HOW TO TREAT PATIENTS WITH ADULT STEM CELLS WITHOUT SPECIFIC FDA APPROVAL AND WITHOUT THE NECESSITY OF CONDUCTING ANY PRIOR CLINICAL TRIAL

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Dedication

To my beloved husband Israel, my precious children, my son Abel Enrique and my daughter Adaia Isabel. To my mom Gladys, my aunt Sonia and my family, thank you all for the support along these years.
HOW TO TREAT PATIENTS WITH ADULT STEM CELLS WITHOUT SPECIFIC FDA APPROVAL AND WITHOUT THE NECESSITY OF CONDUCTING ANY PRIOR CLINICAL TRIAL

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Abstract: This research reviews the new study categories in which, a patient, can receive treatment using autologous adult stem cells. These new categories are legal under the FDA regulations: “Patient Sponsored”, “Open Label” and “Institutional Review Board” studies. This research also explore the modality of Point of Treatment Centers in which adult stem cells are isolated from the patient and re-injected the same day.

Keywords: autologous adult stem cells; adipose tissue; mesenchymal stem cells; point of treatment center; FDA; Patient Sponsored; open label; institutional review board.
I. Introduction

The U.S. Food and Drug Administration on the regulation of adult stem cells is “…since the intention of stem cells is similar to a medical product (treat, cure or prevent a disease) it generally requires FDA approval before they can be marketed” [1]. According to their website, the FDA warns the consumers about any stem cell treatment they might use; the agency wants the general public to only use those stem cells treatments that are approved by them and/or are under clinical investigations that has been submitted to and are allowed to proceed by the agency [2]. The only approved treatment by the FDA is the use of Hemacord which is a cord-blood derived product (hematopoietic progenitor cells) to be used for those diseases that affects the blood-forming system including blood cancers, some inherited metabolic and immune system disorders [2].

The opposing view is that the use of autologous adult stem cells should not be regulated by the agency. According to Dr. Joseph Mercola there are a great potential and benefit with the use of adult stem cells; as he mentioned “a truly exciting part of the future medicine, with seemingly limitless potential for anti-aging, arthritis and far more…”there is a huge history of the FDA attacking natural products and procedures that seems to be a threat to the big businesses (pharmaceuticals, agriculture, etc.)” [3]. Author Richard Epstein concluded on his report The FDA’s Misguided Regulation of Stem-Cell Procedures: How administrative overreach blocks medical innovations that the FDA has to change their traditional uniformed paternalism style and that the choice of which treatment to take has to be on the patient’s hands, not the FDA [4]. Congressman Berkley Bedell pointed out on his article Why can’t we use our own stem cells to heal our bodies? that FDA regulation at 21 CFR 1271.3 is bad for adult stem cells [5].

Physicians might want to incorporate therapy with adult stem cells to treat their patients. There are three new categories of studies that scientists and physicians are using that are legal under FDA regulation, yet avoid the need for specific approval and prior clinical trial. These categories are:

1. “Patient Sponsored” study: What seems to be happening is that a physician or company attempts some treatment modality with autologous stem cells and instead of government sponsorship, the patients pay for the study, usually a relatively small amount, and every patient receives the treatment, no placebo involved.
2. “Open Label” study: When you look at masking, they say, open label. This means no randomized or double blind study, but rather, both physician and patient know they are receiving the treatment and what the treatment is and involves.
3. “IRB” approval (which stands for Institutional Review Board): If you get institutional review board approval, like from any hospital or non-profit, or maybe even for profit entity, one can conduct a patient study without specific FDA approval.

The objective of this research review is to explain how to treat patients with adult stem cells using the new categories of studies that are legal under the FDA regulation.
II. Materials and Methods

Information regarding FDA regulations on stem cells, patient sponsored, open label and IRB studies were search using Google. Stem cell protocols and treatments were searched on different databases. These databases included Google Scholar and Research Gate. Webpages from stem cell treatment centers were accessed in order to obtain information regarding the stem cells isolation, expansion and application to patients. Stem cells treatment centers were contacted via email and, in the case of U.S. Stem Cell Clinic, a meeting with the Chief Scientific Officer (CSO) Kristin Comella was held. The objective of this research review is to explain how to treat patients with adult stem cells using the new categories of studies that are legal under the FDA regulation; also to discuss different approaches and protocols to isolate and expand stem cells, thus determine which protocol is the best to be used by physicians.

