

Infusion of Autologous, Culture-Expanded, Adipose-Derived Mesenchymal Stem Cells in the Treatment of Coexisting Autoimmune Disease

Sarah V. Kelly, MD, and Jane S. Young, MD, PhD



CELLTEX®

To further Celltex Therapeutic Corporation's commitment to lead the United States in the advancement of regenerative medicine through the use of autologous stem cells, we are continually pursuing research, studies and clinical trials to help the world learn more about the application of adult stem cells for various medical conditions. Recently, a case report that highlights Celltex's proprietary technology was published. **Celltex Chief Scientific Officer, Dr. Jane Young, MD, PhD**, together with a physician, Sarah V. Kelly, MD, authored the case study, which focused on a rheumatoid arthritis patient with coexisting autoimmune disease who is responding favorably to autologous mesenchymal stem cell therapy using stem cells processed by Celltex. Read on to learn more about the clinical research in which Celltex is involved.



Sarah V. Kelly and Jane S. Young

Infusion of Autologous, Culture-Expanded, Adipose-Derived Mesenchymal Stem Cells in the Treatment of Coexisting Autoimmune Disease

Abstract

Over the last two decades, stem cell therapy has shown remarkable disease-controlling, immunomodulatory effects in a number of human and animal studies of immune-related conditions, including rheumatoid arthritis, multiple sclerosis, and Parkinson's Disease; however, at present the FDA has not approved use of stem cell therapy for clinical use in humans, and, therefore, administration of such therapies can only be performed in the United States as a part of a clinical trial. Clinical trials in a variety of diseases are currently underway and publishable results are pending completion of such trials.

Here, we present a case of medically refractory rheumatoid arthritis (RA) with coexisting autoimmune thyroiditis (Hashimoto Disease) that showed subjective and objective improvement in disease findings following systemic administration of autologous Adipose-derived Mesenchymal Stem Cells (AdMSCs). The safety and immunomodulatory and therapeutic efficacy of AdMSCs in the treatment of autoimmune disorders are discussed, as are the barriers to widespread clinical use of such therapy. Our goal is to increase awareness of the evolving role of such therapies among medical specialists who manage autoimmune disease.

Introduction

Worldwide, the prevalence of RA, classically described as a chronic, idiopathic, symmetric, inflammatory polyarthritis, is estimated at 0.4 to 1.3% [1,2] and these rates appear to be increasing among women [3]. If left untreated, RA eventually leads to widespread joint destruction, disability and inability to perform necessary Activities of Daily Living (ADLs). Symmetrical joint erythema, swelling and pain are common disease findings, but RA can also affect other organ systems, such as the respiratory system. Contemporary standard treatment for RA consists of aggressive medical therapy with non-biologic Disease-modifying Antirheumatic Drugs (DMARDs) with newer biologic DMARDs becoming increasingly available; however, ultimately, there is no cure for RA or for the destructive joint erosion that it causes.

RA is commonly associated with other autoimmune conditions, including autoimmune thyroiditis which may present clinically as eu-, hypo- or hyperthyroid states. Concomitant association of autoimmune thyroid disease and RA appears to have a genetic predisposition that can be traced through certain Human Leukocyte Antigen (HLA) types, most often occurring in HLA-DR expressing individuals [4].

Over the last ten to fifteen years, the world has witnessed an explosion in stem cell research and the field of regenerative medicine field that focuses on the development of biomolecular techniques of generating and repairing aging and/or damaged tissues. Stem cells from a number of human tissue sources have been reported to have a positive effect on disease in a variety of degenerative and autoimmune conditions, including rheumatoid arthritis, multiple sclerosis, Parkinson's disease and osteoarthritis. [5]. As a result, Stem Cell Therapy (SCT) has gained worldwide attention for potential use in tissue engineering, gene therapy, and immunomodulation.

Here, we present a case of medically refractory rheumatoid arthritis with coexisting autoimmune thyroiditis (Hashimoto disease) that showed subjective and objective improvement in autoimmunity following systemic administration of autologous, Adipose-derived Mesenchymal Stem Cells (AdMSCs). In this report, we discuss the immunomodulatory effects and therapeutic efficacy of AdMSCs in the treatment of autoimmune disorders, and we present a discussion of the current state of stem cell therapy in the United States, including the barriers to widespread clinical use of such therapy.

