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REVIEW ARTICLE

How to introduce MSC-based therapy for the developing lung safely into clinical care?

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Extreme prematurity is associated with an increased risk to develop bronchopulmonary dysplasia (BPD). Severe BPD is associated with a significant long-term burden for the affected infant, families and society. Currently there are limited prevention and treatment options. Regenerative approaches using mesenchymal stromal cells (MSC) are associated with promising benefits in animal experiments. First clinical studies, using MSC in humans, suggest safety. To accelerate the process of bench to bed-side development of MSC-based therapies, a global and collaborative approach is needed that includes all key stakeholders. Results of a workshop that was held during the Pediatric Academic Societies meeting in 2019 are summarized. A roadmap is provided discussing next steps of bringing MSC-based interventions into clinical practice.

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INTRODUCTION

Prematurity accounts for approximately half of all deaths in infants below 5 years of age. It is associated with severe short- and long-term morbidity, which also adversely impacts health-care resources.[1] Despite change in neonatal care, the rate of prematurity-associated lung injury has remained unchanged over the past decade.[2] Almost 50% of all very low-birth-weight infants still suffer from bronchopulmonary dysplasia (BPD).[3] Severe BPD is associated with neurodevelopmental impairment, pulmonary hypertension, cor pulmonale, death or the later development of chronic pulmonary insufficiency of prematurity such as asthma and/or repeated respiratory infections.[4,5]

As preventing preterm birth has had limited success to date, research has focused on ameliorating the consequences of prematurity including BPD. Currently, regenerative approaches based on mesenchymal stromal cells (MSC) seem to hold great promise. Whereas MSC are successfully used in adult diseases,[6,7] clinical translation into neonatology has been quite limited. To accelerate the process of bench to bed-side development of cell-based therapies, a global and collaborative approach is needed. A successful and fast but also safe translation mandates a collaboration of all key stakeholders, including scientists, clinicians, industry, regulatory authorities, and patient/parent organizations.

A workshop was held during the Pediatric Academic Societies meeting in 2019 to discuss next steps of bringing MSC-based interventions into clinical practice. Key results are summarized below.

MSC-BASED INTERVENTIONS FOR BPD

Mesenchymal cells play a crucial role in fetal lung development and subsequent acute and chronic lung disease. The exposure of the extra-uterine fetus (preterm infant) to a hyperoxic environment disrupts normal lung development and interferes with pathways that promote alveolar and vascular growth in lung resident MSC.[8] In vitro human fetal lung MSC (hflMSC) that are exposed to extra-uterine conditions show a pattern very similar to MSC from preterm neonates that have developed BPD. These hflMSC exhibit excessive proliferation, alterations in the cell's surface marker profile, reduced colony-forming ability, and disturbed secretion of factors important for lung growth.[9] Isolation of MSC in tracheal aspirate predicts the development of BPD, which suggests that MSC play an important role in the pathogenesis of this disease.[10]

There is convincing preclinical evidence that MSC administration can prevent hyperoxia-induced lung injury in rodents, a conclusion that has been pooled in a meta-analysis.[11] In adult humans, several phase I/II clinical trials have shown promising results[6,7] and a meta-analysis of human clinical data from 36 studies has demonstrated the safety of MSC-based therapy.[12]

These observations provide strong rationale to use MSC in preterm neonates at risk of developing BPD. In a phase I dose-escalation trial, allogeneic umbilical cord blood-derived MSC were administered intratrachealy with the study demonstrating the approach was feasible and safe in nine preterm neonates.[13] Follow-up at 18−24 months showed no obvious signs of toxicity. The authors suggested that larger trials are warranted.[14] A second phase 1 dose-escalation trial of intratracheal admin-

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istration of MSC in 12 extremely low-birth-weight neonates at a mean postnatal age of 10.6 days was published recently. The treatment was well tolerated and appeared safe and feasible.[15] Similarly, safety of allogeneic human amnion epithelial cells (hAEC), administered intravenously at a dosage of 1×10^6 cells/kg, was reported, when given to very preterm neonates at a corrected age of 36 weeks with severe BPD.[16]

STUDY DESIGN AND ETHICAL ISSUES OF FUTURE NEONATAL STUDIES WITH MSC

In the past, several therapeutic interventions in neonatology were introduced into clinical practice based on scant preclinical studies. To prevent a similar scenario in MSC-based therapy, resources should be invested in well-designed clinical trials that aim to demonstrate scientifically supported mechanisms of action and the optimal patient population while carefully monitoring the effect of the intervention. The trials should use specific definitions of BPD severity in order to select the highest risk patients and to assess the effect on different components of disease.

