

Review

Stem cells and Bronchopulmonary Dysplasia - The five questions: Which cells, when, in which dose, to which patients via which route?



Martin Mueller^{a,b,c,*}, Boris W. Kramer^{d,e,f}

^a Department of Obstetrics and Gynecology, University Hospital Bern, Bern, Switzerland

^b Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA

^c Department of Clinical Research, University of Bern, Bern, Switzerland

^d Department of Pediatrics, Maastricht University Medical Center (MUMC), Maastricht, The Netherlands

^e School for Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands

^f School of Oncology and Developmental Biology (GROW), Maastricht, The Netherlands

EDUCATIONAL AIMS

The reader will come to:

- Understand the definition of stem cells.
- Understand different sorts and sources of stem cells.
- Understand the basic concept of stem cell therapy.
- Appreciate the use of stem cells as a promising new therapy for the prevention of the BPD.
- Realise that testing in clinical medicine is happening, although essential questions about efficacy and ethics remain to be clarified.

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SUMMARY

Preterm birth is the leading cause of death in newborns and children. Despite advances in perinatology, immature infants continue to face serious risks such as chronic respiratory impairment from bronchopulmonary dysplasia (BPD). Current treatment options are insufficient and novel approaches are desperately needed. In recent years stem cells have emerged as potential candidates to treat BPD with mesenchymal stem/stromal cells (MSCs) being particularly promising. MSCs originate from several stem cell niches including bone marrow, skin, or adipose, umbilical cord, and placental tissues. Although the first MSCs clinical trials in BPD are ongoing, multiple questions remain open. In this review, we discuss the question of the optimal cell source (live cells or cell products), route and timing of the transplantation. Furthermore, we discuss MSCs possible capacities including migration, homing, pro-angiogenesis, anti-inflammatory, and tissue-regenerative potential as well.

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INTRODUCTION

One of the most complex multifactorial syndromes is preterm birth and, not surprisingly, the leading cause of death in newborns and children [1,2]. Despite advances in perinatology, immature

infants continue to face serious risks during pregnancy, parturition and adaptation after birth. Advances in perinatal care have reduced mortality but morbidity still represents a major burden which is evident in very low gestational age infants (< 32 weeks gestation) [3,4]. The magnitude of this burden to both society and family is enormous. Besides prematurity related cerebral morbidities including spastic cerebral palsy and behavioral deficits [5,6], approximately 25% of immature infants are confronted with long-lasting consequences such as chronic respiratory impairment in the form of bronchopulmonary dysplasia (BPD) [7]. Changes in clinical management have reduced the incidence

* Corresponding author. Maastricht University Medical Center, Department of Pediatrics, P. Debyeelaan 25, 6202 AZ Maastricht, The Netherlands.
Tel.: +31 43 387 4202; fax: +31 43 387 5246.

E-mail addresses: martin.mueller@insel.ch (M. Mueller), b.kramer@maastrichtuniversity.nl (B.W. Kramer).

of BPD significantly, with a shift towards extreme low gestational age newborns developing BPD. Thus nowadays, the pathogenesis of BPD is as a developmental disorder consisting of immaturity and impaired alveolar and capillary development leading to incomplete regeneration of the lung [8].

The basic concept of stem cell administration is the prevention of BPD. However, BPD is a term which is currently used to describe the chronic problems of preterm infants that survived up to 36 weeks post menstrual age, [PMA] [9]. In other words BPD is based on a single time point in the continuum of disease and does not take into account the babies that have already died because of lung problems. The time point of 36 weeks PMA is questionable with respect to its predictive value for the future risk and retrospective value in omitting newborns that may have already died of more severe lung disease. The clinical management of BPD in the perinatal period is focused on gas exchange. However, it is not the most common problem. The pathogenesis of BPD is driven by chronic inflammation in the airways which may have its origin antenatally. Further, postnatal triggers such as mechanical ventilation, sepsis, and oxygen toxicity may sustain and aggravate the existing pulmonary inflammation [10]. Since systemic steroids modulate inflammation in the airways, but have detrimental side effects, a local approach would seem preferable. For example, the local application of glucocorticoids has been tested with encouraging effects [11].

