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Concise Review: Adipose-Derived Stromal Vascular Fraction Cells and Stem Cells: Let's Not Get Lost in Translation

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ABSTRACT

Subcutaneous fat has emerged as an alternative tissue source for stromal/stem cells in regenerative medicine. Over the past decade, international research efforts have established a wealth of basic science and preclinical evidence regarding the differentiation potential and regenerative properties of both freshly processed, heterogeneous stromal vascular fraction cells and culture expanded, relatively homogeneous adipose-derived stromal/stem cells. The stage has been set for clinicians to translate adipose-derived cells from the bench to the bedside; however, this process will involve "development" steps that fall outside of traditional "hypothesis-driven, mechanism-based" paradigm. This concise review examines the next stages of the development process for therapeutic applications of adipose-derived cells and highlights the current state of the art regarding clinical trials. It is recommended that the experiments addressing these issues be reported comprehensively in the peer-review literature. This transparency will accelerate the standardization and reproducibility of adipose-derived cell therapies with respect to their efficacy and safety. STEM CELLS *2011;29:749–754*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION—WHAT IS THIS ABOUT?

Subcutaneous fat is an abundant and accessible source of both uncultured/heterogeneous stromal vascular fraction (SVF) cells and cultured/relatively homogeneous adipose-derived stromal/stem cells (ASCs). The peer-reviewed literature focusing on SVF cell and ASC research has expanded exponentially over the past decade. This body of work has excited the international stem cell community as demonstrated by the registration of 36 clinical trials in 11 different countries on the NIH (http://www.clinicaltrials.gov) identified with the key words "adipose stem cells"; this compares to 143 studies under the term "mesenchymal stem cell." Regulatory authorities require more than mechanism-based evidence before authorizing "investigational new drug" (IND) studies with cell-based therapies. Additional "development" studies must be provided to complete the "research and development" required to support IND proposals. Despite the collection of this information in both public (academic) and private (biotech) sector, little of this data has appeared in the scientific literature. Increased distribution of such data through peer-reviewed papers would accelerate the pace of translation for ASCs and SVF cells to the clinic. Such studies would document the reproducibility of outcomes-based evidence regarding adverse events, safety, and efficacy from independent sources. Disseminating information on isolation and culture methods, surgical approaches, challenges, and their solutions would foster international cooperation and standardization. Despite financial incentives and intellectual property concerns to the contrary, all parties in the stem cell community could benefit from a greater public awareness of the development side of the picture. This concise review evaluates current and future experiments designed to minimize the likelihood that the clinical value of SVF cells and ASCs will get "lost in translation."

PRECLINICAL SAFETY AND EFFICACY DATA—WHAT (AND HOW) HAVE WE BEEN DOING?

Regulations

The regulations controlling the delivery of adipose-derived cell therapeutics to the clinic parallel many of those developed for the pharmaceutical industry [1]. Guidelines governing the development of cell-based products can be found on websites for the U.S. Food and Drug Administration (FDA: http://www.fda.gov/), the European Medicines Agency

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(EMEA: http://www.ema.europa.eu/ema), and related governmental regulatory authorities. Similarly, the United States Pharmacopeia (USP) is an internationally recognized resource defining the currently accepted industry standards for product purity, potency, and quality assurance (http://www.usp.org/). The use of USP-based assays for each step in the ASC and SVF cell manufacturing process ensures the reproducibility and reliability of the final product. To date, most laboratories use several common steps to process cells from adipose tissue [2]. These are: (a) washing; (b) enzymatic digestion/mechanical disruption; (c) centrifugal separation for isolation of SVF cells which can used directly, cryopreserved, or (d) culture expanded for the generation of ASCs (see figure in [3]).

GLP, cGMP, and Standard Operating Procedures

Most academic research laboratories do not produce adipose stem cells in accordance with the criteria for either Good Laboratory Practices (GLP) or the more stringent current Good Manufacturing Practices (cGMP). Both GLP and cGMP require strict operational and certification records relating to all laboratory equipments used in the cell manufacture process [1, 4]. Additionally, all operational procedures, from the mopping of floors to the maintenance of incubators and biological safety cabinets, must be performed and recorded routinely in accordance with defined and validated standard operating procedures. Lot specific manufacturing records should be developed to ensure standard practices and provide a written document validating quality assurance and quality control by the operators. In a recent manuscript, Sensebé et al. [5] provide a comprehensive and thorough review of this topic. Since any adipose cell–based therapeutics destined for clinical use must meet cGMP standards, there are multiple reagents and procedures that merit special attention.

