Mesenchymal Stromal Cells for the Treatment of Interstitial Lung Disease in Children: A Look from Pediatric and Pediatric Surgeon Viewpoints

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Abstract: Mesenchymal stromal cells (MSCs) have been proposed as a potential therapy to treat congenital and acquired lung diseases. Due to their tissue-regenerative, anti-fibrotic, and immunomodulatory properties, MSCs combined with other therapy or alone could be considered as a new approach for repair and regeneration of the lung during disease progression and/or after post-surgical injury. Children interstitial lung disease (chILD) represent highly heterogeneous rare respiratory diseases, with a wide range of age of onset and disease expression. The chILD is characterized by inflammatory and fibrotic changes of the pulmonary parenchyma, leading to gas exchange impairment and chronic respiratory failure associated with high morbidity and mortality. The therapeutic strategy is mainly based on the use of corticosteroids, hydroxychloroquine, azithromycin, and supportive care; however, the efficacy is variable, and their long-term use is associated with severe toxicity. The role of MSCs as treatment has been proposed in clinical and pre-clinical studies. In this narrative review, we report on the currently available on MSCs treatment as therapeutical strategy in chILD. The progress into the therapy of respiratory disease in children is mandatory to ameliorate the prognosis and to prevent the progression in adult age. Cell therapy may be a future therapy from both a pediatric and pediatric surgeon’s point of view.

Keywords: mesenchymal stromal cells; children; interstitial lung disease; pediatrics

1. Introduction

Stem cell therapy represents a prospective approach in regenerative medicine for the repair, replacement, and rejuvenation of tissue [1,2]. Mesenchymal stromal cells (MSCs) expanded in vitro have been proposed as potential therapy to treat congenital and acquired lung diseases [2–11]. MSCs are multipotent cells that can differentiate into multiple tissue-forming cell lineages, such as osteoblasts, adipocytes, chondrocytes, tenocytes, and myocytes. In addition, MSCs regulate immune and inflammatory responses. MSCs are characterized by an innate self-renewal capacity, and they can be in vitro expanded without losing their differentiation potential [12].

The lung originates from the endodermal and mesodermal germline. Each phase in lung development is reliant on inductive cues and reciprocal interactions between the pulmonary epithelium and the surrounding mesenchyme. Loss of or abnormalities in
cells and in their interactions can lead to severe anatomical and functional defects in
the airway and alveoli [13–16]. MSCs are key cells in the connective pulmonary tissue
hierarchy, supporting the crucial relationship between the epithelium and mesenchyme
during branching morphogenesis [13–16].

Interstitial lung disease in children (chILD) is a group of highly heterogeneous and rare
respiratory diseases with wide ranges in the age of onset and disease expression [2,17–19].
chILD is characterized by inflammatory and fibrotic changes in the pulmonary parenchyma,
leading to gas exchange impairment and chronic respiratory failure associated with high
morbidity and mortality. chILD disorders often overlap, and the classifications remain
difficult. chILD is characterized by abnormality of the lung interstitium, alveoli, and distal
air spaces, leading to chronic respiratory failure. Even though chILD pathogenesis remains
unclear, the aberrant activation of the alveolar epithelium and mesenchyme has been
proposed as a crucial player [18,20]. A multidisciplinary approach to the diagnosis and
follow-up is mandatory to provide improved care. Surgery may represent a critical step
for management. The therapeutic strategy is mainly based on the use of corticosteroids,
hydroxychloroquine, azithromycin, and supportive care; however, the efficacy is of these
methods is variable. The role of MSCs as a state-of-the-art treatment has been proposed in
clinical and preclinical studies [4,21–29].

This narrative review reports the information currently available on MSCs treatment
as a new therapeutic strategy in chILD. To evaluate the efficacy of MSCs treatment, the
pre-clinical and clinical results on bronchopulmonary dysplasia (BDP) were also included
as model of chronic respiratory diseases characterized by interstitial lung abnormalities
leading to chronic failure. The need for research to advance the therapy of pulmonary dis-
eases is mandatory to improve the prognosis and to prevent their progression in adulthood.
Cell therapy, as reparative and regenerative treatment, may be innovative from both the
pediatric and pediatric surgeon’s viewpoint.

2. Methods

A narrative review of the English literature published in the past 10 years was con-
ducted. We independently identified the most relevant published manuscripts including
original papers, metanalyses, clinical trials, and reviews. Case reports or series and letters
were excluded. Human and experimental studies were included. Papers were searched
using the following keywords in combination: “children interstitial lung disease and
mesenchymal stromal cells”, “children idiopathic pulmonary fibrosis and mesenchymal
stromal cells” and “infants bronchopulmonary dysplasia and mesenchymal stromal cells
and clinical trials”. The following electronic databases were searched: PubMed, Scopus,
EMBASE, and Web of Science. In this study, we included clinical studies recorded on
ClinicalTrial.gov searched with the following keywords in combination: “stem cells” and
“infants bronchopulmonary dysplasia”. The contributions were critically reviewed and
collected. The final version was approved by all authors.

3. Mesenchymal Stromal Cells and MSCs-Derived Extracellular Vesicles

Cell therapy has recently received considerable interest as a treatment of respira-
tory system diseases. Several lines of evidence encourage the use of MSCs in lung dis-
eases [30,31]. The ease of isolation, the lack of immunogenicity, and the ability to expand
ex vivo and to differentiate in multilineages make MSCs an attractive therapeutic tool. The
therapeutic potential of MSCs is due to their paracrine effect, supported by their secretion
of extracellular vesicles (EVs), transferring genetic material, and releasing of soluble factors
such as cytokines. The conditioned medium (CM) or secretome is defined as the products
secreted by MSCs in their cultured medium [32–34]. In Vivo administration of MSCs, as
well as of their products, appears a suitable approach for injured lung tissue repair by
reducing fibrosis and stimulating proper alveolar and vascular repair [35,36].
3.1. Mesenchymal Stromal Cells (MSCs)

MSCs are a population of multipotent nonhematopoietic stromal cells, capable of differentiating into tissue derived from the three main mesodermal lineages. MSCs represent a cell population with secretion [37], homing [38], and immunomodulatory [39] properties.

MSCs can be isolated from various sources, including bone marrow, adipose tissue, skeletal muscle, synovium, spleen, thymus, lung, and amniotic fluid. The International Society for Cell and Gene Therapy reported, as minimal criteria for their definition: plastic adherence when in vitro cultured; the expression of surface markers such as CD105, CD73, and CD90; and the absence of the expression of endothelial and hematopoietic markers such as CD11b, CD14, CD19, CD34, CD45, CD79a, and class II human leukocyte antigen-DR (HLA-DR) [40,41]. They have to show the capability to differentiate in vitro into tissues of mesodermal origin, such as osteoblasts, adipocytes, and chondroblasts. Subsequently, it was shown that MSCs can transdifferentiate into neural cells, pancreatic cells, liver cells, and cardiomyocytes [42].

MSCs can exert several functions including immunomodulatory effects through inhibition of T-cell proliferation and secretion of anti-inflammatory cytokines and growth factors [43,44]. In Vivo, their effects were reported to be mediated by paracrine mechanism with the release of growth factors, which stimulate endogenous repair pathways [12] and host inflammation sites that express anti-inflammatory cytokines to dampen the host’s immune response [45].

It is a long-established fact thatMSCsinfuseintravenously; while circulating within the lungs, they remain partially entrapped, producing the pulmonary first-pass effect. This effect represents one of the hurdles faced by MSCs therapy when targeting other organs, but may be an inherent advantage when biotherapy with MSCs is directed to the lungs. The ability to deliver MSCs to the lungs via a simple intravenous approach has the potential for large-scale retention [11,46,47]. Even more attractively, retained cells appear to target areas of injured lung where they differentiate into specific cell types and start regeneration [48]. Both in vitro and In Vivo, MSCs have been observed to differentiate into alveolar epithelial cells, indicating their potential as a regenerative therapy for lung diseases [49–51].

Isolation and culture make MSCs suitable candidates for preclinical and clinical studies. Effectively, clinical trials phase I in patients with chronic obstructive pulmonary disease (COPD) confirmed the safety of MSCs; outcomes from phase I/II clinical trial administration and investigation showed their potential anti-inflammatory effects, reporting a reduction in C-reactive protein levels [52].

3.2. Extracellular Vesicles (EVs)

MSCs also exert their therapeutic effects through the release of EVs. MSCs-derived EVs are small membrane-bound vesicles that contain biomolecules including proteins, lipids, microRNA, and mRNA, which play a role as mediators in cellular communication to maintain physiological homeostasis [53].

EVs provide intercellular communication by transferring their membrane contents to the target cells through the binding of surface receptors [54,55]. EVs, according to their origin, are divided into microvesicles and exosomes [56]. Microvesicles are generated by direct budding of the plasma membrane toward the extracellular environment through a calcium-induced asymmetric reorganization of phospholipids [57]. Membrane germination is induced by calpain and gelsolin, proteases that cut the protein network of the cytoskeleton [58]. The exosomes instead originate from the intracellular fusion of the endosomes with the endocytic vesicles, thus incorporating their contents. After maturation, endosomes merge with the plasma membrane, becoming exosomes that are released into the extracellular space [59]. The microvesicles are larger, ranging from 50 to 1000 nm; exosomes are smaller, with a size ranging from 40 to 120 nm [60,61]. Western blot and mass spectrometry analyses allowed the identification of proteins expressed in EVs [60,61]. In particular, microvesicles contain phosphatidylserine, metalloproteinases, some integrins, and P-selectin [62]. Exosomes contain proteins with GTPase activity involved in transport
and fusion [63], heat shock proteins (Hsp 60, Hsp70, Hsp90) [59,63], and tetraspanins (CD63, CD81, and CD9), which are necessary in the fusion between exosomes and recipient cells [64]. EVs’ composition and structure are different according to the mother cell. Moreover, EVs mediate horizontal mRNA transfer to a recipient cell [59,65]. As such, EVs are able to change the phenotype and function of the recipient cells, regulating different cellular pathways and activating regenerative mechanisms. Therefore, MSC-derived EVs could be capable of restoring the homeostasis of damaged tissues [66] and interacting with immune system cells [67], demonstrating regenerative and anti-inflammatory properties [68]. The use of EVs confers some advantages compared to the use of original stem cells, such as a higher safety profile, an increased ability to cross biological barriers, lower immunogenicity, and poor immune rejection. Despite the advantages shown by MSC-derived EVs, their use in clinical studies requires further investigation to resolve critical issues related to the methods of production, characterization, quantification, pharmacokinetics, and transfer to target sites [69].

