Immunotherapy of Viral Infections and Malignancy

Helen Heslop

Methodist The Methodist Hospital System

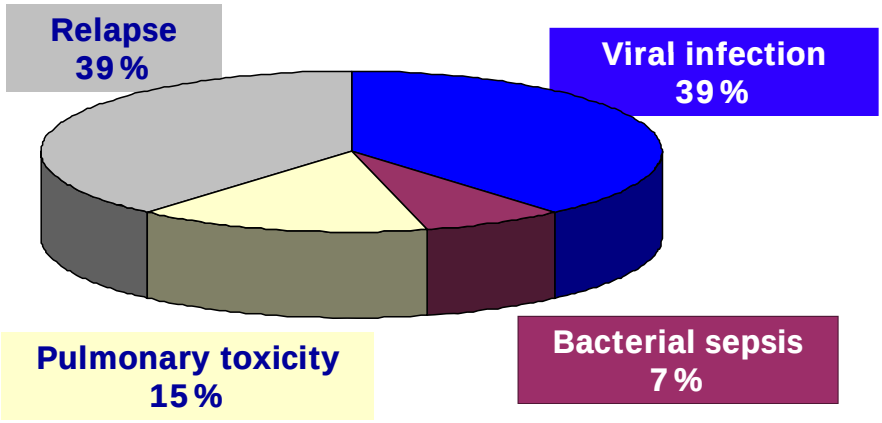
BCM Baylor College of Medicine

Texas Children's Hospital

Financial Disclosure:

Cell Medica- licensing agreement

Critical Problem: Viral Infections and Relapse Post-Alternative Donor SCT



Alana Kennedy-Nasser, BBMT 2008

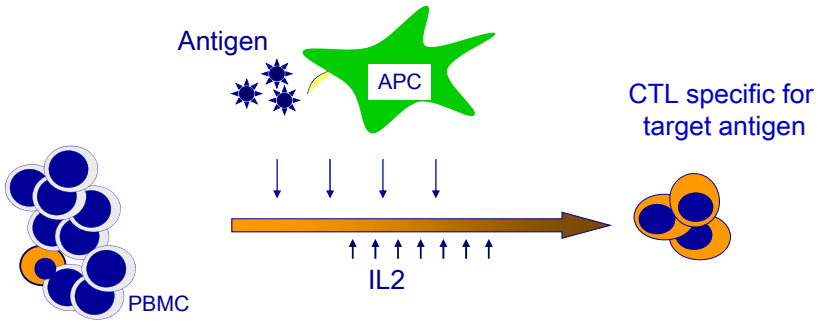
Approaches to Reconstituting Immunity

- Reconstituting antiviral and anti-tumor immunity using unmanipulated donor T cells → risk of GVHD
- T cell precursor frequencies in allogeneic transplantation:

  alloreactive > anti-viral > anti-tumor
- Option to generate antigen specific T cells

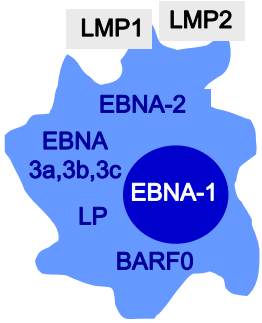
Generating Antigen Specific T Cells

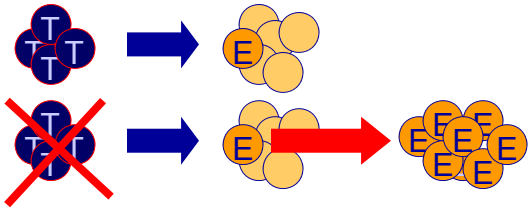
- Repeated stimulation with antigen expressed on antigen presenting cell
- Expand antigen specific T cells
- T cells with specificities for other antigens will not survive



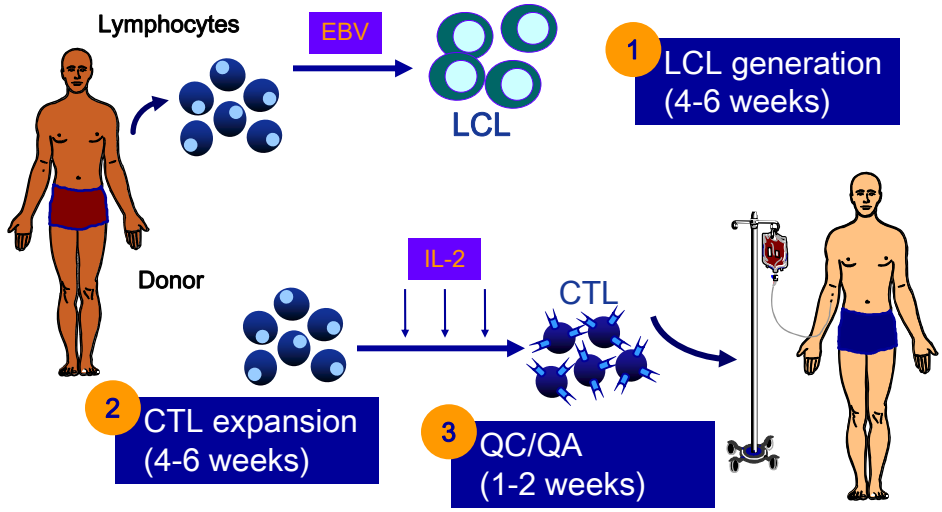
EBV Lymphoma post BMT

- Incidence 1-25% following mismatched or unrelated donor BMT
- Predisposing factors:

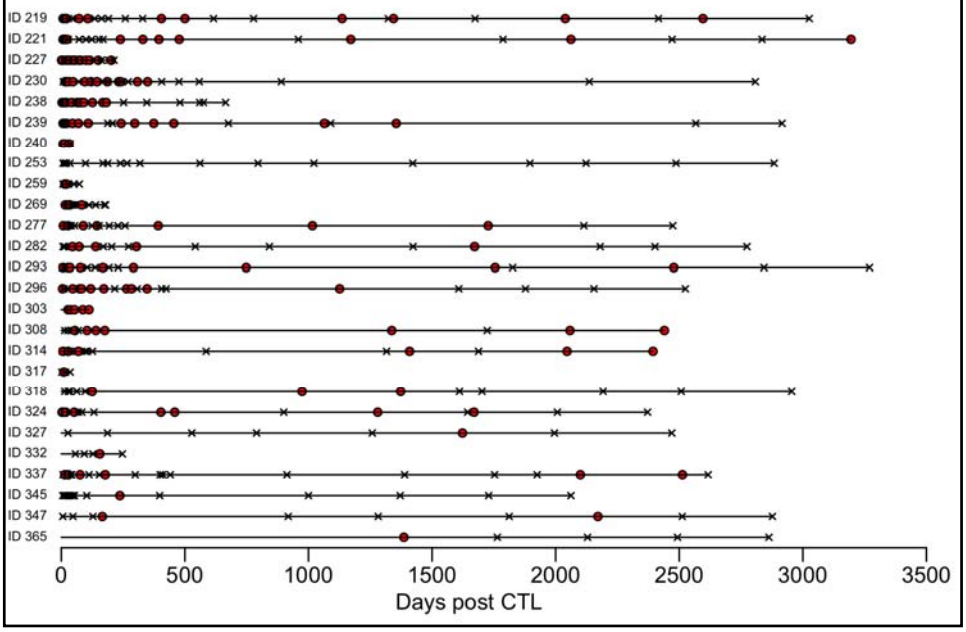




EBV-specific T-cell Generation



Long Term Detection Of Neo By Real Time PCR In PBM



Donor EBV-Specific CTL Post HSCT

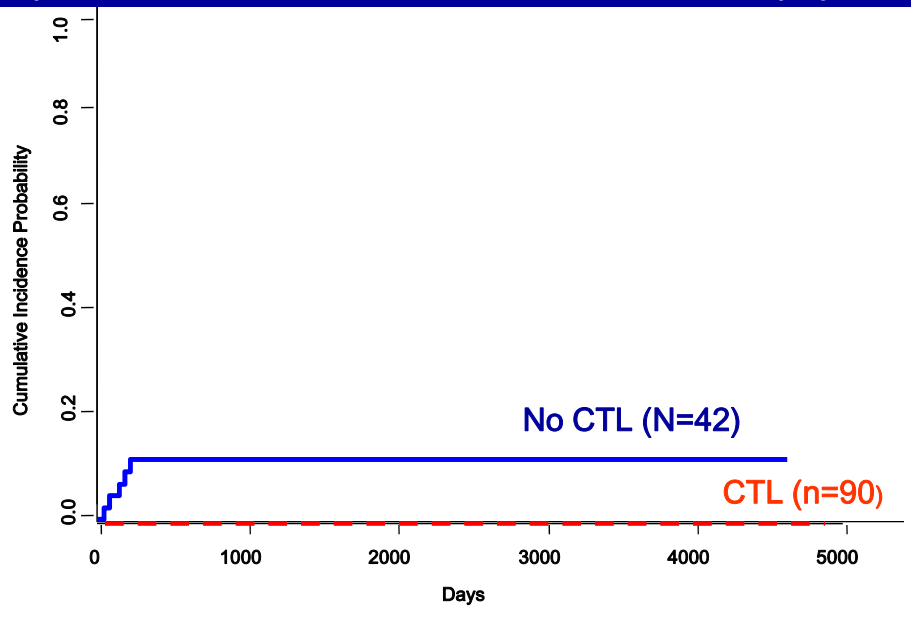
- **Prophylaxis:**
  - 90 patients after CD6/CD8 depleted graft
  - 11 very high risk patients (XLP, previous EBV lymphoma)
  - None developed EBV lymphoma

St Jude Children's Research Hospital
Baylor College of Medicine

St Jude - Slobod/Hurwitz
Great Ormond St - Amrolia

Heslop et al Blood 2010

Incidence Of LPD In Patients Receiving CD6/CD8 Depleted Marrow With Or Without EBV CTL Prophylaxis



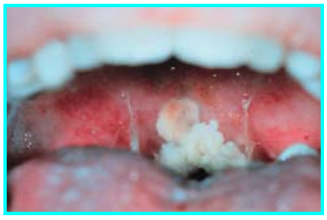
EBV Specific CTLs as Therapy

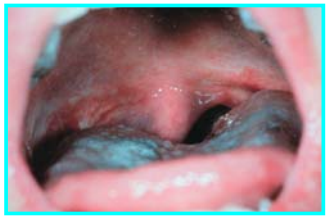
Therapy

- 13 patients treated with active disease
- 2 failed to respond
  - 1 with extensive disease died 5 days
  - 1 died with progressive disease
    - Line restricted specificity and epitopes deleted in tumor (Gottschalk et al Blood 2001)
- 11 attained CR
  - No recurrences

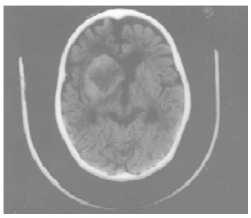
Clinical Responses

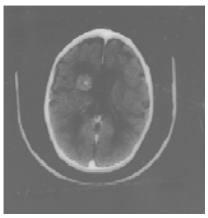
Peripheral Disease





CNS Disease: Slower response

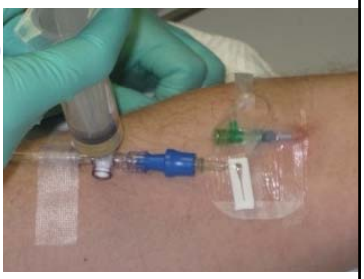


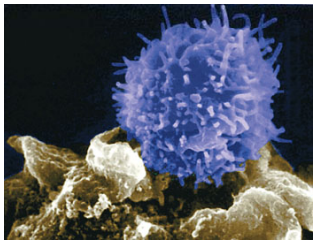


EBV CTLs Post HSCT

Small numbers (10^4 –10^5 / kg)

- Restore virus-specific immunity
- Reduce virus load
- Cure Disease in over 80%
- Long-lasting protection
- Low toxicity





Reasons For Long Term Persistence

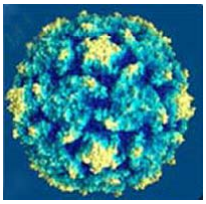
- Lines contained CD4 and CD8 cells
- EBV latent antigen
- Administration early post transplant
- Starting population contained precursors with both central and effector memory phenotype

Can We Extend Approach To Other Viruses?