In very general terms, we have to consider the development of BPD as an imbalance of chronic injury and incomplete resolution of inflammation coupled with imperfect repair/regeneration. In the context of a lung developing outside the uterus, it is an environment of high oxygen exposure, mechanical stress, and sepsis with a nutritional supply that may be suboptimal. Together, the chronic inflammation may affect both the alveolo- and vasculo-genesis of the developing lung which forms the basis of a disease spectrum that we encounter on the ward and in the out-patient setting.

Although the pathophysiology of the preterm birth syndrome is complex, survivors share morbidities with a common link. It is

accepted that inflammatory responses explain the common features of multi-organ injuries in these infants. For example, the initial influx of neutrophils, followed by increased numbers of macrophages, represents the characteristic inflammatory changes in BPD [12]. Further, altered growth factor signaling such as the release of cytokines and disturbance of growth factor signaling is present. One of the key factors is transforming growth factor (TGF)- β which induces apoptosis [13]. Other examples are angiogenic growth factors, such vascular endothelial growth factor (VEGF) and its receptors, which modulate dysmorphic capillaries and influence the subsequent development of pulmonary hypertension [14]. Additional remodeling or early alveolar epithelial dysfunction promote lung inflammation [15]. Although the pathogenesis of BPD is well defined, current treatment approaches such as supportive therapies in combination with vitamin A and caffeine are insufficient and the results often remain unsatisfactory [16].

Consequently, new therapeutics addressing inflammatory responses and promoting lung regeneration are desperately needed. Mesenchymal stem/stromal cells (MSCs) possess this potential [17]. They were shown to modulate innate and adaptive immune responses, to have anti-apoptotic effects, to decrease inflammation, and to enhance tissue repair, mostly through the release of paracrine factors (Figure 1) [18,19].

STEM CELLS

Stem cells are defined by their properties with respect to their ability to undergo self-renewal (proliferation) and to give rise to more differentiated cells (differentiation) [17]. Proliferation means the cells renew in principle for an indefinite period. Differentiation implies changing of the cell properties in cells derived from the stem cells. These cells that are no longer stem cells have different functional and phenotypic properties than their parent (stem) cell. The extent of differentiation of cell types in which stem cells can differentiate is called potency [20]. In contrast, totipotent or embryonic stem cells are cells that can differentiate in all cell types, namely into placental and embryonic tissues. Such individual stem

