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Experimental Basis and New Insights for Cell Therapy in Chronic Obstructive Pulmonary Disease

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Abstract Chronic Obstructive Pulmonary Disease (COPD) can be briefly described as air flow limitation and chronic dyspnea associated to an inflammatory response of the respiratory tract to noxious particles and gases. Its main feature is the obstruction of airflow and consequent chronic dyspnea. Despite recent advances, and the development of new therapeutic, medical and clinical approaches, a curative therapy is yet to be achieved. Therapies involving the use of tissue-specific or donor derived cells present a promising alternative in the treatment of degenerative diseases and injuries. Recent studies demonstrate that mesenchymal stem cells have the capacity to modulate immune responses in acute lung injury and pulmonary fibrosis in animal models, as well as in human patients. Due to these aspects, different groups raised the possibility that the stem cells from different sources, such as those found in bone marrow or adipose tissue, could act preventing the emphysematous lesion progression. In this paper, it is proposed a review of the current state of the art and future perspectives on the use of cell therapy in obstructive lung diseases.

Keywords Chronic obstructive pulmonary disease · Cell therapy · Stem cells · Bone marrow

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Chronic Obstructive Pulmonary Disease - COPD

According to the Global Initiative for Chronic Obstructive Lung Disease – GOLD – "Chronic Obstructive Pulmonary Disease (COPD), a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and lung to noxious particles or gases. Exacerbations and comorbities contribute to the overall severity in individual patients" [1].

COPD includes two nosological entities: obstructive bronchiolitis and emphysema. Obstructive bronchiolitis, a small airway disease, is characterized by airway inflammation, fibrosis, luminal plugs and increased airway resistance. On the other hand, emphysema has as main feature the enlargement of air spaces distal to terminal bronchiole. The contributions of the small airway (bronchiolitis) and parenchymal (emphysema) components of COPD to the resultant pathology may vary from patient to patient and the pathologic manifestations and clinical course of the disease will depend on the interaction and the individual contribution of each of these factors [1–7].

The inflammatory response of the lung tissue to inhaled cigarette smoke and other noxious particles is exacerbated in COPD patients and may induce parenchymal tissue destruction, resulting in emphysema, and/or inadequate repair and defense airways mechanisms, resulting in bronchiolitis [1–5]. The inappropriate repair of lung tissue after injury may also contribute to the development of emphysema. The fibroblasts isolated from lung tissue of patients affected by COPD present defective repair process, such as inadequate modulation of extracellular matrix and production of growth factors that affect other cell types at the lung parenchyma. As a consequence, there is a worsening of the lesion and loss of tissue function [8].

The occurrence and progression of COPD is, in fact, resultant from the interaction of multiple etiological aspects,

a multifactorial process involving environmental and genetic factors. Approximately 1–3 % cases of emphysematous element of COPD are caused by deficiency of α-1-antitrypsin, an enzyme responsible for controlling the proteolytic action of factors released by inflammatory cells on the lung tissue. The deficiency of this enzyme results in an imbalance and exacerbation of aggressive factors, with gradual destruction of lung tissue [9, 10]. The main environmental aggressors include oxidative stress, air pollution and cigarette smoke. The smoking habit is undeniably the main risk factor, responsible for about 85 % of deaths worldwide due to pulmonary emphysema [1, 11–15]. Other risk factors include: aging, infection, as well as economic and social factors [9, 16]. Degeneration of lung tissue, regardless the predominant etiology, leads to the limitation of gas exchange and progressive dyspnea with subsequent disability and premature death.

The current therapeutic approaches to COPD mainly involve the control of symptoms, without a significant change in the natural history of the disease. The pharmacological therapeutic approach is mainly comprised of bronchodilator drugs, corticosteroids and anticholinergics. Oxygen therapy and physiotherapy are important for improving the quality of life of patients [1, 17–20].

As the proposition enunciated by Rojas et al. (2005), when the lung tissue is affected by COPD, there is an intense production of inflammatory signaling molecules able to recruit progenitor cells and stem cells (SC) to the site of injury. These aspects of intense cellular signaling and chemotaxis, as well as the results obtained with cell therapy (CT) in experimental models and human patients in different tissues and disorders, represent the rationale for the use of CT with SC as a new therapeutic approach for COPD [10, 21–25].