What Is The Role Of Antigen in Maintaining Persistence?

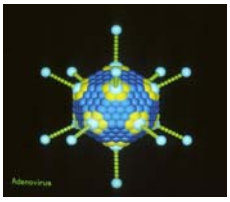
Trivirus-Specific T Cells EBV, CMV and Adenoviruses

- 3 most common viral complications after HSCT
- Most donors immune
- Have detectable levels of T cells



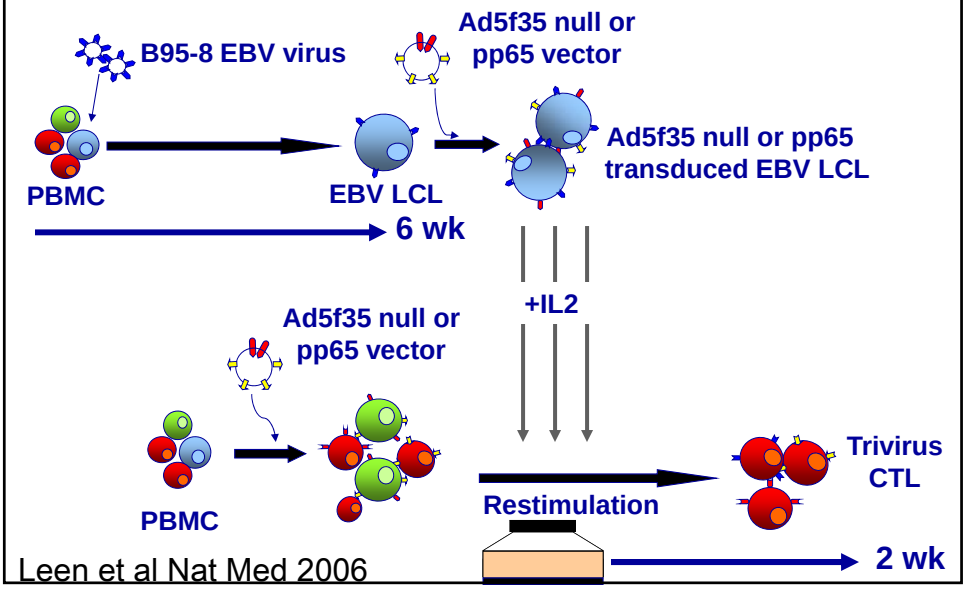
EBV

CMV



Adv

Generation Of Multivirus-specific CTL Using Ad5f35 Vectors

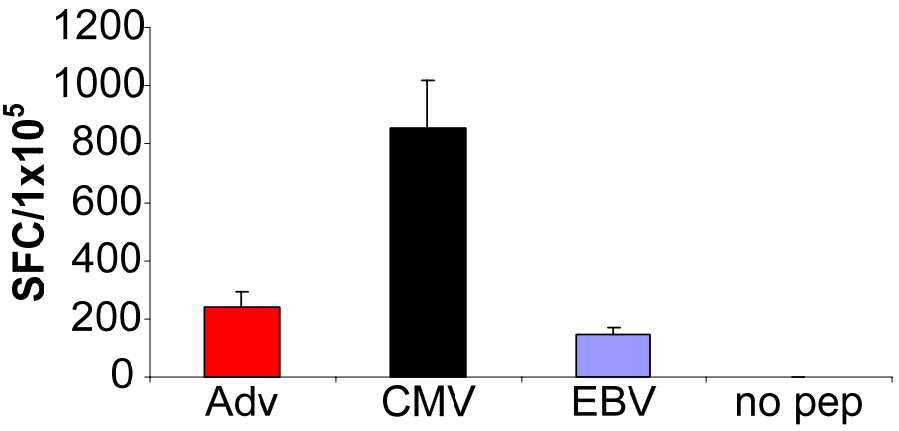


Leen et al Nat Med 2006

Analysis of CTL Lines

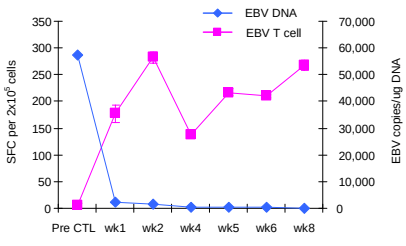
- 56 CTL lines analyzed
  - 20 Bi-virus specific
  - 36 Tri-virus specific (CMV+ donors)
- Phenotypic analysis
- Specificity for Antigen:
  - Cytotoxicity assay
  - ELISPOT assay for IFN-γ secretion
  - Pentamer analysis

Trivirus Specificity Of CTL



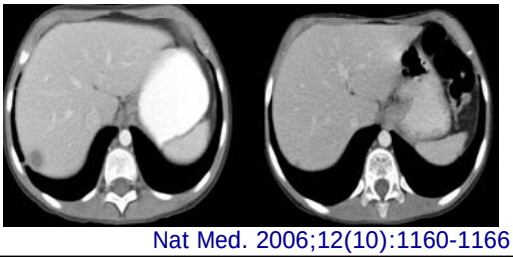
Multivirus -specific CTL Protect against EBV after HSCT

- 9/40 patients had EBV reactivation
- 9/9 patients had decrease in EBV viral load with corresponding elevation in EBV-specific CTL detected in PB
- No antiviral therapy required



Diagnosis of PTLD

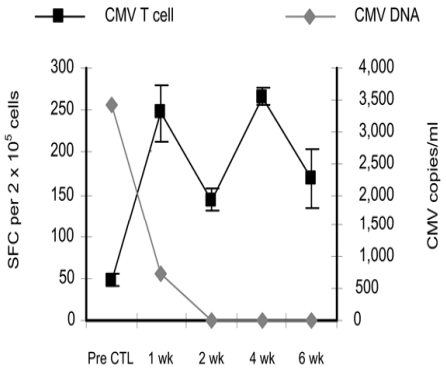
2 months later



Nat Med. 2006;12(10):1160-1166

Multivirus-specific CTL Protect against CMV after HSCT

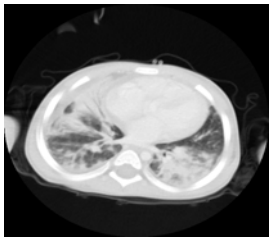
- 10/26 patients developed CMV reactivation immediately pre or post CTL infusion
- 7/10 cleared virus
  long term (>12mths)
  without requiring
  anti-viral therapy
  →1 had repeat CTL
  -cleared virus
  →1 fall in viral load
  -before foscarnet
  →1 pt died of CMV



