T-cell Therapies for Malignancies

Stephen Gottschalk

Epstein Barr Virus

- Infects >90% population
- Acute infection is followed by life-long latency
- Expression of limited array of viral latency proteins
- Usually benign BUT
- Latent virus associated with diverse group of malignancies

EBV-associated Malignancies

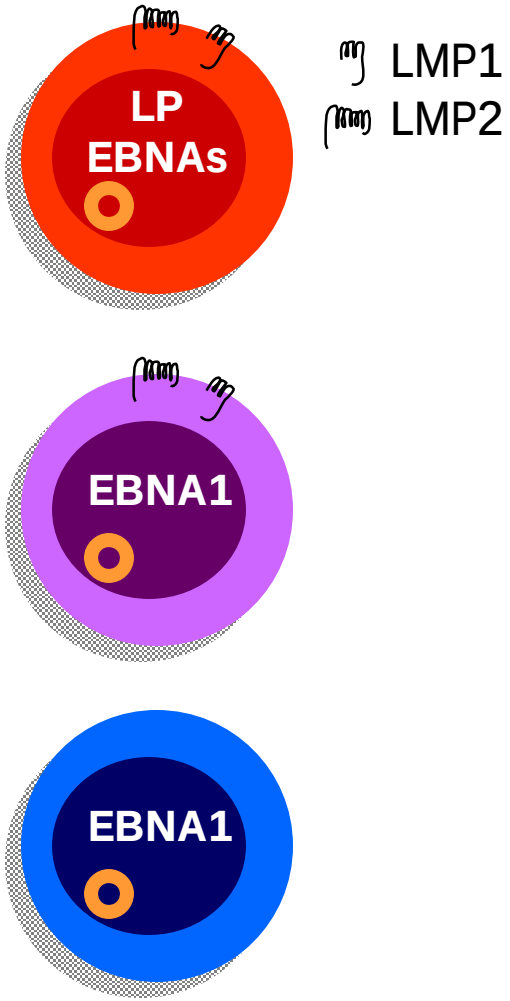
Latency/Malignancy

Type III
Post Transplant lymphoma
HIV-associated lymphoma

Type II
EBV+ Hodgkin's disease
EBV+ NHL
Nasopharyngeal Carcinoma

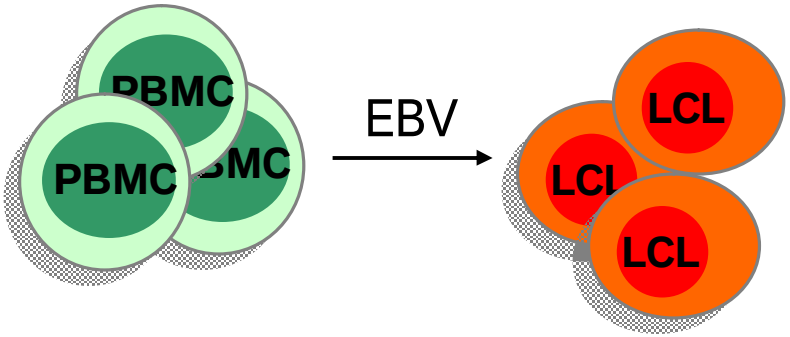
Type I
Burkitt's lymphoma
Gastric adenocarcinoma