Closed System Manufacturing Devices

Contamination by infectious agents presents a fundamental challenge to any cell or tissue product. Several companies have developed self-contained lipoaspirate processing devices that collect, wash, digest, and separate cells without exposing them to the environment (http://www.cytori.com and http://www.tissuegenesis.com/) [6]. Closed culture/expansion systems have been developed to exchange medium in large capacity tissue culture flasks using stopcocks and gravity-based flow to minimize the risk of operator error during the culture expansion process [4]. Bioreactors with controlled flow rates and built in monitors for cell viability, lactate production, pH/pO_2, and glucose levels need to be made efficient, practical, and scalable to current and future needs [7]. The use of automated, computer-controlled devices has the potential to reduce the risk of operator error during the culture period.

Donor Considerations

The age, depot site, and sex of the adipose tissue donor have the potential to impact the functionality and quality of the derived cells. For example, a recent murine study found that a subpopulation of adipocyte progenitor cells are most frequent in visceral as opposed to subcutaneous adipose depots, increase with advancing age, and are more frequently observed in female donors [8]. A limited number of human studies provide similar findings. While an analysis of breast tissues specimens from >180 women donors aged 16–73 did not observe an age dependent difference in stromal cell numbers or adipogenesis, increased body mass index correlated significantly with reduce cell numbers and differentiation [9]. Clinical studies examining subcutaneous adipose tissue from 12 to 52 donors have reported reduced ASC adipogenesis, angiogenesis, osteogenesis, and/or proliferative capacity as a function of advancing donor age [10–12]. Similarly, a detailed comparison of five different subcutaneous depots determined that ASC isolated from the arm and thigh best maintained adipogenic potential as a function of advancing age [12]. Further studies in larger cohorts will be necessary before patient demographics can be used to predict the functionality and recovery of SVF cells and ASCs from donors as well as the relative utility of specific depot sites. Besides, future studies will need to compare the efficacy of SVF cells versus ASCs from the same donor based on function in vivo.

Sourcing of Reagents

The quality of all cell-processing reagents must be validated by in-house assays. Each lot of growth factor, medium, or serum (e.g., fetal bovine serum; FBS) should be tested for potency using quantifiable metrics such as cell proliferation rates, viability, and/or differentiation potential. Even if a sole source provider is used for a particular reagent, there is no guarantee that they will not make changes in their manufacturing process to reduce costs or in response to another customer. Indeed, there is a risk to using a single supplier for any particular reagent. Changes in ownership, ordering backlogs, or other uncontrollable external factors can prevent access to critical materials.

Enzyme Quality

Collagenase, dispase, and hyaluronidase are some of the enzymes used to disrupt lipoaspirate tissue. In their crude form, these reagents often contain contaminating amounts of endotoxin, other peptidases, and xenoproteins [13]. The steps involved in the manufacture of "sterile" or cGMP grade enzymes increases their cost by over 10-fold. The development of an efficient and reproducible mechanical-based tissue disruption process would remove the need for enzyme reagents and merits further investigation. There is evidence that functional ASCs can be expanded directly from lipoaspirate fluids without the need for collagenase digestion [14]. Similarly, multiple groups routinely use porcine-derived trypsin to passage plastic adherent ASCs, and recent studies have documented the equivalent performance of bacterial-derived or corn-derived trypsin products [15–17]. Thus, the removal of enzyme reagents is achievable.