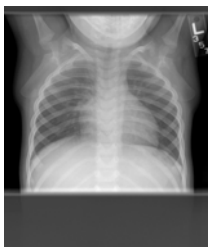
Nat Med. 2006;12(10):1160-1166

Multivirus-specific CTL Protect against Adv after HSCT

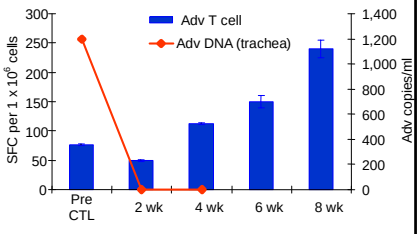
- 0/29 developed adenoviral infection post CTL infusion
- 8/8 patients with adenoviral infection (stool/blood) cleared virus after 1-2 doses CTL
- 3 patients with adenovirus disease →treated for progressive Adv pneumonia
  - 2/3 cleared the infection from lungs post CTL



Pre CTL



Post CTL



Nat Med. 2006;12(10):1160-1166

Multivirus Specific CTLs

- Expansion and persistence of CTLs specific for latent viruses EBV and CMV
- Adenovirus-specific CTL expand only in presence of adenovirus infection
  - Can reactivate adenovirus-specific response ex vivo by stimulation
- Responses to infections with all 3 viruses – overall response rate 93%

Leen et al Nat Med. 2006;12:1160-1166

How to incorporate as standard of care?

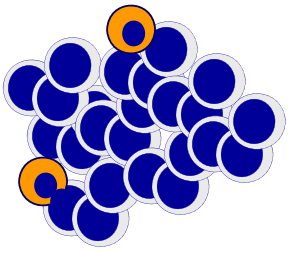
How Do We Extend Applicability?

Limitations are

- Cost
- Complexity
- Time

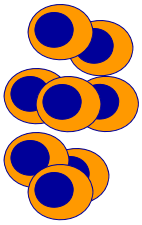
Rapid Selection Procedures

Select frequency antigen-specific CTLs

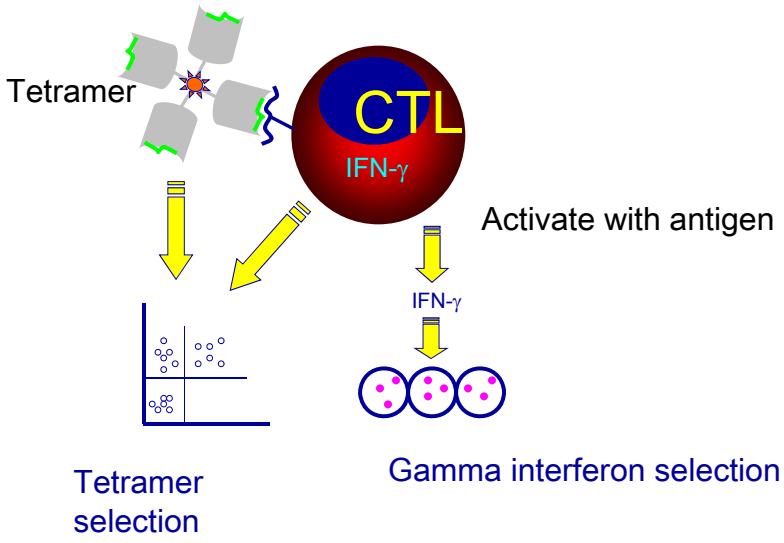


Transfer small numbers selected cells

Rely on expansion in vivo



Methods to Select T-cells



Rapidly Available CTLs

- CMV promising results
  - Later phase studies in Europe
- EBV
  - Lower response rates
- Adenovirus
  - Lower CTL frequency limiting

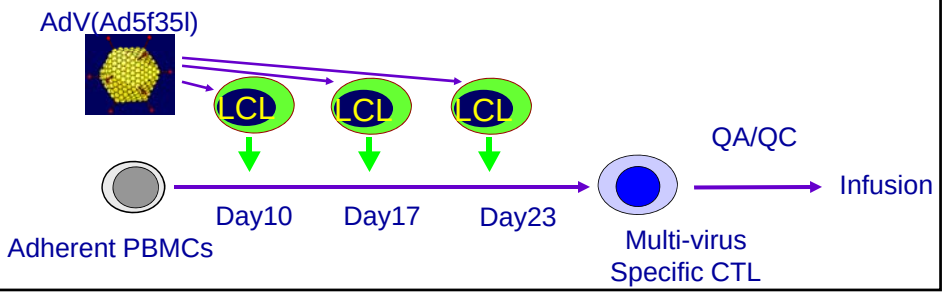
Manufacturing Costs of Trivirus-Specific CTL Production

Cost Item	
GMP facility	$2,280
Trained technician	$2,000
CTL line manufacture	$3,076
Release testing	$3,203
TOTAL	$10,559

Standard Treatment Charges
Rituximab for EBV-PTLD $9,000-$11,000
Ganciclovir for CMV $15,000

Current Method To Generate Multivirus-specific T Cells

- Lengthy process
  - EBV-transformed lymphoblastoid cell lines (LCL) production (~4 to 6 weeks)
  - CTL expansion (3-4 weeks)
  - Quality Assurance /Quality Control (14 days)



Rapid Generation of CTLs

- Eliminate viral vectors
  - EBV to manufacture LCLs
  - Ad5/35 to transduce APCs
- Shorten CTL generation time

