

Section VII

Clinical Guidelines for the Practice of Cell Based Medicine

Chapter 1-Introduction and Historical Context

The International Cellular Medicine Society (ICMS) is a medical science organization, composed of physicians, scientists, and laboratory personnel, dedicated to the development and dissemination of laboratory and clinical guidelines for minimal culture expansion of autologous, adult stem cells (A-ASCs). The ICMS maintains a Clinical Best Practices Committee (CBPC) consisting of physicians and medical research scientists.

The CBPC began the process of guideline development by reviewing existing guidelines and other publications pertaining to the clinical translation of stem cells. Because most clinical translation publications and discussions have focused on the relatively greater risks of allogeneic transplants such as umbilical cord blood and embryonic stem cells the CBPC had to essentially start from scratch in developing guidelines for the substantially smaller risk techniques used for A-ASC minimal culture expansion.

These guidelines were created to balance the minimization of risk with the availability of clinical care within the framework of current treatment paradigms for various conditions. For example, the risks of a hypothetical treatment for severe type II diabetes with A-ASC therapy would be weighed against the risks of conventional therapy as well as the risk of morbidity and mortality in untreated patients. Another example would be the risks posed by NSAID therapy in cases of orthopedic trauma (>60 days of therapy results in death in 1 in 1200) versus the risks and benefits of A-ASC therapy for the same condition. As part of this risk to benefit assessment the CBPC has had to include the type of tissue intended for treatment as the risk of therapy is proportional to the tissue receiving the treatment. For example, A-ASC transplants of orthopedic and cosmetic tissue carry less treatment risk than transplants involving cardiac and central nervous system tissue.

As a historical context, the evolution of In-vitro Fertilization (IVF) illustrates the development of a minimal culture expansion process. The first successful IVF procedures were performed in the late 1970's. By the 1990's fertility specialists had decided that extending cell culture to the blastocyst stage allowed them to improve conception outcomes by not only choosing the embryo that demonstrated the best in-vitro proliferation potential, but also by implanting a more mature cell mass into the patient. This marked the transition of IVF from a simple tissue transplant procedure to a cell culture technique. Further advances

in genetic screening will likely require more extended cell culture methods, as cells from the growing blastocyst are usually sacrificed for genetic testing.

Fertility specialists have maintained that Assistive Reproductive Technology (ART) and the cell culture techniques that it uses are in fact the practice of medicine and not the production of biologic drugs. While the FDA has proposed regulations to regulate IVF clinics (21 CFR parts 50, 56, and 312-Oocyte cytoplasm transfer), it has failed to classify IVF culture as the production of a biologic drug. The CDC has published guidelines for Assistive Reproductive Technology (ART) clinics, intended to assist states in producing their own certification programs. However, federal oversight is not mandated for such clinics. As a result, the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology have put forth their own credentialing criteria for ART . Most ART labs are accredited through the College of American Pathologists' Reproductive Laboratory Accreditation Program or through the Joint Commission on Accreditation of Healthcare Organizations. The ICMS approach to guidelines and ultimately credentialing development presented herein has been designed along the lines of the successful model developed ASRM and SART for the clinical application of ART.

Chapter 2-Practice Guidelines

Section A-Patient Eligibility Requirements

A-ASCs are commonly derived from bone marrow (BM), blood products (BP), or solid tissues. Since donation of marrow or whole blood can impact blood counts, minimum blood requirements apply to donation. For a BM or BP donation, minimum requirements are as follows:

- Hematocrit of at least 40% (both genders)
- No evidence of acute or chronic infection
- Identification of disease transmission risks (such as HIV, Hepatitis, etc) by the collecting clinic

Since loss of solid tissue impacts on functionality of the donor tissue, minimum requirements apply to donation. For Solid Tissue donation, minimum requirements are as follows.

- Appropriate sample size determined by the physician based on the type of tissue harvested. As examples, cosmetic procedures such as liposuction may procure larger amounts of fatty tissue whereas other solid organ donation sites such as muscle or skin may be limited to smaller samples.
- No evidence of acute or chronic infection
- Identification of disease transmission risks (such as HIV, Hepatitis, etc) by the collecting clinic

- Whole marrow or blood donation should not be within 6 weeks from the last draw without physician documentation supporting the safety and necessity of an earlier draw

Ultimately it is left up to the treating physician to determine if exceptions to these minimum requirements can be made safely. As an example, surgery in burn patients has been carried out where donor tissue amounts to the remaining viable tissue of the individual. In another example, surgery can be performed with substantially lower hematocrit levels than is given here for minimum donor requirements when deemed appropriate.

A-ASCs may be harvested from sites other than those containing bone marrow, such as the synovial fluid or tissue found in the knee, skeletal muscle, dermis of the skin, and subcutaneous fat. The specific sites of bone marrow, blood tissues, skeletal muscle, and dermis harvest must be first assessed for eligibility, including the following criteria:

- No indication of infection in the area
- The planned collection procedure should not inordinately risk the tissue being harvested or the donor site.

Finally, the A-ASC therapy physician must conduct a comprehensive assessment of the patient prior to donation which includes:

- History and physical examination specific to the problem being considered for treatment
- Assessment of general health status and ability of the patient to participate safely in the A-ASC therapy
- Discussion with the patient concerning the risks and benefits of the planned A-ASC procedure

Proper informed consent should be given to, documented and signed by the patient. The consent and subsequent patient discussion should include a discussion with the patient of the risks and benefits of the procedure as well as alternatives to the procedure.

Section B-Harvesting of Tissue Samples

A-ASCs can be obtained from many sites including bone marrow, mobilized peripheral blood using apheresis, synovial fluid or tissue, dental pulp, skeletal muscle biopsy, fat liposuction, fat biopsy, and dermal biopsy. Each harvest site should be prepped in a sterile surgical fashion using anti-septic prep with sterile gloves and drapes. The harvesting surgeon should wear proper surgical attire which should include at a minimum sterile gloves and a face mask with surgical hat. The collection facility should be clean with a documented and scheduled

anti-septic cleaning regimen. The site(s) and volume of tissue obtained from each site should be recorded as well as any complications.

Harvesting requirements are listed here by tissue source:

Bone Marrow: The most common collection site for bone marrow is near the posterior superior iliac spine (PSIS). However, this procedure can often yield significant variability in yield based on the variability in the thickness of the marrow cavity. As a result, C-arm fluoroscopy is recommended for marrow collection. General or local anesthetic procedures should be used to provide anesthesia to the donor area. A sterile trocar should be used and inserted into the marrow cavity, just penetrating the cortex. Whole marrow should be drawn into a sterile, heparinized syringe. No more than 400 ml of whole marrow should be drawn at any one time. Usually, marrow draw volumes are in the 50-70 ml range. Pre-heparinization of the bone site may be used to help prevent marrow clotting in the collection syringe.