Stem Cells and Cell Therapy

Cell therapy can be defined as the set of therapeutic procedures that employ cells or cellular components for the treatment of diseases. The implementation of the use of SC in the CT in human patients was initiated with the work of Thomas and Murray in 1956, when the authors observed the remission of leukemia in patients after infusion of bone marrow from healthy donor. However, due to rejection of the transplanted tissue by the recipient, this procedure was effective only between identical twins. Studies in histocompatibility complex, pioneered proposed by Jean Dausset, in 1952, associated with the development of more effective antibiotics, allowed Thomas and Murray to perform, in 1969, the first successful bone marrow transplantation in which the donor and recipient were not twins. In 1990, Thomas and Murray shared the nobel prize in physiology or medicine for the discoveries concerning organ and cell transplantation in the treatment of human diseases [10, 21–23, 25–30].

A significant advance for the consolidation of SC as well as to establish the concept of SC, was initiated with the work of Till and McCulloch in 1961. The authors observed the reconstitution of the hematopoietic system in lethally irradiated animals submitted to bone marrow transplantation from syngeneic healthy animals. The bone marrow reconstitution in irradiated animals indicated the presence of cells capable of recover the hematopoietic system and give rise to all kinds of blood cells. These capabilities, later called self-renewal and differentiation, are some of the characteristics attributed to those defined, after the work of Till and McCulloch, as stem cells [31–33].

Therapies involving the use of autologous or donor-derived bone marrow SC present a promising alternative in treating degenerative diseases and injuries. Existing cellular therapies basically involve cell replacement, such as bone marrow transplantation for patients with disorders related to blood tissue. However, there are still challenges to be overcome, such as the difficulty in obtaining cells for transplantation, immunological and compatibility issues, as well as precise control of cell fate in vivo under defined conditions [32, 34–36]. It should be emphasized that the bone marrow transplantation represents, up to date, the only form of CT with SC consolidated in human therapy [31–33].

Despite recent advances, little is known about SC resident in lung tissue and about the characteristics and functional abilities related to regeneration and repair processes in the lung tissue [37–39]. Although several studies have suggested the existence of lung SC, there are no consolidated methods of isolation and culture for these cells.

Hegab et al. (2010) isolated and characterized, from the pulmonary tissue of mice, a population of multipotent SC. These cells were positive for bronchioalveolar SC markers SCA1 and CD34, and negative for CD45 and CD31, typical markers of endothelial cells and hematopoietic SC. Also showed extensive self-renewal capacity in culture and ability to differentiate into several cell types in vitro. The transfer of these cells to animal models of lung injury induced by high doses of elastase significantly improved survival levels and amelioration of lung tissue. These findings have important implications for understanding the lung tissue homeostasis and the tissue response to injury, as well as the study of the CT in the pulmonary tissue [25, 40, 41].

Potential candidates to act as a pulmonary SC are the trachea basal cells, which express cytokeratin 5/14, have the property of self-renewal and are capable of originating ciliary secretory Clara cells after epithelium injury. It has also been described in the tracheal epithelium basal layer, a “side population” characterized by the ability to generate basal, ciliated and secretory cells and maintain the epithelial tissue homeostasis. The authors also evidenced cells in lung tissue

niches which are stimulated after injury, such as neuroendocrine bodies and the bronchoalveolar duct [25, 37, 42].

Kim et al. (2005) identified a group of cells in the bronchoalveolar duct capable of maintaining the homeostasis of bronchial and alveolar epithelial cells, the bronchoalveolar stem cells - BASC's. By means of immunofluorescence, the presence of specific markers, such as CD34 and Sca-1, among others, were detected in this group of cells, as well as expression profiles of micro-RNA's (miRNAs) responsible for the negative regulation of gene expression. The authors suggest that these miRNA's play a key role in the maintenance of BASC's self-renewal ability. It is also suggested that the altered expression of miRNA's would be responsible for turning BASC's into cancer cells. These data indicate that a delicate balance of regulatory signals may be related to the performance of SC in injured tissues that undergo CT [22, 43].