Case Presentation

A 36-year-old woman with no significant past medical history presented to her primary care doctor complaining of progressive, moderate to severe fatigue, loss of libido, unintentional weight gain and symmetrical joint pain, swelling and redness. Review of systems was notable for alopecia, heat and cold intolerance, and hot flashes. Family history was significant for a history of rheumatoid arthritis in the patient's father. Initial physical examination revealed a normotensive, adult female with a body mass index of 24.9 kg/m² and mild to moderate bilateral, symmetrical synovitis involving the hands, wrist and knees.

Laboratory workup consisting of complete blood cell count and differential, electrolyte panel and liver function profile was unremarkable. Comprehensive serum hormone evaluation revealed normal levels of estradiol, progesterone, total testosterone, and Dehydroepiandrosterone Sulfate (DHEA-S)

for a premenopausal female, and normal blood levels of serum 25-hydroxyvitamin D and vitamin B12 were also reported.

Although additional testing revealed normal levels of thyroid stimulating hormone (TSH), free-triiodothyronine (T3), and free thyroxine, (T4), elevations in Thyroid Peroxidase (TPO) and thyroglobulin antibodies were reported, consistent with subclinical autoimmune thyroiditis (Table 1). Likewise, an elevated antinuclear antibody (ANA) was detected (1:80 in a speckled pattern), in conjunction with elevated levels of rheumatoid factor (RF; 66 IU/mL, normal < 30 IU/mL). There was no laboratory evidence of active infection with either Epstein Barr Virus (EBV) or Cytomegalovirus (CMV). Fine Needle Aspiration (FNA) of a 1-centimeter thyroid lesion revealed scattered lymphocytes and germinal centers in a background of normal follicular cells and colloid, consistent with lymphocytic thyroiditis

Laboratory Test (normal value)	Initial presentation	11 months after presentation; prior to AdMSC therapy	2 weeks after start of AdMSC therapy	2 months after start of AdMSC therapy
Rheumatoid factors (<30 IU/ML)	66	98	56	37.8
Thyroid Peroxidase AB (<9 IU/ML)	1461	815	NA	434
Thyroglobulin AB (< 4 IU/ML)	964	1069	NA	570
ESR (<20 MM/HOUR)	2	20	2	2
ANA titer	1:80	1:80	1:40	Negative

Table 1: Markers of autoimmune disease pre-and-post therapy with AdMSCs.

Within several weeks of her initial presentation, the patient's clinical status deteriorated, and she developed increasing joint inflammation, synovitis and fatigue, resulting in significant impairment in her ability to perform Activities of Daily Living (ADLs). A diagnosis of RA was given and standard medical therapy for autoimmune thyroiditis and RA was initiated with little resultant improvement in her symptoms. At the advice of her doctor, the patient began an anti-inflammatory, Paleolithic-style diet and was started on oral glucocorticoids for better control of her arthritis.

Despite these measures, the patient was, ultimately diagnosed with biopsy-proven interstitial lung disease attributed to collagen vascular disease and she again experienced unacceptable symptomatic disease progression. At the recommendation of her physicians, she opted to pursue compassionate treatment

with systemically infused, autologous, Adipose-derived Mesenchymal Stem Cells (AdMSCs) produced by Celltex Therapeutics (Houston, Texas).

This is the first published case documenting the resolution of coexisting autoimmune disease in a single patient through treatment with AdMSCs. However, despite these promising results, significant barriers exist to the widespread use of stem cell therapy in the treatment of autoimmune disease.

Here we discuss the current state of stem cell therapy in the United States and the barriers that exist to more widespread use of such therapy. Our goal is to increase awareness of the potential evolving role that stem cell therapy has in the management of autoimmune disease and other immune-mediated forms of disease.

