Cell Sorting and Cell Selection

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Director, Cell Therapy Laboratory
Technical Director, Cord Blood Bank
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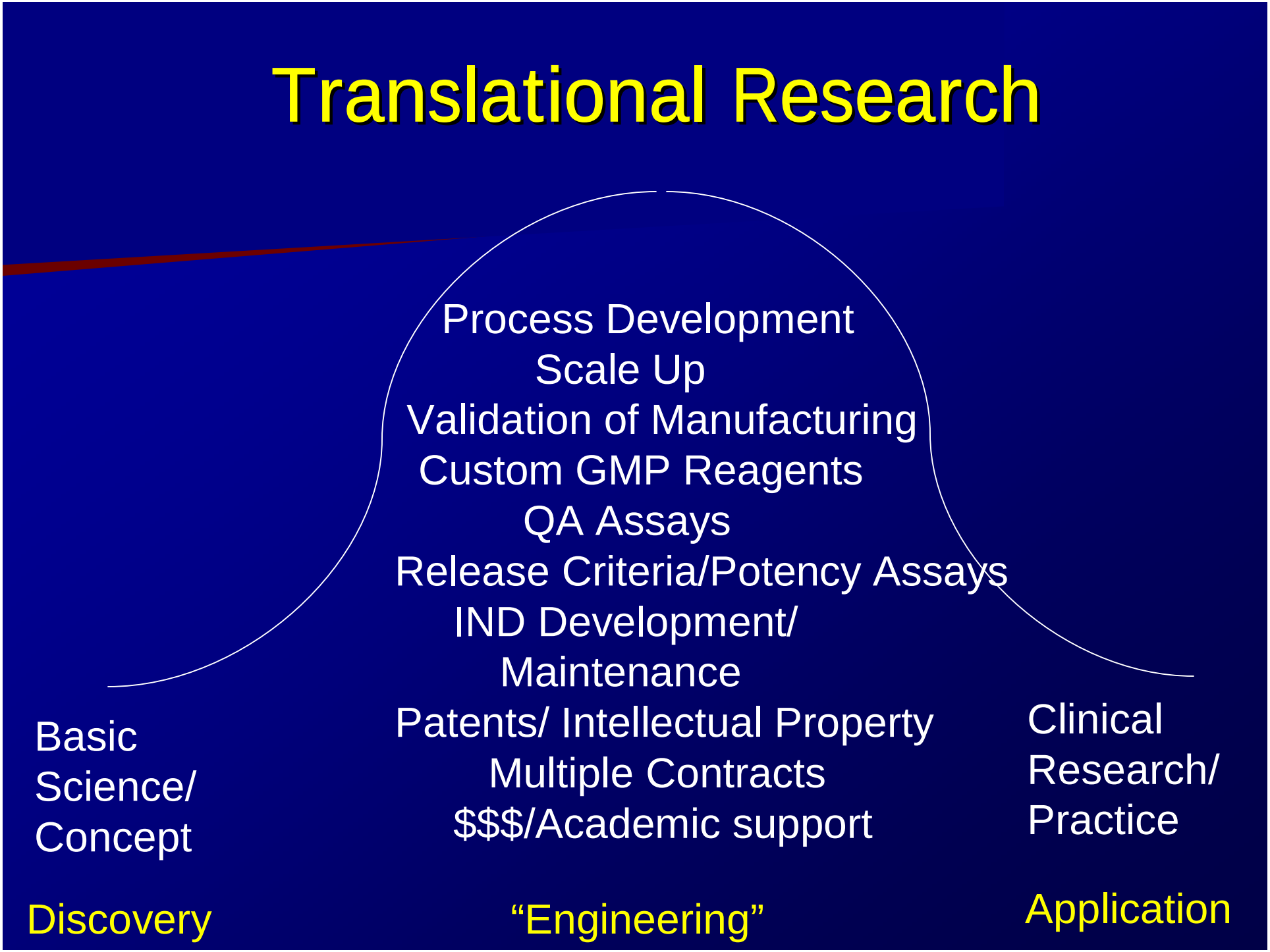
Goals