Latent Gene Expression

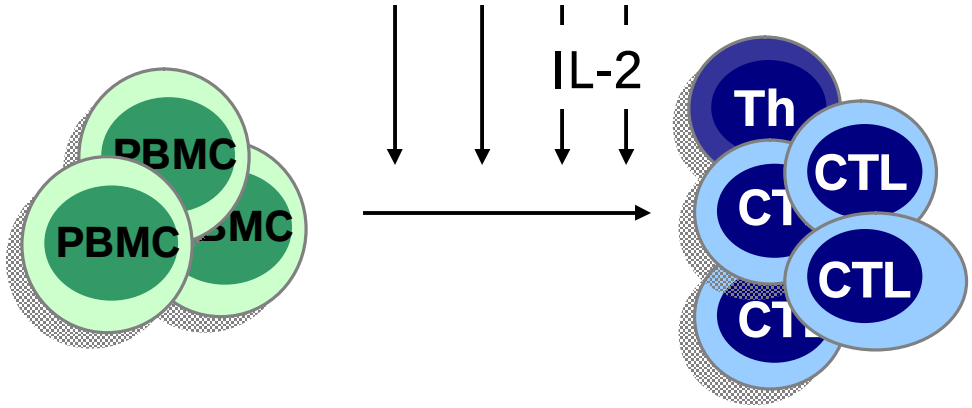


Immunogenicity

EBV-CTL Generation for Clinical Trials



Step 1: LCL generation
4-6 weeks



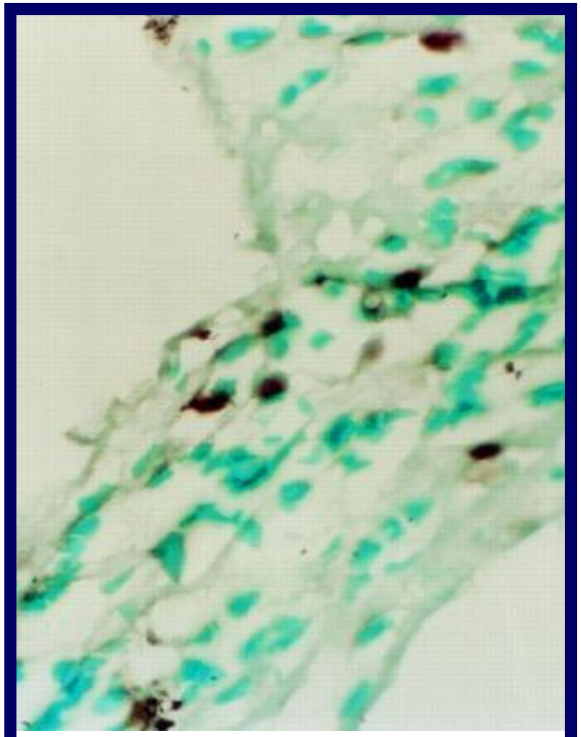
Step 2: CTL expansion
4-7 weeks

Step 3: QA/QC
1-2 weeks

Donor-derived EBV-specific CTL for Stem Cell Transplant Recipients

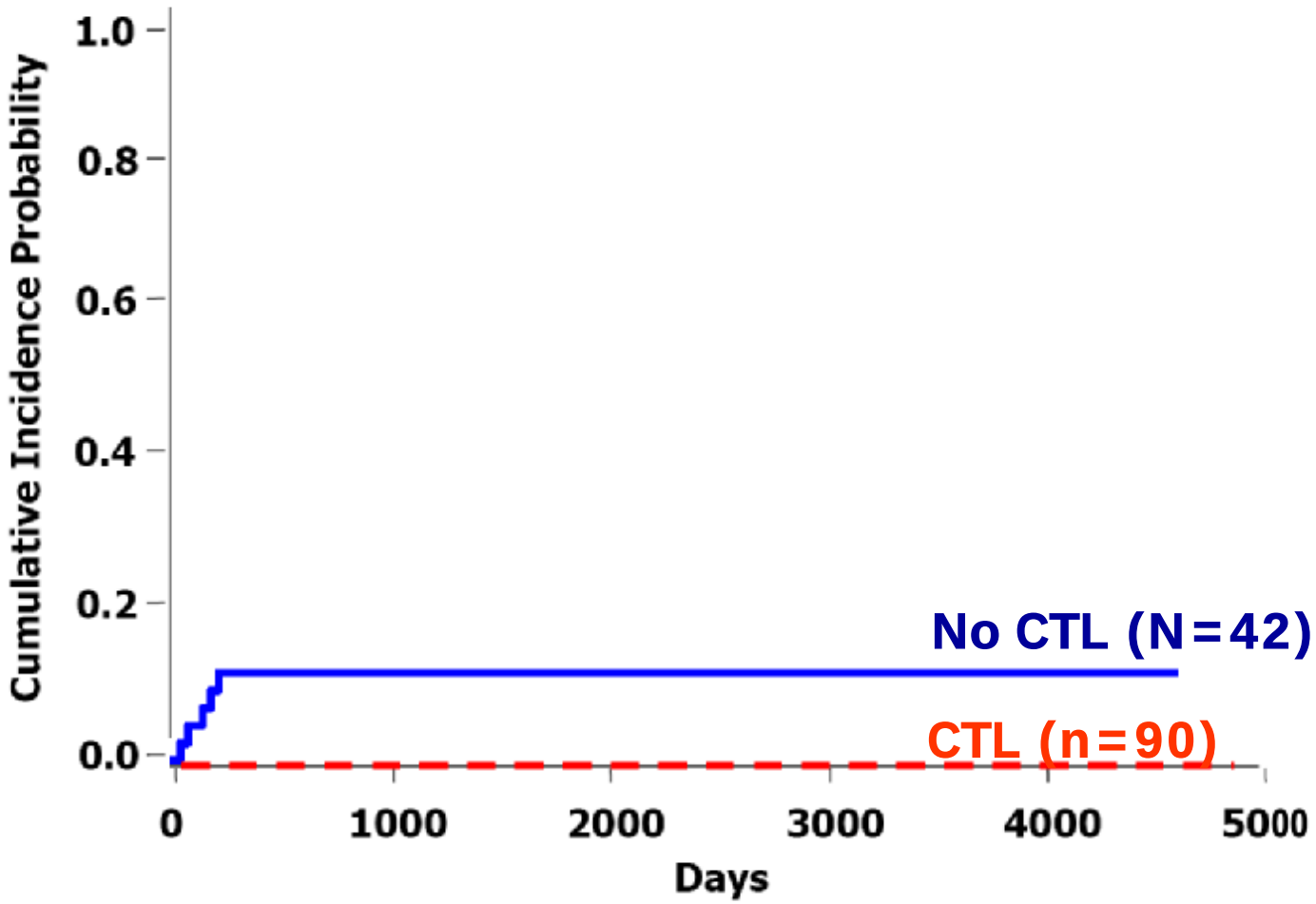
(Heslop et al, Nature Medicine 1996; Rooney et al, Blood 1998)

- >100 allogeneic bone marrow transplant recipients
- Persist up to 7 years
- Remain functionally active and localize to tumor sites



Marked CTL by in situ PCR at tumor site

Incidence of PTLD in patients receiving CD6/CD8 depleted marrow with or without EBV CTL prophylaxis



EBV Specific CTLs as Therapy for EBV-PTLD

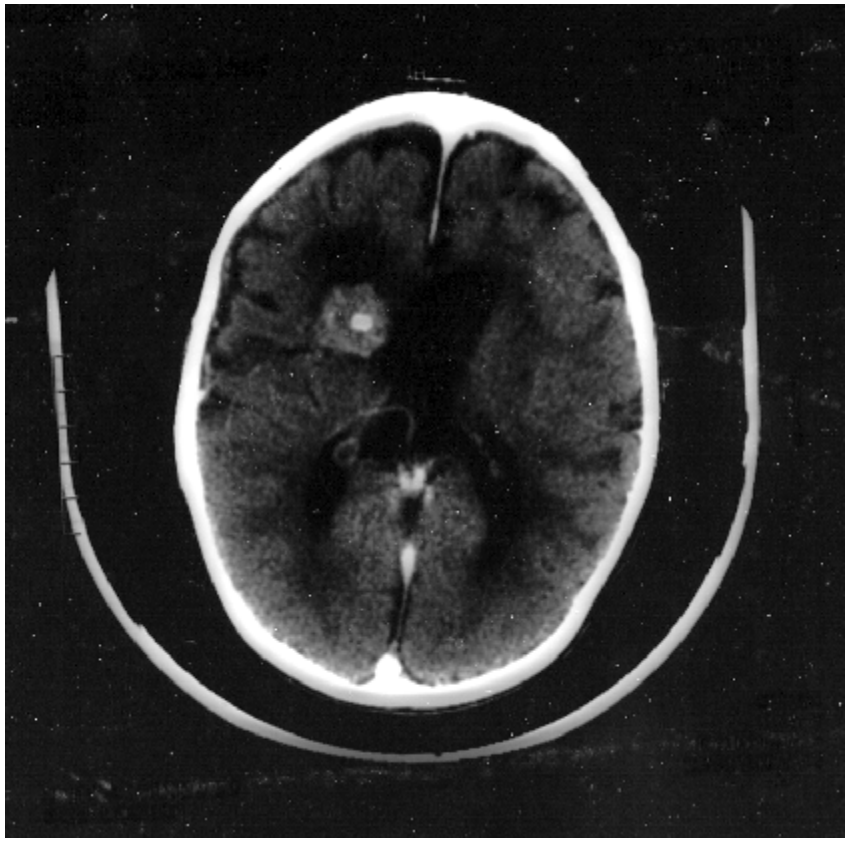
- 13 patients treated with active disease
- 2/13 failed to respond
  - 1 with extensive disease including CNS disease died 5 days after infusion
  - 1 died with progressive disease 3 weeks later
    - Donor CTL line recognized two immunodominant HLA 11 restricted epitopes in EBNA 3B that were deleted in tumor