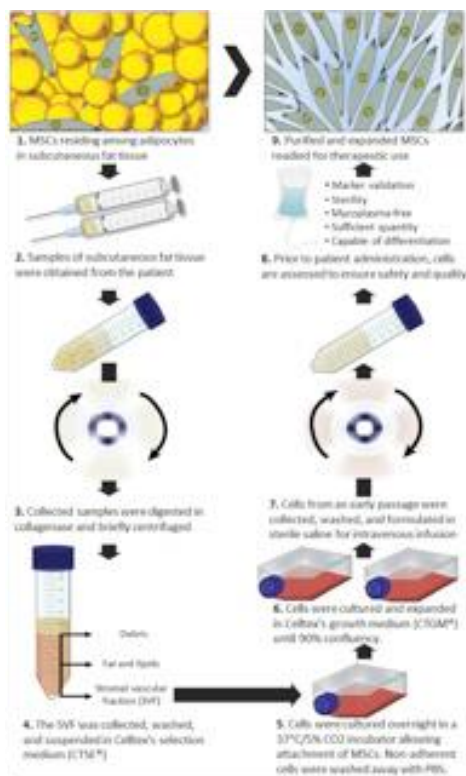


Figure 1: Steps involved in AdMSCs process.

Approximately one year after her initial presentation, the patient underwent a series of four intravenous systemic infusions of AdMSCs (2×10^8 cells) over the course of two months (produced by Celltex Therapeutics, Houston, Texas). Infusions were administered every 7 to 14 days and were then followed by six additional booster infusions administered approximately every two to four months.

Immediately following the initial infusion cycle, the patient reported remarkable subjective improvement in her energy level and functional status with complete resolution of joint pain and swelling. Laboratory evaluation two weeks and 2 months after the start of therapy provided objective laboratory evidence of sequential improvement in several inflammatory markers with decreased levels of circulating RF, TPO, and antithyroglobulin antibodies and a reduction in the Erythrocyte Sedimentation Rate (ESR) from 20 mm/hr. to 2 mm/hr. (Table 1).

At the time of this publication, approximately seven months since her last infusion, the patient reports that she has no joint pain or swelling, her energy level is “normal and she feels well”. Computed Tomography (CT) imaging has documented stable interstitial lung disease, and the patient has not noticed any treatment-related side effects. She reports that she is in good to excellent health and requires no regular medications.

Materials and Methods

After being evaluated by an outside physician for consideration of AdMSC therapy, the patient was referred to Celltex Therapeutics Corporation (Celltex®) for consideration of AdMSC therapy. In preparation for stem cell banking, the patient reviewed and signed informed consent documents with Celltex®, outlining the unproven and experimental nature of the recommended treatment protocol, systemic infusion of autologous, culture-expanded AdMSCs. Protocols and retrospective medical record review were approved by the appropriate institutional review board (Galenia Hospital, Cancun, Mexico).

Subsequently, the process for stem cell banking, including qualifying blood tests was initiated. A single tumescent liposuction procedure was performed using a microcannula under local anesthesia to obtain approximately 20 mL of autologous abdominal subcutaneous fat tissue. Adipose tissue, collected into two 20 mL sterile syringes (10 mL per syringe), was packed into a pre-validated cold shipping box ($2^{\circ}\text{C} - 8^{\circ}\text{C}$) for delivery to the Celltex® laboratory facility within a 24-hour time window.

From these samples, autologous human adipose tissue derived MSCs were isolated and cultured following proprietary manufacturing Standard Operation Procedures (SOPs) in Celltex’s cGMP laboratory facility. Briefly, adipose tissue was digested with collagenase I. Digested tissue was centrifuged and washed to obtain the Stromal Vascular Fraction (SVF). The SVFs were re-suspended in Celltex’s selection medium (CTSE®) and cultured overnight in a $37^{\circ}\text{C}/5\% \text{CO}_2$ incubator in order for the MSCs to attach to the flask. After 24 hours, all non-adherent cells were washed away with Phosphate Buffered Saline (PBS), and the adherent cells were cultured in Celltex’s growth medium (CTGM®). CTGM was changed every 48 hours. For each passage, cells were cultured and expanded until they reached approximately 90% confluence. An early cell passage was harvested, washed, and then formulated in sterile saline for intravenous infusion. The final cellular products were tested by Celltex’s Quality Control unit (QC). These QC tests included cell counts, viability, purity/identity, and microbials to ensure all cell products met test specifications (Figure 1).

Following extraction, expansion and banking of AdMSCs, the initial cycle of MSC therapy was initiated, consisting of three separate weekly systemic, intravenous infusions of 200 million autologous AdMSCs. Additional, booster intravenous infusions (200 million MSCs) were administered two weeks and two months after completion of the initial cycle, for a total of ten treatments (Table 2), and follow-up medical examinations were performed at 2 weeks and 2 months after the start of treatment.

