Published in final edited form as:

J Bone Miner Res. 2018 October ; 33(10): 1721–1728. doi:10.1002/jbmr.3578.

Continuing Challenges in Advancing Preclinical Science in Skeletal Cell-Based Therapies and Tissue Regeneration

Joseph Featherall[1,2,3], **Pamela G Robey**[3], **David W Rowe**[4]

[1]Cleveland Clinic Lerner College of Medicine, Cleveland, OH, USA

[2]Medical Research Scholars Program, Clinical Center, National Institutes of Health, Department of Health and Human Services, Bethesda MD, USA

[3]Skeletal Biology Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda MD, USA

[4]Center for Regenerative Medicine and Skeletal Development, UConn School of Dental Medicine, Farmington, CT, USA

Abstract

Cell-based therapies hold much promise for musculoskeletal medicine; however, this rapidly growing field faces a number of challenges. Few of these therapies have proven clinical benefit, and an insufficient regulatory environment has allowed for widespread clinical implementation without sufficient evidence of efficacy. The technical and biological complexity of cell-based therapies has contributed to difficulties with reproducibility and mechanistic clarity. In order to aid in addressing these challenges, we aim to clarify the key issues in the preclinical cell therapy field, and to provide a conceptual framework for advancing the state of the science. Broadly, these suggestions relate to: (i) delineating cell-therapy types and moving away from "catch-all" terms such as "stem cell" therapies; (ii) clarifying descriptions of cells and their processing; and (iii) increasing the standard of in vivo evaluation of cell-based therapy experiments to determining cell fates. Further, we provide an overview of methods for experimental evaluation, data sharing, and professional society participation that would be instrumental in advancing this field. © 2018 American Society for Bone and Mineral Research.

Keywords

CELL-BASED THERAPIES; TISSUE ENGINEERING; PRE-CLINICAL MODELS; EXPERIMENTAL REPRODUCIBILITY

Address correspondence to: Pamela G Robey, PhD, SBS/NIDCR/NIH/DHHS, Building 30, Room 228, 30 Convent Drive, MSC: 4320, Bethesda, MD 20892, USA. probey@dir.nidcr.nih.gov.

Disclosures

The authors declare that they have no conflicts of interest.

Introduction

Cell-based therapies are a new frontier in musculoskeletal medicine, and are often heralded as holding much promise for modifying disease progression and repairing or replacing damaged or degenerating tissues. Cell-based therapy is an emerging concept that encompasses the fields of engineered tissues, direct cell application, and cell-derived products (eg, platelet rich plasma, extracellular vesicles). Within the bone and cartilage fields, cell-based therapies are mainly permanent cell replacement therapies, whole-tissue engineering, transient cell therapies, and conventional tissue grafts, particularly for the treatment of injury or degeneration of the skeletal system.[1]

The scientific, public, and biomedical healthcare industry excitement for cell-based therapies has grown exponentially over the past decade. Over 18 billion US dollars have been invested in publicly traded cell therapy companies between 2011 and 2016.[2] As of 2016, there were over 500 clinics in the United States alone marketing “stem cell” therapies.[3] Between 2008 and 2012, the growth rate of stem cell scientific publications grew at greater than twice the rate of all publications worldwide, with nearly 30,000 manuscripts published in 2012.[4] This flourishing field not only presents growth and potential therapeutic promise, but increasingly presents the scientific and medical communities with new challenges.[5,6]

The clinical problems associated with cell-based therapies are becoming increasingly acute, particularly in applications unrelated to the skeleton. A prominent case series early in 2017 of blindness in three patients after “stem cell” injections to treat acute macular degeneration marked a crescendo in safety concerns for the field.[7] In one report sampling 1052 publications regarding stem cell clinical trials, of the 393 completed cell-based trials, only 45% had reported their results, with some trials disclosing results directly through press releases, bypassing peer review, contrary to the recommendations of the International Society for Stem Cell Research.[8,9] Further, many stem cell tourism clinics register trials to provide the appearance of legitimacy without the intention of trial completion or disclosure of data, making the actual disclosure rates of stem cell clinical trial data significantly lower.

The intent of this article is to rekindle the professional discourse initiated by Manolagas and Kronenberg[10] in 2014, as to how cell-based therapies and clinical trials for skeletal applications are in need of improvements such as increased regulation, better and more standardized trials, and reduction in profiteering from experimental therapies. Importantly, the discussion and concern also needs to include the basic science community. Although there is a degree of separation between basic scientists and the most pressing public issues of cell-based therapies, scientists working in the areas of stem and progenitor cell biology and tissue engineering hold a unique position in the landscape of cell-based medicine. Basic and translational scientists can, therefore, help clarify the capabilities and limitations of these therapies, and aid in more directly and rigorously advancing the field toward the ultimate goal of clinical benefit to patients with musculoskeletal disease.

Key Issues

Mechanistic complexity

Cell-based therapies, reflecting the rapid advancement of biomedical science, are rooted in exceedingly complex systems. Cell-based therapies rely on multiple traditional fields: biomaterials development, cell harvesting and selection, cell modification techniques, surgical repair techniques, and immune modulation. Understanding cell-based therapies involves understanding the interaction between any or all of these components of a particular therapy.