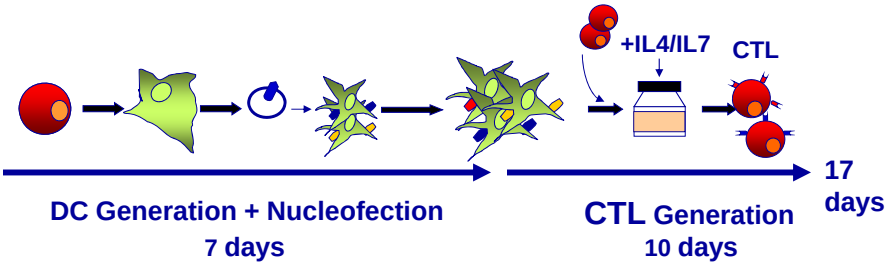
Clinical rCTL Generation Procedure

Source of antigens : DNA Plasmids

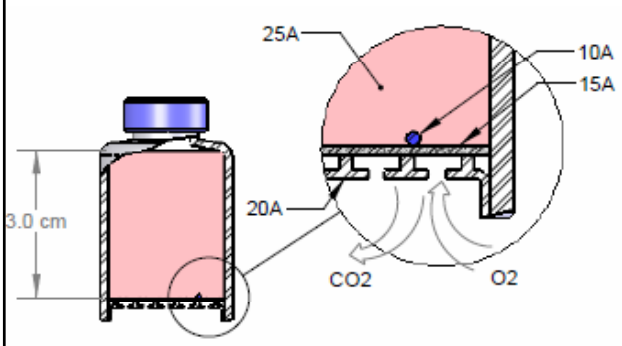
EBV: EBNA1, LMP2, BZLF1

CMV: IE1, pp65

Adv: Hexon, Penton



Wilson Wolf Manufacturing
Gas Permeable Rapid Expansion Device



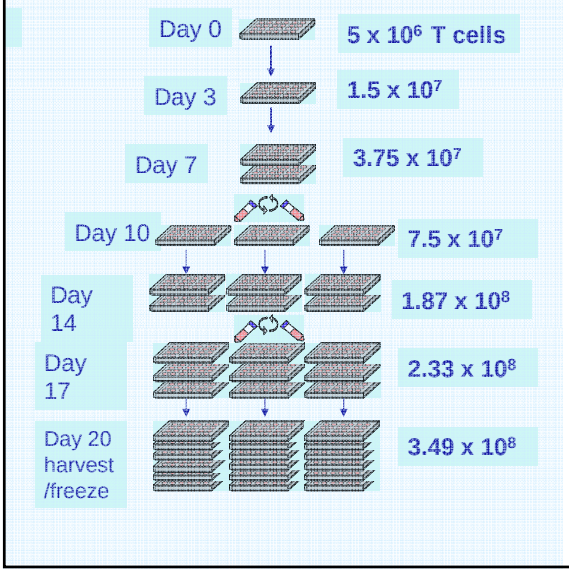
Gas permeable membrane allows optimal exchange of CO2 and O2

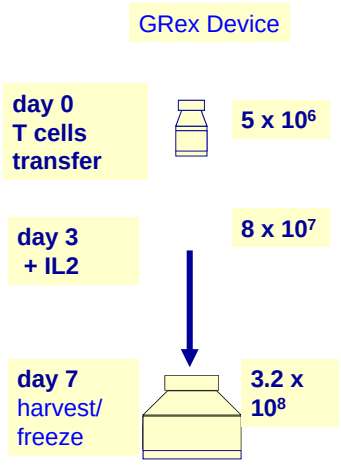
Supports cell growth with large volumes of media

No rocking or stirring

Vera et al J Immunother 2010

Conventional Culture versus G-Rex

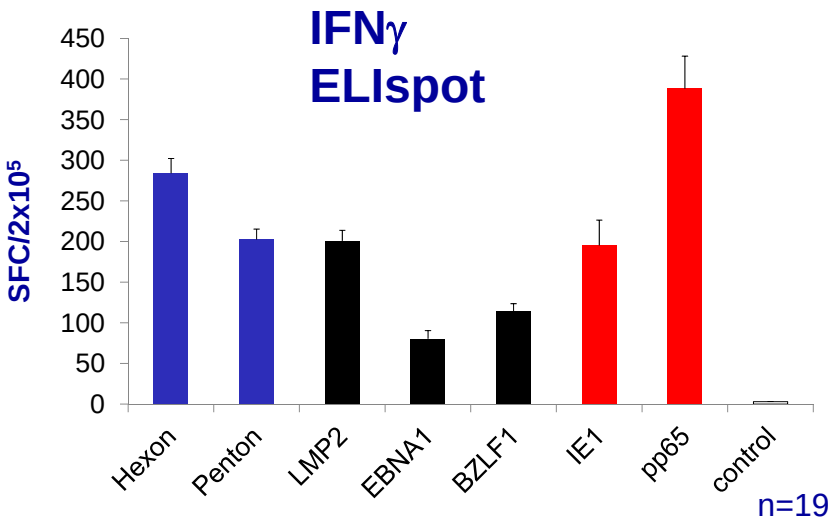




Manufacturing Costs of Each Production of CTLs

Cost Item	Conventional CTL	Rapid CTL
GMP facility	$2280	$300
Trained technician Hours	$2,000	$200
CTL line manufacture	$3,076	$1,334
Release testing	$3,203	$1,671
TOTAL	$10,559	$3,505

