Editorial Commentary: Cell-Based Therapies for Articular Cartilage Repair Require Precise Progenitor Cell Characterization and Determination of Mechanism of Action

Claire D. Eliasberg, M.D., and Scott A. Rodeo, M.D.

Abstract: Biologics and cell-based therapies, in particular, have come to the forefront of orthopaedic sports medicine as agents with therapeutic and regenerative potential. Autologous chondrocyte implantation has been used successfully for many years, but a recent focus on autologous progenitor cells derived from bone marrow aspirate, adipose tissue, and/or synovium has garnered significant interest. Mobilized peripheral blood mononuclear cells [PBMCs or connective tissue progenitors (CTPs)] represent a promising cell population for potential use in articular cartilage repair. The term "stem cell" has become widely popularized, but more specific language identifying the cell type by donor type, tissue of origin, cell surface marker profile, culture conditions, and other cell behavior/characteristics should be used. In 2019, Murray et al. proposed a five-item "DOSES" tool in an effort to encourage standardized reporting for cell-based therapies emphasizing donor, origin of tissue, separation from other cell types/preparation method, exhibited cell characteristics associated with behavior, and site of delivery. The advantages of the DOSES tool include both simplicity and ability to be applied to cell types not yet discovered. However, a universally accepted list of criteria for biologics does not yet exist. Additional research is necessary to better elucidate the precise mechanisms by which cell therapies have a clinical effect and define whether the therapies for the treatment of cartilage pathology merely help alleviate symptoms or actually provide structural improvements. There are few data to suggest exogenous cell therapies directly engraft, so identifying the paracrine mediators produced by these cells would be an area of further interest.

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With increasing interest paid to biologic therapies and the augmentation of surgical procedures with biologic agents, significant attention has been given to cell-based therapies. Cartilage injuries are of particular interest given the poor intrinsic healing potential of articular cartilage. Autologous chondrocyte implantation has been used successfully for many years, but a recent focus on autologous progenitor cells derived from bone marrow aspirate, adipose tissue, and/or synovium has garnered significant interest.

We read the article entitled "Mobilized Peripheral Blood Stem Cells Are Pluripotent and Can Be Safely Harvested and Stored for Cartilage Repair" by Anz, Torress, Plummer, Siew-Yoke Jee, Dekker, Johnson, and Saw[1] with great interest. The authors describe their method for harvesting, processing, and storing these "peripheral blood stem cells" (PBSCs), also known as peripheral blood mononuclear cells (PBMCs), for potential use in articular cartilage repair. The authors harvested PBMCs from 10 healthy subjects using a previously established leukapheresis protocol, and found that post-processing cell viability did not decrease significantly at up to 2 years postprocessing. There was no evidence of contamination on sterility testing. Samples from five participants were tested for differentiation potential, and all samples were deemed to demonstrate multipotency using chondrogenic, adipogenic, osteogenic, endoderm, and ectoderm assays. We would like to commend the authors for describing this process thoroughly and demonstrating sterile storage and preservation of cell viability at a two-year time point.

New York, New York

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The authors acknowledge this study was designed as part of a larger planned manufacturing validation study as part of an Investigational New Drug application for a multicenter phase 2 clinical trial (ClinicalTrials.gov: NCT03101163), the goal of which is to determine whether PBSC injections can improve functional outcomes and pain relief in patients with knee articular cartilage lesions. The authors successfully completed their study's aims, but this article brings up several additional points of discussion.

As the field of OrthoBiologics continues to expand rapidly, there have been many recent calls from within the orthopaedic community to better define the types of biologics being used in both translational and clinical research studies. In particular, nomenclature for cell-based therapies has been very inconsistent in the literature. The term "stem cell" has become widely popularized, but more specific language identifying the cell type by donor type, tissue of origin, cell surface marker profile, culture conditions, and other cell behavior/characteristics should be used.[2] The cells in this study do not meet the formal criteria for "stem cells" and, alternatively, should be referred to as PBMCs or connective tissue progenitors (CTPs). The authors assessed the cells' differentiation potential with colony-forming unit (CFU) assays, which is a standard assay for CTPs, as each CTP cell is the founding cell for a CFU. We believe that there should be universally accepted nomenclature for cell-based therapies that should be mandatory and standardized across the orthopaedic literature.

Additionally, clear criteria are needed to better characterize the various biologics that are being used in orthopaedic research. In 2017, Murray et al. published recommended minimum reporting requirements for clinical studies evaluating platelet-rich plasma (PRP) and mesenchymal progenitor cells.[3] Standardized checklists, such as these, across orthopaedic journals would allow for academic transparency, scientific reproducibility, and the ability to more accurately compare biologic studies. We acknowledge that development of individualized, specific checklists for every possible biologic agent may prove arduous. In 2019, Murray et al. proposed a five-item "DOSES" tool in an effort to encourage standardized reporting for cell-based therapies.[4] This communication tool emphasizes five key items: donor, origin of tissue, separation from other cell types/preparation method, exhibited cell characteristics associated with behavior, and site of delivery. The advantages of the DOSES tool include both its relative simplicity and its ability to be applied to future cell types not yet discovered. However, despite these proposals, a universally accepted list of criteria for biologics does not yet exist.

In the current study, this PMBC population demonstrates promise due to its widespread use in the field of oncology and the relative ease of translational potential. However, additional research is necessary to better identify and characterize the specific cell types and their efficacy. There is likely heterogeneity within the cells being harvested. For example, the authors demonstrate that only a small fraction of the total cells harvested were CD34+, and that was the only marker used to characterize the cells in this study. Previous studies suggest that there are likely distinct subpopulations within PBMCs, and these phenotypes may correspond to differences in differentiation and function.[5–7] It would be interesting to further delineate the cell population obtained in this study into hematopoietic versus mesenchymal progenitors and to compare the CFU potential of these cells with that of other cell therapy formulations. Further characterization of the specific cell types harvested in this manner would be very beneficial to the field of OrthoBiologics moving forward.

Finally, additional research is necessary to better elucidate the precise mechanisms by which these PBMCs and other cell therapies are having a beneficial effect. The authors performed multiple differentiation assays to demonstrate multipotency, but the assays did not allow for quantification, nor do these five assays definitively prove "pluripotency" (the ability of a cell to become differentiated into any cell type in the adult body). The term pluripotency is typically reserved for embryonic stem cells and induced pluripotent stem cells. It is also important to note that differentiation of cells in culture does not necessarily predict the *in vivo* response after implantation. It would be interesting to further characterize the cells' relative differentiation potential *in vivo*, to identify which cell characteristics might be important in determining differentiation potential (particularly their chondrogenic potential), and what the minimum number and subtypes of cells is necessary to produce a clinically appreciable effect. At the clinical level, we also need further evidence to better define whether the use of PBMCs and other cell-based therapies in the treatment of cartilage pathology merely help to alleviate symptoms or whether they actually provide structural improvements in cartilage lesions.[8] There are few data to suggest exogenous cell therapies directly engraft, so identifying the paracrine mediators produced by these cells would be an area of further interest. While determining cell viability during the preservation process is an important first step, ultimately, demonstrating these cells' mechanisms of action and clinical efficacy through rigorous, well-designed translational and clinical studies will determine whether this cell-based therapy is worth pursuing.

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