EBV-CTL for CNS EBV PTLD

- T-cell depleted unrelated transplant for Hurler syndrome
- 12 months post BMT presented with lymphoma in brain

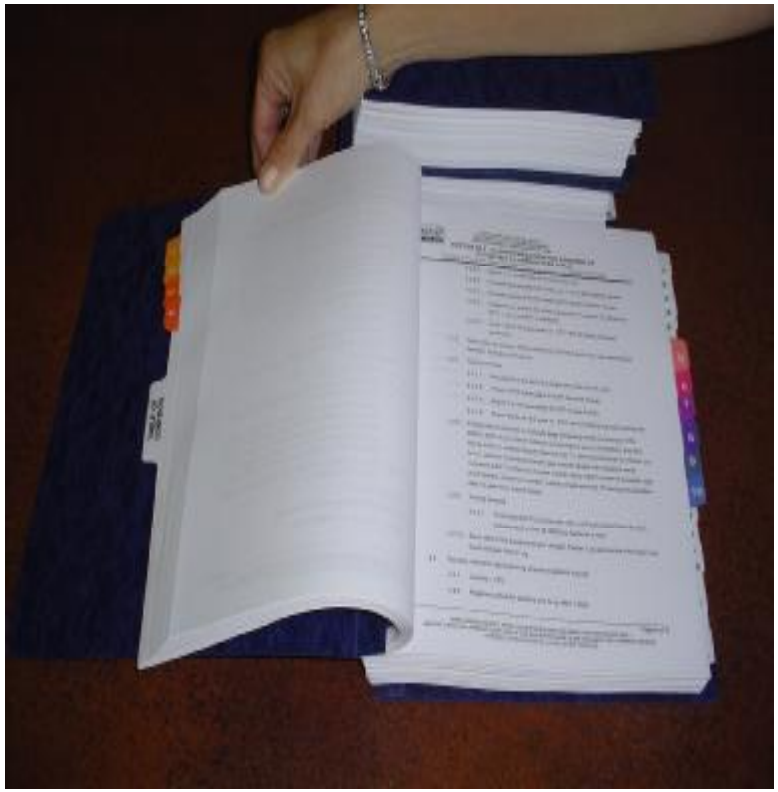
EBV-CTL for CNS EBV PTLD

- Given donor derived EBV- specific CTLs
- Progressive clinical and radiologic response over 6 months



EBV-specific T cells for PTLD

- Successful Orphan drug designation in 2007
- How to obtain orphan drug ***approval***?