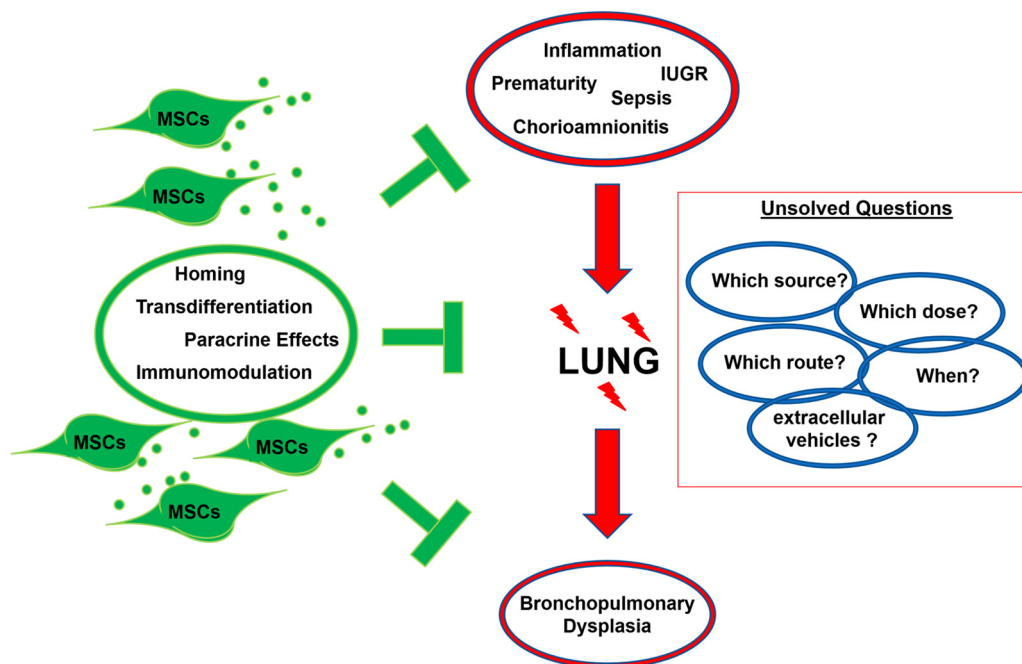


Figure 1. MSCs induced protection of the lung Multiple factors contribute to neonatal lung injury resulting in bronchopulmonary dysplasia (BPD). These factors include inflammation, prematurity, intrauterine growth restriction (IUGR), sepsis, and chorioamnionitis. MSCs protective capacity in prevention/reduction of BPD is based on following factors: homing to the injury sight, transdifferentiation of MSCs, production of paracrine factors, and immune modulation. Although first MSCs clinical trials in BPD are ongoing, multiple questions remain open: Which MSCs source, at which dose, when, and which route is the optimal one.

cells can form complete individuals which explains the ethical considerations and limitation around these cells. Stem cells that can give rise to embryonic tissue are called pluripotent stem cells. Multipotent stem cells can differentiate to adult cell types. The best known adult stem cell is probably the hematopoietic stem cell.

MESENCHYMAL STEM/STROMAL CELLS

Mesenchymal stromal or stem cells are cells that can give rise to tissue types such as cartilage, bone marrow and fat tissue. Stem cells that can differentiate into multipotent MSCs were first described by Friedenstein et al. in 1970 in hematopoietic organs, mainly bone marrow. The term “mesenchymal stem cell” was first used in 1991 by Caplan [21,22]. In recent years clinical and animal studies have focused on mesenchymal stem/stromal cells (MSCs) as a potential cell graft to prevent/repair perinatal acquired injury [17]. MSCs originate from stem cell niches such as bone marrow (BM), skin, or adipose umbilical cord, and placental tissues and therefore are considered somatic stem cells [23]. MSCs must adhere to plastic when cultured in a tissue culture flask and express the surface markers CD73, CD90, and CD105 while lacking expression of CD45, CD34, CD14/CD11b, CD19/CD79 α , and HLA-DR [24] in addition to having potency to differentiate into osteogenic, chondrogenic, and adipogenic lineages. From a clinical point of view, MSCs from placental tissue are of special interest. First, placental tissue provides a broad opportunity to harvest MSCs prenatally or postnatally without ethical concerns and as an autologous source. MSCs can be isolated from placental membranes and tissue [25,26], amniotic fluid [27], umbilical cord blood [28] and umbilical cord connective tissue (Wharton's Jelly) [29,30]. Secondly, the predominant causes of preterm birth, such as preeclampsia, have no detrimental impact on the quality of MSCs [31,32]. MSCs are immune-privileged [33]. Finally, MSCs possess the capacity to adapt to the microenvironment of injured organs and therefore, having the heterogeneous pathophysiology of perinatal injuries in mind, MSCs may target multiple organs at once [17].

The next basic problem is the availability of the cells in sufficient numbers at specific time points. The autologous isolation from cord blood, placenta or Wharton's Jelly is an appealing option but raises serious questions [17]. For example, the process must be performed according to good manufacturing practice which implies the careful and time consuming assessment of potential infectious diseases. From a clinical point of view, the more immature the infant, the higher the risk for BPD. However, the most immature infant has the smallest volume of cord blood, smallest placenta and cord size, impacting on the yield of stem cells. Thus, the following questions need to be addressed:

- (1) If the number of autologous stem cells is insufficient, can we expand the cells in vitro to obtain the needed numbers?
- (2) If we expanded the cells in vitro, under which conditions, and in which time period, should the expansion be completed in order to meet the window of opportunity?

Notably, the autologous production process is not only prone to contamination but also potentially compromised by the loss of the sample which makes the autologous approach rather cumbersome.

