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REVIEW ARTICLE

The Therapeutic Potential of Stem Cells for Bronchopulmonary Dysplasia: "It's About Time" or "Not so Fast" ?

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Abstract: ***Objective*:** While the survival of extremely premature infants has improved over the past decades, the rate of complications – especially for bronchopulmonary dysplasia (BPD) – remains unacceptably high. Over the past 50 years, no safe therapy has had a substantial impact on the incidence and severity of BPD.

***Methods*:** This may stem from the multifactorial disease pathogenesis and the increasing lung immaturity. Mesenchymal Stromal Cells (MSCs) display pleiotropic effects and show promising results in neonatal rodents in preventing or rescuing lung injury without adverse effects. Early phase clinical trials are now underway to determine the safety and efficacy of this therapy in extremely premature infants.

***Results and Conclusion*:** This review summarizes our current knowledge about MSCs, their mechanism of action and the results of preclinical studies that provide the rationale for early phase clinical trials and discuss remaining gaps in our knowledge.

Keywords: Therapeutic potential, stem cells, bronchopulmonary, dysplasia, MSCs, angiogenesis.

1. INTRODUCTION

1.1. Bronchopulmonary Dysplasia: Recognizing the Complexity and Challenge to Treat

Great advances in perinatal medicine have improved the survival of extreme low birth weight infants but this success has been tempered by the fact that these infants have an unacceptably high risk for developing complications such as Bronchopulmonary Dysplasia (BPD). Recent cohort studies report that the incidence of BPD in extremely low birth weight infants ranging between 30 to 70% [1-5]. It has been almost half a century since BPD was first described by Northway and colleagues in 1967 [6] but a cure for BPD still remains unavailable. The complexity of this debilitating chronic lung disease has hampered the discovery of efficient therapies. BPD remains the most common cause of morbidity and mortality in this group of infants [7-9].

Over the past five decades, as a result of the increasing survival of more premature infants, the disease pathogenesis has changed: Compared to the lung histology observed in premature infants before the surfactant era, the "new" BPD is characterized by a significant reduction in alveolarisation and vascularization. BPD now represents a combination of interruption of normal developmental signaling during early stages of lung development and a dysregulated repair process in response to perinatal insults (including inflammation, oxidative stress, and others) [10-12].

Postnatal corticosteroids, vitamin A, caffeine, lung-protective ventilation strategies, have been explored in large clinical trials but proven to be of limited success or unsafe in preventing BPD. Follow-up studies raised concerns about certain therapies such as dexamethasone due to its side effects especially on the premature brain [13-15]. Recent insights into stem cell biology have highlighted the potential use of these cells in regenerative medicine. Indeed, cell based-therapies have now advanced to be one of the most promising avenues for tissue repair including the preterm lung. More recent evidence suggesting dysfunction of resident lung stem cells in human lung diseases and animal models of BPD [16] provide further rationale for exogenous cell-based therapies to supplement the impaired endogenous repair mechanisms.

2. STEM CELLS: THE CONCEPT OF SELF-RENEWAL AND DIFFERENTIATION

Discovered by Till and McCulloch in 1961 [17], stem cells are generally defined as clonogenic cells capable of

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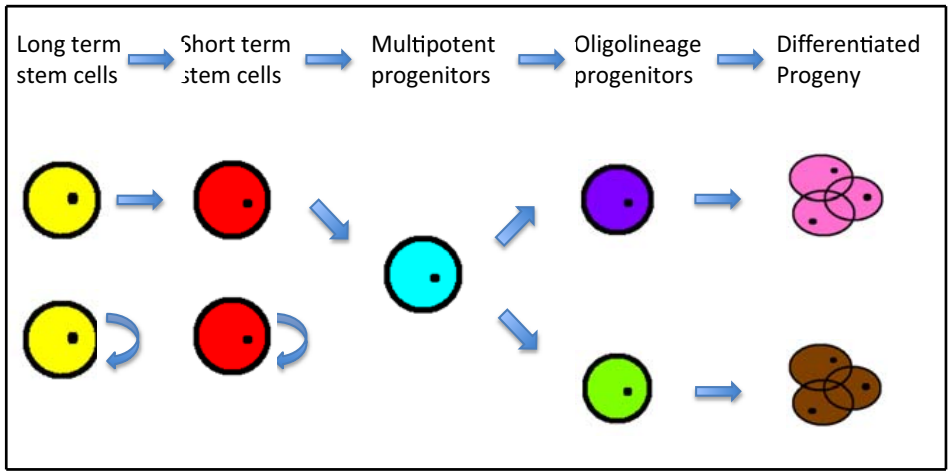