3.3. Conditioned Medium (CM)

The CM/secretome from cultured MSCs, containing both soluble factors and EVs, may represent a significant tool to produce efficacy similar to the original cells [70]. A systematic review and meta-analysis of preclinical studies, including different lung diseases such as bronchopulmonary dysplasia, asthma, pulmonary hypertension, acute respiratory distress syndrome, chronic obstructive pulmonary disease, and pulmonary fibrosis, recorded comparable efficacy between CM and MSCs [71]. To obtain CM from cultured MSCs, different collection timings, confluence grades, and culture passages have been reported in the literature [72–74]. As already underlined, MSC-derived CM consists of all MSC-secreted cytokines, proteins, growth factors, and EVs [75]; it contains several angiogenic growth factors, such as vascular endothelial growth factor (VEGF) [76,77]. In in vitro models, it was shown that MSC-derived CM containing high levels of VEGF promoted angiogenesis and the regeneration of periodontal tissue [78]. In another study, the addition of anti-VEGF antibodies to MSC-derived CM decreased vessel formation and induced poor bone regeneration in a rat calvaria model [77].

In line with these findings, in a mouse model of Escherichia coli endotoxin-induced model of acute lung injury (ALI), administration of MSC-derived CM induced a reduction in septal thickening, alveolar hemorrhage, alveolar infiltrates, and fibrin filaments compared to untreated mice. Similar reductions in neutrophils and lung permeability were observed both after treatment with MSCs or CM [79]. Moreover, in a rat model of bleomycin-induced pulmonary fibrosis, administration of MSC-derived CM reduced the deposition of collagen involved in fibrosis [80].

Therefore, these data encourage the use of the CM as potential cell-free therapy; however, it is necessary to identify and standardize the most advantageous cellular source, the culture method, and the purification method to obtain the maximum therapeutic yield.

4. Routes of Administration of MSCs

The use of MSCs as treatment for respiratory diseases, although promising, needs to overcome some limitations. Some of these include determining the optimal dosage, the cell type, and the appropriate route of administration.

In preclinical and clinical studies, cell therapy envisages systemic administration, such as intravenous or intra-arterial infusion or local administration via intratracheal administration [81]. Intravenous cell infusion causes the accumulation of MSCs in the lungs, an effect known as the first pass [82]. This phenomenon may represent an obstacle when MSCs therapy is aimed toward other organs but may be a notable advantage when MSC therapy is directed to the lungs. However, it has been shown that this entrapment is transient, followed by a distribution of the cells to other organs, such as the spleen and liver, during the following 24–48 h [83–85].
In an In Vivo study performed in a rodent silicosis model with intravenously administered bone-marrow-derived MSCs (BM-MSCs), the first pass event was observed six hours after treatment [86], whereas in a rodent model of myocardial infarction, it occurred within minutes with subsequent embolization [87]. However, the small diameter of the pulmonary microcirculation and the presence of adhesion receptors may prevent MSCs from reaching the targeted lung site [88]. MSCs entrapment in the vascular system can induce the formation of an embolus, as demonstrated by a clinical study in which cell therapy promoted venous thrombosis in two patients with Crohn’s disease [89]. Instead, intra-arterial infusion allows reaching the target organ, avoiding entrapment inside the lung [81,82,90]. However, even this procedure may be harmful due to the possible generation of emboli in microcirculation [91].

The local administration has greater or equal therapeutic potential at lower dosages than systemic administration [92]. For cell therapy, local delivery to the lungs occurs by intratracheal administration [81]. This route, although a possible cause of trauma and injury, has been reported to produce promising therapeutic outcomes in several clinical studies [36,93,94], showing MSCs engraftment into the lung in real time [88]. However, the available data are contradictory and insufficient to identify the most suitable route for MSC infusions both in terms of safety and of targeted delivery to a specific area of the organ, in particular in the lung.

5. Interstitial Lung Disease in Children (chILD)

chILD refers to a heterogeneous group of rare lung disorders, with a wide range of prevalence (from 0.13 to 16.2/100,000 children/year) as a result of a lack of standardized diagnostic criteria, and a heterogeneous clinical presentation and pathological picture [17,18]. chILD is characterized by abnormalities in the lung interstitium, alveoli, and distal air spaces, leading to abnormal gas exchange and chronic failure [17,18].

The pathogenesis of chILD is complex and has not yet been fully elucidated. The central role of the alveolar epithelium and aberrant mesenchymal activation has been proposed [18,20]. The involvement of repeated injuries of vulnerable alveolar epithelial cells (AECs) and the failure of the alveoli to respond to injury, leading to aberrant lung repair and progressive fibrosis, were suggested to be involved in pathogenesis [95]. An acceleration of the ageing process of progenitor cells, leading to stem cell exhaustion, was also proposed [96]. The initial recruitment of inflammatory cells including collagen-producing fibrocytes is not excluded in the pathophysiology of alveolar injury.

chILD comprises more than 200 different conditions, for which different classification systems have been proposed based on the etiology and physiopathology and lung biopsies [18,20]. As reported in Table 1, more recently, a subclassification considering infancy ILD different from other pediatric ILD was introduced, again on the basis of etiologic and pathologic criteria [17,18,97–99].

In most of the chILD cases, no family history is documented; moreover, the occurrence of familial forms with an estimated prevalence ranging from 1.3 to 5.9 per million has been reported [17,18]. Mutations in the surfactant protein (SP) genes, mainly in SP-B and -C genes, are responsible for the familial form [17,18]. As reported in Table 2, other genetic forms have been described.
Table 1. Classification of children’s interstitial lung disease according to Rice et al. [97].

| Disorders More Prevalent in Infants | Disorders More Prevalent in Children |
|-------------------------------------|-------------------------------------|
| Diffuse developmental disorders (acinar dysplasia, alveolar capillary dysplasia, congenital alveolar dysplasia) | Disorders related to systemic disease (Langerhans cell histiocytosis, related to acquired heart disease, storage disease/endogenous lipid pneumonia) |
| Growth abnormalities (alveolar hypoplasia, chronic neonatal lung disease, related to chromosomal disorders, related to congenital heart disease) | Disorders of the normal host (eosinophilic bronchiolitis/pneumoniae, infection/post infectious processes, hypersensitivity pneumonitis, aspiration pneumonia) |
| Specific conditions of undefined etiology (neuroendocrine cell hyperplasia of infancy, pulmonary interstitial glycoegenosis) | Disorders of the immunocompromised host: (opportunistic infection, transplant-related) |
| Surfactant protein disorders | Surfactant protein disorders |

Table 2. Genetic mutations associated with children’s interstitial lung disease.

| Genetic Mutation | Inheritance | Lung Involvement |
|------------------|-------------|-----------------|
| SFTPB (surfactant protein B deficiency) | Autosomal recessive | Surfactant disorder |
| SFTPC (surfactant protein C mutation) | Autosomal dominant | Surfactant disorder |
| CSF2RB (colony stimulating factor 2 receptor β) | Autosomal recessive | Pulmonary alveolar proteinosis |
| CSF2RA (colony stimulating factor 2 receptor α) | X-linked | Pulmonary alveolar proteinosis |
| ABCA3 (ATP-binding cassette-family A-member 3) | Autosomal recessive | Deficit surfactant |
| COPA (coatamer associated protein subunit alpha) | Autosomal dominant | General disorder including lung |
| FLNA (Filamin A) | X-linked recessive | General disorder including lung |
| FOXF1 (forkhead box F1) | Autosomal dominant | Alveolar capillary dysplasia |
| GATA2 (GATA Binding Protein 2) | Autosomal dominant | Pulmonary alveolar proteinosis |
| MAR5 (metionil-transfer RNA sintetasi) | Autosomal recessive | Pulmonary alveolar proteinosis |
| NKK2-1 (NK2 homeobox 1) | Autosomal dominant | Interstitial lung disease |
| NSMCE3 (non-structural maintenance of chromosome element 3 homolog) | Autosomal recessive | Immunodeficiency |
| OAS1 (oligoadenylate synthetase 1) | Autosomal dominant | Pulmonary alveolar proteinosis |
| SLC7A7 (solute carrier family 7 member 7) | Autosomal recessive | Surfactant disorder |
| TBX4 (T-box transcription factor 4) | Autosomal dominant | Acinar dysplasia |
| TMEM173 (transmembrane protein 173) | Autosomal dominant | Lung fibrosis with general inflammation |

The clinical presentation of chILD varies, ranging from mild nonspecific symptoms to a very severe clinical picture. Usually, the earlier the disease onset, the more severe the presenting symptoms [100].
During the neonatal period, in term neonates, chILD may occur shortly after birth, with unexplained respiratory distress requiring intubation and ventilation [101]. In born preterm infants, chILD presents with acute respiratory distress that is more severe and is expected because of prematurity [101].

During the first two years of life [102,103], common presentations include asymptomatic forms or nonspecific respiratory signs and symptoms, such as dyspnea, polypnea, dry cough, wheezing, recurrent respiratory infections, and exercise intolerance [101]. Moreover, severe respiratory distress usually triggered by viral infections may occur. Older children can show tachypnoea, hyperoxia, digital clubbing, and/or cyanosis during exercise or at rest [101,104,105]. As reported in Table 3, a severity score of disease was proposed by Fan et al. [106].

**Table 3.** Severity-of-illness score according to Fan et al. [106].

| 1. Asymptomatic |
|------------------|
| 2. Symptomatic, normal room air oxygen saturation under all conditions |
| 3. Symptomatic, normal resting room air saturation but abnormal saturation (90%) with sleep or exercise |
| 4. Symptomatic, abnormal resting room air saturation (90%) |
| 5. Symptomatic with pulmonary hypertension |

At the initial investigation, the chest radiograph may be normal or reveal nonspecific alterations [107]. The role of functional lung testing in infants is unclear: in older children, a restrictive pattern is usually detected. Within the diagnostic workup, blood tests including genetic evaluation, immunological profile, and autoantibody studies are recommended, and environmental organic dust exposures could be considered.