Date	Approximate Time Interval	Number of AdMSCs (x 10 ⁶)
Oct 30, 2014	0	200
Nov 06, 2014	1 week	200
Nov 14, 2014	1 week	200
Dec 03, 2014	2 weeks	200
Feb 11, 2015	2 months	200
Jun 20, 2015	4 months	200
Jun 24, 2015	4 days	250
Dec 20, 2015	6 months	200
Apr 1, 2016	4 months	200
Jun 10, 2016	2 months	200

Table 2: Administration of systemically-infused, intravenous AdMSCs.

Discussion

Stem cells, by definition, maintain their ability to divide and differentiate throughout their lifespan and can be classified as adult or embryonic. Regardless of their tissue source, all stem cells are capable of proliferation, self-renewal, and regeneration of damaged tissue. Adult (i.e. postnatal, non-embryonic) stem cells, because of their general acceptability and safety profile, have emerged as the primary candidate (over the more controversial embryonic stem cells) for use in a variety of SCTs. Adult stem cells can be harvested from a wide variety of mature tissue types, including bone marrow, umbilical cord, and adipose tissue and have been shown to have a variety of immune modulating effects [6].

Systemic and/or site-specific administration of mesenchymal stem cells, a form of nonhematopoietic adult stem cells, has shown tremendous promise, particularly in immune-mediated and/or autoimmune disease [6].

Although bone marrow was the first and, to date, is the most extensively studied source of multipotent stem cells utilized clinically in regenerative medicine, Adipose-derived Mesenchymal Stem Cells (AdMSCs), first isolated by researchers at University of California, Los Angeles (UCLA) in 2002, have become an attractive therapeutic alternative as a result of their relative abundance and accessibility [7]. As a multilineage stem cell, the AdMSCs can be cultured and isolated from the stromo-vascular fraction of adipose tissue and are, not only capable of dividing and differentiating both *in vivo* and *in vitro* into many mesodermal tissue types, including cartilage, bone, and muscle, but are also postulated to have pluripotent regenerative capabilities or the ability to divide into tissue types derived from all three embryonic germ cell layers [8].

However, current thinking regarding the regenerative capabilities of stem cells emphasizes that systemically infused MSCs have immunomodulatory and trophic properties *in vivo* which allows them to home to injured tissues where they release growth factors, cytokines and chemokines that induce endogenous tissue-specific stem cells to differentiate cells that regenerate the damaged tissues [9-12].

These unique cells, phenotypically characterized by their expression of CD105, CD73, and CD90 and lack of expression of CD45, CD34, CD14, CD11b and CD79a or CD19, do not express HLA class II antigens which contributes to their ability to elude immune surveillance further improving their clinical utility [13].

Adipose-derived Mesenchymal Stem Cells (AdMSCs)

Adipose tissue, which has a relatively high concentration of stem cells (approximately 100,000 MSCs per gram of harvested fat) [14], is an ideal source of therapeutic adult mesenchymal stem cells for culture, as harvesting can be done through a simple, minimally invasive, relatively painless procedure (micro liposuction under local anesthesia) [15]. Following harvesting, a cell pellet is obtained by treatment with collagenase and centrifugation, producing the Stromal Vascular Fraction (SVF). The SVF contains a mixture of cell types, including red and white blood cells, fibroblasts, endothelial cells and AdMSCs [16]. Unlike the other cell types within the SVF, AdMSCs are capable of adhering to and growing on plastic surfaces, such as tissue culture dishes, and can be readily identified by fluorescence-activated cell sorting based on the expression of their characteristic cell surface markers [13] (Figure 1).

Although the exact mechanism by which AdMSCs exert their clinical effect on damaged tissues is currently unknown, these cells appear to promote healing of damaged and/or inflamed tissues without engrafting, exerting their effects through a combination of direct cell-to-cell interactions and through the production of soluble chemical anti-inflammatory mediators such as Prostaglandin E2 (PGE2), Indoleamine 2, 3-dioxygenase (IDO), Transforming Growth Factor (TGF) beta and Interleukin-6 (IL-6) [17,18]. AdMSCs are also reported to have an immunosuppressive effect in conditions such as Graft-versus Host Disease (GVHD) [19]. This effect appears to be mediated through inhibition of both the innate and adaptive immune responses. *In vivo*, MSCs have been shown to inhibit the cellular activity of a variety of immune cells, including T cells, B cells, dendritic cells and natural killer cells [20].