Fig. (1). Schematic diagram to illustrate the subsets of stem cells [18]:
Long term (LT) stem cells: highly self-renewing cells that reconstitute an animal for its entire lifespan, for example: LT-HSCs (Thy-1.1^{lo}Lin^{-} Sca1^{+}c^{-}Kit^{+})
Short term (ST) stem cells: self-renewing cells that cannot give rise to LT stem cells but reconstitute an animal for a limited period, for example: ST-HSCs (Thy-1^{lo}Lin^{-}Mac1^{lo}Sca1^{+}c^{-}Kit^{+})
Multipotent progenitors (MPP): arise from ST stem cells, for example: MPP-HSCs (Thy-1.1^{lo}Lin^{-}Sca1^{+}c^{-}Kit^{+}Mac1^{lo}CD4^{lo})
Oligolineage progenitors: Oligolineage-restricted cells, for example: common lymphocyte progenitors (CLP) and common myeloerythroid progenitors, alveolar epithelial progenitor cells such as alveolar type(AT)2 pneumocytes, dual-lineage bronchioalveolar stem cells [12].
Differentiated progeny: for example: CLP give rise to T lymphocytes, B lymphocytes and natural killer cells progeny, AT2 pneumocytes give rise to AT1 and mature AT2 pneumocytes progeny.

both self-renewal and multilineage differentiation (potency). Totipotent stem cells are the earliest cells in the ontogeny and extend from the zygote to the inner cell mass of the blastocyst. They are capable of differentiating into all adult, embryonic and extraembryonic tissues. Embryonic Stem Cells (ESC) are pluripotent stem cells capable of differentiating into derivatives of all three germ cell layers (ectoderm, mesoderm and endoderm). Multipotent stem cells are more restricted and give rise to multiple cell types of one lineage, such as Hematopoietic Stem Cells (HSCs). Residual pools of multipotent or unipotent stem cells are hypothesized to reside in almost all adult organs, contributing to their ability to repair and regenerate after injury [12, 17-20].

Their ability to self-renew and regenerate after damage maybe limited naturally due to advancing age and extent of the disease [21]. This can occur secondary to depletion of the residual stem cell pools or as a consequence of genetic or microenvironmental changes that prevent proper stem cell renewal and function. These changes can potentially be reversed via stimulation of the endogenous stem cell pools or the replacement/supplementation of exogenous stem cells. Such exogenous stem cell replacement therapies have been used for decades for hematological disorders using Bone Marrow (BM) derived HSCs and increasingly for treating debilitating metabolic disorders [22-27].

HSCs are dependent on the proper function of their niche cells: mesenchymal stromal cells (MSCs). Over the past two decades, the recognition of the repair capabilities of these cells, has advanced MSCs as the most promising cell therapy for organ regeneration. This is in part due to their pleiotropic effects, including immune modulation [28, 29], angiogenesis [30, 31], and anti-oxidant activity [32, 33], appealing properties for the treatment of a multifactorial disease, such as BPD.

3. MSCs: FROM NICHE CELLS TO POTENT REPAIR CELLS

MSCs were first described by Friedenstein and coworkers as adherent, fibroblast-like cells isolated from BM, stroma of the spleen and thymus [34, 35]. MSCs can differentiate into mesodermal lineage cells including osteoblasts, adipocytes, and chondrocytes *in vitro* and have the capability to self-renew. Since, MSCs have been isolated from almost every tissue, including adipose tissue [36], skeletal muscles [37], synovium [38], circulatory system [39], dental pulp [40], spleen, liver, kidney [41], umbilical cord [42], amniotic fluid [43], fetal blood, lung, liver and BM [44, 45].