When chILD is suspected clinically, chest computed tomography (CT) scanning is the gold standard to evaluate the presence and extent of lung damage; however, only in some cases can it be diagnostic [107]. Common radiologic patterns in chILD are widespread ground-glass attenuation, sometimes coupled with intralobular lines; irregular interlobular septal thickening; honeycombing; and, less frequently, large subpleural air cysts (usually located in upper lobes adjacent to areas of ground-glass opacities) [18,108–110]. Invasive testing, such as bronchoscopy with bronchoalveolar lavage (BAL), may be diagnostic in pulmonary hemorrhage syndromes, alveolar proteinosis, and eosinophilic lung disease, and a normal cell differential can rule out hypersensitivity pneumonitis [17]. Endobronchial and transbronchial biopsies are rarely performed in chILD and are not recommended unless a specific diagnosis is suspected, such as pulmonary alveolar microlithiasis or sarcoid granulomas. Lung biopsy is the last step in the diagnostic workup. The timing and need for lung biopsy in chILD remain controversial; biopsy prior to steroid treatment is recommended to minimize risk to wound healing and to expedite specific chILD treatments.

A multidisciplinary approach for diagnosis and follow-up is mandatory in chILD. Pediatricians, pediatric pulmonologists, radiologists, geneticists, and pediatric surgeons are the crucial players [17,18].

The prognosis of chILD is variable, ranging from complete recovery in neuroendocrine cell hyperplasia during infancy and pulmonary interstitial glycogenosis to a mortality rate that approaches 100% in alveolar capillary dysplasia. The overall mortality rate is around 15%, with a variable outcome in infants [106,111–114].

The standard treatment of chILD is mainly supportive and based on oxygen supplementation, and/or ventilation, and respiratory physiotherapy [17,18]. Nutritional support is mandatory to maintain an adequate caloric intake to prevent failure to thrive. From a pediatric surgery point of view, most of these patients require tracheostomy in the few first months of life, followed by enteral nutritional supply by gastrostomy. With time and in order to protect the lung tissue, most of the children need an antireflux procedure. In
some patients, when bullous emphysema and hypertensive pneumothorax occur, leading
to ventilation failure, surgical resection may be necessary to ensure lung growth.

Empirical medical therapy with anti-inflammatory and immunomodulatory drugs
including corticosteroids, hydroxychloroquine, and azithromycin is usually used with
varying degrees of efficacy [17,18]. New antifibrotic drugs currently proposed in adults,
including pirfenidone and nintedanib, may represent new therapy options for certain forms
of chILD in the future. Lung transplant is a therapeutic option for children with end-stage
chronic respiratory failure. The outcome and survival are similar to those reported in other
lung conditions [115].

Preclinical and clinical studies support a potential beneficial role of cell therapy to
prevent the chILD progression [116]. Additionally, cell therapy could be useful to promote
the repair and regeneration of the impaired lung.

6. Preclinical and Clinical Studies
6.1. Preclinical

ILD is a group of diseases that includes several chronic lung diseases characterized
by varying degrees of inflammation and fibrosis. Most ILDs are idiopathic, including
idiopathic pulmonary fibrosis (IPF). In order to understand the pathogenetic mechanisms
of pulmonary fibrosis and identify possible therapeutic targets, several animal models have
been developed that are capable of mimicking the human characteristics of the disease. The
bleomycin model is the most used and best characterized model that reproduces different
cellular and molecular mechanisms involved in IPF and in other fibrotic ILDs [117,118].
Bleomycin is a complex glycopeptide [119] isolated from Streptomyces verticillus, an acti-
nobacteria strain [120]. This drug showed usefulness as an anticancer in several carcinomas
and lymphomas [121]. However, the use of this drug has been limited due to its toxicity in
organs such as the lung. These adverse events promoted the use of this agent to induce
animal models of pulmonary fibrosis [122]. Initially, drug administration induces direct
damage of alveolar epithelial cells, promoting the production of alveolar inflammatory
cells within the first seven days [123]. Subsequently, these cells are eliminated and the
proliferation of fibroblasts is induced, with the consequent development of pulmonary
fibrosis [124]. Notably, the bleomycin model is characterized by an overlap between the
inflammatory and fibrotic models. Initially, the model induces an early inflammatory phase
that switches to the fibrotic state after 5–7 days. The strong inflammatory response induced
by bleomycin may take up to 10 days before complete elimination. The fibrotic phase
begins when the inflammatory process has subsided. After two weeks, the resolution of
the fibrosis starts. Therefore, in this animal model, the “time window” during which it is
possible to test the fibrogenic mechanisms and the antifibrotic drugs action’s is relatively
short [125,126]. Another experimental model that is used is induced by fluorescein isothio-
cyanate (FITC), which involves the intratracheal administration of FITC in both C57Bl/6
and Balb/c mice [127,128]. FITC caused infiltration of mononuclear and neutrophil cells
into the lung interstitium, inducing pulmonary fibrosis by day 21, as also observed in the
bleomycin model [128]. Silica-induced lung fibrosis is another model used to reproduce
fibrotic nodules that resemble lesions that develop following occupational exposure to
mineral dust and particulate aerosols [129]. Silica is trapped in the lung where a toxic and
inflammatory response is activated, inducing the alveolar accumulation of proteins and neu-
trophilia, responsible for activating a fibrotic response [130,131]. Several transgenic strains
mimic the characteristics of pulmonary fibrosis, such as human collagenase directed by the
haptoglobin promoter [132], PDGF-B directed by the surfactant protein C (SP-C) promoter,
human transforming growth factor-alpha (TGF-α) directed by the SP-C promoter [133],
interleukin (IL)-11, and IL-13 directed by the bronchiolar exocrine/club cells 10-kDa (CC10)
protein promoter [134,135]. The transgenic model, however, has the disadvantage of not
accurately recreating the multigenic environment of natural pulmonary fibrosis. Addition-
ally widely used are models involving the use of adenoviral vectors, which exploit
gene transfer mediated by adenoviruses to overexpress cytokines and chemokines such as
granulocyte-macrophage colony-stimulating factor (GM-CSF) [135], tumor necrosis factor-
alpha (TNF-α) [136], transforming growth factor-beta 1 (TGF-β1) [137], and IL-1β [138].
These transgenes persist for 21 days and cause major fibrotic lesions in rodents, proving
useful models for studying the pathogenesis of this disease [139]. Contrarily, the use of
lentiviral vectors permanently transfers the transgenes in different cells of the lung, thus
allowing analysis of the effect of the overexpression of the transgene in the long term.
These models are useful for studying the effect of the gene product at the onset and during
pulmonary fibrosis [140].

6.1.1. Animal Models of Pulmonary Fibrosis

The use of MSCs in clinical practice is now a field of considerable interest. However,
most preclinical studies employed human BM-MSCs to treat asthma [141], acute lung
injury [142], pulmonary fibrosis [143], and acute respiratory distress syndrome [144]. In
this context, Balogh et al. [145] evaluated the immunomodulatory role of MSCs taken from
bronchoalveolar lavage fluid (BALF) from patients with hypersensitivity pneumonitis (HP)
compared to normal MSCs isolated from healthy subjects. Immunophenotyping by flow
cytometry and confocal laser scanning microscopy demonstrated that BALF MSCs have
reduced levels of CD105, CD73, and CD90. Therefore, these cells, compared to the MSCs
isolated from control subjects, showed a loss of immunosuppressive activity. In order to
evaluate the role of bronchoalveolar MSCs on T-cell function in vitro, phytohemagglutinin
(PHA)-stimulated peripheral blood mononuclear cells (PBMCs) were co-cultured with
normal or HP-derived BALF MSCs. Measurement of 5,6-carboxyfluorescein-diacetat succ-
inimidyl ester (CFSE) positivity by flow cytometry demonstrated that normal MSCs were
able to reduce T-cell proliferation, whereas HP-derived MSCs did not have a significant
immunosuppressive effect on normal or HP T lymphocytes. When T cells were cultured
with normal MSCs or HP, reduced proliferation and activation of CD4+ and CD8+ T cells
were observed with reduced CD25 positivity mediated by normal MSCs. In contrast,
HP-derived MSCs did not induce division energy in either T-cell subtype. Therefore, these
results demonstrate that healthy MSCs can affect HP-derived activated T cells, suggesting
the use of these cells as a potential therapeutic approach for this type of ILD [145].

Given the positive role of MSC transplantation in reducing pulmonary fibrosis [146],
Chen et al. [147] evaluated the effects of adipose-derived MSCs (AD-MSCs) in rats in
which pulmonary fibrosis was silica-induced. For the study, AD-MSCs were taken from
the adipose tissue of rats and cultured in vitro. In order to study the antifibrotic effects
of AD-MSCs, the animals underwent oral-tracheal intubation with a silica suspension
(50 mg/mL) to induce the pulmonary fibrosis pattern. After 24 h, the animals were treated
intravenously with AD-MSCs (1 × 10^6 cells/kg). Twenty-eight days after the transplant, the
animals were sacrificed and the organs were isolated for histopathological investigations.
In lung tissue, treatment with AD-MSCs markedly reduced the expression levels of TNF-α,
IL-6, and IL-10; conversely, it increased IL-1β expression levels. Treatment with AD-MSCs
reorganized the alveolar structure that had been severely destroyed by exposure to silica,
reducing inflammatory cell infiltration phagocytic cells and silicon nodules. Additionally,
AD-MSCs reduced silica-induced apoptosis in lung tissue cells. A decrease in caspase-3 was
observed due to the decrease in the B-cell lymphoma protein-2-associated X (Bax)/B-cell
lymphoma protein 2 (Bcl-2) ratio in the treatment group. These results further confirmed
that AD-MSCs appear to exert a lung protective effect and reduce the apoptosis process in
the animal model of pulmonary fibrosis [147].