Current Regulations and Controversy Regarding Stem Cell Therapy

The development and expansion of non-embryonic SCTs, coupled with the growth of regenerative medicine, has led to tremendous controversy and regulatory uncertainty within the United States on the issue of cellular-based therapies. Central to this debate is the classification and regulatory oversight of SCT. Are autologous stem cells considered human cells, or are they better classified as drugs? And does this designation change if the cells undergo clonal expansion outside the body prior to administration [21]?

In the United States, the use of stem cells in regenerative medicine is complicated by regulations imposed at the federal and state levels. Although umbilical cord-blood-derived hematopoietic progenitor cells are the only stem-cell based products currently approved for specific use by the United States Food and Drug Administration (FDA) (www.fda.gov, accessed 11/30/16), several state medical boards, including California and Texas, have proactively passed legislation regulating and outlining acceptable practices regarding use of SCTs in their states, preventing disciplinary action against physicians who administer SCT according to state law. This protection under state law, however, is not extended to the federal level.

Under section 361 of the Public Health Service (PHS) Act, premarket review by the United States Food and Drug Administration (FDA) is not required of “human cells, tissue or cellular and tissue-based products (HCT/Ps).”

As defined by the PHS Act, HCT/Ps:

1. Must be “minimally manipulated”
2. Cannot be combined with other therapeutic substances
3. Are intended for homologous use
4. Do not have systemic effects, and
5. Do not require the metabolic activity of living cells for their therapeutic effects

In regard to cells or nonstructural tissues, minimal manipulation refers to “processing that does not alter the relevant biological characteristics” of the cells and includes actions such as density or gradient separation, cell selection, centrifugation, and cryopreservation. The FDA has deemed that cellular expansion in culture, however, represents more than minimal manipulation, and, therefore, disqualifies ex-vivo expanded cellular therapies as being classified as HCT/Ps, requiring them to receive FDA approval for use in the U.S. Such approval by the FDA would require costly, time-consuming clinical trials clearly demonstrating safety and efficacy for each intended clinical use.

Unfortunately, most forms of SCT are still largely considered experimental with a paucity of rigorous, controlled scientific data available to define or support their medical use. Although clinical trials in a variety of conditions are currently underway, results are not currently available. Meanwhile, demand for SCT has grown tremendously within the United States. Patients suffering from many forms of severe, refractory and/or incurable disease, including rheumatoid arthritis, multiple sclerosis and Parkinson’s Disease, are frustrated that regulation by the FDA does not grant the autonomy to freely pursue SCT for compassionate use in the United States, and many Americans opt to pursue SCT in other countries where regulations do not exist or are less stringent [21].

Use of STC in the Treatment of Autoimmune Disease

Autoimmune Disease (AD), which results from an inappropriate, self-directed immune-response, is a cause of tremendous morbidity and mortality worldwide, presenting in over sixty unique conditions and affecting as much as 6 percent of the population [22]. Affected individuals typically have abnormal circulating antibodies that target various organs and/or tissue types and multiple coexisting autoimmune conditions commonly occur in a single individual and/or within families. The most common forms of AD in the United States today include rheumatoid arthritis and autoimmune thyroiditis (also known as Hashimoto’s thyroiditis) [23].

The standard treatment of most autoimmune conditions has not changed substantially in several decades and rests heavily on immunomodulation and/or immunosuppression, utilizing medications such as corticosteroids, cyclophosphamide, azathioprine, and methotrexate. Unfortunately, such medical therapies are not uniformly effective and are associated with significant side effects and/or toxicity. In recent years, SCT has emerged as a promising new tool in the treatment of immune-mediated disease with the potential of providing sustained results with little toxicity and/or side effects.

The immunomodulatory effects and capacity of MSCs to avoid immune surveillance make them attractive clinical tools in immune-mediated disease. Since the 1990s, autologous Hematopoietic Stem Cell Transplantation (HSCT) has been associated with durable, sustained remission and loss of autoantibody expression in a fraction of treated patients [24]. However, due to the risk of myeloablation and subtotal response rates, such therapy can only be justified in individuals with severe, refractory disease [25]. With recent advancements in SCT, however, the AdMSC has emerged as a powerful tool in the treatment of immune-mediated disease, having both immunomodulatory and anti-inflammatory effects, with far less toxicity or inherent risk than HSCT.