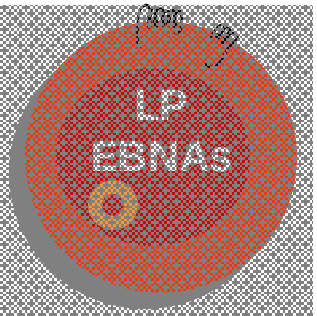
EBV-associated Malignancies

Latency/Malignancy

Latent Gene Expression

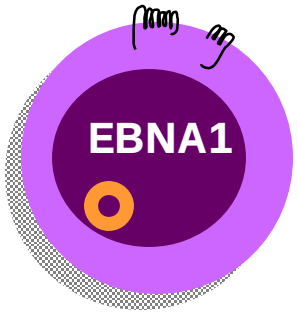
Immunogenicity

Type III
Post Transplant lymphoma
HIV-associated lymphoma

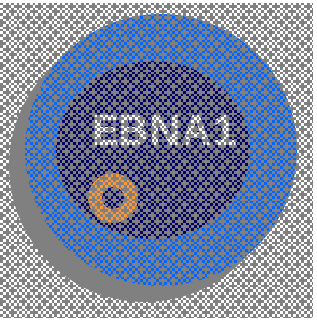


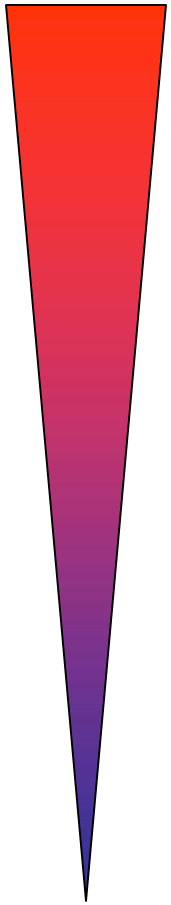
LMP1
LMP2

Type II
EBV+ Hodgkin's disease
EBV+ NHL
Nasopharyngeal Carcinoma

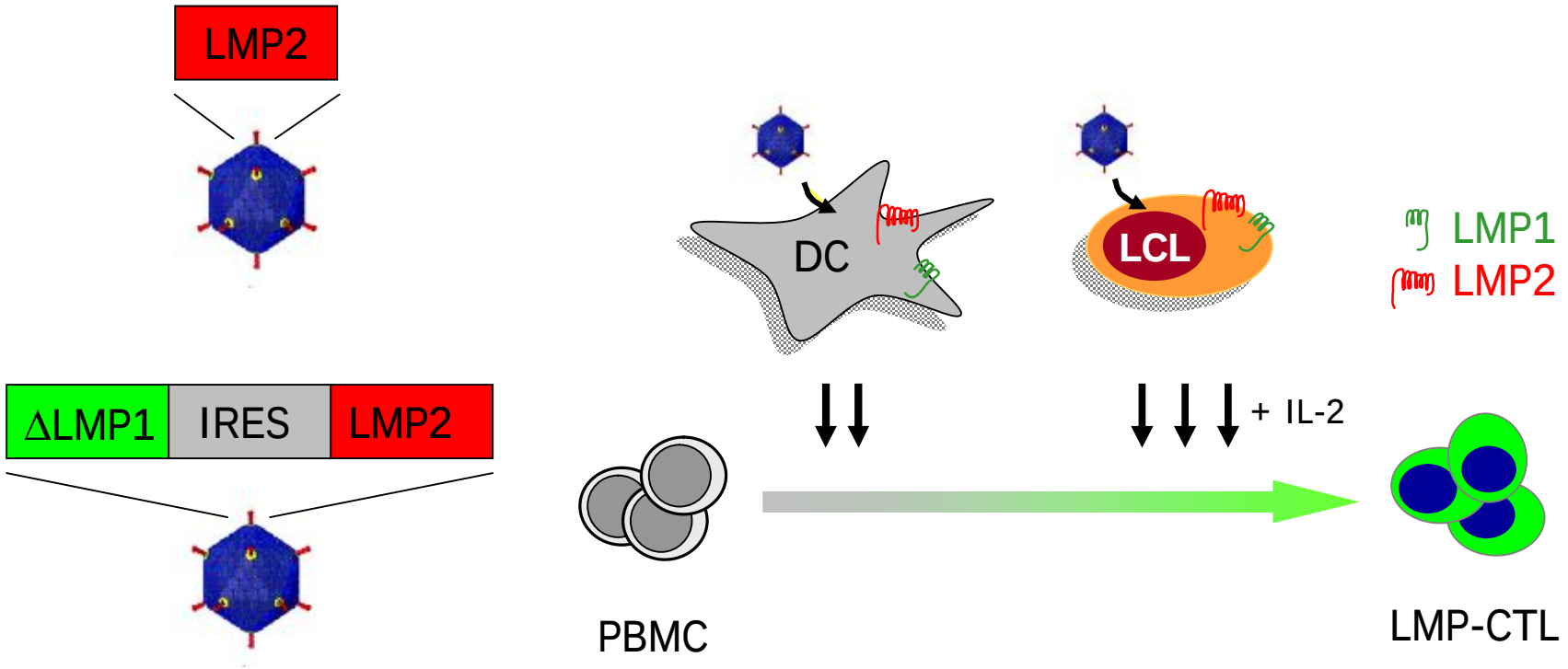


Type I
Burkitt's lymphoma
Gastric adenocarcinoma