Most preclinical and clinical studies evaluated the efficacy of BM-MSCs and AD-
MSCs in IPF. Cores et al. [148] instead investigated the effects of adult lung spheroid
cells (LSCs) as a source of stem cells for allogeneic cell therapy in pulmonary fibrosis.
For the study, lung outgrowth cells from the distal region of the lungs were harvested
from male Wistar-Kyoto rats (MHC haplotype, RT1), used as LSCs donors. In order to
evaluate the proangiogenic effects in vitro, human umbilical vein endothelial cells
(HUVECs) were cultured in media conditioned with LSCs. The data showed that LSCs,
by means of paracrine action, stimulated angiogenesis by reducing fibrosis. To reproduce an allogeneic cell transplantation model, Wistar-Kyoto and Brown Norway female rats (MHC haplotype, RTII) as syngeneic and allogeneic recipients, respectively, were used for the experiment. The animals were treated with bleomycin (1.5 U/kg) intratracheally on day 0 to induce the pulmonary fibrosis pattern. The following day, the rats were treated with LSCs (5 × 10^6 cells) intravenously via the tail vein, while the control group received saline. Both allogeneic and syngeneic LSCs alike attenuated the onset of fibrosis and the deposition of connective tissue and collagen in the post-injury lungs. LSC treatment also protected the pneumocytes from bleomycin-induced injury, reduced apoptosis in the lungs, and induced angiogenesis. Expression data on cytokine levels, such as inflammatory cytokine, immune response, wound healing, and epithelial proliferation, demonstrated that the treatment creates no systemic immune or inflammatory response. Furthermore, data on the expression levels of T lymphocytes in the lung showed that cell therapy did not induce any local immune response. Therefore, this evidence suggests that allogeneic LSC treatment can be considered safe and efficacious in slowing the progression and severity of fibrosis [148].

In the above-described studies, MSCs were administered intratracheally or intravenously. MSCs administration appears to decrease inflammation and fibrosis in several chronic lung disease models of newborn rodents. Despite the benefits of this therapeutic approach, the route of administration and the optimal dose in newborns still need to be determined. Liu et al. [149] surveyed the dose-dependent effects of intranasal MSCs release versus the intraperitoneal route in an early neonatal lung injury model. The long-term mechanical and histological effects of human umbilical cord derived MSCs (hUC-MSCs) were evaluated in the study. In order to induce lung damage, neonatal mice with severe combined immunodeficiency were exposed to hyperoxic conditions from birth until postnatal day 7, while one group was maintained in normoxic conditions as a control group. On the fifth postnatal day, during the phase of intense acute lung injury, the pups were subjected to the administration of cell suspension (0.1, 0.5, or 1 × 10^6 cells/kg in 20 µL of phosphate-buffered saline) via the intranasal or intraperitoneal route. Instead, sham normoxic controls received 20 µL of phosphate-buffered saline either intranasally or intraperitoneally. Animals were sacrificed either 48 h or 8 weeks after cell transplantation to assess the short- and long-term effects of cell therapy. Systemic (intraperitoneal) administration of hUC-MSCs in neonatal mice exposed to hyperoxia restored lung compliance, pressure-volume loop, and elastance in a dose-dependent manner 8 weeks after transplantation. At the highest dose (1 × 10^6 cells/kg), intraperitoneal MSC transplantation effectively increased the thickness of the alveolar septum, probably remodeling the interstitial matrix. Conversely, transplantation of hUC-MSCs via the intranasal or intraperitoneal route at lower doses had no significant effects on lung function or alveolar remodeling. Therefore, the results of this study highlight the beneficial effects of hUC-MSCs transplantation on tissue recovery and lung function following chronic neonatal injury. However, future studies are needed to evaluate the long-term safety of systemic transplantation with MSCs, especially when considered for pediatric use [149].

Mansouri et al. [150] investigated the therapeutic effects of EVs isolated from BM-MSCs in an animal model of IPF. In order to induce the IPF model, C57BL/6 mice were subjected to a single intratracheal administration of bleomycin sulfate (50 µL, 3 U/kg), while the control group received saline (50 µL). Simultaneously, the animals were treated with a single dose of EVs (200 µL; dose, 5 × 10^6 MSC equivalents; ~8.6 × 10^8 particles) or with EVs-free iodixanol vehicle only (control group). EVs were intravenously administered via the tail vein. Animals were evaluated on days 7, 14, or 28 by cytometric, histological and/or quantitative PCR analysis. A single dose of BM-MSCs-derived EVs attenuated bleomycin-induced damage, improving lung morphology, reducing collagen deposition, and restoring lung architecture. After both 7 and 14 days, treatment with BM-MSCs-derived EVs increased the populations of alveolar macrophages and infiltrated monocytes, while reducing the classical proinflammatory monocytes. The same immunomodulatory
effect was demonstrated in myeloid cells derived from the BM. In order to investigate the modulatory effect of EVs on the BM-myeloid/monocyte cell lineage phenotype, these cells were preconditioned with BM-MSCs-derived EVs ex vivo. Proteomic analysis of this cell line, performed by liquid chromatography–tandem mass spectrometry (LC-MS/MS), demonstrated that treatment with BM-MSCs-derived EVs exerts its protective effects, at least in part, by reprogramming the phenotypic profile of myeloid cells derived from BM toward a nonclassical phenotype. Subsequently, BM-derived myeloid cells preconditioned with EVs ex vivo were administered to mice with pulmonary fibrosis. After receiving bleomycin, animals received two doses of BM-derived myeloid cells preconditioned with EVs on days 0 and 3. Treatment with this cell population reduced collagen deposition, restored lung architecture, and reduced inflammation. These data demonstrate that the effects of BM-MSCs-derived EVs mediated, at least part, the myeloid cells reprogram toward a proregulatory phenotype, thus reducing infiltration into the lungs of proinflammatory and profibrotic monocytes [150].

6.1.2. Animal Models of BPD

Bronchopulmonary dysplasia (BPD) is a complex chronic lung disease that is common in premature infants, especially in very low birth weight and extremely low birth weight infants with an incidence of 30–40% and 54.1%, respectively [2]. In the early postnatal period of preterm infants, the pulmonary cells in the late canalicular or early saccular stage are in a highly proliferative state, leading to the lack effective alveolar gas exchange [2]. BPD results in a lung injury and abnormal lung repair, characterized by a decrease in the number of alveoli, abnormal morphology, uneven ventilation distribution, and abnormal development of the vascular system of the lung [2]. Similarly to chILD, the consequences of lung immaturity remain through-out childhood and can often lead to chronic respiratory diseases characterized by parenchymal fibrosis and alveolar and vascular growth block [2,151]. For this reason, BPD may represent a model to document the efficacy of MSCs treatment in ILD.

Moreira et al. [152] investigated the efficacy and safety of intranasal MSCs infusion in a BPD model. The cells were obtained from the gelatinous tissue of Wharton’s umbilical cord from a healthy human infant. The rat pups at postnatal day 4 were randomly divided into four groups. One group was maintained in normoxic conditions (21% O₂) for 21 days (controls group). The remaining groups underwent continuous hyperoxia (60% O₂) for 4 days to induce the BPD model. In the study, one group of BPD rats received nothing (BPD group), one group was treated with vehicle, and one group received MSCs (20 µL containing 5 × 10⁵ cells/kg) on days 4, 12, and 20. Morphometric pulmonary analyses showed that the administration of MSCs restored alveolarization, vascularization, and pulmonary remodeling, which, on the contrary, are altered in hyperoxic conditions. In pulmonary homogenates, treatment with MSCs increased vascular endothelial growth factor mRNA expression compared to the control group, which did not change compared to the BPD group. An analysis of lung tissue genes and proteins suggested that MSCs exert their beneficial effects, at least in part, by modulating genes involved in angiogenesis, immunomodulation, wound healing, and cell survival. Therefore, the data from this study showed that MSCs can improve the outcomes of BPD infants. Notably, these findings demonstrated that the experimental intranasal route of administration is a feasible, noninvasive, and efficacious route that may have clinical applicability [152].

The beneficial effect of MSCs-derived EVs was also demonstrated by Portionato et al. [153] in a mouse model of BPD induced by exposure to hyperoxia in the first 2 weeks postnatally. The newborn rats were randomly divided into four groups: one group of animals was raised for six weeks in normoxic conditions, as a control group. The experimental groups were exposed to 60% hyperoxia for 2 weeks, and for another 4 weeks to normoxic conditions. Half of the animals in the experimental group received saline (sham-treated animals), while the others were treated with MSC-derived EVs (0.64 × 10¹⁰ particles) or with MSCs (6 × 10⁶ cells/kg) on postnatal days 3, 7, and 10 via intratracheal administration.
MSCs-derived EVs delivery increased the number of alveoli, their surface area, and the proliferation index, which were decreased in the hyperoxic conditions. Furthermore, the treatment reduced mean alveolar volume, mean linear intercept, and fibrosis. The medial thickness index for vessels <100 µm reduced in MSCs-derived EVs, which increased in hyperoxic rats compared to the normoxic group. EVs also prevented the reduction in CD163-positive macrophages in both interstitial/alveolar and perivascular populations, which was instead induced in hyperoxic conditions. Intratracheal administration appears to be intriguing for translation in the future, probably due to its capacity to directly target organs for treatment. These data support the use of MSCs-derived EVs as a cell-free approach to improve altered alveolarization and remodeling of the pulmonary artery even after long-term treatment in preterm-born human infants [153]. These findings suggested that most of MSCs’ beneficial effects are mediated by paracrine signaling, pointing to the feasibility of using MSC secretome as a therapeutic tool. However, both the toxicity and biodistribution of MSCs-derived EVs need further investigation in animal models before they can be used as first-in-human patients.

