Proceedings of the ISCT scientific signature series symposium, “Advances in cell and gene therapies for lung diseases and critical illnesses”

Ting, Anthony E.; Baker, Elizabeth K.; Champagne, Josee; Desai, Tushar J.; dos Santos, Claudia C.; Heijink, Irene H.; Itescu, Silviu; Blanc, Katarina Le; Matthay, Michael A.; McAuley, Daniel F.

Published in:
Cytotherapy

DOI:
10.1016/j.jcyt.2021.11.007

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher’s PDF, also known as Version of record

Publication date:
2022

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Review article

Proceedings of the ISCT scientific signature series symposium, “Advances in cell and gene therapies for lung diseases and critical illnesses”

International Society for Cell & Gene Therapy, Burlington VT, US, July 16, 2021

Anthony E. Ting1, Elizabeth K. Baker2, Josee Champagne3, Tushar J. Desai4, Claudia C. dos Santos5, Irene H. Heijink6, Silviu Itescu7, Katarina Le Blanc8,9, Michael A. Matthay10, Daniel F. McAuley11, Lauralynn McIntyre12, Shirley H.J. Mei13, Biju Parekkadan14,15, Patricia R.M. Rocco16, John Sheridan17, Bernard Thébaud18, Daniel J. Weiss19,*

1 Bone Therapeutics, Gosselies, Belgium
2 Newborn Research Centre, Royal Women’s Hospital, Melbourne, Victoria, Australia
3 Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
4 Stanford University School of Medicine, Stanford, California, USA
5 Interdepartmental Division of Critical Care, Department of Medicine and the Keenan Center for Biomedical Research, St. Michael’s Hospital, University of Toronto, Toronto, Canada
6 Medical Center Groningen, Department of Pathology and Medical Biology, University of Groningen, Groningen, the Netherlands
7 Mesoblast, Melbourne, Australia
8 Department of Laboratory Medicine, Karolinska Institutet, Sweden
9 Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Stockholm, Sweden
10 University of San Francisco, San Francisco, San Francisco, California, United States
11 Wellcome-Wolfson Institute for Experimental Medicine, Queen’s University Belfast, NI, UK
12 Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
13 Sinclair Centre for Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
14 Sentien Biotechnologies, Lexington, Massachusetts, USA
15 Rutgers University, Piscataway, New Jersey, USA
16 Laboratory of Pulmonary Investigation, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
17 Cystic Fibrosis Foundation, Bethesda, Maryland, USA
18 Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
19 University of Vermont College of Medicine, Burlington, Vermont, USA

ARTICLE INFO

Article History:
Received 2 November 2021
Accepted 5 November 2021

Keywords:
Cell therapy
Critical illness
Gene therapy
Lung disease
Mesenchymal stromal cell

ABSTRACT

The ISCT Scientific Signature Series Symposium “Advances in Cell and Gene Therapies for Lung Diseases and Critical Illnesses” was held as an independent symposium in conjunction with the biennial meeting, “Stem Cells, Cell Therapies, and Bioengineering in Lung Biology and Diseases,” which took place July 12–15, 2021, at the University of Vermont. This is the third Respiratory System–based Signature Series event; the first 2, “Tracheal Bioengineering, the Next Steps” and “Cellular Therapies for Pulmonary Diseases and Critical Illnesses: State of the Art of European Science,” took place in 2014 and 2015, respectively. Cell- and gene-based therapies for respiratory diseases and critical illnesses continue to be a source of great promise and opportunity. This reflects ongoing advancements in understanding of the mechanisms by which cell-based therapies, particularly those using mesenchymal stromal cells (MSCs), can mitigate different lung injuries and the increasing sophistication with which preclinical data is translated into clinical investigations. This also reflects continuing evolution in gene transfer vectors, including those designed for in situ gene editing in parallel with those targeting gene or cell replacement. Therefore, this symposium convened global thought leaders in a forum designed to catalyze communication and collaboration to bring the greatest possible innovation and value of cell- and gene-based therapies for patients with respiratory diseases and critical illnesses.

© 2022 International Society for Cell & Gene Therapy. Published by Elsevier Inc. All rights reserved.
Introduction

Rapid developments in cell and gene therapy for pulmonary diseases and critical illnesses require continuous updates for experts in the field and other interested parties. Real-time updates are even more critical in the context of the COVID-19 global pandemic, where a significant percentage of patients in intensive care units are suffering from acute respiratory failure. Thus, the International Society for Cell and Gene Therapy (ISCT) commissioned a Scientific Signature Series Symposium entitled “Advances in Cell and Gene Therapies for Lung Diseases and Critical Illnesses,” which was held as an independent symposium in conjunction with the biennial meeting, “Stem Cells, Cell Therapies, and Bioengineering in Lung Biology and Diseases,” which took place July 12–15, 2021, at the University of Vermont. This is the third Respiratory System–based ISCT Scientific Signature Series event; the first 2, “Tracheal Bioengineering, the Next Steps” and “Cellular Therapies for Pulmonary Diseases and Critical Illnesses: State of the Art of European Science,” took place in 2014 and 2015, respectively [1,2].

The meeting kicked off with a warm welcome from ISCT President Bruce Levine, who provided a background of ISCT and how the society supports the field of cell and gene therapy. Daniel J. Weiss and Anthony Ting, Chief Scientific and Commercialization Officers for ISCT, respectively, co-chaired the meeting. The morning session was focused on the basic mechanistic and translation studies of cell and gene therapies, while the afternoon session was focused on clinical trials using cell therapy and ended with a lively discussion around recent trials for the treatment of patients with COVID-19 respiratory disease.

Significant lung damage accompanies many lung diseases including chronic obstructive pulmonary disease (COPD), lung fibrosis including idiopathic pulmonary fibrosis (IPF), bronchopulmonary dysplasia (BPD), pulmonary hypertension, cystic fibrosis (CF), acute respiratory distress syndrome (ARDS), and viral infections such as COVID-19. These diseases are in urgent need of novel regenerative treatment strategies, including treatment with mesenchymal stem cells (MSCs). There are several conceivable approaches, each being intensively investigated, including but not limited to immunomodulation for inflammatory diseases, in situ gene editing, in situ cell replacement strategies, and ex vivo lung bioengineering. These are of significant importance given the prevalence of respiratory diseases and critical illnesses and the lack of curative therapies for many of these. For example, according to the World Health Organization, COPD is currently the third leading cause of death, and its prevalence will continue to increase with the aging population [3]. While other diseases such as ARDS, IPF, BPD, and CF are less prevalent, the absence of curative therapies enhances the urgency of new regenerative medicine–based approaches.

There is an extensive preclinical literature investigating cell- and gene-based therapies in models of pulmonary diseases and critical illnesses. These have both demonstrated efficacy of a variety of approaches and provided evolving mechanistic information on how cell- or gene-based therapies can be best used [4,5]. These have provided a platform for a growing number of clinical investigations, particularly those using MSCs. This has accelerated during the COVID-19 pandemic, in which >50 clinical investigations involving predominantly MSC administration occurred or are currently taking place [6].

As such, the goal of the Signature Series symposium was to convene global leaders in academia, respiratory disease foundations, and industry to extensively discuss and debate recent advances in cell- and gene-based therapies for respiratory diseases and critical illnesses and to devise continuing plans and collaborative efforts for further advances. A summary of each presentation is given below, followed by a summary of the overall discussion and recommendations for future progress.