Resident SC in the lung tissue were also described by Fujino et al. (2011). A population with the ability of giving rise to colonies, self-renewal and differentiation into type II pneumocytes was isolated from the lung tissue. By means of immunofluorescence, it was observed that these cells presented surface markers characteristic of mesenchymal SC, such as CD90, CD73 and CD105, as well as characteristic markers of type II pneumocytes, such as pro-SP-C, SP-A and SP-B . These SC showed characteristics similar to bone marrow mesenchymal SC, such as the ability to give rise to osteocytes and adipocytes, but not chondrocytes, and showed high commitment to the pulmonary lineage. The authors also concluded that altered levels of proliferation, differentiation or senescence in this cell type could play an important role in the pathogenesis of lung diseases and therefore is a potential target for therapies, and the study of pathogenesis of different diseases [44].

Wada et al. (2011) compared the tissue regeneration after lung injury in animals with or without the infusion of type II pneumocytes 1 day after pneumonectomy. The authors postulated that the transplanted cell stimulated regeneration and cell growth in lung tissue subjected to pneumonectomy, showing that this cell type play a role in cell replacement procedure [45].

A population of Alveolar Epithelial Progenitor Cells (AEPC) with regenerative potential was described by Chapman et al. (2011). The authors noticed, ex vivo, that these cells expanded clonally and also differentiated toward mature cell types. By means of in vivo lung organoid assay, the authors showed that the AEPC population was able to give rise to distinct secretory clara cells. More interestingly, it was observed, through bleomicin lung injury model, that AEPC differentiate after parenchymal injury, maintaining the alveolar epithelial cells population during lung repair, identifing a strong candidate population for maintenance of alveolar epithelial cells after lung injury [46]. This lung SC candidate cell lineages are summarized in Fig. 1.

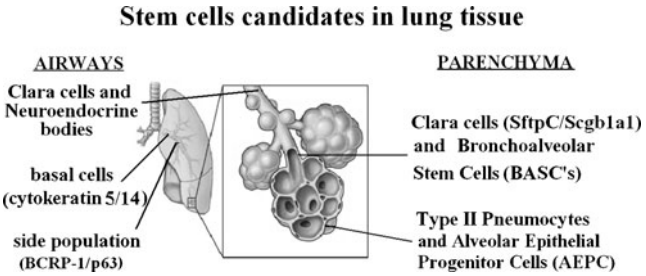

Fig. 1 Stem cells candidates in lung tissue

Despite major advances in knowledge on lung SC, there are still a number of gaps, questions and propositions to be properly confirmed. For example, the better characterization of lung SC and SC from different potential donor tissues, as well as the role of the different SC in the COPD etiopathogenesis and int the SCT for COPD. Thus, further studies are needed to understand the role of these cells in the etiology of different lung diseases, as well as their therapeutic potentia [25].

Cell Therapy in COPD

Pereira et al. (1995) initiated the studies involving SC and the lung tissue. The authors verified the migration of SC derived from bone marrow into the pulmonary tissue of recipient mice by means of PCR. The cells could be observed for a period of up to 5 months, indicating that they would be incorporated into the host tissue and diffused through the parenchyma. Migration of SC to the lung tissue after systemic infusion was also observed by Krause et al. (2001) and Kotton et al. (2001), using molecular labeling techniques, such as FISH and Lac-Z [47–49].

The migration of bone marrow derived SC to the lung tissue was also observed in humans after marrow transplantation by Suratt et al. (2003). Through the FISH technique, the authors detected the presence of cells with the Y gene in lung tissue of women who received, for different reasons, bone marrow transplant from male donors. Subsequently, Lama et. (2007) also detected the presence of SC in lung tissue of patients after bone marrow transplantation, using different techniques, such as RT-PCR and immunohistochemistry [50, 51].

The role of SC in both, pathophysiology and therapy of pulmonary emphysema, was evidenced by the work of Adachi et al. (2006). The authors infused, intramedullary, bone marrow cells from Tight-skin (animal model of emphysema) mice into irradiated C3H mice. The mice that received transplants [Tsk → C3H] developed emphysema, highlighting the role of stem cells in the pathogenesis of the disease. The authors also prevented the progression and even regressed lung injury through the intramedullar transplantation of hematopoietic

SC [C3H → Tsk]. These data are favorable to the use of cell therapy in the treatment of emphysema [52].

Jungebluth et al. (2011) observed a significant and lasting improvement of clinical parameters after intratracheal infusion of bone marrow derived mesenchymal stem cells – MSC in allogeneic rats with chronic thromboembolic pulmonary hypertension - CTEPH. Also observed significant changes in protein expression patterns in the pulmonary and hepatic tissues from treated animals. These results suggest that BMMC could be capable of restoring lung function and that the therapeutic effects of mesenchymal SC could be related to paracrine stimulation of protein-based reconstructive processes [53].

