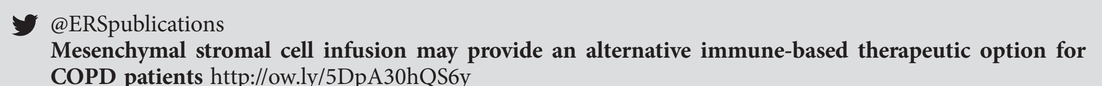
Mesenchymal stromal cell infusion modulates systemic immunological responses in stable COPD patients: a phase I pilot study

To the Editor:

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 their administration to COPD patients; however, it was reported that C-reactive protein (CRP), a marker for systemic inflammation, was reduced 1–3 months after infusion. Earlier time-points were not assessed in detail in these trials, which limits further investigation of these changes [4, 5]. Identifying the fate of intravenously infused MSCs 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 number EC2012/103) and all patients had provided written informed consent. A single site, phase I study (Australian clinical trials registry number 12614000731695) was conducted to determine 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 Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I, n=2 GOLD II, n=3 GOLD III, n=3 GOLD IV). All recruited patients had not experienced an exacerbation for at least 3 months prior to trial commencement with no change in regular medications. Patients received two infusions of low passage (p4–5) allogeneic bone marrow-derived MSCs of approximately 2×10^6 MSCs per kg, 1 week apart, with the first infusion comprising radiolabelled cells and the second infusion using unlabelled cells. MSCs used for the first infusion were labelled with indium-111, a low energy radioisotope with a half-life of 68 h to enable tracking across several days. Labelled MSCs were able to adequately suppress peripheral blood mononuclear cell (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 were monitored up to 1 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, indium-111 was detected in the lung within 30 min by computed tomography (CT) scan and remained detectable after 24 h, after which uptake was detected in the liver, spleen and bone marrow up to 7 days after infusion (figure 1a and 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 h after the first infusion and superimposed with low dose CT to determine indium-111 activity and MSC localisation in the lungs. There was reduced indium-111 in emphysematous lung (red arrows; figure 1c) compared to normal lung (blue arrows; figure 1c). Retention of indium-111 in the lungs was also positively correlated with baseline forced



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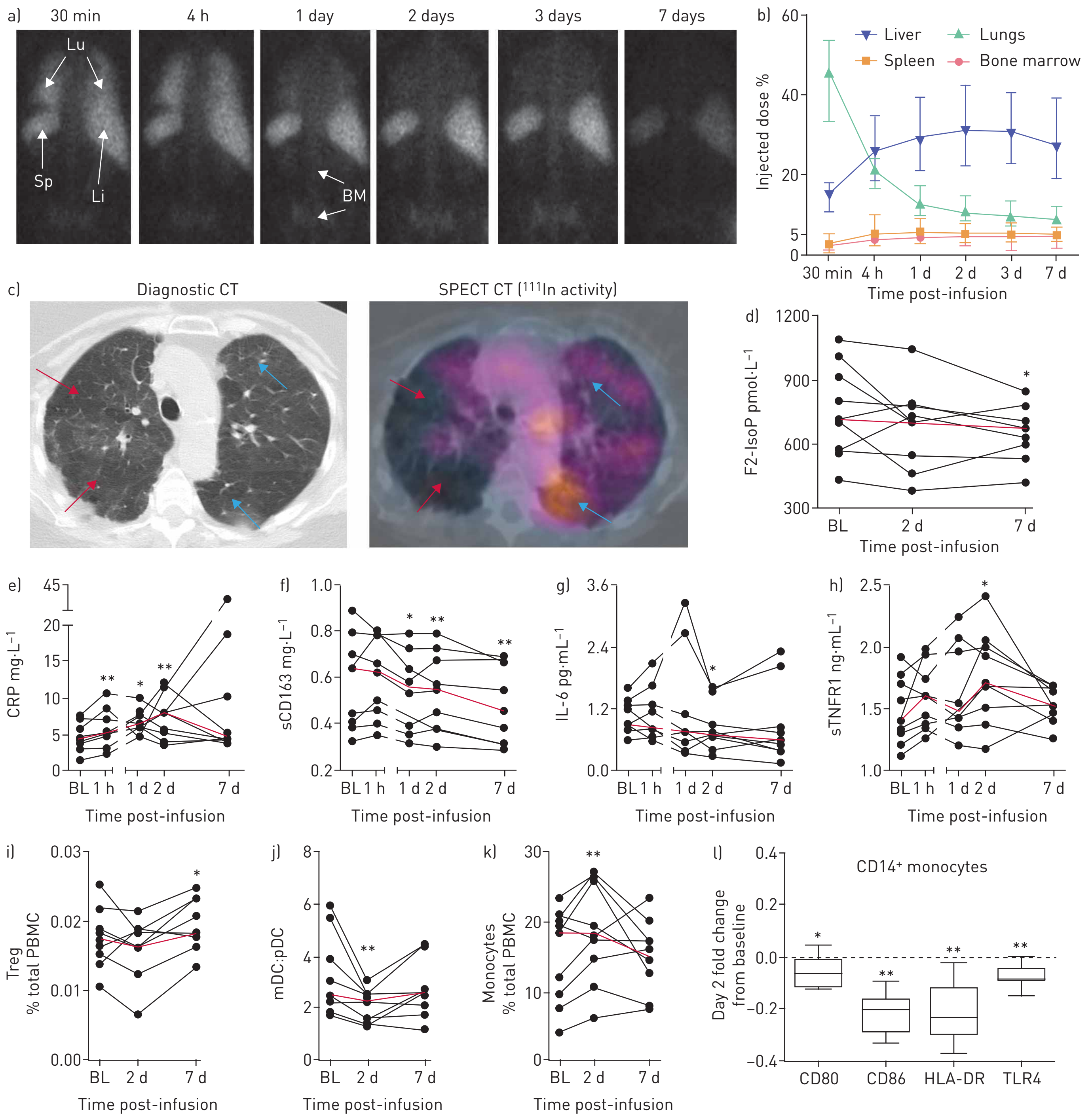