Since its discovery in 1970s, MSCs have generated increasing interest but reported studies used different methods of isolation and expansion and different approaches to characterize the cells. In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposed minimal criteria to define human MSCs [46]:

1. MSC must be plastic adherent when maintained in standard culture conditions using tissue culture flasks.
2. Phenotype:
   - ≥ 95% of the MSC population must express CD105, CD73, and CD90, as measured by flow cytometry.
   - Must lack expression (≤ 2% positive) of CD45, CD34, CD14, or CD11b, CD79α or CD19 and HLA class II.

3. Cells must be able to differentiate to osteoblasts, adipocytes and chondroblasts under standard *in vitro* differentiating conditions.

While the more detailed characterization of MSCs remains a topic of intense investigation, studies on the repair potential of MSCs have been equally intense. Extensive preclinical experiments have demonstrated the capacity of MSCs to ameliorate tissue damage and to improve organ function after injury.

A milestone indicating the potential of MSC replacement in organ repair occurred in 1999 when Makino S. and colleagues isolated a Cardiomyogenic Cell Line (CMG) from murine BM stromal cells. After treatment with 5-azacytidine, these cells changed morphology and acquired cardiomyocyte-like ultrastructure, including synchronous beating rhythm and expression of atrial natriuretic peptide and brain natriuretic peptide [47]. Shake J. G. and colleagues showed that MSCs engrafted in host myocardium, expressed muscle specific proteins and improved contractile dysfunction in the swine model of left ventricular wall infarction [48]. Although it was subsequently recognized that tissue engraftment is not the main mechanism of action of MSCs, their therapeutic benefit has been demonstrated extensively in acute lung injury [49, 50], acute and chronic kidney injuries [51-53], acute pancreatitis [54], liver fibrosis [55] and autoimmune encephalitis [56, 57].

Currently, there are more than six hundred clinical trials utilizing MSCs being conducted for adult diseases, such as in heart failure, inflammatory bowel disease, asthma, acute respiratory distress syndrome, cystic fibrosis, idiopathic lung fibrosis, osteoarthritis, diabetes mellitus, and various neurological diseases.

4. MSCs AND PRECLINICAL STUDIES IN BPD

4.1. Animal Models

In order to determine the beneficial effect of MSCs in human premature lung, various animal models have been explored to mimic the pathological features of BPD [58]. Rodents (rat and mice) exposed to hyperoxia have been widely used. While these rodents are not delivered preterm and are otherwise healthy, the advantages of this model include birth at the saccular stage (equivalent to 26-28weeks of human gestation) of human lung development. [59, 60], low cost, fast turnaround, low maintenance, making this model ideal for rapid proof of concept experiments. Premature rabbits, lambs and piglets have also been employed to mimic BPD. The need for preterm delivery requires the need for mechanical ventilation and other life-sustaining interventions, making these models more clinically relevant, but labor intense and expensive [61-64]. The other interesting model using prematurely delivered baboon at 125 days (equivalent to 27 weeks of human gestation) and mechanically ventilated for 2 weeks offers great opportunity to test the effect of MSCs therapy in a model very close to the human setting [65]. These large animal models allow ultimate feasibility, safety and efficacy studies that may be required for regulatory approval of cell products.

4.2. Proof of Concept in Rodent Models of BPD for Testing the Therapeutic Benefits of MSCs

In 2007, Tian and colleagues has shown that Intravenous (IV) injection of $5x\ 10^4$ (approximately 5×10^6/kg) BM derived MSCs (BM-MSCs) improves radial alveolar count and reduces inflammatory cytokines [Tumor Necrosis Factor Alpha (TNFα) and Transforming Growth Factor Beta-1 (TGFβ-1)] in the rat model exposed to 95% O_2 [66]. This finding is supported by Aslam and colleagues in 2009, who showed normalized lung structure with attenuation in vascular remodeling in hyperoxic mice by IV BM-MSC injection [67]. Subsequent studies confirmed the same beneficial effects in the hyperoxic rat model [68-71].

Besides the IV route, intratracheal (IT) administration has been investigated by van Haaften and colleagues in 2009. In this prevention study, BM-MSCs improve survival, exercise tolerance and lung structure and reduce vascular changes secondary to pulmonary hypertension [72]. The benefits of this route are further supported by Chang and colleagues in 2011, 2013, Ahn and colleagues in 2015, Sung and colleagues in 2015 and Kim and colleagues in 2016 [73-77]. Most of these animal studies seem to indicate that early MSC replacement confers better lung protection than late therapy.

