Using Gene Transfer to Retarget Cytotoxic T lymphocytes

Malcolm Brenner

Epstein Barr Virus

- Infects >90% population
- Acute infection is followed by life-long latency
- Expression of limited array of viral latency proteins
- Usually benign

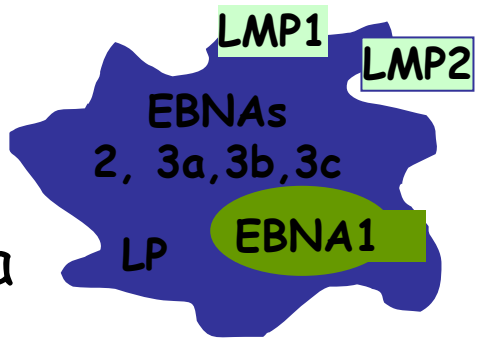
Epstein Barr Virus

- Infects >90% population
- Acute infection is followed by life-long latency
- Expression of limited array of viral latency proteins
- Usually benign
- Latent virus can produce malignant transformation in B/T lymphocytes and epithelial cells

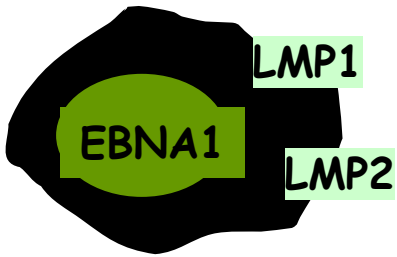
EBV-associated Malignancies

Latency/Malignancy **Gene Expression** **Immunogenicity**

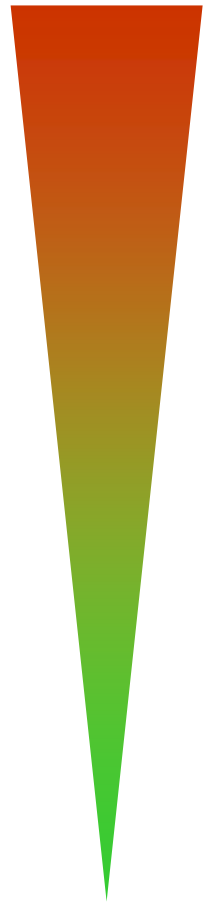
<u>Type 3</u>
Post transplant lymphoma
HIV-associated lymphoma



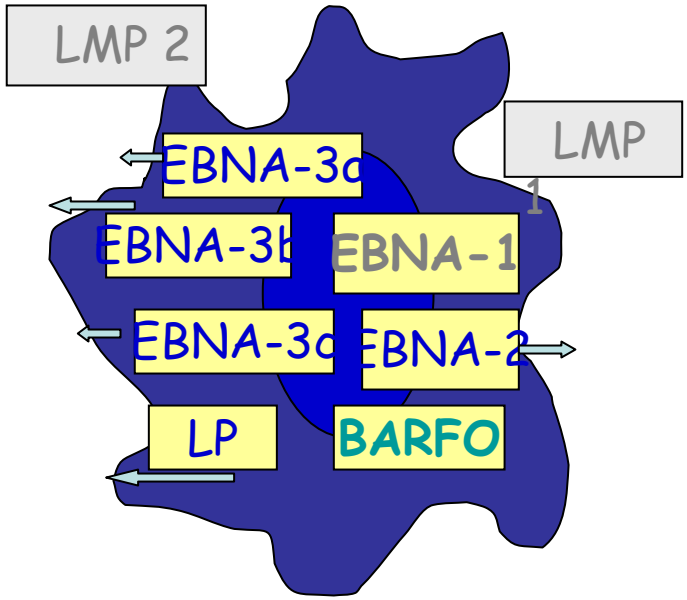
<u>Type 2</u>
Hodgkin's lymphoma
NHL
Nasopharyngeal carcinoma