The paracrine effects of MSCs are mediated by the release of immunomodulatory and growth factors that have been identified in the CM [154]. In this regard, Hansmann et al. [155] evaluated the effects of the CM in BPD management. In order to induce the BPD pattern, newborn FVB mice were exposed to 75% O₂ for 14 days, while a control group was kept in normoxic conditions. After 14 days of exposure to hyperoxic conditions, the animals were treated with a dose of BM-MSCs-derived CM isolated from the femurs and tibias of FVB mice, at a concentration of 10 µg of BM-MSCs-derived CM protein per mouse. A group of animals subjected to hyperoxic conditions, instead, was treated with mouse lung fibroblasts (MLF)-derived CM as a control group. CM was administered intravenously either through the superficial temporal vein or the jugular vein. Compared to treatment with MLF-derived CM, the administration of BM-MSCs-derived CM improved the hyperoxia-induced pathogenesis of BPD. BM-MSCs-derived CM reduced alveolar damage, septal thickening, and myofibroblast infiltration, and improved lung function. Treatment with BM-MSCs-derived CM attenuated pulmonary hypertension, right ventricular hypertrophy, and peripheral pulmonary artery pressure muscularization related to hyperoxia-induced BPD. Furthermore, pulmonary artery pruning lead by hyperoxia was ameliorated by a single intravenous dose of MSC-derived CM, highlighting the angiogenic and vasculogenic effect of MSC-derived CM. Therefore, the use of CM derived from MSCs could be a valid therapeutic option for BPD and other chronic lung diseases; however, future studies will be needed to understand the mechanisms underlying their beneficial effects [155].

The results of all these studies (Table 4) showed the beneficial effects MSCs transplantation in tissue recovery and lung function following pediatric fibrotic lung disease. These findings also highlight that MSCs’ beneficial effects are mediated, at least in part, by paracrine signaling, demonstrating the feasibility of using MSC secretome as a therapeutic tool. However, further preclinical studies are required to evaluate the long-term safety of systemic transplantation with MSCs and the toxicity and biodistribution of MSCs secretome before it can be used in humans, especially when considered for pediatric use.
Table 4. Synthesis of the studies that evaluate the role of MSCs in several animal models of pediatric pulmonary fibrosis (PF).

| In Vivo Models | Cell Therapy | Dose | Route of Administration | Intervention | Results | Ref. |
|---------------|--------------|------|-------------------------|--------------|---------|------|
| Newborn Sprague-Dawley rats | Human umbilical cord Wharton’s Jelly-derived MSCs transplantation | $5 \times 10^5$ cells/kg | Intranasal delivery | Multiple administration on days 4, 10, and 20, after 3 weeks from induction | Improvement of alveolarization, vascularization, and pulmonary remodeling | [152] |
| Severe combined immunodeficiency–beige mice | hUC-MSCs transplantation | 0.1, 0.5, or $1 \times 10^6$ cells/kg | Intranasal or intraperitoneal administration | Single dose on postnatal day 5, during induction | Recovery of lung compliance, pressure-volume loop and elastance, Enhancement of the thickness of the alveolar septum | [149] |
| Wild-type Sprague-Dawley rats | MSCs-derived EVs or MSCs administration | $0.64 \times 10^{10}$ particles | Intratracheal delivery | Multiple administration on days 3, 7, and 10 after induction | Enhancement of the number of alveoli, alveolar surface area, and proliferation index, Decrease of the mean alveolar volume, mean linear intercept, and fibrosis was decreased, Reduction the medial thickness index for vessels | [153] |
| Mouse pups FVB | BM-MSCs-derived CM or MLF-derived CM | CM containing 10 µg protein | Intravenous administration | A single dose after 14 days of induction | Reduction of the alveolar damage, septal thickening, myofibroblast infiltration, Improvement of the lung function, Reduction of the pulmonary hypertension, right ventricular hypertrophy, and peripheral pulmonary artery pressure muscularization | [155] |

AD-MSCs—adipose-derived mesenchymal stem cells; TNF-α—tumor necrosis factor-alpha; IL—interleukin; Bax—B-cell lymphoma protein 2-associated X; Bcl-2—B-cell lymphoma protein 2; LSCs—lung spheroid cells; BPD—bronchopulmonary dysplasia; MSCs—mesenchymal stromal cells; hUC-MSCs—human umbilical cord-derived MSCs; BM-MSCs—bone-marrow-derived mesenchymal stem cells; EVs—extracellular vesicles; CM—conditioned medium; MLF—mouse lung fibroblasts; PF—pulmonary fibrosis.
6.2. Clinical Studies

Recent evidence showed that MSCs represent a valid therapeutic approach for the treatment of pulmonary diseases in adult patients, including chronic obstructive pulmonary disease, silicosis, acute respiratory distress, and idiopathic pulmonary fibrosis [146,156–159]. To date, in the pediatric population, the therapeutic effects of MSCs on respiratory diseases were evaluated in BPD that may be considered as potential model of interstitial diseases and thus discussed.

6.2.1. Phase 1 Clinical Trials

Chang et al. [160] in a phase I clinical trial (NCT01297205), evaluated the safety, feasibility, and efficacy of the transplant of human umbilical cord blood derived MSCs (hUCB-MSCs) in premature BPD children. In the study, the recruited nine infants of gestational age 24–26 weeks (extremely premature) and at high risk of developing BPD, with damaged respiratory conditions and on ventilatory support. Three infants received a low dose of hUCB-MSCs ($1 \times 10^7$ cells/kg), and six received high-dose hUCB-MSCs ($2 \times 10^7$ cells/kg). Cells were intratracheally delivered into the left and right lungs. Cell transplantation was found to be safe: neither serious adverse effects nor dose-limiting toxicity up to 84 days following transplantation were observed. Only three out of nine children developed moderate BPD, highlighting the beneficial effects of cell transplantation. hUCB-MSCs transplantation also reduced the duration of intubation, the mean respiratory index 3 days after transplantation, steroid use, and positive airway pressure. Additionally, compared to baseline or day 3 after cell infusion, at day 7 in tracheal aspirates, treatment reduced levels of IL-1, IL-6, IL-8, IL-10, matrix metalloproteinase (MMP)-9, TGF-β, and TNF-α. Therefore, the data from this study demonstrated that intratracheal hUCB-MSCs delivery may be safe and feasible in preterm infants, and may be effective in reducing BPD severity [160].

A long-term follow-up study is underway (NCT01632475) to evaluate the safety and feasibility of the long-term treatment with these cells of premature infants with BPD enrolled in the previous trial. The primary aim of the study led by Ahn et al. [161] is to evaluate the long-term safety of MSC transplantation in infants up to 24 months of age. Only one in nine children died six months after treatment, but this adverse event was not related to MSC transplantation. None of the remaining infants experienced treatment-related adverse events. Therefore, intratracheal transplantation with hUCB-MSCs would appear to be safe in premature infants at high risk of developing BPD up to 2 years of age. Compared to the comparison group, infants treated with hUCB-MSCs no longer needed supplemental oxygen. After 18–24 months, the infants demonstrated weight gain that could be related to better long-term neurodevelopmental outcomes [161].

6.2.2. Phase 2 Clinical Trials

In order to further evaluate the efficacy and safety of stem cell therapy for BPD, a long-term clinical trial is underway for children up to the age of 5 years (NCT02023788) for patients who completed the earlier part of the previous phase I clinical trial (NCT01632475). Ahn et al. [162] performed a larger, double-blind, randomized, phase II clinical trial aimed at evaluating the therapeutic efficacy of transplantation with hUCB-MSCs (NCT01828957). The study enrolled 60 extremely premature infants, aged between 23 and 28 gestational weeks, with BPD. In the experimental group, infants received hUCB-MSCs in step 6 ($1 \times 10^7$ cells/kg), administered intratracheally via a gavage tube in two fractions in the left and right lungs. The placebo group only received an equal volume of saline. Treatment with hUCB-MSCs reduced levels of IL-1β, IL-6, IL-8, TNF-α, and MMP-9 at day 7 compared to the control group. However, the treatment did not improve the death outcome or disease progression. Treatment with hUCB-MSCs improved the outcome of severe BPD from 53% to 19% in the 23–24 gestational weeks subgroup of infants. Conversely, the severity of the disease was not improved in the subgroup of patients aged from 25 to 28 gestational weeks. Therefore, these data showed that hUCB-MSCs transplantation is effective and feasible;
however, the small number of samples does not allow establishing the efficacy in preterm infants aged from 23 to 28 gestational weeks [162]. Therefore, a further larger phase II study is underway recruiting infants aged from 23 to 24 gestational weeks (NCT03392467). Sixty premature infants within 13 days of postnatal age were recruited in the trial. The experimental group will receive hUCB-MSCs ($1 \times 10^7$ cells/kg), while one group will receive a saline solution (control group). The results of this study will help to understand the efficacy and safety of hUCB-MSCs for the treatment and prevention of severe BPD in premature infants. The subjects who passed phase II of the trial (NCT01828957) were recruited into the follow-up study, which aims to monitor participants up to the age of 5 years (NCT01897987). The main trial aim is to evaluate the respiratory outcome following transplantation with hUCB-MSCs compared to the control group.

Another open-label dose-escalation trial (phase I/II), led by Powell et al. [163] aimed to investigate the safety of single-dose intratracheal administration of hUCB-MSCs in extremely low birth weight preterm infants with BPD (NCT02381366). The study enrolled 12 premature infants born at 23–27 weeks of gestation with a high risk of BPD. One group of infants received hUCB-MSCs at the lowest dose ($1 \times 10^7$ cells/kg), and the other group at the highest dose ($2 \times 10^7$ cells/kg). The administration of hUCB-MSCs, at both doses, was well-tolerated by all patients with no signs of toxicity during the 72 h of observation. No serious adverse events were recorded during the 84 days of the study. Therefore, treatment with hUCB-MSCs proved to be safe and feasible [163].

Based on these promising results, Wu et al. [9] performed a phase II study, with respective control groups, to further investigate the safety and efficacy of the use of allogeneic hUCB-MSCs in children with severe BPD (NCT03601416). The study recruited 72 children up to the age 1 year with moderate or severe BPD undergoing traditional supportive treatments. Participants were treated intravenously with low-dose ($2.5 \times 10^6$ cells/kg) or high-dose ($5 \times 10^6$ cells/kg) hUCB-MSCs. The end date of the trial is expected to be December 2021. The study results will help prove the long-term safety and efficacy of hUCB-MSCs. They will also allow the ability of treatment to improve lung structure impairment by exploring its potential therapeutic use in the management of severe childhood BPD [9].