The efficacy and safety of Human Umbilical Cord/Cord Blood (HUC/B) derived MSCs in these animal models (xenogeneic therapy) suggest that allogenic transplantation is feasible in the clinical setting. The concept of harvesting HUC/B-MSCs after extreme premature delivery for autologous transplantation later to prevent or rescue BPD is worth considering, without jeopardizing the benefits of delayed cord clamping or umbilical cord milking. Alternatively, a ready to use, off-the-shelf MSC product may be logistically easier. Interestingly, Di Bernado and colleagues found that placenta derived MSCs (PL-MSCs) are as potent as BM-MSCs in terms of increasing alveolar surface area, branching morphogenesis and stimulating surfactant production in an *in vitro* study [78]. In an *in vivo* study using the combined perinatal inflammation and hyperoxic lung injury rat model, IT PL-MSCs reduces inflammatory cytokines and improves lung structure and vascular density [79]. The beneficial effects of MSCs are not only limited to the lung as IT injection of HUCB-MSCs has been shown to reduce brain inflammation and neuronal apoptosis [77].

4.3. Long Term Effects of MSCs

It is prudent to consider the potential long-term side effects of MSCs replacement therapy in the preclinical setting before pursuing clinical trials. In 2013, Pierro and colleagues have studied the short- and long-term effects of IT injection of both HUCB-MSCs and HUC derived perivascular cells (HUC-PCs, putative precursors of MSCs) as both preventive or rescue therapy in the hyperoxic rat model. Their study shows improved alveolar growth, improved lung compliance and reduced pulmonary hypertension in the short term, with long term preserved exercise tolerance and alveolar structure at 6 months of age. There were no observed side effects including tumor formation at 6 months [80]. The same safety profile is observed in the studies by Sutsko and colleagues at

postnatal day 100 and by Ahn and colleagues at postnatal day 70 [81, 82].

4.4. Paracrine Effect of MSCs: Medicinal Signaling Cells [83] with Pleiotropic Effects

4.4.1. Low Cell Engraftment

The initial working hypothesis stipulated that MSCs would replace injured cells by engrafting and differentiation into the target cell type (heart, kidney, lung *etc*). However, contrary to this cell replacement hypothesis, very few engrafted cells could be found [84]. In the newborn rat BPD model, a low number of MSCs engraft as visualized by deconvolution microscopy and quantified by qRT-PCR, respectively [72, 80]. The observed engraftment of MSCs in the lung ranges from 0 to 20 percent in the majority of studies [52-55]. There is emerging evidence that most of the beneficial effects of stem cell therapy are due to cell-derived paracrine factors, rather than cell engraftment [85]. Tropea and colleagues in 2012 show that treatment of bronchoalveolar stem cells with MSCs derived conditioned media increases their growth efficiency, which might contribute to lung repair after injury [68]. However it is still unknown whether utilizing conditioned-media replacement alone will be superior to MSCs transplantation.

4.4.2. MSCs as Potent Immune Modulators

MSCs interact with cells of the innate and adaptive immune systems [86]. MSCs suppress inflammation, *via* their soluble factors, including interleukin(IL)-6, M-CSF, IL-10, TGFβ and prostaglandin E2 [87-89]. MSCs upregulate anti-inflammatory Th2 cytokines, including IL-3, IL-5, IL-10 and IL-13 and downregulate proinflammatory Th1 cytokines, including IL-1α and β, interferon-gamma(IFNγ) and TNFα [90, 91], culminating in reduced fibrosis. In the series of *in vivo* studies by Prockop *et al.*, MSCs secrete TNFα stimulating gene/protein (TSG)-6 that reduces the inflammatory response [92-95]. Furthermore, MSCs display important anti-apoptotic properties in vital organ injury [96]. This immune modulation effect has been shown in most of the rodent studies of BPD [66, 67, 69, 71, 73, 76, 77, 79, 85] and is particularly important to attenuate inflammation and cell deaths in BPD and to restore lung and vascular growth.