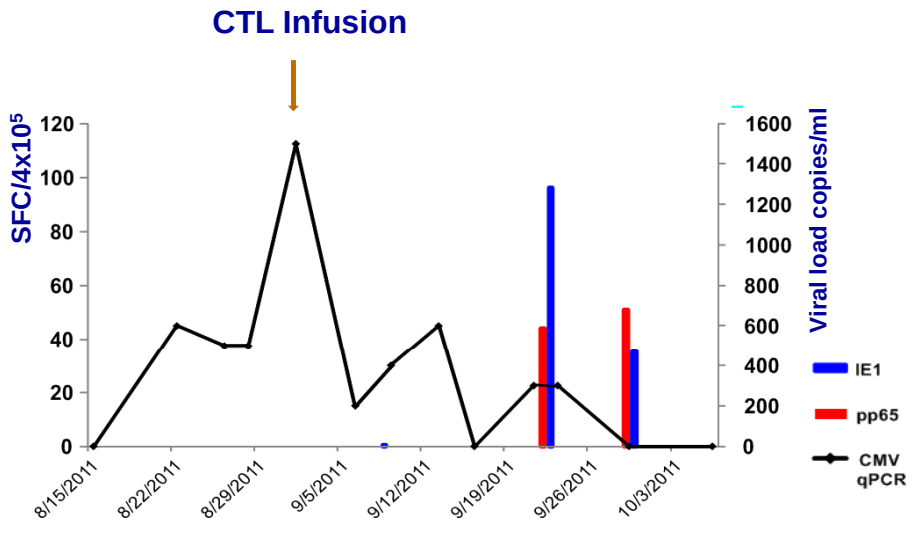
rCTL Specificity



Clinical Trial Of Rapid CTLs

- FDA required we only treat patients with disease in dose escalation phase
- 5 patients treated
  - 2 with CMV complete response
  - 1 with adenovirus complete response blood and urine
  - 1 with EBV and adenovirus complete response both viruses
  - 1 too early

Patient 2 CMV



Extending Applicability
Banked Allogeneic Matched CTLs

- 50% response rate in Phase II study for PTLD post solid organ transplant

  Haque et al Blood 2007

- EBV CTLs induced CRs in 4/5 patients with PTLD

  Barker et al Blood 2010

  Doubrivina et al Blood 2011

Most Closely HLA Matched Allogeneic Virus Specific Cytotoxic T-Lymphocytes (CTL) to Treat Persistent Reactivation or Infection with Adenovirus, CMV and EBV after Hemopoietic Stem Cell Transplantation

PACT
Production Assistance for Cellular Therapies

SCCT
Specialized Centers for Cell-Based Therapy
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Most Closely HLA Matched Allogeneic Virus Specific Cytotoxic T-Lymphocytes (CTL) to Treat Persistent Reactivation or Infection with Adenovirus, CMV and EBV after Hemopoietic Stem Cell Transplantation

CAGT
Helen Heslop
Ann Leen
Clio Rooney
Cath Bollard
Malcolm Brenner
Adrian Gee

Other Sites	
MDACC	EJ Shpall
Harvard	Joe Antin, B Dey
Duke	Paul Szabolcs
CHLA	Neena Kapoor
Childrens Boston	Sun Yun Pai
Miami	Gary Kleiner
Hackensack	Scott Rowley

PACT
Production Assistance for Cellular Therapies

SCCT
Specialized Centers for Cell-Based Therapy
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Product

- Most closely HLA-matched multivirus specific CTLs (CHM-CTL)
  - CTL lines already made for previous studies
  - New CTL lines made from donors with common alleles (PACT)
- 32 lines currently available
- Multicenter study)

Clinical Protocol

- Treatment of refractory EBV, CMV, or Adv
- Patients receive 2 x 10^7 CHM-CTL/m^2 as a single infusion.
- If partial response may receive up to 4-5 additional doses at 2+ weekly intervals

Screening

- 77 patients screened
- Line identified for 68/77
  - Suitable line if matched at least one antigen with activity against infecting virus
- 9/77 no suitable line

Screening

- 23 patients with line not on study
  - subsequently not eligible due to other infections
  - improved
  - progressed and died prior to infusion
  - declined

Patients Treated on Study

- 48 patients enrolled
  - 23 with CMV
  - 9 with EBV
  - 16 with adenovirus

Matching of CTL Line

	Recipient	Donor 1
• 1/6 match	26%	24%
• 2/6 match	45%	45%
• 3/6 match	26%	26%
• 4/6 match	3%	5%

Are The CTL Safe?

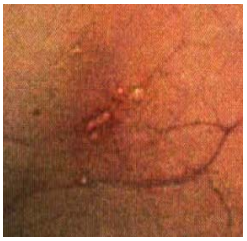
Adverse Events

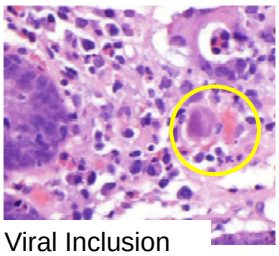
- Acute GVHD within 45 days first infusion
  - 33 none
  - 5 Grade 1 skin
  - 1 Grade 3 liver (also adenovirus)
  - 1 chronic GVHD flare (discontinued immunosuppression)
- 2 developed transplant-associated microangiopathy (both on Rapamycin)

Do The CTL Produce Clinical Benefit?

