Chapter 14
The Future of Regenerative Medicine

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Background on the Burden of MSK Conditions

Musculoskeletal diseases place a significant burden on the United States (US) healthcare system and contribute significantly to rising costs. In 2014, 66 million people sought medical care for a musculoskeletal injury [1, 2]. Current medical costs of musculoskeletal diseases are estimated at 873.8 billion US dollars (USD) annually. Osteoarthritis (OA), an example of a degenerative musculoskeletal disease with a significant impact on the US healthcare system, was responsible for raising aggregate annual medical care expenditures by 185.5 billion USD [3–5]. OA currently affects more than 27 million people in the United States and is forecasted to affect 25% of the adult US population or nearly 67 million people by the year 2030 [3, 5, 6]. At this time, there is no known cure for OA. With the potential to prevent or reverse disease progression, regenerative medicine provides an opportunity to reduce the financial burden of degenerative diseases like OA. This would significantly impact the overall financial burden of musculoskeletal diseases.

One model to describe regenerative medicine and the engineering of tissues divides the underlying component categories into three parts, analogous to a garden that requires seeds, dirt, and fertilizer: (1) cells or cellular components, (2) biomaterial scaffolds, and (3) chemical and physical growth factors including cytokines like those in PRP [7]. This triad involves cells which are cultured on either a natural or synthetic scaffold where attachment and differentiation or proliferation can take place.

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The future of regenerative medicine will focus on research and science on the efficacy and specific mechanisms of action of regenerative therapies (as broadly broken down into the above categories). A respect for Food and Drug Administration (FDA) regulations will be required. There will be an improved understanding of genetics pertaining to musculoskeletal diseases, and genetic targets involved with different degenerative diseases will be identified. Questions pertaining to the appropriate level of tissue loading and the appropriate post-procedure rehab protocols will need to be answered. Ultimately, controlled trials demonstrating efficacy, standardization in reporting, improved data collection processes, and improved outcome metrics will give merit to the field and allow physicians to feel confident recommending regenerative medicine treatments to patients.

Definitions/Nomenclature

Regenerative medicine and "stem cells" can be confusing and misleading terms, especially with regard to culture-expanded cells, cell products, and live or attenuated growth factors such as amniotic membrane-derived products. Names are used haphazardly, and nomenclature can be misleading and disconnected from the science and identity of cells in native tissues [3]. According to the National Institutes of Health (NIH), stem cells are defined by their ability to divide and renew themselves for long time periods, by their lack of specialization, and by their ability to give rise to specialized subtypes [8, 9]. Essentially, the current cell therapies offered in the United States involve transplanting adult cells obtained through harvest and minimal manipulation of native tissues (blood, bone marrow, and fat), which contain stem and progenitor cells [8]. While the concentration of these cells can be increased at the point of care [8, 10], stem and progenitor cells are the least plentiful cell type in these preparations. Specifically, only one in one thousand to one in one million cells harvested from healthy tissues is stem or progenitor cell capable of differentiating into one or more types of connective tissue [8, 11–13]. Another issue that contributes to confusion surrounding the nomenclature of stem cells is that both "mesenchymal stem cell" and "mesenchymal stromal cell" are abbreviated "MSC" and used to describe culture-expanded cells. Chu et al. suggested that the term "stem cell" has been overused to include uncharacterized minimally manipulated cell preparations as well as tissue-derived culture-expanded cell populations. It has been suggested, therefore, that these cell preparations and expanded cell populations be referred to as "cell therapy" [8]. While the term "stem cell" has become common, future work will need to clearly define what is meant when this term is used. The future of regenerative medicine will need to have a standardized and accurate nomenclature for descriptive, classification, and billing purposes but most importantly for the science and clinical applicability to move forward.

