Perinatal Stem Cell Society (PSCS) Advisory Recommendations for Perinatal Tissue Product(s) Safety and Quality

Attending members of the 4th Annual Perinatal Stem Cell Society Congress, held March 1-2, 2018 in Salt Lake City, Utah, resolved to develop a voluntary code of responsible conduct as a guidance for manufacturers, suppliers, and end-users of perinatal HCT/Ps (Human Cell, Tissue, and Cellular and Tissue-Based Products), with the purpose to raise the bar for the entire industry and to produce safer products. This advisory document contains non-binding recommendations and are in no way substitutes to the pertinent FDA regulatory mandates.

These recommendations aim to inform the reader of some of the key elements involved in the evaluation and understanding of perinatal cell and tissue products. In addition, we wish to encourage manufacturers, suppliers, and/or authorized medical professional users of perinatal HCT/Ps to adhere to existing FDA HCT/P regulations.

Examples of Currently Manufactured, Supplied, and Used Perinatal HCT/Ps (alone and in combination); not necessarily approved by FDA.

Amniotic fluid (cell-depleted)
Amniotic fluid cells
Amniotic membrane sheets (including cells and cell-depleted)
Amniotic membrane derived products (including cells and cell-depleted)
Placental-derived cells
Placental membranes (amnion & chorion; including cells and cell-depleted)
Placental-derived extracellular matrix (ECM; cell-depleted)
Umbilical cord Wharton's Jelly cells
Umbilical cord Wharton's Jelly derived extracellular matrix (cell-depleted)
Umbilical cord blood products (including cells and cell-depleted)
Derived exosomes from HCT/Ps

Product Safety

Many basic elements of product safety have already been well-established by existing FDA regulations and guidance documents. These documents, for example, ensure that HCT/Ps are procured under donor-informed consent and, where appropriate, approved by Institutional Review Boards (IRBs). Furthermore, HCT/Ps are prepared and handled using aseptic techniques, are free of pathogenic organisms that transfer communicable diseases, and are free of levels of chemical and biological agents that might cause adverse effects after administration to patients. Accordingly, PSCS certification for responsible conduct will require agreement to follow these existing FDA regulations and guidances for safety ("Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments; Inspection and Enforcement" [69 FR 68612]"). Briefly, from Current Good Tissue Practice (cGTP) Dec. 2011 covers:

"Core CGTP requirements (§ 1271.150(b)) are those requirements that directly relate to

preventing the introduction, transmission, or spread of communicable disease by HCT/Ps.

The core CGTP requirements include requirements for:

- facilities (§ 1271.190(a) and (b));
- environmental control (§ 1271.195(a));
- equipment (§ 1271.200(a));
- supplies and reagents (§ 1271.210(a) and (b));
- recovery (§ 1271.215);
- processing and process controls (§ 1271.220);
- labeling controls (§ 1271.250(a) and (b));
- storage (§1271.260(a) through (d));
- receipt, predistribution shipment, and distribution of an HCT/P (§ 1271.265(a) through (d)); and
- donor eligibility determinations, donor screening, and donor testing (§§ 1271.50, 1271.75, 1271.80, and 1271.85)."

Additional Considerations for FDA 361 Designation

Presently, the PSCS is aware of some perinatal HCT/P products being sold without FDA New Drug Application (NDA) approval and having the potential of being harmful to patients and of indeterminate efficacy. This represents the biggest challenge in the perinatal industry where many companies are self-registered 361, when they should be included under 351 as per the guidelines.

According to the FDA guidance, 21 CFR 1271.10, an HCT/P is regulated solely under section 361 of the PHS Act and the regulations in this part if it meets all of the following criteria:

(1) The HCT/P is minimally manipulated;

(2) The HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent;

(3) The manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P; and

(4) Either:

> (i) The HCT/P does not have a systemic effect and is not dependent upon the metabolic activity of living cells for its primary function; or
>
> (ii) The HCT/P has a systemic effect or is dependent upon the metabolic activity of living cells for its primary function, and:

(a) Is for autologous use;
(b) Is for allogeneic use in a first-degree or second-degree blood relative; or
(c) Is for reproductive use.
(d) If you are a domestic or foreign establishment that manufactures an HCT/P described in paragraph (a) of this section:

(1) You must register with FDA;
(2) You must submit to FDA a list of each HCT/P manufactured; and
(3) You must comply with the other requirements contained in this part.
[66 FR 5466, Jan. 19, 2001, as amended at 69 FR 68681, Nov. 24, 2004]

Although not covered under the FDA regulations, the safety issue may not be with the perinatal HCT/P itself, but instead with the choice of administration route, dosage or lacking relevant safety studies/data for example. For instance, the presence of extracellular matrix components when injected intravenously could be harmful (e.g., activation of coagulation cascades). In contrast, localized injection into the synovial space of joints may not produce such adverse side effects. We exert companies to follow appropriate measures to ensure patient safety in this regard.

Instituting Product Quality Criteria
The nature and novelty of many perinatal products present unique challenges for meeting FDA regulatory standards. Previous FDA regulations and guidances have addressed good manufacturing standards (1,2) but have not addressed a detailed criterion for testing the quality or effectiveness of HCT/Ps. Ensuring HCT/Ps' safety requires the product to be free of infectious or toxic agents. In addition to the safety standard, the PSCS recommends that HCT/Ps should be characterized for their identity (ingredients) and potency, based on their proposed therapeutic mechanism. When stem cells are an important principle for the treatment, their specific dose should be determined. Treatments performed without any scientific evidence of efficacy have potential for detrimental effects to the field of regenerative medicine and should be considered unacceptable and unethical. Similarly, the risk associated with treatments performed without the use of available HCT/P quality assurance is unacceptable. An important element of the PSCS code of responsible conduct would be performance of quality assurance testing to ensure the quality of perinatal HCT/Ps. These tests would be applied along the perinatal HCT/P supply chain as appropriate to the stage of HCT/P production and use.

Some elements that we suggest should be considered when evaluating HCT/Ps is to determine their purity, identity, potency, and in the case of tissue stem cell-based treatments, the stem cell-specific dose. This last evaluation basis is in keeping with the FDA's Standards Coordinating Body for Regenerative Medicine's (SCB) 2019 listing of determination of stem cell-specific dose as a needed standard for stem cell therapies (Reference FDA SCB's "Landscape" 2019)

Contaminants: This component refers to the product being free of unwanted material, including contaminants (non-therapeutic), communicable disease agent, toxins, animal

components and microorganisms in the product. Communicable diseases include, but are not limited to those transmitted by viruses, bacteria, fungi, parasites, and transmissible spongiform encephalopathy agents. The FDA has outlined current Good Tissue Practices (cGTP) in Subpart D of 21 CFR 1271 (REF) and companies should be able to provide evidence that their products are cGTP compliant. Information regarding additives to products (e.g., cryoprotectants, preservatives, etc.) must be clearly included in the product information/description.

Presently, all HCT/P manufacturers claim cGTP compliance and this is confirmed by FDA inspections of these facilities. The PSCS believes that further certification of manufacturing facilities is necessary, and we suggest that current Good Laboratory Practices (cGLP) are followed and certified and that current Good Manufacturing Practices (cGMP) be followed and certified. The American Association of Tissue Banks (AATB), American Association of Blood Banks (AABB), and the International Organization of Standardization (ISO), among others, provide standards, audits and certification of facilities. The PSCS suggests that manufacturers seek appropriate certifications to further demonstrate the standards, under which these products are manufactured.

