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Abstract

The Signature Series Symposium "Cellular Therapies for Orthopaedics and Musculoskeletal Disease Proven and Unproven Therapies—Promise, Facts and Fantasy" was held as a pre-meeting of the 26th International Society for Cellular Therapy (ISCT) annual congress in Montreal, Canada, May 2, 2018. This was the first ISCT program that was entirely dedicated to the advancement of cell-based therapies for musculoskeletal diseases. Cellular therapies in musculoskeletal medicine are a source of great promise and opportunity. They are also the source of public controversy, confusion and misinformation. Patients, clinicians, scientists, industry and government share a commitment to clear communication and responsible development of the field. Therefore, this symposium convened thought leaders from around the world in a forum designed to catalyze communication and collaboration to bring the greatest possible innovation and value to patients with musculoskeletal conditions.

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Introduction

The following areas were identified as key priorities for communication and action: (i) understanding market forces and trends; (ii) confronting the issue of extensive direct-to-patient marketing of unproven therapies; (iii) defining the value of current therapies for patients and payers; (iv) defining constructive regulatory paths and priorities; (v) defining industry and clinical standards; (vi) clarity and transparency in publications, regulatory affairs and marketing claims; (viii) enabling clinical networks and registries; and (ix) identifying knowledge gaps and innovation opportunities.

These topics were addressed by a series of sessions and speakers. The content below provides a focused synopsis of the outcome of each of these sessions and associated panel discussions.

Market trends and market practices

Proven–unproven therapies, Massimo Dominici, MD

The current spectrum of cellular therapies can be divided objectively into two general models. On one end are precisely characterized cellular medical therapies provided to patients by intellectually rigorous caregivers with appropriate informed consent (e.g., first in humans, phase 1, phase 2 and phase 3 trials). Studies of this kind have been the scientific and ethical foundation of medical innovation and new medical/surgical treatments for decades. On the other end of the spectrum are a broad range of unregulated cell and tissue-based products and medical procedures for which claims of efficacy are made with little or no scientific evidence. These therapies are often offered to patients on a cash-only basis and are provided to patients without clarity in the informed consent. There is broad consensus that this unproven cellular therapy (UCT) end of the spectrum represents a large and growing, but unjustified, business model [1].

The size of the UCT world-wide business has been estimated to be around 2.4 billion US dollars (USD)/y [1–6]. This market touches upon almost every medical discipline, particularly neurological and musculoskeletal conditions [6]. However, UCTs are not embraced or endorsed by leading professional societies, and care is often provided by practitioners who are not board certified in the relevant medical specialties.

Several factors seem to be driving UCT, including the following: (i) the unmet demand for effective therapies for many common diseases. For example, in the case of osteoarthritis of the knee, many patients reach a point where conventional therapies have failed to control symptoms, but the symptoms are not so severe that joint replacement is considered desirable; this results in a large demand and placed hope for new injectable therapies; (ii) enormous public hope and expectations, particularly for long-anticipated "stem cell therapies" reflecting inadequate public education, (iii) poor marketing communication, if not deliberate miscommunication regarding the nature and track record of individual therapies; (iv) the wide availability of technology (centrifuges, culture systems, reagents etc.), and (v) patient ability and willingness to pay for therapies out of pocket for care that is not covered by traditional payers or health care systems.

The UCT strategies that are marketed to patients frequently make reference to legitimate scientific developments, and imply directly or indirectly that they are aligned with or based on advancements in the field. This time-honored marketing strategy is used to establish the perception of quality and value by association. However, this is not reality. Both patient information and product design and specifications are rarely more than business decisions based on cost and regulatory loopholes, without real contributions of innovation, product validation or rigorous clinical evidence of efficacy.

Social media, regulatory gaps and loopholes and the legitimate ethical and liability concerns of larger established companies, which might normally be required to bring new therapies to market, have opened the door for small targeted clinics and even small manufacturers to rapidly bring forward business models in niche markets on a "pay cash to be treated" basis, without performing controlled clinical studies.

Clinics often adopt the "charitable dress," meaning "we care enough about you to cut through the red tape that keeps greedy rich people in big pharmaceutical companies and timid less talented traditional doctors in ivory tower institutions from offering you these therapies." However, the environment of direct-to-consumer marketing and disinformation presents substantial risk for patients. Physical harm can occur, as illustrated in several high-profile incidences [7,8]. However, the frequency of true and direct harm may be small. More likely is that a patient loses time and money and experiences added emotional frustration of investing in something that later might prove to be ineffective.

The professional literature has begun to address the challenge of UCT more assertively in the past few years [9]. This has been picked up by a number of attentive and dedicated journalists who have begun to contribute greatly to address deficits in public knowledge and awareness related to "stem cells" and other unproven cellular and acellular therapies [10].

There is an obvious need to increase patients' access to promising investigational therapies. There is also a spirited debate over how this can best be accomplished in a way that serves both current and future patients in an environment of safety and respect. "SMAC", which stands for Science evidence, rigorous Manufacturing process, Accurate information for patients and Consistent product (content and delivery), is a clearly articulated, albeit greatly simplified acronym, that can be applied to guide this challenge. SMAC puts data, quality, transparency and consistency squarely at the center of the discussion, which may be considered minimum requirements for progress. SMAC embraces traditional clinical trials, compassionate use, clinical outcomes networks, as well as the concept of well-designed clinical registries and biorepositories. However, these approaches demand rigorous design, execution (communication, recruiting, quality care, data collection, follow-up), statistical support and ethical oversight.

We already have examples of evolution in policy designed to facilitate compassionate use, hospital exemption and priority pathways for clinical implementation. The recent Food and Drug Administration (FDA) position paper offers guidance on ways to get an investigational product into settings where they may benefit a large number of patients [11–13]. Legislation has also been used in some countries to support medical innovation based on cell therapies. For example, Japan passed a law to stimulate the regenerative medicine industry by conditional approvals. The 21st Century Cures Act in the USA also has provisions designed to accelerate approvals of cell therapies and the recent "right-to-try" law to provide products to terminally ill patients. However, these efforts should not be interpreted as a relaxation toward deregulation, nor a tolerance for loose controls in manufacturing platforms or compromises in patient safety on the altar of innovation

Hope, hype, investment and rewards, Nicolas Piuzzi, MD

Between 1994 and 2016 more than 23 billion USD have been invested in stem cell companies. More than 18 billion USD has been invested between 2011 through 2016 alone [14]. However, this growth has not been matched by similar growth rates in earnings, reflecting a field that is only emerging from infancy [14].

Direct-to-consumer marketing of unproven cellular therapies marketed as "stem cells" is a well-known phenomenon. Musculoskeletal conditions, particularly osteoarthritis (OA) of the knee, are among the most frequent conditions for which these therapies are marketed. A recent publication aggregated the claims of "stem cell" clinics, suggesting that a mean of 80% of the patients have "good results" or "symptomatic improvement" [15]. However, there is a substantial gap between these claims and published literature [15].