III. The Three Categories of Allowed Therapies that are FDA Compliant

Example 1: Open Label Study [6]

On this study adipose derived stem cells are going to be used to treat patients who suffered of human Chronic Obstructive Pulmonary Disease (COPD). This is a study in which the main goal is to prove it safety and how effective is in improving health of patients that COPD. For the purpose of this study the primary outcome measure is the functional capacity improved compared to baseline; with a timeframe of 3 and months. The second outcome measure is quality of life improved compared to baseline; with a timeframe of 3 and 6 months as well. The estimated enrollment is 200 patients. The autologous stem cells are derived from the patient’s adipose-derived tissue. The intervention of the patients to extract the stem cells is as follows: 120cc of fat are extracted from the patients using liposuction using local anesthesia and syringe collection. This adipose tissue is transferred to a laboratory in order to separate the adipose tissue-derived stem cells. Once this is achieved the stem cells are transferred for IV delivery into the patient. This is an open label study, meaning that it is a no randomized or double blind study; both the patient and physician know that the treatment the received and what it is. A great example of the use of autologous stem cells and how this treatment can be available in the United States by working around the FDA.

Example 2: Patient-Sponsored Study [7]

This is a study which use autologous stem cells harvested from adipose tissue to treat Type 2 diabetes. It is being conducted by the US based company Bioheart (now called U.S. Stem Cell Clinic) and they estimated that two-thirds of participants will experience a significant improvement and symptom reduction. Patients that are eligible for this treatment are any that suffered of diabetes type-2. The procedure is divided in four stages and it is performed at the doctor’s office. The stages are:
- Adipose harvest: A mini liposuction is performed in the stomach to extract a small amount of adipose tissue; this tissue contain tens of millions of adipose stem cells [7].
- Laboratory processing: The extracted stem cells will be isolated, analyzed, clean and concentrated [7].
- Stem Cell Implantation: Up to 60 million stem cells will be transplanted to the patient. Rejection risk is minimal since the cells are autologous [7].
- Postoperative care: Participants will leave after the procedure and RSCI will check the progress of the patient on a monthly basis for the first year after the treatment [7].

This is a patient-sponsored study and the cost of the treatment and the follow-up care is $5,000. This treatment can be done in other centers worldwide but it will cost more. The participation is limited to 100 Type-2 Diabetes patients.

**Example 3: Open Label/Patient-Sponsored Study** [8]

Integrative Stem Cell Institute (ISCI) developed an integrative medical approach in which combines autologous adipose derived mesenchymal cells or bone marrow mesenchymal and hematopoietic cells with other preconditioning medical therapies (parenteral cellular activation, hyperbaric oxygen therapy and physical therapy). This approach is under evaluation in a series of open-label, non-randomized, patient sponsored clinical trials in order to measure effectiveness and safety. These trials are divided into two different arms:

Arm 1: Are non-differentiated, unexpanded mesenchymal stem cells which are harvested from the patient and processed the same day [8]. These cells are re-injected back to the patient using direct injection or fluoroscopic guidance, or via endovascular catheterization.

Arm 2: Adipose mesenchymal cells are harvested from the patient adipose or bone marrow tissue. These cells are culture expanded and differentiated, processed in ISCI laboratory and re-injected back to the patient using direct injection or fluoroscopic guidance, or via endovascular catheterization [8].

Patients are treated in the ISCI facility in Cancun Mexico. Follow up will be conducted for two years at 2 and 4 weeks, 3, 6, 12, 18 and 24 months after the procedure. Data will be collected and reported by patient/study employee of ISCI or by the treating physician. Data will be compared against a control segment of standard, non-treated patients. And to establish differentials in therapy modalities, safety data from Arm 2 patients will be compared against Arm 1 patients. The article also mentions that “Each condition will have a unique and custom inclusion and exclusion criteria” [8]. “Inclusion criteria” are those factors that allow someone to participate in a clinical trial while “exclusion criteria” are the ones that disallow someone to participate in a clinical trial. This means that, depending on the condition that is been treated, the participants will be chosen.
Again, we can see here how things can be worked around the FDA: Arm 1 of the study, since the cells are not culture and re-injected the same day to the patient, it can be performed in the United States. On the other hand, Arm 2 of the study has to be performed in Mexico since the cells are cultured and processed, something that the FDA does not allow in the United States.

