The Use of Mesenchymal Stem Cells in Orthopedics: Review of the Literature, Current Research, and Regulatory Landscape

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ABSTRACT

Mesenchymal stem cells (MSCs) show promising clinical potential as multi-potent therapeutic agents in regenerative medicine, including a number of orthopedic applications. A comprehensive review of the medical literature regarding the pre-clinical and early clinical use of MSCs demonstrates that they are likely to be effective cellular repair agents for cartilage and joint injuries.

Cultured MSCs were injected into the knee joints of 153 patients with moderate to severe osteoarthritis (OA) of the knee. The study included 24 untreated patient candidates who were recruited as controls. At a mean follow-up of 11.3 months, knee patients reported mean pain relief as +53.1% (n=133), and -5.0% relief was found in the untreated control (n=25 at 12.0 months post-op) ($P<.001$ for control vs. knee comparison). Significant decreases were seen in four out of five of the Visual Analog Scale (VAS) score metrics and in most functional metrics in the knee group. There were no serious complications reported.

MSCs may reduce the need for joint replacement in knee osteoarthritis. Despite the great potential of the use of autologous MSCs as the practice of medicine, the Food and Drug Administration (FDA) has attempted to regulate MSCs as a drug. This policy is inconsistent with its policy on other matters including tissue re-implantation and in vitro fertilization, and will delay the development of this type of therapy.

Introduction

While news media have prominently featured embryonic stem cells as the main therapeutic agent in the developing field of regenerative medicine, less has been reported on adult stem cells. Adult stem cells are plentiful in the body, and are responsible for tissue maintenance functions. Mesenchymal stem cells (MSCs) are multi-potent, adult stem cells that show great potential as therapeutic agents in regenerative medicine.[1-5] They are also known as marrow stromal cells and are derived from the mesoderm. Recently it has been shown that bone marrow MSCs are actually a heterogeneous population of cells in a certain class rather than one distinct cell type.[6]

Review of the Literature

Animal and human studies have demonstrated the multi-potency of MSCs, and how they can differentiate into muscle, bone, cartilage, tendon, and various cells of internal organs, and also exhibit paracrine effects to assist in tissue repair.[13] In this context, paracrine means that MSCs release certain growth factors.[7] These include transforming growth factor beta (TGF-beta), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and other signaling factors that can help recruit other cells to the local area. Many authors have questioned whether most of the positive repair effects observed in experimental MSC therapies are due more to this paracrine signaling than to differentiation of cells.

MSCs can be easily isolated from many different parts of the body, including marrow aspirate, marrow mobilized blood, muscle, adipose, and other tissues.[1] For orthopedic purposes, many authors have compared these cells for their ability to heal bone and cartilage, and there are measurable differences in this regard. For example, Vidal compared equine MSCs obtained from bone marrow (bm-MSCs) vs. adipose tissue (a-MSCs) for chondrogenic potential and found that bm-MSCs produced a more hyaline-like matrix and had better glycosaminoglycan production.[8] Additional animal studies published by Niemeyer et al. demonstrated that bm-MSCs produced better repair of a tibial osteochondral defect when compared to a-MSCs.[9]

A limited number of cells can be obtained from any tissue—often fewer than the critical quantity needed for tissue repair. One method of obtaining more cells is culture expansion, or multiplying cells in culture to larger numbers. MSCs are usually culture expanded via monolayer culture, a procedure that involves seeding a certain density of cells onto a specialized flask, where the cells attach to a plastic surface and begin to form colonies. The cells are fed by means of a nutrient broth that is maintained above the plastic surface. Since MSCs are contact inhibited, they will multiply in culture until they become confluent and then abruptly stop growing.

To keep cells proliferating in culture, when the colonies are near confluence the non-adherent cells are discarded and an enzyme, such as trypsin, is used to remove the MSCs from the plastic surface. They are then re-plated in a similar flask and the media changed, a process known as a "passage." Most MSCs in culture are grown to the second to the fifth passage, as some studies have shown reduced differentiation and a higher chance of genetic rearrangement and aberrations if MSCs are grown for protracted periods in culture (see Figure 1).[10-12] Note that the percentage of adherent cells vs. non-adherent cells increases with each passage, with most labs considering a "pure" MSC population being obtained after approximately the second passage.