Several studies in animal models of acute lung injury and pulmonary fibrosis, as well as in human patients, have demonstrated that mesenchymal SC have the capacity to modulate the local immune responses, thus, it is possible that the bone marrow mesenchymal SC could prevent the lesion progression in the emphysematous lung tissue. This would occur due to the immunomodulatory property of mesenchymal SC on the inflammatory component of the lesion in the pulmonary emphysema and other inflammatory diseases of the lung tissue. It is proposed that the action of MC would be through expression of antagonists of interleukin 1 (IL-1) receptors or expression of Tumor Necrosis Factor - α (TNF-α) and other pro-inflammatory factors by activating resident macrophages and causing a negative feedback in the inflammatory cascade. It would also increase the expression of protective factors, such as antiproteases [4, 6, 8, 23, 42, 54–58].

Many other potential sources of SC have been investigated. Despite these studies, several aspects of CT with SC are yet to be elucidated. One of the main challenges in establishing new cellular groups for CT is the characterization and selection of populations responsible for tissue remodeling during the repair process after injury [22, 39]. In addition to these aspects, the mechanisms by which infused SC would act on the repair process of damaged tissue are not well determined and require further studies. Several hypotheses have been proposed to explain the SC mechanisms of action. Among the different mechanisms proposed, it is worth mentioning: 1) production and release of paracrine factors, such as lateral transfer of miRNA, which stimulate the cell renewal process of the affected tissue cells, 2) cell fusion, which would attribute high levels of cell proliferation to heterocárion resulting from the merged cells, restoring the damaged tissue, 3) differentiation, whereby the SC would be responsible for cell replacement of the target tissue, giving rise to a "pool" of progenitor cells and 4) transdifferentiation, the mechanism by which the infused SC "pool" directly give rise to differentiated cells, repairing the damaged tissue [42]. However, there is no consensual explanation of the processes by which the infused SC would act over the target tissue in CT. The proposition of our group is that the process of tissue repair after CT is quite complex and multifactorial, involving different mechanisms and/or different types of SC, resident or not in the lung tissue itself. Our proposal in presented in Fig. 2.

In our laboratory, several animal models and methodological approaches for the pulmonary emphysema were established in order to evaluate the effects of CT with bone marrow mononuclear cells - BMMC - in lung tissue affected by emphysema. Initially, we adopted the intranasal instillation of papain model. This model consists on the intranasal administration of the protease. Lung injury resulting from the proteolytic action of papain on pulmonary parenchyma resembles the emphysematous lesion in humans. After establishing the animal model of pulmonary emphysema, the group evaluated the effects on the damaged lung tissue of a systemic infusion of BMMC. The bone marrow from donor femurs were removed and the BMMC isolated through a Ficoll gradient. The isolated cells were then infused in the teil vein of the emphysematous host mice. The regeneration of lung tissue from animals exposed to papain and infused with BMMC was observed in a qualitative and quantitative way. The mean alveolar intercept was statistically different between treated and control animals, indicating a alveolar destruction diminish [10, 59].

From these studies, were established, in our laboratory, the methodology of experimental emphysema induced by intranasal instillation of protease, as well as harvesting and infusion of BMMC. In order to accesses the etiopathogenesis of pulmonary emphysema in a more effective way, as well as the effects of the proposed CT, new morphophysiological models of pulmonary emphysema in mice have been suggested. Among these, the intranasal instillation of porcine pancreatic elastase, a protease synthesized during the inflammatory process by neutrophils and macrophages, and its main activity is the degradation of extracellular matrix proteins. Thus, the elastase plays an important role in the destruction of extracellular matrix in patients suffering from COPD [60]. Therefore, in animal models, the intranasal instillation of elastase leads to an inflammatory response, promoting a similar injury to the lung tissue than the one resultant from pulmonary emphysema [61].