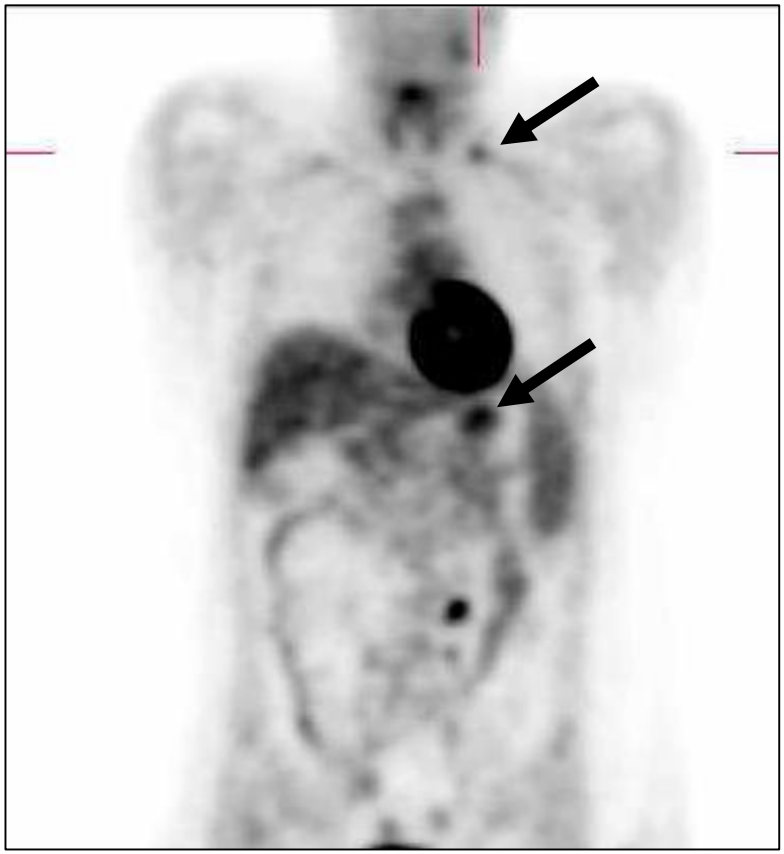


Ex-vivo Activation and Expansion of LMP1 and LMP2-specific CTL

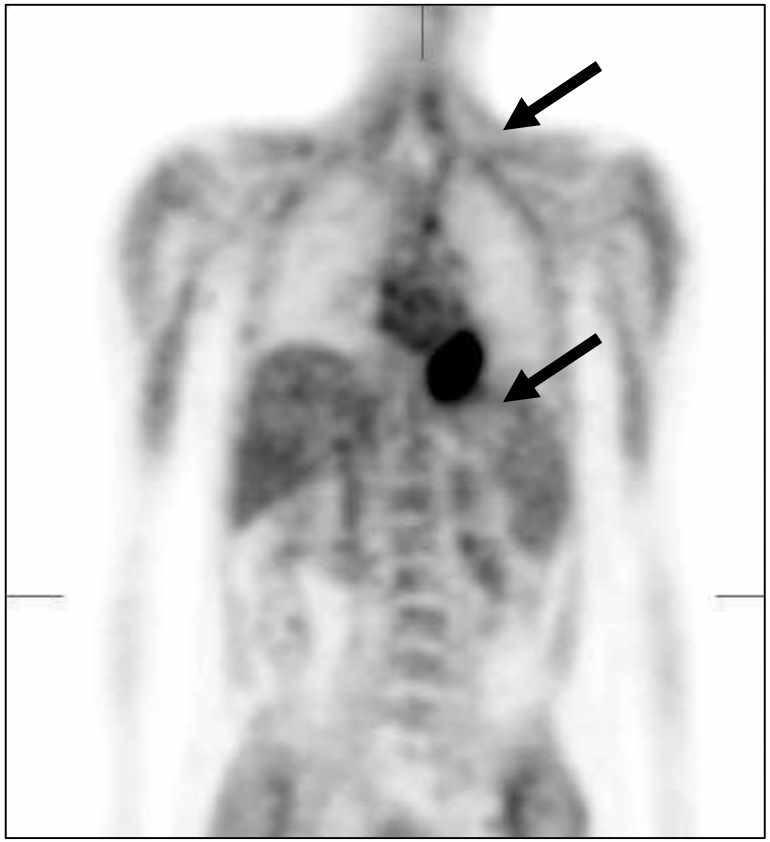


Complete Radiological Response on PET Scan Post LMP-CTL

Pre CTL



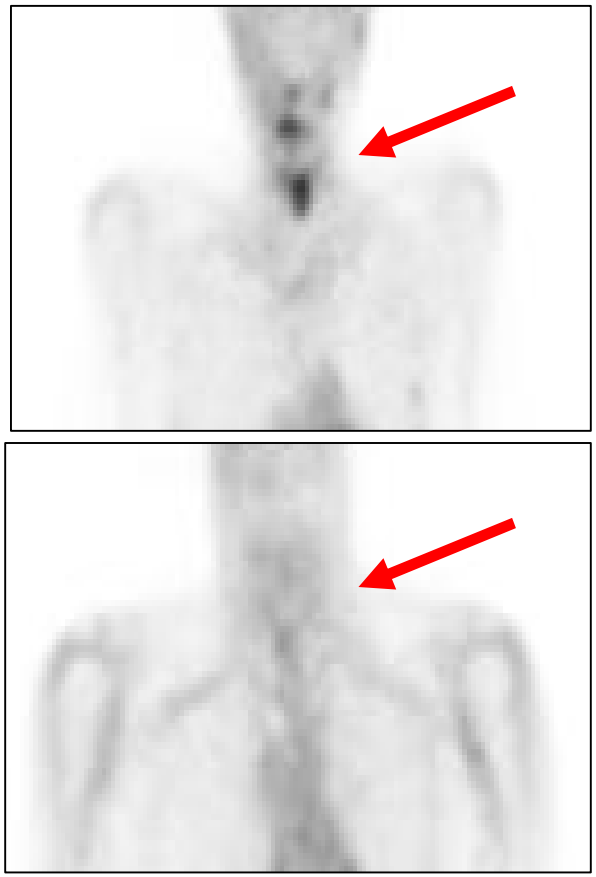
Post CTL

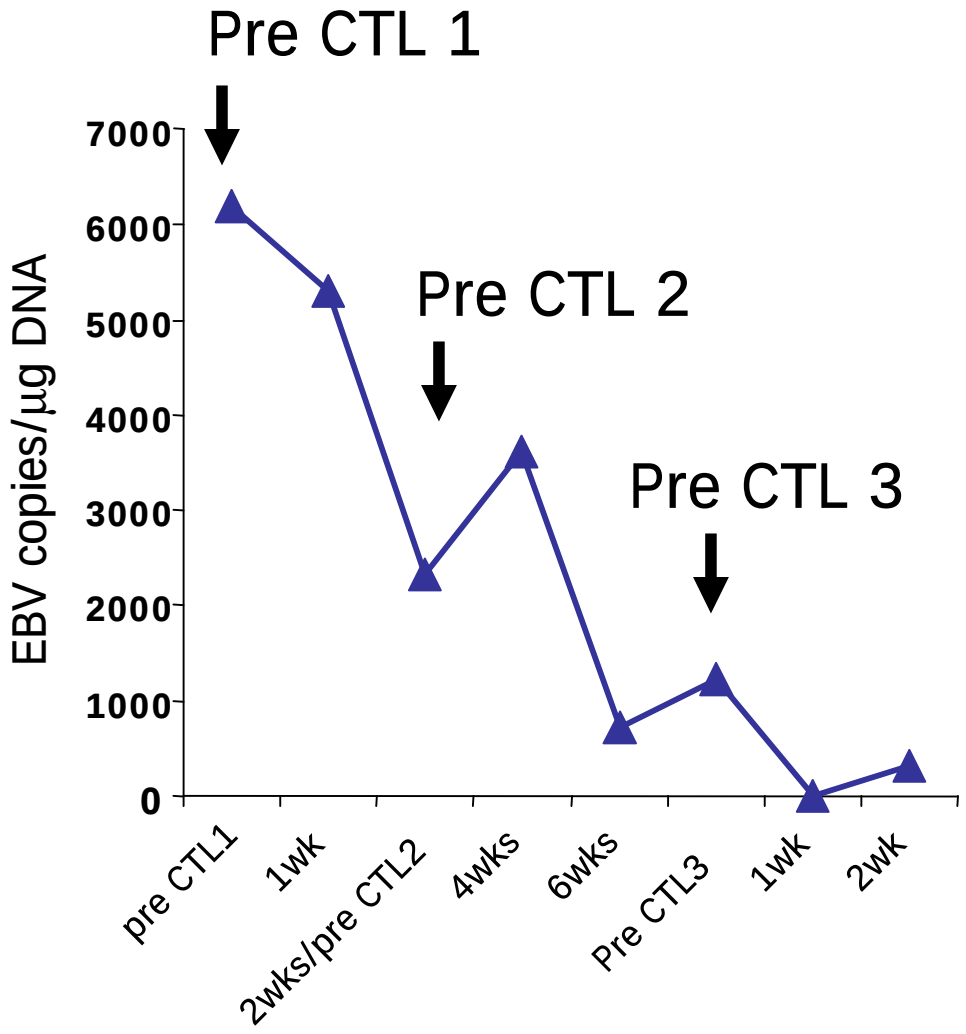


EBV positive NK-T NHL: Complete Radiological Response

Pre CTL

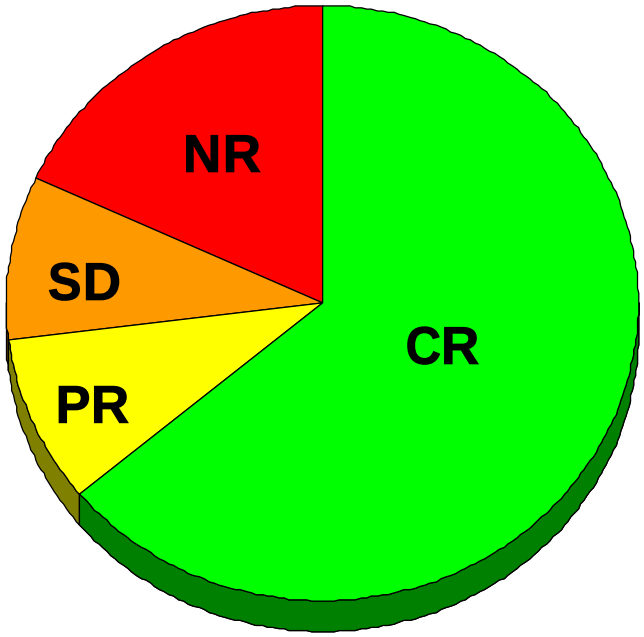
Post CTL





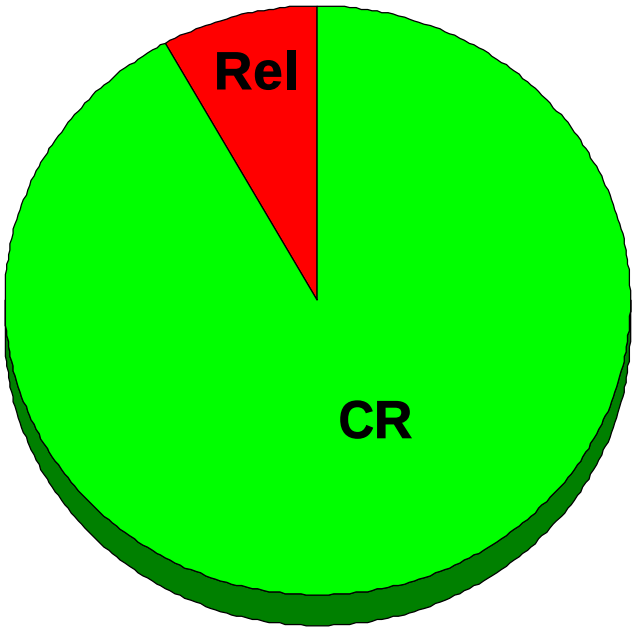