We are mindful that recent clinical trials with MSC in adults have failed to show a benefit—despite encouraging preclinical evidence. About a quarter of "advanced" therapies introduced more broadly in medicine result in discontinued trials.[17] Significant resources are committed to developing such therapies.[18] Since the proposed mode of action in preterm neonates differs from adults, attempts at translation should not be halted, but care must be taken to learn from the interventions in adults. This will reduce unwarranted hopes from animal studies being extrapolated to human care.[19]

In a first step, clinical trials should provide a better understanding of MSC biology and mechanisms of action rather than aiming to obtain statistically significant reductions in clinical endpoints. This first phase could be characterized as "hypothesis generation". This should lead to short- and long-term definitions of disease phenotypes most likely to benefit from MSC-based interventions. For these diseases, a homogeneous patient population should be enrolled and monitored for well-defined outcome measures that helps to further develop MSC-based interventions.[20]

Exposure of preterm neonates to a new intervention is associated with a potential risk of side effects. We suggest it is unethical to treat infants without fully exploring the data to learn more about the mechanism of action. In order to maximize scientific output from clinical studies, data from all neonates exposed to MSC-based interventions should be collected prospectively and made available for additional analysis.

Furthermore, attention to methodological rigor is needed. This includes ensuring adequate randomization to the intervention and placebo. Randomization in clinical studies has generally been perceived as necessary only in large confirmation studies. Early exploratory stage studies of experimental agents, as in stem cell therapy, are predominantly open label case series. Yet in 1977, Chalmers cautioned against the exploding acceptance of then innovative—but experimental—therapy of indomethacin in neonatology, based only on uncontrolled studies.[21–23] Chalmers argued for a robust "randomization from the first patient".[21] This seems appropriate now for stem cell therapy for BPD.

KEY ENDPOINTS FOR CLINICAL STUDIES ON MSC

Problems with existing BPD definitions partly explain why so few therapies have been successfully guided through the discovery and regulatory approval process for BPD prevention. It is now appreciated that development of BPD at 36 weeks PMA does not correlate well with long-term pulmonary health.[5] As well as being a distinct endpoint, a BPD diagnosis can also be viewed as a practical assessment of lung function at a time of transition to home. A major problem is that well-validated endpoints at later time points (e.g. 1 year corrected age) have not been definitively established. New studies are focusing on parental questionnaires to capture the burden of disease after hospital discharge, as well as a validated severity score at 1 year of age based on the need for supplemental oxygen, hospital readmission, or specific respiratory medications.

AN INTERNATIONAL COLLABORATIVE APPROACH TO SOLVE OPEN PROBLEMS IS NEEDED

While preclinical evidence suggests a beneficial role of exogenously administered MSC to prevent BPD, clinical development is currently hampered by a variety of significant obstacles. The major problems impeding translation can be summarized as (i) lack of appropriate cell material that meets regulatory requirements for obtaining subsequent *Marketing Authorization*, (ii) insufficient tools to monitor treatment efficacy, and (iii) lack of definition of the optimal population to target for therapy.

In order to overcome these problems and promote translation while ensuring patient safety, the Collaboration to use **M**SC to **A**meliorate **S**evere **C**omplications of Prematurity (***MASC-Collaboration***) was founded. The collaboration coordinates the expertise of key stakeholders such as scientists, clinicians, parent/patient organizations, regulatory authorities, and industry to overcome some of the obstacles.

By addressing the following issues in particular, the MASC-Collaboration strives to make MSC-based regeneration an integral part of neonatal care within the next decade.

Appropriate cells

MSC are ubiquitous in human connective tissues and can be isolated from different anatomical sources, with bone marrow being most commonly used in the past. Depending on the source, MSC have a different developmental origin and thus exhibit various biological functions, transcriptomic patterns, and differentiation potential.[24] Comparing MSC derived from bone marrow (BM), adipose tissue (AT), Wharton's Jelly of the umbilical cord (UC), and placenta (PL), it has been demonstrated that UC-MSC had the strongest immunomodulatory and immunosuppressive potential.[25] While UC-blood-derived MSC showed the highest rate of cell proliferation and clonality when compared with BM- or AT-MSC, they had lower expression of various senescence markers.[26] Furthermore, UC-MSC express more genes involved in angiogenesis and neurogenesis, which was associated with better neural differentiation and neural cell migration and better neuroprotection in vivo.[27] Finally, a gender effect has recently been described, with MSC obtained from female donors being more efficient in an animal model of neonatal hyperoxia-induced lung injury.[28]