Models of MSC-based Orthopedic Repair

Some of the earliest models of cartilage repair used autologous, cultured chondrocytes. However, the complications of using chondrocytes for cartilage repair include hypertrophy, graft failure, long culture times, and the invasiveness of the implant procedure.[13] Since MSCs are multi-potent, animal models of cartilage healing using MSCs started to appear in the literature in the early 1990s.[14] In many of these studies, an osteochondral defect (OCD) is created experimentally, and the MSCs are implanted into the lesion, usually in a hydrogel or other carrier. Repair of the defect occurs over weeks to months.[1] The cartilage produced by these cells is very much like native hyaline cartilage, but subtle differences have been observed.[15]

Repair of the knee meniscus is a potential use for MSC-based interventions. The challenge in repairing the meniscus is largely the poor blood supply of the inner two-thirds (white zone) of the structure versus the excellent blood supply of the outer third (red zone).[16] Interestingly, Izuta et al. demonstrated that cultured MSCs may be able to overcome this problem of poor repair in the avascular zone. His group was able to demonstrate meniscus repair in the white zone when MSCs were transplanted into this area using a fibrin matrix.[17] Of note, Agung reported a murine model of intra-articular injection after acute injury of multiple knee structures, including the meniscus. This model established that for blind intra-articular injection (rather than the local adherent model proposed by Koga), the likelihood of finding cells in the meniscus was related to the number injected.[18] For example, at a dose of 10^6 MSCs, none were found in the injured meniscus, but at a dose of 10^7 cells, MSCs were generally found in this area. This may fit with Koga's hypothesis, as a higher number of cells injected into the joint would make it more likely that cells would be able to attach at the site in need of repair. Horie described that synovial-derived MSCs, injected into massive rat meniscus tears, were able to differentiate and repair meniscal tissue.[19] Interestingly, Horie also demonstrated that these cells did not migrate out of the knee to distant organs. Finally, Yamasaki has taken a different approach, freezing the meniscus repeatedly to kill the living cells and then reseeding it with cultured MSCs, validating the ability to repopulate the meniscus with good biomechanical properties approximating the normal meniscus.[20]

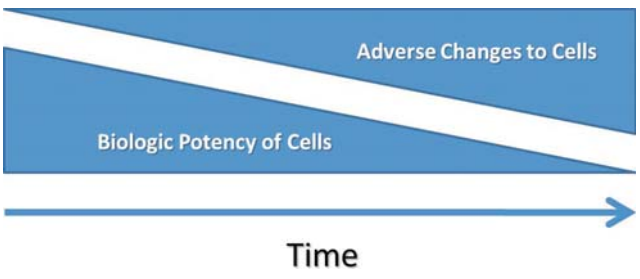

Figure 1. Adverse Changes in Cells Increase with Time in Culture as Biologic Potency Decreases.

Delivery of MSCs to the Lesion

Delivery of cells into a joint to treat musculoskeletal injuries could take two routes, used daily in clinical practice: percutaneous injections and arthroscopic placement. Injection of MSCs into a hydrodynamically confined space, such as by infiltration into soft tissues, is likely to result in MSCs' remaining at the injection site. However, injecting into a large joint presents some challenges, as multiple animal models have shown that cells may or may not home to the damaged areas.[18] Since MSCs have been shown to act through local attachment to the damaged site, data presented by Koga, showing that MSCs dripped on a lesion produce better repair, are encouraging as a model for injection (i.e. slow injection onto a lesion).[21] Other injection-based methods may involve using MSCs tagged with ferrous nanoparticles and/or magnetic fields to encourage attachment to the damaged site.[22] Finally, since MSCs are capable of chemotaxis, placing certain growth factors on the injured tissue may result in more MSCs accumulating at the target site.[23]

Current Research

We have previously described several case studies in which favorable changes on magnetic resonance imaging (MRI) were observed in knees treated with percutaneous, culture-expanded MSCs, corresponding with symptomatic improvement.[24,25] In particular, increases in cartilage and meniscus volume were observed on high-field MRI performed pre- and post-procedure using cartilage-specific sequences. In addition, these objective changes corresponded to subjective improvement.