Although a number of patients worldwide have been treated with AdMSCs in a variety of medical disciplines, rigorous clinical

trial data evaluating their efficacy in autoimmune disease is lacking and the majority of published data on the subject is in the form of case reports and/or non-controlled studies. Improvements in subjective disease-related complaints and biological markers of disease have been demonstrated in a number of immunologic and immune-associated disorders: dysautonomia, Graft-versus Host Disease (GVHD), Idiopathic Thrombocytopenia (ITP), diabetes mellitus type 1, and multiple sclerosis [26-34]. And, as this case presents, multiple autoimmune conditions within a single individual could potentially derive benefit from monotherapy with SCT.

Currently, there are over 350 clinical trials focusing on the regenerative capabilities of MSCs, with nearly 50 of these studies specifically addressing the role of MSCs in autoimmune conditions with a particular emphasis on systemic autoimmune diseases such as inflammatory bowel disease, lupus and RA. The safety and efficacy of AdMSC therapy in the treatment of RA was reported in a 2010 case report in which authors reported remarkable clinical improvement in subjective complaints of joint pain and stiffness as well as a decrease in serum rheumatoid factor in a 67-year-old female with medically refractory RA [35]. However, little if any data exists on the treatment of autoimmune thyroiditis with SCT or the concurrent treatment of multiple coexisting autoimmune disorders. In general, no adverse effects have been reported in patients who have been treated with MSC - based stem cell therapy [6].

Safety of AdMSCs

As worldwide interest in the clinical use of intravascularly-administered AdMSCs in the treatment of human disease has exploded over the last 15 years, concerns regarding the biologic characteristics, clinical function and safety of regenerative therapies has emerged. Cited concerns include risks associated with the cells themselves, such as malignancy, immunogenicity, or vascular occlusion, as well as any potential risks imparted by the reagents used in cell culture [36].

The overall safety of infused MSCs was the subject of a 2012 systematic review and meta-analysis of 36 studies from 14 different countries consisting of over 1,000 study subjects [37]. Reviewed studies included randomized and non-randomized control trials as well as uncontrolled clinical trials designed to examine the safety of intravascularly administered MSCs in both adult and pediatric patients. Sixteen (of 36) studies evaluated the use of autologous MSCs.

In this systemic review, the authors concluded that the use of intravascularly-delivered MSCs was associated with transient, uncomplicated fever, but no association was detected between the use of MSCs and acute infusional toxicity, organ system complications, infection, death or malignancy. Studies included in the review used both autologous and unmatched, allogenic MSCs, supporting the notion that MSCs are non-immunogenic [37,38]

which is considered by many to be a consequence of lacking cell surface Major Histocompatibility Complex (MHC) molecules [38]. And although the authors of the review raised potential concerns about theoretical toxic side effects and/or hypersensitivity reactions to reagents used in cell culture, namely fetal bovine serum and dimethylsulfoxide, the majority of studies in the review did not specifically monitor subjects for adverse events related to the use of these reagents. Nevertheless, there were no reported acute infusional toxicities reported among the reviewed studies to suggest such effects.

As a part of the development process, repetitive dose toxicity and tumorigenicity of intravenously administered AdMSCs prepared by the Celltex® proprietary manufacturing process was assessed in preclinical studies in immunocompromised mice (NOD/SCID mice). No cell-related mortality, adverse clinical observations or change in body weight was observed in mice treated by repetitive intravenous infusion of AdMSC over 13 days (total of 5 infusions, each separated by 3 days). Post-infusion necropsy revealed no clinically or toxicological significant differences in gross pathology, organ weight, or histopathology in either male or female mice throughout the 90-day study period, and no adverse palpable masses were observed to support tumorigenicity of infused AdMSCs.

Ultimately, although the available scientific literature supports the notion that MSC therapy is safe in humans, the limited number of randomized control trials designed to specifically evaluate the potential toxic and adverse effects of stem cell infusions precludes an absolute statement of low risk. Additional data on adverse events associated with MSC therapy from large scale-controlled studies and clinical trials is currently being generated and, in the future, will undoubtedly lead the way towards standardized treatment protocols.