Much of the medical scientific community's experience in therapy development has come from single-molecule drug development for modification of a biological molecular pathway. In contrast to traditional pharmaceutical research, which investigates a single or limited numbers of agents in combination and their effects on a biological entity, cell-based therapies rely on an immensely complex microsystem—the cell—which produces and secretes thousands of possible effector molecules, changes its activities over time, and, itself, is incompletely understood. Because of this complexity, traditional methods for developing cell therapies will need to be augmented in order to safely and rationally bring to bear cell-based therapies for the benefit of patients.

Largely unproven applications

Adding to the challenges of understanding and rationalizing cell-based therapies, is the fact that there are very few cell-based therapies proven to be effective. Other than bone marrow transplantation, the US Food and Drug Administration (FDA) has approved only a single cell-based therapy for skeletal disease: matrix-associated autologous chondrocyte implantation (MACI). However, studies are ongoing to determine whether MACI is superior to microfracture, the current standard of care in which an orthopaedic surgeon perforates the subchondral bone, allowing for bone marrow cells to infiltrate the cartilage lesion and regrow fibrocartilage.[11,12] Clinical studies addressing the remaining applications of cell-based therapies in bone and cartilage applications are mostly small, poorly controlled, or merely suggestive of benefit (eg, improvement on imaging).[13] Objective reporting of clinical studies is remarkably sparse. A cursory search of ClinalTrials.gov using search criteria of bone disease and cells identifies over 300 entries, of which ~60 relate to permanent cell therapy (see definition below in the "Delineating cell-based therapy types" section). Only two of the 30 completed studies reported results ((https://register.clinicaltrials.gov/), (https://register.clinicaltrials.gov/)) that were subsequently published.[14,15] The more frequent result of this search is a "completed" or "unknown" status with no reported results, some of which have direct clinical relevance to patients with osteogenesis imperfecta ((https://register.clinicaltrials.gov/)). Reporting disappointing results is as important as reporting those with an incremental or indeterminate outcome. Because the clinical data remains minimal and equivocal, claims of therapeutic potential in basic and translational experiments have become inflated and disconnected from the limitations, challenges, and level of maturity of these techniques. Academic review articles tout "promise," editorially inflate minor positive results, and cite company press releases of unpublished data.[5,8,16] Likewise, questionable companies use publications in dodgy

("predatory") journals to support grand claims of efficacy. Such an environment has promoted unchecked public expectations and unevidenced clinical practice.

Regulatory challenges

The FDA has had difficulty in formulating regulation of human cells, tissues, and cellular and tissue-based products (HCT/P). For over a decade, a number of clinicians have exploited the ambiguity in the Code of Federal Regulations (CFR) Part 1271 in order to profit from untested and underdeveloped cell-based therapies.[13,17,18] The regulatory ambiguity has allowed for cell-based therapy clinical trials and basic science to become uncoupled; ie, clinical trials have progressed without adequate preclinical evidence. Such scientific conduct has heightened public hopes, marred the cell-based therapy field, and created a gap in the translation of science from bench to bedside.

Definitional inaccuracies

The definitions of cell types and therapy modalities have become "catch-all" terms that provide researchers and patients with little understanding of the actual mechanism of action of the therapy or experiment, leading to further confusion. The definitions and markers of cells (mesenchymal stem cells [MSCs], tissue progenitors, etc.) commonly used in preclinical and clinical regenerative experiments are not consistent, leading to differing biological potentials of cells categorized within these broad, nonspecific terms.[19]

Stem cell therapy has become a term that includes nearly any application of poorly defined cellular material to treat human disease. For example, the frequently used adipose-derived stromal vascular fraction (often called adipose-derived MSCs) includes adipose stromal cells, endothelial cells, fibroblasts, lymphocytes, and monocyte/macrophages, among other cell types.[20] Further, these cells, depending on the tissue source and isolation method, may or may not even meet current minimal criteria for "MSC" definitions, which are nonspecific and tentative at best.[21]

Biological and technical variability

In addition to loose definitions, variability in the biologic potential of the cells themselves further contribute to issues of experimental reproducibility. For example, bone marrow stromal cells (BMSCs) vary significantly in their clonogenic potential and phenotype based on donor gender and age[22]; and only a small subset of expanded BMSCs demonstrate full differentiation potential.[23] Cells somewhat imprudently grouped together and referred to as MSCs are derived from over a dozen different tissue types, and are tremendously biologically variable in nearly all quantifiable aspects.[24]

Technical variability in the methods with which cells are procured, processed, and preserved contribute to the cells' in vitro and in vivo performance.[25] Some authors argue that this technical variability in cell "manufacturing" procedures may be largely responsible for the wide variation in clinical trial outcomes of cell based therapies.[26,27] Osteogenic cells derived from induced pluripotent stem cells (IPSCs) created by a vast array of complex induction protocols may have epigenetic memory of their tissue of origin and great variability in their biological performance, and their in vivo potential is difficult to predict.

[28] Some research groups, particularly private entities, keep the details of cell preparation techniques closely guarded, contributing to further confusion regarding efficacy and reproducibility.

Issue summary

Reproducibility is of broad concern in the basic and translational science communities[10]; this concern is amplified in the field of cell-based therapies. Cell-based therapies as a whole are immature and immensely complex technologies with a limited clinical track record. The cell-based therapy fields can address these challenges, at the basic and translational levels, to enhance the social value of science leading more consistently to effective and safe interventions for patients (see Table 1).