Endogenous lung MSCs and their therapeutic potential in lung disease, with a focus on COPD

Irene H. Heijink PhD, University Medical Center Groningen, The Netherlands

Heijink started off the session with an introduction into the biology of MSCs and their history in clinical trials. Their beneficial effects have been attributed mainly to paracrine mechanisms, secreting trophic factors, immunomodulatory factors (including microRNAs, aka miRNAs), anti-apoptotic and anti-fibrotic factors [7], and being able to donate their mitochondria to damaged epithelial cells [8]. However, to improve the effectiveness of MSC-based strategies in lung disease, several hurdles and vital questions need to be addressed, e.g., the optimal source, administration route, and retention time of MSCs in the lungs. Limited effectiveness of MSCs in clinical trials may be due to the short retention time of IV injected MSCs in the lung, as demonstrated in an animal model of emphysema [9]. Preclinical studies comparing MSCs from different sources and administration routes have shown that intratracheally injected MSCs may be retained longer in the lungs than IV injected MSCs. Accordingly, intratracheal administration induced a further reduction of hyperinflation compared with IV administration [10]. As such, there are many unanswered questions with respect to use of MSCs from sources such as bone marrow, adipose tissue, placenta, and elsewhere for use in lung diseases and critical illnesses.

Heijink then discussed the role of endogenous lung MSCs (LMSCs). In healthy lungs, stromal cells within the microenvironment, the niche, are vital for regenerative responses. Stromal cells support activation and differentiation of alveolar epithelial stem/progenitor cells and produce ECM components to maintain the alveolar architecture and regulate adequate repair mechanisms. LMSCs are increasingly recognized as a key stromal element in the lung microenvironment. Existing both as pericytes and as stromal elements, LMSCs share some but not all characteristics with MSCs from other sources [11]. For example, recent transcriptomic profiling has shown that in comparison to human bone marrow (BM)- or adipose tissue (AD)-derived MSCs, LMSCs specifically express high levels of fibroblast growth factor (FGF)10 and hepatocyte growth factor (HGF), while other factors such as FGF7, vascular endothelial growth factor (VEGF), and various extracellular matrix (ECM) genes were more highly expressed in BM-MSCs or AD-MCs [12]. The specific gene signature for LMSCs includes genes encoding transcription factors that regulate epithelial-mesenchymal cross talk during lung development and repair, such as the sonic hedgehog pathway. Of note, when comparing LMSCs from COPD and non–COPD donors, Heijink has observed that LMSCs were specifically compromised in the expression of the ECM molecule decorin and growth factors FGF10 and HGF [12].

Together, these findings suggest that LMSC release of FGF10 and HGF may be critical factors for lung repair and that defective production of these factors may contribute to failing lung tissue repair in COPD. Indeed, it has been reported that MSCs can promote lung tissue regeneration via HGF-stimulated proliferation of alveolar epithelial progenitors and that both HGF and FGF10 mediate alveolar epithelial cell proliferation and repair [13–16]. Further, Wang et al. [17] showed that HGF increases alveolar epithelial organoid formation in vitro. Here, HGF was regulated by Hedgehog (Hh) signaling, which was shown to be mainly active in proximal cells, but with elevated activity in distal epithelial cells in a mouse model of emphysema. Ectopic Hh signaling resulted in lower HGF release in the distal airways and loss of distal alveoli and airspace enlargement. Of interest, FGF10 expression is also regulated by Hh signaling, and the gene encoding for Hh interaction protein (HHIP), an antagonist of the Hh pathway, is a susceptibility gene for COPD [18]. This may, at least in part, explain the intrinsic defects observed in COPD-derived LMSCs [12]. In line with this, a CD90+/CD146+ mesenchymal cell subtype was found to be localized in close proximity to EpCAM+ cells in the...
alveolar region that exerts an immune modulatory and perivascular function in the human lung. These cells supported alveolar organoid formation of EpCAM\textsuperscript{+} cells and microvessel self-assembly, but cells from COPD patients failed to support this and displayed downregulated expression of FGF10, GLI1 and PDLP1 [19].

Based on their in vitro properties, exogenously added LMSCs are expected to have beneficial effects for distal lung tissue repair. For example, IV injected LMSCs may be retained longer in the lungs than IV injected BM-MSCs because of LMSC-specific adhesion molecules [20]. Whereas BM-MSCs were superior in reducing systemic effects, including better cardiovascular function, adipose-derived MSCs (AD-MSCs) and LMSCs each induced a larger reduction in fractional area of alveolar collapse [10]. In addition, conditioned medium from LMSCs reduced oxidative stress—induced cell death and improved recovery from electric field or scratch wounding—induced injury in human alveolar epithelial cell lines. Furthermore, Heijink has observed that LMSCs are able to transfer mitochondria to alveolar cells using LMSCs expressing a mitochondrial-localization construct.

However, the LMSC field is still evolving, including identification of possible multiple different LMSC subtypes. For example, while endogenous MSCs isolated from distal lung tissue ubiquitously express surface markers that define multipotent MSCs [11], heterogeneity exists between cultures from different lung tissue donors with respect to both clonogenic and differentiation potential [12]. Additionally, heterogeneity is likely to exist within cultures from the same lung tissue, and LMSCs from different alveolar niches may have specialized roles in lung regenerative responses. More insight into LMSC subtypes and cellular states, as obtained by single-cell sequencing studies, will further guide the use in clinical trials. LMSC expansion in vitro is indispensable to obtain sufficient cells for transplantation in patients, but may alter the phenotype of cells. For the use of autologous LMSCs, it is important to obtain sufficient volume of lung tissue for LMSC isolation. In patients undergoing lung volume reduction surgery, resected tissue may be a potential source, but its use will be limited to these patients, and treatment will be applicable only months after the surgery. Whether LMSCs obtained from bronchial biopsies are well equipped to support distal lung repair and can be expanded to obtain sufficient cell numbers needs further investigation. In addition, when considering the use of autologous MSCs, it will be important to consider the abnormalities in COPD-derived LMSCs [12], which may be even more prominent in MSCs derived from extrapulmonary sources.

Strategies to optimize paracrine support for regenerative responses of autologous LMSCs in COPD patients can be considered: for instance, genetic manipulation, in vitro preconditioning, or the use of bioactive scaffolds [21]. It is tempting to speculate that culturing in a 3-dimensional microenvironment mimicking the distal human lung can direct the expression of growth factors in MSCs from different sources toward a lung-specialized profile. Whether a diseased or healthy environment would optimally prepare MSCs for lung repair needs further investigation. Furthermore, in future strategies, the exceedingly small retention window of MSCs should be taken into account, as MSCs may poorly adhere to affected lungs with damaged ECM structure, and cells may migrate away from the damaged alveolar tissue and may be cleared via macrophage phagocytosis. Here, the use of active bio-scaffolds may also offer a solution, using hydrogels with protective and supportive action to encapsulate the cells. A first-of-its-kind technology with potential to prolong MSCs’ residence time in the lung has been developed recently [22] upon endobronchial delivery of MSCs, and a similar strategy has been successfully used in a preclinical study to reduce fibrotic lung injury [9] and enhance retention of IV injected MSCs. Such strategies may hold great promise for future cell-based strategies to treat lung damage in different disease using either autologous or allogenic LMSCs.

Structural/functional engraftment of iPSCs and lung progenitor cells

Tushar J. Desai, MD, Stanford University

There is a long history of attempting to engraft exogenous cells into lungs to replace dysfunctional resident epithelial, vascular, or stromal cells. These attempts evolved in large part from an initial report in 2001 in which bone marrow—derived cells were found to have ostensibly engrafted as airway epithelial cells following systemic administration [23]. Multiple subsequent investigations followed in which different populations of bone marrow—derived cells were investigated. However, most of the findings of these reports were felt to represent technical artifacts with respect to immunohistochemical and/or microscopy techniques used [24]. In those few studies in which ostensibly engraftment was found, the number of cells engrafted was too small to have any meaningful physiologic or clinical significance [25,26]. However, there has been a recent resurgence of interest in cell engraftment with focus on that involving exogenous administration of either endogenous lung progenitor or induced pluripotent stem cell (iPSC)-derived lung progenitor cells. This is yielding more promising results than did the original studies using bone marrow—derived cells and has provided a platform for considering clinical investigations administering endogenous lung progenitors, iPSC-derived lung progenitors, or even differentiated lung cells, for example, type 2 alveolar epithelial cells [27,28,29]. This is of particular interest for patients with COPD or CF.