Peripheral Blood: The most common site for peripheral blood draw is the median cubital vein, crossing the cubital fossa of the ventral elbow joint. If that vein is impaired, other venous structures such as the basilic vein can be used for peripheral blood harvest. A sterile needle is inserted into the vein. Whole blood should be drawn into sterile heparinized or EDTA-coated collection container(s). No more than one pint (500-ml) of blood should be removed from a normal healthy patient. Contraindications include body weight of less than 100 pounds, pregnancy, and chronic disease such as hypothyroidism, or acute diseases such as colds, flus, and other viral or bacterial infections.

Synovial Fluid (SF): SF is collected via percutaneous means where a needle is used to draw native fluid from the knee. Local anesthetic procedures should be used to provide anesthesia to the donor area. The lateral approach is associated with a greater chance of fluid recovery in a blind setting. A sterile 18 or 20 gauge needle should be inserted through a lateral inferior portal just superior to the tibia and lateral to the patella. Depth to obtain fluid in most patients is 1-1.5 inches, but obese patients may require a draw depth of 2 inches or more. The needle should pass into the joint without resistance and care should be taken in avoiding needle placement into mid-line structures in the trochlear groove such as the ACL or PCL. The average knee contains approximately 2-3 ml of native synovial fluid from which approximately 1 ml SF harvest can be expected. In a knee with inflammation or edema, a significantly larger volume of SF can be harvested (10-20 ml of fluid depending on the condition of the knee). If additional synovial fluid is needed it can be obtained from the suprapatellar bursa by “milking” the knee joint (by repeated superior to inferior compression over the supra-patellar area). The fluid for transport to the lab should be clear and viscous. If turbid fluid or pus is encountered, the sample should be considered to be contaminated and there should be a high index of suspicion for an infection of the knee. Appropriate

steps should be taken to discard the SF sample and to rule out knee infection prior to reattempting another SF draw for A-ASC therapy.

Skeletal Muscle (SM): SM is collected from a donor site after general or localized anesthesia. Following an incision through the skin, a piece (or strip) of skeletal muscle is removed and the area closed by sutures. Maximal sample size should be no more than 25 ml from any single site. If the site is warm and red to the touch, indicating a possible infection, the sample should be considered contaminated and there should be a high index of suspicion for an infection of the sample area. Appropriate steps should be taken to discard the SM sample and to rule out localized infection prior to reattempt at SM biopsy for A-ASC therapy.

Fat Liposuction (FL): FL is collected from the donor site after general or localized anesthetic. Following a small incision through the skin, a cannula is inserted and fat tissue is removed by suction and collected into heparinized or EDTA-containing collection vessels with sterile transport fluid. Maximal sample size is dictated by the amount of fat to be removed, agreed beforehand by the patient and physician. If the site is warm and red to the touch, indicating a possible infection, the sample should be considered contaminated and there should be a high index of suspicion for an infection of the sample area. Appropriate steps should be taken to discard the FL sample and to rule out localized infection prior to reattempt at FL biopsy for A-ASC therapy.

Fat Biopsy (FB): FB is collected from a donor site after general or localized anesthesia. Following incision through the skin, a piece (or strip) of fat tissue is removed and the area closed by sutures. Maximal sample size should be determined by the physician and patient. The tissue is placed in a sterile transport container with sterile transport medium. If the site is warm and red to the touch, indicating a possible infection, the sample should be considered contaminated and there should be a high index of suspicion for an infection of the sample area. Appropriate steps should be taken to discard the FB sample and to rule out localized infection prior to reattempt at FB biopsy for A-ASC therapy.

Dermal Biopsy (DB): DB is collected from a donor site after general or localized anesthesia. The dermis can be obtained by either punch biopsy or incisional biopsy. A punch biopsy is made through the skin after local anesthesia, from the epidermis down to the hypodermis, and the tissue punch is inserted into a sterile collection tube containing sterile transport medium. An incisional biopsy is made through the epidermis after local anesthesia and a piece (or strip) of dermis is removed after which the area is closed with sutures. The sample is placed into a sterile container with sterile transport medium. Maximal sample size should be no more than 25 cm² from any single site. If the site is warm and red to the touch, indicating a possible infection, the sample should be considered contaminated and there should be a high index of suspicion for an infection of the sample area. Appropriate steps should be taken to discard the DB sample and to rule out localized infection prior to reattempt at DB biopsy for A-ASC therapy.

Transport between lab and Clinic:

The collected tissue sample should be sealed and placed into a sterile transport bag using sterile technique and transported to the lab facility. All samples should be labeled with the items noted in the ICMS Lab Guidelines, signed and dated by the attending lab personnel.

If clinic or lab personnel detect a break in the sterile transport bag or if the exterior of sample becomes contaminated, then chemical sterilization procedures using bleach, phenol acid-based disinfectant, and/or a 70% ethanol solution should be utilized in the lab to protect the integrity of the sample. If actual sample contamination is suspected, the sample should be discarded by clinical or lab personnel and the harvesting physician notified.

Section C-Clinical Translation of Adult Stem Cell Lines

The ISSCR (International Society for Stem Cell Research) states, “The level of regulation and oversight should be proportional to the degree of risk raised...and intended use (autologous versus allogeneic use, minimally versus highly manipulated cell products, use for homologous versus non-homologous functions).” (See <http://www.isscr.org/clinicaltrans/pdfs/ISSCRGLClinicalTrans.pdf> accessed April 10, 2009). The ICMS concurs with this position, and thus the focus of these guidelines is on autologous cell therapy in which the source of the A-ASC is functionally homologous with the therapeutic target. In addition, in order to balance patient safety with the availability of therapy, the ICMS has purposely narrowed its focus to cells which have been minimally culture expanded using techniques which mimic normal physiology. For the purposes of these guidelines **minimal culture expansion** is defined by both period of incubation and number of culture passages of stem cells, in that the former may not exceed 60 days and the latter may not exceed 10 passages after colony formation).