Another report examined messaging related to cell-based therapies for musculoskeletal conditions on social media and found that these messages were dominated by businesses that portray an almost exclusively positive tone, without providing a "fair balance" on the risks, benefits and limitations, reflecting an environment of hype that contributes to public misunderstanding related to the extent to which efficacy has been documented [16].

While efficacy remains in question, the volume of reporting on limited trials of autogenous therapies for OA of the knee, focal cartilage defects of the knee and osteonecrosis do not suggest a substantial safety concern in current practice. However, the quality of the published data is limited and there is a high risk of reporting bias. The current literature is highly inconsistent with respect to reporting standards. There is a critical need for refinement of definitive disease-specific clinical indications and standardization of reporting related to cell sourcing, cell characterization, use of adjuvant therapies, assessment of outcomes and use of appropriate controls [17–22].

Allograft bone graft matrices with viable cells, Marc Long, PhD

The variable quality, limited availability, inconsistent handling properties and donor site morbidity associated with the harvest of autograft bone has led to the development of a broad range of allogeneic and synthetic bone graft matrices (fibers, chips, powders, blocks) and a substantial track record of safety and efficacy. The value of adding osteogenic cells to a matrix has been demonstrated (e.g., from autogenous local bone or bone marrow aspirate) in many studies [23–27]. In fact, several current synthetic matrices are approved for use only when combined with autogenous marrow.

The value of adding cells has been reported by pre-clinical data, suggesting that allograft cells can be transplanted with low risk and possible efficacy [28], stimulating the concept of fabricating allograft bone graft materials with viable cells (BGMVCs). In these materials, living cells are maintained during processing and cryopreservation of human tissues [29]. In theory, therefore, BGMVCs provide the combination of osteoconduction (matrix for cell attachment and migration), osteoinduction (bioactive factors intrinsic to bone matrix) and osteogenic

potential (bone-derived cells including progenitors with bone-forming potential).

Donated human tissues are processed in accordance with FDA and American Association of Tissue Banks (AATB) requirements to generate BGMVCs. There are now several commercially available BGMVCs, which have different donor-screening criteria, processing techniques and formulations. These differences, as well as differences between donors, may result in variation in biological quality, handing properties and regulatory status as Human Cellular and Tissue Products (HCT/Ps). Most follow FDA regulations published in 21 Code of Federal Regulations (CFR) Part 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) and meet all required criteria for 361 HCT/Ps [30]. However, some BGMVCs have been found to involve more than "minimal manipulation" and, therefore, do not meet all 361 HCT/Ps criteria. These must be regulated as drugs or biological products. BGMVCs may also vary in the viability, number and potency of the osteogenic cells present in the matrix.

Level I evidence to suggest that inclusion of viable cells improves efficacy is currently lacking. However, some large prospective and retrospective clinical cohort studies for one BGMVC suggest promise (spinal fusion rates >90% at 1-year follow-up) [31–33]. Appropriately controlled clinical trials are needed.

The good news and bad news in current product/therapy domains

Bone regeneration, George Muschler, MD

Bone healing and bone regeneration rely on generalizable tissue engineering approaches [34–36]. Bone regeneration represents one of the most successful domains of musculoskeletal care. Orthopedic surgeons have access to a withering array of scaffold materials for bone regeneration (more than 70 existing products). These materials fill voids and prevent the encroachment of other tissues into a region where bone is desired. In general, scaffolds provide a porous interconnected surface that enables revascularization and the attachment and migration of bone-forming cells throughout the desired tissue volume (osteoconduction). Matrices can provide soluble or tethered factors that enhance the proliferation, differentiation and survival of osteogenic cells (osteoinduction). In many cases, cell transplantation is not required to accomplish successful bone regeneration because the local tissue environment provides a sufficient population of osteogenic stem/progenitor cells. In those cases, scaffolds or inductive factors simply "target" the local stem/progenitor population. However, in some settings (for example, segmental fractures where bone and periosteum is missing, fracture non-unions, irradiated tissue beds, sites compromised by scarring from previous infection or injury, revision spine fusions [37–40]), the success of bone regeneration is limited by a suboptimal tissue bed and local progenitor pool. In these settings, strategies to enhance bone regeneration require a strategy for sourcing osteogenic stem and progenitor cells. The local delivery of "homing" signals has been considered, but the most effective strategies to date involve the harvest of autogenous cells from a healthy tissue location (e.g., cancellous bone, periosteum or bone marrow from the pelvis or metaphyseal bone) and "transplantation" of those cells to a site where bone is desired. Transplantation will optimally involve a step of concentration of the osteogenic cells (i.e., removing fluid volume such as serum) and selection of the osteogenic stem/progenitor population (i.e., removing cells that do not contribute to tissue regeneration or that may inhibit or compete with the osteogenic population, while selecting for or retaining the osteogenic cells) [34–36,41–43].

The good news in bone regeneration is that the vast majority of bone-healing challenges can be addressed with success rates of more than 90% using available strategies. The bad news is that, despite all the available alternatives, the treatment failure rates remain high in challenging settings, and development has been stalled by three limitations.

The first limitation is that virtually all existing and new bone grafting products seek approval through the 510K mechanism. This means that they aspire to be "substantially equivalent" to existing predicate products. This may be viable if the cost is low and handling and performance properties are desirable, but it can be argued that developing additional 510K products actually squanders research and development (R&D) capital that would benefit patients more if it were used to advance efficacy in complex settings.

The second limitation is that the small and large animal models that have been used to date have essentially reached their "ceiling effect." Segmental defects of 5 cm in healthy young dogs, sheep and goats can be healed with near 100% efficacy using combinations of available scaffolds, growth factors (i.e., bone morphogenetic protein [rhBMP-2]) and/or processed autogenous cells. As a result, existing models are ineffective as tools to measure further improvement in efficacy. More rigorous models are needed. The chronic caprine tibial defect (CCTD) model has been developed to addresses this limitation and to ''raise the bar'' for rigorous assessment of bone-grafting strategies. The CCTD model includes

the biological features of muscle injury, loss of periosteum, bone marrow reaming and local scar formation, which are missing in traditional acute large animal models, and offers an opportunity to examine advances in cell concentration, selection and transplantation strategies, as well as advanced biomaterials [42].

Finally, the third limitation to the advancement of bone regeneration therapies is the limited availability of post-marketing data through prospective cohort registries and through prospective clinical trials, which can only be adequately powered through the coordination of clinical networks.

Cartilage repair and regeneration, Christian Jorgensen, MD, PhD and Cecilia Pascual-Garrido, MD

OA is a common and debilitating disease that affects 27 million people in the US [44,45]. Forecasts indicate that by the year 2030, 25% of the adult US population, or nearly 67 million people, will have symptomatic OA [46]. Furthermore, OA raised aggregate annual medical care expenditures by $185.5 billion USD [44,45].