In this setting, Desai presented work from his multidisciplinary group at Stanford University Medical School working toward transplantation of gene-corrected upper airway basal epithelial progenitor cells as a durable treatment for sinus disease resulting from monogenic mutations such as CF and primary ciliary dyskinesia (PCD). Their group has determined that upper airway basal cells express telomerase reverse transcriptase (hTERT), suggesting that endogenous adult basal stem cells may have the capacity to maintain telomere length over multiple divisions. They developed highly efficient CRISPR targeting for correction of the common delta F508 mutation as well as a universal approach that can correct all mutations [30]. Gene-corrected human basal cells demonstrate appropriate multilineage differentiation in air–liquid interface (ALI) culture and appear to be no more or less proliferative in vitro than uncorrected basal cells [30]. Currently, the group is testing different biomaterials in which the gene-corrected cells can be surgically delivered to achieve efficient engraftment in the sinuses of immunodeficient rodents. Initial results of transplanting luciferase and green fluorescent protein (GFP)-expressing mouse basal cells into mouse sinuses have demonstrated durable (>190 days) and stable engraftment with differentiation and integration into the recipient epithelial monolayer. Corresponding human-to-mouse transplants are currently underway. If successful, autologous basal epithelial stem cell transplantation therapy may be valuable for chronic sinusitis in CF patients who have mutations not amenable to CFTR modulators and as an adjunct after lung transplantation if modulators are no longer indicated for extrapulmonary disease [31]. In the future, a parallel approach may be developed for treatment of chronic sinusitis in PCD patients, and perhaps even for bronchial Airways in CF if a safe and effective transplantation approach can be developed.

Mechanisms of MSC actions in vivo

Katarina Le Blanc MD, Karolinska Institute, Stockholm, Sweden

Le Blanc discussed the growing appreciation of mechanisms by which MSCs provide therapeutic benefit. This includes release of paracrine mediators by MSCs but also effects of the host immune system on the MSCs that subsequently alter inflammatory and immune pathways. For example, it has recently been demonstrated that after IV infusion, MSCs lodged in the pulmonary vasculature are phagocytosed by immune cells, which triggers a cascade of events with...
monocyte priming and induction of T regulatory cells [32]. Additionally, cytotoxic cells can lyse MSCs, and the phagocytes that engulf membrane particles start secreting anti-inflammatory factors. Notably, high cytotoxic activity against infused MSCs correlates with responsiveness to MSC therapy in patients with graft-versus-host disease [33]. Among other host responses, MSCs trigger both the coagulation and the complement cascades [34], both important for mediating MSC effects with complement activation important for efficient phagocytosis [35]. The procoagulant properties are mainly dependent on tissue factor expression, which differs depending on the MSC tissue of origin. It is high in MSCs derived from placenta compared with bone marrow, with MSCs from umbilical cord and adipose tissue somewhere in between [36]. As such, the risk of triggering thromboembolism needs to be taken into consideration when patients in a hypercoagulable state, including potentially COVID-19 patients, are treated with MSCs.

In 2013, Le Blanc and colleagues used allogeneic bone marrow–derived MSCs on a compassionate-use basis to treat 2 patients with ARDS and multiorgan failure [37]. In an important example of combining mechanistic studies with clinical investigations, the MSCs were characterized in vitro before administration and potently suppressed monocytes, neutrophil, and T cell activation. Although this was not a clinical investigation of MSC efficacy and thus no direct conclusions can be made, both patients subsequently improved and were able to be discharged. At 5-year follow-up, both patients had full recovery of lung function and worked full time [38]. This experience laid the ground for a phase 1 study of patients with COVID-19–induced lung disease (ClinicalTrials.gov identifier: NCT04447833). Eligible patients were screened if they required 2 liters of oxygen to receive a saturation of 90% and later included if they developed severe ARDS according to the Berlin criteria, lasting for >48 h. A total of 7 patients were included in the trial and received 1 infusion of allogeneic MSCs in a dose-escalation format. The primary end point was safety, defined as the absence of a number of prespecified adverse events within 7 days of infusion. Secondary endpoints included survival and pulmonary function. Results of the trial were to be reported late in 2021.

Mechanisms of exosome actions in lung diseases

Claudia dos Santos, University of Toronto dos Santos presented a talk on the mechanisms of extracellular vesicle actions in acute lung diseases, with a focus on the role of EV miRNA content. An initial important point was made based on the consensus statement from the most recent (2018) International Society for Extracellular Vesicles (ISEV) proposed Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines [39]. This states that since consensus has not emerged on specific markers of EV subphenotypes, such as endosome-origin “exosomes,” studies need to diligently report operational preparation features including biophysical characteristics (e.g., size “small 50–200 nm”); biochemical composition (surface markers, e.g., CD63*), and conditions of cell origin (e.g., MSC origin). Seminal work from Sai Kiang Lim [40] has clearly outlined criteria for defining EVs of MSC origins: these are published and were recently presented at the International Society for Cell & Gene Therapy meeting in New Orleans on May 26, 2021.

A brief review of the mechanisms involved in internalization of EVs was presented [41], highlighting 4 major mechanisms including (1) receptor-mediated, (2) direct membrane fusion, (3) phagocytosis and micropinocytosis, and (4) clathrin-mediated endocytosis. In addition to a discussion on MSC EV content a link to Vesiclepedia (http://microvesicles.org/index.html), a manually curated compendium of molecular data (lipid, RNA, and protein) identified in different classes of EVs was provided. dos Santos then discussed multiple studies that have demonstrated the salutary effect of EVs in preclinical lung disease models including a compelling study showing that MSCs outsource mitophagy to recipient macrophages while in parallel shuttling miRNAs that are able to down-regulate Toll-like receptor 4 signaling in recipient cells [42]. Two other examples discussed included the therapeutic benefit of MSC-derived EVs in mitigating inflammation in a murine model of pneumonia [43] and MSC EV-mediated restoration of lung function through macrophage immunomodulation in a murine model of BPD [44].

Reference was also made to a systematic review of EV-based treatments in preclinical models of lung injury in which 4,925 studies were screened and 39 articles deemed adequate based on the criteria that the EVs used in the studies had to be of “stem cell” origin. These were predominantly studies of systemic rather than direct airway EV administration and demonstrated overall attenuation of inflammation (reduction of pro-inflammatory cytokines, neutrophil infiltration, and M2 macrophage polarization); regeneration of alveolar epithelium (decreased apoptosis and stimulation of surfactant production); repair of microvasculature permeability (increased endothelial cell junction proteins); and prevention of fibrosis (reduced fibrin deposition) [45]. Thus there is a growing platform for clinical investigation of EVs, particularly MSC-derived EVs in lung diseases and critical illnesses [46]. A search of clinicaltrials.gov revealed various completed and ongoing studies including those for severe acute respiratory syndrome due to SARS CoV 2 infection. Route of administration include intratracheal and IV, dose ranges from 10^9 to 10^12 EV particles, and different dosing strategies including <5–7 consecutive doses.

Notably, several of these investigations use administration of nebulized EVs, although there is no preclinical data as yet to support this approach. The only published human clinical trial to date used a proprietary product, ExoFlo, derived from Bone Marrow MSC-derived EVs (https://directbiologics.com/). This was a phase 1, nonblinded, nonrandomized study using a single dose (800 billion EVs) delivered IV in patients with moderate to severe ARDS [47]. While the main point for this study was safety, the authors reported that oxygenation improved with an average pressure of arterial oxygen to fraction of inspired oxygen ratio (PaO_2/FiO_2) increase of 192% (P < 0.001) in patients who received the EV preparation. Although no causality can be inferred from this study, it has provided a platform for a phase 2 study of 120 patients that is currently underway. The company has recently announced U.S. Food and Drug Administration (FDA) approval for proceeding with second ExoFlo Investigational New Drug Application for Post-Acute COVID-19 Syndrome and Chronic Post-COVID-19 Syndrome [48]. However, these studies have provoked controversy, as little information is available on the nature of the EV particles used [49]. This is contrary to the recent MISEV guidelines and highlights an ongoing difficulty with transparency in EV studies, particularly when attempting to compare and contrast different EV studies and to get a better handle on mechanisms by which MSCs might be acting.