**Example 4: Open Label/Patient-Sponsored Study [9]**

An open-label, non-randomized, multi-center and patient-sponsor clinical study for the assessment of treatment safety and effectiveness of Adipose-Derived Stem Cells (ASC) delivered via intravenous push for those patients that suffered of type II diabetes mellitus is ongoing. This study will be performed by The Ageless Institute in Miami, Fl. The methodology for the collection of ASC is via liposuction. Adipose-derived tissue will be collected and transferred to a laboratory in order to separate the adipose tissue derived stem cells. For the purpose of this study platelet rich plasma will be isolated from the peripheral blood of the patient, this will be used in a combination with the ASC for intravenous push administration.

The masking of this study is open-label and it will assessed safety and efficacy of the treatment. Trial size for this study is 500 participants. Another great example in how patients can have access to this treatments, without the need of further funding since they are paying for the treatment. Most important, all the 500 participants will receive the actual treatment.

**Example 5: Open Label Study [10]**

This study was discussed above and the intention is to assess safety and efficacy of autologous stem cells implantation for the treatment of COPD. Using a liposuction (tumescent syringe) in the abdomen area the adipose tissue will be collected; then this tissue will be transferred to the laboratory for separation of the autologous stem cells (isolation). These cells are then transfer for IV delivery. The number of cells injected will vary depending of the amount of tissue and number of cells obtain. The inclusion criteria are those patients with prior diagnosis of moderate or severe COPD – GOLD III and IV in a range of age of 25 and 80 years. The exclusion criteria are those females that are pregnant or nursing; females with childbearing potential unwilling to maintain contraceptive therapy for the duration of the study. Also those patients with exposure to any investigational drug or procedure within 1 month prior the study; those with active infectious disease have to consult their physician. Patients on chronic immunosuppressive drugs; patients with drug or alcohol problem; severe asthma and with history of cancer in the last five years.
Example 6: Open Label/Patient-Sponsored Study [11]

The Miracle Mile Outpatient Surgery Center (MMOSC) announced the creation of Los Angeles Stem Cell Institute which is going to provide open-label patient-sponsor SVF Investigational Protocols for the rejuvenation of joints, reversal of inflammation and the promotion of healthy tissue. The procedure to be used is as follows: Using a 20 minutes liposuction procedure, 50cc of fat are extracted to harvest 15cc of SVF rich in mesenchymal stem cells. The procedure takes about 40 minutes. What I like about this announcement is that they mentioned: “Using YOU to Rejuvenate YOU” [11].

Example 7: Open Label/Institutional Review Board Study [12] [13]

MD Stem Cells announced a new study using stem cells for retinal and optic nerve eye diseases. According to their website this is the “largest stem, cell eye treatment study registered with NIH” [12]. This is an open label/IRB study. The IRB approved this study since it satisfied every requirement. The Stem Cell Ophthalmology Treatment Study (SCOTS) will performed this study evaluating autologous bone marrow derived stem cells (BMSC) for treatment of retinal and optic nerve damage or diseases [13]. Patients are going to be injected with BMSC; these injections may include retrobulbar, subtenon, intravitreal, intraocular, subretinal and intravenous [13]. After the administration, patients will have 12 months of follow up with serial comprehensive eye examinations [13]. Here is an example of how to perform a study using an IRB. Another way to administer this treatments within the FDA regulations.

IV. Some Examples of Current Protocols for Physicians that Want to Treat Patients

Platelet Rich Plasma, Stromal Vascular Fraction and AdiStem

Before going deeper into the adult stem cells isolation and expansion and the examples of current protocols, it is pertinent and important to discuss what is Platelet Rich Plasma (PRP) and Stromal Vascular Fraction (SVF).