<u>Type 1</u>
Burkitt's lymphoma
Gastric adenocarcinoma

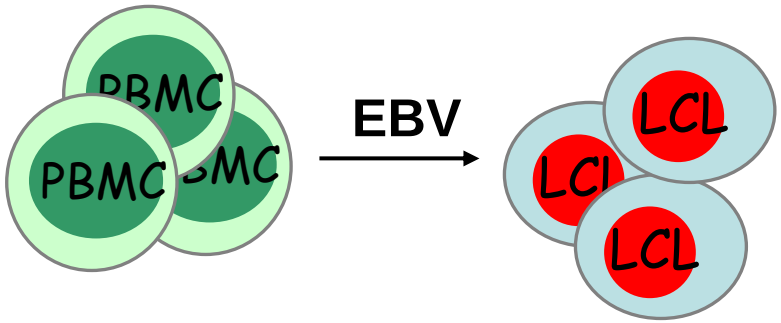


Types of EBV Latency

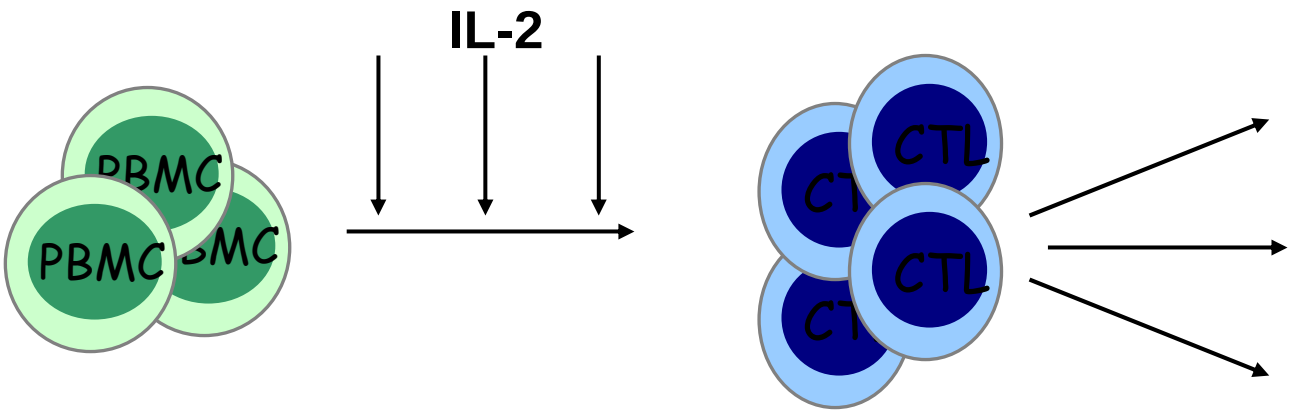


Type 3 Latency
Post Transplant Lymphoma
5-25% of T cell depleted SCT recipients

Generation of EBV Specific Cytotoxic T lymphocytes (CTLs)



