Reviewer's report

Title: Bone Marrow-Derived Progenitor Cells in End-Stage Lung Disease Patients.

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Reviewer: Ivan Bertoncello

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This is a well structured and coherent study which has carefully quantified the relative incidence and content of bone marrow-derived fibrocytes and epithelial-like CCSP-positive cells in the bone marrow and peripheral blood of end-stage lung disease patients undergoing transplantation for different lung disease indications. The study identifies significant differences in the relative incidence of fibrocytes and CCSP-pos cells in the marrow and peripheral blood in fibrotic lung diseases (elevated fibocytes) and in cystic fibrosis (elevated CCSP-pos cells). Further multiplex array analysis of plasma cytokine levels in different disease indications reveals a significant correlation between the plasma level of stem Cell Growth Factor-beta and the incidence of circulating CCSP-pos cells; and Monocyte Chemotactic Protein-1 and the incidence of circulating fibrocytes, providing circumstantial evidence of the involvement of these factors in the trafficking and recruitment of these cell types to the diseased lung.

The study is unique in that: (a) the large number of lung donors (36), and end-stage disease patients (154) comprising large cohorts of patients undergoing transplant for cystic fibrosis and each of the fibrotic disease cohorts allowed robust comparative statistical analysis of measured variables; and (b) the sampling of bone marrow from the exposed sternum of both lung transplant donors and recipients allowed standardised collection of marrow with no (or minimal) peripheral blood contamination. The study is largely descriptive, and inferences that each cell type contributes to the pathophysiology of different lung diseases assumes a causal relationship between their relative incidence and lung disease indication. But, it is an informative and valuable dataset of great utility in modelling and designing future mechanistic studies analysing the role of these bone-marrow derived cells in intractable lung disease, and understanding how fibrocytes and CCSP-pos cells might be targeted to attenuate lung injury at earlier stages of disease progression.

I have the following specific comments and questions for the authors to consider:

1. The analysis of fibrocytes and CCSP-pos cells in bone marrow and peripheral blood are robust and statistically validated. However, the authors should clarify the gating strategy used to quantify relevant subsets: in particular the quadrant gating used to quantify CCSP-pos cell subsets in Figure 5. Isotype control labelled cells are not shown, so it is not possible to properly assess gating of minor subsets against background fluorescence although I presume this was
done for each antibody using the appropriate IgG subtype. Accepting that, close scrutiny of the bivariate plots shows that double-positive populations are not uniform. For example, in Figure 5B, the upper right quadrant captures two subsets with clearly different levels of expression of both CCR4 and CCSP. I wonder whether non-rectilinear gates would be more appropriate.

2. Can the authors confirm that this gating does not include autofluorescent cells in one or other parameter? I ask because the quadrants are set on bivariate density plots. Were list files of the same number of events used to do so to exclude the possibility that events abutting the positive quadrant boundaries are “true” positive cells – and therefore that the comparative incidences of minor subsets are accurately quantified. Likewise for Figure 1E and F.

3. The authors should provide a more detailed description of FACS analysis and sorting. For example: The fluorochromes used in bivariate analyses should be identified in the methods and/or relevant figure legends – only Alexa Fluor 488 is cited. How was spectral overlap of fluorescence emissions compensated for bivariate analysis? Was doublet discrimination used to exclude analysis of spurious events? How large were the list files acquired for quantitation of relevant cells?

4. In the concluding discussion (pg.18), the authors comment on the link between “loss of epithelial-like progenitors” and impaired epithelial repair in COPD or IPF. On reviewing the data in Figure 1, it seems to me with the exception of peripheral blood levels in BO, the incidence of CCSP-pos cells is not significantly different from donor control levels. I question whether this is evidence of a “loss” of epithelial-like progenitors, or an inability of signals from the fibrotic lung to expand/mobilise/recruit those progenitors to contribute to repair if indeed that is their function. Conversely, is the increased mobilisation and trafficking of epithelial-like bone marrow-derived cells associated with a hyperproliferative epithelium in CF, a cause or a consequence of the hyperproliferation? Do the authors have, or know of, any data showing that bone marrow derived CCSP-pos cells are integrated and proliferate in CF airways? It is an intriguing question which I concede cannot be resolved in this study.....but worth broaching in the discussion.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests