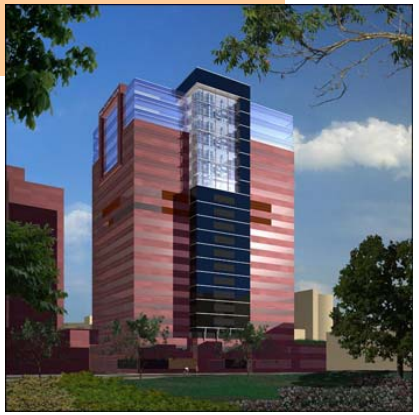
PACT Educational Workshop
Pittsburgh May 2008

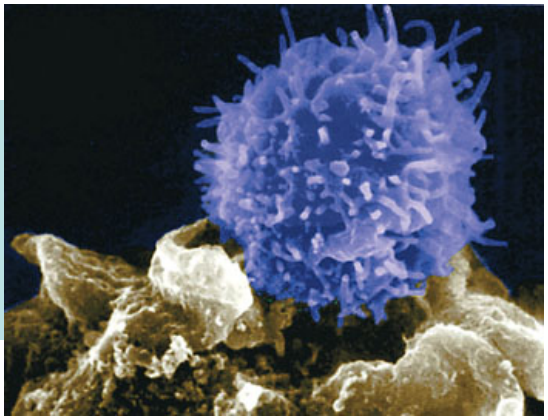
Production Assistance for Cellular Therapies

T Cell Therapies
The What, the Why and The How

Cliona Rooney
Baylor College of Medicine



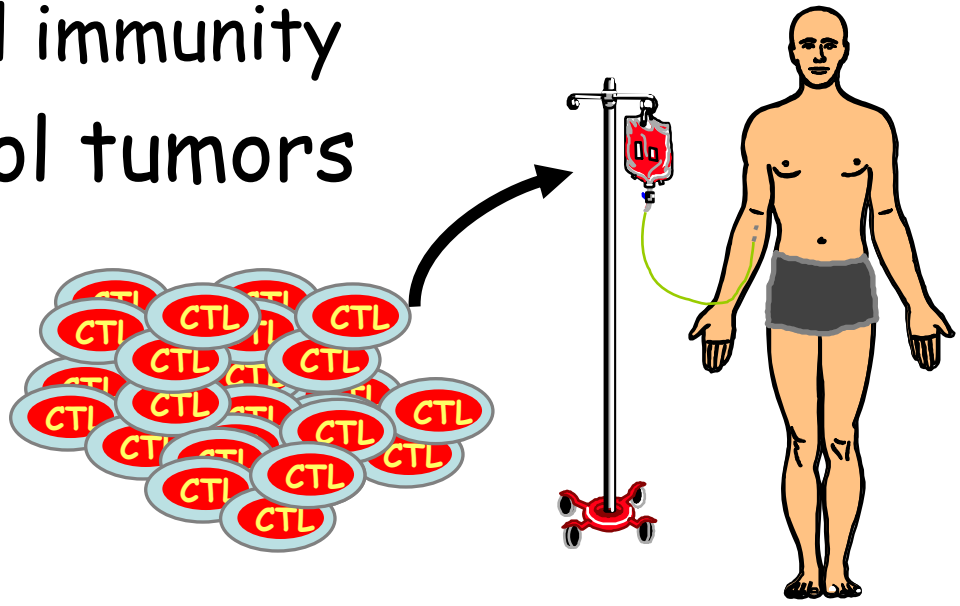
What are T cells?



- Recognize foreign proteins expressed in cells
  - Virus or parasite derived
  - Caveat – can be modified self antigens or fetal antigens or cancer testis antigens
    - Can also target cancer
- Have cytotoxic (killer) functions (CTLs)
  - CD8 and CD4
- Have "helper" functions
  - CD4 (help killer T cells and B cells)

Why Do We Need T cell Therapies?

- Immunosuppressed patients are at high risk from viral infections
  - Viruses are controlled by T cells
  - Infusion of virus-specific T cells can restore anti-viral immunity
- T cells can control tumors
  - T cells fail



Who Needs Virus-Specific T cells?