It should be noted that at this time, that due to a lack of data regarding safe usage, the ICMS does not believe the following stem cell types are ready for clinical translation:

- Induced Pluripotent Cells (IPS)
- Embryonic or Cord Stem Cells
- Allogeneic Adult Stem Cells
- Autologous Adult Stem Cells where genes have been manipulated

Translation of Stem Cells to Clinical Work:

The ICMS recognizes that pre-clinical trials and early clinical work should focus on safety. While clinical outcomes are important, since the use of stem cells are in early pre-clinical status for many disease applications, complications tracking

is currently of paramount importance. Safety tracking can be broken into two basic components:

1. Transplant safety: The focus here is tumorigenesis, transplant site monitoring, and new disease monitoring. While these risks are believed to be minimal for therapy involving A-ASCs (as defined by ICMS), they are nonetheless inherent risks of A-ASC transplants.
2. Treatment specific risks: Every A-ASC transplant can pose specific risks based on transplant location, the disease being treated, and cell type. As an example, a cardiac treatment focused on implanting cells in the coronary artery would pose more inherent physical risks than a cosmetic transplant.

Clinical Safety Record:

ICMS has established a process to balance safety with the availability of treatment. This process involves clinical translation staging for cell lines. The cell line is first broken into one of two categories:

1. Established in Prior Human Testing: The cell line has passed the staging below in all categories. Once a cell line is established for one tissue type, it's known as "Clinical Grade" or CG
2. Unestablished in Prior Human Testing: The cell line has not passed the staging below in all categories.

The ICMS then breaks "Un-established in Prior Human Testing" cell lines into the following use categories for the purposes of clinical translation.

Clinical Staging for Cell Lines:

Pre-Investigational Cell Line (PICL): No animal data is available. These cell lines should not be used in humans until animal data is available.

Early Investigational Cell Line (EICL): This is an un-established stem cell line being used in a new tissue where several animal models exist that show efficacy and safety, but no human data exists. An un-established cell line can be used in early stage human studies where 5-10 patients are treated and followed for a minimum of 6 months. The physician should be able to document subjective and objective outcome measures. Once these criteria are met and no significant complications have been reported, the cell line moves to the next grade. Note that before LICL patients can be treated, a minimum of 6 months follow-up is required at EICL.

Late Investigational Cell Line (LICL): This is an un-established stem cell line being used in a new tissue and is being tested in humans in larger numbers, typically 20-50 patients who are followed for a minimum of 6 months. The physician should be able to document subjective and objective outcome measures. Once these LICL criteria are met and no significant complications have been reported, the cell line moves to the next grade. To move onto treating

ECCL patients, at least 20 of the LICL patients should be at the 6 month follow-up stage and have no complications.

Early Clinical Cell Line (ECCL): This is an un-established stem cell line being used in a new tissue and is being used for early stage clinical treatments in 50-200 patients that are followed for a minimum of 6 months. The physician should be able to document subjective and objective outcome measures. Once these criteria are met and no significant complications have been reported, the cell line moves to the next stage. To move on to treating LCCL patients, at least 50 of the EICL patients should be at the 6 month follow-up stage and have no complications.

Late Clinical Cell Line (LCCL): This is an un-established stem cell line being used in a new tissue and is being used for early stage clinical treatments in 100-300 patients that are followed for a minimum of 6 months. The physician should be able to document subjective and objective outcome measures. Once these criteria are met and no significant complications have been reported, the cell line moves to the next stage. To move on to treating CG patients, all phases of the staging must be completed.

Clinical Grade (CG): This is an established cell line that has completed all stages above and is being used in patients in an unrestricted fashion. All patients being treated must still be entered into the ICMS Re-implantation Registry.

Summary Table of Clinical Staging for Cell Lines:

Clinical Staging Grade	Months of Treatment at the Clinical Stage	Number of Patients Treated in this Stage	Number of Patients at this Stage and Duration of f/u needed to begin Treating Patients in Next Stage	Preceding Stage and Duration in months of Follow-up Needed to Finish Stage
EICL	6	5-10	5-10 at 6 months	
LICL	6	20-50	20 LICL at 6 months	EICL-12
ECCL	6	50-200	50 ECCL at 6 months	EICL-18 LICL-12
LCCL	6	100-300	All phases of staging must be completed	EICL-24 LICL-18 ECCL-12
CG				

Exceptions

- A CG cell line does not have to pass the PICL/EICL/LICL process when used in a new tissue. With such use the cell line will be considered ECCL. The rationale is that for a homologous use, an autologous cell line that has shown durable safety in one homologous tissue type, is likely to show similar safety characteristics in other homologous tissue types as well. The cases would be entered into the re-implantation registry in the same fashion as other cases.
- Note that small culture changes made in an ECCL/LCCL or CG stage cell line that has been used with no complications and are in accordance with these guidelines (mimicking normal physiology) do create a “new cell line”, but this begins the staging in the ECCL stage. As an example of these small culture changes, this might include a change of basal media from A-MEM to D-MEM, a change in oxygen tension in culture, a change from serum free media to a documented, non-inductive FBS (Fetal Bovine Serum), or a change of adherence substrate from plastic to collagen with the concomitant switch from the use of trypsin to collagenase.

Certification of Clinical Cell Line Staging

Note that the ICMS Reimplantation Registry will be the final arbiter of the number of cases followed in each stage and if those cases have shown significant complications. In order for a cell line to be certified in any clinical stage, a letter is required from the ICMS Reimplantation Registry stating that the required number of cases have been documented for the requisite follow-up periods.

For the first 12 months after the acceptance by the ICMS membership of these guidelines, a clinical stem cell line can be “grandfathered” or accepted into the ICMS staging only if it:

1. Meets the definitions described herein (autologous, adult, minimal culture expansion, exposure to physiologic parameters in culture)
2. Has complications tracking data that meets the ICMS Reimplantation Registry’s standards.

The “grandfathered” line will enter into the clinical staging based on follow-up safety data as defined by the ICMS Reimplantation Registry with the issuance of a letter as described above. For example, a stem cell line with 50 patients in 12-24 month follow-up and 10 patients in 24-36 month follow-up that has shown no complications would enter at ECCL status. As end-point safety is the most important parameter, a cell line can be “grandfathered” as long as the line has current outcome data that meets the registry’s specifications. As an example, if a cell line has 100 cases with at least 24 month follow-up (all as single data points collected in the several months prior to ICMS certification), then the line can be “grandfathered” in the LICL stage.

Definition of Cell Lines

The core of safe stem cell therapy is a definition of the therapeutic agent, the stem cell line. As described above, each cell line must move through the clinical translation staging. ICMS defines a cell line by its:

1. State of Differentiation: Describes the ability of the cell to produce various cell lineages through differentiation.
2. Tissue Origin: The tissue type and location where stem cells originate.
3. Cell Line Type: Describes the source of the stem cell and its general ability to differentiate into specific tissues as well as its ability to mobilize to either local or distant tissues.
4. Tissue Culture Parameters: Records the major effects that the ex-vivo cell culture process can have on stem cells.
5. Tissue Use: The general tissue type where the cells will be used. This includes the embryonic origin of the tissue as well as its general function.