In approaching the isolation of autologous adipose derived stem cells, there are different techniques. These techniques will depend if the stem cells are going to be expanded or immediately administered to the patient. Here will be discussed different approaches and techniques used around the world including the United States.

Platelet Rich Plasma (PRP)

The use of Platelet Rich Plasma (PRP) as a source of growth factors for stem cells has been growing with the years. PRP is blood plasma enriched with platelets (Figure 1). It is important to mention that it is a concentrated source of autologous platelets and it contained several growth factors and cytokines which helps stimulate healing of bone and soft tissue [14]. It is use in different medical fields (i.e. cosmetic surgery, dentistry, sports medicine and pain management)
The use of PRP in tissue repair has a basis in the healing properties and the efficacy of certain growth factors that can be found on it. Platelets are collected in PRP and are activated by adding thrombin and calcium chloride, inducing the aforementioned factors from alpha granules. According to YU, W et al., the growth factors and cytokines that can be found on PRP are the following: platelet-derived growth factor, transforming growth factor beta, fibroblast growth factor, insulin-like growth factor 1 and 2, vascular endothelial growth factor, epidermal growth factor, Interleukin 8, keratinocyte growth factor and connective tissue growth factor [15].

PRP can be obtained by using one of the two methods that are approved by the FDA (as of 2009). The process is as follows: whole blood from the patient is collected and anticoagulated using citrate dextrose previous to go in a two-stage centrifugation (TruPRP) (Harvest), this process separates PRP aliquot from platelet-poor plasma and red blood cells [14]. PRP concentrates the platelets five-fold. PRP has been investigated and use in different medical treatments which includes nerve injury, chronic tendinitis, osteoarthritis, cardiac muscle injury and androgenic alopecia, bone repair and regeneration, plastic surgery, oral surgery and to treat sports injuries in professional athletes [14]. The cost of this procedure alone is approximately $1,000US.

![Platelet Rich Plasma (PRP) after blood centrifugation](image)

**Figure 1:** Platelet Rich Plasma (PRP) after blood centrifugation [16].

**Stromal Vascular Factor (SVF)**

SVF (Figure 2) is a rich source or cocktail of cells in which one can find “preadipocytes, mesenchymal stem cells (MSC), endothelial progenitor cells, T cells, B cells, mast cells and adipose tissue macrophages” [17]. SVF is a component of the lipoaspirate which is the waste product of liposuction contains a large amount of adipose derived stem cells (ADSCs); these cells have characteristics similar to bone marrow stem cells (multilineage differentiation) [17]. These cells also show high colony-forming unit frequencies, apparent pluripotent ability to differentiate to cells of neuronal phenotype and a high quantity of cells can be obtained from the lipoaspirate [18].

SVF can be obtained using the following protocol from Miltenyi Biotec. Lipoaspirate from human thigh or abdomen is washed in phosphate-buffered saline (PBS), this is done before enzymatic digestion with collagenase in order to obtain a single-cell suspension [18]. Then digestion is performed and the centrifuged pellet (which is the SVF) is resuspended in NH
Expansion Medium, this is prior to filtration through 100µm and then 40 µm nylon filters. The cells are counted and the sample is taken into culture in NH Expansion Medium. Within 24 hours, MSCs adhere to the plastic surfaces; the medium should be exchanged with fresh NH Expansion Medium. MSCs can be further enriched by magnetic separation using MSC Research Toll Box – CD271 (LNGFR), CD271 (LNGFR) Microbeads Kits or CD146 MicroBeads; these products are offered by Miltenyi Biotec.

Figure 2: Stromal Vascular Fraction (SVF) after centrifugation [17].

AdiStem

Through the years different products for adult stem cell isolation has been developed. One of these products is called AdiStem. This product is used for adult stem cell isolation from adipose tissue. It is widely known that a high concentration of adult stem cells can be found in adipose tissue, especially in the abdominal region due to sheer volume of availability; this ensures abundance in number of ASCs which ranged in the millions per unit volume [19]. This sheer number gives an advantage since the cells does not need to be cultured in a laboratory over days in order to reach the desired number of ASCs, this is called “therapeutic threshold” (therapeutic benefit) [19].

