Proceedings: Using Stem Cell Therapies to Reestablish Osteogenic Capability for Bone Regeneration

NEIL LITTMAN, ARIE ABO

California Institute for Regenerative Medicine, San Francisco, California, USA

SUMMARY

The California Institute for Regenerative Medicine (CIRM) has invested approximately $70 million in programs targeting various orthopedic indications, including osteoporosis, bone fracture healing, vertebral compression fractures, and several others. The present article serves to outline the current state of CIRM’s more advanced programs, comparing and contrasting them with the current standard of care and several other novel approaches under development.

INTRODUCTION

Most people will experience some form of musculoskeletal-related injury or disease during their lifetime. These injuries become particularly acute and problematic in the elderly population, for whom delayed skeletal healing and osteoporosis-related fractures are commonplace. Orthopedic medical devices targeting these injuries currently generate more than $30 billion in annual worldwide revenue and include the following four subsegments: reconstructive devices ($14.5 billion), spinal implants and instruments ($7.5 billion), fracture repair ($6.0 billion), and arthroscopy/soft tissue repair ($4.5 billion) [1]. In addition to orthopedic devices, various prescription and over-the-counter medications are available that can help relieve pain and reduce the swelling that typically results from bone fractures and injury.

Considerable efforts are underway to either augment or replace many of these devices, procedures, and drugs with novel therapeutic approaches, with several treatments having already been approved by the U.S. Food and Drug Administration (FDA). Many new approaches involve the use of stem cells to either regenerate or repair the damaged or fractured tissue and bone, most of which involve the use of mesenchymal stem cells (MSCs) obtained from living adult tissue, typically bone marrow. These approaches aim to provide MSCs capable of differentiating into cells that can repair the musculoskeletal system, including those comprising bone, tendon, articular cartilage, ligaments, and a variety of other tissue types [2].

In contrast to current approaches, California Institute for Regenerative Medicine (CIRM) projects are focused on the enhancement of the osteogenic potential of MSCs. These approaches aim to either increase the homing of the cells to the injured bone or activate and differentiate MSCs to osteogenic lineage. All the described projects were selected and peer reviewed by a panel of 15 expert members, in addition to at least one patient advocate, which together constitute CIRM’s Scientific and Medical Research Funding Working Group. The mandate of this working group is to make recommendations to the Institute’s 29-member governing body, the Independent Citizens Oversight Committee, with respect to research grants funded by the Institute, including consideration of the scientific merit of each project. Among the criteria for funding and selecting an application for funding approval is whether the project uses a stem cell-based approach and targets an unmet medical need. For example, preclinical and clinical proposals are evaluated and scored using the following key criteria:

1. Significance and potential for impact and practical value proposition for patients and/or health care provider
2. Sound scientific and/or clinical rationale supporting the development of the therapeutic candidate
3. An appropriate planned and designed proposal to meet the objective of the program announcement and achieve meaningful outcomes to support further development of the therapeutic candidate.

4. The feasibility of the intended objectives to be achieved within the proposed timeline with the appropriate team to execute the plan.

A typical project is funded for 3–5 years and, depending on the scope of the project, receives $3–$10 million dollars during the life of the grant.

**Treatment of Osteonecrosis With a Biphasic Molecule That Recruits Endogenous MSCs to the Osteonecrotic Bone**

Bone marrow MSC numbers decline significantly with age and also become impaired in their ability to home to the bone surface, thus attenuating their ability to repair damaged bone. Several MSC-based therapeutic approaches to address this deficiency are currently under clinical development, including a CIRM-funded project led by Dr. Nancy Lane at University of California, Davis. Dr. Lane seeks to enhance MSC function by using a biphasic molecule to recruit endogenous MSCs to the bone surface, thereby accelerating osteogenesis at an injury site. The active pharmaceutical ingredient, LLP2A-Ale, is a biphasic molecule with two ligands that are covalently joined by a linker. One ligand moiety, LLP2A, is a highly derivatized synthetic tripeptide with high affinity and specificity for the integrin α4β1. The other ligand is a bone-targeting bisphosphonate, alendronate. Dr. Lane has demonstrated that LLP2A-Ale can increase bone formation, mass, and strength in mouse models of osteoporosis and osteonecrosis similar to that seen with parathyroid hormone (PTH). In addition, in experiments when LLP2A-Ale was coadministered with exogenous MSCs, rapid recruitment of MSCs to the injured cortical bone was observed [3, 4].