Factors Limiting the Clinical Use of AdMSCs

Although limited preliminary data suggests that systemically-infused stem cells may provide therapeutic immunomodulation and disease control, data from confirmatory, rigorous randomized controlled studies is lacking, and a number of significant barriers to the widespread use of these therapies exist. Compassionate, unregulated use of SCT has produced promising results in many disease states, however, significant variability in study design (including differences in MS Corigin, preparation, expansion, dosing and administration) does not allow for head-to-head comparison or reliable meta-data from which to draw conclusive, evidence-based treatment recommendations. It is generally accepted, however, that most forms of stem cell therapy, particularly those that are minimally manipulated, have a high safety index and low risk of major side effects. Clear, conclusive evidence of the safety and efficacy of SCT in autoimmune disease will require a significant amount of scientific data from well

structured, statistically powerful, randomized control studies involving a large number of study subjects.

Conclusions

As a consequence of their simple collection requirements, multipotency and acceptable safety profile, AdMSCs are an ideal source of stem cells for use in clinical practice. However, currently, standardized treatment protocols are lacking, as limited data is available to provide the basis for such protocols and FDA approval has not yet been granted in the United States. Nevertheless, for many forms of immune-mediate and/or autoimmune disease that remain either incurable or refractory to conventional treatments, AdMSC therapy appears to have an acceptable safety profile and early reports suggest favorable outcomes in many forms of immune-mediated disease. And although the exact biologic mechanism by which stem cells exert their clinical effects remains elusive, large, multicenter, double-blinded clinical trials are currently underway for a variety of medical conditions many of which have proposed autoimmune mechanisms. In time, such research may lead the way for more comprehensive, evidence-based treatment protocols and expanded use of stem cell therapies in regenerative medicine.

Acknowledgements: Illustration (Figure 1) by David Parker, Ph.D., dparker@privatehealth.com, Private Health Management, Inc., 1880 Century Park East, Suite 425, Los Angeles CA 90067, USA.

References

1. Graudal NA, Jurik AG, de Carvalho A, Graudal HK (1998) Radiographic progression in rheumatoid arthritis: a long-term prospective study of 109 patients. *Arthritis Rheum* 41: 1470-1480.
2. Sacks JJ, Luo YH, Helmick CG (2010) Prevalence of specific types of arthritis and other rheumatic conditions in the ambulatory health care system in the United States, 2001-2005. *Arthritis Care Res (Hoboken)* 62: 460-464.
3. Myasoedova E, Crowson CS, Kremers HM, Therneau TM, Gabriel SE (2010) Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007. *Arthritis Rheum* 62: 1576-1582.
4. Staykova ND (2007) Rheumatoid arthritis and thyroid abnormalities. *Folia Med (Plovdiv)* 49: 5-12.
5. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I (2005) Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood* 106: 1755-1761.
6. Munir H, McGettrick HM (2015) Mesenchymal Stem Cell Therapy for Autoimmune Disease: Risks and Rewards. *Stem Cells Dev* 24: 2091-2100.
7. Zuk PA (2010) The adipose-derived stem cell: looking back and looking ahead. *Mol Biol Cell* 21: 1783-1787.
8. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, et al. (2001) Multi-lineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 7: 211-228.
9. Baraniak PR, McDevitt TC (2010) Stem cell paracrine actions and tissue regeneration. *Regen Med* 5: 121-143.
10. Joyce NG, Wirthlin AL, Olson S, Bauer G, Nolte JA (2010) Mesenchymal stem cells for the treatment of neurodegenerative disease. *Regen Med* 5: 933-946.
11. Caplan AI, Correa D (2011) The MSC: an injury drugstore. *Cell Stem Cell* 9: 11-15.
12. Hofer HR, Tuan RS (2016) Secreted trophic factors of mesenchymal stem cells support neurovascular and musculoskeletal therapies. *Stem Cell Res Ther* 7: 131.
13. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315-317.
14. Sen A, Lea-Currie YR, Sujkowska D, Franklin DM, Wilkison WO, et al. (2001) Adipogenic potential of human adipose derived stromal cells from multiple donors is heterogeneous. *J Cell Biochem* 81: 312-319.
15. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, et al. (2008) IFATS collection: *in vivo* therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. *Stem Cells* 26: 2705-2712.
16. Brown SA, Levi B, Lequeux C, Wong VW, Mojallal A, et al. (2010) Basic science review on adipose tissue for clinicians. *Plast Reconstr Surg* 126: 1936-1946.
17. Trivanović D, Kocić J, Mojsilović S, Krstić A, Ilić V, et al. (2013) Mesenchymal stem cells isolated from peripheral blood and umbilical cord Wharton's jelly. *Srp Arh Celok Lek* 141: 178-186.
18. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143-147.
19. Yañez R, Lamana ML, García-Castro J, Colmenero I, Ramírez M, et al. (2006) Adipose tissue-derived mesenchymal stem cells have *in vivo* immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells* 24: 2582-2591.
20. Askenasy N (2013) Enhanced killing activity of regulatory T cells ameliorates inflammation and autoimmunity. *Autoimmun Rev* 12: 972-975.
21. Stem Cell Therapies: Opportunities for Ensuring the Quality and Safety of Clinical Offerings: Summary of a Joint Workshop. Washington (DC) (2014) National Academy of Sciences.
22. Siatskas C, Chan J, Field J, Murphy K, Nasa Z, et al. (2006) Gene therapy strategies towards immune tolerance to treat the autoimmune diseases. *Curr Gene Ther* 6: 45-58.
23. Jacobson DL, Gange SJ, Rose NR, Graham NM (1997) Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol* 84: 223-243.
24. Tyndall A (2009) Cellular therapy of systemic lupus erythematosus. *Lupus* 18: 387-393.
25. Choi EW (2009) Adult stem cell therapy for autoimmune disease. *Int J Stem Cells* 2: 122-128.
26. Fang B, Song YP, Liao LM, Han Q, Zhao RC (2006) Treatment of severe therapy-resistant acute graft-versus-host disease with human

