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Early View

Research letter

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Mesenchymal stromal cell infusion modulates systemic immunological responses in stable COPD patients: A phase I pilot study

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Summary: Mesenchymal stromal cell infusion may provide an alternative immune-based therapeutic option for COPD patients

Chronic obstructive pulmonary disease (COPD) is a leading cause of global morbidity and mortality due to limited therapeutic options for the persistent pulmonary and systemic inflammation that characterises this condition [1]. Recently, pre-clinical studies of mesenchymal stromal cells (MSCs) in COPD demonstrate efficacy in alleviating inflammation and reducing emphysema following either systemic or intra-tracheal administration [2, 3]. Human trials have demonstrated that MSCs did not improve spirometry following administration of MSCs to COPD patients, however it was reported that C-reactive protein (CRP), a marker for systemic inflammation was reduced between 1-3 months post-infusion. Earlier timepoints were not assessed in detail in these trials which limits further investigation of these changes [4, 5]. Identifying the fate of intravenously-infused MSC and the potential implications of their biodistribution, as well as short-term MSC-induced systemic changes that were not explored in previous trials will better delineate the utility of MSC treatment for COPD.

The study was approved by the Royal Perth Hospital ethics committee (approval no. EC2012/103) and all patients had provided written informed consent. A single site, phase I study (Australian clinical trials registry no. 12614000731695) was conducted to determine the MSC biodistribution, inflammatory and clinical endpoints following systemic MSC infusion in a cohort (n=9) of mild to very severe stable COPD patients (n=1 GOLD I, n=2 GOLD II, n=3 GOLD III, n=3 GOLD IV). All recruited patients had not experienced an exacerbation for at least three months prior to trial commencement with no change in regular medications. Patients received two infusions of low passage (p3) allogeneic bone marrow-derived MSC of approximately $2x10^6$ MSCs/kg, one week apart with the first infusion

comprised of radiolabelled cells and the second infusion using unlabelled cells. MSCs used for the first infusion were labelled with ^{111}indium, a low energy radioisotope with a half-life of 68 hours to enable tracking across several days. Labelled MSCs were able to adequately suppress PBMC proliferation *in vitro* compared to unlabelled MSC ($p>0.05$) and retained regular morphological characteristics. Safety and hospitalisations attributed to acute exacerbations of COPD (AECOPD) were monitored up to a year later. Wilcoxon matched pairs tests were used for comparison of pre- and post-infusion levels of cell subsets and circulating plasma biomarkers.

MSC infusion showed no attributable adverse side-effects and was well tolerated. Following infusion, ^{111}indium was detected in the lung within 30 minutes by CT scan and remained detectable after 24 hours after which uptake was detected in the liver, spleen and bone marrow up to 7 days post-infusion (Figure 1A-B). In keeping with mouse studies assessing MSC localisation [6], this pattern may be explained in part by leakage of indium from MSCs, which are then bound to transferrin and taken up by the reticulo-endothelial system, accumulating in the liver and spleen. Patients were assessed by Single-photon emission computed tomography (SPECT) 4 hours after the first infusion and superimposed with low dose CT to determine ^{111}indium activity and MSC localisation in the lungs. There was reduced ^{111}indium in emphysematous lung (red arrows; Figure 1C) compared to normal lung (black arrows; Figure 1C). Retention of ^{111}indium in the lungs was also positively correlated with baseline FEV_1 ($R^2=0.68$, $p=0.02$) and diffusing capacity of carbon monoxide (DLCO; $R^2=0.81$, $p=0.01$) by linear regression analysis, suggesting that patients with mild disease retained MSCs in the pulmonary vasculature longer than more severe disease which exhibit poor perfusion in remodelled emphysematous lung. This distribution may protect healthy lung tissue.

We first determined if ^{111}indium induced an inflammatory response independent of MSCs by co-culturing PBMC from healthy controls (n=3) and stable COPD (n=3) with plasma containing 0.001-0.1 MBq of indium since these concentrations reflected the range of indium exposure in our cohort. In media alone and following stimulation with inflammatory stimulants aCD3 and lipopolysaccharide (LPS), there was no difference in cytokine production (IL-1β, IL-6, IL-8, IL-10, TNFα) in indium-exposed PBMC versus non-indium exposed controls demonstrating that indium did not induce an inflammatory response. From the same cultures, lymphocyte subsets and cell viability based on phosphatidylserine expression were also assessed by flow cytometry, and we observed that ^{111}indium had no effect on cell proportions or viability *in vitro* (data not shown).