We have also reported on the complication rate of human culture-expanded MSCs used for orthopedic purposes, noting a rate no greater than with other needle-based interventional techniques directed at peripheral joints.[26] In submitted, but yet unpublished data on 339 patients, this safety profile was continued at up to three years post-MSC re-implantation. This was despite more than 200 separate 3.0 Tesla MRIs of approximately 50 re-implant sites followed for 2-3 years. In particular, these MRIs showed no evidence of tumor formation. In addition, hundreds of follow-up contacts at various times showed no evidence of complications exceeding those typically encountered with other injection-based care (see Figures 2a and 2b).

Other authors have described similar results using surgical implant techniques: Wakatani described effective treatment of cartilage defects in nine knees with culture-expanded MSCs.[27] These authors followed up with an 11-year prospective study of 45 knees (in 41 patients) treated with autologous bone marrow-derived MSCs, with results representing both safety and efficacy.[28]

Nejadnik and colleagues recently described a comparison between surgically embedded chondrocytes versus MSCs placed by needle in 72 knees of older patients. They demonstrated good safety, less donor-site morbidity, and better efficacy for the MSC treatment when compared with an autologous chondrocyte procedure.[13] Haleem noted that autologous, cultured bm-MSCs re-implanted into articular cartilage defects in platelet-rich fibrin demonstrated evidence of healed cartilage in most patients at 12 months post-operative.[29]

Percutaneous Use of MSCs in Osteoarthritis

Minimally invasive injection procedures have the potential to forestall or eliminate a significant proportion of the more invasive and increasingly common alternative of arthroplasty surgeries. Knee arthroplasty has been used increasingly over the past decade to treat symptomatic degenerative joint disease. For example, it is estimated, using discharge data from the Nationwide Inpatient Sample (NIS) of the Healthcare Cost and Utilization Project (HCUP), that the number of partial and total knee replacement procedures among U.S. patients 65 years and older increased from 178,653 in 2000 to 357,472 in 2008, a 100% increase.[30] Knee arthroplasty is associated with some mortality and morbidity; in 2008 there were an estimated 4,964 deaths, 2,788 pulmonary emboli, 2,908 myocardial infarcts, and 4,670 cases of pneumonia associated with the procedures among the Medicare population alone.[31]

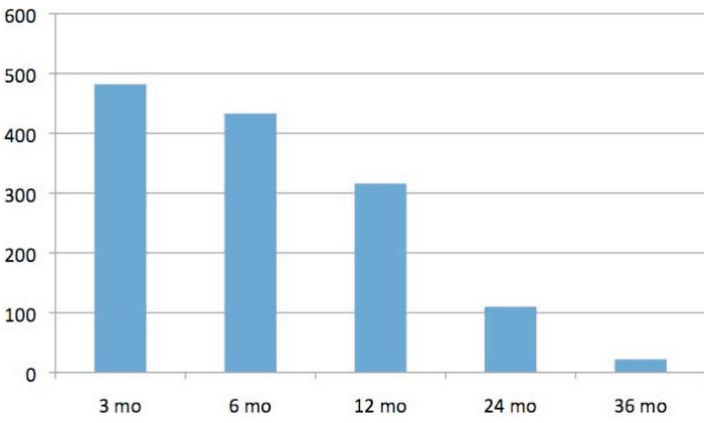

Figure 2a. Number of Follow-up Contacts at Each End Point (for Groups 1 and 2).