By using the proposed animal model, our group carried out several qualitative and quantitative assays regarding the emphysematous lung tissue response of animals subjected to infusion of BMMC. It was observed the presence of infused cells into the host lung tissue by means of immunohistochemical analysis. It was also possible to observe statistically significant differences regarding the morphology of the lung tissue of animals, in other words, the mean alveolar diameter found for the treated animals was lower than the one found for the emphysematous controls. Our team also

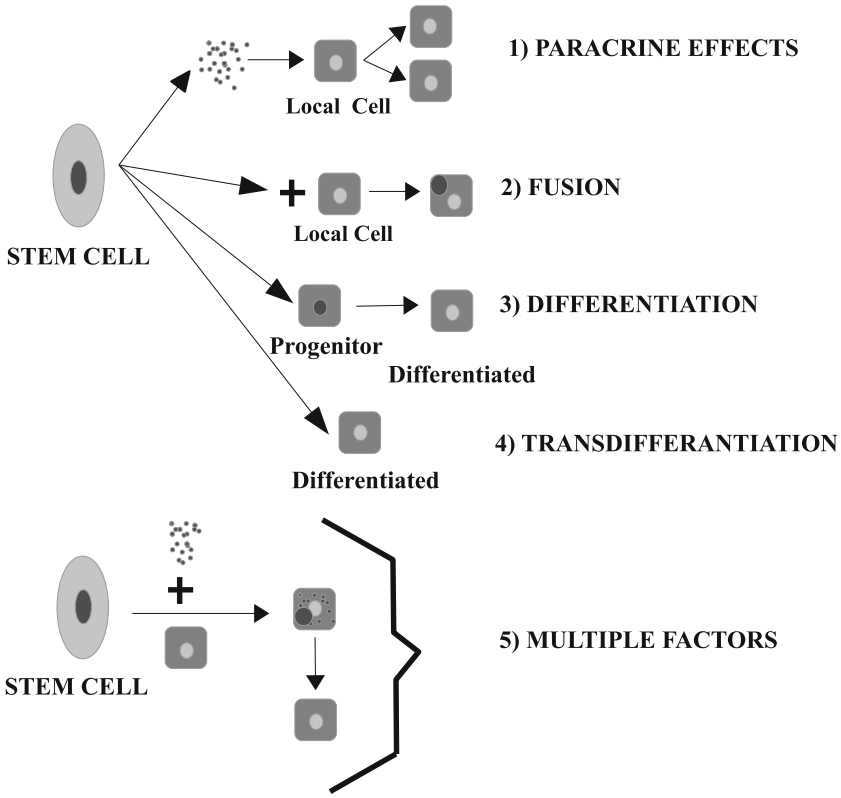

Fig. 2 Possible mechanisms of action of stem cells in tissue repair after cell therapy

observed, by means of immunohistochemistry, differences in the inflammatory response patterns of the lung tissue, in terms of expression of molecules characteristics of the inflammatory process, such as the metalloproteinases 9 and 12. These results showed that the systemic infusion of BMMC promotes regenerative changes in the morphometric and inflammatory patterns of lung tissue from animals with experimentally induced pulmonary emphysema [24, 39, 62].

New analyzes are ongoing in our laboratory using bone marrow-derived MSC. The first morphometric evaluations evidence that animals exposed to elastase and subjected to infusion of bone marrow derived MSC have regenerative response compared to control groups (unpublished data).

In order to obtain an animal model of pulmonary emphysema that tries to mimic the physiological condition of patients affected by pulmonary emphysema, our team has developed an apparatus for inhalation of cigarette smoke in mice and rats. The advantage of the developed apparatus is the lower cost compared to the marketed models (10 to 20 times lower), making it is extremely viable and attractive to application in research projects aiming the analysis of pathophysiological processes and development of new therapies for pulmonary diseases. Tests are already underway with the prototype built to evaluate the proposed model (unpublished data).

Based on these and other data from in and out of the research group, our team conducted the first study in human patients to assess the safety and feasibility of the procedure. This study was based on intravenous infusion of autologous BMMC. The bone marrow was selected as the SC source due to the fact that the harvesting and isolation of SC from this tissue are feasible and a well established methodology in animal models and in humans. Previous findings in animal models and human patients suggests that systemically infused BMMC play a role in the regeneration and/or protection of the lung tissue affected by COPD [8, 10, 18, 22, 24, 39, 52, 54]. The study was approved by the National Ethics in Research Committee (Comitê Nacional de Ética em Pesquisa - CONEP) in 2009 (reg. 14 764, 233/2009) and registered in Clinical Trials - NIH - USA (NTC01110252). The patients underwent cardiac and lung function tests before and after infusion. The patients received Granulocyte Colony-Stimulating Factor (G-CSF) 3 days prior to the bone marrow harvest and isolations of BMMC. Immediately after the isolation, the BMMC were infused through the brachial vein and the patients were followed during 12 months, showing a clinical improvement and stable condition. The patients were evaluated, showing spirometry and blood gas parameters amelioration. Although the suggested change in the natural history of the disease, due to the small number of patients, it was not possible to formulate consistent propositions on the procedure effectiveness. The results allow, however, to conclude that it is a safe procedure. The study design is presented on Fig. 3 [24, 39]. The patients continued to be monitored for a period of 3 years after the procedure. An interesting result