Traditional OA treatments have focused on modifying symptoms of pain. What is needed is disease-modifying treatments that restore lost cartilage tissue or prevent cartilage degeneration.

A variety of cartilage repair and replacement strategies have shown promise. These have included the following: en bloc transplantation of autograft or allograft osteochondral tissues [47,48], transplantation of culture-expanded cells beneath or within a biological polymer (e.g., collagen or hyaluronan) or synthetic matrix. The success of cell transplantation will inevitably be dependent on the concentration, prevalence, biological attributes and biological potential of the cell population involved. However, no consensus has evolved regarding the optimal source of cells for cartilage repair, harvest or processing techniques, or critical quality attributes (CQAs) that predict future performance [49]. A particular limitation in cell transplantation is selection of a cell population that maintains an articular cartilage phenotype and does not undergo endochondral ossification over time [49–52].

Preparations of culture-expanded bone marrow stromal cells (BMSCs; both autologous and allogeneic) have been assessed in many pre-clinical and clinical study settings [18,21,48,53,54]. In addition to contributing to the formation of chondrocytes, BMSCs may have other functions derived from the diversity of bioactive factors that they secrete, including the following: hepatocyte growth factor (HGF), transforming growth factor β (TGFβ), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). Insulin-like growth factor (IGF)1, interleukin (IL)-6 and stanniocalcin-1 are essential for apoptotic reversal in fibroblasts, whereas VEGF, HGF and TGFβ1 have been shown to protect endothelial cells from apoptosis [55]. For example, BMSCs may reduce scar tissue formation [56]. However, the heterogeneity and batch-to-batch variation in BMSCs are challenges. Moreover, clinical studies vary widely with respect to clinical methodology, inclusion of appropriate controls, cell dose (range one million to 180 million cells per intra-articular injection), methods of cell expansion and characterization and choice of carrier [17,19,20,57]. Despite the heterogeneity of BMSC preparations, in aggregate, these studies suggest a reduction in pain and improved function is possible using cellular therapies. However, larger treatment effects and improvement in reproducibility are needed before regulatory approval, clinical adoption and reimbursement can be expected [58,59].

Several strategies are available to improve the efficacy of BMSC or similar cellular therapies. For example, cells might be activated before injection (e.g., using Rapamycin, which inhibits mammalian target of rapamycin (mTOR) signaling and mimics interferon stimulation) [60]. Alternatively, inhibition of Peroxisome Proliferator-Activated Receptor (PPAR) Beta/Delta transcription factor may enhance anti-inflammatory effects [61]. Cell combinations may be used (e.g., autologous chondrocytes with BMSCs) [62]. Knowledge of CQAs that can be measured *in vitro* and predict *in vivo* efficacy have not been defined but are critical to the long-term development of this approach.

The potential for disease-modifying osteoarthritis drugs (DMOADs) that preserve cartilage in the setting of early OA is opening up on several new fronts. The strategy for use of these agents is linked to a "personalized" patient-specific approach in which biological or gene expression markers are used to identify joints at risk and justify pre-emptive intervention, even before symptoms might justify current invasive intervention approaches [63]. Several biological targets have been evaluated (e.g,. inhibition of IL1β or Nerve growth factor (NGF) receptor), and may reduce pain, but thus far with no effect on OA progression [64,65].

Future therapeutics may consider both the patient's genetic susceptibility as well as environmental risk factors (e.g., injury). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genome engineering technology enables strategies like Stem cells Modified for Autonomous Regenerative Therapy (SMART) to selectively reduce inflammation caused by arthritis and other chronic conditions through local production of anti-inflammatory molecules. If durable engraftment can be obtained, such cells may be used like a long-term

vaccine-limiting progression of OA from early-stage disease [66].

One of the biggest limitations in establishing the safety and efficacy of these new cartilage therapies is the cost of clinical trials, particularly randomized controlled phase 2 and 3 trials (RCTs). The organization of large multicenter registries for cartilage repair may be critical to reducing these barriers to progress. Given the well-documented natural history of OA, and its progression to expensive interventions of arthroplasty surgery, prospective cohort studies may provide a practical and rigorous means to improve clinical research generally and set the stage for an alternative approval pathway [67].

Effective clinical trials of DMOADs will focus on early OA with the goal of limiting progression of OA (e.g., reducing inflammation and bone edema). The musculoskeletal community has not yet defined consensus of the most appropriate methodology for such trials. However, it is likely that trials will include both clinical measures of pain and function as well as imaging to measure structural attributes of the joint (e.g., cartilage thickness/volume, cartilage surface integrity, meniscus volume, effusion volume and synovial thickness).

Tendon repair and regeneration, Scott Rodeo, MD

Cellular therapies for tendon repair are less well developed than for bone and cartilage [68]. The process of determining the optimal protocol for cell therapy for tendon disorders must begin by defining the underlying tendon pathology being treated. The optimal cell therapy for treatment of chronic, degenerative tendinopathy will likely differ from the approach needed for repair of an acute tendon tear. Moreover, the anatomic location of tendon repair will vary, along with the biology. Repair may be tendon-to-tendon (e.g., flexor tendon repair in the hand or Achilles tendon repair), but other clinical settings require tendon-to-bone repair (such as in rotator cuff tendon repair).

Further information is needed about the cellular and molecular mechanisms of tendon degeneration and healing in each of these settings, to identify the rate-limiting steps and key biological targets that may be addressed using cell therapy [69–74]. For example, the optimal cell type and dose (concentration, volume, frequency) may be different in each setting. In some settings, the goal may be to increase tendon cell proliferation and to accelerate matrix synthesis in a healing tendon repair. In other settings, optimal methods may target the inhibition of matrix-degrading proteases and inflammatory mediators in a chronically degenerative tendon.

There are important outstanding questions about optimal timing and dosing of cell therapy. For example, cell therapy may be most valuable if delivered at the time of surgery. On the other hand, efficacy, retention and survival may be better if delivered days or weeks after injury or surgery. Furthermore, it is possible that repeat cell dosing will optimize outcome.

The tissue volume that requires repair is less defined and more variable in the setting of tendon or ligament repair than for bone and cartilage. Optimal methods for delivery to optimize cell retention and survival at the tendon repair site will likely be different. A carrier vehicle may be required to localize the cells to the repair site for a relevant period of time. The microstructure, composition and chemistry of such a carrier vehicle will inevitably affect the biological activity of the implanted cells.

Like bone and cartilage therapies, robust clinical studies, including prospective cohort studies and multi-center registries, will be critical to assess and optimize cellular therapy for treatment of degenerative tendinopathy and surgical tendon repair.

Muscle repair and regeneration, Johnny Huard, PhD

Muscle tissue contains progenitor populations that can be stimulated to form new muscle tissue. However, muscle regeneration is frequently limited by scar formation, rather than muscle regeneration [75].