AdiStem uses PhotoActivation technology. They have researched the effect of different monochromatic light intensities and frequencies in the colored spectrum on MSC and white blood cells populations from human and animal sources. What they noticed with these experiments was that “low-level light photoactivation or photomodulation can be utilized for significant benefit in stimulating the proliferation, differentiation, and inhibition/induction release of growth factors/cytokines of cells from any living organism” [19]. This photoactivation is possible with the AdiLight-2 system.

Once the PRP is prepared it is activated using the AdiLight-2 system before injection into the area. According to AdiStem, in most cases, when the photoactivation is performed using the AdiLight-2 it increases Interleukin-1 Receptor Antagonist (IL-1RA) which decreases the pain and inflammation associated with PRP injections, thus duration of the pain is significantly reduce [19].

Benefits that the use of photoactivation has include: process duration (10 minutes); simple use of the AdiLight-2 it does not required monitoring; no training needed; it can be used with any high quality PRP kit; can be used for both orthopedic and cosmetic applications and it only requires
one injection per week for a period of three weeks [19]. It is important to mention that photoactivation is also used with Adult Stem Cells derived from Adipose Tissue.

AdiStem also have the cell extraction medium. This is a proprietary lecithin emulsifier that allows safe and easy extraction of stromal cells. The use of this medium ensures that the cells won’t be affected (cell viability, cell count and clinical efficiency) since it is plant-based. Lipids and connective tissue fragments from the adipose tissue are dissolved thus the concentration of stem cells is increased [19].

**AdiStem Protocol**

It is important to mention that AdiStem does not interfere with the therapeutic potential conferred by the ingredients (cells, proteins and growth factors) of the Stromal Vascular Fraction. For the purpose of AdiStem’s Fat Transfer protocol the composition of the extracellular matrix (different types of Collagen such as Types 1, 3-4, 14-15, 18 and 27 among others. Here is the protocol using adipose tissue (Figure 3):

First, a small amount of fat, approximately 100 cc is harvested from the patient’s waist area. This fat contains millions of dormant stem cells. Fat is then placed into test tubes. AdiStem extraction solution is added and then the stem cells are gently separated from the fat cells using a centrifuge. The separated stem cells are at the bottom of the test tube. Stem cells are then transferred into another test tube and set aside. 50 cc of blood are drawn from the patient to obtain PRP. Whole blood is centrifuged and the PRP is at the top of the test tube. The yellow PRP is removed and added to the patient’s dormant stem cells. The solution is now ready for Photoactivation using the photoactivation unit (AdiLight-2). This process of photoactivation takes approximately 15 minutes. Then the activated stem cells are placed into a syringe and then added to normal saline drip bag. The patient’s own activated stem cells are returned to the patient via standard intravenous drip this can last from one to two hours. A natural healing process occurs. Stem cells respond to injured or damaged tissue, this is due to the chemokines the injury release (stem cells are attracted to chemokines). This process is called homing. Stem cells helped regenerate damaged tissue and turn into new tissue. If the cells are going to be used to treat joint pain, the stem cells are injected directly into the joint.
Stem Cell Expansion and Point of Treatment Centers

U.S. Stem Cell Clinic is a company which works with the following cell types: adipose, bone marrow and muscle cells. From adipose and bone marrow cells, Mesenchymal Stem Cells (MSC) are harvested while from muscle cells myoblasts are harvested. U.S. Stem Cell Clinic has published papers on treatments with stem cells for the following diseases: COPD, osteoarthritis, cardiac, degenerative disease (vertebral column), traumatic brain injury and Parkinson’s.