- 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



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

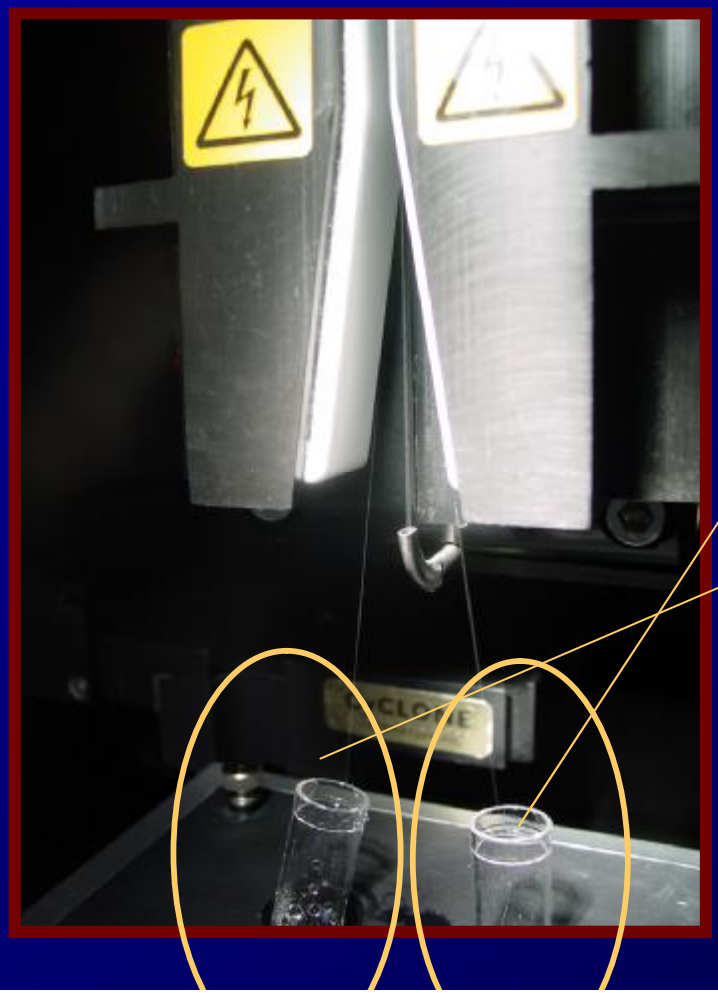
FDA issues

- 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

- Unclassified laboratory

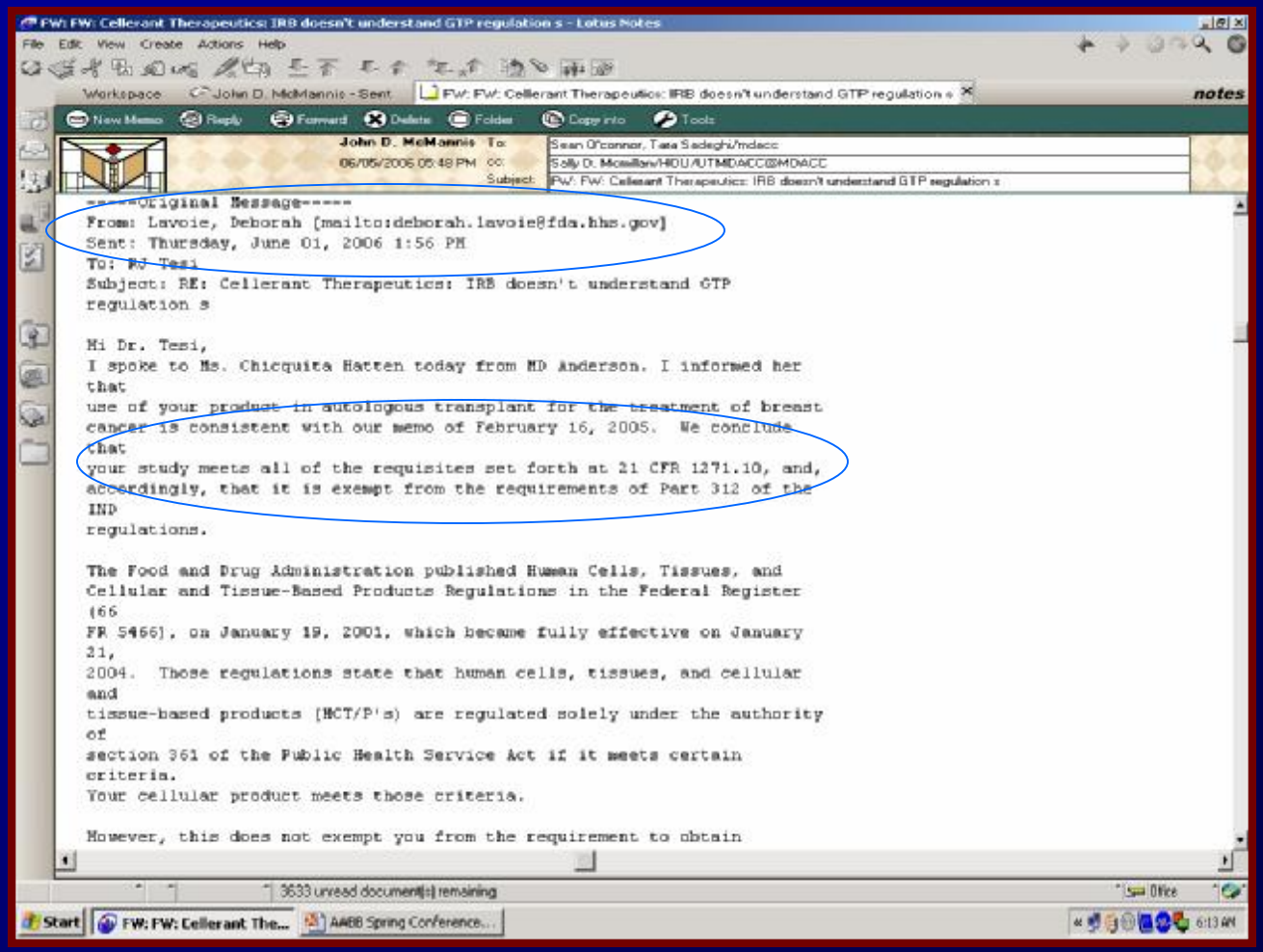
Cellerant : Applications

- 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

FDA Correspondence



FW: FW: Cellerant Therapeutics: IRB doesn't understand GTP regulation s - Lotus Notes

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Workspace John D. McMannis - Sent FW: FW: Cellerant Therapeutics: IRB doesn't understand GTP regulation s notes

New Memo Reply Forward Delete Folder Copy into Tools

John D. McMannis 06/05/2006 05:48 PM

To: Sean O'connor, Tara Sadeghi/mdacc
cc: Sally D. Mcmillan/HOU/UTMDACC@MDACC
Subject: FW: FW: Cellerant Therapeutics: IRB doesn't understand GTP regulation s

-----Original Message-----
From: Lavoie, Deborah [mailto:deborah.lavoie@fda.hhs.gov]
Sent: Thursday, June 01, 2006 1:56 PM
To: MJ Tesi
Subject: RE: Cellerant Therapeutics: IRB doesn't understand GTP regulation s

Hi Dr. Tesi,
I spoke to Ms. Chicquita Hatten today from MD 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 requisites 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 5466), 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/P's) are regulated solely under the authority of section 361 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

3633 unread document[s] remaining

Office

Start FW: FW: Cellerant The... AABB Spring Conference... 6:13 AM

CLT-001 – Manufacturing Process

Mobilized Leukapheresis Product from Patients/Donors → CD34+ enrichment using Isolex Device → Cell Staining & Sorting using MoFlo Sorter → 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

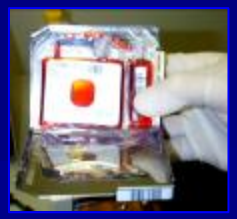
Potential Solutions:

- *Double Cord Transplantation*
- *Ex Vivo Expansion*

Gamida Expansion Trial

MDACC Expansion Trial

20 – 50% fraction



100% fraction

CD133+ enrichment

CD133+ enrichment

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

Ex-Vivo Expansion
with G-CSF, SCF, and TPO

21 days

14 days

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

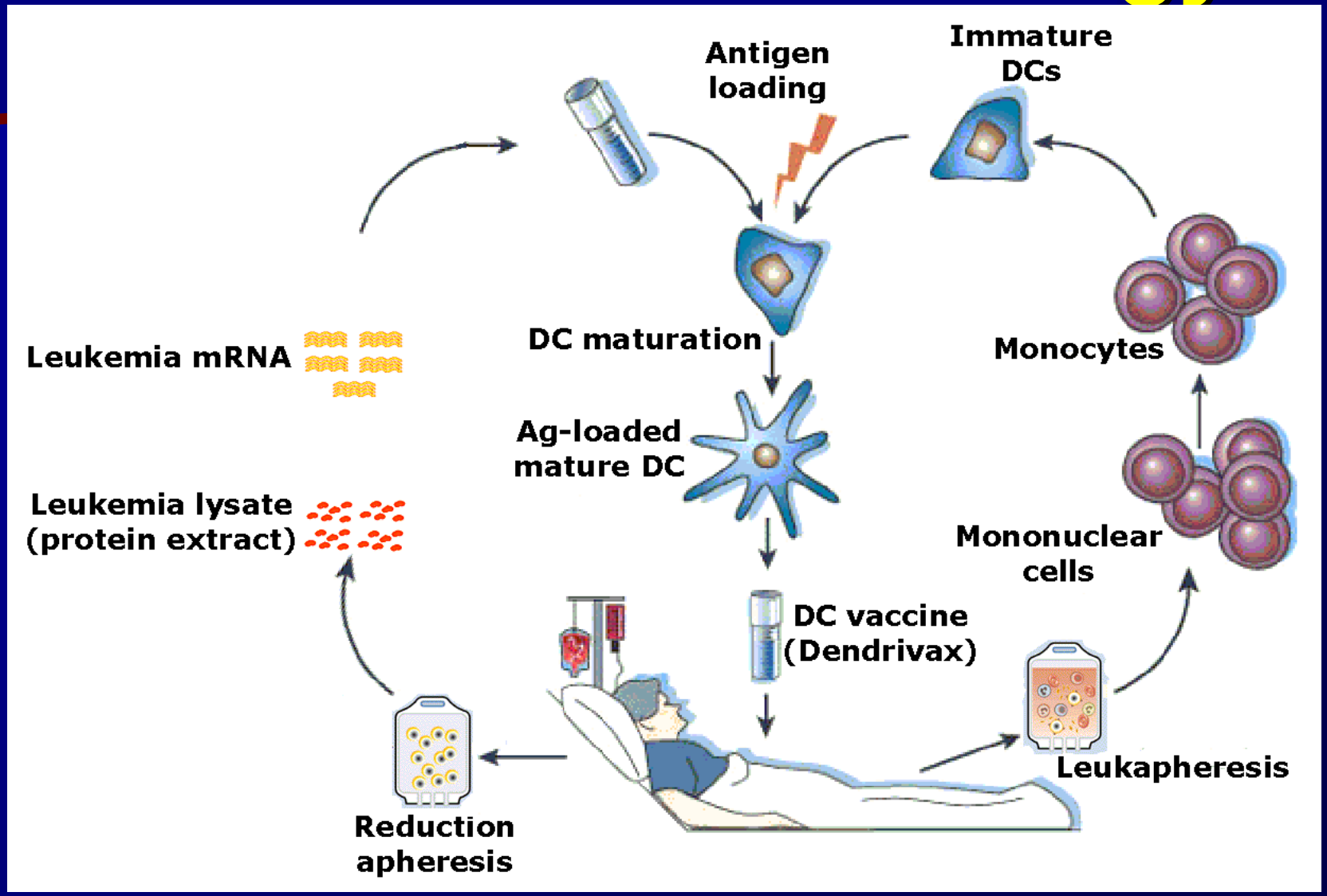
NK Protocol

- 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.