Regulations and Standardization

There have been two general approaches to cellular therapies within regenerative medicine [3]. The first approach involves specifically characterized cellular medical therapies provided by physicians who are diligent and committed to the scientific innovative process of first studying a product in animals and then through three phases of trials where appropriate informed consent is executed. Alternatively, the second approach utilizes unregulated cell- and tissue-based products and associated procedures that are unproven, offered without appropriate informed consent including an explanation of scientific limitations, and offered on a cash-only basis. It is estimated that these unproven therapies have a yearly financial impact of 2.4 billion USD [3, 14–19]. The demand of effective treatment for common diseases, hope from the public (and providers), poor and inaccurate marketing communications regarding the expectations, strengths and limitations of these therapies, availability of various technologies and systems for culturing, and patient ability and willingness to pay for care not covered by insurance companies have contributed to the hype around "stem cells" [3]. The surge of social media, gaps in regulation, and ethics and liability concerns of larger, more established companies have allowed small targeted clinics and manufacturers to bring forth lucrative business models without backing of controlled clinical studies [3]. This is concerning given reports of serious adverse events with treatments that at this point are not fully understood [20–22]. This second, unscrupulous approach highlights the need for regulations in the field of regenerative medicine to not only ensure patient safety but also allow potential strengths of these therapies to be demonstrated.

In response to these unregulated clinics, the FDA issued a guidance document on November 16, 2017, that had two directives: (1) identify and subsequently prosecute unscrupulous regenerative medicine clinics and (2) streamline the approval pathway for legitimate therapies [23]. The majority of regenerative medicine products is regulated under title 21 of the Code of Federal Regulations (21 CFR 1271), and there are two separate descriptions under part 1271: Section 361, which is reserved for tissues that are "minimally manipulated" and intended only for homologous use, and Section 351 used for a new drug or biologic product requiring FDA premarket review process that is more time intensive. If they originate from autologous bone marrow or adipose, stem cell preparations have traditionally been regulated under Section 361; however, recent guidance documents from the FDA caution that products from adipose, such as those created by mechanically processed lipoaspirate for orthopedic indications, are not considered minimally manipulated or homologously used and would therefore fall under Section 351 and have to undergo the rigors of an "investigational new drug." This would require appropriate regulatory submissions for the conduct of clinical trials and marketing [20].

While the FDA is targeting the unregulated practices of smaller clinics by necessitating approval standards, it demonstrated a sense of urgency by incorporating a mechanism for expediting the development of new therapies with an emphasis on

those aimed at serious or life-threatening conditions [20]. For example, the 21st Century Cures Act enacted in December 2016 introduced an additional expedited program in which a product is designated as regenerative medicine advanced therapy (RMAT). This designation gives sponsors of a qualified regenerative medicine product intended for treating serious or life-threatening conditions an advantage in that it requires preliminary clinical evidence that the therapy addresses unmet medical needs as opposed to the requirement of preliminary clinical evidence of a substantial improvement over existing therapies [20]. In addition, RMAT-designated products that receive accelerated approval have potential eligibility for use of an expanded range of options, including the use of traditional studies along with submitting patient registries to fulfill post-approval commitments. Ultimately, the November 2017 policy from the FDA has given developers of lower-risk regenerative medicine products 36 months to determine if their products have undergone more than homologous use or minimal manipulation and if they need to submit an application for investigational new drug or marketing [20, 23]. Within the FDA's framework in thinking about musculoskeletal applications, if investigators are able to collaborate among different sites and agree on common manufacturing protocols and a common clinical trial protocol and the data along with the manufacturing information show a positive benefit-risk profile, there would be potential for receipt of biologics licenses at each of these sites by pooling the data [20]. This approach would be appropriate for developing products that, despite being more than minimally manipulated, would not be highly complex and would be able to be applied in simple trial designs.

The collaborative strategy outlined above highlights a need for standardization. There is an inconsistency in the literature with regard to reporting standards [3]. Direct-to-consumer marketing has allowed for erroneous claims. For example, aggregated claims of "stem cell" clinics suggested an average of 80% of patients experience "good results" or "symptomatic improvement," but published literature would suggest that there is a gap between what is reported and reality [3, 24]. Similarly, messages on social media about cell-based therapies are dominated by positive tone without discussing risks [3, 25]. Standardization is also needed from a research standpoint in terms of disease-specific clinical indications, reporting on how cells are sourced and characterized, the use of adjuvant therapies, the use of appropriate controls, trial methodology, and assessment of outcomes [3, 11, 12, 26, 27].