Gas chromatography-mass spectrometry was performed for quantitation of F2-Isoprostanes (F2-IsoP) [7], as a marker of oxidant load from neutrophils and macrophages. F2-IsoP were reduced 7 days following MSC infusion (Figure 1D), which is in line with a reduction in oxidative stress after allogeneic human umbilical cord-derived MSC infusion in a rat model of LPS-induced acute lung injury [8]. We assessed several inflammatory mediators by ELISA and multiplex that are important in COPD, however several mediators including IL-1β, IL-10, IL-12p70 and IL-17A were undetectable in our cohort. CRP levels increased from 1 hour to 2 days after MSC infusion (Figure 1E), which contrasts data from other trials where systemic administration of allogeneic bone marrow-derived MSCs in COPD patients reduced CRP levels between 1-3 months post-infusion [4, 5]. Notably, there were changes in certain inflammatory mediators including soluble CD163 (sCD163), a biomarker for macrophage activation that correlates with disease severity in COPD [9] that was significantly reduced between day 1-7 post-infusion (Figure 1F). In addition, there was a fall in IL-6 (7/9 patients), a major mediator in COPD that which stimulates the secretion of matrix metalloproteinases and T cell responses which can contribute to airway remodelling [10]. (Figure 1G).

Furthermore there was an increase in the anti-inflammatory circulating sTNFR1 (Figure 1H), a decoy non-cell associated receptor that binds to and sequesters excess circulating TNFα thus reducing systemic inflammation [11]. This data is supported by the upregulation of sTNFR1 by MSCs in endotoxaemic mice [12].

MSC administration also shifted the balance to a more anti-inflammatory circulating cellular profile as assessed by flow cytometry. Circulating Tregs that are central to resolution of inflammation and usually reduced in COPD, were increased 7 days after MSC infusion (Figure 1I) [13]. MSC infusion also altered the proportions of DC subsets at day 2, favouring plasmacytoid (p)DC over myeloid (m)DC (Figure 1J). pDCs are the largest producers of interferon-α which can augment the anti-viral response in COPD, while mDC provides a major source of T cell stimuli and inflammatory cytokines. The reduction in mDC:pDC ratio by MSC infusion may have direct benefits in COPD since an increased ratio of mDC:pDC subsets has been associated with higher grade emphysematous damage in COPD patients. [14]. Furthermore, $CD14^+$ monocytes were significantly increased at day 2 (Figure 1K). These monocytes also displayed a reduced expression of co-stimulatory molecules (CD80, CD86, HLA-DR) and pro-inflammatory receptors (TLR4), adopting an immunoregulatory phenotype (Figure 1L). Indeed co-culturing of umbilical cord- and adipose-derived MSCs with purified monocytes had downregulated their co-stimulatory receptors, and were able to suppress T cell responses *in vitro* [15].

There was a reduction in hospital admissions for AECOPD from 11 events occurring within a year before MSC infusion to 6 events occurring within a year post-infusion. No additional interventions in the year following infusion were used, unless treatment was required following an exacerbation in this period. Lung function measured 3 weeks following the second infusion did not change compared to pre-infusion levels; median (range) FEV_1 [%predicted] = 37 (23-87) *vs.* 41 (41-98), p=0.48 and FVC [%predicted] = 80 (59-106) *vs.* 82

(56-101), p=0.84. These findings are also consistent with other MSC trials in COPD which also showed no statistically significant improvement in spirometry.

Our study describes rapid systemic immunological changes which were not explored in previous MSC trials for COPD and we have related this to the biodistribution of MSCs following intravenous infusion. We hypothesise that systemically administered MSCs reach the lung within 30 minutes and probably releases trophic factors including extracellular vesicles that results in immunomodulation and a reduction in important inflammatory mediators. Despite a lack of improvement in spirometry, systemic MSC infusion may be useful in the attenuation of inflammation in COPD patients.

References

1. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, Lopez Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. *Am J Respir Crit Care Med* 2017: 195(5): 557-582.

2. Gu W, Song L, Li X-M, Wang D, Guo X-J, Xu W-G. Mesenchymal stem cells alleviate airway inflammation and emphysema in COPD through down-regulation of cyclooxygenase-2 via p38 and ERK MAPK pathways. *Scientific Reports* 2015: 5: 8733.

3. Li X, Wang J, Cao J, Ma L, Xu J. Immunoregulation of Bone Marrow-Derived Mesenchymal Stem Cells on the Chronic Cigarette Smoking-Induced Lung Inflammation in Rats. *BioMed Research International* 2015: 2015: 10.