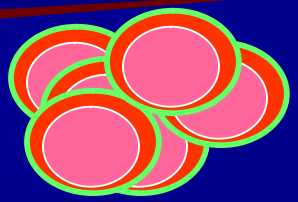
NK Procedure

- Non-mobilized HPC-A cells collected
- CD3 depletion
- CD56 enrichment
- Culture overnight with IL-2
- Harvest and infuse

DC Vaccine Strategy



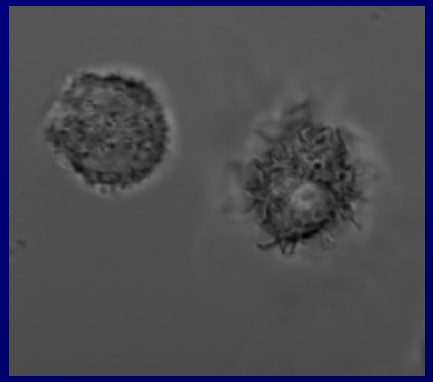
Preparation of AML-Loaded DCs



CD14 selected Monocytes
(Adherence, Elutra)

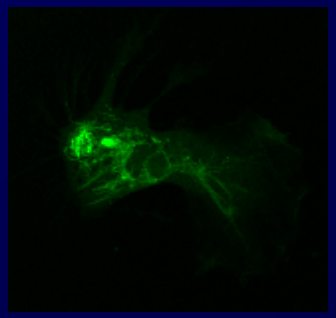
GM-CSF, IL-4

6 days



Immature DC

Antigen Loading 2 hours



Poly (I:C),
TNF-a,
IL-1 b,
IL-6,
PGE2

2 days

Mature DCs
Analyze:CFC, Elispot
chromium release, ^{3}H
or
Inject into patient

Preparation of AML-Loaded DCs

CSF, IL-4

days

2 days

Poly (I:C),
TNF-a,
IL-1 b,
IL-6,
PGE2

Dendritic Cell Trials

- AML
- Myeloma

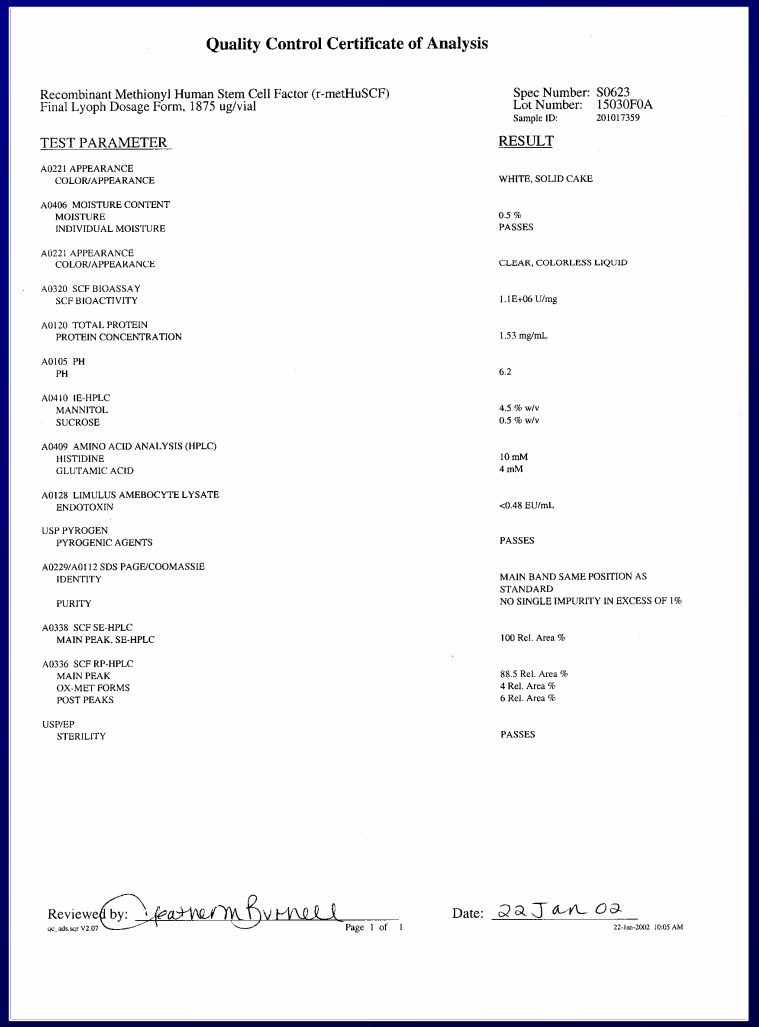
How do we choose an instrument?

