Mesenchymal Stromal Cells in ARDS: More Questions than Answers

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An effective treatment for acute respiratory distress syndrome (ARDS) remains elusive. Pre-clinical studies have demonstrated therapeutic potential of multiple MSCs and MSC-related therapies in the context of ARDS, including autologous, allogenic, xenogenic, and induced pluripotent stem cell (iPSC) derived MSCs, multipotent adult progenitor cells, alongside MSC-derived extracellular vesicles and exosomes(1). These cells, or cell-free products, have been sourced from various tissues, including bone marrow, umbilical cord, perinatal tissue, and adipose. However, few pre-clinical studies, and no clinical trials, have compared these head-to-head. Considerable debate persists as to what is the most efficacious cell product. The situation is further complicated when factors such as scalability, storage, delivery, dose, safety, and regulatory compliance are considered.

Here, Batchinsky and colleagues use a porcine model of smoke inhalation injury to compare the therapeutic efficacy of autologous bone marrow aspirate concentrate (BMAC) (n=10), allogenic bone marrow derived MSCs (n=10), and sham injection (n=12)(2). Their well-established model combined wood smoke inhalation, targeting a carboxyhemaglobin > 70%, with a full-thickness burn affecting 40% of the total body surface area, inducing moderate ARDS in the sham group by 24 hours. Animals were supported with protocolized intensive care until death or 72 hours. Autologous BMAC was harvested post-injury, at 24 and 48 hours, and concentrated at the bedside, using a point-of-care, automated, closed-loop apheresis device. Allogenic MSCs were procured from bone marrow aspirate (BMA) prior to the experiments and were expanded by serial passage. Each animal group received a total of three treatments: immediately post-injury, at 24 hours, and at 48 hours via a pulmonary artery catheter.

The study showed that BMAC, and to a lesser extent allogenic MSCs, effectively delayed the decline in PaO_2/F_IO_2 ratio associated with injury, postponing the onset of ARDS. Notably, four of the ten animals treated with BMAC did not meet ARDS criteria by 72hours. The BMAC-treated group demonstrated longer mean survival time and a reduced mortality rate compared

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with sham and allogeneic MSC groups. Furthermore, the BMAC-treated group had a lower incidence of acute kidney injury, lower systemic IL-6, IL-8 and HMGB-1 levels, and higher levels of normally aerated lung tissue on CT imaging.

This was a complex and resource-intensive study with key strengths. The challenges of successfully executing a large-animal model which requires clinical-level intensive care and at the same time, deeply characterizing the effect of the interventions should not be underestimated. The study was clinically relevant by testing MSCs and BMAC after injury occurred (albeit before onset of ARDS).

While the authors conclude that autologous MSCs (from BMAC) appear more potent than allogenic MSCs, the situation is more nuanced. Allogenic cells in this study had some protective effects, but the dose used was much lower than those used in previous large animal studies or clinical trials of ARDS (usually 1-10 million cells per kg compared with total dose of approximately 0.4million cells/kg in this study). The relatively modest therapeutic effect in the allogenic MSC-treated group is therefore unsurprising. It is also an oversimplification to describe autologous BMAC as “autologous MSCs”. BMAC is an enriched mixture of mononucleated cells, platelets, cytokines, and growth factors, many of which have immunomodulatory effects in ARDS(3, 4) . MSCs account for ~ only 0.001-0.01% of the total mononuclear cell population of bone marrow(5). In this study, the dose of MSCs in the BMAC in this study was not known, but rather a total white cell count was available. Notably this was less than the dose of allogeneic MSCs in the comparator group. It is plausible that some beneficial effects demonstrated by BMAC in this study relate to the non-MSC components (table 1).