Platelet-rich plasma (PRP) is an emerging biological tool with particular relevance to the field of regenerative medicine because it contains an abundance of autologous growth factors and is easy to obtain and manipulate. Although the use of PRP has become an increasingly popular treatment option for various musculoskeletal conditions, published clinical results have been inconsistent. PRP contains many important growth factors that may accelerate tissue healing (including platelet-derived growth factor [PDGF], VEGF, IGF and TGF-β1, among many others). However, PRP also contains large quantities of substances that are known to pro mote further inflammation and subsequent tissue damage (such as inflammatory cytokines, reactive oxygen species [ROS], and matrix metalloproteinases [MMPs]).

A combination of PRP injection and oral administration of losartan (an antifibrotic agent) has been shown to enhance muscle healing by stimulating muscle regeneration and angiogenesis and to prevent fibrosis in contusion-injured skeletal muscle [75]. Muscle regeneration and muscle function were significantly promoted with the combined PRP + losartan treatment in contusion injuries created in the tibialis anterior muscles of mice compared with the

other groups. Combined PRP + losartan treatment significantly decreased the expression of phosphorylated Smad2/3 and the development of fibrosis compared with PRP treatment alone, and it increased VEGF expression and the number of CD31-positive cells compared with losartan treatment alone. Follistatin, a positive regulator of muscle growth, was expressed at a higher level in the PRP + losartan group compared with the other groups. PRP + losartan combinatorial therapy improved overall skeletal muscle healing after muscle contusion injury by enhancing angiogenesis and follistatin expression and by reducing the expression of phosphorylated Smad2/3 and the development of fibrosis. These results suggest that blocking the expression of TGF-β1 with losartan improves the effect of PRP therapy on muscle healing after a contusion injury [76].

Neutralizing TGF-β1's action in PRP has also been shown to improve muscle repair [76]. PRP was isolated from in-bred Fisher rats. TGF-β1 neutralization antibody (Ab) was used to block the TGF-β1 within the PRP prior to injection. The effects of customized PRP on muscle healing was tested on a cardiotoxin (CTX)-induced muscle injury model, proving that neutralizing TGF-β1 within PRP significantly promotes muscle regeneration while reducing fibrosis. Not only did the neutralization reduce fibrosis, it enhanced angiogenesis, prolonged satellite cell activation and recruited a greater number of M2 macrophages to the injury site, which also contributed to the efficacy that the customized PRP had on muscle healing. These findings could contribute to the development of biological treatments that aid in the healing of skeletal muscle after injury [76,77].

Intervertebral disc, Jérome Guicheux, PhD

Intervertebral disc (IVD) is the central player of spine kinematics. Its degeneration is one of the major causes of low back pain (LBP). Discogenic LBP is primarily managed using pharmacological treatments. Surgical procedures (fusion or disc replacement) are reserved for severe debilitating LBP. To clinically address LBP early in the degenerative cascade of IVD, cell-based regenerative strategies could offer less-invasive alternatives to spinal reconstructive surgery.

Different cell-based approaches have been proposed [78]. First, the intradiscal injection of undifferentiated MSCs, mostly from bone marrow or adipose tissue, has been tested in pre-clinical animal models with some success [79,80]. Several clinical trials have described improvement in pain but with no clear evidence of a structural effect [81]. MSC therapies using a biomaterial cell carrier have more recently been considered (e.g., an injectable hydrogel). A recent clinical trial reports that this could be safe and well tolerated. Several trials are ongoing [82].

Finally, the production of regenerative cells derived from induced pluripotent stem cells (iPSCs) is being considered. The generation of such engineered IVD cells and their association with scaffolding biomaterials mimicking the natural environment of a healthy IVD holds great promise for IVD regeneration [83].

Clinical adoption gap—value, regulations and standards

Reporting standards for cell therapies, Nicolas S. Piuzzi, MD

There have been many calls for standardization in the field of cell-based therapies and in the treatment of musculoskeletal diseases [17,19–22,57,84,85]. Many standards exist for classification of disease, biological and bioactive composition in tissues, chemical reagents and materials. However, currently there is a dearth of standards for quantitative identification and characterization of cell populations and for methods of cell harvest, processing and fabrication.

Standards are essential for clear, precise and rigorous communication, and to allow reproducibility in measurement and process management. Standards are usually established by authority, custom or general consent, and provide models or rules for measurement and reporting on the identity, quantity, quality or purity of a material or the efficiency, effectiveness or variability in a task or process.

The field of cell biology, especially related to culture-expanded cells, is filled with a confusing and competing litany of names for various culture-expanded cells and cell products. Names are frequently coined arbitrarily and nomenclature is frequently disconnected from the biology and identity of cells in native tissues or a specific and consistent measure identifying phenotypic or functional attribute. Moreover, publications vary widely with respect to the relevant metrics of cell processing and characterization that are reported: tissue source, isolation/selection method, expansion conditions, surface marker attributes, concentration, prevalence, gene expression profile, morphological features, proteome signature, etc. As a result, articles frequently lack information that would be critical to enable work to be repeated or effectively compared with work by another investigator or laboratory.

However, progress in standards development and use is being made. For example, the International Society for Cellular Therapy (ISCT) committee on

mesenchymal stromal cells (MSCs) has proposed minimal criteria for defining the term MSC in an attempt to advance communication in the field [86]. Standards for reporting are being proposed for PRP preparations and isolation of bone marrow–derived populations [17,20,57,84,87]. Furthermore, validating assays that assess cellular composition and prevalence, concentration and biological potential of stem and progenitor cells will be required as test methods and analysis methods are developed. As an example, the colony-forming efficiency unit assay, which constitutes a key feature in the assay and identification of almost all stem cell and progenitor populations, has been standardized. To advance the quantitative use of colony-forming assays, the ASTM International has published Standard Test Method for automated colony-forming unit (CFU) assays; Image Acquisition and Analysis Method for Enumerating and Characterizing Cells and Colonies in Culture has been developed (ASTM International–F2944-12) [88]. Automated methods for colony analysis have been applied in recent publications [41–43,49,89].

The adoption and use of these standards and the ongoing development of additional standards enumerating quantitative metrics that measure CQAs for cellular therapies (product standards) will be essential elements in advancing the field of cellular therapy in general and particularly cellular therapies for musculoskeletal disease [17–20,87,90].

Standards–value proposition and gaps, Richard McFarland, PhD, MD

Existing consensus standards efforts in the area of cellular therapies for musculoskeletal disease have focused on the development of standardizing terminology of various cell types, pre-clinical models and analytical methods; in essence, they are focused on measurement of the tissue-engineered product [91]. These are important areas for standardization for academic reasons, but this focus is problematic for transition of the space from an academic field with the occasional artisanal product and widespread non-clinically proven, off-label and practice of medicine treatments to an industry with a wide array of well-characterized and understood products available from which the practicing orthopedic surgeon can chose according to their patient's specific clinical needs. Therefore, manufacturers should characterize, understand and, therefore, reproducibly control the clinically relevant variabilities inherent in their specific musculoskeletal medical product.