- adipose tissue derived mesenchymal stem cells. *Bone Marrow Transplant* 38: 389-390.
27. Fang B, Song Y, Lin Q, Zhang Y, Cao Y, et al. (2007) Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-versus-host disease in two children. *Pediatr Transplant* 11: 814-817.
 28. Fang B, Song Y, Zhao RC, Han Q, Lin Q (2007) Using human adipose tissue derived mesenchymal stem cells as salvage therapy for hepatic graft-versus-host disease resembling acute hepatitis. *Transplant Proc* 39: 1710-1713.
 29. Trivedi HL, Vanikar AV, Thakker U, Firoze A, Dave SD, et al. (2008) Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin. *Transplant Proc* 40: 1135-1139.
 30. Fang B, Song Y, Li N, Li J, Han Q, et al. (2009) Mesenchymal stem cells for the treatment of refractory pure red cell aplasia after major ABO-incompatible hematopoietic stem cell transplantation. *Ann Hematol* 88: 261-266.
 31. Fang B, Song YP, Li N, Li J, Han Q, Zhao RC (2009) Resolution of refractory chronic autoimmune thrombocytopenic purpura following mesenchymal stem cell transplantation: a case report. *Transplant Proc* 41: 1827-1830.
 32. Riordan NH, Ichim TE, Min WP, Wang H, Solano F, et al. (2009) Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. *J Transl Med* 7: 29.
 33. Fang B, Mai L, Li N, Song Y (2012) Favorable response of chronic refractory immune thrombocytopenic purpura to mesenchymal stem cells. *Stem Cells Dev* 21: 497-502.
 34. Numan MT, Kamdar A, Young J, Butler IJ (2017) Autologous Adipose Stem Cell Therapy for Autonomic Nervous System Dysfunction in Two Young Patients. *Stem Cells Dev* 26: 391-393.
 35. Ichim TE, Harman RJ, Min WP, Minev B, Solano F, et al. (2010) Autologous stromal vascular fraction cells: a tool for facilitating tolerance in rheumatic disease. *Cell Immunol* 264: 7-17.
 36. Prockop DJ, Brenner M, Fibbe WE, Horwitz E, Le Blanc K, et al. (2010) Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy* 12: 576-578.
 37. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, et al. (2012) Safety of cell therapy with mesenchymal stromal cells (Safe-Cell): a systematic review and meta-analysis of clinical trials. *PLoS One* 7: e47559.
 38. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O (2003) HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 31: 890-896.