated with nonfatal pediatric interstitial lung disease. Am J Physiol Lung Cell Mol Physiol 2008;295(4):L698–L707.
52. Wambach JA, Yang P, Wegner DJ, et al. Functional characterization of ATP-binding cassette transporter A3 mutations from infants with respiratory distress syndrome. Am J Respir Cell Mol Biol 2016;55(5):716–721.
53. Hayes D, Jr., Lloyd EA, Fitch JA, et al. ABCA3 transporter deficiency. Am J Respir Crit Care Med 2012;186(8):807.

54. Hallik M, Annilo T, Ilmoja ML. Different course of lung disease in two siblings with novel ABCA3 mutations. Eur J Pediatr 2014;173(12): 1553–1556.

55. Hamvas A, Nogee LM, Mallory GB, et al. Lung transplantation for treatment of infants with surfactant protein B deficiency. J Pediatr 1997; 130(2):231–239.

56. Clark JC, Wert SE, Bachurski CJ, et al. Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. Proc Natl Acad Sci U S A 1995;92(17):7794–7798.

57. Melton KR, Nesslein LL, Ikegami M, et al. SP-B deficiency causes respiratory failure in adult mice. Am J Physiol Lung Cell Mol Physiol 2003; 285(3):L543–L549.

58. Lin S, Na CL, Akinbi HT, et al. Surfactant protein B (SP-B)$^{-/-}$ mice are rescued by restoration of SP-B expression in alveolar type II cells but not Clara cells. J Biol Chem 1999;274(27): 19168–19174.

59. Nesslein LL, Melton KR, Ikegami M, et al. Partial SP-B deficiency perturbs lung function and causes air space abnormalities. Am J Physiol Lung Cell Mol Physiol 2005;288(6): L1154–L1161.

60. Ban N, Matsumura Y, Sakai H, et al. ABCA3 as a lipid transporter in pulmonary surfactant biogenesis. J Biol Chem 2007;282(13):9628–9634.

61. Cheong N, Zhang H, Madesh M, et al. ABCA3 is critical for lamellar body biogenesis in vivo. J Biol Chem 2007;282(33):23811–23817.

62. Fitzgerald ML, Xavier R, Haley KJ, et al. ABCA3 inactivation in mice causes respiratory failure, loss of pulmonary surfactant, and depletion of lung phosphatidylglycerol. J Lipid Res 2007; 48(3):621–632.

63. Hammel M, Michel G, Hoefer C, et al. Targeted inactivation of the murine Abca3 gene leads to respiratory failure in newborns with defective lamellar bodies. Biochem Biophys Res Commun 2007;359(4):947–951.

64. Besnard V, Matsuzaki Y, Clark J, et al. Conditional deletion of Abca3 in alveolar type II cells alters surfactant homeostasis in newborn and adult mice. Am J Physiol Lung Cell Mol Physiol 2010;298(5):L646–L659.

65. Rindler TN, Stockman CA, Filuta AL, et al. Alveolar injury and regeneration following deletion of ABCA3. JCI Insight 2017;2(24):e97381.

66. Kabore AF, Wang WJ, Russo SJ, et al. Biosynthesis of surfactant protein C: Characterization of aggresome formation by EGFP chimeras containing propeptide mutants lacking conserved cysteine residues. J Cell Sci 2001;114(Pt 2):293–302.

67. Brasch F, Griese M, Tredano M, et al. Interstitial lung disease in a baby with a de novo mutation in the SFTPC gene. Eur Respir J 2004;24(1):30–39.

68. Maguire JA, Mulugeta S, Beers MF. Endoplasmic reticulum stress induced by surfactant protein C BRICHOS mutants promotes proinflammatory signaling by epithelial cells. Am J Respir Cell Mol Biol 2011;44(3):404–414.

69. Beers MF, Hawkins A, Maguire JA, et al. A nonaggregating surfactant protein C mutant is misdirected to early endosomes and disrupts phospholipid recycling. Traffic 2011;12(9):1196–1210.

70. Beers MF, Zhao M, Tomer Y, et al. Disruption of N-linked glycosylation promotes proteasomal degradation of the human ATP-binding cassette transporter ABCA3. Am J Physiol Lung Cell Mol Physiol 2013;305(12):L970–L980.

71. Hu JY, Yang P, Wegner DJ, et al. Functional characterization of four ATP-binding cassette transporter A3 gene (ABCA3) variants. Hum Mutat 2020;41(7):1298–1307.

72. Wambach JA, Yang P, Wegner DJ, et al. Functional genomics of ABCA3 variants. Am J Respir Cell Mol Biol 2020;63(4):436–443.

73. Munis AM, Hyde SC, Gill DR. A human surfactant B deficiency air-liquid interface cell culture model suitable for gene therapy applications. Mol Ther Methods Clin Dev 2021;20:237–246.

74. Leibel SL, Winquist A, Tseu I, et al. Reversal of surfactant protein B deficiency in patient specific human induced pluripotent stem cell derived lung organoids by gene therapy. Sci Rep 2019; 9(1):13450.