Currently, manufacturers must develop most methods to control their manufacturing process and product characterization procedures independently because of a lack of such consensus standards. Nonetheless, many of these unit operations and analytical tests could be established and shared without jeopardizing the intellectual property of any of the manufacturers. This situation results in increased cost to developers in terms of time and resources when everyone is forced to work independently. Fortunately, this situation was recognized by the FDA, National Institute of Standards and Technology (NIST) and the regenerative medicine industry group, Alliance for Regenerative Medicine (ARM), and several steps have been taken to ameliorate it. ARM spawned an independent non-profit from its Standards and Technology Committee known as the Standards Coordinating Body (SCB) whose mission is to coordinate the accelerated advancement and improved awareness of standards and best practices that address the rapidly evolving needs of the global regenerative medicine advanced therapy community. The FDA and NIST are working collaboratively to focus their respective resources to facilitate standards efforts forward through many avenues, including through efforts to interact and support SCB. For example, the standards landscape report referenced above and an approach for identification, prioritization and feasibility analyses of standards gaps that were due in September 2018 are the result of 21st Century Cures Act mandated efforts. Over the next few years, implementation of these processes will provide new tools for transitioning the space from almost entirely an academic and individual practitioner-based pursuit to a growing industry with numerous products and a vibrant R&D base.

Insights from veterinary medicine

Regenerative medicine in equine and canine musculoskeletal injuries, Laurie Goodrich DVM, PhD, ACVS Diploma and Stéphane Maddens, PharmD, PhD, MBA

Biologically and clinically relevant animal models are an essential component in pre-clinical screening for virtually all biomaterials, drugs and cellular therapies. However, veterinary practice in large and small animals also represents the vanguard setting for clinical application of many musculoskeletal therapies. Naturally occurring injuries, such as degenerative joint disease, OA and tendinopathy, are common clinical settings with substantial demand for new therapeutic approaches, particularly in the canine and equine setting. Race horses represent an intensely managed patient pool with a high incidence of tendinopathy and whose return to service and racing performance are rigorously and quantitatively measurable.

Culture-expanded MSCs derived from marrow or fat are among the most commonly used autologous cell therapies. Clinical use is climbing based on reported efficacy in equine tendinopathy and articular joints [92–94]. Doses of 10 to 20 million cells per site are typically delivered. Regional perfusion via intra-venous or intra-arterial delivery (without a tourniquet) have been used [95], but intra-lesional implantation appears to be most effective with respect to clinical response and cell survival at the site of the lesion [95]. In horses, multiple evidence-based studies have suggested improved function and athleticism and reduced re-injury rates compared with non-treatment [92–94].

MSC joint therapy has suggested success in cases of meniscal disease, OA and cartilage damage [94,96,97]. The value of injected MSCs in various stages of joint disease is under intense investigation and clinical studies are in progress. The safety and efficacy of allogeneic MSCs are also being explored with neonatal or adult tissues [98].

Many questions remain regarding cellular therapies for musculoskeletal disease in the horse, including the following: cell tissue source, allograft versus autograft, dose, timing, disease severity and anatomic location. The equine model is highly relevant to each of these questions, as well as the assessment of other biologics, used alone or in combination with MSCs. Biologics such as PRP [99,100], Interleukin Receptor Antagonist Protein (IRAP) [99,101] and autologous protein solution (APS) [102] are also extensively used in equine practice.

The health market for non-equine companion animals (primarily canines) differs greatly from its clinical human counterpart. It is much smaller in size and care is not covered by a developed health insurance system. Given these limitations, the veterinary commercial industry has mainly focused on allogeneic MSC products with a high manufacturing scalability. However, autologous MSC-based services are often provided by academic centers.

Several characteristics may make MSCs from neonatal tissues (placenta or cord-derived cells) preferable to the use of allogeneic adult tissues or postnatal tissues: (i) donors are healthy, (ii) harvest is not invasive or compromising to the animal well-being, (iii) there is low risk of biological hazard, (iv) donor age is minimized and standardized, (v) neonatal MSCs have a higher proliferation potential before replicative senescence and (vi) neonatal MSCs have a lower immunogenicity and a higher immunomodulation potential [103,104]. Moreover, standardizing source materials enables streamlining of regulatory constraints, scale-up technologies (i.e., dynamic culture systems) and automation. These are critical parameters in optimizing the product development process and limiting the risk of variation in product composition and quality. All of these factors also help to minimize fabrication cost. Lower costs will make therapy more acceptable to a greater proportion of practitioners and animal owners.

Prospective registries will be instrumental in developing an improved understanding of the indications, contraindications, risks and benefits of cellular therapies in veterinary practice in the same way that they will benefit human clinical practice.

Cell therapy communication–nomenclature–"The Tower of Babel"

Defining the "MSC" populations, Massimo Dominici, MD

Progenitor cells in bone marrow (BM) were described in the 1960s by Alexander Friedenstein *et al.* to explain the bone-forming potential of a rabbit BM cell suspension [105]. The concept of rare, elusive bone-forming cells was explored by many investigators over the past 50+ years using many definitions and systems of nomenclature. Friedenstein *et al.* initially used the term "mechanocyte" for the plastic adherent fibroblast-shaped mechanocyte progenitors, due to their osteogenic (and, therefore, eventually "mechanical") properties [106,107]. Parallel investigations outlined the role of adherent BM-derived cells in generating the stromal cells that were necessary to provide the microenvironment needed to support hematopoietic tissues *in vitro* [108].

These attributes of adherent fibroblastic cells were merged in 1988 by Owen and Friedenstein who defined culture-expanded BM-derived stromal cells also as progenitors for the osteogenic lineage [109]. Building on the studies of the attributes of culture-expanded marrow stromal cells, in 1991, Caplan coined the term "mesenchymal stem cells" (MSCs), using terms from embryonic development to reflect the origin, multipotentiality as well as apparent proliferative potential or "stemness" that progressively prevailed the MSC "stromality" [110]. A few years later, Prockop described culture-expanded cell populations with MSC properties from non-hematopoietic tissues, including inducible expression of bone, cartilage, fat and muscle markers *in vitro* [111]. Pittenger *et al.* elegantly added deeper phenotypical characterization [112].

A much higher level of stemness has been subsequently attributed to a possible subset of culture expanded "MSCs" up to pluripotency, with almost infinite proliferation and differentiation capacities with a terminology shift to multipotent adult progenitor cells (MAPCs) [113]. Unfortunately, *in vivo*

investigations in a variety of regenerative medicine approaches revealed a low-engraftment rate and suboptimal therapeutic impacts [114].