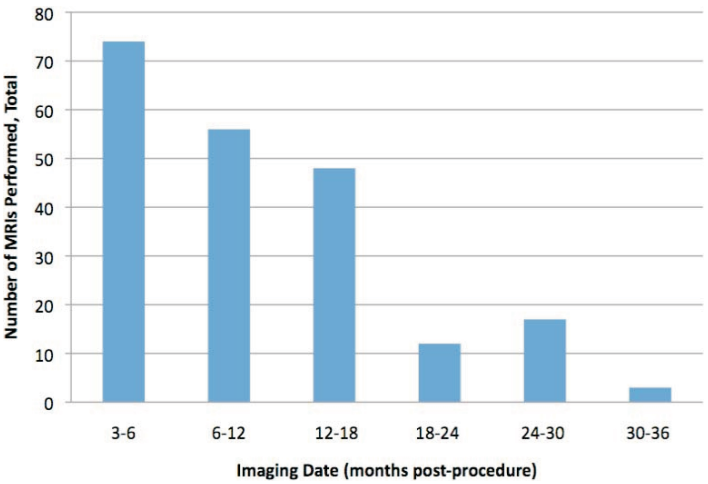

Figure 2b. Number of MRI Follow-ups at Each End Point (for Group 1). All were read as negative for tumorigenesis or ectopic tissue formation.

In a recently submitted and yet unpublished case control study, cultured MSCs were injected into the knee joints of 153 patients with moderate to severe osteoarthritis (OA) of the knee. The study included 24 untreated patient candidates who were recruited as controls. At a mean follow-up of 11.3 months, knee patients reported mean pain relief as +53.1% (n=133), and -5.0% relief was found in the untreated control (n=25 at 12.0 months post-op) ($P<.001$ for control vs. knee comparison). Significant decreases were seen in four out of five of the visual analog scale (VAS) score metrics and in most functional metrics in the knee group. There were no serious complications reported.

Approximately 66% of the knee patients were total joint arthroplasty (TJA) candidates prior to treatment (120 cases total). During an average total surveillance period of >2 years a total of five knee patients reported having TJA, 14.2% of the patients determined to be at greatest risk of having the procedure in the short term. Based on this result, if percutaneous transplantation of autologous stem cells could replace just 10% of the TJA procedures of the knee in the >65-year-old population, based on data from 2008, there would be approximately 500 lives saved, and 280, 290, and 470 cases of pulmonary embolus, myocardial infarction, and pneumonia prevented, respectively.

Regulatory Issues

The Food, Drug and Cosmetic Act (FDCA) was established in 1938 to reduce and manage public health risks of food, drugs, and (later) devices sold and distributed in interstate commerce.[32] In 1962 the Act was modified to include drug and device pre-market approval, resulting in the present regulatory scheme of the FDA in which regulation is directed at patentable drugs and devices.[33] With the advent of tissue transplant technology, the FDCA has been amended to include the regulation of tissue transplants with regard to the potential for communicable disease transmission (Public Health Service Act).[34]

The FDCA, as administered by the FDA, parallels the regulation of the practices of health care providers. The FDCA draws its regulatory mandate from the United States Congress, but Congress and federal courts have determined that control over medical practice is dictated by individual states.[35] A good illustration of the difference between the way the states and the FDA regulate is seen in the existence of compounding pharmacies. The FDA has for many years recognized that physicians are free to prescribe non-FDA approved medicinals or to use FDA-approved drugs for applications that are not included in the labeling of the drug. The latter is known as "off-label" use.[36] Off-label use means that a physician can prescribe an approved

drug in a different dose range than that approved by the agency, or for a different disease than the approved indication.

The FDA has stated repeatedly that it does not regulate the practice of medicine. In a Federal Register Notice for proposed rulemaking regarding labeling of prescription drugs, the FDA recognized that Congress did not intend the FDA to interfere with the practice of medicine, and the legislative history of the 1938 Federal Food, Drug and Cosmetic Act ("FDCA") and the 1962 drug amendments to the FDCA demonstrate that the FDCA did not purport to regulate the "practice of medicine as between the physician and the patient."[37] The FDA went on to explain that under the 1962 amendments, the agency must review the labeling of every new drug, but at the same time noting that labeling "is not intended either to preclude the physician from using his best judgment or to impose liability if he does not follow the package inserts." In addition, the 1962 amendments contain specific exemptions that exclude the practice of medicine from the Act. For example, while section 374 gives the FDA extensive powers, it also exempts licensed practitioners who administer, prepare, or manufacture drugs or devices "solely for use in the course of their professional practice."

