Starting Materials Considerations for Cell and Gene Therapy Manufacturing

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Good Beginnings Make For Good Endings

Introduction

- Cellular starting material presents considerable challenges in developing and manufacturing cell-based products
  - Complex, heterogeneous, and variable cellular and non-cellular elements
    - Inter- and intra-individual variability
  - Effects of environmental conditions, time, demographics, etc.
  - Autologous products – potential effects of prior treatment

- Starting material is a major determinant of manufacturing process success -- high-quality cellular starting material is necessary for a high-quality final product
  - Sufficient number(s) of the desired cell populations/subpopulations, without excessive cellular and non-cellular contaminants
  - But specifications for these cell populations and contaminants may not be well understood

Cellular Starting Materials for CGT Manufacturing

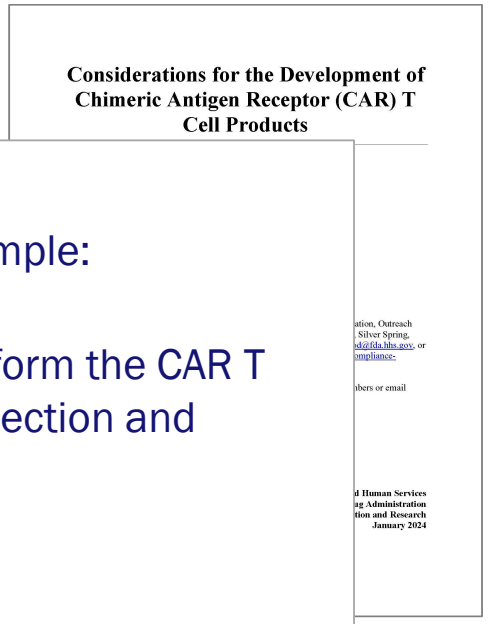
- Apheresis products
  - Unmobilized leukopaks
    - Lymphocytes ⇨ CAR-T cells, NK cells, activated T-cells, etc.
    - Monocytes ⇨ dendritic cells
  - Mobilized PBPCs
    - PBPCs for transplant, *ex vivo* gene therapy, etc.
- Bone marrow aspirates/biopsies
  - MSCs, transplant
- Non-hematopoietic tissue biopsies /explants
  - Skin, cartilage, muscle, etc.
- Multiple cell sources can be used for iPSC generation

Mononuclear Cell Collection by Leukapheresis

- Selecting an apheresis services provider
  - Apheresis product yield, purity, consistency
  - Size and characterization of donor pool
  - Collection protocols
- Apheresis services provider is a critical vendor
  - Audit, and put a quality agreement in place
  - Apheresis collections should be performed under an appropriate Quality system to ensure quality of apheresis product

Apheresis Services Provider Audit – Key Points

- Allogeneic donor screening and qualification per 21CFR1271?
- Procedures and equipment appropriately documented, qualified/validated?
  - If multiple apheresis collection sites, same procedures for all
- Vendors, supplies, and apheresis sites qualified?
  - Quality agreements for their critical vendors?
- Appropriate equipment and environmental monitoring?
- Adequate staff training and qualification?
- Acceptable documentation and records management?
- Management of exceptions and deviations, CAPA
- Tracking and trending quality indicators
- Management of donor reactions
- Internal and external inspections
  - AABB, FACT


Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products

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Cellular Starting Material

- Establish and refine acceptance criteria for the apheresis product. For example:
  - Minimum mononuclear cells, % viable cells, and % $CD3^+$ cells
- "Additional characterization of the leukapheresis starting material… may inform the CAR T cell manufacturing process as these characteristics may influence T cell selection and expansion and final CAR T cell quality."
  - % and absolute number of $CD4^+$ and $CD8^+$ T cells, NK cells, monocytes, B cells
- Autologous CAR T cell therapy
  - "**Particular consideration should be given to patients who have received CAR T cells previously.**"
  - Failure to respond to prior CAR-T cell therapy, relapse, or second malignancy
  - Residual CAR T cells in the newly-collected cellular starting material (i.e., apheresis product)
    - Potential for "unexpected effects on CAR T cell manufacturing (e.g., expansion or transduction rates), potency, *in vivo* expansion, safety, and efficacy"
  - Evaluate levels of previously-administered CAR T cells in the cellular starting material
    - "…detection of common vector or CAR features to evaluate the presence of previously administered CAR T cells."
  - Keep retains of the new apheresis product, in case additional analysis is needed

Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
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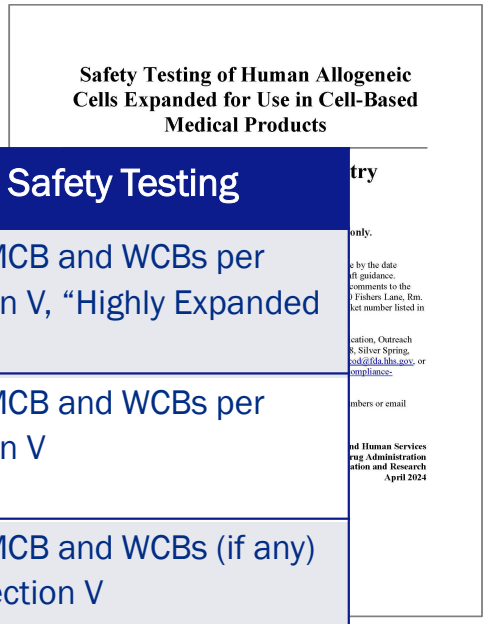
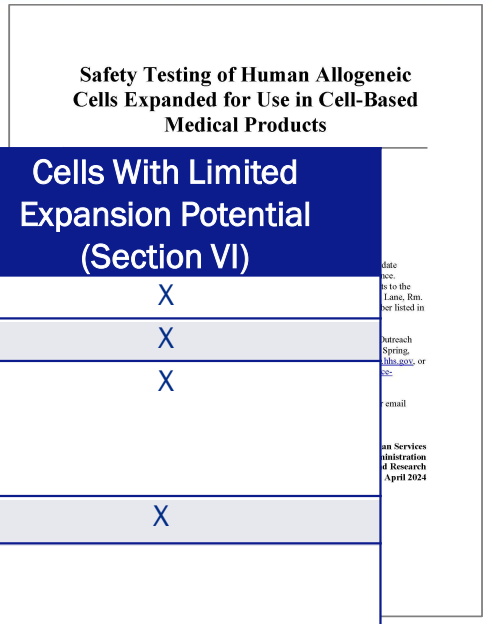

Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products

Cells	Cell Culture and Preparation	Product Use	Safety Testing
ES cells and allogeneic iPSCs	Cells expanded into MCB and WCBs. WCBs differentiated into final cell therapy product.	Potentially, many individuals	Test MCB and WCBs per section V, “Highly Expanded Cells”
Immortal cancer cell lines and transformed cell lines	Cells expanded into an MCB and WCBs. Cell-based product derived from WCBs.	Potentially, many individuals	Test MCB and WCBs per section V
Primary allogeneic cells capable of extensive expansion (highly expanded)	Cells expanded to make MCB. MCB vials thawed and expanded to make final product.	Potentially, many individuals	Test MCB and WCBs (if any) per section V
Primary allogeneic cells, including some genetically engineered cells, capable of limited expansion before loss of cell quality	Cells expanded several passages to make a small to midsized MCB or a single lot of cells that is the cell therapy product.	Limited number of individuals	Test MCB or lot of expanded cells, or EOP cells per section VI
Primary allogeneic cells expanded in culture	Cells expanded to make product lots of cells for a few subjects or a single subject.	A few individuals or a single individual	Test expanded cells for sterility, mycoplasma, and endotoxin

Partial Summary of Required/Recommended Testing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Testing	Extensively Expanded Cells (Section V) MCB	WCB	Cells With Limited Expansion Potential (Section VI)
Sterility	X	X	X
Mycoplasma	X	X	X
Specific pathogens: HIV 1&2, HTLV 1&2, HBV, HCV, CMV, EBV, Parvo B19, HPV, HHV-6, -7, -8, JCV (human polyomavirus 2), BK virus "as appropriate"	X		X
In vitro adventitious virus testing	X	X	X
In vivo adventitious virus testing	If "specific risk factors that are not fully mitigated by other types of testing"		
TEM	X		
Retroviral testing	If cultured on non-human cell feeder layers		
Species-specific virus testing	X		
Bovine- or porcine-derived virus testing (CFR 113.47, CFR 113.53(d))	If bovine– or porcine-derived reagents are used.		Additional safety testing if animal-derived reagents are used
Residual viral and plasmid reprogramming vectors -- iPSC lines	Cell bank, DS, or DP		
Whole genome sequencing and analysis	Cell banks of continuous cell lines and genome edited cells		

Solid tissue-derived cellular starting material

- Muscle, cartilage, pancreas, skin, etc.
- Quality of tissue biopsy/harvest is a major determinant of manufacturing process success
  - Manufacturing process begins in the OR/procedure room
  - Surgeons performing tissue biopsy are, in effect, manufacturing operators
  - Quality system must oversee the biopsy/harvest procedure and the surgeons performing it
- Collection method/technique, consistency
  - Identity and purity considerations apply
  - Ex. Is cartilage biopsy weight/size within spec? Cartilage : bone ratio? Trace back to collection sites and surgeons.
  - Re-training and other interventions sometimes needed. Can be challenging, particularly when surgical personnel are not directly connected to the therapeutic use of the product
- Transport conditions and duration
- Storage conditions and duration
- Initial tissue processing – cell isolation

Summary

- Quality of cellular starting material is a major determinant of manufacturing process success and product quality
  - Cell numbers, identity, and purity
- Apheresis products (leukopaks) are starting material for many cellular immunotherapies.
  - An apheresis services provider is a critical vendor and must be carefully selected and audited prior to arranging services and quality agreements
- Recent FDA guidance documents outline considerations for collection and testing of cellular starting material, including allogeneic cells expanded for use in cell-based therapies.

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