MSCS AND BPD

MSCs based therapies are an attractive strategy since the strategy can potentially replace/restore lost tissue and/or protect/salvage injured cells. Firstly, clinical MSCs studies addressed the pro-angiogenic, anti-inflammatory, or tissue-regenerative

potential in adults [34]. Animal studies confirmed prophylactic and therapeutic MSCs' capacity to ameliorate BPD [35] leading to piloting clinical trials in neonates [36]. This clinical concept was tested in Korea with cells isolated from umbilical cord tissue. The cell populations were expanded in vitro to achieve sufficient cell numbers which were then given “off the shelf” from unrelated donors. In the pilot study, two different doses (in 9 preterm infants at 25 weeks of gestation with a mean birth weight of 790 g) via the intratracheal administration route were chosen [36]. This strategy was successful and longer-term follow-up of these pilot patients is proceeding. Larger studies testing the clinical feasibility are underway. Nevertheless, questions such as the mode of actions, the desired cell types or products, and the route of transplantation need to be addressed.

MSCs have been successfully studied in both preclinical and clinical studies and in several disorders such as cardiovascular, neurological, haemato-oncological, and auto-inflammatory conditions [34]. The approach of cell graft transplantation developed over the years has led to the first basic question: What type of transplant and application route is appropriate? Basically this concept includes the direct or peripheral transplantation of whole cells, conditioned medium (CM), or purified and defined components of CM (extracellular vehicles: EV). For example, both intratracheal and intravenous administration of BM-MSCs is protective in murine models of BPD [37,38]. Given that the protective effects were attributed in part to paracrine immunomodulation, both BM-MSCs and CM were successful [38]. Other cell sources and both short and long term outcomes were tested as well, showing comparable beneficial results [39]. The question as to whether whole cells or rather cell products are most suitable for BPD remains open and several factors need to be considered. The peripheral approach is based on key properties of MSCs including delivery of the cells “homing” to the site of injury. Interestingly, migration and homing are influenced by multiple factors including age, passage, and number of cells, culture conditions, and delivery method [40]. This migration and homing potential without tumorigenic potential has been described in different disease [41,42] and in BPD models previously [38,43]. Chemokines with chemokine receptors CXCR3, CXCR4 and CXCR6 were identified as the key players responsible for cell homing [44]. Further, CXCR4 ligands such as stromal cell-derived factor-1 α (SDF-1 α) promote MSCs' migration to the injury site [45]. Apart from migration and homing, recent studies have focused on cell free approaches such as EV [46]. Multiple vehicles are present in the extracellular space, including shedding vesicles, apoptotic bodies and exosomes. Exosomes (40–100 nm in diameter) are secreted by cells and may transfer proteins and functional genetic materials (i.e. mRNA and microRNAs). They are involved in cell-to-cell signaling/regulation [47]. Although this approach is very promising, it is still in its infancy. We also have to consider that stem cells encourage the growth and even supplement (host) cells [48]. Additionally, the immunomodulation potential of MSCs may depend on the bidirectional communication between the injured host cells and the graft [49]. Together multiple transplantation routes and graft types have been successfully tested in diverse injury models including BPD. Regardless of the transplantation route, the basic mode of action is paracrine immunomodulation [17].

MSCS AND MODE OF ACTION

MSCs response to injury is a crucial advantage over non-cell based therapies. A vast amount of data has originated from adult pulmonary diseases or in-vitro studies. Studies have identified key factors including cytokines, interleukins, and trophic factors predominately involved in angiogenesis, hematopoiesis or neuronal and cardiovascular regeneration [50–52]. Classical examples