Serum Alternatives

Historically, ASCs have been expanded in culture medium supplemented with FBS. The European regulatory agencies have particular concerns regarding any use of FBS due to the widespread presence of bovine spongiform encephalopathy (BSE). While rare cases of BSE have been identified in North American cattle herds, the use of irradiated FBS is allowed for cell expansion. Nevertheless, it is likely a matter of time before FBS will be phased out for use in clinical products. There is evidence that the presentation of FBS proteins, such as albumin, to the recipient immune system results in subsequent antibody-based responses with the risk of serum sickness [18, 19]. A number of laboratories have found that human serum or platelet-derived supplements can serve as alternatives (reviewed in [20]). Some groups have relied on autologous serum, donated by the subject at the time of tissue collection, for ASC expansion [16]. The future may witness the development of commercial grade, infectious agent-free allogeneic serum sources for the generation of cGMP cell products. An optimal human serum reagent would be depleted of antibodies and complement proteins to reduce the risk of cell damage or adverse events. There is the possibility of removing serum entirely from the culture medium [15]. The Regea Institute has demonstrated the use of a commercially

available xenoprotein-free product for ASC expansion [15]. While the proprietary nature of the medium leaves the public with questions about its active ingredients, the deposition of a confidential master file with a regulatory agency (FDA and EMEA) would address this concern.

Product Definition

There remains some dispute over the criteria defining an SVF cell or an ASC. While there is a general consensus that the SVF cells are a heterogeneous population, no specific ranges for each subpopulation have been agreed upon formally. The International Society for Cell Therapy (ISCT) has provided guidelines for the definition of mesenchymal stromal cells (MSCs) based on their plastic adherent properties, immunophenotype ($CD73^+$ $CD90^+$ $CD105^+$ $CD11b/14^-$ $CD19/CD73b^-$ $CD34^-$ $CD45^-$ $HLA\text{-}DR^-$), and multipotent differentiation potential (adipogenic, chondrogenic, and osteogenic) [21]. While some have attempted to apply these criteria to ASC, there is a reason to doubt their applicability because early passage ASCs are routinely $CD34^+$ [22, 23]. Investigators continue to search for ASC specific surface markers. Some have used the protein Pref1, first identified on murine 3T3-L1 preadipocytes, as a putative ASC marker [24]. Others have reported the use of pericytic markers such as platelet-derived growth factor receptor β and 3G5 [23, 25–28]. Finally, combinatorial phage display approaches have associated the presence of α_5 β_1 integrin with ASCs [29]. It is recommended that the ISCT, the International Federation of Adipose Therapeutics and Science, or an equivalent society establish a task force of academic, biotechnology, and regulatory agency representatives to issue a consensus statement on minimal acceptance criteria for both SVF cells and ASCs. These criteria should be based on cell viability and/or proliferation rates, immunophenotype, and differentiation potential. Wherever possible, criteria that can be collected in process and without the destruction of the final cell product should be considered, for example, measurement of secreted proteins in the conditioned medium. Additional parameters based on transcriptomic or proteomic approaches can be considered. Finally, the criteria must be practical, reproducible, and robust to meet future industry and manufacturing demands.

Contamination Testing

Assays must document that all cell products for human clinical applications are free of bacterial, endotoxin, mycoplasma, and viral (B19, cytomegalovirus, Epstein-Barr virus, hepatitis B and C, human immunodeficiency viruses 1 and 2, as well as human T-cell leukemia viruses 1 and 2) contamination. The adipose tissue donors may themselves be carriers of infectious agents or these can be introduced during the manufacturing process despite precautions implemented under the cGMP process. For example, the inclusion of antibiotics and antimycotics in the culture medium can mask the presence of contaminants. There is reduced, but not absent, concern for infectious agents, when autologous adipose-derived cells are used. All allogeneic cell products must be defined rigorously as infectious agent-free and this introduces considerable cost and time to the manufacturing process.

Cryopreservation

Long-term storage will be critical to ensure a reliable supply and delivery of ASCs and SVF cells to point of care providers. The majority of published ASC and SVF cell cryopreservation procedures rely on the use of dimethyl sulfoxide (DMSO) as a cryoprotectant agent (CPA), often in combination with serum protein components. While DMSO is used routinely with blood cell products, it has potential adverse effects on the recipient and may not be optimal for all cells. Alternative CPAs for ASCs and SVF cells include hydroxyethyl starch, trihelose, and polyvinyl and some can be used under serum free conditions [30–32]. These alternative options should be explored, validated as reproducible, and considered as future industry standards. While most academic laboratories store cryopreserved cells submerged in liquid nitrogen, cGMP grade products must be maintained in liquid nitrogen vapor phase storage containers. This removes any risk of cross-contamination between individual containers. It is unlikely that hospitals and clinics will routinely have access to liquid nitrogen storage containers at the point of care. Instead, it is likely that cell products will be kept at $-70°C$ to $-80°C$ and further data on the shelf life of adipose cell products at these temperatures is needed.