Hence current knowledge suggests that MSC obtained from UC may be preferable for several reasons: (1) UC is available in large quantities from ethical and acceptable sources; (2) UC-MSC appear superior to BM-MSC with regard to the number of colony-forming units and the immunomodulatory potential[29]; (3) there is some evidence suggesting that UC-MSC are even more immunomodulatory than BM-MSC in their ability to interfere with the function of antigen-presenting cells.[30,31]

Although the choice of UC as a source of MSC is attractive from a practical point of view, several questions remain.[32] Although cord blood represents an easily accessible source, it is also used for allogeneic transfusions in preterm neonates or as a source for hematopoietic stem cells. Several groups have used Wharton's Jelly of the umbilical cord for production of MSC. However, the yield was relatively low requiring substantial ex vivo expansion up to passage 4. To address this limitation, a method was developed to isolate MSC from UC tissue which does not require excessive ex vivo expansion.[33]

Table 1. Potential issues to be addressed to bring cell-based therapies into clinical routine.

Disease
Serious or life-threatening
Unmet medical need
Patient
High risk to develop the disease
Intervention
Better and potentially safer than any other available therapies
Preliminary nonclinical and/or clinical evidence suggesting the possibility of substantial improvement of a clinically meaningful endpoint
Challenges related to cell-based products
Significant cellular heterogeneity and multiple potential mechanisms of action with different cell types
Keeping cells viable for prolonged periods of time
Lack of appropriate reference standards
Difficulty in controlling variability, inability to sterilize the products, and the use of small lot sizes

Assuming UC tissue will be the primary source of MSC for future studies, manufacturing still represents a complex process that depends on isolation methods, culture time, and media composition.[34] Thus clinical use of freshly prepared MSC is feasible, but may not be practical for widespread use.[35] However, an approach that reduces variability and focuses on a standardized manufacturing process could be highly beneficial. This would enhance development of a GMP-quality product that can be made available off-the shelf, after freeze-storage, and, ultimately subsequent thawing at the bed-side. To obtain the *Marketing Authorization* for this cell product, it will be helpful to base all preclinical work on cells which will be used in future efficacy trials.

Monitoring efficacy of treatment

Current knowledge regarding the beneficial effects of MSC is mainly based on animal models. However, the mechanism of action is still poorly understood mainly due to the limitations associated with some of these animal models that may not truly reflect preterm neonates. Most rodent experiments were performed in term animals exposed to hyperoxia, a situation that differs from the immature infant exposed to extra-uterine conditions.

Prior to wide introduction of this intervention into humans, data would be needed from a nonhuman primate model of prematurity in order to better understand the mode of action and to find the optimal parameters to measure safety and efficacy in high-risk preterm neonates.

The optimal target population

In order to identify the optimal target population for MSC-based therapies, the MASC-collaborators are prospectively planning phase I trials in different populations. A phase I trial is starting soon in Canada in preterm neonates at 2 weeks of age (still requiring significant respiratory support), European collaborators will study safety in extremely preterm neonates within the first few days of life, and US collaborators will study preterm neonates at 36 weeks PMA who have developed severe BPD. All available clinical data will be collected in a prospective meta-analysis and patient registry.

MERGING DIFFERING INTERNATIONAL REGULATIONS

The first priority in developing an MSC-based intervention is patient safety, which will require collaboration between investigators, sponsors, and regulatory agencies to obtain high-quality preclinical and clinical safety and efficacy data. Furthermore, a harmonized approach between different agencies will ensure a quick transfer of knowledge, prevent unnecessary delay, and reduce costs. Regular regulatory cluster consultations between FDA, Health Canada, the European Medicines Agency, and many others are taking place on a routine basis to help facilitate these types of studies.

Phase I trials will focus on safety. In preterm neonates, there is a highly variable rate of background complications (in different parts of the world), making a safety analysis very challenging. To expedite the development of high profile treatments such as cell-based therapies, multiple issues must be addressed (Table 1). It is critically important that we bring effective new therapies such as MSC to neonates as quickly as possible. Improvements in speed and efficiency will require engaging multiple global key stakeholders. The infrastructure, regulatory guidance, and collaborative efforts exist and need to be leveraged.

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AUTHOR CONTRIBUTIONS

All authors have equally contributed to the manuscript, have read the final version of the manuscript and agreed on the final version.

ADDITIONAL INFORMATION

Competing interests M.R. and members of his working group hold a patent on isolating mesenchymal stromal cells; all other authors have nothing to disclose.

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