When asked to decide disputes between the FDA and health care practitioners over alleged regulatory overreach, U.S. courts have adopted a similar approach of differentiating between medical practice and large-scale drug manufacture. In *United States v. Evers*, 453 F. Supp. 1141 (M.D. Ala. 1978), the United States government, on behalf of the FDA, filed proceedings against a licensed physician, H. Ray Evers, M.D. The FDA alleged that Evers had promoted and administered a drug for a use that was not approved by the FDA. Evers's defense was that he was a licensed physician and as such had a right to prescribe drugs for his patients in accordance with his best professional judgment. Further, Evers asserted that the FDCA does not prohibit physicians from using a drug in any manner not contraindicated on the labeling.

The court agreed with Evers, stating, "Congress did not intend the Food and Drug Administration to interfere with medical practice as between the physician and the patient." The court went further, expanding on why the FDA should not interfere with the practice of medicine. In its opinion, the court noted that a drug's package insert is not the most up-to-date information on the drug's uses. New uses are often discovered, reported through medical journals or seminars, and may become widely used in the medical profession; however, the drug manufacturer may not have sufficient financial or other interests to pursue FDA approval for the new uses. Further, if a doctor must prescribe and treat only within "federally sanctioned" methods, this would result in stagnation of medical progress, as physicians await drug manufacturers' initiative and FDA approval.[38] The court concluded that, "*a free, progressive society has an enormous stake in recognizing and protecting this right of the physician*."

As the court in *Evers* made clear, Congress did not intend the FDA to regulate the practice of medicine; rather, individual states have the mechanisms and resources in place and with closer supervision of the physician to ensure proper education and other qualifications for licensing, continued educational requirements, and proper review and remedies. This delineation between federal and state regulation of the practice of medicine has been reiterated by the courts and repeatedly acknowledged by the FDA.[39]

FDA Changes Its Regulatory Focus

The FDA first issued guidance and asserted its jurisdiction over cell therapies in 1993.[40] In 1996 and again in 1997, FDA issued additional guidance documents that proposed a regulatory framework for human cellular and tissue-based products.[41] In the 1997 document, the agency explained that it would regulate cell-based products with a "tiered approach based on risk and the necessity for FDA review."[42] This ill-defined "tiered" approach to the regulation of an emerging and rapidly evolving class of therapy introduced a new level of vagueness to the boundaries of FDA oversight.

In 2005 the FDA expanded its oversight to include both allogeneic and autologous "articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer ***into a human recipient***."[43] This was a new development, as prior to 2005, the agency had specified that regulation was only applicable to cells or tissues "…intended for transplantation *into* ***another human***…" as a means of monitoring for, and thus reducing, risk of disease transmission. This relatively minor adjustment of language allowed the FDA, for the first time in its history, to assert jurisdiction over medical procedures that carried no risk beyond that of the individual patient receiving the therapy. In essence, this change in regulation now overlapped FDA's authority with common medical procedures involving autologous tissue.

Is FDA's Cell Policy Consistent?

The FDA has two different risk tiers for oversight of handling of biologic tissues that are sold in interstate commerce. Transplant tissues intended for one-to-one or one-to-few applications, such as ligaments or other cadaveric structural tissues, have relatively minimal FDA oversight, with policy focused on donor screening and current Good Tissue Practices (cGTP) for tissue processing. If these tissues are used for autologous re-implantation, such as a vein from a leg used in coronary bypass, there is no FDA oversight. In contrast, human cells can either be regulated by the agency or not regulated, depending on the degree of manipulation of the cells. Cells that are more extensively processed and sold in interstate commerce, even if targeted for one-to-one therapies, are subject to a much more onerous Biologic License Application (BLA). The FDA states:

Biological products are approved for marketing under the provisions of the Public Health Service (PHS) Act. The Act requires a firm who manufactures a biologic *for sale in interstate commerce* to hold a license for the product. A biologics license application is a submission that contains specific information on the manufacturing processes, chemistry, pharmacology, clinical pharmacology and the medical effects of the biologic product. If the information provided meets FDA requirements, the application is approved and a license is issued allowing the firm to market the product.[44]

Thus biologic tissues requiring a BLA are treated by the agency like a mass-produced drug. This approach makes sense for an allogeneic product intended for a large number of recipients, which represents a magnified public health risk. For the risk level associated with autologous cell therapies, however, this increased level of surveillance is not only inconsistent with previous policy, it is unprecedented. There is no risk of spread of communicable disease when the cells and cell progeny from a particular patient are only used on that same patient.