### 6.2.3. Active Clinical Trials

Currently, 13 additional clinical trials of MSCs therapy for BPD have been registered with ClinicalTrials.gov. Among them, five phase I studies (NCT02443961, NCT03631420, NCT01207869, NCT04255147, and NCT03683953) are active in not-recruiting status. All five registered clinical trials involve the administration of MSC via intratracheal or intravenous routes at doses ranging from 1 to $30 \times 10^6$ cells/kg of body weight in children (1–37 weeks) at high risk of BPD. Seven phase I/II studies (NCT03774537, NCT03558334, NCT03873506, NCT04003857, NCT03378063, NCT04062136, and NCT03645525) are in recruiting status. These trials foresee the administration of hUCB-MSCs by an intratracheal or intravenous route at doses ranging from $1 \times 10^6$ to $2 \times 10^7$ cells/kg of body weight in children (from 3 days to 5 years of age) at high risk of BPD. The study results will better assess the safety, feasibility, and efficacy of MSCs for the prevention and treatment of premature infants at high risk of BPD.

### 6.2.4. Terminated Clinical Trial

A placebo-controlled trial (NCT03857841) intended to evaluate the safety of intravenous infusion of BM-MSCs-derived EVs (UNEX-42) in preterm neonates (from 3 to 14 days postnatal) at high risk of BPD. The BM-MSCs-derived EVs were administered at doses of 20, 60, or 200 pmol phospholipid/kg body weight. The results of this study will be useful for understanding the safety and tolerability of BM-MSCs-derived EVs up to 10 months after treatment. Furthermore, at the end of this post-treatment phase, patients will be included in the next phase and will be monitored until they reach 1 year of age to also evaluate the long-term effects of pediatric treatment with cell derivatives.
6.2.5. Clinical Trial in Child with Chronic Respiratory Failure

In a brief report, Calcaterra et al. [164] isolated MSCs from the lung tissue of a male infant presumed to have congenital lobar emphysema and filamin A (FLNA) gene mutation. MSCs isolated from this child’s lung tissue exhibited the same characteristics as MSCs and the ability to differentiate into osteoblasts. Instead, these MSCs, compared to the control BM-MSCs, showed low migration capacity, which could be linked to FLNA deficiency. Therefore, these data highlight the important role that may have been played by dysfunctional lung MSCs in impaired lung development and in the formation of emphysematous lesions. In addition, they may also have been responsible for the altered matrix remodeling that induced progressive fibrotic lung disease [164]. Based on this evidence, in a clinical study performed by Pelizzo et al. [11], the effects of repeated intravenous administration of allogeneic BM-MSCs were evaluated in a child with progressive obstructive pulmonary disease associated with an FLNA gene mutation [11]. The mutation in this gene was recently associated with lung growth abnormalities, which, in several cases, progress to interstitial lung disease [165]. In this work, a 32-day old male child was hospitalized with respiratory distress and suspected congenital lung malformation. He underwent lobar resection of the damaged lung segments, noninvasive mechanical ventilation at 11 months, followed by a tracheostomy after 1 month. Therefore, at the age of 18 months, due to severe and irreversible chronic respiratory failure, the child underwent salvage therapy with allogeneic BM-MSCs. The allogeneic BM-MSCs were isolated and expanded ex vivo from healthy donor bone marrow. The child received four infusions of MSCs (1 × 10^6 cells/kg) intravenously 4 weeks apart. Before each infusion and 1 month after the last one, peripheral blood and mononuclear cells were collected. Treatment with allogeneic MSCs greatly improved the child’s respiratory condition. Furthermore, the positive effects observed after the second dose suggested the need for serial administrations rather than single injections. The mechanisms used by MSCs to exert these beneficial effects are, at least in part, mediated by paracrine immunomodulatory capacity on the recipient lung tissue. After the second MSCs infusion, decreased levels of Th17 and the consequent normalization of the T_{reg}/Th17 balance were observed, which is usually dysregulated in various lung diseases. The increase in PHA-induced PBMC proliferation after injections of MSCs and the increase in the percentages of B lymphocytes support the involvement of MSCs in immune-mediated processes. The data from this work also showed that intravenous administration was well-tolerated: no serious adverse events were reported in the child. Therefore, the intravenous route of administration, taking advantage of the pulmonary first-pass effect that characterizes MSCs, could represent the optimal route of administration in lung diseases’ treatment. In conclusion, this clinical study supports the use of serial infusions of MSCs in the pediatric treatment of mutated FLNA-associated respiratory failure [11].

In all studies, treatment with MSCs was authorized after receiving informed parental consent of enrolled children. The clinical trials (phases I/II) described above (Table 5) aim to show the safety and/or efficacy of MSC use as a therapeutic tool in pediatric BPD and chronic respiratory failure.
Table 5. Synthesis of the clinical trials of stem cell therapy in pediatric pulmonary diseases (https://clinicaltrials.gov/, accessed on 10 November 2021). The table shows the efficacy and safety of stem cell therapy in the management of BPD and mutated FLNA-associated respiratory failure.

| Identifier   | Phase          | Subjects                                                                 | Cells Therapy       | Route of Administration | Intervention/Treatment                  | Efficacy                                                                                      | Security                                                                                   | Ref. |
|--------------|----------------|--------------------------------------------------------------------------|---------------------|-------------------------|------------------------------------------|---------------------------------------------------------------------------------------------|---------------------------------------------|------|
| NCT01297205 | Phase 1 (completed) | 9 premature infants (up to 14 days) at high risk for BPD | hUCB-MSCs transplantation (PNEUMOSTEM) | Intratracheal delivery | $1 \times 10^7$ or $2 \times 10^7$ cells/kg | Improvement of the respiratory condition Reduction of IL-1, IL-6, IL-8, IL-10, MMP-9, TGF-β, and TNF-α levels, in tracheal aspirates | Well-tolerated and no serious adverse events | [160] |
| NCT01632475 | Phase 1 (active, not recruiting) | 9 premature infants (up to 14 days) at high risk for BPD | hUCB-MSCs transplantation (PNEUMOSTEM) | Intratracheal delivery | $1 \times 10^7$ or $2 \times 10^7$ cells/kg | Improvement of the respiratory condition Weight gain | No adverse events | [161] |
| NCT02023788 | Phase 1 (completed) | 8 premature infants (from 45 to 63 months) at high risk for BPD | hUCB-MSCs transplantation (PNEUMOSTEM) | Intratracheal delivery | $1 \times 10^7$ or $2 \times 10^7$ cells/kg | - | - | - |
| NCT01828957 | Phase 2 (completed) | 69 premature infants (up to 14 days) at high risk for BPD | hUCB-MSCs transplantation (PNEUMOSTEM) | Intratracheal delivery | $1 \times 10^7$ cells/kg | Decrease of the IL-1β, IL-6, IL-8, TNF-α, and MMP-9 levels. Improvement of the outcome of severe BPD | No adverse events | [162] |
| NCT03392467 | Phase 2 (recruiting) | 60 premature infants (up to 13 days) with severe BPD | hUCB-MSCs transplantation (PNEUMOSTEM) | Intratracheal delivery | $1 \times 10^7$ cells/kg | - | - | - |
| NCT01897987 | Phase 2 (completed) | 62 premature infants at high risk for BPD | hUCB-MSCs transplantation (PNEUMOSTEM) | Intratracheal delivery | $1 \times 10^7$ cells/kg | - | - | - |
| NCT02381366 | Phase 1/2 (completed) | 9 premature infants (up to 14 days) at high risk for BPD | hUCB-MSCs transplantation (PNEUMOSTEM) | Intratracheal delivery | $1 \times 10^7$ or $2 \times 10^7$ cells/kg | - | Well-tolerated without signs of toxicity No serious adverse events | [163] |
| Identifier   | Phase                      | Subjects                                                                 | Cells Therapy                  | Route of Administration         | Intervention/ Treatment                   | Efficacy | Security | Ref. |
|--------------|---------------------------|-------------------------------------------------------------------------|--------------------------------|----------------------------------|------------------------------------------|----------|----------|------|
| NCT03601416  | Phase 1 (not yet recruiting) | 72 children (up to 1 year) with moderate and severe BPD                 | Allogenic hUC-MSCs transplantation | Intravenous administration       | 2.5 $\times$ 10^6 or 5 $\times$ 10^6 cells/kg | -        | -        | [9]  |
| NCT02443961  | Phase 1 (active, not recruiting) | 10 preterm newborns (from 1 month to 28 weeks) at high risk of BPD      | MSCs transplantation             | -                                | 3 doses of 5 $\times$ 10^6 cells/kg      | -        | -        |      |
| NCT03631420  | Phase 1 (active, not recruiting) | 9 infants (up to 51 days) at high risk for BPD                          | hUC-MSCs transplantation        | -                                | 3 $\times$ 10^6, or 10 $\times$ 10^6, or 30 $\times$ 10^6 cells/kg | -        | -        |      |
| NCT01207869  | Phase 1 (active, not recruiting) | 9 extremely premature infants (up to 6 months) with severe BPD           | hUC-MSCs transplantation        | Intratracheal delivery           | 3 $\times$ 10^6 cells/kg                | -        | -        |      |
| NCT04255147  | Phase 1 (not yet recruiting) | 9 extremely premature infants (up to 21 days) at risk of BPD            | Allogeneic UC-MSCs transplantation | Intravenous administration       | 1 $\times$ 10^6, or 3 $\times$ 10^6, or 10 $\times$ 10^6 cells/kg | -        | -        |      |
| NCT03683953  | Phase 1 (not yet recruiting) | 200 infants (28–37 weeks) with BPD                                      | MSCs transplantation             | Intratracheal delivery           | 25 $\times$ 10^6 cells/kg, administrated on 14 days after birth | -        | -        |      |
| NCT03774537  | Phase 1/2 (recruiting)      | 20 preterm infants (up to 14 days) at high risk for BPD                 | hUC-MSCs transplantation        | Intravenous administration       | 1 $\times$ 10^6 or 5 $\times$ 10^6 cells/kg | -        | -        |      |
| NCT03558334  | Phase 1/2 (recruiting)      | 12 premature infants with moderate and severe BPD                       | hUC-MSCs transplantation        | Intravenous administration       | 1 $\times$ 10^6 or 5 $\times$ 10^6 cells/kg | -        | -        |      |
| NCT03873506  | Phase 1 (recruiting)        | 30 premature infants (from 1 month to 5 years) with moderate and severe BPD | hUC-MSCs transplantation        | Intravenous administration       | 1 $\times$ 10^6 or 5 $\times$ 10^6 cells/kg | -        | -        |      |
| Identifier | Phase | Subjects | Cells Therapy | Route of Administration | Intervention/ Treatment | Efficacy | Security | Ref. |
|------------|-------|----------|---------------|-------------------------|-------------------------|----------|----------|------|
| NCT04003857 | Phase 1 (recruiting) | 60 premature infants (6–60 months) with BPD | hUC-MSCs transplantation | Intratracheal delivery | $1 \times 10^7$ cells/kg | - | - | - |
| NCT03378063 | Early Phase 1 (recruiting) | 100 preterm infants (1–3 months) with BPD | hUCB-MSCs transplantation | - | - | - | - | - |
| NCT04062136 | Phase 1 (recruiting) | 10 infants (1–6 months) with BPD | hUC-MSCs transplantation | Intravenous administration | Two injections at a dose of $1 \times 10^6$ administered one week apart | - | - | - |
| NCT03645525 | Phase 1/2 (Recruiting) | 180 extremely preterm infants at high risk for BPD | hUC-MSCs transplantation | Intratracheal delivery | $2 \times 10^7$ cells/kg | - | - | - |
| NCT03857841 | Phase 1 (Terminated) | 3 preterm neonates (3–14 days) at high risk for BPD | BM-MSCs-derived EVs (UNEX-42) administration | Intravenous administration | 20, 60, or 200 pmol phospholipid/kg | - | - | - |
| - | - | 1 infants (32-day-old) with mutated FLNA-associated respiratory failure | Allogeneic BM-MSCs transplantation | Intravenous administration | 4 infusions of MSCs at $1 \times 10^6$ cells/kg dose, 4 weeks apart | Improvement of the respiratory condition. Reduction of the Th17 levels and normalization of the Treg/Th17 balance | Well-tolerated No serious adverse events | [11] |