From a scientific standpoint, it is critical to develop a standardized and consistent approach to reporting in publications how cells are processed and characterized. Specifically, it is important to report the source of tissue, the selection or isolation method, expansion conditions, cell surface markers and their attributes, concentration, prevalence, gene expression profile and morphological features, and proteome profile. Publications vary widely with regard to relevant metrics of how the cells or components were processed and characterized [3]. When articles lack this information, it becomes difficult to communicate and repeat or compare one study to another. For example, Piuzzi et al. attempted to review the use of bone marrow aspirate concentrate in musculoskeletal disorders but, after reviewing 46 studies, found that no study gave enough details so that the methods could be repeated [3, 28]. Similarly, the composition of PRP can change depending on the time of day it is

obtained or can vary when prepared using systems from different manufacturers [8, 29–31]. Demographic information is important to report as well because it has been noted that growth factor and cytokine concentrations vary by donor age, health status, and sex [8, 31, 32]. In a similar way, progenitor and MSC populations isolated from a given donor also differ widely from one preparation to another, along with being different in terms of age, sex, tissue source, harvest, and processing [8, 11–13, 28–30, 33–38]. Ultimately, the Delphi consensus approach describes a multidisciplinary group of investigators who defined minimum information for studies evaluating biologics in orthopedics (MIBO), specifically related to the use of PRP and MSCs, that serve as a checklist of the minimal requirements to guide study design and reporting [3, 39].

Registries

Registries can be a significant vehicle to direct the future of regenerative medicine toward standardization and facilitate outcomes-based research. There is a need for registries which include demographics (age, sex, medications, underlying medical conditions, and smoking status). Each patient who undergoes a procedure is very different. Would an older patient with multiple medical comorbidities respond to an injection of PRP, for example, the same way as a healthy patient with no comorbidities? A registry can be linked to a biorepository to capture and preserve clinical samples for future analysis and create cohorts that can help to power clinical trials [3, 8]. With cartilage, for example, one of the biggest barriers to establishing the safety and efficacy of these new therapies is the cost of clinical trials [3]. This is where the organization of multicenter registries for cartilage repair can be critical to reducing barriers to progress and allowing for multicenter trials to take place [3]. Overall, registries provide opportunities for collecting standardized data on both how the patient was doing clinically and what their outcome was for a variety of different interventions performed to treat the same disease [8].

The American Joint Replacement Registry [8, 40], the Kaiser Registries [8, 36], and the PRP registry at Veterans Hospital in Palo Alto, California, are model registries that have contributed important data on practice patterns, shown the potential issues from a particular treatment, or illustrated the potential for clinical evidence pertaining to PRP. The biorepository-linked PRP registry at the Veterans Hospital in Palo Alto, California, addressed the gap between the differing composition of PRP from patients and clinical outcomes [8]. Patients that received PRP injections for knee OA completed patient-reported outcomes (PROs) before treatment and at specific time points after treatment. At the same time, a sample of the PRP was stored for patients who consented to federally funded research and who additionally underwent functional and structural assessments of gait and quantitative MRI. In doing so, the registry supports correlating PRP proteomics with PRO and quantitative clinical outcome metrics in the interest of learning about potential mechanisms of action and clinical efficacy [8].

Effective registries require commitment and a team approach from physicians, clinics, and hospitals to recruit all qualifying patients, appropriate incentives for participation, and a process for financial support of the human resources required to accrue and report clinical and baseline outcomes data [8]. In addition, there will need to be a defined assessment of quality, technique of preparation, device used, and clinical laboratory data on the administered biologic [8]. Tissue specimens may also be collected to aid in stratifying the patient's disease state along with analyses of biomarkers, molecules, and genomes. These data could be required to help identify which patients would most likely respond to therapy and define the critical quality characteristics of a cell or biologic therapy.