Tools and Methods for Improving the Scientific Value of Cell-Based Therapy Work

Delineating cell-based therapy types

Providing specificity in the applications of cells to treat human disease will add clarity to scientific fields and public discourse.

Tissue engineering is a historically well-defined field that uses scaffolds, cells, and/or biologically active molecules to construct functional tissues.[29] This canonical application of cells for human therapeutics is well established and has minimal controversy regarding its capability. The clarity of capability of tissue engineering is largely driven by its aim of reconstructing organized tissue and its rigorous evaluation being the testing and analysis of whole-tissue constructs that can be relatively easily evaluated.

Permanent cell replacement therapy has its conceptual roots in the bone marrow transplant field, in which stem and progenitor cells are harvested and administered without an organized construct. These cells then permanently replace the depleted bone marrow, and, via proliferation and differentiation, replenish the full spectrum of functional hematologic cell types. However, this conceptual model of circulating and tissue-homing cells that engraft and regenerate skeletal tissues has proven more difficult to document in highly organized, three-dimensional tissues, such as in the skeleton.[30–34] Studies of skeletal development and tissue injury/repair indicate that stem/progenitor cells are tissue-resident with a limited migratory potential in the circulation, but within their domain, these cells have remarkable regenerative capacity.[35,36]

Transient cell therapies describe the majority of therapies currently in clinical trials. In this noncanonical conceptual model, cells secrete effector molecules or have direct interactions with the target tissue, which modify the course of disease or injury. Examples range from augmentation of host bone repair to modulation of graft versus host disease. The mechanisms of such treatments are difficult to pinpoint, because there are thousands of possible interactions and effector molecules, possibly modifying recipient tissue homeostasis.

The classifications described herein (Table 2), can help clarify therapy types to move away from the legacy terminology of “stem cell” therapies. This historical term should be avoided because it is largely misleading to both the scientific community and broader public alike. The vast majority of “stem cell” therapies are heterogeneous cell populations that may or may not include a significant number of stem or progenitor cells applied transiently, and have no proven stem or progenitor function; ie, engraftment, production of functional tissue/cell types, and self-renewal. More nuanced terminology that better reflects the actuality of these therapy modalities will assist in improving the communication of findings, developing realistic expectations, and translating basic science findings.

Clarity in defining cell types and modifications

The classical definition of skeletal stem cells is “postnatal, self-renewing, and multipotent stem cells giving rise to all the skeletal tissues (cartilage, bone, stroma and marrow adipocytes).” The cell types termed MSCs, used widely in both clinical and preclinical investigations, generally express a variety of putative stem cell markers that have turned out to be nonspecific, may not necessarily exhibit multipotent properties in vivo, and are heterogeneous in nature. Even in well-characterized cell types with established purification methods such as BMSCs, there is significant heterogeneity in both population subtypes and in vivo potential.[25,37] Newer technologies such as single-cell RNA sequencing will help characterize these subpopulations, further elucidating the crucial cell populations, as well as their functions.[38]

Based on the differences between “MSCs” derived from different tissues, investigators should move away from using broad, nondescript terminology, such as MSCs, and gravitate toward more detailed descriptions of cell sources and processing to enhance the specificity of cell descriptions, scientific conclusions, and experimental reproducibility. Such information is often lacking even in well-regarded journals[10]; investigators, reviewers, and editors, alike, should place increasing emphasis on clear, detailed protocols for all aspects of cell-based experiments from cell sourcing through transplantation.

Increasing in vivo validation

In vitro experimentation classically holds less accuracy than in vivo experimentation, and this discrepancy is magnified in the case of cell-based therapies. Although there are some gold standard in vitro assays, such as cartilage pellet cultures or myotube formation assays, many in vitro assays have limited ability to predict in vivo behavior.[28] Although in vivo testing is inherently more resource and time intensive, the wide availability of robust, severely immunocompromised mouse models, such as NSG and SHC mice, and a number of well characterized simple-to-implement assays, increase the feasibility of widespread in vivo validation.

Standards of in vivo evaluation

Because of the complexity of cell-based therapies, as described above in the “Key Issues” section, the efficacy of the cell therapy should be determined by characterizing the in vivo fates of the transplanted cells and the response of the resident tissue cells. In order to determine cell fates and responses, the use of multiple complementary techniques is

necessary. Histological, biochemical, and molecular techniques should be used together to provide sufficient evidence such that experimental outcomes are unequivocal.

The presence of the transplanted cells at the experimental site can indicate either paracrine action (cell non-autonomous, noncanonical, transient cell therapy) or direct tissue regeneration (cell autonomous, canonical, permanent cell therapy), or both. Tracking resident cells during the healing process and evaluating the manner in which these cells are influenced by exogenously added cells will help clarify the efficacy and putative mechanisms of action of the cell therapy. Despite its importance, historically, few studies of cell-based therapies perform cell tracing or describe the localization or persistence of donor cells in the recipient tissue. In studies where donor cell fate is followed, a variety of techniques are used including: fluorescent membrane labeling, reporter trans-genes, or species-specific markers/antigens in xenogeneic transplantation. Each approach has its own unique artifacts and can mislead when used singly.

As a few examples of technique-specific artifacts, cells labeled by fluorescent chemicals or a GFP protein that binds to the plasma membrane or cytosolic components can be engulfed by macrophages.[39] Non-Cre-expressing cells adjacent to apoptotic cells that have released a Cre protein can activate a dormant Cre reporter, or GFP/β-galactosidase (β-gal)-expressing cells may have a broader range of expression than is appreciated. Additionally, antibodies can have unpredictable levels of antigen recognition depending on the lot.[40] Thus, it is necessary to map the fluorescent reporter signal with a traditional histological feature of a specific cell type so that accurate identification can be assured.