As *in vitro* and *in vivo* data have accumulated related to culture-expanded plastic adherent fibroblastic cells, the accuracy of the word "stem" has been called into question [115]. At the same time, ongoing work has become more focused on biological functions more attributable to stromal and secretory function than to differentiation [86,116,117].

Further work doubled back on history to include the concepts of MSC heterogeneity among MSC populations and variation in functional plasticity [118]. Culture-expanded populations are not homogeneous, as initially assumed. They contain multipotent populations together with less potent sub-types whose nature, identity and *ex vivo* isolation/expansion potential has yet to be defined. In addition, MSCs retain variable attributes of stromal function that can be influenced by environmental cues. All this is certainly increasing the complexity, making it difficult to unify a definition for culture-expanded MSC populations.

The current nomenclature uses the term "multipotent mesenchymal stromal cell" and is based on position papers of almost 15 years ago [86,117]. Characterizations by using new technologies for biological definition of culture-expanded MSC populations are needed for an updated nomenclature. Investigation also needs to be traced back *in vivo* to unveil the heterogeneous underlying colony-founding population(s) in native tissues, from which culture expanded "MSC" populations can be derived. Tangible links to tissue-specific niches, tissue health (formation and remodeling) and disease are needed. All these approaches should be combined for future MSC definitions rather than in a sterile revision of the nomenclature alone.

Tissue-specific "connective tissue progenitors"—a generalizable paradigm for tissue resident stem/progenitors populations in musculoskeletal tissues, George F. Muschler, MD

The concept of tissue-specific connective tissue progenitor cells (CTPs) has been used as a valuable paradigm to explore and to define the heterogeneous populations of stem and progenitor cells that are present *in vivo* in native tissues, and to distinguish the native cell populations that may be available for point-of-care cell harvest and processing methods from highly selected culture expanded populations (e.g., MSCs) and cell lines that are commonly used in research. This distinction is necessary for the understanding of stem and progenitor cell function during tissue formation and remodeling, and to identify and define the populations of cells that may be targeted or used as cell source populations for tissue engineering and regenerative therapies [17,34–36,41–43,88,119].

CTPs are defined as the heterogeneous population of "tissue-resident" cells that can be harvested from native tissues, induced to proliferate *in vitro* and that can generate clonal progeny that can differentiate into one or more connective tissue phenotypes (e.g., bone, cartilage, adipocyte, fibroblast, stromal cell or blood) [35,119]. The heterogeneous population of cells that meets the criteria for a colony-founding CTP includes true resting (G_0) or self-renewing stem cells. These true stem cells may be present in one or more niches within a given tissue. The CTP population also includes cells within native tissues that may already be proliferating (transient amplifying populations) or progenitor cells. In fact, the dynamics of stem cell activation and downstream transient amplifying populations (TAP) that precede differentiation into a stable mature cell phenotype predict that far more CTPs (colony founding cells) will be derived from downstream TAP populations than from true stem cells in any growing or remodeling tissue [34,35,119].

The CTP paradigm assumes that each tissue will contain one or more different CTP cell types representing different niches or compartments in that tissue [35]. The hierarchy of cells that form the connective tissue of blood have been best characterized [120,121]. The hierarchy of stem and progenitor populations in BM began with Friedenstein and Owen and others [109,122–125]. CTPs with different biological properties can be found in the marrow space and the trabecular surface [41]. Some CTPs are derived from aspirates of BM that retain hyaluronan (HA) on their surface, suggesting that they are derived from a niche characterized by an HA-rich extracellular matrix [43]. Bianco and Robey have defined the "Skeletal Progenitor Cell" as a tissue-specific CTP population on the trabecular surface, which appear to express CD271 and CD146 [126,127]. Periosteum, synovium, cartilage, fat, muscle, IVD and virtually all solid organs can be sources of CTP populations. Perivascular cells, likely among the pericyte population, are a source of CTPs in all tissues, except cartilage [128]. However, evidence suggests that not all pericytes are CTPs and that the pericytes in different tissues have tissue-specific properties [126,127].

The prevalence of CTPs in native tissues varies widely from patient to patient and from tissue to tissue, from a mean of nearly one in 20 000 in native BM to one in 2000 in healthy fat, synovium and cartilage [41,89,129,130]. CTPs, even in low numbers,

represent an available target cell population, with biological potential that may be elicited or induced by local or systemic drug delivery or local bioactive biomaterials [4]. However, the low prevalence of CTPs limits some tissue-engineering approaches. This can be overcome by efficient harvest and processing methods that are designed to concentrate or select CTPs for transplantation [1,9,14]. In many cases, it may be desirable to isolate and culture expand the progeny of CTPs *in vitro* to increase the number of available cells and to select for or optimize their biological properties prior to use. Application of selected CTP populations include the following: basic research in exploring the hierarchy of tissue-specific CTPs and differentiation pathways, drug discovery/screening and biofabrication of tissues for re-implantation.

Under appropriate conditions, the culture-expanded progeny of some CTPs may meet the criteria that have been defined for a culture expanded "MSC" population [86,117]. However, it is not clear that MSC criteria are either necessary or specific for prediction of biological potency or efficacy for any particular application [89,131]. Defining the CQAs for the culture-expanded progeny from the various tissue-specific CTP populations for specific research and clinical applications is a highly active area of basic and translational research [132].

The skeletal stem/progenitor cell: a tissue-specific stem/progenitor cell population in BM, Pamela G. Robey, PhD

There are two defining features of stem cells: (i) the ability to functionally reconstitute the parenchyma of a tissue, and (ii) the ability to self-renew (i.e., generate progeny that retain all of the biological properties and potential of the original cell). Based on the early work of Friedenstein and those that followed, it is apparent that BM contains a population of clonogenic, non-hematopoietic, rapidly adherent cells that have the ability to form cartilage, bone, hematopoiesis supportive stroma and marrow adipocytes (functional skeletal tissues), and that can self-renew based on serial transplantation assays.

Culture-expanded cells that would have these properties have commonly been labeled as "mesenchymal stem cells," grouping them together with culture-expanded fibroblastic cells from many tissues that have fibroblastic cell surface markers. However, the use of MSC nomenclature in this way is a misnomer. Mesenchyme is an embryological term that is used to define embryonic connective tissue that forms not only connective tissues, but also blood vessels and blood (no post-natal stem cell retains this biological potential). Furthermore, bone forms from three embryonic sources (neural crest, ventral paraxial mesoderm of the sclerotome and somatic lateral plate mesoderm). So-called MSCs from non-skeletal tissues arise from other mesodermal specifications. As a result, colony-forming cells in adult tissues do not arise from a single common ancestor, and are not a lineage. Furthermore, when the progeny of colony forming units-fibroblasts (CFU-Fs) are culture-expanded from different tissues, their transcriptomes are indicative of their tissue source, as is their differentiation capacity. The culture-expanded cells derived from a selected population of $CD45^-/CD34^-/CD146^+$ cells will make bone and cartilage. However, the progeny of $CD45^-/CD34^-/CD146^+$ muscle-derived cells do not make bone or cartilage, but do make muscle. These data reinforce the concept that CFU-Fs in native tissues represent populations of tissue-specific stem/progenitor cells. For these reasons, standards for nomenclature will need to incorporate metrics that enable differentiation of tissue-specific populations of colony-founding CFU-Fs. One of these important subpopulations is the $CD45^-/CD34^-/CD146^+$ cell population on the surface of BM sinusoids that has been defined by the term skeletal stem/progenitor cell. In spite of their differences in transcriptome and differentiation capacity, many $CD146^+$ cells from different tissues have the ability to associate with blood vessel walls, to form pericytes and to remain quiescent until activated by an injury or liberated by a bone turnover event [126,133,134].