This highlights an issue that despite advances into the clinic, the “active ingredient” in EVs remains unclear. The likelihood is that any putative benefit is multifactorial, with various EV components, including protein and miRNA contents, mitochondria, and lipids, playing important roles in the biology of these nanoparticles. For the remainder of her presentation, dos Santos focused on what’s currently known about the role of EV miRNAs. The current state of the literature indicates that MSC-derived exosomes contain ~120 miRNA species per exosome [50]. Quantitative and stoichiometric analysis of the MSC-derived exo-miR content [51] suggests that the copy number for each miRNA species may be <1; more recent studies suggest this may be as high as 6 per exosome [52]. Importantly, MSCs from different origins may have similar small RNA expression profiles. Exo-miRs represent ~2–5% of the small RNA content in a cell, and multiple highly expressed cellular miRs are precluded from EV sorting, suggesting that packaging of miRs into EVs is deliberate and may be manipulated for therapeutic purposes [53]. While there is debate
in the literature as to whether the low copy numbers of miRNAs contained within an EV are enough to impart any therapeutic effect, a recent manuscript was discussed showing that EVs containing miR-486-5p promote angiogenesis after myocardial infarction in mice and nonhuman primates [54]. Here the authors used a miRNA inhibitor to demonstrate they could abrogate the therapeutic effects of EVs by co-administering the therapeutic EVs with an miR inhibitor. From a systems biology perspective, the profound effect of a single miR on gene expression networks may underscore their biological significance even at low copy numbers [55], dos Santos also shared work from her own group showing that delivery of bioengineered microparticles containing miRNA inhibitors can be used for therapeutic purposes. Her group has identified endogenous miRNAs that are regulated following MSC administration: miR-27a-5p [56] and miR-193b-5p [57] inhibition ameliorates lung injury in experimental models of lung injury. Finally, dos Santos presented unpublished results from the work performed in collaboration with Daniel Weiss, Michael Matthay, and Patricia Rocco and others sequencing miRNAs contained within EVs derived from MSCs exposed to bronchoalveolar lavage fluid from patients with ARDS as a step toward furthering our understanding of the effects of the inflammatory microenvironment on MSCs and EV structure and function in anticipation of advanced individualized cell-free therapeutics.

**Gene therapies for lung diseases**

John Sheridan PhD, Cystic Fibrosis Foundation, Bethesda, MD

The Cystic Fibrosis Foundation has had a longstanding interest in gene therapy approaches for lung disease. This has most recently incorporated advances in gene transfer vectors and in gene editing technologies. Sheridan, Director of Research at the US Cystic Fibrosis Foundation, discussed advances in gene therapy approaches for treating cystic fibrosis. While small molecule therapies that modulate CFTR have significantly improved lung function and quality of life for people with cystic fibrosis [58], roughly 10% of the CF patient population lacks access to highly effective therapies due to their genotype. Alternative strategies, such as cell and genetic therapies, will need to be developed to restore normal CFTR function for these patients [59]. While these therapies represent potentially curative treatments for CF and other airway diseases, such as PCD, there are significant challenges to their clinical use. First, although the lung appears to be a relatively easy organ to target as it is readily accessible, it is anatomically and biologically complex and has a robust epithelial barrier. Airway mucus traps pathogens and particulate matter and represents a physical barrier to aerosolized delivery [60]. CF mucus is dehydrated and thick and becomes chronically infected and inflamed, decreasing the efficiency of aerosolized delivery. Second, specific cells may also need to be targeted selectively. Single-cell transcriptomic studies of the proximal airway reveal a diverse population of epithelial cell subtypes that differentially express CFTR. Secretory cells and ionocytes have the highest level of CFTR expression, whereas ciliated cells have been found to express very little CFTR [61–64]. While the functional role of these different cell types is not fully understood, they do represent potential cellular targets for transient therapies. Finally, for a long-lasting and potentially permanent restoration of CFTR function, it will be necessary to correct the mutation by editing or replacing the CFTR gene in airway basal cells. Gene editing technologies may treat multiple mutations with a single therapy through prime editing or site-specific gene insertion [65,66]; however, additional research and optimization is necessary. Additionally, efficiently delivering the gene editing technology to the airway basal cells is a significant barrier to clinical use. Alternatively, engraftment of genetically corrected airway stem cells may also permanently restore CFTR function. A cell-based therapy must appropriately engraft and persist long term and maintain the capacity to differentiate into the CFTR-expressing epithelial cells [67]. To develop a successful genetic or cell-based therapy for CF, these technical challenges must be overcome, and each represents a current research and funding focus for future therapeutic development.

**Pediatric pulmonary diseases: cell-based therapies for BPD**

Elizabeth Baker, University of Melbourne, Australia
Bernard Thébaud, MD, University of Ottawa, Canada

Baker and Thébaud discussed the use of cell therapy to treat neonates suffering from BPD. BPD is a chronic lung disease associated with preterm birth. The phenotype of BPD has evolved from that which was originally described by Northway in 1967 [68]. BPD is now considered predominantly an illness of the extremely preterm infant born in the late canalicular or earlier saccular stage of lung development. BPD is multifactorial, and in some instances contributed to by necessary, life-sustaining treatments. Inflammation, ventilation-associated lung injury, and oxidative stress culminate in “arrested lung development.” [69] The resulting alveoli are large and simplified, and the pulmonary vascular bed is abnormal. Infants diagnosed with BPD are at greater risk of not just poorer respiratory health through childhood and into adulthood but also adverse neurodevelopmental outcomes such as cerebral palsy and cognitive impairment [70,71]. The multifactorial nature of BPD presents therapeutic challenges, and while the survival rates of the most immature infants has increased, unfortunately few advances have been made in the treatment of BPD. Cell therapy, with its capacity to regenerate and modulate many of the pathways that contribute to BPD, presents a unique and promising therapeutic option.

Numerous cell therapies have been tested in experimental BPD, with various rationales and levels of biological plausibility [72]. MSCs are by far the most investigated cell, because their putative pleiotropic effects target many pathophysiologic mechanisms contributing to BPD. Proof-of-concept experiments demonstrated first the lung-protective effect of bone marrow–derived MSCs in oxygen-induced neonatal lung injury in rodents [73,74]. MSCs can be easily and safely obtained from perinatal tissues after birth and represent a clinically relevant source for the treatment of neonatal diseases. Numerous investigations critically reviewed in a systematic review of all preclinical studies with MSC in experimental preclinical BPD models confirm that MSCs isolated from cord or cord blood improve lung structure and function, attenuate pulmonary hypertension, and mitigate lung inflammation and fibrosis, while enhancing lung vascular growth [75]. These effects persist into adulthood and have not been associated with long-term adverse events such as tumor formation [76]. These promising preclinical data have rapidly led to early-phase clinical trials. There are currently 2 published phase 1 clinical trials testing the feasibility and safety of the same proprietary cord blood–derived MSC product in extreme preterm infants at risk for BPD [77,78]. In the first, a total of 21 preterm infants received a single intratracheal injection of 1 or 2 × 10⁷ cells/kg a mean of 10 days after birth. The procedure was well tolerated, and no adverse events were reported. A follow-up study on the first 9 patients treated confirmed no adverse effects at age 18–24 months [79]. More recently, the results of a phase 2 trial using the same proprietary cell product were published [80]. Sixty-six preterm infants were stratified by gestational age (23–24 weeks and 25–28 weeks) and received a single intratracheal injection of MSCs (1 × 10⁷ cells/kg) or vehicle control. Inflammatory cytokines in tracheal aspirates were significantly reduced with MSCs, but there were no differences in the primary outcome of death or BPD (placebo 18/33, 55%; MSCs 17/33, 52%). However, a subgroup analysis in the 23–24-week cohort showed a reduction in death resulting from BPD [53% (8/15) to 19% (3/16)]. Although underpowered, these results suggest a potential benefit in lower gestational ages, and this is currently being examined in a new phase 2 trial. As of July 2021, a total of 15 MSC trials were registered under clinicaltrials.gov, including a phase 1 trial examining the
feasibility and safety of MSC-derived extracellular vesicles based on promising preclinical observations [81].