1-State of Differentiation:

There are multiple adult stem cell types present in adult tissues. These adult stem cells range from the most differentiated cell types (functioning parenchyma

and stroma) to the least differentiated cell type (totipotent stem cells). Each cell type within this group has its own unique attributes and cell culture requirements.

- A-Differentiated Cells (DCs)
- B-Progenitor Cells (PCs)
- C-Germ Layer Lineage Stem Cells (GLSCs)
- D-Pluripotent Stem Cells (PSCs)
- E-Totipotent Stem Cells (TSCs)

A cell can be classified as a differentiated cell (DC) (i.e., parenchyma or stroma) if it is derived from a single cell, has a lifespan limited to less than 50 population doublings, and is a functioning portion of the tissue.

A cell can be classified as a progenitor cell (PC) if it is derived from a single cell, it has a defined biological clock of 50-70 population doublings, and is the immediate precursor for adult differentiated cell types.

A cell can be classified as a germ layer lineage stem cell (GLSC) if it is derived from a single cell, it has unlimited proliferation potential and forms all cell types within a single germ layer lineage, i.e., ectoderm, mesoderm, and endoderm. GLSCs are also known as multipotent stem cells.

A cell can be classified as a pluripotent stem cell (PSC) if it is derived from a single cell, it has unlimited proliferation potential, it can form multiple cell types from all three germ layer lineages, but cannot produce gametes (sperm or ova) or placenta cells.

A cell can be classified as a totipotent stem cell (TSC) if it is derived from a single cell, it has unlimited proliferation potential, it can form multiple cell types from all three germ layer lineages, and it can produce gametes (sperm & ova) and placental cells.

2-Cell Line Type

The definition of stem cell lines is further broken down into a specific cell line type based on categories of adult, autologous cells. ICMS defines cell lines by their common embryonic origin, state of differentiation, and cell types that can be produced by differentiation.

Mesenchymal Stem Cells (MSCs): A multipotent stem cell (GLSC) that is self-renewing and adherent, which make up a very small fraction of the marrow stroma. These non-hematopoietic stromal cells are usually harvested from a patient's bone marrow or other tissues of mesodermal origin (fat, joint synovium, dental pulp, etc...).[1-13] They can be isolated based on adherence and can be culture expanded in vitro. These cells may differentiate into various

mesenchyme-derived cell types. They generally do not circulate in the peripheral blood, but rather are resident in the mesenchymal tissues they serve.

Hematopoietic Stem Cells (HSC's): A multipotent stem cell (GLSC) that is self-renewing and non-adherent. This stem cell gives rise to all blood types (including myeloid and lymphoid lineages) and is usually derived from either the patient's bone marrow or mobilized peripheral blood.[14] They can be isolated with centrifugation or antibody selection and can be culture expanded in vitro (usually only in the presence of other marrow stromal cells, in particular MSCs). These cells can circulate in the peripheral blood.

Neural Stem Cell (NSC): A multipotent stem cell (GLSC) found in adult neural tissue that can give rise to neurons and glial (supporting) cells.[15] These cells do not circulate in the peripheral blood, but serve their resident tissues.

Endothelial Progenitor Cell (EPC): A multipotent stem cell (GLSC) found in bone marrow that participates in vasculogenesis.[16] These cells do circulate in the peripheral blood in response to vascular injury.

Epithelial Stem Cell (ESC): A multipotent stem cell (GLSC) that resides in the dermis and is controlled by epithelial-mesenchymal interactions.[17-20] This cell gives rise to new dermal tissue and other tissues of epithelial origin. These cells are resident in the tissues they serve.

Very Small Embryonic Like Cell (VSEL): A totipotent stem cell (TSC) that resides in the bone marrow and also circulates in the peripheral blood during times of tissue injury.[21-23]

More specific descriptors (phenotype and genotype) of different cell populations produced by a specific cell culture process are possible, but often problematic. As an example, cell surface markers are variable even with the same stem cell line. For example, mesenchymal stem cells have variable markers with very few markers being generally agreed upon as likely MSC specific.[24-29] HSC's are better defined by cell surface markers (usually CD34+), but CD34- HSC's have been identified.[30] VSEL's can be identified by cell surface markers (usually CXCR4⁺ Sca-1⁺ Lin⁻ CD45⁻, expressing SSEA - 1/4, Oct - 4 and Nanog). EPC's and ESC's are less well defined by markers. In addition, new stem cell surface markers to assay are still being discovered. Even genetic profiling is difficult as a definitive identification tool, as different levels of gene expression are also seen within the same stem cell line.[31]. In addition, many adult cells have paracrine functions that would be difficult to assay.[32-37]

3-Tissue Origin:

A-ASCs can be isolated from a number of tissues as described in the Harvesting of Tissues Section of these guidelines.[25, 38] A specific adult stem cell line harvested from one tissue may have certain differences in properties when compared to the same type of adult stem cell harvested and isolated from

another tissue. As an example, it's known that MSCs harvested from bone marrow, synovial fluid or tissue, and adipose tissue express different levels of chondrogenesis.[38] In addition, even MSCs harvested from the marrow cavity of the vertebral body show slightly different differentiation characteristics toward intervertebral disc tissue, than those harvested from a distant site to the spine.[39, 40] As a result, tissue origin is important in defining a cell line.

As part of the cell line definition, both the general source (i.e. bone marrow aspirate, synovial fluid, muscle, dermis, etc...) must be listed as well as the specific location (bone marrow aspirate-PSIS, synovial fluid-knee, muscle-deltoid, dermis-posterior auricle, etc...).

4-Cell Culture Parameters

Once a stem cell is isolated from a specific tissue, changes in cell culture conditions can alter cell type.[41-43] As a result, at a minimum the ICMS believes that for any cell line described by its state of differentiation (for example GLSC) and specific category as defined above (for example MSC), the cell culture process further defines that cell line. Therefore, a cell line is further defined by the following cell process parameters.[42] The cell culture parameters that can lead to shifts in phenotypes in adult stem cells include:

1. Cell Isolation Method: Some process must be used to isolate and select adult stem cells from tissue. The most common methods include:
 - a. Centrifugation: Usually multiple steps whereby cell populations are separated from each other by gravity.
 - b. Gradient: The use of a substance (usually Ficol) that amplifies the differences in gravity dependant cell movement with centrifugation. These differences are used to select a layer from the substance where the desired cell population is more likely to settle.
 - c. Adherence: The use of plastic or other surfaces to select adherent cells from a mixed culture with non-adherent cells.
 - d. Filtration: The selection of cells based on size differences.
 - e. Surface Antigen(s): The selection of cells based on surface markers via antibody binding.
2. Seeding Density Range: Seeding density can impact the identification of the final cell line. A range must be specified. Seeding density is further broken down by range:
 - a. Low-less than 5,000 cells/cm²
 - b. Medium-Between 5,000-12,000 cells/ cm²
 - c. High-Greater than 12,000 cells/ cm²
3. Serum Type and Concentration Range: The concentration of serum used (autologous serum or FBS) can also vary phenotype. A serum type and concentration range must be specified, the concentration range must not vary more than +/- 10% fluid concentration. As an example, if autologous platelet lysate were used as a serum type with a mean concentration of 20%, it should not vary more than 10% concentration at a minimum and 30% as a maximum concentration.

4. **The Total quantity and Type of Media:** The type and total quantity of media used can have a lesser impact on cell phenotype. As a result, media type and volume per culture flask size must be specified and must not vary by more than +/- 10%. As an example, if a T-75 monolayer culture flask was usually filled with 20 ml of A-MEM media, the volume of media placed in that flask size should be no less than 18 ml and no more than 22 ml.
5. **Incubation Time:** Time in culture can vary phenotype. The time in culture should not exceed ICMS standards and should not vary for each cell line by more than 7 days. As an example, if several samples were cultured using the same cell line technique, then the minimum time in culture could be 14 days with a maximum of 21 days. However, it is understood that very young patients may demonstrate rapid proliferation and may need to have shorter incubation times as a practical measure. Therefore, the emphasis for this definition is on the maximum time in culture. As an example, a cell line with a usual incubation time of 14-21 days may have a small percentage (less than 10%) of patients with lesser incubation times such as 10-14 days.
6. **Other Factors:** To reduce the chance of other factors having a negative impact on the cell line, any processes or other cell exposures (i.e. chemical) should reflect the physiology present in the body. To be more specific, literature must exist that the basic process or chemical family is used in the body to manage or modulate stem cell populations and that this process normally occurs in the body. For a medication or chemical, the dose should be in the same or comparable range as is used by the body in normal physiology. As an example, MSCs are naturally expanded in the hypoxic region of subchondral bone, so MSC exposure to short periods of 1-5% physiologic hypoxia would be considered consistent with this policy.[44-48] Another example is seen with corticosteroid exposure. As corticosteroids are found in nanogram concentrations in many areas of the body to modulate inflammation, exposure of stem cells to physiologic doses of this drug family would be consistent.[49] Other examples include the use of naturally derived growth factors from platelets or other sources.[50-54] In addition, recombinant growth factors would only be allowed if their use in the body is considered physiologic. As an example, the body normally uses TGF-beta to help differentiate mesenchymal stem cells into cartilage; hence as long as recombinant TGF-beta is used in the dose ranges that naturally occur in the body during this process, this recombinant growth factor would be allowed.[55] Other examples would be preconditioning HPC's with physiologic ranges of VEGF.[56] Other physiologic factors that may be introduced to stem cells in culture would include pressure, tension, or other physical factors that mimic the physical in-vivo stem cell environment.[6, 57, 58] As discussed in the ICMS Lab Practice Guidelines, if FBS (Fetal Bovine Serum) is used, it must not contain inductive factors.

As examples of “other factors” that are not permitted by these guidelines:

- Recombinant growth factors that are used outside of their functions or doses in normal physiology. As a hypothetical example, the use of milligram doses of TGF-beta or corticosteroids to induce vasculogenesis Insertion of any genetic material into stem cells.

These “other factors” that are introduced to the cell line in the culture expansion process should be clearly specified as part of the cell line definition.

5-Tissue Use

Each cell line that passes each stage of development should be used for one general tissue category. The decision to generalize the use of a stem cell line to other tissues should be made first and foremost with consideration of the degree of risk. The embryonic origin of the tissue should be considered as well as its final differentiated state. In addition, risk should be determined based on the implications and remedy in the event of a negative outcome. As an extreme example, the risk of blindness in treating nearsightedness with stem cells would always be considered more than minimal risk and studies should proceed cautiously, whereas treating knee pain in a knee replacement candidate would be considered minimal risk. Other examples include treating NIDDM with stem cells where a negative outcome could include loss of all pancreas function (more than minimal risk) versus treating degenerative disc disease in a patient contemplating intervertebral disc fusion where a negative outcome is increased pain (minimal risk). Further examples include the minimal risk of a negative cosmetic application where stem cells are used as tissue fillers versus the more than minimal risk of treating a mild case of pediatric cerebral palsy where a negative neurologic outcome could be permanent worsening of the remaining neurologic function.

To assist with the decision making process of generalizing a stem cell line to treat various tissues, the following risk stratification system for tissue translation was developed. These tissue categories are based on divisions within their embryonic origins, final differentiated tissue types, the overall patient health risk of damage to the tissue, and the general availability of alternate treatment remedies if the stem cell treatment produces a negative outcome. These tissue categories are defined as:

- Ectodermal Integument: Skin (epidermis), hair, nails, sweat glands, teeth enamel, inner ear, eye lens
- Ectodermal Upper/Lower Digestive Tract: Mouth, Pharynx, Terminal Rectum
- Ectodermal Neurological: Peripheral Nervous System, Central Nervous System, Autonomic Nervous System, Retina, Pineal Body, Posterior Pituitary, Cranial and Sensory Ganglia
- Mesodermal Vascular: Heart, Vascular Smooth Muscle, Artery, Vein

- Mesodermal Orthopedic: Muscle, Tendon, Cartilage, Bone, Ligament, Intervertebral Disc
- Mesodermal Organ: Spleen, Kidney, Adrenal Glands
- Mesodermal Integument: Skin (Dermis)
- Mesodermal Urogenital: Oviducts, Uterus, Epididymis
- Endodermal Organ: Endocrine-Pancreas, Thyroid
- Endodermal Organ: Digestive-Liver
- Endodermal Respiratory: Trachea, Bronchi, Alveoli of Lungs
- Endodermal Urinary: Bladder-Urethra
- Endodermal Digestive Tract: Esophagus, Stomach, Small Intestine, Colon

Risk categories for cell line tissue translation:

Minimal Risk: The cell line is translated from one tissue type to another in the same general tissue category.

More than Minimal Risk: The stem cell line is being translated from one tissue category to another category.

If a CG stem cell line is used within one tissue category, then it is considered minimal risk and Clinical Translation Staging would not have to be repeated; however the cases would be entered into the ICMS re-implant registry. Examples would include the tissue translation of a stem cell line within the Mesodermal-Orthopedic category, such as from joint tissue to ligament tissue. Another example would be the tissue translation of a stem cell line within the Mesodermal-Vascular category, from coronary arteries to peripheral arteries.