75. Kikkawa Y, Yoneda K, Smith F, et al. The type II epithelial cells of the lung. II. Chemical composition and phospholipid synthesis. Lab Invest 1975;32(3):295–302.

76. Williams MC, Mason RJ. Development of the type II cell in the fetal rat lung. Am Rev Respir Dis 1977;115(6 Pt 2):37–47.

77. Yamamoto Y, Gotoh S, Korogi Y, et al. Long-term expansion of alveolar stem cells derived from human iPS cells in organoids. Nat Methods 2017;14(11):1097–1106.

78. Galetskiy D, Woischnik M, Ripper J, et al. Aberrant processing forms of lung surfactant proteins SP-B and SP-C revealed by high-resolution mass spectrometry. Eur J Mass Spectrom (Chichester) 2008;14(6):379–390.

79. Alapati D, Zacharias WJ, Hartman HA, et al. In utero gene editing for monogenic lung disease. Sci Transl Med 2019;11(488):eaav8375.

80. Alysandratos K-D, de Alba Rivas CG, Yao C, et al. Impact of cell culture on the transcriptomic programs of primary and iPSC-derived human alveolar type 2 cells. bioRxiv 2022:2022.02.08.479591.

81. Weaver TE, Whitsett JA. Function and regulation of expression of pulmonary surfactant-associated proteins. Biochem J 1991;273(Pt 2): 249–264.

82. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001;11(7):1156–1166.

83. Klink D, Schindelhauer D, Laner A, et al. Gene delivery systems—Gene therapy vectors for cystic fibrosis. J Cyst Fibros 2004;3:203–212.

84. Katz M, Fargnoli A, Gubara S, et al. Targeted gene delivery through the respiratory system: Rationale for intratracheal gene transfer. J Cardiovasc Dev Dis 2019;6(1):8.

85. McCarron A, Cmielewski P, Drysdale V, et al. Effective viral-mediated lung gene therapy: Is airway surface preparation necessary? Gene Ther 2022; DOI: https://doi.org/10.1038/s41434-022-00332-7.

86. Strayer MS, Guttentag SH, Ballard PL. Targeting type II and clara cells for adenovirus-mediated gene transfer using the surfactant protein B promoter. Am J Respir Cell Mol Biol 1998;18(1): 1–11.

87. Matsuzaki Y, Xu Y, Ikegami M, et al. Stat3 is required for cytoprotection of the respiratory epithelium during adenoviral infection. J Immunol 2006;177(1):527–537.

88. Liu Y, Sadikot RT, Adami GR, et al. FoxM1 mediates the progenitor function of type II epithelial cells in repairing alveolar injury induced by Pseudomonas aeruginosa. J Exp Med 2011; 208(7):1473–1484.

89. 277. Efficient transduction of alveolar type II epithelial cells in nonhuman primates by helper-dependent adenovirus 5-fiber 35 chimeric vectors. Mol Ther 2011;19:S108.

90. Muruve DA. The innate immune response to adenovirus vectors. Hum Gene Ther 2004;15(12): 1157–1166.

91. Shirley JL, De Jong YP, Terhorst C, et al. Immune responses to viral gene therapy vectors. Mol Ther 2020;28(3):709–722.

92. Dicks MDJ, Spencer AJ, Edwards NJ, et al. A novel chimpanzee adenovirus vector with low human seroprevalence: Improved systems for vector derivation and comparative immunogenicity. PLoS One 2012;7(7):e40385.

93. Crystal RG. Adenovirus: The first effective in vivo gene delivery vector. Hum Gene Ther 2014;25(1): 3–11.

94. Nathwani AC, Rosales C, McIntosh J, et al. Long-term safety and efficacy following systemic administration of a self-complementary AAV vector encoding human FIX pseudotyped with serotype 5 and 8 capsid proteins. Mol Ther 2011; 19(5):876–885.

95. Sabatino DE, Lange AM, Altynova ES, et al. Efficacy and safety of long-term prophylaxis in severe hemophilia A dogs following liver gene therapy using AAV vectors. Mol Ther 2011;19(3): 442–449.

96. Nathwani AC, Reiss UM, Tuddenham EGD, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. N Engl J Med 2014; 371(21):1994–2004.

97. Zabner J, Seiler M, Walters R, et al. Adeno-associated virus type 5 (AAV5) but not AAV2

binds to the apical surfaces of airway epithelia and facilitates gene transfer. J Virol 2000;74(8): 3852–3858.