- 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???



Quality Control Certificate of Analysis

Recombinant Methionyl Human Stem Cell Factor (r-metHuSCF)
Final Lyoph Dosage Form, 1875 ug/vial

Spec Number: S0623
Lot Number: 15030F0A
Sample ID: 201017359

TEST PARAMETER	RESULT
A0221 APPEARANCE COLOR/APPEARANCE	WHITE, SOLID CAKE
A0406 MOISTURE CONTENT MOISTURE INDIVIDUAL MOISTURE	0.5 % PASSES
A0221 APPEARANCE COLOR/APPEARANCE	CLEAR, COLORLESS LIQUID
A0320 SCF BIOASSAY SCF BIOACTIVITY	1.1E+06 U/mg
A0120 TOTAL PROTEIN PROTEIN CONCENTRATION	1.53 mg/mL
A0105 PH PH	6.2
A0410 IE-HPLC MANNITOL SUCROSE	4.5 % w/v 0.5 % w/v
A0409 AMINO ACID ANALYSIS (HPLC) HISTIDINE GLUTAMIC ACID	10 mM 4 mM
A0128 LIMULUS AMEBOCYTE LYSATE ENDOTOXIN	<0.48 EU/mL
USP PYROGEN PYROGENIC AGENTS	PASSES
A0229/A0112 SDS PAGE/COOMASSIE IDENTITY PURITY	MAIN BAND SAME POSITION AS STANDARD NO SINGLE IMPURITY IN EXCESS OF 1%
A0338 SCF SE-HPLC MAIN PEAK, SE-HPLC	100 Rel. Area %
A0336 SCF RP-HPLC MAIN PEAK OX-MET FORMS POST PEAKS	88.5 Rel. Area % 4 Rel. Area % 6 Rel. Area %
USP/EP STERILITY	PASSES

Reviewed by: Jeanne M Burnell
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Date: 22 Jan 02
22-Jan-2002 10:05 AM

What do we do?

Documentation

- Documentation from companies

What do we do?

Internal Validations/ Qualifications

DEPARTMENT OF BLOOD & MARROW TRANSPLANTATION
CELL THERAPY LABORATORY

UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER
HOUSTON, TEXAS

VALIDATION PLAN: Memgen ISF35 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 ISF35 transduced cell product for use in the clinical trial: "A Phase I Trail of Autologous CLL B Cells Transduced to Express Chimeric CD154 (ISF35)."

2.0 PRINCIPLE

Patients with CLL have B cells 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 adenoviral 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 to into the patient.

3.0 SPECIMEN

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

3.2 Final product: ISF35 Transduced cells

4.0 REAGENTS, SUPPLIES AND EQUIPMENT

4.1 Equivalent reagents, supplies, and equipment may be substituted as required. Substitutions must be documented and final procedures must be in agreement with the validation.

4.2 All reagents, supplies, and equipment required for the manufacture of the ISF35 cells can be found in the Procedure 10.14.1: Memgen CLL Percoll to AdISF35 Transduction-Draft (Appendix A).