The criteria by which FDA considers A-ASC (Adult-Autologous Stem Cell) therapy to constitute the use of BLA is when the cells are more than "minimally manipulated" prior to re-implantation [21 CFR §1271.3(f)(2)] (see Figure 3). FDA's policy toward medical therapy involving minimal manipulation of autologous cells would be easier to understand if it were consistent, but this is not the case. Since the agency's 2005 declaration of regulatory authority over all human tissue conflicted in many instances with common medical practice, the agency issued multiple exemptions. One of these was issued for assisted reproductive techniques (ART), and this exclusion illustrates the inconsistency in the FDA's newly defined regulatory framework for cell-based therapies. In vitro fertilization fits all of the technical and risk criteria as other more than minimally manipulated cell therapies. Cells are removed, often heavily manipulated ex vivo, combined with other cells, at times expanded in vitro, in some cases cryogenically preserved, and then re-implanted to the donor.

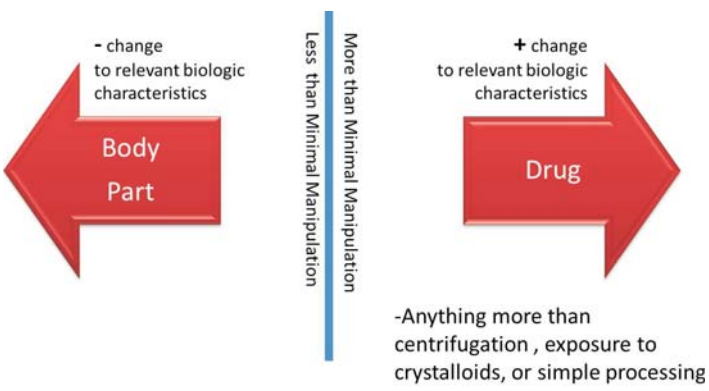

Figure 3. Determination of FDA Regulatory Status. The definition of "more than minimal manipulation" of human cell and tissue products (HCT/Ps) determines what will be regulated as a drug.

Despite the similarities to other autologous cell therapies (MSC-based cartilage repair or myocardial regeneration, for example), in vitro fertilization enjoys a specific exclusion from the BLA definition and thus falls outside the FDA regulatory framework.

Similarly, platelet-based wound care is also excluded from the BLA requirement, despite the fact that the procedure involves manipulation and re-implantation of autologous cells. Procedures using platelet-rich plasma (PRP) employ a concentration of platelets that have been separated via centrifuge from whole blood in serum. Such procedures are gaining wide acceptance in the medical community as a means of enhancing healing.[45] PRP is typically activated prior to use by ex vivo exposure to calcium and thrombin. Wide variations in platelet concentrations exist between patients, and there are no standardized levels of calcium and thrombin for clinical application, even though it has been demonstrated that even slight alterations in dose of these activation agents can produce wide variations in platelet growth factor release and kinetics.[46] Since activation of PRP alters the biologic characteristics of platelets, this type of processing would also meet FDA's 2005 definition of a biologic drug, yet therapy involving re-implantation of PRP is currently excluded from BLA requirements as well.

A final example, illustrating the problems in FDA's definition of "more than minimal manipulation" of cells as any alteration made to the "relevant biologic characteristics" of the cells, is the use of diagnostic MRI with cell therapy. It has been recently determined that the exposure of adult stem cells to the magnetic field of an MRI scanner significantly alters their biologic characteristics.[46] For MSCs, this includes a reduction in alkaline phosphatase, resulting in a decrease in the osteogenic differentiation capabilities of the cells. A variety of other alterations have been described in A-ASCs exposed to the magnetic fields in MRIs, including up-regulation of CD93 mRNA, lipocalin 6 mRNA, sialic acid acetylesterase mRNA, and olfactory receptor mRNA, and down-regulation of ubiquilin 1 mRNA.