As discussed above, translating a stem cell line between tissues can be a lower risk or a higher risk proposition. As a result, the tissue translation should follow these guidelines and the risk of the specific tissue translation should be considered. As a general rule, if a CG stem cell line is used between categories, then cell line staging would have to be repeated for each category, but the cell line would enter the process in ECCL, since its general safety has been established. If an earlier stage stem cell line (EICL, LICL, ECCL, LCCL) is translated from one tissue category to the next, then it must repeat most of the stages and start as EICL. An exception is a more established LCCL, which would start as LICL. An example would include the tissue translation of an unestablished mesenchymal ECCL from the Mesodermal-Orthopedic category (joint tissue) to the Mesodermal-Vascular category (coronary arteries), which would need to repeat all staging categories. Another example would include the tissue translation of an unestablished VSEL LCCL from the Endodermal Organ category (Liver) to the Ectodermal Integument category (skin). This LCCL would start the clinical staging again at LICL.

NB- At the present time ICMS only considers homologous use of cell lines as meeting minimal safety for clinical application. By “homologous” it is meant that

the cell line is therapeutically applied to perform the same general function that it performed in its donor site. As examples:

1. EPC's and HPC's circulate in response to vascular injury to cause neovasclogenesis.[59] As such, their use for vascular and cardiac purposes would be homologous.
2. Bone Marrow MSCs participate in the repair of cartilage, tendon, bone, ligament in cases of multi-trauma.[4, 6, 11] These cells area also used throughout surgery to repair these tissues (micro fracture for cartilage) or to augment repair of these same tissues (same procedure BMAC centrifuge systems).[60] As such their use for these purposes would be homologous.
3. VSEL's circulate in response to various multi-system insults.[21-23, 61] As a result, their application as homologous would be broader.
4. There is little evidence that bone marrow derived MSCs participate in repairing brain tissue, so their use for this purpose would be non-homologous.

Examples of Cell Line Definitions:

In summary, the definition of a unique cell line for the purposes of these guidelines and clinical translation is defined by:

1. A state of differentiation (PC, GLSC, PSC, TSC)
2. A specific category of cell line type (MSC, HSC, EPC, ESC, VSEL)
3. A tissue origin (bone marrow aspirate-PSIS, synovial fluid-knee, muscle-deltoid, dermis-posterior auricle, etc...)
4. Cell culture parameters including seeding density, serum type and concentration, media type and volume, incubation time, and "other factors".
5. Tissue Use: The general tissue type where the cell line will be used (i.e. Mesodermal-Orthopedic).

As discussed in the ICMS Lab Practices Guidelines, the phenotype and/or genotype must also be periodically tested to assure stability in the cell line.

Example 1: A mesenchymal stem cell line could be defined as:

1. State of differentiation: GLSC
2. Cell line type: MSC
3. Tissue Origin: Bone marrow aspirate-PSIS
4. Cell culture parameters:
 - a. Cell Isolation: Adherence
 - b. Seeding density: Medium
 - c. Serum type/concentration range: Platelet lysate at 5-20%
 - d. Media type/volume: A-MEM at 20ml
 - e. Incubation time: 10-17 days
 - f. Other factors: Hypoxic colony formation

5. Tissue Use: Mesodermal Vascular

Example 2: A hematopoietic stem cell line which could be defined as:

1. State of differentiation: GLSC
2. Cell line type: HSC
3. Tissue Origin: Bone marrow aspirate-PSIS
4. Cell culture parameters:
 - a. Cell Isolation: Centrifugation
 - b. Seeding density: Low
 - c. Serum type/concentration range: Non-inductive FBS at 5-20%
 - d. Media type/volume: A-MEM at 20ml
 - e. Incubation time: 3-5 days
 - f. Other factors: Normoxic, VEGF activated
5. Tissue Use: Mesodermal Vascular

Each distinct cell line based on the above parameters will receive an ICMS registry identifier, which is comprised of the cell type, and the registry number. As an example, an MSC line might be known as ICMS Registry MSC134. An HSC line might be known as ICMS Registry HPC021. Ten exemplary samples, eight taken from unique patients and two from the result of two culture expansions of the same patient, will be stored on a permanent basis in -150 C cryo-storage. These will be used to further identify the cell line if complications arise or for further research study if one cell line proves superior to others in treating certain diseases. The ICMS Reimplantation Registry will track all outcomes and complications related to each stem cell line by its ICMS registry identifier.

A physician may have a clinical rationale for using more than one cell line at a time. ICMS does not consider this to be the creation of a new cell line. However, for the purposes of outcomes and complications tracking, the patient receiving more than one cell line as treatment must have the function of each line be homologous. In addition, the outcomes and complications tracking should be under all cell lines being used. For example, using the same cell line in the same patient would raise the following issues:

1. The clinical translational staging of each line must be considered. For example, this may be EICL for MSC134 and LCCL for HPC021.
2. The cell lines would be entered into the ICMS Reimplantation Registry as entries for each cell line as well as the combination of the two cell lines. For example, the patient would be tracked under MSC134 and HPC0021. A separate entry in the registry would be for MSC134/HPC021.

Cell Line Nomenclature:

In order to help physicians quickly recognize key facts about a cell line, the ICMS cell line nomenclature breaks a cell line definition into a string of characters that

identifies most of the information discussed above. The string contains the following information in five components separated by commas:

1. State of Differentiation: Either DC, PC, GLSC, PSC, or TSC
2. Cell line type: Either MSC, HSC, NSC, EPC, ESC, or VSEL
3. Tissue Origin: Either BM-Bone Marrow, SF-Synovial Fluid, AD-Adipose Tissue, MU-Muscle, DE-Dermis, or PB-Peripheral Blood.
4. Cell culture parameters: The whole section in brackets “[]” and preceded by CULT-, and then separated by commas in the following order:
 - a. Isolation: I followed by a “-” and then either CF-Centrifugation, GR-Gradient, AD-Adherence, FL-Filtration, or SA-Surface Antigen. If multiple steps are used, all should be listed and separated by commas.
 - b. Seeding Density Range: SDR followed by a “-” and then either L-Low, M-Medium, or H-High. If multiple seeding density ranges are used in the cell culture, then all should be listed and separated by commas.
 - c. Serum Type and Concentration-Only serum type by SER followed by a “-” and then either:
 - i. FBS-Fetal Bovine/Calf Serum
 - ii. PD-Platelet Derived-Platelet lysate, platelet rich plasma
 - iii. S-Human serum
 - iv. SF-Serum Free-No animal or human serum is used
 - d. Total quantity and type of media is omitted from the nomenclature.
 - e. Incubation time: TIM followed by a “-” and then either S-Short duration-defined as less than 7 days, M-Medium duration-defined as more than 7 days and up to 28 days, or L-Long duration-defined as more than 28 days and up to 60 days
 - f. Other Factors: These are listed in parentheses “()” which is begun with the designator OTH- and then the following are separated by commas:
 - i. Oxygen level: O2 followed by a “-” and then either NORM-Normoxic, HYPO-Hypoxic, HYPE-Hyperbaric
 - ii. Growth Factors: GF- and then one or more of the following in {} separated by commas:
 1. TGF-any of the transforming growth factor beta family
 2. PDGF-any of the platelet derived growth factor family
 3. IGF-any of the insulin like growth factor family
 4. VEGF-any of the vascular endothelial growth factor family
 5. FGF-any of the fibroblast growth factor family
 6. SDGF-any of the stromal derived growth factor family
5. Tissue use: One of the following:
 - ECTO/INTEG-Ectodermal Integument
 - ECTO/DIG-Ectodermal Upper/Lower Digestive Tract
 - ECTO/NEURO- Ectodermal Neurological