- Stem cell transplant recipients
  - Effective for CMV, EBV and adenoviruses
- Solid organ transplant recipients
  - Benefit for EBV
  - Problem of continuing immunosuppression
- Patients with virus-associated cancers
  - NPC, lymphoma (EBV), cervical cancer (HPV)
- Non-virus-associated cancers
  - Melanoma, lymphoma/leukemia

EBV Specific CTL As Therapy

- 12 year old 7 months post MUD BMT for PNH
- Massive lymphadenopathy and incipient respiratory obstruction due to EBV lymphoma
- Received donor EBV-CTLs

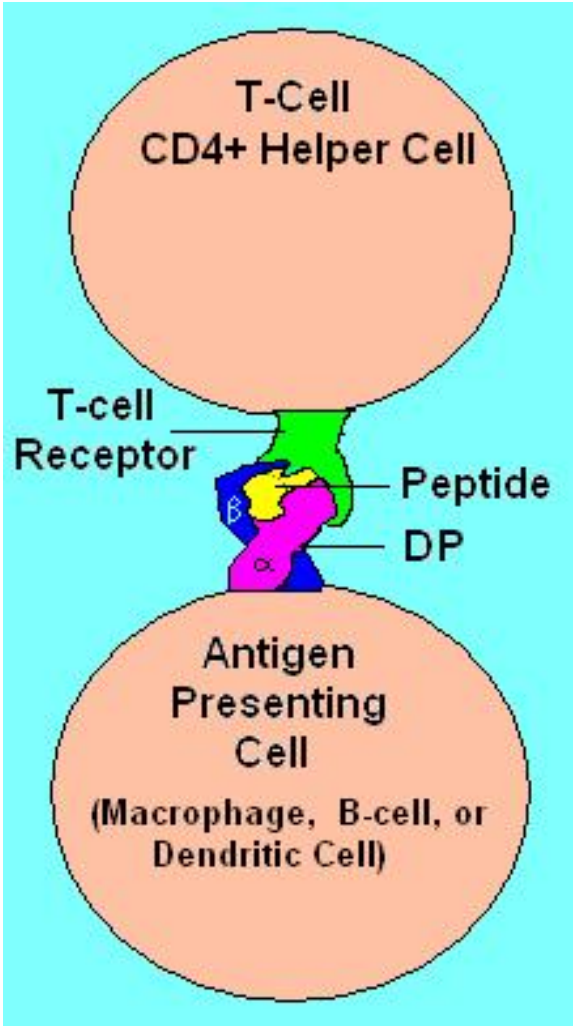
EBV Specific CTL As Therapy

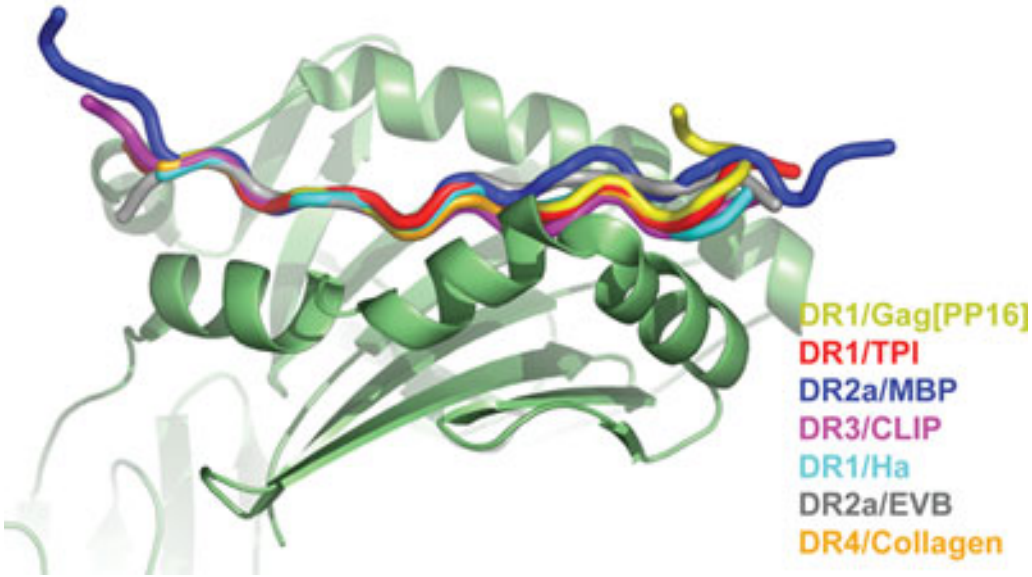
- All symptoms resolved
- Remains in remission 10 years later

How Can You Grow Antigen-Specific CTLs?