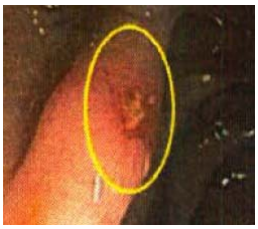
CMV Colitis Responds to CTLs – pt69

Pre CTLs

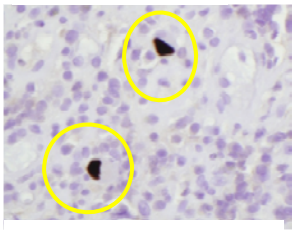




Viral Inclusion

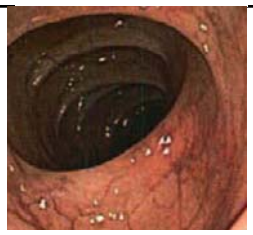


Ulcers on endoscopy

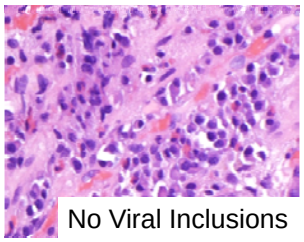


Immunostain for CMV

Post CTLs

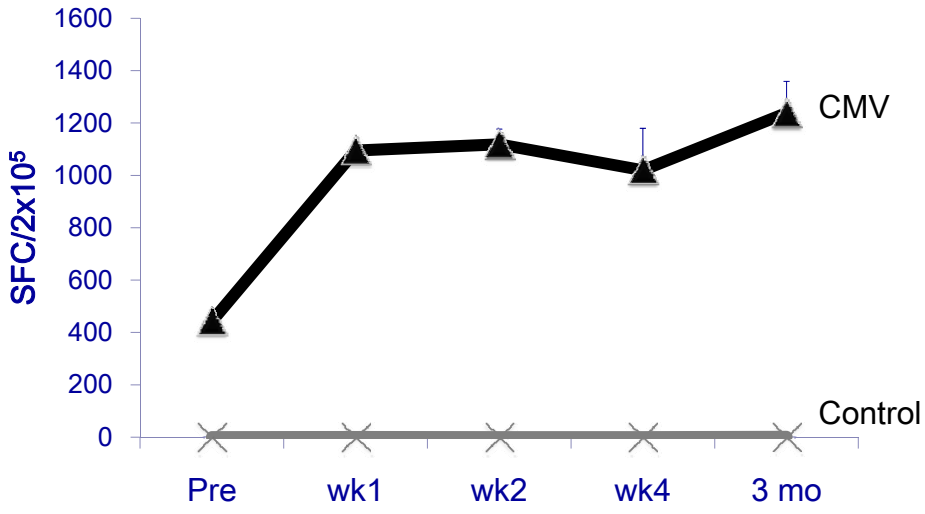


Normal endoscopy

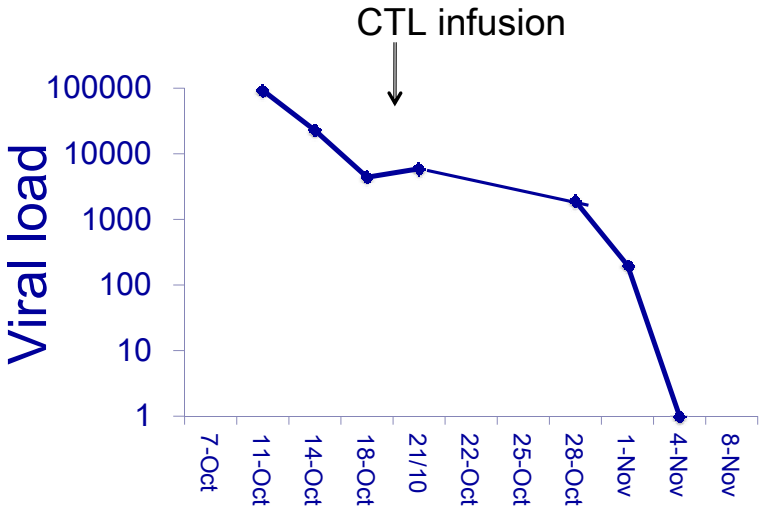


No Viral Inclusions

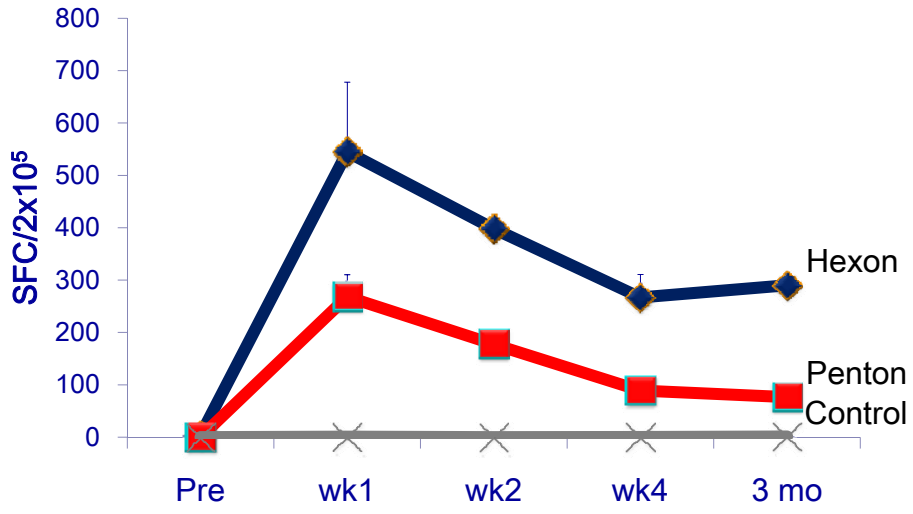
Clinical Response Correlates With Increase In Virus-specific T Cells - pt69



Clinical Response pt69 – Adv

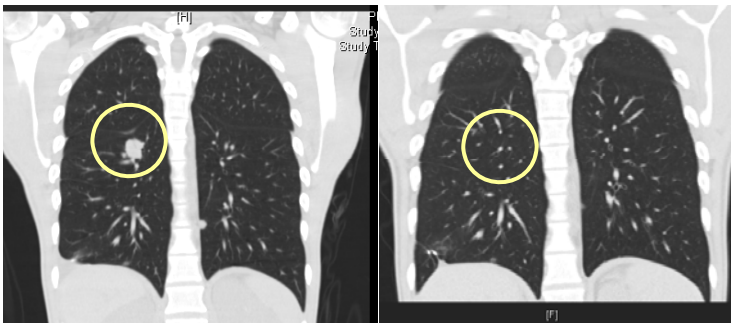


Clinical Response Correlates With Increase In Virus-specific T Cells - pt69



Do The CTL Produce Clinical Benefit?

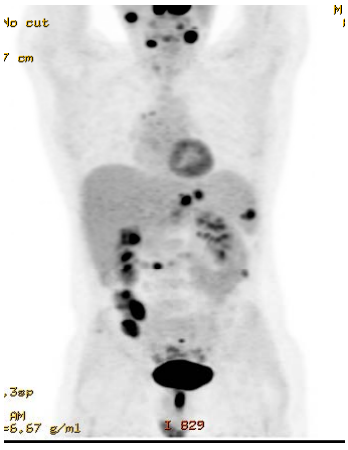
CD20- EBV+ Lymphoma Responds to Allogeneic Trivirus CTLs (pt16)



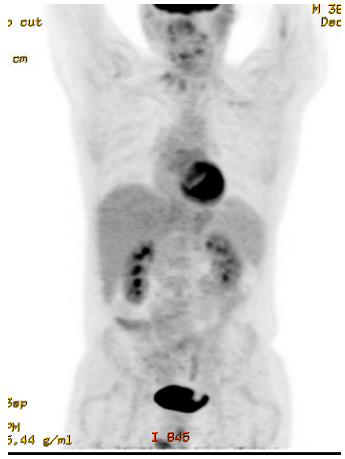
Pre 4 months

Clinical Responses –EBV (pt37)