Although osteonecrosis can occur at any age, it is most common in the elderly. Without treatment, which can include non-surgical or surgical interventions, most people with the disease will have severe pain and limited movement within approximately 2 years [5]. Nonsurgical treatments include medications such as nonsteroidal anti-inflammatory drugs, used to reduce pain and swelling; limiting activity or use of the affected joint to slow bone damage and allow time for healing; range-of-motion exercises; and electrical stimulation [6]. Additional treatment options include standard osteoporosis treatments, including antiresorptive medications (e.g., bisphosphonates, calcitonin, denosumab, and estrogen), anabolic drugs (e.g., teriparatide), and dietary prevention (e.g., calcium supplements, vitamin D).

Surgical intervention typically includes any of four options: core decompression surgery to lower the pressure inside the bone and increase blood flow; osteotomy to reshape the bone and relieve stress on the injured joint; bone grafting to replace the diseased bone with healthy bone from another part of the body; and total joint replacement. Because most people with osteonecrosis will need surgery, an agent that mobilizes endogenous MSCs to build bone (Fig. 1) could be used either as monotherapy or as combination therapy to augment the existing standard of care. This project is currently completing investigational new drug-enabling studies and is expected to begin a phase I clinical trial in the next 2 years.

**Autologous Bone Graft for the Treatment of Bone Defects (Osteonecrosis)**

Osteonecrosis is caused by restriction of the blood supply to the bone and can ultimately lead to destruction of the hip joint and arthritis. As described, most people will require surgical intervention. Currently, autologous bone graft material (BGM), which contains hematopoietic, vascular, and osteogenic stem cells, is the standard of care for the surgical treatment of bone defects and diseases. Although BGM is effective in young patients, a reduction in the number of MSCs in the elderly affects its osteogenic capacity. Furthermore, although BGM is used extensively to treat diverse bone disorders, it is not standardized and should not be applied to patients with limited autogenous tissue.

A CIRM project led by Dr. Jill Helms at Stanford University seeks to improve and further develop this approach by ex vivo expansion of the osteogenic stem cells in BGM by treatment with recombinant liposomal Wnt3A protein (L-WNT3A). It is well documented that the Wnt pathway plays a key role in the regulation of survival and pro-osteogenic signals in stem and progenitor cells. Numerous therapeutic agents targeting the Wnt pathway are currently in clinical development, including antibodies against the Wnt inhibitors sclerostin and Dkk1. These studies provide strong validation that elevating Wnt signaling stimulates bone formation.

Using mammalian cells, Dr. Helms was able to generate sufficient quantities of recombinant Wnt3A protein, purified and packaged in liposomes, to study its efficacy in animal models. The material was used to demonstrate that osteogenic activity can be restored in BGM by treatment with a potent stem cell activator, Wnt3A, and that the protein could be generated in reconstituted liposomes with unparalleled stability.

Dr. Helms’ approach is predicated on activating an aged patient’s own stem cells for the purpose of bone regeneration. When these autografts are treated with L-WNT3A (autograftWNT), their osteogenic potential is restored. This pro-osteogenic response has been validated across small and large animal models of spinal fusion, osteonecrosis, segmental bone defects, critical size calvarial...
defects, implant osseointegration, and fractures. In all cases, autograft-WNT generated a significantly greater volume of bone, at significantly earlier time points, relative to the controls.

Although Wnt ligands are potent agents in the activation of the transcriptional program in adult stem cells, chronic administration of a Wnt agonist carries with it a substantial risk of neoplastic transformation owing to its effects on the delicate balance of normal Wnt-mediated functions. This makes the ex vivo administration of the Wnt ligand in Dr. Helms’ project particularly attractive.

The CIRM-funded portion of Dr. Helms’ project was successfully completed, and the technology was licensed from Stanford University by Ankasa Regenerative Therapeutics, Inc. (La Jolla, CA).

Ankasa is focused on developing pharmaceuticals for the reactivation of stem cells for organ and tissue regrowth, tissue repair, and healing. Ankasa intends to initially develop a proprietary localized therapy involving WNT3A for spinal fusion surgery patients. The company anticipates initiating a clinical trial within approximately 30 months targeting spinal fusion and envisions its product becoming a key therapy in a current market estimated at more than $1 billion in annual sales. The company also intends to investigate the use of WNT3A in additional bone and other tissue repair applications.

Ankasa raised an initial $8.5 million out of the first tranche of a total $17 million Series A financing round to fund the development of WNT3A and build on the initial work funded by CIRM.

**Effective Systemic Mesenchymal Stem Cell Therapy for Vertebral Compression Fractures**

In the United States, osteoporosis-related vertebral compression fractures (VCFs) occur at a rate of 750,000 annually. Importantly, the 3-year mortality rate is nearly 50% in patients with acute VCFs. Although limited treatment options are available for these individuals, the most common approach is open surgery with implants, which often fail in patients with osteoporosis.