Suggested Methods for Cell-Based Regenerative Experiments

Although there is no gold standard for the evaluation of cell-based experiments, a number of established techniques are available to make investigations systematic, and as rigorous as possible. This section provides a number of suggested techniques and considerations derived from review of the literature of cell-based therapy experiments and the authors' experiences.

Immunity considerations

Recipient immunity to transplanted cells can be a significant confounder in cell-based experiments, and evidence that stem cells are immune-privileged (in particular, "MSCs") is not adequately established.[41] In mouse to mouse experiments, recipient and donor isogeneity mitigates this potential confounding effect.[41] In human to rodent experiments, NSG (Jackson Labs, Bar Harbor, ME, USA) and NOG and SHC mice (Charles River, Wilmington, MA, USA) have well-established tolerance to human grafts.[42] In order to mitigate the size constraints of these mouse models, Hera BioLabs (Lexington, KY, USA) has developed a Rag2/Il2rgamma double knockout rat using the CRISPR/Cas9 system called the SRG strain. This rat has an immune profile analogous to the NSG mouse; however, the model is currently in limited supply, and is largely untested in cell-based therapy experiments.

Determining recipient and donor contributions

Identifying the roles of recipient and donor cells in modifying the target tissue will improve the research community's ability to gain mechanistic insight and refine techniques. Differentiation-restricted promoters driving GFP reporters will allow for the identification of early progenitors and fully differentiated and persistent chondrocytes, osteoblasts, osteoclasts, etc.[43]; ubiquitously expressed reporters and membrane labeling techniques are subject to greater artifact, indistinguishable cell types, and dilution or engulfment. Co-localization of reporter signal with traditional histological features strengthens such methods. In human to mouse models, donor cell labeling with a lentiviral-delivered GFP reporter prior to transplantation, antibody staining for human nuclear or mitochondrial antigens, and human specific bone matrix staining help clarify contributions.

Functional outcomes

The end goal of cell-based therapies is to improve function in human patients. Studies using small animal models should be designed to provide early understanding of the functionality of repaired tissues. In therapies targeting bone repair, integration of host and donor-derived matrix as demonstrated by the intermingling of the corresponding GFP-positive cells, particularly at the margins of the repair field, provides histological evidence for a function repair. Mechanical studies of the repaired bone provide additional evidence for a successful outcome. However, the use of a weight-bearing model that allows removal of the fixation hardware in the living animal is probably the best demonstration of functional success.[44] In therapies targeting cartilage repair, weight-bearing analysis or exercise endurance help clarify functional improvements.[45]

Evaluation time points

Examination of the relevant tissues at both early and late stages of the healing process is necessary to establish that the donor-derived cells are still present in the target tissues. Early time points (<2 weeks) aid in understanding cell migration and interaction, transient cell effects, possible immune response modification, and early tissue changes. Mid-term time points (2 weeks to 6 months) clarify functional success, tissue repair parameters, and progenitor potential of cells through observation of retention or gradual loss of donor cells. Long-term time points (>6 months) investigate the durability and stability of the tissue repair, as well as the ability to modify longer-term degenerative processes.

Ex vivo structural analysis

Detailed micro-computed tomography (μCT) studies of both bone and cartilage can provide high-resolution structural detail and can aid in estimating structural properties.[46,47] Mechanical tests, including microindentation, microcompressive, micro-tensile, and microbending tests, characterize bone structural integrity.[48] Testing of cartilage lubricity and stiffness, including confined, unconfined, and indentation testing, may be considered.[49]

Special considerations for transient cells experiments

If all tissue formation is host-derived, and the experimental group has superior tissue repair or function compared to control, this indicates the possible presence of a non-autonomous,

transient cell-based mechanism. In this case, a ubiquitously expressed promoter driving a GFP reporter will aid in determining the length of time the donor cells are within the repair field. Determining what cell type and what factors these cells produce that affect repair are additional experimental directions that need to be considered.[50] Having a GFP reporter marking the human cells within the repair field provides the opportunity to identify and even isolate these cells to discover potential candidate cell products that promote the host repair.

Cross-laboratory comparisons

There are large-scale efforts underway to enhance the repeatability of biomedical experiments; cell-based therapies are an area that can greatly benefit from such efforts.[10] Sharing of reporter mice, proven antibodies, cell procurement, selection, culture protocols, scaffold materials, and analytical technologies increases the broader scientific value of each of these resources, and will greatly enhance experimental repeatability and corroboration. Critical evaluation of progenitor potential utilizing functional,[25] cytometric,[51] molecular,[52] or proteomic criteria need to be tested across multiple laboratories.[53] Ideally, promising strategies should be evaluated in an environment that is independent of the primary laboratory to ensure that the outcomes are reproducible and unbiased in their interpretations.

Data and technique transparency

Tiled histological images of the entire repair field in original resolution should be made available to journals and subsequently to readers. Alternatively, investigators may deposit the original histology images on a public or laboratory Web site and a link can be provided in the article. Histology plays a central role in the interpretation of transplantation experiments. The generation and distribution of images should be likened to microarray or sequencing data, or open source software, with universal access to primary data. Detailed cell processing protocols and scaffold/vehicle construction protocols should be made universally available in either methods sections, or, if extensive, as supporting materials.