Shipping

It is not only unlikely but also financially undesirable to maintain GMP facilities at all hospitals and clinics for the preparation of either SVF cells or ASCs. Consequently, adipose tissue and cell products will be shipped between the donor/recipient site and the processing laboratory. Data suggests that viable and functional ASC can be recovered from adipose tissue stored for up to 24 hours after liposuction [33, 34]. Studies need to be published relating to the viability of SVF cells and ASCs after shipment by either vehicle or air freight for extended periods of time. All studies must monitor the ambient temperature of the product. Outcome measures should include the minimal acceptance criteria for the cell products outlined above.

Animal Studies

There is a wealth of published evidence in animal models evaluating the safety and efficacy of adipose-derived cells (reviewed in [35]). The majority involves the use of rodents but a substantial number have used canine, ovine, porcine, and other large animal models. Nevertheless, is this body of evidence sufficient to satisfy regulatory authorities? The drug industry must perform trials in large numbers of male and female animals of varying ages monitored over periods ranging from a few days to $\geq$1 year. Monitoring studies need to evaluate the migration of cell implants to major organs (brain, heart, liver, lung, and kidney). Animal recipients must be monitored closely for evidence of tumor formation. There are few if any long-term, large animal studies with adipose-derived cells reported in the literature and this literature needs to be expanded in the near future. Needless to say, all animal studies require veterinary oversight and must be reviewed and approved by an institutional animal care and safety committee before implementation.

Tumorigenesis

There is precedent documenting the ability of human ASCs to transform during in vitro passage based on karyotypic changes in genotype and the development of nonadherent growth characteristics in agar cultures [36]. Furthermore, when these transformed ASC were implanted in immunodeficient mice, they formed sarcomas in vivo. Similar evidence from work using bone marrow MSCs has led to a policy statement by the ISCT regarding tumorigenesis [37]. While there is evidence suggesting that not all reported transformations in culture were actual events, it is incumbent on the stem cell community to take the most conservative approach with respect to patient safety. Studies should be published that specifically monitor for the absence or presence of tumorigenesis using SVF cells and ASCs. Moreover, simply evaluating the cells alone is not sufficient. Whenever these cells are further

Table 1. Clinical studies and targets

Disorders	References
Soft tissue—breast augmentation and reconstruction, craniofacial lipoatrophy, decubiti ulcers, postirradiation fibrosis	[48–52, 54]
Hard tissue—craniofacial reconstruction	[16, 55]
Immune—Crohn's disease, multiple sclerosis, rheumatoid arthritis	[56–62]
Ischemia—limb ischemia, myocardial infarction, stroke	[63, 64]

Table 2. Levels of evidence according to Center for Evidenced Based Medicine

Level	Description
5	Expert opinion without support from physiological bench science or first principles
4	Poorly controlled case series
3	Individual case controlled study
2	Retrospective cohort study
1	Randomized case controlled prospective trial

manipulated, either through a differentiation step or by combination with a scaffold or gene therapeutic, additional in vivo safety monitoring is necessary. The adipose-derived cell's immunomodulatory and immunosuppressive properties present additional concerns. Recent reports document the ability of ASCs to promote the proliferation of active breast cancer cells in vitro and in vivo via paracrine mechanisms [38–42]. Comparable studies indicate that ASCs promote the growth of prostate cancer cell lines transplanted into immunodeficient mice [43]. These results are consistent with a larger body of evidence relating to the interaction of bone marrow MSCs with the tumor microenvironment [44–46]. Bone marrow MSCs can migrate to tumors and can release tumor-promoting chemokines upon arrival [44, 46]. While there is a promise that this homing property can be exploited by using transduced or transfected bone marrow MSCs as antitumor delivery vehicles, this, nevertheless, remains a potential risk [45]. This has particular relevance with respect to the expanded use of SVF cells and ASCs for postmastectomy reconstruction in breast cancer patients [38, 42]. Consequently, additional preclinical studies evaluating SVF cells and ASCs interactions with a wider range of tumor types should be undertaken in the near future. There is a strong argument to be made that clinical studies should be closely regulated and monitored for outcomes until the tumorigenic actions of adipose-derived cells are better understood.

Clinical Studies—Are We There Yet?