Although systemic administration of MSCs might provide therapeutic benefit, the CIRM-supported approach led by Dr. Dan Gazit at Sedar Sinai Medical Center is focused on the enhancement of bone repair by administration of MSCs combined with the osteogenic drug PTH to increase homing of MSCs to sites of bone fracture. PTH would promote the terminal differentiation of MSCs into osteoblasts, thus leading to enhanced bone formation and fracture repair. The combined MSC plus PTH therapy would produce bone regeneration that would be significantly superior to either treatment alone.

The CIRM-supported project isolated human MSCs from bone marrow and expanded them in tissue culture. Human bone marrow-derived MSCs were labeled with Luciferase reporter gene, and multiple vertebral defects were created in the lumbar spine of osteopenic rats. Bone voids were created in lumbar vertebrae of nude rats. Treatment included multiple intravenous injections of labeled cells and daily injections of PTH for 4 weeks. Dr. Gazit then monitored cell survival and homing to the defect site in the rats.

The CIRM-supported project demonstrated that vertebral defects in osteopenic rats treated with the combined stem cell and PTH therapy resulted in a twofold increase in bone volume density 2 months after treatment compared with defects treated with PTH only. The vertebrae in the untreated rats had not healed after 8 weeks. The combined stem cell and PTH therapy regenerated the defect much more efficiently than each treatment alone. Importantly, no signs of neurological side effects, bone growth into the spinal canal, or immunogenicity were detected [7].

These data strongly support the idea that vertebral defects in osteopenic animals are efficiently repaired when treated with human MSCs and PTH compared with the controls. In addition, labeled MSCs were detected in the lumbar region of the animals treated with PTH. This project provided evidence for future therapies that could bring new treatment of vertebral and other complex fractures, especially in osteoporotic patients. This is a critical step toward the development of allogeneic therapeutic candidate to treat VCFs. This project was recently completed, and further proposals to advance this technology to clinical development are expected to be submitted for evaluation.

**Autologous Perivascular Stem Cells (MSCs) Together With an Osteoinductive Protein (NELL) on an Acellular Scaffold for Treatment of Spinal Disorders**

Spinal fusion is a procedure used to address an array of disorders of the spine, including degenerative conditions, deformities, trauma-based disorders, and spinal tumors. More than 600,000 spinal fusion procedures are performed annually in the United States, with fusion failures continuing to pose a significant challenge [8]. It has been estimated that the spinal fusion market will reach $6.9 billion by 2020, with a compound annual growth rate of 5.6% [5]. Much of this growth is expected to be driven by an increasing elderly population with age-related degenerative disorders of the spine, which will play a significant part in the driving demand for spinal fusion procedures. It is projected that between 2010 and 2020, the population in the United States aged 65 years and older will increase 8.6%, from 309.2 million to 336.0 million [9].

MSCs, currently the only candidate autologous cells for bone repair in the absence of known osteogenic progenitors, are heterogeneous, long-term cultured cells. Prolonged exposure to animal products and the risks of genetic alterations could be circumvented by MSC prospective purification, a possibility now opened by a recent identification of ubiquitous perivascular stem cells (PSCs) as human MSC originators (Fig. 2). Unlike conventionally cultured MSCs, PSCs can be obtained from human adipose tissue much more rapidly and, unlike the stromal vascular fraction (SVF) from fat, PSCs are a cellular product with defined identity, purity, and potency. Biologically, adipose-derived PSCs are in situ progenitors of cultured MSCs and are a more ideal cellular starting material than MSCs/SVFs for multiple cell therapy/tissue engineering applications, including bone formation, owing to their ability to be purified in real time (PSCs can be harvested and applied in the same surgery) and their ability to satisfy FDA requirements for cellular product identity, purity, and potency.

CIRM’s supported scientists at the University of California, Los Angeles, Drs. So and Peault developed a unique approach to isolate PSCs for the treatment of spinal bone disorders. PSCs span (a) pericytes, which ensheathe capillaries and microvessels and are purified on expression of CD146, NG2, or platelet-derived growth factor receptor-β; and (b) adventitial cells, which surround larger arteries and veins, and are typed by exclusive CD34 expression. In long-term culture, both pericytes and adventitial progenitors give rise to bona fide MSCs. For bone development- and tissue engineering-based applications using cells, growth factors, and scaffolds, one is critical for appropriate cell seeding and growth factor dosing. The rapidly isolated PSCs are superior to the less-defined conventional MSC/SVF preparations, as assessed in mice (calvarial regeneration), rats (spine fusion, nonunion fracture repair), and larger mammals (spinal fusion). Similar to theirMSC progeny, PSCs mediate wound healing/tissue repair via several distinct mechanisms: progenitor activity, “niche” effects on specific stem cells, profibrotic potential, immunosuppression, and stimulation of angiogenesis. Not exclusive to bone repair, PSCs
represents a more general cell therapy platform and are currently being explored for applications toward tendon, cartilage, and cardiovascular regeneration.