Example of communicable diseases (FDA 21 CFR 1271 Subpart A, 2):

- Human immunodeficiency virus, types 1 and 2; Hepatitis B virus; Hepatitis C virus; Human transmissible spongiform encephalopathy, including Creutzfeldt-Jakob disease; and Treponema pallidum, Human T-lymphotropic virus, type I; and Human T-lymphotropic virus, type II. For reproductive cells or tissues, a disease agent or disease of the genitourinary tract listed as follows: Chlamydia trachomatis; and Neisseria gonorrhea.

- In addition, products should include sterility testing (micro-organisms/fungi/yeast growth) and mycoplasma testing. Please refer to USP<85> The Bacterial Endotoxins Test and USP<71> The Sterility Test.

Cellular and Molecular Constitution: Perinatal tissue contains a heterogeneous mixture of cells, extracellular matrix proteins, cytokines and other components. It is incumbent upon all manufacturers of HCT/Ps from perinatal tissues to disclose the constituents and/or characteristics of those products. The PSCS recommends that the presence of extracellular matrix materials, bioactive cytokines and growth factors, and cellular components be characterized and disclosed. This disclosure will provide basis for avoiding elements that could be the basis for contra-indications in patients, present due to the nature of their harvest and processing.

The essential constituents of the product should be properly labelled according to FDA regulations. Labeling provides not only important information about ingredients, but other information such as intended use, route of administration (i.e. intra-articular only etc.), dosage, warnings, storage temperature etc., which should be adhered to for safety purposes. For more information please refer to the examples provided in this document.

Potency: The US FDA describes potency tests as measures of appropriate biological activity (3). Potency assay should be a test(s) that captures the key activities of the HCT/Ps product. Bioactivity assays are an available method to demonstrate the therapeutic activity of products. Examples include but are not limited to: T-cell activation, macrophage polarization, and in vitro angiogenesis. It is the recommendation of the PSCS that a pertinent bioactivity assay be utilized as part of the lot release criteria during the manufacturing process (to minimize lot-to-lot variability). For products whose essential therapeutic principles are tissue stem cells, the stem cell-specific fraction should be utilized.

Registry: The PSCS firmly believes in accountability of the manufacturers of HCT/Ps to ensure safe products to end-users. When possible, manufacturers should provide a means of tracking results in patients following the use of these products. With IRB consent, Manufacturers should be willing, able, and committed to follow results of products at short-term, mid-term and long-term timepoints. We recognize that compliance with this recommendation may be difficult, since it must also involve the treating entity. For example, it would be hard for a producer of an HCT/P to obtain patient outcome data from confidential clinical trials. The entity administering HCT/P treatments to patients and research subjects MUST be primarily responsible for ensuring their safety and ethical treatment. Manufacturers can reasonably only be held to providing HCT/P products that meet standards, certifications, and regulations specified by the treatment providers and the FDA.

Application of these perinatal HCT/P recommendations will improve the overall quality of manufactured HCT/Ps, improve end-user confidence in manufactured HCT/Ps, and further standardize the manufacturing processes for these products. These perinatal HCT/P qualifications will be crucial denominators for future treatment outcome analyses. In addition to increasing ethical treatments for end-users and enabling better investigations of treatment efficacy, the addition of quality assessments is likely to increase the safety and efficacy of perinatal products.

References:

1- Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use Guidance for Industry and Food and Drug Administration Staff. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM585403.pdf
2- 21 CFR 1271
3- FDA summary for basis of approval: BLA Ref.No. 96–0372, Carticel

Examples:

Note: Examples are provided for discussion purposes and are not necessarily indicative of final recommendations or products

Example 1. – Process Example- Non-Enzymatic Processing of umbilical cord tissue to derive a heterogeneous population of cryopreserved native cells