4. Weiss DJ, Casaburi R, Flannery R, LeRoux-Williams M, Tashkin DP. A placebo-controlled randomized trial of mesenchymal stem cells in COPD. *Chest* 2013: 143.

5. de Oliveira HG, Cruz FF, Antunes MA, de Macedo Neto AV, Oliveira GA, Svartman FM, Borgonovo T, Rebelatto CL, Weiss DJ, Brofman PR, Morales MM, Lapa ESJR, Rocco PR. Combined Bone Marrow-Derived Mesenchymal Stromal Cell Therapy and One-Way Endobronchial Valve Placement in Patients with Pulmonary Emphysema: A Phase I Clinical Trial. *Stem Cells Transl Med* 2017: 6(3): 962-969.

6. Allers C, Sierralta WD, Neubauer S, Rivera F, Minguell JJ, Conget PA. Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. *Transplantation* 2004: 78(4): 503-508.

7. Barden AE, Mas E, Croft KD, Phillips M, Mori TA. Minimizing artifactual elevation of lipid peroxidation products (F2-isoprostanes) in plasma during collection and storage. *Analytical Biochemistry* 2014: 449: 129-131.

8. Li J, Li D, Liu X, Tang S, Wei F. Human umbilical cord mesenchymal stem cells reduce systemic inflammation and attenuate LPS-induced acute lung injury in rats. *Journal of Inflammation (London, England)* 2012: 9: 33-33.

9. Baines KJ, Backer V, Gibson PG, Powel H, Porsbjerg CM. Impaired lung function is associated with systemic inflammation and macrophage activation. *European Respiratory Journal* 2015: 45(2): 557.

10. Jarvinen L, Badri L, Wettlaufer S, Ohtsuka T, Standiford TJ, Toews GB, Pinsky DJ, Peters-Golden M, Lama VN. Lung Resident Mesenchymal Stem Cells Isolated From Human Lung Allografts Inhibit T Cell Proliferation via a Soluble Mediator. *Journal of immunology (Baltimore, Md : 1950)* 2008: 181(6): 4389-4396.

11. Tan DBA, Fernandez S, Price P, French MA, Thompson PJ, Moodley YP. Impaired CTLA-4 responses in COPD are associated with systemic inflammation. *Cellular and Molecular Immunology* 2014: 11(6): 606-608.

12. Yagi H, Soto-Gutierrez A, Navarro-Alvarez N, Nahmias Y, Goldwasser Y, Kitagawa Y, Tilles AW, Tompkins RG, Parekkadan B, Yarmush ML. Reactive Bone Marrow Stromal Cells Attenuate Systemic Inflammation via sTNFR1. *Molecular Therapy* 2010: 18(10): 1857-1864.