Large animal models

Large animal models, although essential, are expensive and technically demanding. Large animal studies currently are limited to autologous-based experiments, thus methods for distinguishing recipient from donor are limited.[54] The National Swine Resource and Research Center (NSRRC; https://nsrrc.missouri.edu/) is a National Institutes of Health (NIH)-supported facility that can provide GFP reporter pig lines, and also has pigs with immune profiles similar to the NSG murine strain. Cell therapies successful in mouse or rat models could be scaled to the immunocompromised pig as a route to an FDA-approved clinical trial and subsequent benefit to human patients.

The Importance of Professional Societies, Academic Institutions, and National Funding Agencies in the Standardization of Cell-Based Regenerative Science

Professional societies can aid in advancing the state of cell-based therapy science by actively promoting efforts to increase scientific rigor, standardization, and collaboration. For

example, the ASBMR and ORS recently convened a task force to provide standard recommendations for preclinical cell-based therapy experiments. A consensus task force report is anticipated in the coming year. Professional societies may also contribute by providing workshops on surgical models, histological techniques, and cell processing methods that would help standardize experimental implementation and interpretation. How the current and next generation of cell-based scientists are being trained requires examination. In too many institutions, there are physical and operational barriers between advanced training programs in the clinical, material, and biological sciences that preclude optimizing the advances and prolong the limitations of each discipline, which in turn is an impediment to standardization. Equally important is the need for the major federal funding agencies to support the development of criteria for assessing the success and cellular mechanism of action of a cell-based therapy. Once established, the basis for demonstrating transparency, rigor of methodology, and frankness of experimental interpretation can be assessed by grant review panels. The current emphasis on publication number, safe science, pseudo-translational potential, and principal investigator (PI)-driven science all act as an impediment toward a critical interlaboratory consensus-building process that will be needed to develop meaningful cell-based regenerative strategies.

Summary

Currently, the basic and translational scientific foundations of cell-based therapies lag behind clinical trials and unregulated, questionable clinical use. Increasing the field’s understanding of cell-based therapies at the tissue, cellular, and molecular levels will greatly aid in intelligent translation of these techniques to human patients.

An optimal experimental approach to evaluating cell-based therapies for enhancing musculoskeletal tissue repair/regeneration would be to initiate studies in small animals, focusing on cellular, molecular, functional, and mechanical outcome measures. Once these models provide proof-of-principle in multiple laboratories for the utility of a specific cell preparation in augmentation of repair, additional investigation would be completed in larger animal models such as the recently developed immunocompromised pig. Subsequent successful outcomes in the large animal models, with inclusion of appropriate safety and efficacy profiles, would identify prime candidates for human clinical trials.

Identification of cell fates and the interpretation of the role of donor cells in the repair process is critical. Donor cells may undergo terminal differentiation and remain in the tissue as mature cells, or may have a critical early and more transient role in driving the repair process. Cell-based therapies should be investigated in relevant models across the various skeletal tissues. Because skeletal tissues (cartilage, bones, and associated tissues such as marrow, tendon, ligament, and intervertebral disc) each require unique mechanical properties, the model should permit assessment of whether an appropriate functioning tissue forms. Due to the highly variable nature of protocols for cellular work, increased transparency and sharing of techniques and materials is needed. There is a need for development of additional in vitro and in vivo models, as well as computational approaches. In particular, noninvasive assessments of tissue composition, structure, and function need further development as specific cell-based therapies are extended to human trials.

Professional societies, academia, and national funding agencies all need to contribute by developing experimental standards and technique-focused workshops.

Concluding Remarks

Cell-based therapies are an area of public confusion and are subject to increasing regulatory, scientific, and public safety scrutiny. To ensure that the promise and scientific potential of this field are met, and that public and regulatory trust in the field is upheld, basic and translational scientists can implement currently available technologies to increase the scientific rigor supporting cell-based therapies. Further, increasing focus on mechanism and cell fate determination can improve the utility and accuracy of the scientific conclusions drawn from these experiments. Such advancements will inform intelligent clinical trial design, strengthen the scientific foundation for clinical translation, and drive the discovery of cell-derived products that may be used for treatment of musculoskeletal conditions. Such an approach, using currently available techniques, will greatly enhance the societal value of the scientific efforts put forth in these fields, and will more rapidly lead to safe, proven, and efficacious therapies for musculoskeletal disease.

Acknowledgments

This research was made possible through the National Institutes of Health (NIH) Medical Research Scholars Program (to JF), a public-private partnership supported jointly by the NIH and generous contributions to the Foundation for the NIH from Pfizer Inc., The Leona M. and Harry B. Helmsley Charitable Trust, and the Howard Hughes Medical Institute, as well as other private donors (listed at http://www.cc.nih.gov/training/mrsp/index.html), and by support from the Division of Intramural Research, NIDCR (ZIA DE000380, to PGR), a part of the Intramural Research Program, NIH, DHHS, and by AR064381, DOD W81XWH-111-0262, AR052374, AR043457 (to DWR). The expressed opinions are those of the authors and not necessarily those of the National Institutes of Health.