- MESO/VASC- Mesodermal Vascular
- MESO-ORTHO- Mesodermal Orthopedic
- MESO-ORG- Mesodermal Organ
- MESO-DERM- Mesodermal Integument
- MESO-URO- Mesodermal Urogenital
- ENDO-ORG- Endodermal Organ
- ENDO-RESP- Endodermal Respiratory
- ENDO-URO- Endodermal Urinary
- ENDO-DIG- Endodermal Digestive Tract

Any component that doesn't apply to that particular cell line should be omitted from the string. If two cell lines share the same string, they should be followed by a space and then a designator 01, 02, 03, etc.

Nomenclature Examples

To continue the example discussed above (MSC and HSC cell lines), the following strings describe these lines:

Example 1: GLSC, MSC, BM; CULT-[I-AD, SDR-M, SER-PD, TIM-M, OTH-(O2-HYPO)]; MESO/VASC

Example 2: GLSC, HSC, BM, CULT-[I-CF, SDR-L, SER-FBS, TIM-S, OTH-(O2-NORM, {GF-VEGF})], MESO/VASC

Using a Specific Cell Line in Practice:

Cell Line Quality Assurance:

To ensure that any negative treatment outcome of an autologous stem cell culture process can be appropriately tested and tracked, ICMS recommends the use of a QA sample saved in cryo-storage (-150 C) for every completed cell culture. This is in addition to the exemplar lines stored as part of the ICMS Reimplantation Registry.

Clinical Use Recommendations:

As discussed above, once the stem cell line is established by its parameters, it must go through the clinical staging as described above. The ICMS guidelines do not permit the use of PICL, EICL, or PICL cell lines in routine clinical practice. ECCL and LCCL cell lines can be used in early clinical practice with the above noted restrictions. However, only "Clinical Grade" stem cell lines would have unrestricted clinical use with regard to numbers of patients allowed for treatment. Even patients being treated with a CG cell line must be entered into the ICMS re-implantation registry for long-term tracking and follow-up.

Significant Complications:

Significant complications are defined as an impairment of health or a condition of abnormal functioning that is directly caused by stem cell re-implantation. This does not include direct procedural complications such as infection, inflammation, tissue damage physically caused by the re-implantation, or manipulation of tissue. While these procedural complications should be recorded and could be

used in the overall assessment of procedure safety, they are not considered as directly related to the stem cell line.

Other Considerations

In addition to the above, the ICMS considers the following to be basic tenants of clinical translation:

- All research protocols using EICL and LICL cell lines should be approved by an Institutional Review Board (not required for an established (CG) or LICL cell line used within the same tissue category).
- ICMS takes no position on how research is funded and due to the dire need for treatment options for patients approves the use of “pay for trial” type research.
- A blinded standard is not necessary for this phase of research. However, there should be measurement of both clinical outcomes and objective imaging or other objective changes.

We encourage all ICMS physicians to conduct blinded clinical trials. ICMS physicians will pool resources to allow these trials to be undertaken so that insurance reimbursement can be sought for therapies that pass this level of evidence.

Section D-Tumorigenesis and Contraindications

A theoretical risk of A-ASC therapy is tumorigenesis. While this risk is believed to be reduced for A-ASC therapy relative to allogeneic stem cell therapy, the risk should still be mitigated. **As a result, the ICMS does not approve of allogeneic stem cells transplants at this time. In addition, the ICMS does not approve of more than *minimal* culture expansion as of the date of these guidelines.** Minimal cell culture expansion is defined here as less than 10 culture passages or less than 60 days in culture. These precautions are designed to minimize the probability of malignant transformation in the cell line.

Research on adult stem cells and cancer has focused mostly on mesenchymal stem cells.[51, 62-64] Several authors have argued that culture expansion of MSCs for less than approximately 60 days (up to approximately 10 culture passages), poses no significant risk of malignant transformation.[29, 65]. In addition, appropriate telomere shortening (indicating lower risk of neoplasm) has been shown to be present in MSCs in culture.[65] Given the balance of the data, while a risk is present, the ICMS balances that risk by strict clinical translation guidelines and complications tracking.

In particular, mesenchymal stem cells when injected directly into a tumor in experimental animal models can lead to tumor propagation.[66-69] As a result, the re-implantation of any A-ASC line directly into a region of active malignancy is contraindicated. The ICMS expressly recommends against implantation of an

A-ASC line in a patient who has had an active malignancy within the 5 years prior to the planned treatment. An exception can only be made if the planned treatment has shown that the specific cell line being utilized will not leave the re-implant site and the re-implant site is distant to neoplasm site. Even in these circumstances, a significant interval of time should have passed between the last documented active neoplasm and the treatment. There should also be negative imaging or other studies for the presence for active neoplasm. As an example, MSCs have been shown to stay resident in a joint when injected into that joint. In a patient with a prior prostate cancer, injection of MSCs may be allowed into a distant knee joint only in situations where PSA and imaging have shown no active neoplasm for a reasonable interval (12-24 months) and the physician has no reason to believe that there is any clinical evidence of recurrence.

Other absolute contraindications to A-ASC therapy include:

- Severe anemia
- Blood dyscrasias
- Active infection
- Systemic illness that would increase the risk of an adverse outcome

Section E-Medication Restrictions

The state of knowledge regarding the effect of medications on A-ASCs is evolving. At the time of dissemination of these guidelines, the ICMS will maintain an updated list of such medications and their effects on A-ASCs. It must be emphasized, however, that it is incumbent on each and every physician who uses these guidelines to be responsible for keeping track of the most recent literature on the cell line being used concerning the effects of medications on that cell line.