Several companies have developed mesenchymal stem cell combination products. For example, NuVasive (San Diego, CA, http://www.nuvasive.com) markets Osteocel Bone Graft, a cellular allograft bone graft substitute for spinal fusion surgery. Osteocel contains mesenchymal stem cells and is meant to mimic a bone autograft, providing a scaffold for new bone growth, responding to endogenous signals and promoting the formation of new bone [10]. Another MSC-based cell combination product, sold by Orthofix (Lewisville, TX, http://www.orthofix.com), is called Trinity Evolution, an allograft of cancellous bone containing osteogenic cells and osteoprogenitor cells within a matrix and a demineralized cortical bone component.

The Australian-based Mesoblast (Melbourne, Victoria, Australia, http://www.mesoblast.com) is developing an MSC-based therapeutic and has completed a phase II clinical trial for posterior lumbar fusion. The trial evaluated Mesoblast’s allogeneic mesenchymal precursor cells surgically implanted posteriorly. The trial was successful in generating intervertebral lumbar spinal fusion and did not have any associated adverse events [8].

CIRM’s supported project is unique, because it tested in vitro and in a preclinical model, the efficacy of PSCs combined with NELL-1, an osteogenic protein. NELL-1 significantly increased the osteogenic potential of human PSCs (hPSCs) in both osteoporotic and non-osteoporotic donors. A high dose of hPSCs plus NELL-1 significantly improved the fusion rates among osteoporotic rats and confirmed solid bone fusion.

The increasing cases of spine-related diseases, coupled with the increasing aged population, will significantly increase the need for spinal fusion surgery. Although bone grafts are currently used to treat musculoskeletal defects, bone graft substitutes have undesirable side effects or lower efficacy, presenting the opportunity for novel alternative therapeutic approaches. This project was recently completed. The investigators are seeking additional funds to take the approach to clinical development.

CONCLUSION

Although a multitude of treatment alternatives are available for orthopedic-related injuries, ranging from surgical interventions and medical devices to drugs used to treat pain and swelling, a critical unmet need remains for novel, less-invasive approaches to treat the underlying injuries themselves. Many of the approaches funded by CIRM are focused on recruiting MSCs to aid in the healing process itself. The goal of CIRM’s current efforts is to generate safe and effective therapeutic alternatives to complement and improve the currently available surgical and nonsurgical outcomes.

AUTHOR CONTRIBUTIONS

N.L. and A.A.: conception and design, manuscript writing.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

REFERENCES

1. The QiC Group. The Orthopaedics Market. Available at http://www.qiggroup.com/orthopaedics-market.aspx. Accessed July 11, 2015.
2. OrthoInfo, American Academy of Orthopaedic Surgeons. Stem cells and orthopaedics. Available at http://orthoinfo.aaos.org/topic.cfm?topicid=4005001. Accessed July 12, 2015.
3. Guan M, Yao W, Liu R et al. Directing mesenchymal stem cells to bone to augment bone formation and increase bone mass. Nat Med 2012;18:456–462.
4. Yao W, Guan M, Jia J et al. Reversing bone loss by directing mesenchymal stem cells to bone. Stem Cells 2013;31:2003–2014.
5. National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). Available at http://www.niams.nih.gov/health_info/osteonecrosis/osteonecrosis_ff.asp. Accessed August 5, 2015.
6. Jing W, Smith AA, Liu B et al. Reengineering and inducing bone formation in vivo with cells that resemble mesenchymal stem cells. Biomaterials 2015;46:29–40.
7. Sheyn D, Cohn Yakubovich D, Kallai I et al. PTH promotes allograft integration in a calvarial bone defect. Mol Pharm 2013;10:4462–4471.
8. Mesoblast website. Spinal fusion. Available at http://www.mesoblast.com/products/orthopedic-diseases-of-the-spine/spinal-fusion. Accessed July 22, 2015.
9. Medtech Insight. United States Markets for Instrumented Spinal Fusion and Posterior Dynamic Stabilization Products. Available at http://www.medtechinsight.com/ReportA319.html. Accessed August 13, 2015.
10. NuVasive website. Available at http://www.nuvasive.com/patient-solutions/nuvasive-integrated-surgical-solutions/osteoecel-bone-graft/. Accessed July 22, 2015.

To join the conversation, please visit the Stem Cells Portal at http://StemCellsPortal.com/content/2015-0202