Clinical Responses After LMP-CTL Therapy in Lymphoma

Patients with disease at CTL infusion



n = 11

Patients high risk for relapse at CTL infusion

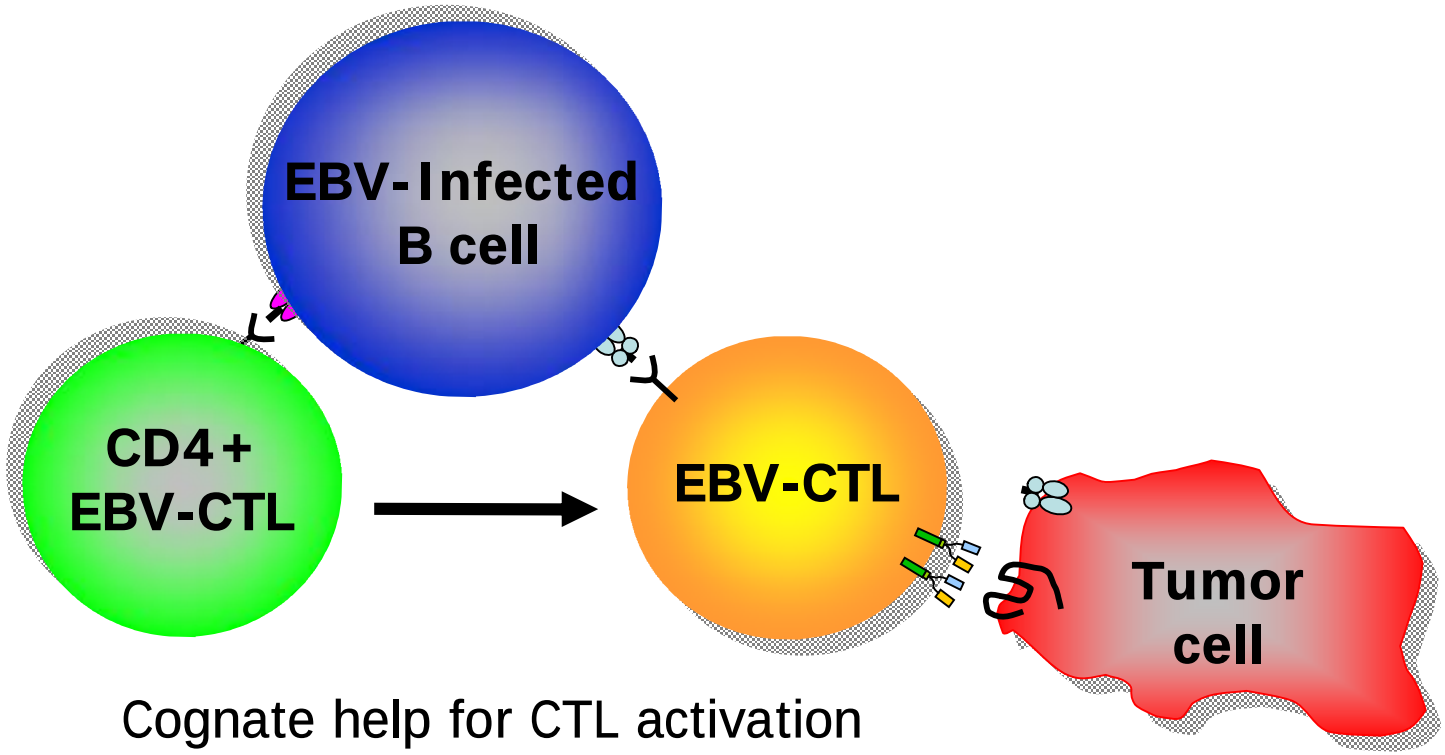


n = 13

Genetic Modification to enhance the efficacy of T-cell Therapies

- Genetic engineering of T cells to
  - Target non viral tumor antigens
  - Enhance T-cell function
    - Homing to tumor sites
    - Render cells resistant to inhibitory tumor microenvironment