98. Auricchio A, O'Connor E, Weiner D, et al. Noninvasive gene transfer to the lung for systemic delivery of therapeutic proteins. J Clin Invest 2002;110(4):499–504.
99. Van Lieshout LP, Domm JM, Rindler TN, et al. A novel triple-mutant AAV6 capsid induces rapid and potent transgene expression in the muscle and respiratory tract of mice. Mol Ther Methods Clin Dev 2018;9:323–329.
100. Chen OG, Mather SE, Brommel CM, et al. Transduction of pig small airway epithelial cells and distal lung progenitor cells by AAV4. Cells 2021;10(5):1014.
101. Yang M-S, Park M-J, Lee J, et al. Non-invasive administration of AAV to target lung parenchymal cells and develop SARS-CoV-2-susceptible mice. Mol Ther 2022;30(5):1994–2004.
102. Limberis MP, Wilson JM. Adeno-associated virus serotype 9 vectors transduce murine alveolar and nasal epithelia and can be readministered. Proc Natl Acad Sci U S A 2006;103(35): 12993–12998.
103. Meyer-Berg H, Zhou Yang L, Pilar De Lucas M, et al. Identification of AAV serotypes for lung gene therapy in human embryonic stem cell-derived lung organoids. Stem Cell Res Ther 2020; 11(1):448.
104. Kang MH, Van Lieshout LP, Xu L, et al. A lung tropic AAV vector improves survival in a mouse model of surfactant B deficiency. Nat Commun 2020;11(1):3929.
105. Rindler TN, Brown KM, Stockman CA, et al. Efficient transduction of alveolar type 2 cells with adeno-associated virus for the study of lung regeneration. Am J Respir Cell Mol Biol 2021; 65(1):118–121.
106. Halbert CL, Rutledge EA, Allen JM, et al. Repeat transduction in the mouse lung by using adeno-associated virus vectors with different serotypes. J Virol 2000;74(3):1524–1532.
107. Sumner-Jones SG, Gill DR, Hyde SC. Lack of repeat transduction by recombinant adeno-associated virus type 5/5 vectors in the mouse airway. J Virol 2007;81(22):12360–12367.
108. Borok Z, Harboe-Schmidt JE, Brody SL, et al. Vesicular stomatitis virus G-pseudotyped lentivirus vectors mediate efficient apical transduction of polarized quiescent primary alveolar epithelial cells. J Virol 2001;75(23):11747–11754.
109. Tran E, Shi T, Li X, et al. Development of novel in vitro human alveolar epithelial cell models to study distal lung biology and disease. bioRxiv 2020:2020.12.25.424415.
110. Hendrickson B, Senadheera D, Mishra S, et al. Development of lentiviral vectors with regulated respiratory epithelial expression in vivo. Am J Respir Cell Mol Biol 2007;37(4):414–423.
111. Huang J, Hume AJ, Abo KM, et al. SARS-CoV-2 infection of pluripotent stem cell-derived human lung alveolar type 2 cells elicits a rapid epithelial-intrinsic inflammatory response. Cell Stem Cell 2020;27(6):962.e7–973.e7.
112. Korfhagen TR, Glasser SW, Wert SE, et al. Cis-acting sequences from a human surfactant protein gene confer pulmonary-specific gene expression in transgenic mice. Proc Natl Acad Sci U S A 1990;87(16):6122–6126.
113. Glasser SW, Korfhagen TR, Wert SE, et al. Genetic element from human surfactant protein SP-C gene confers bronchiolar-alveolar cell specificity in transgenic mice. Am J Physiol 1991; 261(4 Pt 1):L349–L356.
114. Glasser SW, Burhans MS, Eszterhas SK, et al. Human SP-C gene sequences that confer lung epithelium-specific expression in transgenic mice. Am J Physiol Lung Cell Mol Physiol 2000; 278(5):L933–L945.
115. Wunderlich S, Gruh I, Winkler ME, et al. Type II pneumocyte-restricted green fluorescent protein expression after lentiviral transduction of lung epithelial cells. Hum Gene Ther 2008;19(1):39–52.
116. Limberis MP, Bell CL, Heath J, et al. Activation of transgene-specific T cells following lentivirus-mediated gene delivery to mouse lung. Mol Ther 2010;18(1):143–150.
117. Griesenbach U, Inoue M, Meng C, et al. Assessment of F/HN-pseudotyped lentivirus as a clinically relevant vector for lung gene therapy. Am J Respir Crit Care Med 2012;186(9):846–856.
118. Wilson AA, Murphy GJ, Hamakawa H, et al. Amelioration of emphysema in mice through lentiviral transduction of long-lived pulmonary alveolar macrophages. J Clin Invest 2010;120(1):379–389.
119. Sinn PL, Arias AC, Brogden KA, et al. Lentivirus vector can be readministered to nasal epithelia without blocking immune responses. J Virol 2008;82(21):10684–10692.
120. Hardee CL, Arévalo-Soliz LM, Hornstein BD, et al. Advances in non-viral DNA vectors for gene therapy. Genes (Basel) 2017;8(2):65.
121. Uchida E, Mizuguchi H, Ishii-Watabe A, et al. Comparison of the efficiency and safety of non-viral vector-mediated gene transfer into a wide range of human cells. Biol Pharm Bull 2002;25(7):891–897.
122. Katsumiti A, Ruenraroengsak P, Cajaraville MP, et al. Immortalisation of primary human alveolar epithelial lung cells using a non-viral vector to study respiratory bioreactivity in vitro. Sci Rep 2020;10(1):20486.

Received for publication June 8, 2022;
accepted after revision August 29, 2022.

Published online: September 26, 2022.