Editorial practices—challenges and responsibilities

The Journal of Bone and Joint Surgery—*editorial practices, Thomas Bauer, MD, PhD*

The potential benefits of cell therapy are commonly discussed, many clinical trials are in progress and hundreds of clinics offer "stem cell therapy" for various musculoskeletal conditions. However, attempts to judge and report cogently and definitively on the efficacy of stem cell treatments have been largely unsuccessful, in part because many details of cell characterization, cell enrichment, cell processing and indications for use have been missing in published reports.

Piuzzi *et al.* [57] attempted to review the use of BM aspirate concentrate in musculoskeletal disorders but found that, of 46 reviewed articles, no single study provided sufficient details to allow the methods to be repeated. Only 30% of studies even provided

the number of nucleated cells that were present after processing. The authors proposed minimum information that should be included in future publications.

Other publications have also suggested minimal criteria needed to define the critical generalizable parameters when discussing MSCs [86], stromal cells from adipose tissue [135] and composition of PRP [87]. All have emphasized the need for more standardized content in cell therapy publications [17–20,90]. Murray *et al.* [87] compiled the results of a Delphi consensus approach by e-mail, in which a multidisciplinary group of investigators defined Minimum Information for Studies Evaluating Biologics in Orthopedics (MIBO), specifically related to the use of PRP and MSCs, and have posted those recommendations on line.

The mission of *The Journal of Bone and Joint Surgery* (*JBJS*) is to improve musculoskeletal health across the globe by delivering gold standard information resources for clinicians, researchers and orthopedic care teams. It strives to be the best place to publish high-quality musculoskeletal research, and is constantly seeking ways to improve publication quality and the peer review process. Through its web site, *JBJS* offers links to the checklist developed by the **CON**solidated **S**tandards of **R**eporting **T**rials (CONSORT) Group [136], the format for cohort studies suggested by the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) panel [137], and meta-analysis criteria from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement [138,139]. *JBJS* has not yet developed a link to any specific site that recommends minimal recommended content with respect to cell therapy publications but, in principle, supports that concept. Fulfilling such recommendations within the 3000 word limit for a *JBJS* manuscript may be difficult but, in principle, the *Journal* would promote adherence to reporting standards developed by the ISCT or other appropriate organizations. Within limits, standardized reporting should help maintain consistency and the high quality that readers expect from *JBJS*.

Cytotherapy—*editorial practices, John Barret, MD*

Cytotherapy is the official journal of the ISCT. It was created by the founding members of the ISCT in 1999 and its editors are ISCT members. This association shapes the aims and scope of the journal to conform to the expertise and purpose of the society. *Cytotherapy* publishes reviews, commentaries, position statements, white papers, original articles and correspondence on all aspects of cell therapy in humans from relevant laboratory science through translational research to clinical implementation. The journal is particularly a home for cell therapeutics involving tissue-specific CTPs, culture-expanded MSCs, other well-defined culture expanded cell populations and immune cells. *Cytotherapy* prominently features authoritative articles from ISCT leadership (e.g., the Presidential Task Force on Unproven Therapies, white papers on regulatory issues and nomenclature). Aside from its association with the ISCT, the journal serves a diverse readership. It is a unique forum for publications on cell science, clinical cell therapy trials, regulatory affairs, product manufacture and commercialization of cell therapy.

The editor's challenge is to serve this diverse interest group, while maintaining an upward momentum for the journal, as measured by the international breadth of its readership, its authority among peer-reviewed journals and, of course, by its impact factor. This diversity of responsibility can cause dilemmas: not all society-related manuscripts are well cited and this can be detrimental to the journal's impact. Conversely, some papers of potential impact are not handled because the topic is outside the journal's scope, in terms of reviewer expertise. The rapid developments in new technologies behoove the editor to constantly review, extend and modify the journal scope in the light of important developments (e.g., the burgeoning science of cell exosomes). The rapid growth potential of cellular therapies for musculoskeletal care is another example.

Fortunately for *Cytotherapy*, the field of cell therapy is one of the most exciting and rapidly evolving domains in the treatment of human disease. This and the innovative and creative energy of our members and readers are the essential forces that drive our journal upwards.

Research gaps—hidden gems—collaboration opportunities—strategic initiatives

Obstacles to translation, Frank Barry, PhD

Over the past two decades a considerable body of pre-clinical data was generated that suggested that cellular therapy using culture-expanded MSCs would find application in human medicine. However, that promise for human medicine has not yet been fully realized. To date, MSC treatments have received approval for only a handful of indications. There are several major obstacles to translation that still exist relating to understanding the mechanism of the host response following transplantation. There is also a general lack of technical competence in rigorous, seamless, efficient and reproducible product

manufacturing and characterization strategies. As long as these gaps are unfilled, it can be argued that MSC therapy will not become a mainstream option for patient care.

In broad terms, it is possible to identify a number of major issues that hinder the therapeutic application of MSCs. These include questions of fundamental biological importance such as the precise therapeutic mechanism(s) of action, an understanding of the interaction between transplanted cells and cells of the host immune system and the nature, accuracy and sensitivity of potency tests. For example, it is apparent that many of the widely used markers of MSCs, such as CD73 and CD105, lack biological relevance and specificity and are not sensitive indicators of phenotype or therapeutic activity [89,131]. In addition, there are severe challenges in terms of the logistics of manufacturing and supply, including the use of biologically specific methods for prospective isolation, scalable manufacturing systems and reliable cryopreservation. Further, the current dependence on fetal bovine serum for cell expansion represents a clear but unaddressed vulnerability. Of these, unravelling the therapeutic mechanism of action seems to be the most pressing and, as clinical proof of concept emerges, it will be possible to achieve this. Only then will it be possible to devise quantitative, disease-specific potency tests and quantitative CQAs for use as release criteria and in calibrating therapeutic dose.

Cell and engineered tissue survey of ISCT members, Ivan Martin, PhD

As cellular therapies in orthopedics become more widespread for a variety of indications and modalities, it is challenging to maintain a clear perspective on the effectiveness of the treatments performed, and to provide a basis of objectivity and transparency in defining developing trends.

