Cell Sorting and Cell Selection

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Goals

- Discuss the role of cell selection devices in clinical labs
  - Quick overview of several clinical trial
    - Goal
    - Procedure
    - No Clinical outcome data
- Discuss the decision process of what to use
- Discuss what is required from manufacturer before initiation of trial (from academic POV)
- Discuss the “issues”
Translational Research

Basic Science/Concept

Discovery

Process Development
Scale Up
Validation of Manufacturing
Custom GMP Reagents
QA Assays
Release Criteria/Potency Assays
IND Development/
Maintenance
Patents/Intellectual Property
Multiple Contracts
$$$/Academic support

“Engineering”

Clinical Research/Practice

Application
High Speed Cell Sorting
Dako MoFlow
High Speed Cell Sorting Trials at MDACC

- ALDH+ cell enrichment for cardiac repair
- ALDH+ cell enrichment for chronic critical limb ischemia
- CD34+90+ for Breast Cancer transplantation
- CD34+90+ for Sickle cell disease (Allogeneic)
- ALDH+ cell enrichment for cord blood expansion
- Elimination of alloreactive T cells for transplant
- Isolation of Antigen specific T cells (PR1)
- Isolation of myeloma cells for vaccine production
Laboratory Procedure
Aldegen Procedure

- 100ml HPC-M
- Ammonium chloride lysis
- CD15 and Gly-A depletion
- Cell Sorting of ALDH<br>cells
- Release assays
  - 65% ALDH+ cells
  - Viability >70%
  - Gram Stain negative
  - Endotoxin <5EU/ml
- Return cells to Hospital for reinfusion
  - Within 48 hours of collection
Benefits of High Speed Cell Sorting

- High purity of cell population
  - ‘elimination’ of tumor cells
  - Elimination of T cells
- Enrichment of a subpopulation
  - Cardiomyocyte progenitors
  - Gene modified cells
  - Tumors for vaccine production
Disadvantages of High Speed Cell Sorting

- Single use disposables
- Time...time...time
- Cost of equipment
- Cost of procedure
- Regulatory Issues
  - Reagents
  - Instruments
FDA issues

- Use of Mouse Monoclonal antibody
- Depletion of RBC procedure
- Use of antibiotics
- Lot release characterization
- Validation of route of administration
  - Catheter
- Sterility and endotoxin testing
Maintaining Sterility
Class 10,000 Suite
Monitoring Sterility

1. 1ml of supernatant from last spin
2. 1ml from negative fraction (including cells)
3. Pooled and sent for 14 day sterility
Cellerant Technology

n Unclassified laboratory
Cellerant : Applications

n Cancer – Breast Cancer
“361” Product

- Minimally Manipulated
- Homologous use only
- Not a combination product
- No systemic effect or if it does
  - Autologous, or 1st, 2nd degree relative
Hi Dr. Tani,
I spoke to Ms. Chicquita Hatten today from MB Anderson. I informed her that use of your product in autologous transplant for the treatment of breast cancer is consistent with our memo of February 16, 2005. We conclude that your study meets all of the requirements set forth at 21 CFR 1271.10, and, accordingly, that it is exempt from the requirements of Part 312 of the IND regulations.

The Food and Drug Administration published Human Cells, Tissues, and Cellular and Tissue-Based Products Regulations in the Federal Register (#66 FR 5460), on January 19, 2001, which became fully effective on January 21, 2004. Those regulations state that human cells, tissues, and cellular and tissue-based products (HCT/Ps) are regulated solely under the authority of section 301 of the Public Health Service Act if it meets certain criteria. Your cellular product meets those criteria.

However, this does not exempt you from the requirement to obtain
CLT-001 - Manufacturing Process

1. Mobilized Leukapheresis Product from Patients/Donors
2. CD34+ enrichment using Isolex Device
3. Cell Staining & Sorting using MoFlo Sorter
4. Formulation & Cryopreservation

QC QC
‘361’ Product

- No pre-approval of the study by FDA
- GTPs apply
  - Full validation
    - Definition of ranges
    - Reagents
    - Final specifications
  - Environmental monitoring
  - Emphasis on Core GTPs
Final Conclusion

- This trial required more thought (and maybe more work) for the laboratory than any IND we are currently doing.
Background

Disadvantages of Cord Blood vs. Bone Marrow

- Low Cell Dose
- Delayed Engraftment
- Delayed Immune Reconstitution
- Increased Graft Failure

Potential Solutions:

- Double Cord Transplantation
- Ex Vivo Expansion
Ex-Vivo Expansion
with G-CSF, SCF, and TPO

Ex-Vivo Expansion
with FLT3, SCF, IL6, TPO plus TEPA (Copper Chelator)