Human amnion epithelial cells (hAECs), derived from the amniotic membrane of term placenta, have also shown promise in preclinical studies of BPD. hAECs modulate the immune response by decreasing the proliferation and recruitment of T cells and favoring protective T regulatory cells and M2 macrophages [82–85]. hAECs promote angiogenesis, reduce fibroblast activation and collagen deposition, and activate the bronchialalveolar stem cell niche [86–89]. These protective and reparative actions restore lung architecture in preclinical models of BPD. These promising findings have led to early-phase clinical studies [90–92]. A first-in-human study administered 1.0 × 10^6 hAECs/kg IV to 6 extremely preterm infants with established BPD [88]. This study provided evidence that hAECs were tolerable in this vulnerable population of infants [88,93]. A phase 1 dose-escalation study of IV hAECs is currently recruiting extremely preterm infants with evolving BPD, to evaluate the tolerability of higher doses given earlier in the development of BPD [90]. A maximum dose of 3.0 × 10^7 hAECs/kg administered in 3 divided doses beginning in the first 2–3 weeks of life will be studied.

As these early-phase studies in neonates emerge, a number of challenges are yet to be addressed. These require urgent yet careful consideration. For example, early in the course of the current dose-escalation study, hAECs were found to have settled in the IV line used to deliver the infusion, resulting in a substantial decrease in dose delivery (20% of the dose that had been intended) [94]. The necessity to provide small-volume infusions at slow rates given the small size (<1 kg body weight) and hemodynamic immaturity of the population brings unique challenges. Simple measures, using readily available clinical equipment, resolved the delivery issues. However, this experience highlights the detailed attention required in the conceptualization and conduct of early-phase cell therapy studies [95].

**Adult pulmonary diseases**

Patricia R.M. Rocco, MD, PhD, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Rocco provided an update on the use of cell therapy approaches for potential treatment of asthma and COPD. Asthma is a major non-communicable disease affecting both children and adults, with high morbidity and relatively low mortality compared with other chronic diseases. Around 300 million people have asthma worldwide, and it is likely that by 2025, a further 100 million may be affected. There is a large geographic variation in asthma prevalence, severity, and mortality, and asthma still imposes an unacceptable burden on health care systems and society. Asthma is a complex multifactorial disorder, and its cause is increasingly attributed to interactions between genetic susceptibility, host factors, and environmental exposures. There are no curative approaches for asthma, and anti-inflammatory drugs (e.g., glucocorticoids) and bronchodilators (e.g., β-adrenoceptor agonists) remain the first choices for symptomatic treatment. However, asthma symptoms are still poorly controlled after an optimal treatment in 5%–10% of patients, in large part because of lung parenchyma and airway structural remodeling. Therefore, new therapeutic strategies are required.

Systemic and intratracheal administration of BM-MSCs is effective at reducing airway inflammation and improving lung function in several models of asthma, including those induced by ovalbumin [96], house dust mite extract [97], and *Aspergillus hyphae* extract (mixed Th2/Th17 model) [98]. Moreover, systemic and intratracheal administration of bone marrow–derived mononuclear cells (BMMCs), a heterogeneous pool of cells that includes MSCs, has shown promising results similar to those of BM-MSCs in preclinical models of severe asthma [99,100]. These provided a platform for a case series of 3 patients who presented with severe asthma and were unresponsive to conventional therapy and omalizumab. They each received a single IV dose of autologous BMMCs (2 × 10^7 cells) and were periodically evaluated for 1 year after the procedure. BMMC administration caused no serious adverse events, during or after the procedure, and lung function remained stable throughout. In 1 patient, a slight increase in ventilation of the right lung was observed 4 months after therapy. All 3 patients reported improvement in quality of life in the early course of treatment [101]. Further randomized, double blind, placebo-controlled trials in severe asthma patients using stem cells are required.

COPD is currently the third leading cause of death (3 million worldwide every year) [102]. Despite progress in the treatment of symptoms and prevention of acute exacerbations, few advances have been made to ameliorate disease progression or reduce mortality. Novel treatment strategies aiming to restore damaged lung tissue are urgently required. In this context, administration of MSCs attenuates inflammation, decreases apoptosis, improves lung parenchyma repair, and increases lung perfusion in different models of emphysema [103,104]. Different MSC sources and administration routes had different effects on lung damage in experimental emphysema [105,106]. Intratracheal administration, compared with IV, of BM-MSCs reduced areas of alveolar hyperinflation and collagen fiber content [107]. Based on the data obtained in this preclinical study, Rocco’s group investigated the therapeutic potential of MSC administration through bronchoscopy associated with endobronchial valves (EBVs) in patients with severe COPD [108]. EBVs are able to deflate emphysematous sections, which reduces air trapping, thus allowing better expansion and improvement in lung function and quality of life [109]. However, EBVs may induce granuloma formation and localized inflammation with subsequent mucus hypersecretion, which can increase the risk of infection and contribute to worsening of clinical status in these patients. Considering the anti-inflammatory, microbicial, and antifibrotic properties of MSCs, the combination of EBVs and intratracheal local administration of MSCs at the EBV site was evaluated. EBVs associated with MSCs decreased levels of circulating C-reactive protein at 30 and 90 days and improved quality of life. Thus, combined use of EBVs and MSCs appears to be safe in patients with severe COPD, providing a basis for subsequent investigations using MSCs as concomitant therapy. These studies complement other data assessing effects of MSC administration in patients with COPD and further highlight that certain COPD populations may be more amenable to MSC-based cell therapies. Additional studies need to be performed to analyze whether the local administration of MSCs alone may ameliorate the underlying inflammatory process in patients with homogeneous and heterogeneous emphysema and to determine optimal dose, dosing strategies, and patient populations most likely to receive benefit.

**Cellular immunotherapy for septic shock**

Shirley H. J. Mei, PhD, MSc, Sinclair Centre for Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

Josee Champagne, BMSc, PMP, CCRP, Department of Medicine (Division of Critical Care), University of Ottawa, Ottawa, Ontario, Canada

On behalf of Lauralyn McIntyre, Shirley Mei and Josee Champagne summarized the Cellular Immunotherapy for Septic Shock (CISS) Program and the work that has been done to justify and design the future phases of this translational research program.

The CISS phase 1 study was a single-center, open-label, dose-escalation, clinical trial designed to examine the safety and tolerability of ≤3.0 million cells/kg of freshly administered allogeneic bone marrow–derived MSCs delivered as a single IV infusion [110]. Before launching the interventional arm, the researchers prospectively enrolled 21 participants who met eligibility criteria but did not receive MSCs into an observational arm to examine feasibility of recruitment, provide data to compare adverse events (AEs), and...
serially collect biologic outcome measures against the CISS intervention cohort. After the recruitment of the 21 observational participants, a total of 9 patients were enrolled into the intervention cohort, which included 3 MSC dose cohorts of 3 participants, each receiving a single IV dose of ≤0.3, 1.0, and 3.0 million cells/kg. There were no transfusion-associated AEs in any of the intervention cohorts, nor any unexpected and serious AEs considered possibly or definitely related to the MSCs.