Section F-Rehabilitation

While A-ASC therapy has the potential to repair damaged or injured tissue, consideration of how the tissue became damaged is essential to clinical practice. As an example, if the patient treated were a type II diabetic, then in addition to treating the pancreas, the clinician should also work concomitantly on weight loss and exercise to promote healthy pancreas function.

In particular, the musculoskeletal system is susceptible to biomechanical overload. As a result, it is suggested that treating damaged tissue is not complete without also treating the biomechanical causes of the tissue damage. A comprehensive multidisciplinary rehabilitation evaluation by the appropriately trained physician(s), physical therapist, occupational therapist, nutritionist, social worker and psychologist to create a rehabilitation treatment plan customized for the individual patient and specific tissue pathology repaired through A-ASC therapy will promote optimal recovery and outcome. This approach allows for the

best possible frame of mind, conditioning, diet, therapy, and life style change that will not only benefit the treatment of the damaged or diseased tissue but address the disease itself that led to the tissue impairment. An ongoing preventative way of life thereafter as a result of the comprehensive rehabilitation program will hopefully lead to better disease management, reduced morbidity, extend longevity and avoid further compromise of tissue.

Section G-Physician Training Requirements

All physicians involved in A-ASC therapy must have a significant knowledge of the peer reviewed literature on the cell line used for treatment. . This should include the possible outcomes and complications of treatment.

The peer reviewed literature as of the date of this guideline has demonstrated that many adult stem cell lines can't be delivered with efficacy via a peripheral intravenous route due to a pulmonary first pass effect.[70] This means that for the time being, effective stem cell therapy means delivery of cells to the intended target tissue. As a result, the physician should have the requisite training necessary to deliver the cell line to the intended target tissue. As an example, stem cells delivered via percutaneous means to the intervertebral disc should only be performed by a physician with formal training in disc access procedures and appropriate board certification (pain management, anesthesiology, interventional radiology, etc...). As another example, the delivery of stem cells to the pancreas should only be performed by a physician with formal training in interventional access to the GI system or surgical training to access the same site. This would include physicians boarded in interventional radiology, general surgery, or gastroenterology.

Complications of any procedure must be able to be reasonably managed by the physician performing the procedure. Physicians who can't document formal training in the body system where stem cells are to be deployed should not be involved in treating that tissue. As an example, a physician without formal musculoskeletal training should not deploy cells in the musculoskeletal system just as a physician without formal cardiology training should not deploy cells into the heart or peripheral circulation.

Section H-Complications Tracking

All physician members of the ICMS are required to report any complication of A-ASC therapy to the ICMS Autologous Adult Stem Cell Re-implantation Registry. The ICMS will maintain, monitor, and analyze the data in the Registry and provide regular reports to the ICMS membership as well as any oversight bodies. Failure to report a complication may result in ICMS sanction against a member.

Section I-Re-implantation of A-ASCs

Once A-ASCs have been appropriately transferred from the lab to the clinic, several considerations must be monitored by the treating physician:

1. Time in container: Many A-ASCs have limited life spans outside of the body or outside of optimum in-vitro culture conditions. The time in container must be monitored and must be controlled. The maximum time in container should be known for each cell line.
2. Use of radiographic or other contrast agents: Many A-ASC lines are sensitive to contrast agents. The use of these agents must not exceed the concentrations and doses known to have adverse impacts on A-ASC survival.
3. The use of local anesthetics: Many local anesthetics will also have negative impacts on A-ASCs. The use of local anesthetics agents must not exceed the concentrations and doses known to have adverse impacts on A-ASC survival.
4. Placement of A-ASCs: Implantation of A-ASCs in the target tissue must be performed to maximize:
 - a. Survival: Many poorly vascularized tissues will not or will only minimally support A-ASC survival.
 - b. Containment: Many A-ASC lines need to attach to local tissue to promote engraftment. Placement of these cell lines must focus on conditions which will maximize attachment. If the cell line does not attach to the target tissue, then physical containment may need to be used. In addition, consideration needs to be used regarding appropriate scaffolding materials if needed.
 - c. Dose: Many A-ASC lines have optimal dosing for each tissue. If animal data is unavailable on appropriate dose, formal dosing studies should be undertaken or clinical experience with dose should be used. However, since A-ASCs are living tissues, accurate dosing may or may not be feasible, as biologic activity of each sample will impact dose. As an example, A-ASCs from younger patients who are healthy may need to have their dose lowered compared to older and/or infirmed patients.
 - d. Placement: ICMS recommends local placement of all A-ASCs directly into the target tissue with the least invasive method that is practical.

Section J-Prospective Candidacy Grading

The physician should use very effort to prospectively grade each A-ASC therapy candidate into Good, Fair, or Poor candidate categories. While scientific data on candidacy grading may or may not be available, oftentimes pragmatic grading systems can be used until such data becomes available. This grading should therefore take into account:

- Objective indications of disease severity: For example, a patient who has more severe disease may be less likely to respond than a patient with mild disease.
- Co-morbid conditions: For example, a cardiac treatment may be less effective in a patient with significant co-morbidity such as COPD, type I diabetes mellitus, and PVD.
- Patient history: For example, a patient with a history of severe osteoporosis may be less likely to respond to a fracture healing application.
- Prior physician experience with response rates: For example, the physician may have experience that a cosmetic application is less likely to be effective in patients over 80 years of age.
- Contraindications: For example, the patient may have a history of active malignancy.
- Factors that may reduce outcome: For example, the patient may be taking medications that negatively impact A-ASC yields.

At no time should any physician provide a Good candidacy grading to all patients, unless strong level I evidence exists to support this grading.

Section K-Compassionate Use

The ICMS recognizes that there are patients who cannot wait for stem cell therapies to become a mature treatment. For these patients with terminal illnesses, death or severe disability will almost certainly occur before large scale, randomized controlled trials are complete. As such, ICMS condones the use of compassionate A-ASC stem cell treatments (of the type described in this document, autologous, minimal culture expansion, homologous use, physiologic cell culture) under a narrowly defined set of guidelines. To qualify for compassionate use the patient must:

- Have been given a diagnosis of a likely fatal illness. Examples include COPD, Coronary Artery Disease, and Congestive Heart Failure.
- Have a written statement from a board certified physician in the same area of specialty of the likely fatal disease that states that the patient is end stage with an incurable disease, that no other types of care are available or other reasonable alternatives have failed, and the patient's condition is expected to worsen.

If these conditions are met, the patient can be treated with a PICL or greater without restriction. As with the PICL line definition, multiple animal models must be available showing efficacy with the cell line. The patient must also have his/her treatment approved by an IRB as described in this document (to ensure patient protections).

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