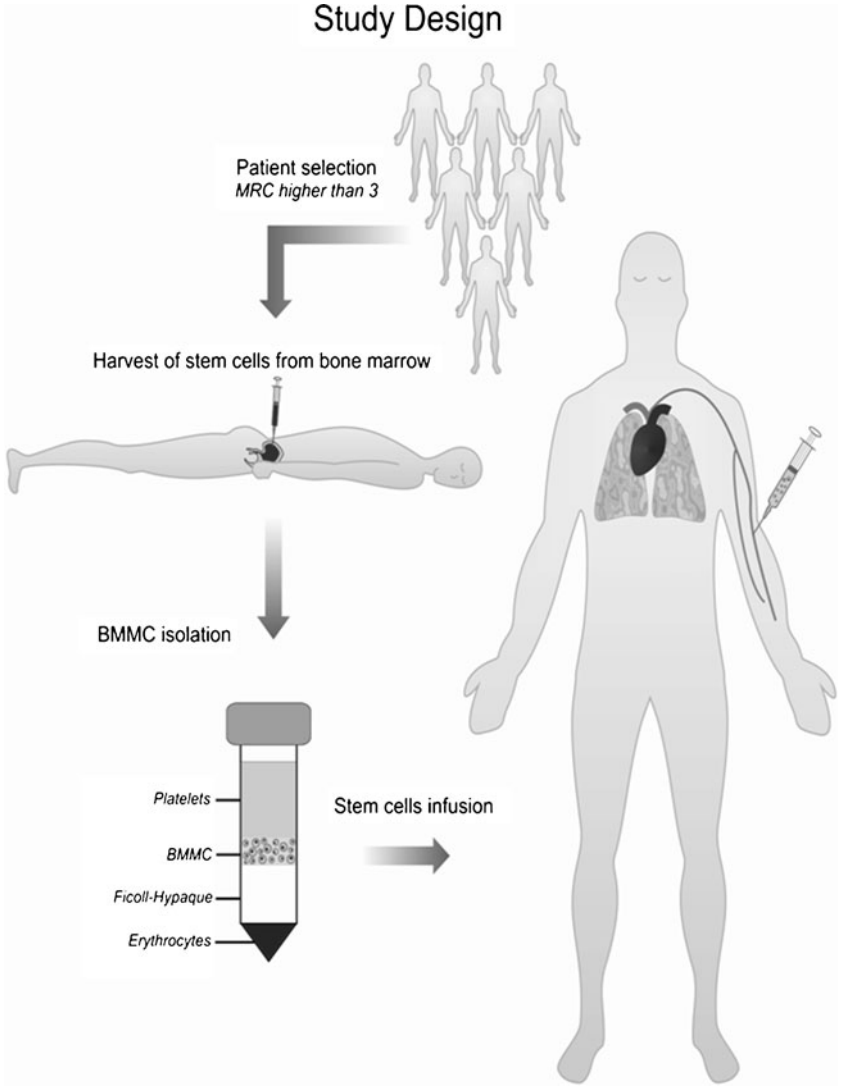

Fig. 3 Experimental design of cell therapy with BMMC for pulmonary emphysema (Ribeiro-Paes et al., 2011)

Table 1 Studies regarding COPD and stem cells registered in the clinical trials database