Umbilical cord tissue (UCT) is collected with proper consent and Institutional Review Board (IRB) approval. The de-identified mother's blood is collected, and anonymous background information is obtained for initial screening. Once passing initial infectious disease screening, the UCT can be processed using a mechanical, non-enzymatic method. Once the native, heterogeneous population of cells are collected and before the addition of any cryoprotectant (e.g., dimethyl sulfoxide; DMSO), a representative sample must be taken for characterization Quality Control (QC). Once the characterization QC sample is taken and placed in an appropriate sterile tube, the remainder of the native, heterogeneous population of cells can be prepared for cryopreservation with the addition of appropriate cryoprotectants, such as DMSO. Once the DMSO is added and the unit is transferred into an appropriate cryovessel (e.g., cryobags, cryovials), an appropriate sample is taken for final bacteriology QC testing. The native cells in the cryovessel can be cryopreserved using an appropriate control-rate freezer (CRF). Once the native cells have been cryopreserved, they can be placed in a quarantine liquid nitrogen (LN2) freezer, until confirmation that the unit has passed bacteriology QC. At that point, the unit(s) can be placed into long-term LN2 freezers. The bacteriology QC sample can be inoculated into appropriate, standardized bacteriology testing or sent out for third-party testing.

The characterization QC sample can be analyzed for viability, cell quantification, and marker expression using fluorescent-activated cell sorting (FACS). The cells can be aliquoted into appropriate FACS tubes and stained with appropriate viability dyes (e.g., 7-Aminoactinomycin D; 7AAD, propidium iodide; PI) and cell surface protein markers of interest (e.g., CD105, CD90, CD73, CD29, CD44, CD31, CD146, CD45, CD34, CD11b, HLA-DR, CD19). Note all samples should have appropriate isotype controls, unstained cells for background autofluorescence emissions, and compensation controls for multi-fluorescent staining. Furthermore, the flow cytometer should have its PMT voltages set using appropriate controls and not changed once validated. Once the units have passed all appropriate viability, cell quantification, cell surface protein markers, and bacteriology thresholds, it can then be released for appropriate use.

For those therapeutic uses based on tissue stem cells in the preparation, a final representative sample(s) for the cryopreserved lot should be analyzed for the tissue stem cell-specific fraction/dose. The determined stem cell-specific fraction/dose should added to the label of all aliquots of the preparation lot.

Example 2. - Potency-release criteria example – Perinatal Stromal Cells

One of the biggest challenges in describing guidelines for practical purposes is the uniqueness and diversity of products being developed for a myriad of applications. However, we would like to demonstrate few practical examples based on some assays currently used in our laboratory.

An important element (biggest challenge) is to understand the therapeutic mechanism of action (TMO) of a product, since the testing has to be pertinent to how it works. For example, if a T cell inhibitory molecule is being manufactured as an anti-rejection drug, then such a molecule should be tested in the laboratory, demonstrating its capability to

inhibit the proliferation of T cells at relevant dosages. Similarly, when working with perinatal-derived products (mainly FDA 351 HCT/Ps), understanding how the product works is of utmost importance.

For example, a laboratory may have an animal model that develops autoimmunity, and uses perinatal stromal cells (PSCs) to ameliorate the symptoms of such a condition. Hence, under the hypothesis, PSCs are capable of inhibiting the proliferation of antigen presenting cells (APCs). Therefore, the laboratory developed a model (**Figure 1**.) capable of measuring the capability of supplied PSCs to inhibit APC proliferation *in vitro*. After evaluating several PSC donors, the laboratory developed a potency criterion for accepting "good" donors versus "bad" donors; the next step would be to correlate the developed *in vitro* testing criteria with clinical/*in vivo* outcomes.

After determining the "acceptable" IC50, the laboratory will monitor every PSC donor cell batch and use that data as potency-release criteria before using it in the animal model.