They are focus in what is known as point of care treatment center. On this type of center the patient’s cells are harvested, isolated and injected the same day being stromal vascular factor (SVF) the preferred treatment. The approximate cost of this treatment is under $10,000 US. Stem cells are isolated from adipose tissue by performing a mini lipo-aspirate procedure using a small cannula and a local anesthetic; when compare with bone marrow one can obtain 500 times more stem cells in adipose tissue. 60 cc’s of adipose tissue are extracted from the patient; the mini lipo-aspirate takes approximately 30 to 60 minutes. From the adipose tissue SVF is separated. SVF has a mixture of different cells such as mesenchymal stem cells, hematopoietic stem cells progenitor and endothelial cells among others. All of these cells has their own growth factors that help promote healing. Then these cells are re-injected back to the patient for therapy purposes. It is important to mention that, additional to the aforementioned services U.S. Stem Cell Clinic gives, they offer training for physicians. The modalities this training is given are: in-person training course (“designed to provide personalized instruction and enable physicians to easily transfer essential stem cell to their practices”), online training course (taught by the Chief Scientific Officer Ms. Kristin Comella, gives the flexibility to learn at the physician’s own pace)
and on-site training course (“personalized hands-on training where one of our stem cell specialists provides one-on-one training at the physician’s clinic”). If a cell expansion is needed, U.S. Stem Cell will perform it for the physician. The cost of a cell expansion facility is above the $10M US. U.S. Stem Cell uses their own growth factors mix which is not published to the general public and are exclusively for their own use. Typical growth factors being used by U.S. Stem Cell Clinic are IGF and FBM. The Invitrogen website have several growth factors available. It is important to mention that U.S. Stem Cell is a registered FDA tissue bank for stem cells banking, meaning that samples from patients can be collected, cultured and expand. These cells are then banked for further use.

In order to have a point of care treatment center physicians should have the following equipment available: centrifuge, laminar flow hood, incubators, clean room and all the laboratory miscellaneous (pipettes, laboratory glassware, etc.).

On the other hand, U.S. Stem Cell Clinic are participating actively advocating against the regulation of stem cells by the FDA. The FDA 2016 hearing on stem cells was moved from April 2016 to November 2016 due to the overwhelming response from the general public. From a one day hearing now it will be a two day hearing. This is pursuing the work performed using SVF. U.S. Stem Cell position is that, one way that the FDA could pursue this type of regulation is through the Medical Boards which are state specific. An example of this is when the FDA tried to regulate Fecal Treatment.

Comparing U.S. Stem Cell with other clinics they offer: training for physicians, treatment for different diseases, point of care treatment centers, cell expansion service, cell banking. Their future plans are to further expand their treatment to other diseases; they are planning to stay in the US and not go international [21].

**Regenestem Network**

Regenestem offers stem cells protocols in adipose, bone marrow and blood stem cells. Regenestem Network offers training to physicians in the latest adipose, bone marrow and blood stem cell protocols. This is to be implanted in the physician’s office. The Network builds the website and all the social media presence for the office along with the maintenance. They offer on-going marketing consultation in order to keep the physician updated in any new protocol that is released.

The following equipment is needed: a centrifuge, bench top laminar flow hood, Adilight and a cell counter. The licensed personnel required for the operation of the clinic are Medical director and a lab technician for cell processing. In the case a person with a PhD in Stem Cell Biology wants to work or help a licensed physician in the clinic, he/she can work as a laboratory technician or as a consultant for the patients on any questions they may have concerning the science behind the treatments. As a hypothetical case: if someone wants to open a clinic in Puerto Rico the total investment would be $50,000 US. This investment includes the following: training, all the necessary equipment, websites, social media pages; as start-up kits: 10 kits of adipose, 10 kits of bone marrow and 10 blood kits. The start-up would be more than enough to recuperate the investment [22].
Since Regenestem uses AdiStem, the stem cell isolation protocol from adipose tissue that Regenestem follows is the same as explain above in the AdiStem section.

On a note; U.S. Stem Cell had a distribution agreement with Regenestem. This agreement was ended.

**Stem Cell Expansion Paper: Culturing Protocols for Human Multipotent Adult Stem Cells** [23].

A cell-based therapy platform for the modulation of inflammatory conditions, immune dysregulation and tissue repair can be created using multipotent adult progenitor cells (MAPC). This paper discusses culture procedures for human MAPC technology-based cell therapy which can be used for the manufacture of human cGMP MAPC technology-based products for use in clinical trials, “with emphasis on culture platforms that accommodate controlled scale-up, standardization, and automation” [23]. Complete details of the protocol may be found in *Culturing Protocols for Human Multipotent Adult Stem Cells* [23].