Clinical Opportunities

Adipose-derived cells have potential applications to a wide range of clinical disorders [47]. These can be categorized based on the target tissue (soft or hard tissues) or the underlying pathology (immunological or ischemic) (Table 1). The greatest number of patients recruited and/or reported have been for breast reconstruction (soft tissue) and fistula repair (Crohn's disease) procedures (reviewed in Table 2 [35]) and outcomes for both therapies appear promising; however, there is a need for caution. In the case of breast reconstruction, autologous SVF cells are recombined with the patient's own lipoaspirate tissue for purposes of fat grafting. Similarly, fat grafting and adipose-derived stem cells can be used to ameliorate skin fibrotic changes following breast or head and neck tumor radiation therapy [48, 49]. Thus, soft tissue defects are a logical therapeutic target for SVF cells and ASCs because they are returned to their tissue of origin. While some plastic surgeons have experienced successful outcomes with fat grafting approaches, adverse events including cyst formation, fat necrosis, and microcalcifications have been observed in a percentage of breast reconstructions [50–52]. In addition, the immunosuppressive and tumor promoting functions of SVF cells and ASCs leads to concerns that their introduction into the breast tissue of a postmastectomy patient could enhance the recurrent growth of residual cancer cells [38]. Consequently, plastic surgeons and related clinical practitioners remain cautious concerning the use of adipose-derived cells in the context of cancer patients at this time [53].

While the clinical literature is extremely limited (Table 1), this presents an opportunity to coordinate future translational efforts regarding adipose-derived cells using the principles of evidenced-based medicine. To date, the majority of peer-reviewed publications on human trials using adipose-derived cells are, at most, Phase I safety and case reports (Table 1). Most, but not all, studies contain written evidence that protocols were reviewed and approved by an institutional review board in accordance with the principles of Helsinki and that all patients provided informed consent. At best, this work would classify as levels 3–4 based on the criteria set out by the Center for Evidenced Based Medicine (http://www.cebm.net) (Table 2). Some randomized controlled clinical trials have been registered with the NIH website. Indeed, it will be challenging to recruit subjects to a nontreatment arm of a randomized cell therapy-based trial. Nevertheless, it is imperative that investigators designing clinical trials position their work not only for their own review but for future retrospective meta-analyses. No single clinical study will enroll a sufficient numbers of subjects to affirm the absolute safety of any adipose-derived cell therapies. Such an evidence-based analysis will require input from multiple independent centers and randomized clinical trials. A partnership between public and private stakeholders in the ASC field could develop guidelines to accelerate everyone's translational efforts. A joint committee of representative basic scientists, bioethicists, biostatisticians, clinicians, and manufacturing/biotechnology representatives could establish a minimum set of safety and efficacy parameters for inclusion in all clinical studies. These data could then be posted to an on-line registry for long-term follow-up. Steps could be taken to identify an appropriate control group for any nonrandomized studies. While there is a cost associated with any effort of this magnitude, it has the potential to reduce the long-term expenses of clinical translation substantially.

Summary—Where Do We Go from Here?

The ASC field is at a critical juncture as it transitions from basic science to a clinical therapy. Clearly, the development process for adipose-derived cells does not always fit the traditional paradigm of "hypothesis-driven, mechanism-based" experimental design common to the stem cell literature. While it is likely that internal studies at biotechnology companies have addressed some, if not all, of the questions raised in this

review, the work has not been extensively reported in peer-reviewed manuscripts. We anticipate that comprehensive reports documenting that SVF cell and ASC safety testing can be replicated will appear in the literature and such evidence will accelerate the clinical translation process. The authors of this review are confident that this can and will be accomplished by a collective international effort that places the highest value on patient safety and product efficacy.

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Disclosure of Potential Conflict of Interest

J.M.G. is the cofounder and has equity in LaCell, LLC, and has consulted for Artecel Sciences and Toucan Capital. F.G. is a cofounder and has equity in Cytex Therapeutics, Inc. B.A.B. and E.S.C. indicate no potential conflicts of interest.

Note Added in Proof

The following article was published since the time of acceptance and provides a comprehensive report of the commercial research and development outcomes for a human ASC product by a biotech company: Ra JC, Shin IS, Kim SH, et al. Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. Stem Cells Dev 2011 (in press).

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