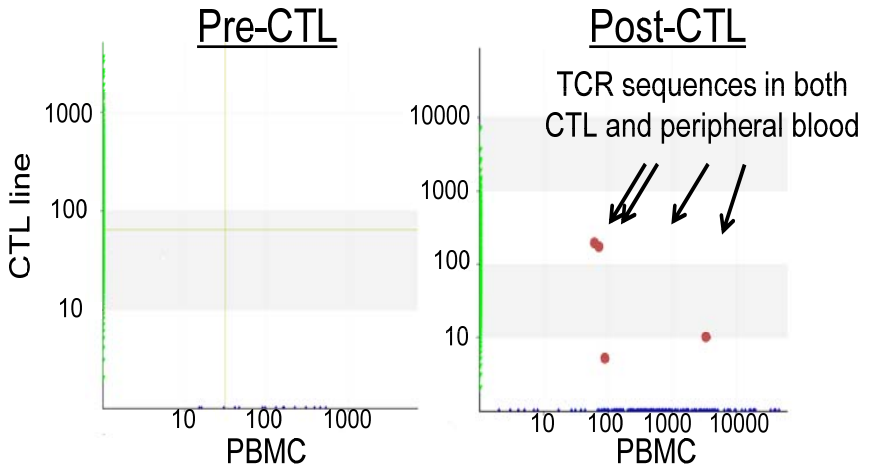
Pre CTLs



1 month post CTLs



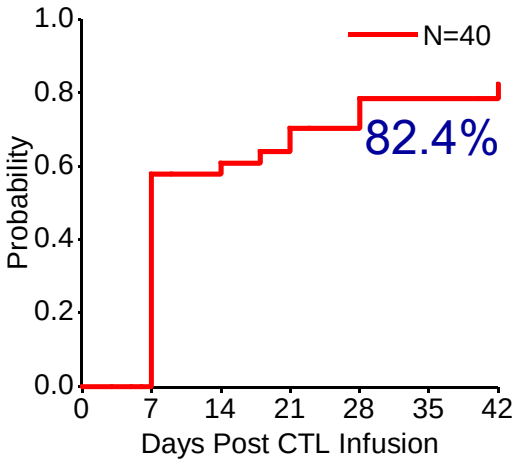
TCR V-beta analysis to track T cell persistence in vivo



Overall response rate

Cumulative incidence of CR/PR

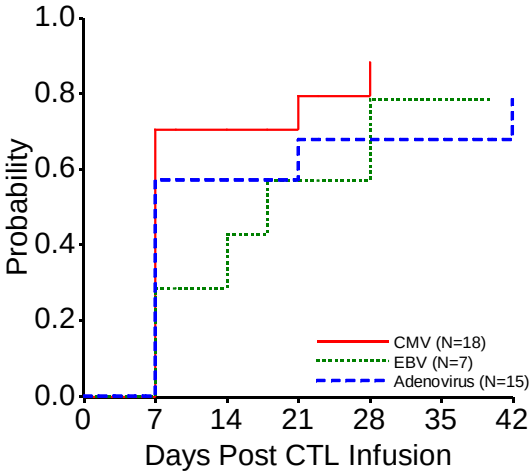
- First 40 pts based on viral load by day 42 post-infusion



Overall Response Rate

CR/PR based on infection

- Overall 82.4%
  - 88.2% - CMV
  - 78.6% - EBV
  - 78.6% - Adv



Conclusions

- Low attributable toxicity
- CTLs effective in clearing EBV/Adv/CMV disease
- T cell expansion often but not always detected
- May require several infusions to sustain benefit

What Are Requirements for Banked Cells?

- Donor evaluation
- Level of testing of banked lines
- Edinburgh group manufacturing new bank with optimal donors

Remaining Questions?

- How many lines do we need?
  - Edinburgh group estimated 22-26 to match 75% at least 3 loci
- Mechanism of action
- What is the best process for CTL manufacture?
  - Plasmids/peptides

Conclusions

Recipient specific CTLs

- Expansion and persistence of CTLs specific for latent viruses EBV and CMV
- Antiviral and anti-tumor effects for EBV, CMV and adenovirus

Partially matched donor CTLs

- Evidence for antiviral activity

Manufacturing

- Process development to shorten & simplify

Acknowledgements

TRL Lab PIs
Malcolm Brenner
Cliona Rooney

Ann Leen
Catherine Bollard
Gianpietro Dotti
Barbara Savoldo
Stephen Gottschalk
Aaron Foster

GMP Laboratory
Adrian Gee
Zhuyong Mei

CTL Laboratory
Oamar Diouf
Tessie Lopez
Joyce Ku
Antonio Di-Stasi
Huimin Zhang

TRL Laboratory
Eric Yvon
Lisa Rollins
Enli Liu
Stephanie Ku
Usha Katari
Jacqueline Kiernan

Clinical Research
Bambi Grilley
Bridget Medina
Alician Brown
Yu-Feng Lin
Vicky Torrano
Tara Gray

Transplant Service
Bob Krance
Kathy Leung
Caridad Martinez
George Carrum
Ram Kamble

TRL Junior Faculty/ Postdocs/PhD students
Chrystal Louis
Carlos Ramos
Nabil Ahmed
Doug Myers
Alana Kennedy-Nasser
Jessica Shafer
John Craddock
Juan Vera
Caroline Arber Barth
Serena Perna
Valentina Hoyos
Ulrike Gerdemann
Leslie Huye
Valentina Hoyos
Russell Cruz
Patrick Hanley
Don Shaffer
Mingtran Ngo

SCCT CHALLAH STUDY
Joe Antin
B Dey
David Avigan
Paul Szabolcs
EJ Shpall
Neena Kapoor
EMMES
Adam Mendizabal
NMDP
Dennis Confer

EBV CTLs post HSCT
St Jude
Greg Hale
Karen Slobod
Julia Hurwitz
Richard Rochester
GOS
Persis Amrolia

Funding: NCI Program Project Grant, NHLBI Somatic Cell Therapy Center, Lymphoma SPORE, Leukemia and Lymphoma Society Specialized Center of Research, Doris Duke Distinguished Clinical Scientist Award, PACT