The authors made a series of important and relevant notes at the end of the paper. Here is the discussion:

- **Serum supplementation:** Even though they use fetal bovine serum (FBS), they acknowledge the fact and the importance of the development of a serum-free defined serum since factors present in animal serum influence a particular properties and/or behavior of human cells [23]. For now they used FBS and decided to identify a lot with optimal serum concentrations since culture medium supplement can provide a significant source of variability. Once this lot is identified a large quantity should be reserved in order to ensure as much consistency as possible.

- **Oxygen Levels:** These cells are cultured under hypoxic conditions (3 – 5% O₂) (MSC are expanded under normoxic conditions or 21% O₂)

- **Cell Density:** MAPC cells are seeded at low densities, approximately 200 – 2,000 cells/cm². They have a passage consistency sub-confluency of 30 – 70%. Meeting these conditions, with the combination of culture media and oxygenation levels describe on the paper, cell population can be routinely expanded for up to 15 – 20 passages, approximately 50 – 70 population doublings [23].

- **Trypsin:** It should be optimal; it is determine for each cell culture protocol, since the cell type and culture plastic surface used will impact the efficiency of cell detachment for expansion. The authors acknowledge the fact that there are xeno-free trypsin alternatives such as Accutase and TryPLE Select.

- **Culture vessel system:** Production of clinical grade material for clinical trials need larger scale expansion platforms, current equipment available for this purposes are 2-dimensional culture technologies (i.e. 10- or 40-tier culture vessels). This will cover clinical product at levels sufficient for early and mid-stage clinical evaluations (10-100 patients) [23]. For Phase 3 (clinical late-stage) production will require cell numbers that will depend on different types of cell expansion platforms providing controlled scale-up [23]. For this purposes the equipment under evaluation includes bioreactor systems and three-dimensional cell culture models based on permeable nanotube configurations [23].
- **MAPC product characterization**: For the development and application of therapeutic stem cell products, specific cell characterization assays are very important. A routine cell comparability testing panel is the way to go to determine donor/batch variation. Among the assays that can be included on the panel are markers (protein, mRNA, miRNA), profiling assays (for cell identity), biologic potency assays for in vivo activity. Also immunosuppression and pro-angiogenic activity is measure. Immunosuppression is evaluated in vitro on mixed lymphocyte reactions while pro-angiogenic activity is measure in vitro and includes the stromal cells or conditioned media ability to induce tube formation of human umbilical vein endothelial cells (HUVEC). Variability to this assay is added due to the need of accessory or target cell types. Surrogates potency assays are needed in order to overcome this. According to the author “MAPC based culture platforms, the expression of the cytokines CXCL5, VEGF, and IL8 has been reported to specifically correlate with their pro-angiogenic capacity” [23]; supporting the development of a matrix of ELISA assays which is a simple and efficient potency screen for specific MAPC cell therapy activity [23].

- **Cryopreservation**: The author acknowledges the availability of different cryopreservation systems such as CoolCell alcohol-free containers.

Here the author offers a scale-up approach which is needed to reach a therapeutical level of stem cells. $1 \times 10^9$ stem cells is the arbitrary number assigned as the number of transplanted stem cells necessary to reach a therapeutic result [24].

We have to keep in mind and in perspective that, the ideal environment for autologous stem cells to growth is autologous plasma. According to Stute et al. “there are reports that MSC’s grow better in autologous plasma than in fetal calf serum” [25]. This will also ensure that there will be no contaminants related to the animal serum entering the autologous cell culture. Even the author of the paper acknowledges the importance of a serum-free defined serum in order to avoid potential contamination.

### V. Conclusion

A physician wishing to treat a patient with autologous stem cells or cord blood stem cells can in fact initiate therapy via either of three mechanisms allowed by the U.S. Food and Drug Administration as exceptions to the traditional requirement for clinical studies. These three mechanisms are:

1. Patient Sponsored Study
2. Open Label Study
3. Institutional Review Board Study

This paper gives examples of such studies and also collects together what some treatment centers are doing right now, intended as a starting point for physicians wishing to pursue adult stem cell therapies.
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