Patient Access

Given the potential of these investigational therapies, there is a need to increase access to these treatments while still maintaining an environment committed to patient safety and respect. The acronym SMAC, which stands for science evidence, rigorous manufacturing process, accurate information for patients, and consistent product in terms of substance and how it is delivered, can be a guide [3]. The FDA, in its recent position paper, has demonstrated its commitment to both proper investigation and patient access to regenerative therapies by giving direction on ways to get an investigational drug into settings where there would be a potential for positively impacting a great number of patients [3, 41–43]. As previously mentioned, in the United States, the 21st Century Cures Act has provisions intended to expedite approvals of cell therapies and the recent "right-to-try" law to allow terminally ill patients access to products. An example from outside the United States can be seen by looking at Japan where a law passed emphasizing the utilization of conditional approvals for the purposes of stimulating the regenerative medicine industry.

Science

With an emphasis on patient registries and increasing patient access, scientists and clinicians need to maintain a sense of urgency in developing a better understanding of the mechanisms behind these regenerative therapies. Improved understanding of the science will allow the appropriate regenerative medicine therapy to be chosen for the appropriate patient. Rodeo (2016) noted that animal studies have been valuable in verifying "proof of principal" for cell-based therapies, PRP, cytokines, and tissue-engineered implants [44–48]. Despite the value of animal studies, there are limitations. In animals, it is challenging to stimulate chronic conditions like tendinopathy or slowly developing OA that is seen in humans [8]. In addition, there is an

inability to control the mechanical loading environment or replicate the loading that takes place with humans. When thinking about humans, there is intrinsic variability in the soft tissues and joint spaces being treated that is poorly understood. The biologic targets need to be better identified [8]. For example, when looking at repair of the rotator cuff, primary targets are thought to be signaling molecules that drive cellular differentiation to reform the organized structure of the enthesis [8, 49]. Identifying biologic targets will necessitate a better understanding of the cellular mechanisms of tissue degeneration and repair for that disease state. Lastly, in terms of the three-part model, there is still much to be understood about the cells, biomaterial scaffolds, cytokines, and growth factors that are unique to the individual patient.

When analyzing stem cells, either marrow derived or adipose derived, there are numerous ways that these cells may work. They may function by way of their own inherent immunomodulatory and anti-inflammatory properties and by directly integrating into the healing tissue thereby directly participating in the healing response or have a local paracrine effect by stimulating and attracting intrinsic host cells [44]. The specific mechanisms by which they work are unknown at this time, however, and will need to be identified for regenerative medicine to progress.

One of the main goals of cell therapy is cartilage repair; however, there are a number of unknown factors involved with this process. Future research will need to work toward addressing current limitations including a lack of consensus regarding the optimal cell source, harvesting and processing techniques, and critical quality attributes (CQAs) that predict future performance [3, 50]. Specifically, when talking about the cell source, cells need to be selected that maintain an articular cartilage phenotype and do not undergo endochondral ossification, which can be a significant adverse effect [3, 50–53].

Scaffolds, as an important part of the tissue-engineering triad, interact with both cells and growth factors [54, 55]. Scaffolds can provide substrate for growth of cells and mechanical integrity for postsurgical implantation. They can also act as drug delivery systems for improved repair in vivo by being coated with bioactive molecules. One promising direction in scaffold production involves nanotechnology, specifically self-assembling peptides [54]. Natural and synthetic biomaterials have been investigated as scaffolds, but self-assembling peptide hydrogel (SAPH) scaffolds combine advantages of both natural and synthetic biomaterials because they are biocompatible and have easily modifiable properties [56]. For example, in a study looking at SAPH for intervertebral disc tissue engineering, after three-dimensional culture of nucleus pulposus cells (NPCs) in the SAPH, upregulation of nucleus pulposus-specific genes confirmed that the system could restore the nucleus pulposus (NP) phenotype in in vitro cultures [56]. The SAPH stimulated time-dependent increases in aggrecan and type II collagen deposition, which are two important NP extracellular matrix components. Overall, the suggestion from this study was that the SAPH could be used as a cell delivery system and scaffold in treating degenerative disc disease. Another promising application in the future of scaffolds will look to utilize 3D printing to achieve a clinically successful

tissue-engineered product. 3D printing offers a way to control scaffold size, shape, pore size, geometry, and mechanical properties [54, 57]. Through the integration of computer-assisted design and modern medical imaging, scaffolds can be individualized to a specific patient and a specific defect [54, 58]. A new development has been biologically relevant bioinks, which are biomaterials that carry cells printed into 3D scaffolds and are an important component of the bioprinting effort [59, 60]. Faramarzi et al. incorporated PRP into an alginate hydrogel scaffold used in bioprinters and demonstrated that this bioink could positively affect the function of two important cell populations (mesenchymal stem cells and endothelial cells) involved in the tissue healing process in vitro [59].