Generation of Bi-specific CTL to target non EBV antigens



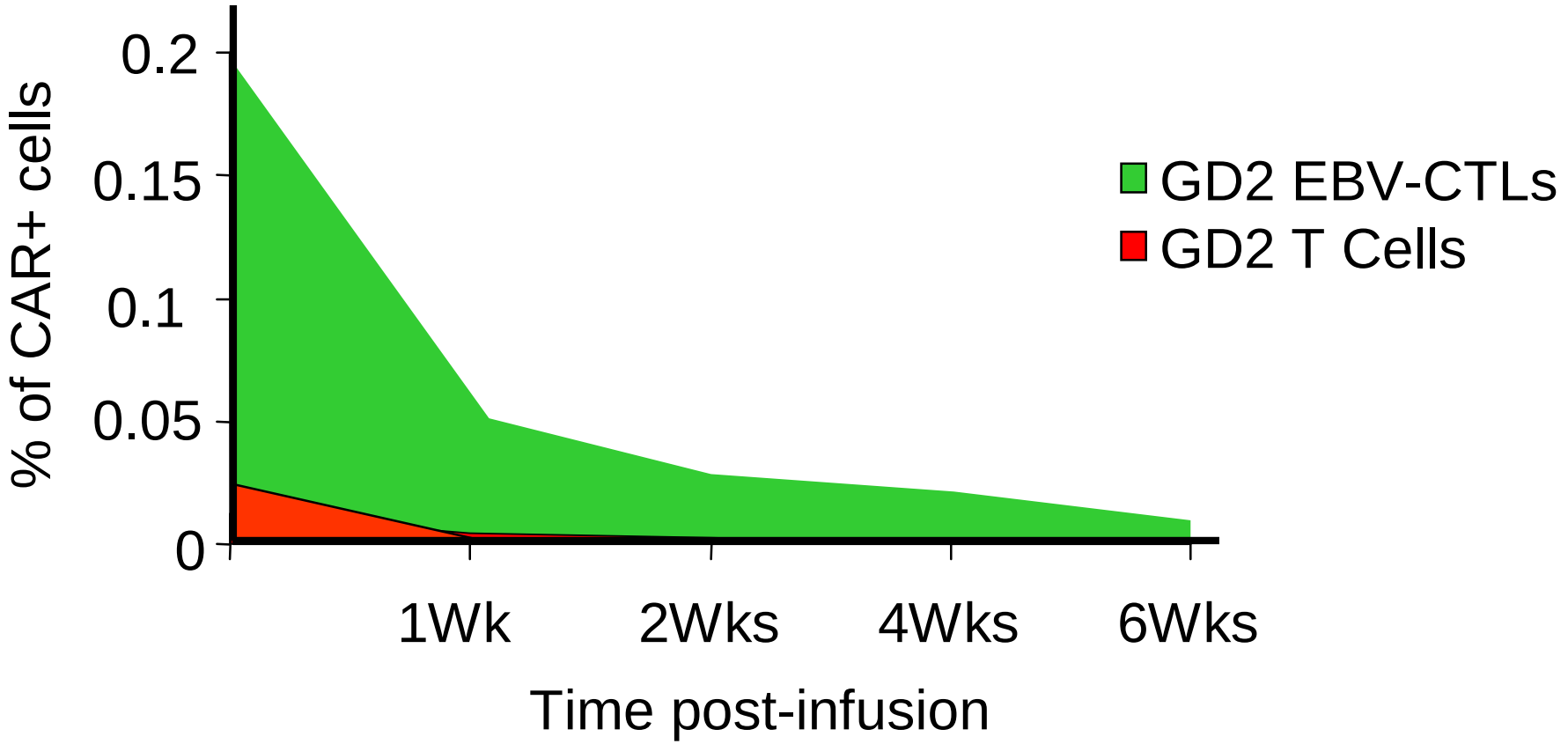
Cognate help for CTL activation and expansion

Rossig et al, Blood 2002

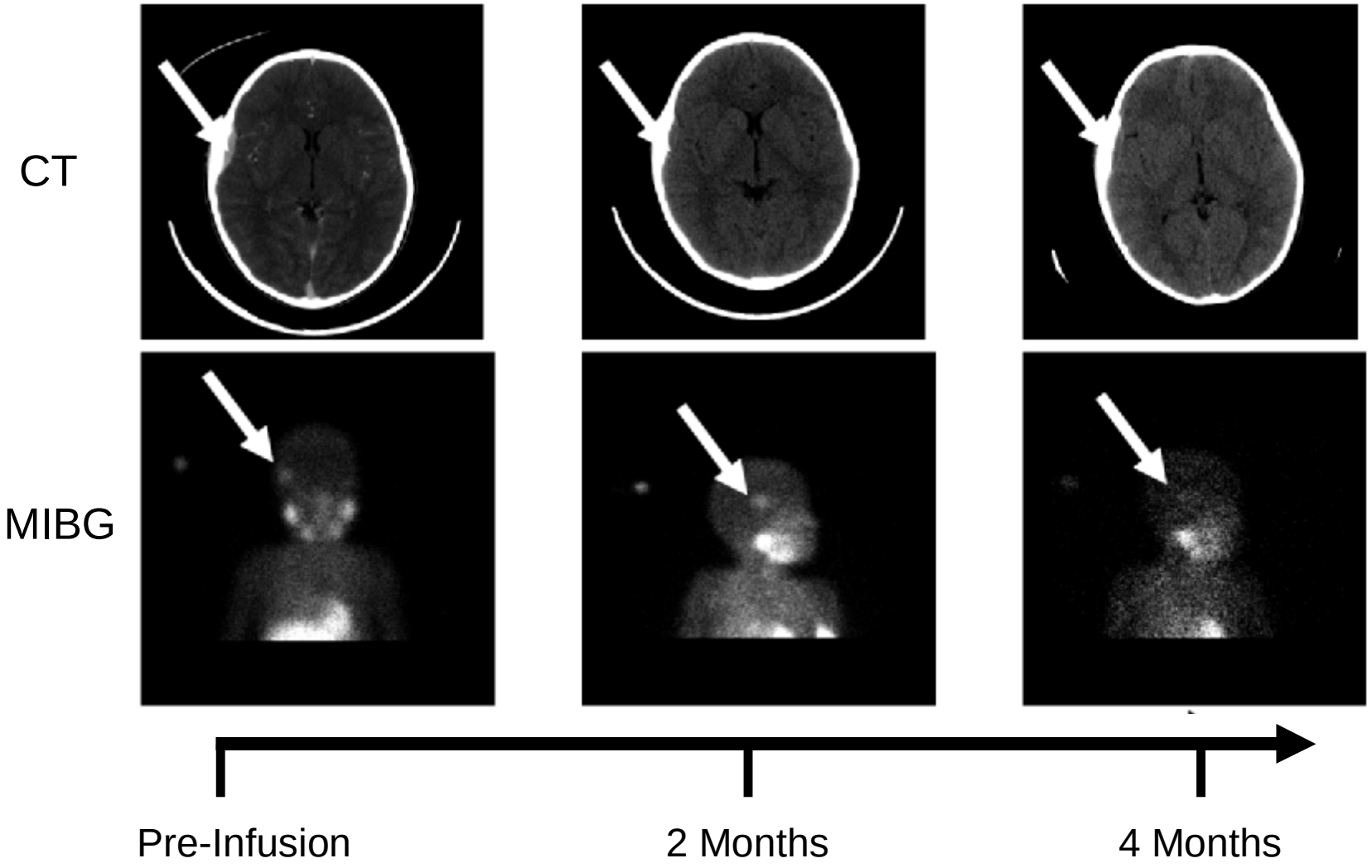
Savoldo et al, Blood 2007

Pule et al, Nature Medicine (in press)