The CISS phase 1 trial was not powered to detect clinical effects of MSC treatment; however, Mei and her team believed that translational studies, such as comprehensive analysis of plasma protein profiles, could provide further information into the safety and biological effects of MSC treatment in critically ill patients. The results gained may be especially relevant given that unbalanced cytokine production contributes to the development and severity of septic shock, and MSCs are known to exert immunomodulatory effects through secretion of paracrine/endocrine factors. Schlosser et al. reported that 49 analytes, including cytokines and biomarkers, were measured in serial plasma samples collected from the CISS I participants before MSC infusion and at 1, 4, 12, 24, and 72 h after MSC infusion. Results show that a single IV infusion of MSCs (<3 million cells/kg) did not cause any gross abnormalities in systemic cytokine levels. Most importantly, no significant increase in levels of known proinflammatory mediators or biomarkers of organ dysfunction were detected in relation to MSC treatment within the 72-h time course of the study. Stratification of the intervention cohort by MSC dose further revealed patient-specific and dose-dependent perturbations in cytokines, including an early but transient dampening of pro-inflammatory cytokines, suggesting that MSC treatment may alter innate immune responses and underlying sepsis biology. Ongoing work is currently being performed to evaluate other biological molecules that, together with the data above, may offer further insight into potential biological mechanisms of MSC treatment and support future investigations in larger randomized controlled trials (RCTs).

Because of the acute onset and rapid deterioration of patients diagnosed with septic shock, the importance of developing a rapidly available MSC product was greatly emphasized. While our fresh MSC product appears safe, some operational challenges related to ensuring the availability of an appropriate dose of a freshly cultured MSC product were encountered. This prompted our team to develop more robust cell manufacturing protocols to produce a validated cryopreserved product. This effort to improve patient selection with prognostic and predictive biomarkers, and articulated that while considerable progress has been made in understanding the pathogenesis of ARDS and mechanisms of repair, treatment remains primarily confined to improved supportive care with lung-protective ventilation, prone position, and a fluid conservative strategy. Several new therapies are being tested, with an effort to improve patient selection with prognostic and predictive enrichments strategies. Among these, there is a strong rationale for investigating MSCs based on numerous small and large animal studies plus experiments done in ex vivo human lung preparations. However, translation of these preclinical findings requires phase 1 and 2 trials before phase 3 trials.

This session brought together academia and industry leaders of current clinical investigations for both non–COVID-based and COVID-based ARDS. Both published and some as yet unpublished data are included in the synopses of each presentation.

Michael A. Matthy, MD. University of California San Francisco

Matthys is a renowned leader in ARDS clinical care and research and articulated that while considerable progress has been made in understanding the pathogenesis of ARDS and mechanisms of repair, treatment remains primarily confined to improved supportive care with lung-protective ventilation, prone position, and a fluid conservative strategy. Several new therapies are being tested, with an effort to improve patient selection with prognostic and predictive enrichments strategies. Among these, there is a strong rationale for investigating MSCs based on numerous small and large animal studies plus experiments done in ex vivo human lung preparations. However, translation of these preclinical findings requires phase 1 and 2 trials before phase 3 trials.

Matthys and colleagues have completed 2 safety trials with allogeneic BM-MSCs for non-COVID ARDS. The phase 1 trial was a standard dose-escalation trial that resulted in confirmation that the highest dose of 10 × 10⁶ MSCs/kg (ideal body weight) appeared to be safe. The phase 2a trial (START) tested safety in 60 patients (40 MSC-treated and 20 placebo) in an FDA-approved, double-blind randomized design. There were no safety issues. The trial was not powered for efficacy, but post hoc analysis suggested a trend for benefit in oxygenation index in a secondary analysis of the patients who received higher viability MSCs. However, the assessments were confounded by major imbalances in the baseline severity of illness and respiratory failure that were greater in the MSC-treated patients. A secondary analysis from this trial that evaluated the BALF from 27 of the 60 enrolled subjects collected at 48 h showed evidence of a reduction in biologic evidence of lung injury in the MSC-treated patients. For example, total protein and angiotensin-2 were significantly lower in the MSC-treated patients. This is an important biologic measure, as previous studies have assessed only circulating inflammatory mediators.
Currently, Matthay and colleagues are conducting a phase 2b randomized, double-blind trial (STAT) with the primary endpoint of oxygenation index, a reliable measure of the severity of respiratory failure. The goal is to enroll 120 patients with ARDS, trauma, or non-trauma on etiology. At the time of Matthay’s presentation, 73 patients had been enrolled. The Data Safety Monitoring Board did an interim review after 60 patients were enrolled and recommended that trial enrollment should continue. Participating hospitals are UCSF San Francisco, Zuckerberg San Francisco General Hospital, University of California at Davis, Vanderbilt Medical Center in Nashville, University of Texas in Houston, Oregon Health Sciences Medical Center in Portland, and Harborview Hospital in Seattle. Enrollment is proceeding well. Approximately 90% of the enrolled patients have been COVID-19 positive to date. The web site for the trial is https://stattrial.com, and the password is StemCells4All.

Daniel F McAuley, MD, Queen’s University Belfast, Northern Ireland, UK

McAuley and colleagues have completed a phase 1 trial with allogeneic UC-MSCs for ARDS. The phase 1 trial was an open-label, dose-escalation trial in which mechanically ventilated patients with moderate to severe ARDS received increasing doses (100, 200, or 400 × 10⁶ cells) of a single IV infusion of UC-MSCs. The primary safety outcome was the incidence of serious adverse events. Nine patients were recruited, with 3 patients in each dose cohort. This trial found that a single IV infusion of MSCs was well tolerated, with no dose-limiting toxicity reported up to 400 × 10⁶ cells [122].

This phase 1 trial informed the design of 2 phase 2 trials. The REALIST-COVID trial was a multicenter, randomized, double-blind, allocation-concealed, placebo-controlled trial in mechanically ventilated patients with moderate to severe ARDS due to COVID-19. Participants were randomized to receive a single IV infusion of MSCs (400 × 10⁶ cells) or placebo (Plasmalyte 148). The primary safety outcome was the incidence of serious AEs. The primary efficacy outcome was oxygenation index at day 7. Sixty participants were recruited, and the results of the trial were to be reported late in 2021.

Currently, the REALIST trial is in setup and will have a design similar to the REALIST-COVID trial. This trial aims to recruit 60 patients with ARDS not due to COVID. It is expected the trial will be completed in 2022.

Silviu Itescu MD, Mesoblast Inc, Melbourne, Australia

Mesoblast Chief Executive Officer Silviu Itescu presented clinical outcomes from the randomized controlled trial of their allogeneic BM-MSC product, remestemcel-L, in ventilator-dependent COVID-19 patients with moderate/severe ARDS. The trial enrolled 222 patients across the United States, of whom 217 were randomized 1:1 and received either standard of care alone or standard of care plus 2 IV infusions, 3–5 days apart, of remestemcel-L at a dose of 2 million cells/kg or vehicle control. The empiric dosing regimen was based on clinical experience with remestemcel-L in patients with steroid-refractory acute graft-versus-host disease (SR-aGVHD) in which a dosing regimen of 8 IV doses of 2 million cells/kg is used, twice per week for 4 weeks.

While the trial was halted early, as it was unlikely to meet its endpoint of 43% reduction in overall mortality at 30 days, remestemcel-L significantly reduced mortality through 90 days in a prespecified subgroup analysis of 123 patients <65 years old. For patients <65 years old, the key secondary endpoints of days alive and free from mechanical ventilation within 60 days was significantly longer in remestemcel-L treated patients than controls, supporting the findings on survival.