21 days

Gamida Expansion Trial

14 days

MDACC Expansion Trial

100% fraction

CD133+ enrichment

CD133+ enrichment

20 – 50% fraction

Gamida Expansion Trial

MDACC Expansion Trial
MDACC Cord Blood Expansion Trial

Day 0
50 ml

Day 7
800ml

Day 14
Harvest
Anergy Trial

- E. Guinan work
- Anergize/tolerize cells to a specific reactivity (HLA) while maintaining all other reactivity
- Haploidentical setting
- Donor T cells tolerized against the recipient’s HLA
  - Reduce GVH
  - Increase immunity post transplant
Anergy Procedure

- **Step 1**
  - CD34 selection of Stem Cell Source

- **Step 2 (d32)**
  - Donor cells are incubated with cells from a family member and anti-B7.1 and B7.2 antibodies
    - Family member cells express HLA antigens in common with the patient but different from the stem cell donor
  - 3 day incubation
  - Harvest and infusion
Veto Cell Project

- Y. Reisner work
- Donor cells are cultured against an irrelevant stimulator population
  - Goal to generate CTL
- These cells then inhibit residual patient cells from rejecting the graft (HvG)
- Benefit
  - Lower number of stem cells required for engraftment
  - Maybe able to use more disparate grafts (9 out of 10 with selection)
  - Reduce the chemotherapy intensity
Veto Procedure

- Day 1: Donor cells and irr EBV cell line
- Day 10: Restimulation
- Day 14: CD4/CD56 depletion
  - >90% CD8+ cells
- Day 21: Restimulation
- Day 28: Harvest
NK Protocol

- Perugia group has noticed that a majority of patients that have responded are Kir mismatched with the donor.
- Evaluate role of Alloreactive NK cells to augment the antileukemia effects of the chemotherapy preparative regimen and allogeneic stem cell transplantation in patients with AML/MDS.
  - engraftment, GVHD, leukemia relapse and survival.
NK Procedure

- Non-mobilized HPC-A cells collected
- CD3 depletion
- CD56 enrichment
- Culture overnight with IL-2
- Harvest and infuse
Preparation of AML-Loaded DCs

CD14 selected Monocytes
(Adherence, Elutra)

GM-CSF, IL-4
6 days

Immature DC
Antigen Loading
2 hours

Mature DCs
Analyse:CFC, Elispot
chromium release, \(^{3}\text{H}\)
or
Inject into patient

2 days
Poly (I:C),
TNF-a,
IL-1 \(\beta\),
IL-6,
PGE2

Inject into patient 2 days
Preparation of AML-Loaded DCs

- CD14 selected Monocytes
  - Adherence, Elutra
- GM-CSF, IL-4
  - 6 days
- Immature DC
  - Antigen Loading
    - 2 hours
    - Poly (I:C), TNF-a, IL-1 b, IL-6, PGE2
- Mature DCs
  - Analyze: CFC, Elispot, chromium release, 3H
  - 2 days
  - Inject into patient
  - 2 days
Dendritic Cell Trials

- AML
- Myeloma
How do we choose an instrument?

- Track record: has it been used in clinical trials before
- Audit of manufacturer
- Documentation
- Discussions with FDA
- Compromise???
What do we do?

Documentation

- Manufacturer Certificate of Analysis
- Instructions for use
- Changes???
What do we do?

Documentation

- Documentation from companies
What do we do?

Internal Validations/ Qualifications

VALIDATION PLAN: Mogen ISFS35 Transduction Procedure

1.0 PURPOSE

This prospective validation provides documented evidence that the BMT Cell Therapy Laboratory has demonstrated that it is able to prepare the ISFS35 transduced cell product for use in the clinical trial: “A Phase I Trial of Autologous CLL T Cells Transduced to Express Chimeric CD154 (ISFS35).”

2.0 PRINCIPLE

Patients with CLL have B cells that are ineffective at stimulating T cells. The goal of this trial is to modify a patient's B cells so that they become effective activators of T cells and result in a systemic immune response to the patient's CLL cells. The strategy uses a replication-defective recombinant adenovirus vector encoding for CD154 (the CD40 ligand receptor) to transduce patient CLL cells into powerful antigen presenting cells (APC). These cells will then be infused back into the patient.

3.0 SPECIMEN

3.1 Starting material: Therapeutic Cells (Autologous cells from a CLL patient)

3.2 Final product: ISFS35 Transduced Cells

4.0 REAGENTS, SUPPLIES AND EQUIPMENT

4.1 Equivalent reagents, supplies, and equipment may be used as required. Substitutions must be documented and final procedures must be in agreement with the validation.
What do we do?
Manufacturer Changes

- Equipment
- Disposables
- Software
What do we do?
Manufacturer Changes

Equipment
- Re-validation/qualification
What do we do?
Manufacturer Changes

Disposables
- Analysis of change
  - Same materials, change in configuration
  - New Material – mini to full revalidation
What do we do?
Manufacturer Changes

Software
- Most common
- Require a summary of changes from QA of manufacturer
  - Evaluate impact on processing
    - Little impact – retrospective analysis
    - Possible/Definite impact – re-validation
Additional Devices Used in Cell Processing
Questions?