PRP and the cytokines contained within it have played a large role in regenerative medicine and are relevant because they contain autologous growth factors that are easy to obtain and manipulate [3]. In a retrospective study by Mautner et al., in which PRP for chronic tendinopathy was evaluated, the majority of patients reported a moderate (>50%) improvement in pain symptoms [61]. However, despite showing an ability to contribute to symptom improvement, there are still many PRP-related questions that require clarification, many related to inconsistencies in published clinical trial results [3]. Due to variabilities in published studys' methods and results, the mechanism of action of PRP based on the various cell types it contains, optimal PRP formulation and system, dose number (single vs. serial), dose timing (intraoperative or delayed), and the impact of adding activating agents or anesthetics needs clarification in the future.

The optimal way of addressing the shortcomings in regenerative medicine is through controlled clinical trials [44]. In addition, it has also been suggested that clinicians carry out translational studies in conjunction with basic scientists to facilitate a thorough assessment of the biologic activity of these agents and then to compare and analyze this activity to clinical outcomes. A major limitation is that with general characteristics of the substance, such as platelet count or white blood cell count with PRP or cell number with stem cells, we do not know the biologic activity of the substance or how these general characteristics relate to that biologic activity. Extensive statistical analyses will be needed to study the interactions between intervention, time point after injury, and injury grade or severity [8]. There will also need to be stratification based on age, sex, and metabolic and systemic factors that may affect treatment response, like diabetes, rheumatologic conditions, and chronic use of anti-inflammatory or antifibrotic medications. At this point, given the amount of "unknowns" in regenerative medicine, has the usage of regenerative therapies outpaced the science supporting them?

Outcomes and Post-Procedure Rehabilitation

As with any treatment in medicine, the desired outcome for each regenerative medicine treatment needs to be clearly defined in controlled clinical trials. "Healed" versus "not healed" may not be the ideal outcome, and instead, the focus should be

on the tissue quality at the site, the time it took to achieve tissue healing, pre- and post-procedure pain levels, and restoration of motion or strength [44]. The ultimate outcome may be to reduce pain or inflammation and not affect healing at all. For acute muscle injury, for example, the primary goal may be prevention of reinjury rather than faster return to sport [8]. Another example pertains to rotator cuff repair, where the goal may be to decrease the rate of retear of the repaired tendon. In addition, and maybe even more importantly, adverse outcomes need to be diligently reported. Given that many regenerative therapies are new, long-term adverse effects are unknown. The first priority is to do no harm to the patient. With a limited understanding of how these regenerative therapies work and limited long-term data available, the clinician is in a precarious position in offering these therapies to patients. Commitment to appropriate informed consent is imperative.

Posttreatment rehabilitation instructions have the potential to contribute to a positive outcome [8]. Mechanical loads are critical for healing tissue. There is a paucity of data on the appropriate timing and progression of rehabilitation after a regenerative medicine treatment. In addition, rehabilitation for shoulder osteoarthritis is very different than rehabilitation for Achilles tendinopathy. Therefore, rehabilitation protocols need to be identified for each location and regenerative treatment. Variables include when and how a tissue should be loaded, active vs. passive range of motion, medications and nutritional factors that may enhance or hinder healing, the role of hyperbaric oxygen, low-level laser therapies, and the types and frequencies of strength training exercises.