4.4.3. MSCs are Pro-angiogenic

In addition, prostaglandin E2 enhances the secretion of vascular endothelial growth factor resulting in improved angiogenesis [86]. HUC-MSCs attenuate remodeling after myocardial infarction by pro-angiogenic, anti-apoptotic and endogenous cell-activation mechanisms. All these effects were paracrine mediated [97, 98]. A recent study by Reiter, J. and colleagues showed that this pro-angiogenic effect is enhanced by stromal derived factor-1 that mediates lung regeneration in the rodent model of BPD [99, 100].

4.4.4. MSCs Enhance Bacterial Clearance

There are also exciting findings regarding the bactericidal properties of MSCs via the secretion of bioactive molecules which are antimicrobial *in vitro* against *Pseudomonas aeruginosa, Staphylococcal aureus* and *Streptococcal pneumonia* [101]. *In vivo*, BM-MSCs infused into septic mice [102] and HUC-MSC into a neonatal *Escherichia Coli* sepsis model in rats, improve survival and enhance bacterial clearance in part through the production of the antimicrobial peptide LL-37 [103].

4.4.5. MSCs Display Anti-oxidant Activity

Anti-oxidant effects have been shown to ameliorate various organ injuries such as connective tissues, intestines and spinal cord [104-106]. Brief hyperoxic pre-conditioning of BM-MSC increases the production of the anti-oxidant stanniocalcin-1 and enhances lung protection in the rodent model of BPD [107].

These promising preclinical study results have prompted early phase clinical trials to examine the feasibility and safety of MSC therapy in preterm infants at risk of developing BPD.

5. CLINICAL TRIALS USING MSC FOR BPD

In 2014, Chang and colleagues conducted the first Phase 1 dose-escalation clinical trial in 9 premature infants (mean gestational age 25weeks, mean birth weight 793g) at high risk of developing moderate to severe BPD (NCT01632475). They administered $1x10^7$ or $2x10^7$ allogeneic HUCB-MSCs *via* IT route from postnatal day 7 to 14 and observed no immediate or short-term toxicity or adverse events compared to a historical control group [108]. Study participants were subsequently followed up for two years (NCT02023788) and no adverse effects in respiratory status, growth and neurodevelopment were reported [109]. There are currently 3 Phase I and 1 Phase II trials being conducted to assess the safety and efficacy of HUCB-MSCs in preterm infants.

Even though early phase of clinical trials have begun, it is critical to further our knowledge on MSC biology to better understand their exact mechanisms of action and thus optimize the manufacturing process of MSCs.

6. REMAINING CHALLENGES FOR THE CLINICAL TRANSLATION

6.1. Uniformity and Safety of MSC Products

There is abundant evidence in the animal literature that MSCs are lung protective, and it is timely to perform well-designed early phase clinical trials to advance our understanding about the safety and therapeutic potential of MSCs in preterm infants. A challenge is the careful characterization of clinical-grade MSCs to be used in these trials, because of the many steps in the manufacturing of a cell product [110, 111]. Causes of variations– impacting efficacy and safety – are plentiful and include amongst others: cell source, isolation process, number of passages/doubling times, freezing method, storage, thawing and administration procedure. For example, cryopreservation and thawing can impede MSC function [112] and bio-distribution [113], whereas a 24-hour culture period post-thawing or IF-γ licensing restores MSC function [114]. These findings highlight just one of the multiple gaps in our understanding of the biology of MSCs and how to harness their healing potential in the clinic. It also emphasizes the importance for the need of reliable potency assays that predict the therapeutic potential of a given cell product before release and infusion into patients.

Table 1. Summary of MSCs clinical trials in BPD.

Phase	Design	Number of Participants (Expected or Final)	Cell Origin	NCT ID
I	Open	10	MSCs (not specified)	NCT02443961
I, II	Open	12	HUCB-MSCs	NCT02381366
I	Randomized, placebo-controlled, double-blind	10	HUC-MSCs	NCT01207869
I	Open	9	HUCB-MSCs	NCT01297205
II	Randomized, placebo-controlled, double-blind	70	HUCB-MSCs	NCT01828957
II	Randomized, placebo-controlled double-blind	70	HUCB-MSCs	NCT01897987
I	Open, observational	9	HUCB-MSCs	NCT01632475
I	Open, observational (2 year follow up from study NCT01632475)	9	HUCB-MSCs	NCT02023788

HUCB-MSCs: human umbilical cord blood derived MSCs.
HUC-MSCs: human umbilical cord derived MSCs.