BPD—bronchopulmonary dysplasia; MSCs—mesenchymal stromal cells; hUCB-MSCs—human umbilical cord blood-derived MSCs; IL—interleukin; MMP—matrix metalloproteinase; TGF-$\beta$1—transforming growth factor-beta 1; TNF-$\alpha$—tumor necrosis factor-alpha; hUC-MSCs—human umbilical cord-derived MSCs; hUCB-MSCs—human umbilical cord blood-derived MSCs; EVs—extracellular vesicles; FLNA—filamin A.
7. Future Perspectives

Repetitive micro injury to the AEC, which leads to aberrant repair during tissue regeneration, is a central paradigm of chILD [95]. Currently medical treatment of chILD is empirical with varying efficacy. Prospective therapies must be developed to ameliorate the prognosis of chILD. MSCs may be considered a promising cellular source to prevent disease progression or to revert established lung fibrosis, suppressing inflammation and supporting alveolar repair. The role of MSCs in the reparative and regenerative processes can also be considered. The plasticity of fibrosis [102] in the pediatric context of pulmonary disorders associated with fibrosis might represent an additional opportunity for cell therapy. A multidisciplinary team approach represents the standard of care for chILD diagnosis and management; it should become a core standard in post-operative treatment of cystic lesions and pneumothorax relapses in chronic pediatric lung disease. Combined treatment including medicaments, cell therapy, and surgery could offer a novel therapeutic approach in children, even in case of severe lung hypoplasia related to complex congenital malformations such as pulmonary malformations, congenital diaphragmatic hernia, or lung volume resection in FLNA gene mutations. The poor prognosis when prenatally diagnosed could be mitigated from birth by the use of combined therapy including surgery and stem cells with the aim of promoting lung growth.

8. Conclusions

Cellular therapy represents a potential treatment for chILD. Even though beneficial effects have been reported in clinical and preclinical studies, the optimal dosage, cell type, cell source, and route of administration have not yet been fully elucidated. Due to their tissue-regenerative and immunomodulatory properties, MSCs combined with other therapy or alone could be considered an innovative approach for the repair and regeneration of the lung during disease progression and/or as post-surgical lung support after pulmonary resection due to severe respiratory complications in chronic lung diseases.

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References

1. Samarelli, A.V.; Tonelli, R.; Marchioni, A.; Bruzzi, G.; Gozzi, F.; Andrisani, D.; Castaniere, I.; Manicardi, L.; Moretti, A.; Tabbi, L.; et al. Fibrotic idiopathic interstitial lung disease: The molecular and cellular key players. *Int. J. Mol. Sci.* 2021, 22, 8952. [CrossRef]

2. Brennan, L.C.; O’Sullivan, A.; MacLoughlin, R. Cellular therapy for the treatment of paediatric respiratory disease. *Int. J. Mol. Sci.* 2021, 22, 8906. [CrossRef] [PubMed]

3. Tong, Y.; Zuo, J.; Yue, D. Application prospects of mesenchymal stem cell therapy for bronchopulmonary dysplasia and the challenges encountered. *BioMed Res. Int.* 2021, 2021, 9983664. [CrossRef]

4. Chia, W.K.; Cheah, F.C.; Abdul Aziz, N.H.; Kampan, N.C.; Shuib, S.; Khong, T.Y.; Tan, G.C.; Wong, Y.P. A review of placenta and umbilical cord-derived stem cells and the immunomodulatory basis of their therapeutic potential in bronchopulmonary dysplasia. *Front. Pediatr.* 2021, 9, 61508. [CrossRef] [PubMed]

5. Hashemian, S.R.; Aliannejad, R.; Zarrabi, M.; Soleimani, M.; Vosough, M.; Hosseini, S.E.; Hossieni, H.; Keshel, S.H.; Naderpour, Z.; Hajizadeh-Saffar, E.; et al. Mesenchymal stem cells derived from perinatal tissues for treatment of critically ill covid-19-induced ards patients: A case series. *Stem Cell Res. Ther.* 2021, 12, 91. [CrossRef]
62. Kalra, H.; Drummen, G.P.; Mathivanan, S. Focus on extracellular vesicles: Introducing the next small big thing. *Int. J. Mol. Sci.* 2016, 17, 170. [CrossRef]

63. Mardpour, S.; Hamidieh, A.A.; Taleahmad, S.; Sharifzad, F.; Taghikhani, A.; Baharvand, H. Interaction between mesenchymal stromal cell-derived extracellular vesicles and immune cells by distinct protein content. *J. Cell. Physiol.* 2019, 234, 8249–8258. [CrossRef]

64. McKelvey, K.J.; Powell, K.L.; Ashton, A.W.; Morris, J.M.; McCracken, S.A. Exosomes: Mechanisms of uptake. *J. Circ. Biomark.* 2015, 4, 7. [CrossRef]

65. Gao, J.; Dennis, J.E.; Muzic, R.F.; Lundberg, M.; Caplan, A.I. The dynamic In Vivo distribution of bone marrow-derived extracellular vesicles. *Semin. Cell Dev. Biol.* 2015, 40, 82–88. [CrossRef]

66. Fierabracci, A.; Del Fattore, A.; Luciano, R.; Muraca, M.; Teti, A.; Muraca, M. Recent advances in mesenchymal stem cell immunomodulation: The role of microvesicles. *Cell Transplant.* 2015, 24, 133–149. [CrossRef] [PubMed]

67. Ragni, E.; Banfi, F.; Barilani, M.; Cherubini, A.; Parazzi, V.; Langhi, P.; Dolo, V.; Bollati, V.; Lazzari, L. Extracellular vesicle-shuttled mRNA in mesenchymal stem cell communication. *Stem Cells* 2017, 35, 1093–1105. [CrossRef]

68. Lai, R.C.; Yeo, R.W.; Lim, S.K. Mesenchymal stem cell exosomes. *Int. J. Mol. Sci.* 2015, 25, 134–149. [CrossRef] [PubMed]

69. Svetlana, S.; Chiricosta, L.; Gugliandolo, A.; Pizzicannella, J.; Diomede, F.; Bramanti, P.; Trubiani, O.; Mazzon, E. Extracellular vesicles derived from human gingival mesenchymal stem cells: A transcriptomic analysis. *Genes* 2020, 11, 118. [CrossRef]

70. Kawai, T.; Katagiri, W.; Osugi, M.; Sugimura, Y.; Hibi, H.; Ueda, M. Secretomes from bone marrow-derived mesenchymal stromal cells after infusion. *Stem Cells Transplant.* 2020, 5555–5569. [CrossRef] [PubMed]

71. Moreira, A.; Naqvi, R.; Hall, I.; Emukah, C.; Martinez, J.; Moreira, A.; Dittmar, E.; Zoretic, S.; Evans, M.; Moses, D.; et al. Effects of mesenchymal stromal cell-conditioned media on measures of lung structure and function: A systematic review and meta-analysis of preclinical studies. *Stem Cell Res. Ther.* 2020, 11, 399. [CrossRef] [PubMed]

72. Kawamura, R.; Hayashi, Y.; Murakami, H.; Nakashima, M. Edta soluble chemical components and the conditioned medium from mobilized dental pulp stem cells contain an inductive microenvironment, promoting cell proliferation, migration, and odontoblastic differentiation. *Stem Cell Res. Ther.* 2016, 7, 1–14. [CrossRef]

73. Murakami, M.; Hayashi, Y.; Iohara, K.; Osako, Y.; Hirose, Y.; Nakashima, M. Trophic effects and regenerative potential of mobilized mesenchymal stem cells from bone marrow and adipose tissue as alternative cell sources for pulp/dentin regeneration. *Cell Transplant.* 2015, 24, 1753–1765. [CrossRef] [PubMed]

74. Ionescu, L.; Byrne, R.N.; van Haaften, T.; Vadivel, A.; Alphonse, R.S.; Rey-Parra, G.J.; Weissmann, G.; Hall, A.; Eaton, F.; Thebaud, B. Stem cell-conditioned medium improves acute lung injury in mice: In Vivo evidence for stem cell paracrine action. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2015, 303, L967–L977. [CrossRef]