NCT number	Study name	Status
NCT00683722	PROCHYMAL™ (Human Adult Stem Cells) for the Treatment of Moderate to Severe Chronic Obstructive Pulmonary Disease (COPD)	Completed
NCT01110252	Safety Study of Cell Therapy to Treat Chronic Obstructive Pulmonary Disease	Completed
NCT01559051	Safety and Efficacy of Adipose Derived Stem Cells for Chronic Obstructive Pulmonary Disease	Recruiting
NCT00826683	Detection of Circulating Endothelial Progenitors Cells (EPCs) in Non-small Cell Lung Cancer (NSCLC)	Unknown
NCT00956358	Study on Systemic and Airway Biomarkers in Haemopoietic Stem Cell Transplantation	Recruiting
NCT00831220	Endothelial Dysfunction in Chronic Obstructive Pulmonary Disease	Completed

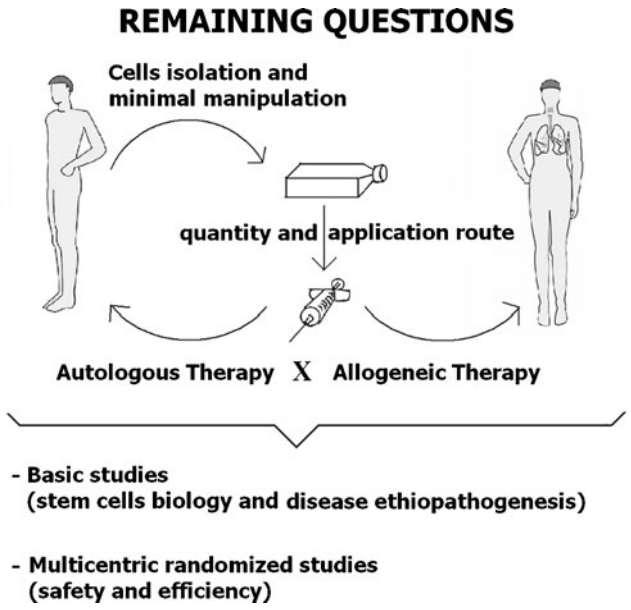

Fig. 4 Questions to be answered on cell therapy in lung diseases

turned out in the long term follow up. The spirometry parameters maintain an regularity and similarity in relation to the evaluation prior the procedure. One of the subjects presented a significant increase in the forced vital capacity 1 year and 3 months after the BMMC infusion, strongly suggesting a change and stabilization in the natural history of the disease. A two-years follow-up report, with the complete data is being submited to publication (unpublished data).

From these assessments, a new protocol was submitted to CONEP, with similar methodology and a larger number of subjects, aiming an initial assessment about the procedure effectiveness. The main hypothesis is that the anti-inflammatory action of mesenchymal SC leads to a decrease in the inflammatory process associated with COPD by promoting improvement in pulmonary function, dyspnea, and especially the quality of life of patients.

A detailed search in the database of Clinical Trials revealed that, besides the safety study from our laboratory, five other clinical trials are listed on the use of CT in COPD, as summarized in Table 1. One will evaluate the use of Prochymal®, human mesenchymal SC derived from adult healthy donors in the treatment of chronic pathologies (Clinical Trials - NTC 00683722). However, there is no data in the literature regarding the results of studies involving the use of Prochymal® in patients suffering from COPD or other chronic lung diseases. Publication of results is limited to the Homepage of the company, among other sites [63].

These and other evaluations in human patients, as well as animal models assays, are underway both in our and other laboratories. These are pioneering studies and bring up new challenges and possibilities for a better understanding of the pathophysiological mechanisms involved in the pulmonary emphysema and the role of SC with CT in the injured tissue, as well as for the optimization of cell isolation processes.

Although animal models and the pioneer study with the use of BMMC performed by our research group have shown potentially interesting results, the clinical application of this new therapeutic approach is surrounded by a series of doubts, questions and methodological difficulties. There are still a number of issues, regarding the process optimization, as well as the selection and minimal manipulation of cells, autologous versus allogeneic therapy, choosing the best route of application and the appropriate number of cells infused, as presented in Fig. 4 [64].

As proposed by Tzouvelekis et al. (2011), large multicentric randomized safety and efficacy studies are needed. These studies will allow the increase of knowledge about the pathogenesis of COPD, as well as the biology of SC, such as the characterization of the cell markers, phenotype and properties of the stem cells from different donor tissues, as well as the role of stem cells in different compartments of the lung. Only the convergence of all this knowledge will allow consolidated advances in the cell therapy in chronic obstructive pulmonary disease and other diseases [65].

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Conflict of interest The authors declare no potential conflicts of interest.

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