References

1. Mason C, Brindley DA, Culme-Seymour EJ, Davie NL. Cell therapy industry: billion dollar global business with unlimited potential. Regen Med. 2011;6(3):265–72. DOI:10.2217/rme.11.28. [PubMed: 21548728]
2. Ng M, Song S, Piuzzi NS, et al. Stem cell industry update: 2012 to 2016 reveals accelerated investment, but market capitalization and earnings lag. Cytotherapy. 2017;19(10):1131–9. DOI: 10.1016/j.jcyt.2017.07.006. [PubMed: 28807603]
3. Turner L, Knoepfler P. Selling stem cells in the USA: assessing the direct-to-consumer industry. Cell Stem Cell. 2016;19(2):154–7. DOI:10.1016/j.stem.2016.06.007. [PubMed: 27374789]
4. Nakatsuji N Stem cell research: trends and perspectives on the evolving international landscape [Internet]. Amsterdam, The Netherlands; Elsevier; 2013 [cited 2018 Aug 27]. Available from: https://www.elsevier.com/__data/assets/pdf_file/0005/53177/Stem-Cell-Report-Trends-and-Perspectives-on-the-Evolving-International-Landscape_Dec2013.pdf.
5. Trounson A, McDonald C. Stem cell therapies in clinical trials: progress and challenges. Cell Stem Cell. 2015;17(1):11–22. DOI:10.1016/j.stem.2015.06.007. [PubMed: 26140604]
6. Marks PW, Witten CM, Califf RM. Clarifying stem-cell therapy's benefits and risks. N Engl J Med. 2017;376(11):1007–9. DOI:10.1056/NEJMp1613723. [PubMed: 27959704]
7. Kuriyan AE, Albini TA, Townsend JH, et al. Vision loss after intravitreal injection of autologous "stem cells" for AMD. N Engl J Med. 2017;376(11):1047–53. DOI:10.1056/NEJMoa1609583. [PubMed: 28296617]

8. Fung M, Yuan Y, Atkins H, Shi Q, Bubela T. Responsible translation of stem cell research: an assessment of clinical trial registration and publications. Stem Cell Reports. 2017;8(5):1190–201. DOI:10.1016/j.stemcr.2017.03.013. [PubMed: 28416287]

9. Mesoblast Limited. Durable three-year outcomes in degenerative disc disease after a single injection of Mesoblast's cell therapy [Internet]. Melbourne, Australia: Mesoblast Limited; 2017 3 15 [cited 2018 Aug 27]. Available from: https://globenewswire.com/news-release/2017/03/15/937833/O/en/Durable-Three-Year-Outcomes-In-Degenerative-Disc-Disease-After-a-Single-Injection-of-Mesoblast-s-Cell-Therapy.html.

10. Manolagas SC, Kronenberg HM. Reproducibility of results in preclinical studies: a perspective from the bone field. J Bone Miner Res. 2014;29(10):2131–40. DOI:10.1002/jbmr.2293. [PubMed: 24916175]

11. Kraeutler MJ, Belk JW, Purcell JM, McCarty EC. Microfracture versus autologous chondrocyte implantation for articular cartilage lesions in the knee: a systematic review of 5-year outcomes. Am J Sports Med. 2018;46(4):995–9. DOI:10.1177/0363546517701912. [PubMed: 28423287]

12. U.S. Food and Drug Administration. Office of Tissues and Advanced Therapies (OTAT) Approved Cellular and Gene Therapy Products [Internet]. Silver Spring, MD: U.S. Food and Drug Administration; 2018 [cited 2018 Aug 27]. Available from: https://www.fda.gov/biologicsbloodvaccines/cellulargenetherapyproducts/approvedproducts/default.htm.

13. Marcucio RS, Nauth A, Giannoudis PV, et al. Stem cell therapies in orthopaedic trauma. J Orthop Trauma. 2015;29 Suppl 12:S24–7. DOI:10.1097/BOT.0000000000000459. [PubMed: 26584262]

14. Kaigler D, Pagni G, Park CH, et al. Stem cell therapy for craniofacial bone regeneration: a randomized, controlled feasibility trial. Cell Transplant. 2013;22(5):767–77. DOI: 10.3727/096368912X652968. [PubMed: 22776413]

15. Kaigler D, Avila-Ortiz G,Travan S, et al. Bone engineering of maxillary sinus bone deficiencies using enriched CD90+ stem cell therapy: a randomized clinical trial. J Bone Miner Res. 2015;30(7):1206–16. DOI:10.1002/jbmr.2464. [PubMed: 25652112]

16. Berebichez-Fridman R, Gomez-Garci'a R, Granados-Montiel J, et al. The Holy Grail of orthopedic surgery: mesenchymal stem cells—their current uses and potential applications. Stem Cells Int. 2017; 2017:1–14. DOI:10.1155/2017/2638305.

17. U.S. Food and Drug Administration. Consumer Affairs Branch (CBER) Regulatory Considerations for Human Cell, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use: Guidance for Industry and Food and Drug Administration Staff [Internet]. Silver Spring, MD: U.S. Food and Drug Administration; 2018 [cited 2018 Aug 27]. Available from: https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM585403.pdf.

18. Taylor-Weiner H, Graff Zivin J. Medicine's Wild West—unlicensed stem-cell clinics in the United States. N Engl J Med. 2015;373(11): 985–7. DOI:10.1056/NEJMp1504560. [PubMed: 26352811]

19. Robey P "Mesenchymal stem cells": fact or fiction, and implications in their therapeutic use. F1000Res. 2017;6:F1000 Faculty Rev-524. Available from: https://f1000research.com/articles/6-524/v1. DOI:10.12688/F1000RESEARCH.10955.1.

20. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;15(6):641–8. DOI:10.1016/j.jcyt.2013.02.006. [PubMed: 23570660]

21. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8-(4):315–7. DOI:10.1080/14653240600855905. [PubMed: 16923606]

22. Siegel G, Kluba T, Hermanutz-Klein U, Bieback K, Northoff H, Schafer R. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. BMC Med. 2013;11:146 DOI:10.1186/1741-7015-11-146. [PubMed: 23758701]

23. Russell KC, Phinney DG, Lacey MR, Barrilleaux BL, Meyertholen KE, O'Connor KC. In vitro high-capacity assay to quantify the clonal heterogeneity in trilineage potential of mesenchymal

stem cells reveals a complex hierarchy of lineage commitment. Stem Cells. 2010;28(4):788–98. DOI:10.1002/stem.312. [PubMed: 20127798]

24. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells—current trends and future prospective. Biosci Rep. 2015;35(2): e00191 DOI:10.1042/BSR20150025. [PubMed: 25797907]
25. Liu S, de Castro LF, Jin P, et al. Manufacturing differences affect human bone marrow stromal cell characteristics and function: comparison of production methods and products from multiple centers. Sci Rep. 2017;7:46731 DOI:10.1038/srep46731. [PubMed: 28447618]
26. Bader P, Kuci Z, Bakhtiar S, et al. Effective treatment of steroid and therapy-refractory acute graft-versus-host disease with a novel mesenchymal stromal cell product (MSC-FFM). Bone Marrow Transplant. 2018;53:852–62. DOI:10.1038/s41409-018-0102-z. [PubMed: 29379171]
27. Munneke JM, Spruit MJA, Cornelissen AS, van Hoeven V, Voermans C, Hazenberg MD. The potential of mesenchymal stromal cells as treatment for severe steroid-refractory acute graft-versus-host disease. Transplantation. 2016;100(11):2309–14. DOI:10.1097/TP.0000000000001029. [PubMed: 26714122]
28. Phillips MD, Kuznetsov SA, Cherman N, et al. Directed differentiation of human induced pluripotent stem cells toward bone and cartilage: in vitro versus in vivo assays. Stem Cells Transl Med. 2014;3–(7):867–78. DOI:10.5966/sctm.2013-0154. [PubMed: 24855277]
29. Berthiaume F, Maguire TJ, Yarmush ML. Tissue engineering and regenerative medicine: history, progress, and challenges. Annu Rev Chem Biomol Eng. 2011;2(1):403–30. DOI:10.1146/annurev-chembioeng-061010-114257. [PubMed: 22432625]
30. Wang L, Liu Y, Kalajzic Z, Jiang X, Rowe DW. Heterogeneity of engrafted bone-lining cells after systemic and local transplantation. Blood. 2005;106(10):3650–7. DOI:10.1182/blood-2005-02-0582. [PubMed: 16081694]
31. Boban I, Barisic-Dujmovic T, Clark SH. Parabiosis model does not show presence of circulating osteoprogenitor cells. Genesis. 2010;48:171–82. DOI:10.1002/dvg.20602. [PubMed: 20127800]
32. Grcevic D, Pejda S, Matthews BG, et al. In vivo fate mapping identifies mesenchymal progenitor cells. Stem Cells. 2012;30(2):187–96. DOI:10.1002/stem.780. [PubMed: 22083974]
33. Pauley P, Matthews BG, Wang L, et al. Local transplantation is an effective method for cell delivery in the osteogenesis imperfecta murine model. Int Orthop. 2014;38(9):1955–62. DOI:10.1007/s00264-013-2249-y. [PubMed: 24384938]
34. Otsuru S, Overholt KM, Olson TS, et al. Hematopoietic derived cells do not contribute to osteogenesis as osteoblasts. Bone. 2017;94:1–9. DOI:10.1016/j.bone.2016.10.003. [PubMed: 27725318]
35. Liu Y, Strecker S, Wang L, Kronenberg MS, Wang W, Rowe DW, Maye P. Osterix-Cre labeled progenitor cells contribute to the formation and maintenance of the bone marrow stroma. PLoS One. 2013;8(8): e71318 DOI:10.1371/journal.pone.0071318. [PubMed: 23951132]
36. Mizoguchi T, Pinho S, Ahmed J, et al. Osterix marks distinct waves of primitive and definitive stromal progenitors during bone marrow development. Dev Cell. 2014;29(3):340–9. DOI:10.1016/j.devcel.2014.03.013. [PubMed: 24823377]
37. Phinney DG. Functional heterogeneity of mesenchymal stem cells: implications for cell therapy. J Cell Biochem. 2012;113(9):2806–12. DOI:10.1002/jcb.24166. [PubMed: 22511358]
38. Dulken BW, Leeman DS, Boutet SC, Hebestreit K, Brunet A. Single-cell transcriptomic analysis defines heterogeneity and transcriptional dynamics in the adult neural stem cell lineage. Cell Rep. 2017;18-(3):777–90. DOI:10.1016/j.celrep.2016.12.060. [PubMed: 28099854]
39. Jensen EC. Use of fluorescent probes: their effect on cell biology and limitations. Anat Rec (Hoboken). 2012;295(12):2031–6. DOI:10.1002/ar.22602. [PubMed: 23060362]
40. Couchman JR. Commercial antibodies: the good, bad, and really ugly. J Histochem Cytochem. 2009;57(1):7–8. DOI:10.1369/jhc.2008.952820. [PubMed: 18854593]
41. Eliopoulos N, Stagg J, Lejeune L, Pommey S, Galipeau J. Allogeneic marrow stromal cells are immune rejected by MHC class I– and class II-mismatched recipient mice. Blood. 2005;106(13):4057–65. DOI:10.1182/blood-2005-03-1004. [PubMed: 16118325]
42. Ito R, Takahashi T, Katano I, Ito M. Current advances in humanized mouse models. Cell Mol Immunol. 2012;9(3):208–14. DOI:10.1038/cmi.2012.2. [PubMed: 22327211]