- T cell + Antigen-presenting cell + Antigen
  - All cells from PBMC of donor or patient
- Antigen-presenting cell
  - Autologous
  - Dendritic cells, monocytes, EBV-transformed B cells (EBV-LCLs)
  - Expressing antigen or interest
    - Viral antigen
    - Tumor antigen
  - Artificial APC
    - Must not express allo antigens
    - Needs to share HLA antigens with donor

The APC Presents Antigen

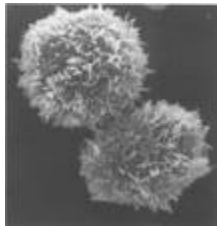


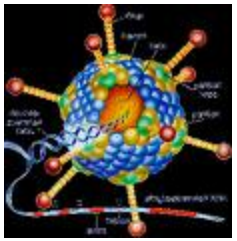


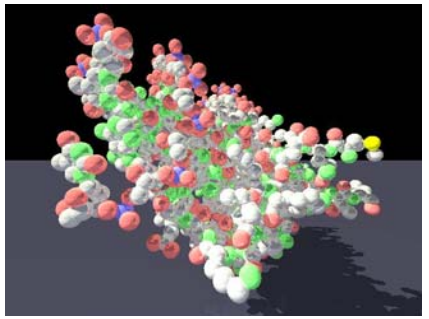
Peptide in groove of
HLA class II molecule

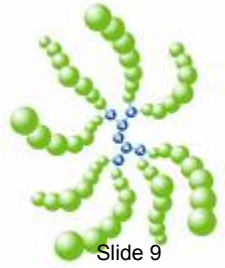
How do you get the Antigen into the APC?

- EBV-LCL
  - Transformed by EBV
  - Express multiple EBV proteins
- Viral vectors
  - Adenovirus, retrovirus, lentivirus
  - Express transgene in APC
  - Transduce monocytes, DC and LCLs
- Protein pulsing
  - Mostly activates CD4+ T cells
- Peptides