Key new findings presented at the ISCT meeting were as follows:

- Remestemcel-L significantly reduced mortality by 48% at 90 days compared with controls in a prespecified analysis of 123 treated patients <65 years old, 26% versus 44%, hazard ratio (HR) 0.52, 95% confidence interval (CI) (0.277, 0.964), P = 0.038. This compares favorably with the 46% mortality reduction reported at 60 days (P = 0.048) and indicates a durable treatment benefit in this patient population (Fig. 1).

- Remestemcel-L was even more effective when evaluated in an exploratory analysis in patients on dexamethasone as part of their standard of care, with 90-day mortality being reduced by 77% compared with controls <65 years old who received dexamethasone, 14% versus 48%, HR 0.23, 95% CI (0.080, 0.681), P = 0.0037 (Fig. 2).

- These survival benefits were accompanied by significant improvements relative to controls in prespecified secondary endpoints of ventilator-free days, respiratory function as assessed by ARDS severity, and overall clinical improvement on a 7-point ordinal scale.

Figure 1. All treated patients <65 years old (n = 123) through 90 days. All P values are descriptive and not adjusted for multiplicity. (Color version of figure is available online).
Across all assessments at days 7, 14, 21, and 30, greater improvement in ARDS severity was observed for remestemcel-L patients compared with controls in a logistic regression model (P = 0.051).

Despite a treatment-related improvement in ARDS severity at day 7, there was no mortality reduction in the 97 treated patients >65 years, suggesting the need for more prolonged or higher dosing of anti-inflammatory therapy in these patients who may have a more exuberant inflammatory response associated with defective immune-mediated viral clearance mechanisms.

Clinical outcomes from meta-analyses of many trials have shown that mortality significantly increases with age for COVID-19 patients on mechanical ventilation [123], and that was also seen in controls in the current study, with those older than 65 years having significantly higher mortality compared with those younger than 65. Robust adaptive naïve T cell responses against COVID-19 are critical for viral clearance, and older patients have reduced numbers of naïve T cells, resulting in less effective viral clearance and persistence of greater viral load. The maladaptive T cell responses associated with greater viral load in older individuals are accompanied by increased immune dysregulation and greater ARDS disease severity [124,125]. In the meta-analysis by Zheng et al. [124], 69 studies were included, describing 57,420 adult patients with COVID-19 who received IMV. Fifty-four of the 69 studies stated whether hospital outcomes were describing 57,120 adult patients with COVID-19 who received IMV. Across all assessments at days 7, 14, 21, and 30, greater improvement in ARDS severity was observed for remestemcel-L patients compared with controls in a logistic regression model (P = 0.051).

It was postulated that it is likely that the exploratory dosing regimen of remestemcel-L used in all patients in the current trial was sufficient to address the exuberant immunological response in younger patients and achieve survival benefits in those <65 years with lower degrees of pulmonary inflammation, while higher dosing may be required to address the greater pulmonary inflammation in older patients. The company is planning to conduct a confirmatory phase 3 trial in COVID-19 ARDS patients <65 years of age with dexamethasone and concurrently explore additional remestemcel-L dosing regimens for patients with ARDS ≥65 years of age.

Biju Parekkadan, PhD, Rutgers University, Piscataway, NJ; and Sentien Biotechnologies, Inc., Lexington, MA

Parekkadan discussed the utility of and a novel methodology for using MSCs ex vivo to treat patients with COVID-19–induced ARDS. MSCs naturally influence local niches by way of secreted factors that shape the differentiation, migration, and effector function of immune cells to maintain homeostasis. These endogenous biological functions have therapeutic effects when MSCs are used in cases of dysregulated immunity. The use of pharmacological models of MSC potency can aid in identifying critical criteria to maintain when scaling to human patients. Using a widely accepted T cell inhibition assay of MSCs as a model system, evidence has shown local concentration of MSCs, time of exposure, and MSC licensing all to be necessary for potent T cell inhibition [126,127]. However, IV infusion of MSCs, although clinically practical to resolve systemic inflammation, is hindered by a short circulating half-life of MSCs [128], limited long-term engraftment [129], clot induction, and a maximum IV dose that can be tolerated safely without lung toxicity. The potency of MSCs on immune cells is thus difficult to control and to evaluate in vivo by systemic administration. Several alternate approaches that investigators are taking to better control for MSC bioavailability include local injections [130], the use of surface-modified MSCs to better home to target organs [131–133], or even engineered MSCs with increased survival signaling [134].

The Parekkadan lab invented a new ex vivo MSC approach to circumvent challenges with systemic administration and to better control MSC dose and duration (Fig. 3) [135]. This combination product, now translated into the clinic by Sentien Biotechnologies, Inc., immobilizes allogeneic human MSCs into a hollow-fiber hemofilter, which maintains adherent MSC viability, while exposing patient blood indirectly to the MSCs via a semipermeable membrane. This device allows MSCs to act outside the body to deliver a potent mixture of secreted mediators including proteins and EVs that can impact regeneration, vascularization, and inflammation concurrently [136]. Moreover, the separation of MSCs to blood by a membrane significantly reduces clotting potential in model assays [137].

A first-in-man study was recently conducted in patients with severe acute kidney injury (AKI) who receive continuous hemodialysis treatment as a standard of care. This study showed pharmacodynamic trends that are supportive of a reduction in systemic inflammation [138]. This clinical experience motivated a new trial multi-center, randomized, case controlled, single-dose study of extracorporeal MSC therapy in COVID-19 subjects with AKI receiving continuous renal replacement therapy (CRRT) (NCT04445220,
manuscript in preparation). In the initial cohort of 3 patients analyzed to date, each was exposed to $250 \times 10^6$ MSCs through the extracorporeal circulation device for up to 24 h. All 3 survived and subsequently no longer required CRRT and 1 no longer required mechanical ventilator. Survival was associated with FiO₂ (%) reduction after treatment in all patients. COVID-19 / SARS-CoV-2 associated circulating inflammatory markers such as C-reactive protein, Ferritin, D-dimer, interleukin (IL)-6, lactate dehydrogenase, platelet count, and lymphocytes all showed improvement when assessed 7 days after treatment. Urine kidney injury biomarkers were also improved. Although no causality can as yet be ascribed to use of the device, the experience suggests that it can be safe and well tolerated. Overall, this initial study supports the potential to further evaluate ex vivo MSC therapy in the treatment of severe COVID-19 patients with or without AKI and suggests other potential uses in other areas where systemic inflammation prevents recovery from severe injury.

**Summary and conclusions**

While our understanding of cell and gene therapy for pulmonary diseases and critical illnesses continues at a rapid pace, attendees discussed several outstanding issues in the field that need further study. Broadly, the challenges encountered by the field can be divided into 3 main categories: first, manufacturing and regulation; second, translating the preclinical science in mechanistic ways to best approach complex clinical conditions to achieve the greatest benefit; and last, optimal clinical trial design and conduct, including patient selection. A further challenge fundamental to these categories is the need to continue efforts to elucidate the mechanisms of action of cell-based therapies.

The development of potency assays for cell-based products, particularly MSCs but also EVs, is necessary to ensure a greater degree of homogeneity across end products. This is important both from a regulatory perspective but also to ensure that clinical trial data is reliable.
A Clinical Study of Mesenchymal Stem Cells (MPCs-Exo) for the Treatment of Pulmonary Infection

| Recruiting | Biological: Dosage 1 of MPCs-derived exosomes |
| Inhaled | Drug-resistant |
| Completed/has results | Biological: Dosage 2 of MPCs-derived exosomes |
| Not yet recruiting | Biological: No MPCs-derived exosomes |
| IV | Drug: EXO 1 inhalation |
| ARDS/OSI, perinatal MSC-derived exosome therapy | Drug: EXO 2 inhalation |
| Biological: MSC-exosomes delivered IV every other day on an escalating dose (2:4:8) |
| Biological: MSC-exosomes delivered IV every other day (8:8:8) |
| Biological: MSCs-derived exosomes |
| Biological: COVID-19 Specific T Cell derived exosomes (CSTC-Exo) |

Drug: Placebo inhalation

Medical Centre Dynasty Samara, Russian Federation

Mission Community Hospital Panorama City, California, United States

Ruijin Hospital Shanghai Jiao Tong University School of Medicine Shanghai, Shanghai, China

Ruijin Hospital Shanghai Jiao Tong University School of Medicine Shanghai, Shanghai, China

Drug: COVID-19 Specific T Cell-derived exosomes (CSTC-Exo)

Coronavirus

Drug: MSC-exosomes delivered IV every other day on an escalating dose (2:4:8)

Drug: MSC-exosomes delivered IV every other day (8:8:8)

Drug: COVID-19 Specific T Cell-derived exosomes (CSTC-Exo)

Drug: COVID-19 Specific T Cell-derived exosomes (CSTC-Exo)

Corona Virus Infection

Pneumonia

Biochemically active cargo, e.g., enzyme activity of CD73; unit activity per μg protein.