Table 2. Summary of the preclinical studies using MSCs/ MSCs derived conditioned media (MSCs-CM).

Experimental Model	Therapeutic Cell or Product	Therapeutic Outcomes	Suggested Mechanisms	Ref.
Tian 2007 Hyperoxic-95% rat	IV 5 x10^4 BM-MSCs	Increased Radial alveolar count	Engraftment Reduced TNFα and TGFβ1	[66]
Aslam 2009 Hyperoxic-75% mice	IV 5x10^4 BM-MSCs or MSC-CM at PN4	Both groups: Normalized alveolar number, improved vascular density, Reduced PHT vascular remodeling, Reduced macrophage and neutrophils count Reduced IL-5, IL-17, TNFα in MSC-CM	Minimal engraftment Paracrine	[67]
van Haaften 2009 Hyperoxic-95% rat	IT1x10^5 BM-MSCs at PN4 and PN14	*In vivo*: Improved survival Improved exercise tolerance Reduced MLI and improved vascular density and PHT *In vitro*: MSC-CM prevented O_2 induced AEC2 apoptosis, accelerated AEC2 wound healing, Enhanced endothelial cord formation	Low engraftment Paracrine effect PN 4 better than PN14 therapy	[72]
Chang 2011 Hyperoxic-95% rat	IT UCB-MSCs comparing 5x 10^3, 5x10^4, 5x10^5 At PN5	For doses of 5x10^4 and x10^5 Reduced mean linear intercept, mean alveolar volume, collagen level Reduced MPO activity, mRNA levels of TNF-α, IL-1β, IL-6, TGF-β, reduced cytosolic and membrane p47	Engraftment Paracrine effect-aninflammatory and modulation oxidative stress	[73]

(Table 2) Contd…

Experimental Model	Therapeutic Cell or Product	Therapeutic Outcomes	Suggested Mechanisms	Ref.
Waszak 2012 Hyperoxic-95% rat	IP BM-MSC-CM or preconditioned CM after 24hrs BM-MSC exposed to 95% O_2 for 21d	Both Improved alveolar growth, MSC-O_2CM PHT prevention Higher level of antioxidant stanniocalcin-1 in MSC-O_2CM	Paracrine and preconditioning More effect seen with MSC-O_2CM in PHT prevention	[107]
Tropea 2012 Hyperoxic-75% mice	IV $5x10^4$ MSCs at PN4 and IV MSC-CM and control at PN4	Increased Bronchoalveolar Stem cells *in vivo* Improved alveolar density and surface area	MSC and MSC-CM are equally effective in promoting BASC growth	[68]
Zhang 2012 Hyperoxic-95% rat	IV 1x 10^5 BM-MSC PN10	Improved weight Increased in radial alveolar count Reduced in TNFα and TGF β-1 but increased in IL-10	Engraftment Paracrine effect Immunomodulation effect	[69]
Hansmann 2012 Hyperoxic-75% mouse	IV BM-MSC-CM	Reversed parenchymal fibrosis, peripheral PA devascularization, partially reversed alveolar injury, normalized lung function, fully reversed the moderate PH and RVH	Paracrine effect via CM	[134]
Pierro 2013 Hyperoxic-95% rat Short term, long term, paracrine effect	*In vivo* cell: IT $3x10^5$ or $6x10^5$ HUC-MSC or HUC-PCs at PN4 (EP P22) or PN14 (EP P35) Long term treated at PN4 and harvested at 6m *In vivo* CM: IP 7ul/kg CM from PN4-21 (EP P22) and PN14-28 (EP P35) Long term Treated PN4-21 and harvested at 6m	Preserved alveolar growth Improved lung compliance Reduced pulm hypertension Long term: Improved exercise capacity Normalized alveolar structure	Low engraftment Both HUC-MSC/HUC-PC are effective Both preventive/rescue therapy are effective Long term safety up to 6m	[80]
Chang 2013 Hyperoxic -90% rat	IT HUC-MSCs $5x10^5$ at PN3, PN10, PN3+10	In PN3 and PN3+10 group: Reduced MLI and mean alveolar volume Reduced TNFα, IL1a, IL-1b, IL-6, TIMP, CXCL7, RANTES, L-selectin, sICAM-1 Reduced cell apoptosis Reduced MPO activity and collagen deposition Increased VEGF and HGF	Early MSC replacement confers better lung protection Engraftment Paracrine effect	[74]
Sutsko 2013 Hyperoxic-90% rat Long term effect	IT $2x10^6$ BM-MSCs and MSC-CM at PN9	Even at P100 (other than P16, P30): Increased vascular density and alveolar area Reduced MLI Reduced RVSP and RVH Reduced IL-6, IL-1β	Minimal engraftment Paracrine	[81]