43. Roeder E, Matthews BG, Kalajzic I. Visual reporters for study of the osteoblast lineage. Bone. 2016;92:189–95. DOI:10.1016/j.bone.2016.09.004. [PubMed: 27616604]
44. Haffner-Luntzer M, Kovtun A, Rapp AE, Ignatius A. Mouse models in bone fracture healing research. Curr Mol Biol Rep. 2016;92(2):101–11. DOI:10.1007/s40610-016-0037-3.
45. Conner JD, Wolden-Hanson T, Quinn LS. Assessment of murine exercise endurance without the use of a shock grid: an alternative to forced exercise. J Vis Exp. 2014;(90):e51846 DOI: 10.3791/51846. [PubMed: 25145813]
46. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Muller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J Bone Miner Res. 2010;25(7):1468–86. DOI:10.1002/jbmr.141. [PubMed: 20533309]
47. Stewart RC, Honkanen JTJ, Kokkonen HT, et al. Contrast-enhanced computed tomography enables quantitative evaluation of tissue properties at intrajoint regions in cadaveric knee cartilage. Cartilage. 2017;8(4):391–9. DOI:10.1177/1947603516665443. [PubMed: 28934883]
48. Vashishth D Small animal bone biomechanics. Bone. 2008; 43(5):794–7. DOI:10.1016/j.bone. 2008.06.013. [PubMed: 18672104]
49. Lakin BA, Snyder BD, Grinstaff MW. Assessing cartilage biomechanical properties: techniques for evaluating the functional performance of cartilage in health and disease. Annu Rev Biomed Eng. 2017;19(1):27–55. DOI:10.1146/annurev-bioeng-071516-044525. [PubMed: 28226218]
50. Nemeth K, Leelahavanichkul A, Yuen PST, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E2-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15(1):42–9. DOI:10.1038/nm.1905. [PubMed: 19098906]
51. Bakopoulou A, Apatzidou D, Aggelidou E, et al. Isolation and prolonged expansion of oral mesenchymal stem cells under clinical-grade, GMP-compliant conditions differentially affects “stemness” properties. Stem Cell Res Ther. 2017;8(1):247 DOI:10.1186/s13287-017-0705-0. [PubMed: 29096714]
52. Larsen KH, Frederiksen CM, Burns JS, Abdallah BM, Kassem M. Identifying a molecular phenotype for bone marrow stromal cells with in vivo bone forming capacity. J Bone Miner Res. 2010;25–(4):796–808. DOI:10.1359/jbmr.091018. [PubMed: 19821776]
53. Mindaye ST, Lo Surdo J, Bauer SR, Alterman MA. The proteomic dataset for bone marrow derived human mesenchymal stromal cells: effect of in vitro passaging. Data Brief. 2015;5:864–70. DOI: 10.1016/j.dib.2015.10.020. [PubMed: 26702413]
54. Berner A, Henkel J, Woodruff MA, et al. Delayed minimally invasive injection of allogenic bone marrow stromal cell sheets regenerates large bone defects in an ovine preclinical animal model. Stem Cells Transl Med. 2015;4(5):503–12. DOI:10.5966/sctm.2014-0244. [PubMed: 25834121]

Table 1.

Key Issues in Cell-Based Therapies

• Because of complexity, firm mechanistic insights often remain elusive.
• Applications are largely unproven in the clinical setting, allowing for unchecked extrapolations of therapeutic potential.
• Unique regulatory structure allows for clinical experimentation with little to no preclinical basis, in some cases leading to harm.
• Cell sourcing, modification, and transplantation methods are highly technically and biologically variable.
• Cell-based therapy researchers have both the responsibility and opportunity to address these issues through current methods and tempering therapeutic claims.

J Bone Miner Res. Author manuscript; available in PMC 2019 August 13.

Table 2.

Cell-Based Therapy Modalities

Tissue engineering	The use of scaffolds, cells, and biologically active molecules to regenerate functional tissues. The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs (canonical).
Permanent cell replacement	The application of free-floating or structurally unorganized cells for the replacement of absent or ineffective native cells.
Transient cell therapies	Cells applied to damaged or diseased tissues that modify the course of disease or healing through effector molecules or direct interaction with resident tissue cells (noncanonical).
Conventional tissue transplantation	The use of graft material, either viable or devitalized, autologous or allogeneic, to treat injury or disease.
Cell-derived products	Materials produced by cells that can be collected for therapeutic applications, including platelet-rich plasma, conditioned medium, and extracellular vesicles.

J Bone Miner Res. Author manuscript; available in PMC 2019 August 13.