MSC origin: concentration of a MSC surface antigen (CD73, CD90, and CD105) and non-MSC surface antigen (CD14, CD34, and CD11b), e.g., nmol CD105 per μg protein.

Number of particles per unit weight protein/membrane lipids and modal diameter within 50–200 nm.

Molar/weight ratio of protein to membrane lipids, e.g., cholesterol, phosphatidylcholine.

Biologically active cargo, e.g., enzyme activity of CD73; unit activity per μg protein.

and able to be generalized. Further, transparency on the cell and gene therapy products being used is important to allow appropriate assessments, including comparison and contrast with other products and approaches as well as with respect to further understanding mechanisms of action. Early engagement with key stake holders such as regulatory and ethics boards is crucial, as is engagement with industry to ensure good manufacturing practices and scalable manufacture and to begin realistic cost analysis.

The enthusiasm for cell therapy brought about by promising preclinical findings must be tempered with consideration for these and other challenges. These further include the identification of biomarkers that can further substantiate the mechanisms of action and allow better understanding of the interactions of cells with immune cell components including T cells, monocytes/macrophages, and neutrophils. The establishment of biomarkers is challenging given the heterogeneity of the patient population with pulmonary diseases and critical illnesses. Discussion was held on potentially using circulating cytokine analysis to identify potential responders versus nonresponders. It was also discussed whether, in the context of biomarker analysis, there can be a standard safety panel for cell therapy that can include baseline and repeated measures such as pulse/oximetry, D-dimer, and other coagulation markers including tissue factor expression. Another broad topic regarding patient selection centered on COVID-19 patients and which categories should be targets for study: severe respiratory failure on mechanical ventilation, those on high level non-invasive ventilation such as high-flow nasal oxygen, those requiring lower level oxygen supplementation, or perhaps even post-COVID survivors. As of yet, there is no cohesive plan or approach. As in all causes of acute respiratory failure, the goal is to find the optimal treatment-responsive patient subgroups for cell therapy.

Another topic of robust discussion was determining the optimal dose and dosing strategy. Most dose-ranging studies are performed in animal models that do not easily translate to human dosing. Because of the lack of pharmacodynamic/pharmacokinetic data in humans, it is difficult to calculate the proper dose based on animal data. As lung is the initial target organ after IV delivery, one suggestion was using cardiac output as a comparator as a reflection of the vasculature. For neonatal studies, the preclinical studies suggested possible ranges of doses, and the studies were based on published studies with adults; several participants mentioned the dose used in the START trial as a basis for dosing strategy. With exosomes, the dosing strategy becomes even more challenging, as there is no extrapolation of how many vesicles the cells would release during the cell residency period, but there is good concordance between mouse and lamb models and dose response. Also, with all neonatal studies, the volume delivered is a limiting factor. Last, the issue of repeated dosing was raised, and Rocco mentioned that in her studies with using MSCs to treat COVID-19 patients, there were no significant changes in D-dimer with repeat dosing. However, overall, there is no consensus as yet on either dose or dosing strategies.

The route of delivery was also discussed. As IV delivery provides a first passage through the lung, this appears to be a standard approach, but there are benefits to intratracheal delivery being more direct and requiring lower doses. This led to a further discussion on
mechanisms of action and whether one needs to have cells present at the site of organ injury or whether the mechanisms of action also include a more systemic effect that includes interactions with immune cells.

The meeting concluded with the recognition that having this closed forum to discuss issues that are critical to the development of cell and gene therapy was quite beneficial to the attendees and that further interactions should continue. Further discussions between participating individuals and groups are anticipated to lead to broader collaborative efforts and advance all of the above issues.

Funding and acknowledgements

Baker: Centre for Research Excellence in Newborn Medicine, Parkville, Australia; University of Melbourne, Parkville, Australia; Desai: TJD is the Woods Family Endowed Faculty Scholar, Stanford Child Health Research Institute; dos Santos: C.C.D.S. is supported by the Canadian Institutes of Health Research (Grant # MOP-130331, MPO-106545, CIHR/NSERC), the Canadian Stem Cell Network and the University of Toronto Robert and Dorothy Pitts Research Chair in Acute Care and Emergency Medicine; Heijink: Research Grant from Dutch Lung Foundation (LongFonds) LF 6.1.15.017; Itescu: Silviu Itescu is an employee of Mesoblast and has no personal disclosures to make; Le Blanc: Swedish Research Council, the Swedish Cancer Society, Stockholm County Council and the Tobias Foundation; Matthay: NHLBI HL134828, Dept of Defense W81XWH-17-1-0631; McAuley: Wellcome Trust Health Innovation Challenge Fund and the Northern Ireland Health and Social Care Research and Development Division; McIntyre: Stem Cell Network Canada, Ontario Institute of Regenerative Medicine; Mei: Stem Cell Network Canada, Ontario Institute of Regenerative Medicine; Parekkadan: Funding for this research was provided by Sentien Biotechnologies, Inc.; Rocco: Brazilian Council for Scientific and Technological Development (CNPq), Rio de Janeiro State Research Foundation (FAPERJ), Department of Science and Technology — Brazilian Ministry of Health (DECT/MS), and the National Institute of Science and Technology for Regenerative Medicine (REGENERA/CNPq); Sheridan: John Sheridan is an employee of the Cystic Fibrosis Foundation (CFF) and has no personal disclosures to make; Thebaud: Canadian Institute of Health Research Foundation Scheme, Stem Cell Network Canada, Ontario Institute of Regenerative Medicine; Ting: Anthony Ting is an employee of Bone Therapeutics, Chief Commercialization Officer of ISCT, and has no personal disclosures to make; Weiss: Dr. Weiss is an employee of the University of Vermont and the University of Vermont Health Network and also the Chief Scientific Officer of ISCT. He has received recent research funding from the NIH, Department of Defense, Cystic Fibrosis Foundation and United Therapeutics Inc. Dr. Weiss has been a paid consultant for NextCell Inc, United Therapeutics Inc., and Vertex Inc (Table 1-3, SESSION I).

Acknowledgment

The authors gratefully acknowledge ISCT for continued support of the Scientific Signature Series and in particular Felix Grignon at ISCT for the immense amount of behind-the-scenes work for both the conference and the final collation of the conference report.

References

A.E. Ting et al. / Cytotherapy 24 (2022) 774–788


tive and stoichiometric analysis of tumor-derived exosomal miRNAs for diagnos


[33] Li Q, et al. Small extracellular vesicles containing mi-486–4p promote angio

[34] MicroRNA (miRNAs), the nucleic frontiers: the hidden masters regulators impacting biological response in body organs due to spaceflight chases vanderburg and afshin beheshti. NASA Am Res Cent 2020.


[38] MicroRNA (miRNAs), the nucleic frontiers: the hidden masters regulators impacting biological response in body organs due to spaceflight chases vanderburg and afshin beheshti. NASA Am Res Cent 2020.


Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID19. 2022 10.1016/j.cell.2021.01.007