Although the current study supports further exploration its therapeutic potential, BMAC presents substantial challenges. The extent of MSC enrichment post-concentration in BMAC production varies according to site of aspiration, donor sex, and the device used to perform concentration(6, 7). The concentration process may induce phenotypic alterations within

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MSCs, modifying their *in-vivo* actions(8) and in swine, the onset of lung injury has been shown to render the MSCs retrieved from autologous bone marrow less effective in terms of regenerative and immunomodulatory properties compared with uninjured controls(9). The issues of batch-to-batch variation and lack of definitive potency assays for efficacy that have plagued the field of MSC therapy, are even more relevant for BMAC. While BMAC offers the potential advantage of retrieving MSCs that will not lose efficacy by undergoing repeated passage *in vitro* and avoids the use of animal products such as FBS or excipients like DMSO, it is difficult to understand how we might standardise BMAC composition at the bedside for clinical trials and therapeutic use.

Furthermore, with increasing age, the cell yield from BMAC falls rapidly. A recent study showed a greater than 67% reduction in mononuclear cell yield per ml of marrow by age 55-60 compared with age 19-20(6). In the Lungsafe study the mean age of patients with ARDS patients was 61 (95%CI 60-62)(10). Even if therapeutically effective in other pre-clinical models of ARDS, clinical trials may not be feasible if the substrate for autologous BMAC is insufficient in most patients.

The heterogeneity of BMAC reflects the heterogeneity evident in the entirety of ARDS cell therapy. Phenotypically different cell populations, obtained from diverse sources using diverse protocols, and applied to a heterogenous syndrome cannot be expected to produce consistent outcomes – a fact evident in the results of recent clinical trials in both COVID and non-COVID related ARDS(11-15). So, how can researchers address the heterogeneity, and can pre-clinical models contribute? First, there should be a concerted effort to better characterize “MSCs” and their related products used in experimentation, ideally beyond the minimal criteria required to identify MSCs, and including assays of function and relevant biological activity. An example is tissue factor/CD142 expression, which varies widely among MSCs, but is implicated in the risk of thromboembolic complications(16) . Second, we need to develop an approach that allows comparison of different cell products in a common model or a consistent product in alternative models. Given the cost and complexity of high-fidelity models, such as the one described in the current study, no single research group is likely to achieve this. The

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solution may be to adopt protocols and collaborations which permit multi-centre platform studies, as is an evolving norm among clinical trials. The recent endeavour of the Stroke Preclinical Assessment Network (SPAN), which tested six treatment candidates across multiple models in 2,615 mice, serves as an exemplar(17).

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Table 1 - Comparison of allogenic MSC products and BMAC

	Allogenic MSC preparations	Autologous Bone Marrow Aspirate Concentrate (BMAC)
Mesenchymal Stromal Cells	Pure population – fixed number per dose	variable % of total cell count
Platelets	-	present - variable amount
Leukocytes	-	neutrophils, erythroblasts, lymphocytes, eosinophils, monocytes and basophils – variable amount
Cytokines	-	IL-1Ra, IL-1β, IL-6, IL-8, GM-CSF, TNFα variably reported
Growth factors	-	TGFβ, VEGF, PDGF, FGF-2/18, IGF-1
Other proteins	-	BMPs, osteoprotegerin reported in some but not all BMAC preparations
Potentially Immunogenic^	yes	no
Persistence of cellular components	rapidly cleared	not yet known, but not foreign
Expansion/passage *in vitro**	yes	no
Freeze/thaw cycle#	usually (for clinical grade product)	no
Excipients	usually DMSO	none
Exogenous animal product used in manufacture	fetal bovine serum	none
Release criteria for clinical grade product	known	unknown
Potency assay	no standard assay	unknown
Is suitability age dependent?	no	likely

^clinical studies have not shown clear anti-HLA antibody development but long-term studies are needed
*serial passage linked to reduction in therapeutic efficacy
#freeze thaw cycle may affect viability
VEGF - vascular endothelial growth factor; PDGF – platelet derived growth factor; TGFβ – transforming growth factor beta; FGF – fibroblast growth factor; IGF-1 – insulin-like growth factor; BMP – bone morphogenic proteins
DMSO – dimethyl sulphoxide

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