75. Rathinasabapathy, A.; Bruce, E.; Espejo, A.; Horowitz, A.; Sudhan, D.R.; Nair, A.; Guzzo, D.; Francis, J.; Raizada, M.K.; Shenoy, V.; et al. Therapeutic potential of adipose stem cell-derived conditioned medium against pulmonary hypertension and lung fibrosis. *Br. J. Pharmacol.* 2016, 173, 2859–2879. [CrossRef]

76. Kean, T.J.; Lin, P.; Caplan, A.I.; Dennis, J.E. Mscs: Delivery routes and engraftment, cell-targeting strategies, and immune modulation. *Stem Cells Transplant.* 2013, 2013, 1247927. [CrossRef] [PubMed]

77. Fischer, U.M.; Harting, M.T.; Jimenez, F.; Monzon-Posadas, W.O.; Xue, H.; Savitz, S.I.; Laine, G.A.; Cox, C.S.; Jr. Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first-pass effect. *Stem Cells Dev.* 2009, 18, 683–692. [CrossRef]

78. Gao, J.; Dennis, J.E.; Muzic, R.F.; Lundberg, M.; Caplan, A.I. The dynamic In Vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 2001, 169, 12–20. [CrossRef] [PubMed]

79. Schreper, S.; Deuse, T.; Reichenspurner, H.; Fischbein, M.P.; Robbins, R.C.; Pelletier, M.P. Stem cell transplantation: The lung barrier. *Transplant. Proc.* 2007, 39, 573–576. [CrossRef]

80. Durand, N.; Mallea, J.; Zubair, A.C. Insights into the use of mesenchymal stem cells in covid-19 mediated acute respiratory failure. *NPJ Regen. Med. Med.* 2020, 5, 17. [CrossRef]

81. Li, X.; An, G.; Wang, Y.; Liang, D.; Zhu, Z.; Tian, L. Targeted migration of bone marrow mesenchymal stem cells inhibits silica-induced pulmonary fibrosis in rats. *Stem Cell Res. Ther.* 2018, 9, 355. [CrossRef]
142. Fang, X.H.; Abbott, J.; Cheng, L.D.; Colby, J.K.; Lee, J.W.; Levy, B.D.; Matthay, M.A. Human mesenchymal stem (stromal) cells promote the resolution of acute lung injury in part through lipoxin a4. J. Immunol. 2015, 195, 875–881. [CrossRef]

143. Jun, D.; Garat, C.; West, J.; Thorn, N.; Chow, K.; Cleaver, T.; Sullivan, T.; Torchia, E.C.; Childs, C.; Shade, T.; et al. The pathology of bleomycin-induced fibrosis is associated with loss of resident lung mesenchymal stem cells that regulate effector t-cell proliferation. Stem Cells 2011, 29, 725–735. [CrossRef]

144. Mei, S.H.J.; Haitsma, J.J.; Dos Santos, C.C.; Deng, Y.P.; Lai, P.F.H.; Slutsky, A.S.; Liles, W.C.; Stewart, D.J. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. Am. J. Respir. Crit. Care Med. 2010, 182, 1047–1057. [CrossRef]

145. Balogh, E.; Nagy, B., Jr.; Gyetvai, A.; Bene, Z.; Hendrik, Z.; Jeney, V.; Nagy, P.; Papp, A.; Balla, J.; Balla, G.; et al. Impaired immunosuppressive effect of bronchoalveolar mesenchymal stem cells in hypersensitivity pneumonitis: Preliminary findings. Cytom. Part B Clin. Cytom. 2018, 94, 363–368. [CrossRef] [PubMed]

146. Geiger, S.; Hirsch, D.; Hermann, F.G. Cell therapy for lung disease. Eur. Respir. Rev. 2017, 26, 170044. [CrossRef]

147. Chen, S.; Cui, G.; Peng, C.; Lavin, M.F.; Sun, X.; Zhang, E.; Yang, Y.; Guan, Y.; Du, Z.; Shao, H. Transplantation of adipose-derived mesenchymal stem cells attenuates pulmonary fibrosis via anti-inflammatory and anti-apoptosis effects in rats. Stem Cell Res. Ther. 2018, 9, 110. [CrossRef]

148. Cores, J.; Hensley, M.T.; Kinlaw, K.; Rikard, S.M.; Dinh, P.U.; Paudel, D.; Tang, J.; Vandergriff, A.C.; Allen, T.A.; Li, Y.; et al. Safety and efficacy of allogeneic lung spherical cells in a murine model of pulmonary fibrosis. Stem Cells Transl. Med. 2017, 6, 1905–1916. [CrossRef] [PubMed]

149. Liu, L.; Mao, Q.; Chu, S.; Mounayar, M.; Abdi, R.; Fodor, W.; Padbury, J.F.; De Paepe, M.E. Intranasal versus intraperitoneal delivery of human umbilical cord tissue-derived cultured mesenchymal stromal cells in a murine model of neonatal lung injury. Am. J. Pathol. 2014, 184, 3344–3358. [CrossRef]

150. Mansouri, N.; Willis, G.R.; Fernandez-Gonzalez, A.; Reis, M.; Nassiri, S.; Mitsialis, S.A.; Kourembanas, S. Mesenchymal stromal cell exosomes prevent and revert experimental pulmonary fibrosis through modulation of monocyte phenotypes. JCI Insight 2019, 4, 4. [CrossRef] [PubMed]

151. Thebaud, B.; Abman, S.H. Bronchopulmonary dysplasia—Where have all the vessels gone? Roles of angiogenic growth factors in chronic lung disease. Am. J. Respir. Crit. Care Med. 2007, 175, 978–985. [CrossRef]

152. Moreira, A.; Winter, C.; Joy, J.; Winter, L.; Jones, M.; Noronha, M.; Porter, M.; Quin, K.; Corral, A.; Alayli, Y.; et al. Intranasal delivery of human umbilical cord tissue-derived cultured mesenchymal stromal cells restores lung alveolarization and vascularization in experimental bronchopulmonary dysplasia. Stem Cells Transl. Med. 2020, 9, 221–234. [CrossRef]

153. Porzionato, A.; Zaramella, P.; Dedja, A.; Guidolin, D.; Bonadies, L.; Macchi, V.; Pozzobon, M.; Jurga, M.; Perilongo, G.; De Caro, R.; et al. Intratracheal administration of mesenchymal stem cell-derived extracellular vesicles reduces lung injuries in a chronic rat model of bronchopulmonary dysplasia. Am. J. Physiol. Lung Cell. Mol. Physiol. 2021, 320, L688–L704. [CrossRef]

154. Aslam, M.; Baveja, R.; Liang, O.D.; Fernandez-Gonzalez, A.; Lee, C.; Mitsialis, S.A.; Kourembanas, S. Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease. Am. J. Respir. Crit. Care Med. 2009, 180, 1122–1130. [CrossRef]

155. Hansmann, G.; Fernandez-Gonzalez, A.; Aslam, M.; Vitali, S.H.; Martin, T.; Mitsialis, S.A.; Kourembanas, S. Mesenchymal stem cell-mediated reversal of bronchopulmonary dysplasia and associated pulmonary hypertension. Palm. Circ. 2012, 2, 170–181. [CrossRef]

156. Zhu, Y.; Chen, X.; Yang, X.; El-Hashash, A. Stem cells in lung repair and regeneration: Current applications and future promise. J. Cell. Physiol. 2018, 233, 6414–6424. [CrossRef] [PubMed]

157. Laffey, J.G.; Matthay, M.A. Fifty years of research in ards. Cell-based therapy for acute respiratory distress syndrome. Biology and potential therapeutic value. Am. J. Respir. Crit. Care Med. 2017, 196, 266–273. [CrossRef] [PubMed]

158. O’Reilly, M.; Thebaud, B. Cell-based therapies for neonatal lung disease. Cell Tissue Res. 2017, 367, 737–745. [CrossRef]

159. Kokturk, N.; Yildirim, F.; Gulhan, P.Y.; Oh, Y.M. Stem cell therapy in chronic obstructive pulmonary disease. How far is it to the clinic? Am. J. Stem Cells 2018, 7, 56–71. [PubMed]

160. Chang, Y.S.; Ahn, S.Y.; Yoo, H.S.; Sung, S.I.; Choi, S.J.; Oh, W.I.; Park, W.S. Mesenchymal stem cells for bronchopulmonary dysplasia: Phase 1 dose-escalation clinical trial. J. Pediatr. 2014, 164, 966–979.e966. [CrossRef]

161. Ahn, S.Y.; Chang, Y.S.; Kim, J.H.; Sung, S.I.; Park, W.S. Two-year follow-up outcomes of premature infants enrolled in the phase i trial of mesenchymal stem cells transplantation for bronchopulmonary dysplasia. J. Pediatr. 2017, 185, 49–54.e42. [CrossRef] [PubMed]

162. Ahn, S.Y.; Chang, Y.S.; Lee, M.H.; Sung, S.I.; Lee, B.S.; Kim, K.S.; Kim, A.R.; Park, W.S. Stem cells for bronchopulmonary dysplasia in preterm infants: A randomized controlled phase ii trial. Stem Cells Transl. Med. 2021, 10, 1129–1137. [CrossRef]

163. Powell, S.B.; Silvestri, J.M. Safety of intratracheal administration of human umbilical cord blood derived mesenchymal stromal cells in extremely low birth weight preterm infants. J. Pediatr. 2019, 210, 209–213.e202. [CrossRef] [PubMed]

164. Calcattera, V.; Avanzini, M.A.; Mantelli, M.; Agolini, E.; Croce, S.; De Silvestri, A.; Re, G.; Collura, M.; Maltese, A.; Novelli, A.; et al. A case report on filamin a gene mutation and progressive pulmonary disease in an infant: A lung tissue derived mesenchymal stem cell study. Medicine 2018, 97, e13033. [CrossRef] [PubMed]

165. Sasaki, E.; Byrne, A.T.; Phelan, E.; Cox, D.W.; Reardon, W. A review of filamin a mutations and associated interstitial lung disease. Eur. J. Pediatr. 2019, 178, 121–129. [CrossRef] [PubMed]