(Table 2) Contd…

Experimental Model	Therapeutic Cell or Product	Therapeutic Outcomes	Suggested Mechanisms	Ref.
Ahn 2013 Long term P70 safety and outcome Hyperoxic-90% rat	IT HUC-MSC $5x10^5$ at PN5	At P70: Reduced MLI, increased vWF, reduced macrophages No abn in heart, liver, spleen	Paracrine effect	[82]
Zhang 2013 Hyperoxic-95% rat	IV $1x10^5$ BM-MSC PN10	Increased survival Increased SP-C expression and AE1 cells survival Inhibit lung apoptosis Increased VEGF expression	Stimulation of potent mediators Reduced apoptosis	[70]
Di Bernado 2014	Incubation of fetal lung with $5x10^5$ PL-MSC, BM-MSC and control	Increased lung surface area, terminal bud formation, branching morphogenesis Increased SP-B, SP-C and AQP-5 in PL-MSC only	PL-MSC as potent as BM-MSC Paracrine effect	[78]
Liu 2014 Hyperoxic-90% mice	Intranasally *vs.* IP 0.1, 0.5, $1x10^6$ HUC-MSCs	Intranasal no effect IP $1x\ 10^6$ has effect on Restoration of lung compliance, elastance, and tissue recoil but assoc with alveolar septal widening	Paracrine modulation of interstitial matrix. Systemically administered MSCs are more beneficial	[135]
Luan 2015 Hyperoxic-60% mice	IV $1x10^6$ BM-MSCs	Increased radial alveolar count Increased VEGF, reduced TGF-b1 Differentiation of MSC into vascular endothelial cells	Engraftement Paracrine effect	[71]
Ahn 2015 Hyperoxic-90% rat and A549 cells	IT $5x10^5$ HUC-MSC HA-MSC HUC-MNC at PN5	*In vivo*: Increased survival HUC/HA-MSC *In vitro*: MLI – reduced in HUC/HA-MSC Angiogenesis improved in HUC-MSC Inflmy cytokines: IL-1α, IL-1β, IL-6 attenuated in HUC-MSC VEGF and HGF recovered by HUC/HA-MSC	HUC-MSC better than HA-MSC to protect hyperoxic lung injury Paracrine effect	[75]
Sung 2015 Hyperoxic-90% rat	IT $5x10^5$ *vs.* IV $2x10^6$ HUC-MSCs	In IT group: Greater decrease in MAV, MLI and ED-1/macrophage pos cells Reduced apoptosis Significant engraftment Reduced MIP-1α, TNFα, IL-6 Downregulate genes assoc with inflammation, cell death, fibrosis and upregulate genes assoc with angiongensis VEGF and HGF expression	IT better than IV HUC-MSCs Engraftment Paracrine	[76]
Gulasi 2016 Hyperoxic-85-95% rat	IT PN11 with NS, CM, RCM, BM-MSC	Increased number of alveoli and decreased in alveolar diameter in BM-MSC group but not CM group Expression of aSMA reduced in BM-MSC	Engraftment	[136]