Since 2008, the ISCT, the European Chapter of the Tissue Engineering and Regenerative Medicine International Society (TERMIS-EU), the International Federation for Adipose Therapeutics (IFATS), the International Cartilage Repair Society (ICRS) and the European group for Blood and Marrow Transplantation (EBMT) have established an instrument to survey and report on the status of cell and engineered tissue therapies in Europe and neighboring Eurasian countries [140]. The program collects data of treated patients sorted by specific therapeutic indications, cell/tissue donor types, processing and delivery modes, without reference to the clinical outcome. The information is thus complementary to published results or to the results available through public clinical trial databases.

Last year's report, related to patients treated in 2015, captured data from 178 clinical teams, related to the treatment of 3686 individuals [141]. The musculoskeletal/rheumatological field represented 32% of all treatments. Cartilage repair was the most frequent indication (53%), followed by reconstructive surgery (19%) and bone repair (13%). Autologous cells were used in 94% of these patients. Culture-expanded MSCs were used in 40% of bone repair and in 19% of cartilage repair procedures. Chondrocytes were used in 37% of bone procedures and in 61% of cartilage repair procedures.

This concise snapshot outlines the type of information that can be gathered from the cell and engineered tissue survey. A yearly report acknowledges the participating teams. The ISCT hosts the instructions and forms required to participate [142] and supports a continuous development of the program, towards extension to other geographical areas and inclusion of additional information (e.g., metrics of cell manufacturing and context of clinical treatment).

Cell therapy—point-of-care quality control and process metrics—feasibility and necessity, David Karli, MD, MBA

Autologous, point-of-care (POC) therapies, including PRP and BM Cell Concentrate (BMC), have continued to increase in clinical use. The overwhelming majority of this care has involved bench-top processing density separation units. However, the process does not include direct cell counting or any other product release criteria as quality control metrics.

Assay of cellular composition is a critical missing element in the responsible advancement and assessment of these therapies. A recent review of published clinical studies using BMCs for orthopedic applications revealed that only 30% of the studies reported on the number of cells injected. When cell concentration or composition was reported, the approach was not standardized with respect to counting method [57]. Despite this inconsistency and gap in the literature, examination of those studies that have quantified cell counts suggest that therapeutic efficacy might be linked to both cellular and platelet concentrations. This reinforces the clinical need for measurement.

Methods to obtain real-time validated cell and platelet counts are available for peripheral blood and PRP preparations using modern automated hemoanalyzers and manual processing techniques. However, the substantial heterogeneity of BM aspirates (BMAs) present a challenge to the validation of accurate and automated hemoanalyzer measurements of

BMC preparations. Efforts are underway to improve automated analysis of BMA and BMC preparations. CFU assays to measure CTP prevalence and flow cytometry assays using markers of hematopoietic stem cells (e.g., CD34) and putative markers of CTP subpopulations (Hyaluronan and CD146) are also under investigation. However, colony assays and flow cytometry methods require technical expertise and add cost, but are generally available at academic medical centers.

Systems and platforms for improved collection and integration of clinical data and quantitative assessment of the composition of cellular therapies are needed. Cost-effective systems can include, at a minimum: (i) patient demographic data; (ii) clinical diagnosis, site and severity data; (iii) cellular composition (at a minimum hemoanalytic data); and (iv) clinical outcomes data (i.e., patient-reported general health and diseases-specific metrics). Prospective scalable practice databases are essential tools to establish formal clinical registries and consortia needed to examine cellular therapies in prospective cohorts or randomized trials.

For profit and non-profit electronic resources exist and are being piloted to collect clinical baseline data as well as follow-up data. Strategic industry and multicenter collaborations using standardized clinical and product metrics will allow capture of the larger datasets needed to provide the statistical power needed to objectively compare the efficacy of different approaches for specific clinical indications.

Development of orthopedic biological registries and clinical trials network, Constance Chu, MD

The disease burden of serious and disabling musculoskeletal conditions, such as OA and degenerative tendinopathy, are high, and restorative treatments are limited. These realities create strong interest for patients to seek new and often unproven therapies. Presently, the clinical use of biologics such as PRP or cell-based therapies greatly outpaces the evidence. Consensus recommendations from a recent American Academy of Orthopedic Surgeons (AAOS) work-group include the strong recommendation that orthopedic biological registries and clinical trial networks be established and supported [143]. Only through collaborative effort can the high-quality evidence that clinicians and patients need become available in a timely and cost-effective manner [144].

The orthopedic community has several established registry models for joint replacement, anterior cruciate ligament reconstruction and other conditions that have contributed important clinical information on practice patterns, patient outcomes and clinical quality based on post-market performance of implants and grafts [145,146]. Leveraging these pathways into a biologics registry could similarly contribute 'real world' clinical data on the use and outcomes of biologics.

Biologics that are derived from the patient's own cells or tissues present the additional challenge of wide variability in composition and bioactivity [147]. In this setting, assessment of processing quality and product biological effects necessitate collection and analysis of samples collected before and after any processing. For example, in the evaluation of PRP, standardized automated laboratory analysis of the complete blood count (CBC) for samples of the patient's whole blood collected to prepare PRP as well as the finished PRP would allow for determination as to what degree the platelets were concentrated. This information could then be used to evaluate quality and consistency of the processing device as well as the relationship between platelet concentration and clinical outcomes.

The non-cellular and proteomic composition of the end product is also highly variable between patients and between samples collected at different times. Variability in proteomic composition and bioactivity likely impact clinical effect. Establishment of biorepository-linked registries where biospecimens are linked to clinical data can support subsequent proteomic analyses to potentially identify proteins and biomarkers that are predictive of clinical outcomes.

Several observational consortia [148,149], as well as a multicenter clinical trials pilot study [150], demonstrate the feasibility of this approach. A framework for systematic gathering and analyses of patient-reported outcomes, establishing a biorepository, as well as development of standardized functional and imaging outcome metrics have been established [144]. Appropriate infrastructure and incentives will be needed to motivate both physician and patient participation in both sample analysis and outcome reporting.

Summary

This Signature Series Symposium achieved its core objectives. A forum of multidisciplinary thought leaders was gathered, including basic and translational scientists, clinicians, regulatory experts, editors and reviewers from the flagship journals in orthopaedic surgery and cellular therapies. The status of the field was rigorously assessed. Key challenges and opportunities were defined in the areas of: marketing of unproven therapies, current clinical effectiveness and value, regulatory affairs, standards development, clarity in nomenclature and

transparency in publication and enabling clinical networks and registries.

Through the symposium a broad-based musculoskeletal community has been initiated within the ISCT with the mission to continue in its effort to enable, engage and expand this leadership forum within ISCT, in collaboration with other relevant groups and societies, to make musculoskeletal cellular therapies a safe and effective reality for patients and a fertile ground for rapid and effective innovation, communication and dissemination of information that advances the field.

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