Step 1: LCL generation
4-6 weeks



Step 2: CTL expansion
4-7 weeks

Step 3: QA/QC
Sterility
HLA type
Phenotype
Cytotoxicity

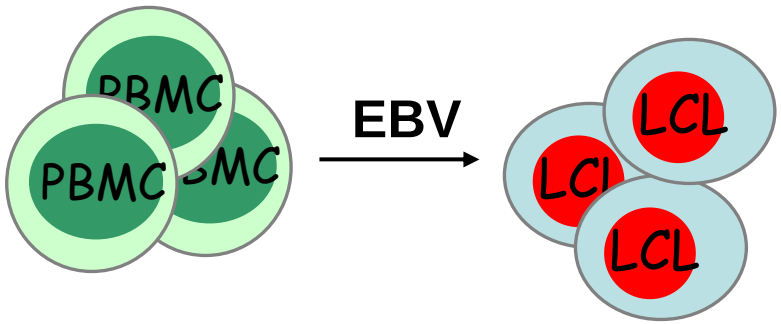
Successful T Cell Therapy of Cancer

Minimal Requirements

Effector Cells need to be

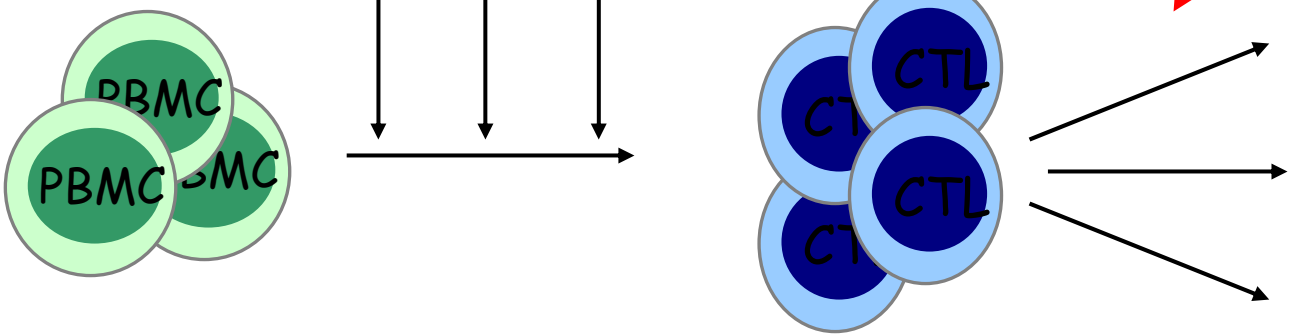
- Plentiful (Proliferate)
- Persistent
- Present in tumor

Generation of EBV Specific Cytotoxic T lymphocytes (CTLs)