(Table 2) Contd…

Experimental Model	Therapeutic Cell or Product	Therapeutic Outcomes	Suggested Mechanisms	Ref.
Chou 2016 IP LPS to pregnant rat +Hyperoxic 85% rat	IT PN5 $3x10^5$ and $1x10^6$ PL-MSCs	Reduced TNFα in high dose Reduced IL-6 in both doses Reduce MLI in both doses Reduced collagen density Increase vascular density Increased VEGF and reduced CTGF	By VEGF and reducing CTGF and cytokines (paracrine effect)	[79]
Sammour 2016 Hyperoxic-85% rat	IT male or female derived BM-MSCs $1x10^6$	Female MSCs has higher VEGF and IL-10 than Male MSC Both: Reduced MLI and increased RAC Improved vascular density Female MSCs is more superior in Reducing RVSP Reducing vasc remodeling Reduced IL-1β and increased IL-10	Female MSC has more impact in terms of protection from PHT	[137]
Kim 2016 Hyperoxic-90% rat	IT $5x10^5$ HUC-MSC at PN5	Reduced neuronal apoptosis Reduced MLI Reduced TNFα, IL-1α, IL-1β, IL-6 Increased VEGF No statistical significance in short term neurofunctional outcome	HUC-MSCs reduced lung and brain injury simultaneously	[77]
Zhang 2016 Hyperoxic-60% mice	IV $1x10^6$ BM-MSCs and IP 5000u/kg rHuEPO 1hr before and at 7d post hyperoxia	Increased RAC Improved BW Increased VEGF Reduced MMP9	More marked response in BM-MSC plus EPO Paracrine, anti-inflammatory effect Low engraftment	[138]

6.2. Potency Assays and Therapeutic Efficacy

In order to further characterize clinical grade MSCs, efforts are focused on redefining MSCs products based on their potency in biological assays rather than their phenotypic characteristics and/or composition [115, 116]. So far, these biological assays have failed to link stem/progenitor properties and effector functions of MSCs and are limited to the anti-inflammatory effect of MSCs [117-119]. Recently, a Clinical Indications Prediction (CLIP) scale has been proposed to predict the therapeutic efficacy of different human MSC isolates for a given disease indication based on TWIST1 expression levels [120]. The CLIP scale overcomes the aforementioned limitations as it predicts differences in growth, survival, stem/progenitor, and effector function of MSCs. The CLIP scale is a promising attempt that can be further expanded to increase its predictability for the therapeutic efficacy of clinical grade MSC lots for different disease indications in the future. Currently the ISCT proposes a "focused analysis of selected MSC markers robustly deployed by *in vitro* licensing and metricized with a matrix of assays" to assess a MSC product's biological potency [121]. This step is vital to ensure consistent validity of stem cells clinical trials so those results can be interpreted and compared with confidence.

6.3. Cell *versus* Cell-Free Products

Most of the MSCs' therapeutic effects are mediated *via* a paracrine activity, as the cell-conditioned media exerts similar (and sometimes superior) therapeutic benefit [85]. Increasing evidence suggests that MSCs derived Extra-cellular Vesicles (EVs) are important players in mediating these paracrine effects [122, 123]. MSCs derived EVs have been shown to promote organ regeneration *via* its anti-apoptotic activities in kidney [124], liver [125], heart [126], suppressing graft-versus-host disease *via* its immune-suppressive effects [127], and ameliorating stroke-associated neurological deficits *via* promoting neurogenesis and angiogenesis [128]. MSCs derived EVs also attenuate pulmonary arterial hypertension in adult mice [129]. Restoration of lung function in the hyperoxia rat BPD model by MSCs derived EVs has also been demonstrated recently [130]. EVs' therapeutic effects are mediated in part *via* miRNAs [131, 132]. These cell-free therapies may serve as a safer alternative cell-free therapy to ameliorate lung injury and restore lung and vascular growth in BPD. However, similar to the cell products, challenges in standardization of EV isolation, purification, industrial scale-up and good manufacturing practice compliance, will need to be overcome.

CONCLUSION

BPD still remains the most challenging complication of preterm birth even half a century after its first description. The multifactorial pathological process that leads to BPD makes small molecules and biologics difficult to tackle. MSC therapy emerges as a promising therapy as these cells are able to interact with their microenvironment and display pleiotropic effects by dispensing a variety of injury modulating substances to orchestrate the repair process [111, 133]. Promising preclinical data and the overall safety profile of MSCs have led to first clinical trial in preterm infants even so current cell products are still imperfect. Manufacturing of therapeutic MSCs and/or MSC free products will need to be standardized with its biological effects being tested and verified with approved potency assays before being released for clinical use.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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