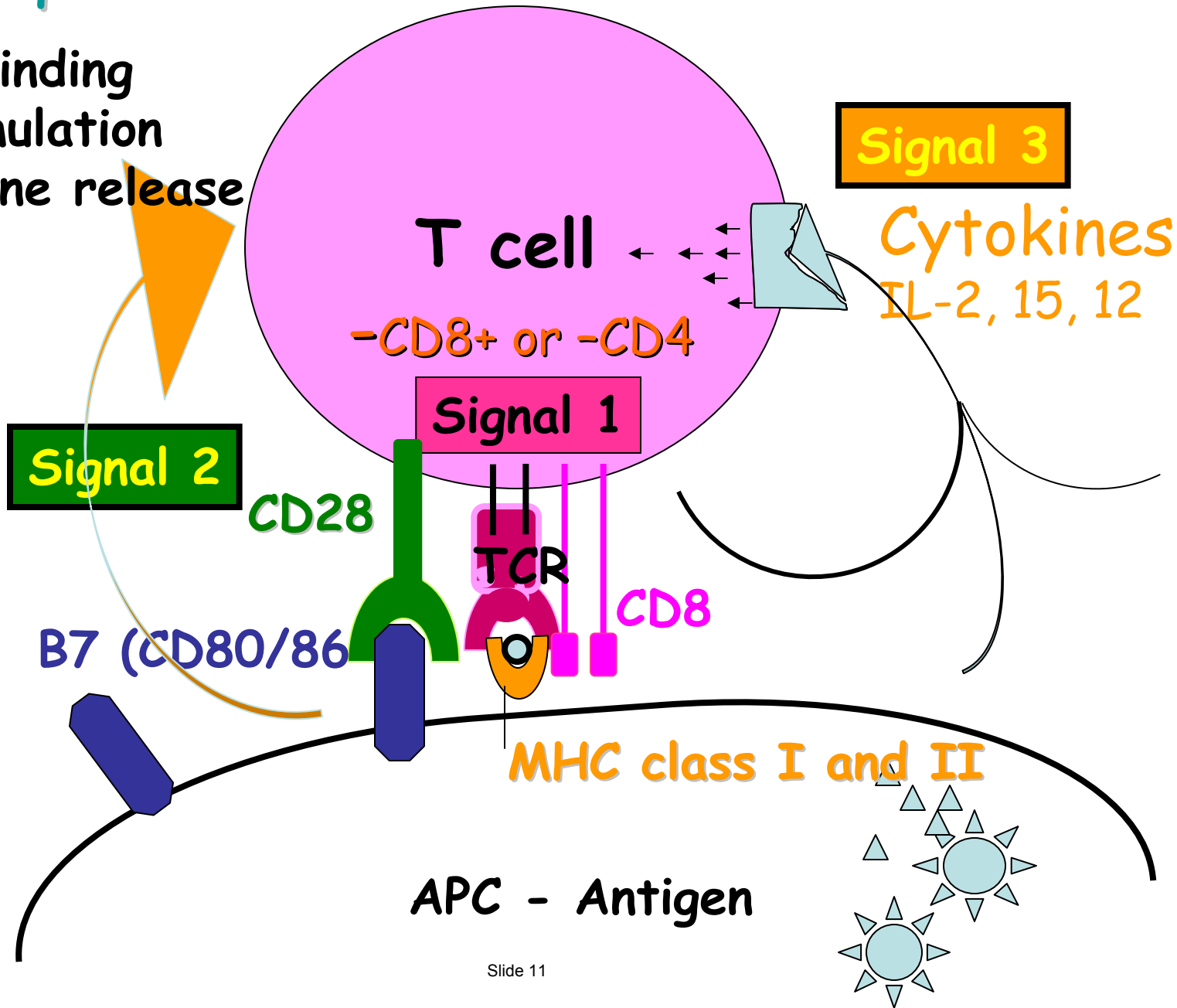


Why can't any cell be an APC?

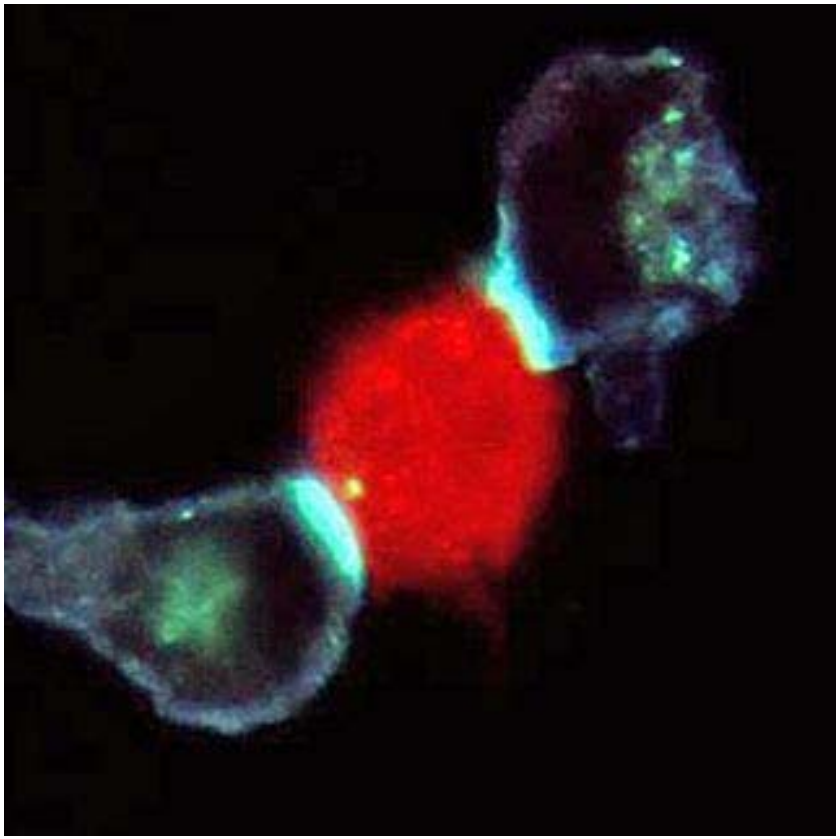
T cells also need costimulation

Requirements for T cell activation

1. TCR binding
2. Costimulation
3. Cytokine release



Infected Cell Examined by Two T Cells



Bright blue areas are where T cell receptors are gathering

The Co-culture

- 4 to 6 weeks coculture of T cells + APCs
  - 1000 to 1,000,000 fold expansion of antigen specific cells
- Weekly restimulation
- The right culture vessel
- The right culture medium
  - Growth factors

Equipment

- Clean room
- Biosafety cabinet
- 5% CO2 incubator
- Centrifuge
- Inverted Microscope