4.3 All reagents, supplies, and equipment required for the testing performed by the Cell Therapy Laboratory can be found in the following SOPs (Documents on file in CTL):

4.3.1 Procedure 3.3 MANUAL CELL COUNT AND VIABILITY MEASUREMENT.

4.3.2 Procedure 3.7: HEMATOLOGICAL ANALYSIS USING AUTOMATED CELL COUNTERS

4.3.2 Flow Procedure F4.1 OPERATION, MAINTENANCE, AND QUALITY CONTROL OF A BD BIOSCIENCES FACSCalibur™.

4.3.4 Procedure 3.13: PYROS KINETIX ENDOTOXIN ASSAY

4.3.5 Procedure 3.12: GRAM STAIN

4.3.6 Procedure 3.8: STERILITY ASSAY

4.4 Flow cytometry panels will be set up according to Appendix B: Immunophenotyping of Chronic Lymphoid Leukemia Cells (CLL) by Flow Assisted Cell Sorting (FACS). The CTL will be using the same antibodies and panels with the substitution of PI for 7AAD.

4.5 All reagents, supplies, and equipment required for the Mycoplasma testing performed by the Baylor College of Medicine is identified in SOP B03.03: Mycoplasma Detection by PCR (Document on file in CTL).

4.6 Additional assays will be performed according to procedures developed in the MDACC laboratory

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DEPARTMENT OF BLOOD & MARROW TRANSPLANTATION
CELL THERAPY LABORATORY

UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER
HOUSTON, TEXAS

VALIDATION REPORT: Memgen ISF35 Transduction Procedure

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3.0 RESULTS

3.1 All procedures, reagents, supplies, and equipment used in the validation runs were in accordance with the Validation Plan.

3.2 A total of five validation runs were performed.

3.3 Table 1 (See below) provides a summary of all samples, tests, specifications, and results for the validation runs. Some tests are for monitoring purposes and do not have required specifications. Not all tests are required for the final release of the product. Refer to the Validation Plan for testing laboratory and whether the test is required for product release.

Table 1

Sample	Test	Specification	Results Run #1	Results Run #2	Results Run #3	Results Run #4	Results Run #5
Pre Processing	TNC (e6)	Not Specified	628656	18099	12630	32226	18840
	Bactec	Negative	Negative	Negative	Negative	Negative	Negative
Bulk ISF 35 Cells	TNC (e6)	Not Specified	888	792	784	724	771
	Flow-ISF35 Expression	≥40% of all cells must be positive for ISF35	65.2	43.5	24.8	54.2	42.0
	Flow-CD5/CD19 Expression	>50% of all cells must be positive for CD5 and CD19	86.9/88.7	96.7/92.6	93.8/96.4	94.7/80.8	81.6/89.7
1st PBS Wash	Free ISF 35 Adenovirus	Not Specified	No Data	No Data	No Data	No Data	No Data
2nd PBS Wash	Free ISF 35 Adenovirus	Not Specified	No Data	No Data	No Data	No Data	No Data

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What do we do?

Manufacturer Changes

- Equipment
- Disposables
- Software

What do we do?

Manufacturer Changes

- Equipment
  - Re-validation/qualification

What do we do?

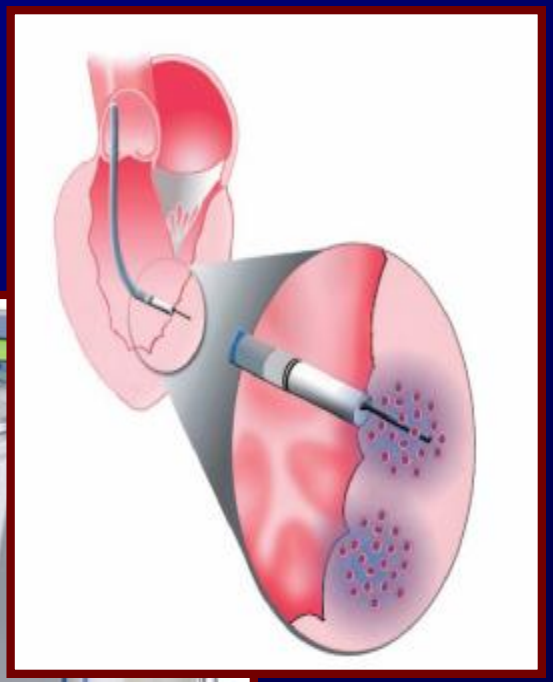
Manufacturer Changes

- 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




CYTOMATE Cell Processing System

Questions?