Persistence of GD2-specific T cells in Neuroblastoma patients



Complete Remission of Refractory Neuroblastoma post GD_2-specific CTL



Summary

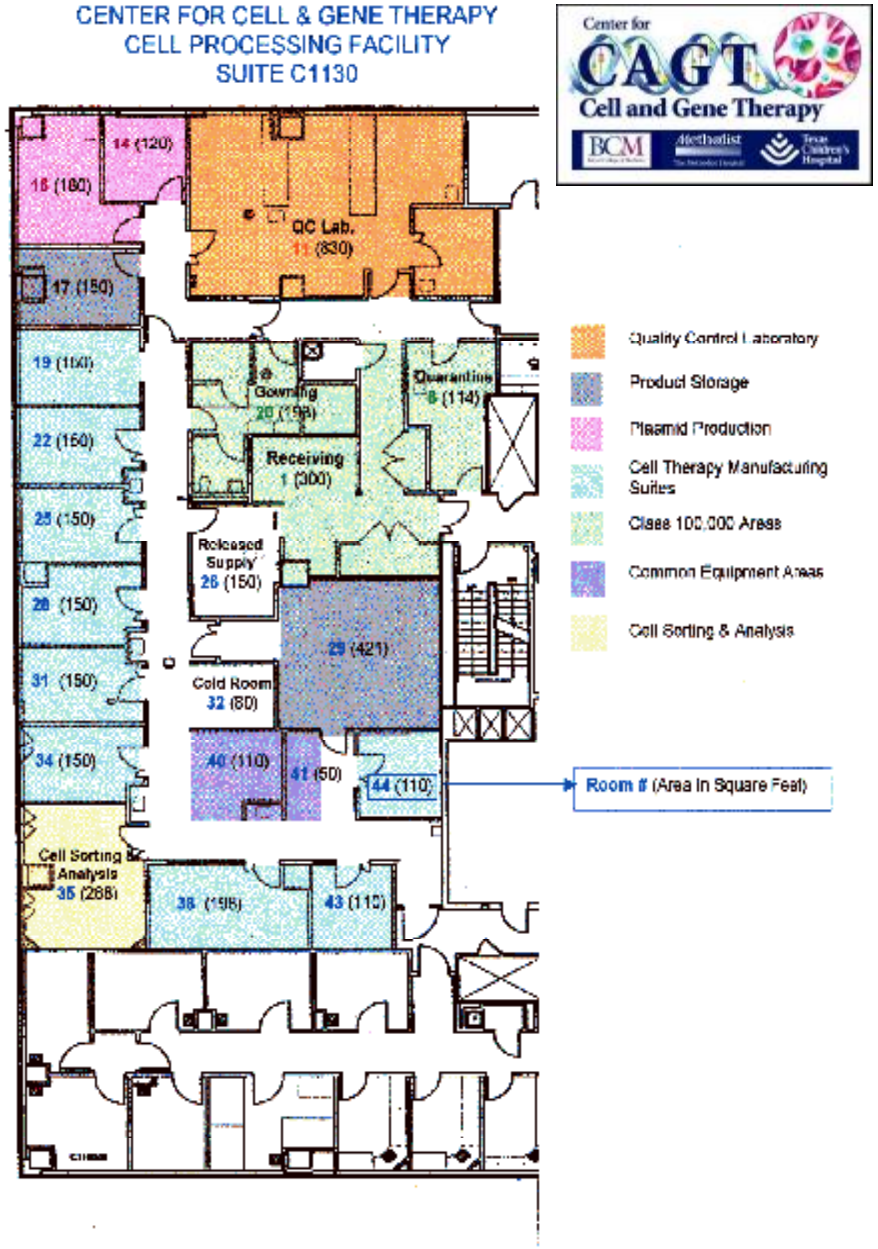
- Adoptive transfer of EBV- and LMP- specific CTL for EBV+ Lymphomas, Nasopharyngeal Carcinoma, Neuroblastoma:
  - Safe
  - Significant antitumor activity

Requirements for T-cell Therapies

- GMP Cell Processing Facility
- Trained staff
- QA/QC Program
- Phase I clinical studies: grant support
- Phase II clinical studies:
  - not possible with standard grant funding
  - need to recover cost of T-cell production

Cell Processing Facility

- Class 10,000 cleanroon facility
- 9 Production Rooms
- Flow Cytometry Laboratory
- Centralized Cryobank
- Central Supply Management
- Barcoding System
- GMP/GTP Compliant



Cell Processing Facility

Center for CAGT Cell and Gene Therapy

BCM

Quality Control Laboratory

- Environmental monitoring
- Bioburden
- Mycoplasma
- Endotoxin
- Viral Titers
- RCA
- EBV PCR
- RCR PCR

T-Cell Products Infused in Clinical Trials at CAGT

EBV specific CTLs for PTLD	125
EBV specific CTLs for Type II latency tumors	54
LMP-specific CTLs	42
CAR-transduced CTLs and activated T cells	25

Cost of Producing T-cell products

	EBV-specific T cells	LMP-specific T cells
GMP charge	3425	3811
Supplies	512.69	1495.09
QC testing	4242.1	4242.1
Total	**8179.79**	**9855.67**

Production cost funding for Phase I clinical studies

Cell and Vector Cores of P01, SPORE, SCOR

Clinical studies with tumor-specific T cells

- Phase I clinical studies promising with complete and sustained responses in multiple diseases

- Phase II clinical studies are cost prohibitive unless T-cell production cost can be recovered

Immunotherapy with antigen-specific CTL

TRL Laboratories

Cliona Rooney
Malcolm Brenner
Helen Heslop
Catherine Bollard
Barbara Savoldo
Stephen Gottschalk
Gianpietro Dotti
Ann Leen
Doug Myers
Nabil Ahmed
Aaron Foster
Chyrstal Louis
Miriam Khalil
John Craddock
Don Shaffer
Alana Kennedy Nasser
Claudia Gerken

Clinical Research

Bambi Grilley
Cynthia Boudreaux
Florence Noel
Yu-Feng Lin
Vicky Torrano

GMP Laboratory

Adrian Gee
Tessie Lopez
Joye Ku
Marina McCeight
Huimin Zhang
Enli Liu
Rong Cai