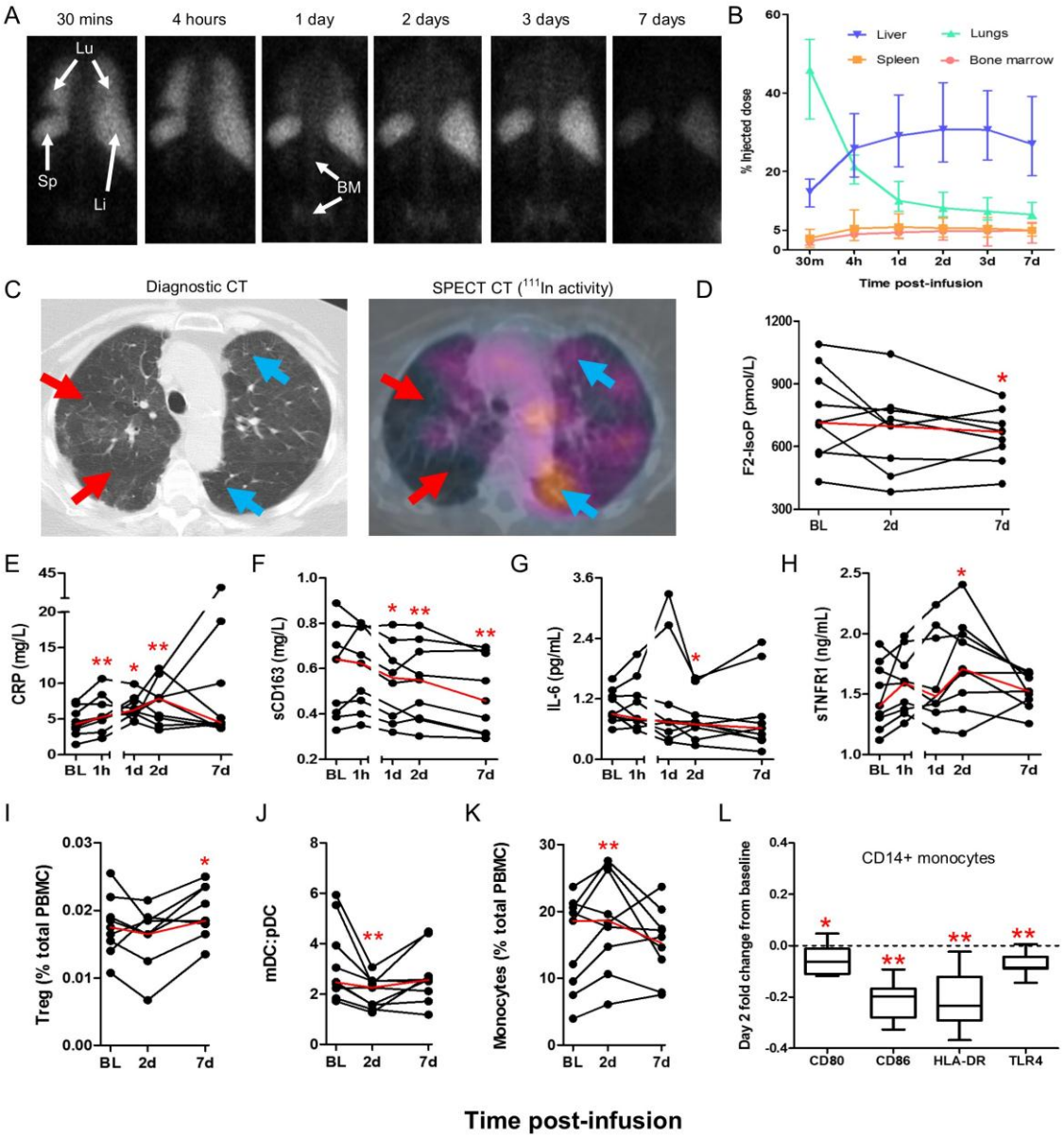
13. Hou J, Sun Y, Hao Y, Zhuo J, Liu X, Bai P, Han J, Zheng X, Zeng H. Imbalance between subpopulations of regulatory T cells in COPD. *Thorax* 2013: 68(12): 1131-1139.

14. Stoll P, Ulrich M, Bratke K, Garbe K, Virchow JC, Lommatzsch M. Imbalance of dendritic cell co-stimulation in COPD. *Respiratory Research* 2015: 16(1): 19.

15. Hof-Nahor I, Leshansky L, Shivtiel S, Eldor L, Aberdam D, Itskovitz-Eldor J, Berrih-Aknin S. Human mesenchymal stem cells shift CD8+ T cells towards a suppressive phenotype by inducing tolerogenic monocytes. *Journal of Cell Science* 2012: 125(19): 4640.

Figure caption

Figure 1. Intravenous infusion of MSCs fail to migrate to areas of emphysematous remodelling in the lung, whilst inducing a number of systemic immunological responses. ^{111}Indium is present in the lungs (Lu), followed by the liver (Li), spleen (Sp) and bone marrow (BM) across 7 days post-infusion (**A**), which was quantatively measured by ROI analysis (mean and standard deviation) which is corrected for background and decay (**B**). Areas of visible emphysematous changes show minimal presence of radiolabel (red arrows), while less obviously affected areas display higher levels of radiolabel accumulation (blue arrows) (**C**). Levels of oxidative stress marker F2-IsoP decreased 7 days post-infusion (**D**). Pro-inflammatory markers such as CRP was significantly increased between 1 hour and 2 days post-infusion (**E**), while levels of sCD163 decreased from 1 day onwards (**F**) and IL-6, 2 days post-infusion (**G**). Circulating sTNFR1 was also increased 2 days post-infusion (**H**). Proportions of circulating immune cells including Tregs were increased 7 days post-infusion (**I**), while there was a reduction in the ratio of mDC:pDC (**J**). Levels of $CD14^+$ monocytes were increased 2 days post-infusion (**K**) and these monocytes at day 2 demonstrated a significant reduction in CD80, CD86, HLA-DR and TLR4 compared to baseline as shown by fold change (**L**). Representative images from 1 patient are shown in each of the radiological images. Black lines represent each individual patient time course while the red line represents the median levels to show the general trend. Statistical analysis was performed using Wilcoxon signed-ranked tests to compare levels between baseline and post-infusion (***p<0.05* ***p<0.01).*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Day 2 fold change from baseline
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