FIGURE 1 Intravenous infusion of mesenchymal stromal cells (MSCs) fail to migrate to areas of emphysematous remodelling in the lung, while inducing a number of systemic immunological responses. Indium-111 is present in the lungs (Lu), followed by the liver (Li), spleen (Sp) and bone marrow (BM) across 7 days post-infusion (a), which was quantitatively measured by region of interest (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-isoprostanes (F2-IsoP) decreased 7 days after infusion (d). Pro-inflammatory markers such as C-reactive protein (CRP) were significantly increased between 1 h and 2 days post-infusion (e), while levels of soluble (s)CD163 decreased from 1 day onwards (f) and interleukin (IL)-6, 2 days after infusion (g). Circulating sTNFR1 was also increased 2 days after infusion (h). Proportions of circulating immune cells including regulatory T-cells (Tregs) were increased 7 days post-infusion (i), while there was a reduction in the ratio of myeloid (mDC) to plasmacytoid (pDC) dendritic cells (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, human leukocyte antigen-DR (HLA-DR) and TLR4 compared to baseline as shown by fold change (l). Representative images from one 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. CT: computed tomography; SPECT: single-photon emission computed tomography; PBMC: peripheral blood mononuclear cell. *: $p<0.05$; **: $p<0.01$.

expiratory volume in 1 s (FEV_1) (R^2=0.68, p=0.02) and diffusing capacity of the lung for carbon monoxide (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 those with more severe disease did, who exhibit poor perfusion in remodelled emphysematous lung. This distribution may protect healthy lung tissue.

We first determined if indium-111 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 αCD3 and lipopolysaccharide (LPS), there was no difference in cytokine production (interleukin (IL)-1β, IL-6, IL-8, IL-10 and tumour necrosis factor (TNF)-α) 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 indium-111 had no effect on cell proportions or viability *in vitro* (data not shown).

Gas chromatography−mass spectrometry was performed for quantitation of F2-isoprostanes [7] as a marker of oxidant load from neutrophils and macrophages. F2-isoprostanes 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 h 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 1–3 months after infusion [4, 5]. Notably, there were changes in certain inflammatory mediators, including soluble CD163, a biomarker for macrophage activation that correlates with disease severity in COPD [9] that was significantly reduced 1−7 days after infusion (figure 1f). In addition, there was a fall in IL-6 (seven out of nine patients), a major mediator in COPD that which stimulates the secretion of matrix metalloproteinases and T-cell responses, which can contribute to airway remodelling (figure 1g) [10]. 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 TNF-α, 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 dendritic cell (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 acute exacerbations of COPD from 11 events occurring within a year before MSC infusion to six events occurring within 1 year following infusion. No additional interventions in the year after 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 37% pred (23–87% pred) *versus* 41% pred (41–98% pred), p=0.48; and forced vital capacity 80% pred (59–106% pred) *versus* 82% pred (56–101% pred), 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 min and probably release trophic factors, including extracellular vesicles, which 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.

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