Tissue Culture Suite at BCM

Culture vessels

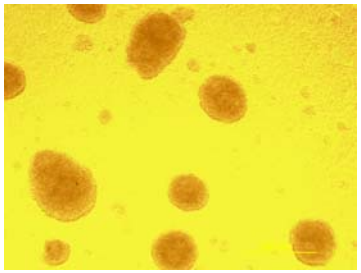
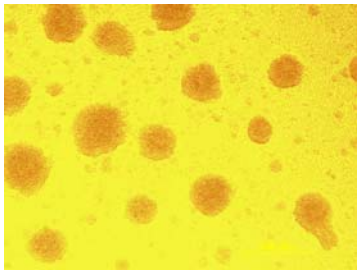
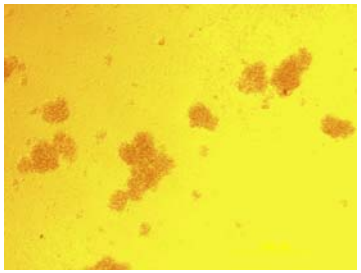
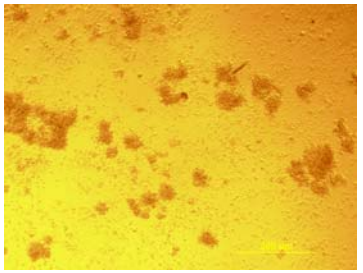
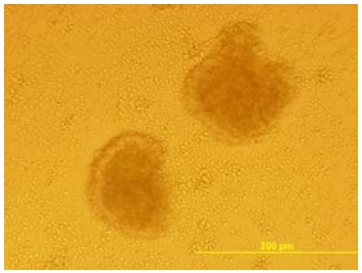
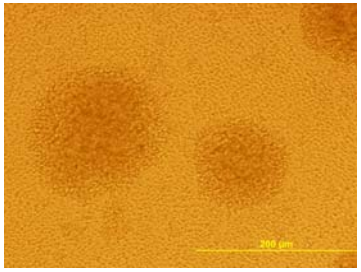
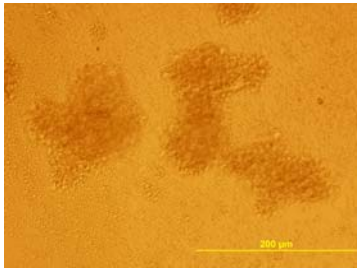
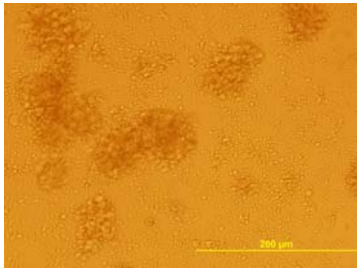
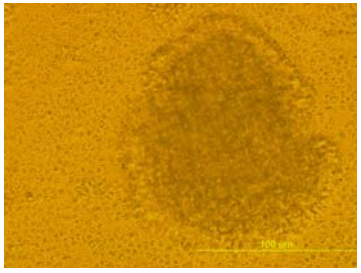
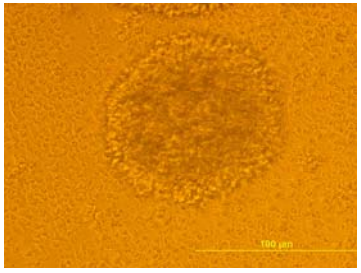
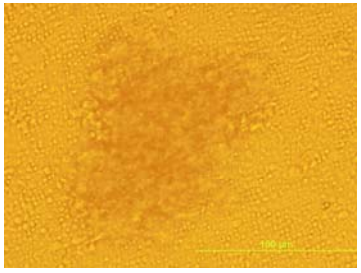
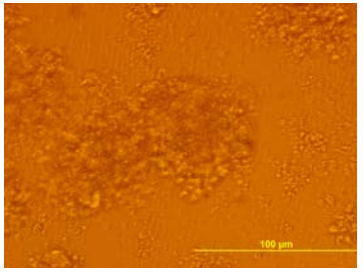
- LCLs grow in flasks
- T cells like 24 well plates