On the other side of the regulatory fence, there is only a handful of examples of cellular "products" that have made it to market that are regulated as are federally regulated drugs. The earliest example of this type of "product" is the autologous cultured cartilage process marketed by Genzyme as Carticel. In this process the surgeon takes a cartilage biopsy and sends this to a central Genzyme lab where it cultured for several months. The culture-expanded sample is then sent back to the surgeon for surgical re-implantation.[47] Despite FDA's concerns over the risks for cultured cells, Carticel was approved in less than a year at a time when the average drug approval lag was 7.3 years.[48] This approval was granted under an accelerated approval process for biologic products, [21 CFR 601.40-46], allowing a manufacturer to submit a surrogate endpoint rather than conduct well-controlled studies to verify clinical benefit or durable outcomes. Curiously, the approval process was dramatically accelerated by classifying a

knee osteochondral lesion as a serious or life-threatening condition in need of accelerated approval.[49]

Long-term Risk Benefit Considerations for Autologous Cell-based Therapeutics

In their guidance documents the FDA indicates the risk of clonal transformation and tumorogenesis, as well as the possibility for cell-based therapies to generate ectopic tissues, as part of the rationale for regulation. While many authors have attempted to induce clonal transformation in human adult stem cell populations, the results have been mixed, with some studies showing no transformation and others showing that it may be possible to induce clonal activity.[50,51] Even when genetic aberrations have been induced experimentally, they haven't led to tumor formation, but rather to nonfunctional adult stem cells. More convincingly, human clinical experience with re-implanted autologous MSCs has shown no evidence of tumor formation or any other adverse effects associated with the use of culture-expanded A-ASCs.[26] In contrast, there is case report evidence of local tumor formation with allogeneic fetal tissue transplants.[52] Other reports have demonstrated tumor formation with Geron's phase I embryonic stem cell trial for spinal cord injury.[53]

The FDA's concern for ectopic tissue formation or migration of cells from the re-implant area is understandable.[54] These risks, however, are shared by other non-A-ASC surgical procedures. As an example, a knee microfracture procedure releases cells from the bone marrow into an osteochondral defect (OCD), with the intent of healing the defect. The procedure doesn't produce native repair cartilage, but a more fibrous substitute with distinct differences in collagen composition that can migrate from the intended therapeutic target.[55] While it is theoretically possible that A-ASC cells may spontaneously transform in the body, long after re-implantation, and cause malignancies, there is at present no evidence of this occurring.[56]

Regardless of the known and potential unknown risks of A-ASC therapy, there can be no disputing the fact that they are of the one-to-one/non-magnified magnitude, and not comparable to the highly magnified risk to public health associated with the mass manufacture of drugs, or even the risk level represented by implantation of cadaver-harvested tissue into multiple recipients.

There Is Risk in All Medical Procedures

The medical literature is rife with reports of therapeutic procedures that came into common use long before the long-term risks were understood. Cardiac bypass surgery, arthroscopic knee surgery, and bone marrow transplantation are just a few procedures that have been modified and even abandoned for some indications as more outcome data were gathered over time.[57-59] The presence of unknown risk is encountered every day in the risk-benefit analysis inherent in the delivery of any medical therapy, and is a key component of the one-on-one delivery of medical care. It is in this category of risk that A-ASC therapy falls, and it is impractical as well as inconsistent to treat it in any other manner.

Conclusions

We can find no justification for the current FDA position on therapeutic use of A-ASCs. There is no readily identifiable public health risk rationale for FDA's current regulatory posture regarding therapy employing adult autologous stem cells. The regulatory "line in the sand" drawn between the therapeutic use of A-ASCs and the cellular manipulation involved with IVF and PRP therapy, inter alia, is inconsistent with the best principles of regulatory science and with the practice of medicine. Clinical use of A-ASCs in the context of therapeutic activity by licensed practitioners is already regulated at the state level, as are all other aspects of medical practice. Picking and choosing additional parts of medical practice for special treatment is capricious, and ultimately harmful to the advancement of medical science and alleviation of human suffering.

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Disclosures: Dr. Centeno holds equity ownership in Regenerative Sciences, LLC. Mr. Faulkner is an employee of the Centeno-Schultz Clinic. The authors declare that they have no other competing interests.

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