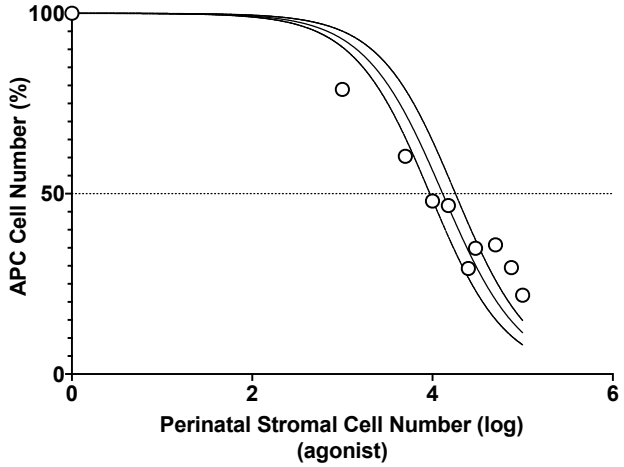

Figure 1. *Cell IC50 curve (Concentration of an inhibitor where the response is reduced by half), showing the inhibitory properties of human PSCs at different concentrations (log) when co-cultured with a fixed number of human APCs.*

Since there is no consensus on the particular mechanism of action of perinatal stromal cells, it is suggested that each laboratory/company should work on understanding how their products work and measure such a bioactivity as an evaluation method.

Example 3. Product example - Uncultured Human Amnion Epithelial Cells (hAECs). Current pre-clinical and clinical applications of hAECs include the treatment of stroke, multiple sclerosis, liver disease, diabetes and chronic and acute lung diseases. Researchers have shown therapeutic efficacy of hAEC in modulating inflammation due to their unique properties (REFS?).

In compliance with cGTP, donors will be tested and screened according to the eligibility requirements described in 21 CFR Part 1271, Subpart C - Donor Eligibility (final rule, "Eligibility Determination for Donors of Human Cells, Tissues and Cellular and Tissue-

Based Products"). The amniotic membrane is collected from donors that meet strict inclusion/exclusion criteria, with proper donor consent. The de-identified donor's blood is collected and Infectious disease testing is performed. Testing includes Hepatitis B, Hepatitis C, HIV-1/2, Human T-Lymphotropic Virus Types I & II , and Syphilis. The donor will complete a donor health history and history collected from medical record to include AFP and Quad screen test results if applicable. If any test results are confirmed positive and results obtained from medical record, the participant's physician will be notified with test results.

The protocol for isolation of hAEC involves a mechanical non-enzymatic isolation method that is to optimized for cell yield, viability and purity. Upon isolaton of hAECs, a suitable number of cells is set aside as a representative sample for Quality Control (QC) characterization or release criteria as determined by final application. This should include bacteriology/viral screening, or other safety or biological criteria. Characterization of cell yield, viability and purity is performed using flow cytometry data or manual counting with trypan blue exclusion. Viability is expected to be above 90%. Characterization of a cell surface marker profile is performed using flow cytometry for markers for epithelial phenotype (e.g., EP-CAM), and non-epithelial phenotype CD90/CD105. The purity of the cell isolate (the percentage of EpCAM positive and CD90/CD105 negative cells) is expected to be 90-95% and <1% respectively. The QC sample will be tested for sterility and endotoxin, and visually inspected as release criteria. Additional release criteria include criteria for potency, either a direct measure or using a surrogate potency biomarker where appropriate. For example, assays will be performed to demonstrate proven anti-inflammatory properties through standardized *in vitro* co-culture assays (e.g., lymphocyte proliferation assay) or *via* a surrogate protein assay based on demonstrated mechanism of action (e.g., Secreted protein/growth factor). Release assays will be performed on validated and/or calibrated testing equipment using approved SOPs. A written procedure for product release has been established and will be followed in order to release the product for use.

Once the characterization QC sample is collected, the remainder of the uncultured cell population can be prepared for cryopreservation with the addition of appropriate cryoprotectants, such as DMSO. The native cells in the cryovessel can be cryopreserved using an appropriate control-rate freezer (CRF).

For those therapeutic uses based on tissue stem cells in the preparation, a final representative sample(s) for the cryopreserved lot should be analyzed for the tissue stem cell-specific fraction/dose. The determined stem cell-specific fraction/dose should added to the label of all aliquots of the preparation lot.