Inconsistent in bags

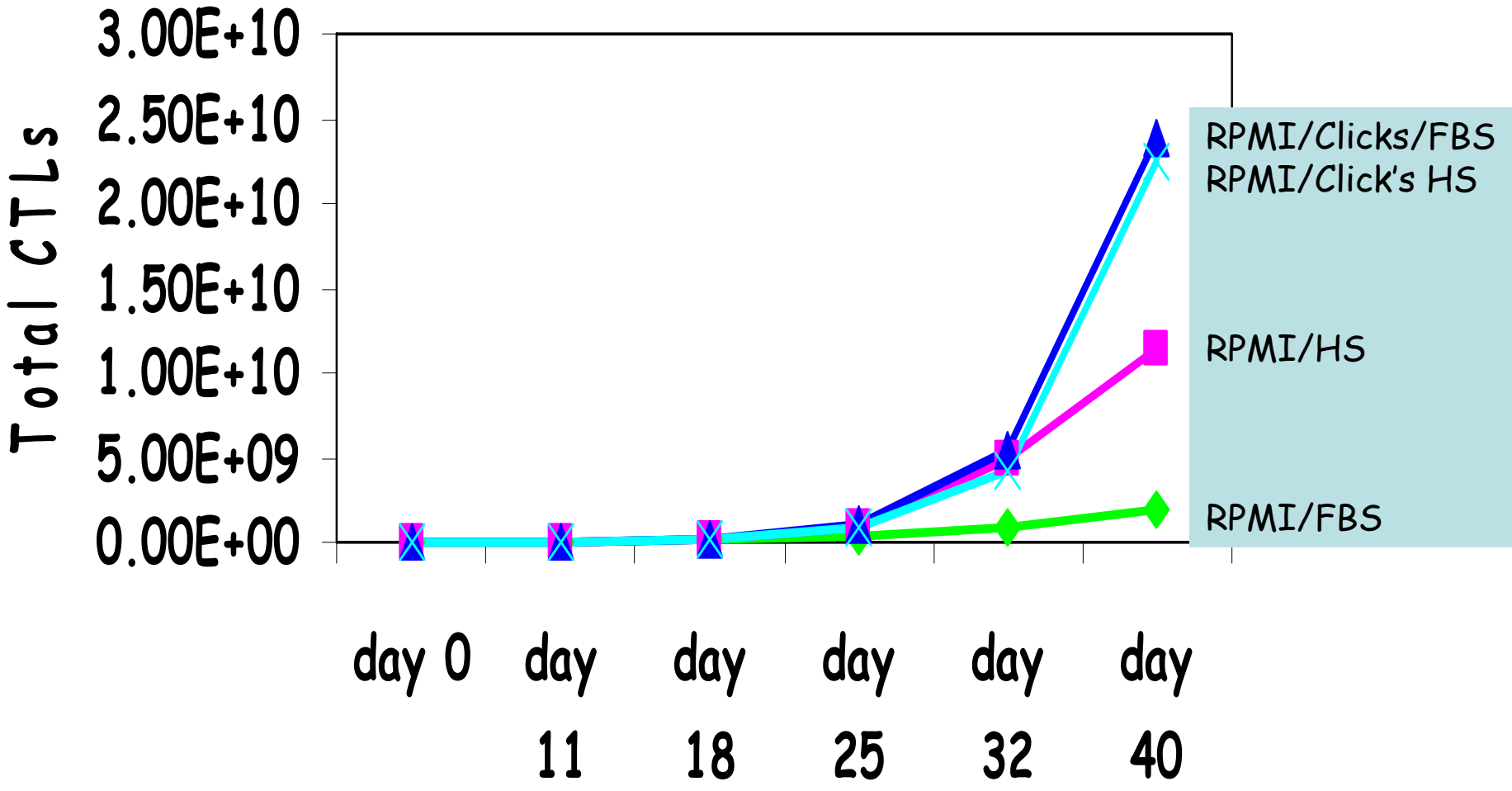
Medium

- Critical to test vendor source and lots
- RPMI 1640 Hyclone
- Fetal calf serum Hyclone
- EHAA Irvine scientific
- Glutamine
- Not clinical grade reagents
  - OK for phaseI/II studies

Testing Different Media

A	B	C	D	
				X 10
				X 20
				X 40

Testing Media Formulations



Growth factors

- IL-2, GM-CSF, PGE1
  - Available as clinical grade
- IL-4, TNF-α
  - R&D Systems
  - 2 viral exclusion steps

Most Common Problems

- Media source
  - FCS and RPMI
  - Tremendous variability between companies
- EBV
  - Stability (temperature sensitive)
- Incubator pH and humidity
  - Look at your cultures
    - They will tell you if they are happy
- Cell concentration
  - Know the upper and lower limits of cell growth

Cell Therapies for Cancer and Regenerative Medicine

- Vaccines
  - Viruses and cancer
- T cells
  - Viruses and cancer
- MSCs
  - Cardiac, stroke, brain injury
- HSCs
  - Cardiac, stroke, brain injury
- Genetically-modified to improve function

CREW

CTL-GMP

Tessie Lopez
Joyce Ku
Huimin Zhang
Rebecca Wolfskill
Daniel Erickson

Production Assistance for Cellular Therapies

CTL-TRL

Ann Leen
Cath Bollard
Barbara Savoldo
Stephen Gottschalk
Juan Vera