are interleukin-10 and TNF-stimulated gene/protein 6 which both possess anti-inflammatory capacity [33]. Stanniocalcin-1, hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) promote lung development or oxidative stress resistance [53–55]. Importantly, MSCs and/or their secretome have effects well beyond promoting lung regeneration as well as the modulation of immune responses [38,47]. For example, MSCs inhibit autoreactive T cell responses and shift the alveolar macrophages from a M1 (pro-inflammatory) to a M2 (protective) phenotype resulting in reduced pulmonary injury [56]. Besides T-cell modulation [57], MSCs inhibit B cell proliferation, neutrophils and monocytes function, and NK toxicity [58–61]. All these paracrine and immunomodulatory properties are present after both whole cell and cell derived product transplantation. However, differences in treatment outcomes were reported. For example, CM seems to be inferior in long-term effects but equal or superior in short-term effects compared to whole cell therapy [38,62]. Thus, a direct interaction with injured cells may potentiate therapeutic efficacy and in the case of CM or CM products multiple treatments may be appropriate [35,63]. The concept of MSCs sensing, integrating, and responding to the surrounding is intriguing as MSCs potential to replace injured cells is not proven and a matter of constant debate [64–66]. For example, MSCs improve myocardial infarction without permanent replacement after intravenous application [67]. However, these cells embolise and cause endothelial damage in the lung as well [67]. In conclusion, MSCs' low rate of in vivo engraftment and differentiation suggest paracrine immunomodulation, but whether factors released by the MSCs or the cells themselves are the best choice remains an open question. Especially as whole cell transplantation carries risks of tumorigenic potential [21,22] this approach needs to be carefully considered. From a clinical perspective two scenarios are possible: (1) a malignant transformation of the cell transplant itself and (2) an immunosuppressive effect of MSCs, which leads to growth of existing malignant cells in a patient [68]. Notably, the tumorigenic potential of the stem cell is primarily affected by the manufacturing process and the cellular source. For example, the risk of tumorigenic potential is higher for autologous cells due to slower or absent clearance by the immune system after transplantation [68]. Although no malignant tumorigenic transformation has been previously reported, the potential for transformation must be further explored and monitored in a clinical setting with long follow-up period, especially as treatments is initiated in immature infants. Additional studies addressing the long term impact on the immune system needs to be addressed as well.

SUMMARY

In summary, MSCs represent new potent therapeutic options to treat multiple disorders associated with preterm birth including BPD. Given that BPD is a developmental disorder with deregulated inflammatory responses, MSCs primary mode of action is to modulate innate and adaptive immunity and decrease inflammation while enhancing tissue repair (Figure 1). While autologous MSCs such as placental tissue are easy available without ethical concerns, the question of sufficient cell number and availability at specific time point remains unanswered. Thus, the concept of cell products such as conditioned media or purified and defined components of condition media (extracellular vehicles) has emerged recently. The protective paracrine and immunomodulatory properties are present after both whole cell and cell derived product transplantation but several questions remain unanswered. Since whole cells may provide a direct interaction with injured cells, cell derived products may require multiple treatments. However, as whole cell transplantation carries risks of tumorigenic potential, this approach needs to be carefully considered. Future basic and clinical research needs to address following the knowledge gaps:

the optimal cell source (autologous versus allogenic), cell type (whole cells in which status versus cell derived products), cell dose (single versus multiple), transplantation route (intratracheal versus peripheral), and finally the patient population.

DIRECTIONS FOR FUTURE RESEARCH

Carefully designed studies with long term follow-up need to give the answers to the essential five questions:

- Which cells have to be given,
- when,
- in which dose,
- to which patients
- via which route?

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CME MCQ QUESTIONS

- 1) Bronchopulmonary dysplasia is defined as:
 - a. 35–37 weeks PMA inclusive
 - b. 36–37 weeks PMA inclusive
 - c. 36–38 weeks PMA inclusive
 - d. Degree of oxygen need/respiratory support at a certain age
 - e. 37–39 weeks PMA inclusive
- 2) Which of the following is true regarding stem cells?
 - a. Ethical dilemmas make their use almost impossible
 - b. Stem cells are defined by their proliferation and differentiation
 - c. Stem cells are an academic myth to generate research funds
 - d. Stem cells are useless outside the womb
 - e. Stem cells can only be used on the donor
- 3) Which of the following is true regarding sources of mesenchymal stem cells?
 - a. Mesenchymal stem cells are a commodity, traded in large quantities and in consistent quality
 - b. Mesenchymal stem cells can only be harvested from embryos
 - c. Mesenchymal stem cells can be isolated from different tissues
 - d. Mesenchymal stem cells can be isolated only from the mother of the baby
 - e. Mesenchymal stem cells can give rise to embryonic tissues such as placenta
- 4) Which difficulties may arise when using MSCs from an autologous source?
 - a. Insufficient numbers of cells
 - b. Cells not available at a specific time point
 - c. Cells are contaminated
 - d. Placenta can't be used because it is embryonic tissue
 - e. Loss of sample
- 5) Which of the following properties is not proposed MSCs' mode of action in BPD models?
 - a. anti-inflammatory
 - b. inflammatory
 - c. paracrine
 - d. homing
 - e. migration