Genomics

Gene therapy administered through viral vectors can serve as a natural "drug store" for the body to help to regenerate tissues, slow aging, or modify disease processes. Improvement in the understanding of genetic and epigenetic factors related to the injury of tissues is needed to facilitate targets for therapy and more predictable results [44]. This improved understanding is also linked to the idea of a "personalized" patient-specific approach in which biological or gene expression markers are used to identify joints at risk and justify preemptive intervention with disease-modifying drugs that can preserve cartilage even before the osteoarthritic process ensues [3, 62]. For example, clustered regularly interspaced short palindromic repeats (CRISPR) genome-engineering technology enables strategies like Stem Cells Modified for Autonomous Regenerative Therapy (SMART), allowing for production of anti-inflammatory molecules that selectively reduce inflammation caused by chronic conditions [3]. With durable engraftment, these cells can then serve the role of vaccine – limiting the progression of OA.

Gene therapy has the potential to deliver proteins to specific tissues and cells for tissue-engineering purposes [1, 63]. Gene therapy involves transferring target genes into cells allowing for protein delivery, growth factors, or other therapeutic gene products to a specific anatomic site. The delivery process of transgenes can

be through in vivo or ex vivo protocols with either viral (transduction) or nonviral (transfection) vectors [1]. Viral vectors can be integrating (retroviral and lentiviral) vectors which stably insert their genome into the DNA of infected cells and provide the best prospects for long-term gene expression as they are passed to both daughter cells during cell division. They also can be non-integrating (adenovirus and recombinant adeno-associated virus (AAV)) and stay in the nucleus as extrachromosomal episomes, which are not replicated during mitosis [1, 64]. The main issue with viral vectors is safety as they have demonstrated the potential to cause cell transformation and carcinogenesis [1, 65–67]. Given these concerns, nonviral vectors have been developed. They are associated with lower gene delivery efficiency compared to viral vector delivery systems [1, 68] but provide advantages with immunogenic response probability and cost-effective manufacturing [1, 69]. To improve the nonviral delivery efficiency problems, nonviral delivery systems have been engineered consisting of chemical or physical transfection systems [1].

There are two different ways of strategizing gene delivery: either in vivo or ex vivo strategies [1]. The vector is directly delivered to the host either systemically or locally with in vivo therapy. In ex vivo gene transfer, target cells are harvested, processed, and genetically manipulated outside the body prior to anatomic implantation. Ex vivo gene therapy is more technically challenging, more invasive, and less cost-effective. However, it is associated with higher transduction efficiency in allowing the delivery of potent cells and the gene product of interest to specific anatomic sites, a selective process of targeting the cell population of interest [1, 70–73]. Ex vivo gene therapy is also safer in only delivering transduced cells and not the actual vectors themselves, allowing for better control of the introduced factor. To overcome the limitations of ex vivo therapy, ex vivo strategies using either allogeneic cells or expedited single-step “same-day” approaches that eliminate the culture expansion step, decreasing the risk of contamination and gene mutations along with the increased cost, are being investigated [1, 74, 75]. Virk et al. evaluated this “same-day” approach using harvested bone marrow cells from a rat along with an osteoconductive scaffold assessing its effect on a critical-sized femoral defect on the rat [1, 75]. Radiographic, micro-CT, histologic, and biomechanical testing at 12 weeks post-implantation demonstrated that “same-day” ex vivo regional gene therapy was able to heal a rat’s critical-sized femoral defect. In addition, for comparison to cultured bone marrow cells, “same-day” cells were associated with earlier radiographic healing and increased bone formation on micro-CT. Safety of this technique was assessed by Alaee et al., and the results indicated that viral vector copies were detected in the defect area following implantation of transduced cells but significantly decreased over time. There were no consistent findings of viral copies in the internal organs and no organ toxicity or histological abnormalities noted [1, 76]. The results suggested that ex vivo therapy, using a lentiviral vector, is safe but required further testing. Given the strengths of this expedited ex vivo approach along with safety, it is likely that this approach will be utilized in future studies.

When looking at possible indications for gene therapy in musculoskeletal diseases, such as articular cartilage repair or osteoarthritis, it is evident that gene therapy has the potential to make an impact on different disease processes. Unlike other therapeutic strategies that focus on alleviating the symptoms of OA, gene therapy focuses on cartilage growth factors and cytokines involved in inflammation and the pathogenesis of osteoarthritis like interleukin-1 (IL-1), IL-10, TNF-α, and TGF-β [1]. Usually, the process involves direct intra-articular administration of genetically manipulated cells or vectors alone. IL-1 is considered the most potent mediator of pain, inflammation, and cartilage loss in OA [1, 77]. IL-1 receptor antagonist (IL-1Ra), by blocking IL-1 and limiting inflammation and cartilage degradation, is a promising option for treatment of OA, and multiple studies in animal models of arthritis have shown efficacy of viral-mediated IL-1Ra gene transfer in inducing subsequent gene expression and biological response [1, 78–81]. Nonviral gene delivery into joints is also an approach that has shown promise. In a rabbit model, Fernandes et al. showed the ability to control progression of OA with intra-articular injection of a plasmid-lipid complex [1, 82]. In addition, using the cDNA of IL-1Ra in combination with TGF-β1 was more effective in cartilage repair than when each is used alone. Safety of in vivo intra-articular gene therapy was addressed by the Wang et al. group in a study that specifically evaluated the biodistribution and toxic effects of recombinant adeno-associated virus (AAV) carrying either rat or human IL-1Ra [1, 83]. In observational, body weight, and pathology studies, administration of this vector caused no local or systemic adverse effects. There was minimal vector leakage into the systemic circulation for the first 4–24 hours after injection, and the vector genome persisted for up to a year with only low levels of vector genomes detected outside the knee. This strategy needs further refinement but shows significant promise and requires future study.

OA is the only orthopedic-related disease being studied in clinical gene therapy trials [1, 84] in the United States and Korea. Phase I and II trials of "TissueGene-C" (TG-C), an ex vivo gene strategy utilizing retrovirally modified allograft chondrocytes in patients with knee OA, have been completed with phase III trials now underway. These patients had Cartilage Repair Society (ICRS) grade IV cartilage damage based on MRI and improved with pain, range of motion, and functional outcomes. Importantly, safety with TG-C has been demonstrated by analyzing peripheral blood in 12 patients treated with TG-C which showed normal levels of TGF-beta 1 and no circulating vector DNA for all patients at all dose levels at every time point [1, 85]. Recently in Korea, TG-C, named Invossa, became the first gene therapy to be approved for musculoskeletal applications and is indicated for moderate knee OA. In addition to Invossa, a single injection of sc-rAAV2.5IL-Ra is being assessed in a phase I clinical trial in patients with moderate knee OA [1, 86].

While there has been successful use of gene therapy in animal models treating difficult bone defects, cartilage defects, and osteoarthritis, there are still obstacles to

clinical application [1]. We need to develop cost-effective, clinically relevant gene therapy strategies. Ideally, gene therapy should not require the clinician to develop a special skill set to prepare the product, and it will be off-the-shelf or easily extractable at the point of care. Safety is a special concern for the future application of gene therapy, and it is important that extensive biodistribution analysis of the transferred genes be consistently completed. The biology of gene therapy including the clinical indications, dose, cell source and scaffold, target gene, vector, and delivery system needs to be better defined.

Conclusion

The outlook on the future of regenerative medicine at this point is one of cautious optimism. Using the triad model framework, including cells, scaffolds, and PRP, along with an improving understanding of the human genome, it is evident that there is promising work being done that could lead to the future ability to modify degenerative diseases instead of simply managing symptoms. The challenge will be balancing patient demands and expectations with the limited evidence base for these therapies and an urgency from an increasing population of older patients. Given the regulations that are being enforced by the FDA, we are at a critical period of time where the onus to show data to support regenerative therapies has never been larger. This can be accomplished through collaboration and the development of registries along with standardization in methodology and outcome measures used in randomized controlled trials. For regenerative medicine to be successful we need an improved understanding of the science behind how stem cell therapy, scaffolds, and cytokines making up PRP work along with a better understanding of the human genome in the context of degenerative diseases like osteoarthritis. Given the immense potential of this field, will regenerative medicine be regarded as its own specialized area of medicine in the future?

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