Step 1: LCL generation
4-6 weeks

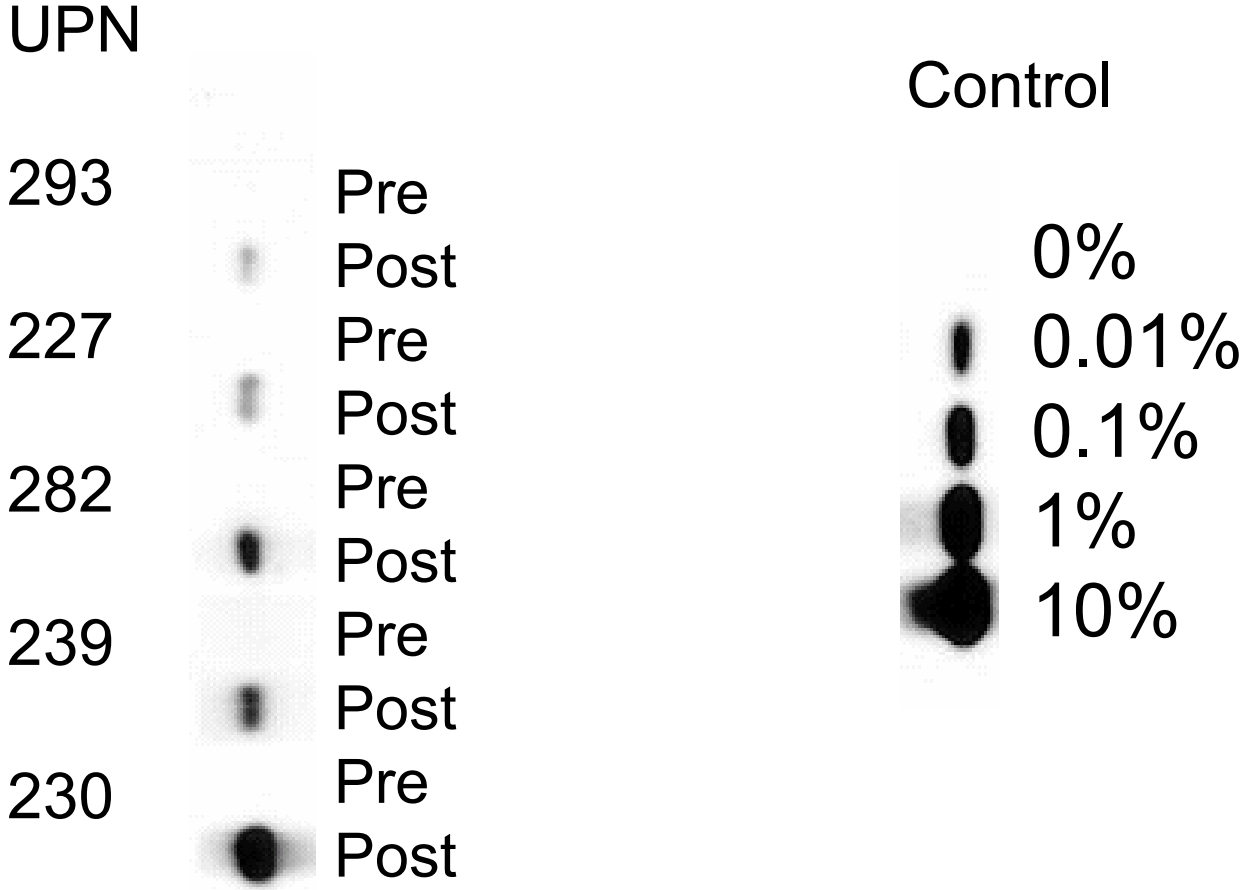
Gene Mark neo-MVV



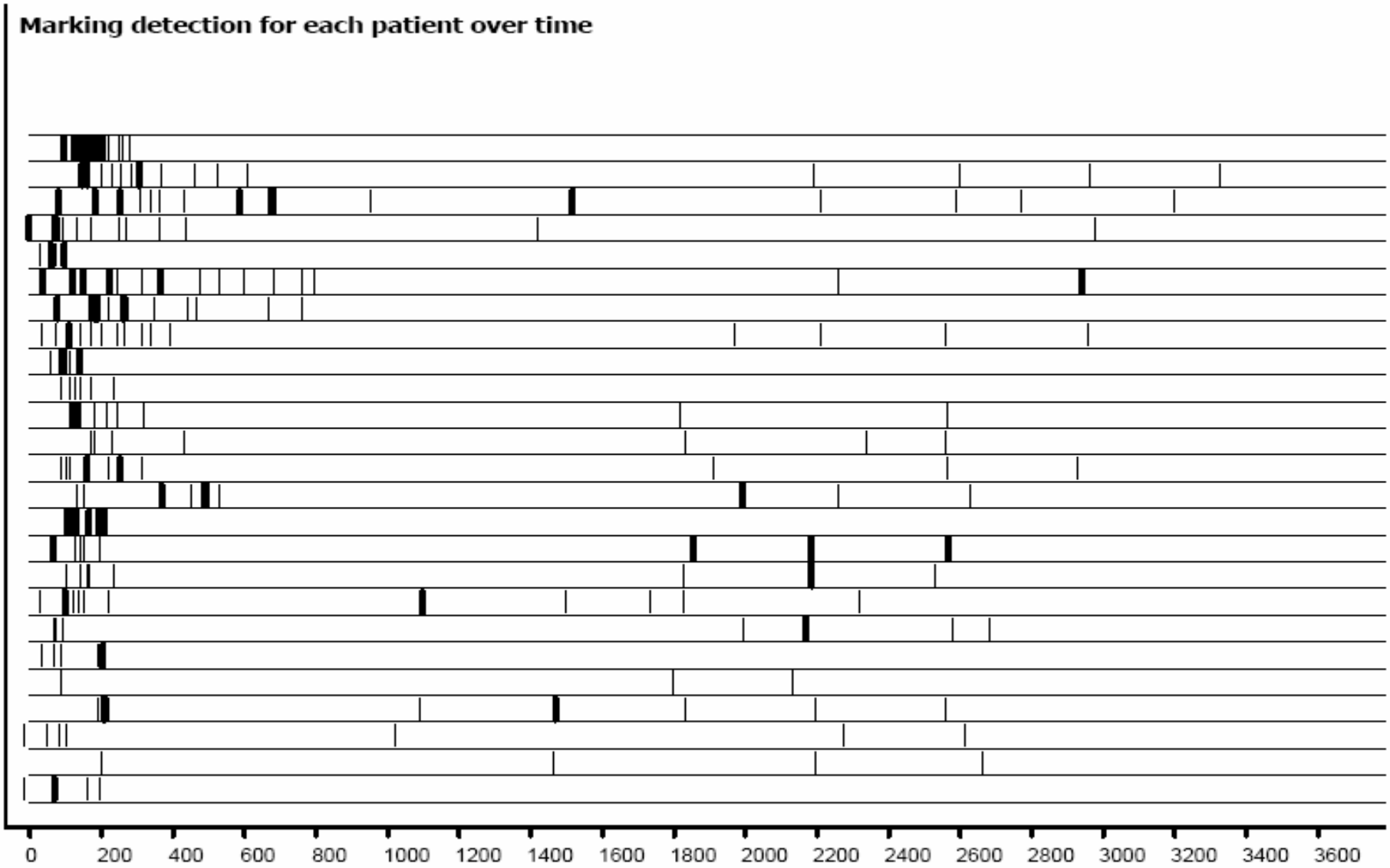
Step 3: QA/QC
Sterility
HLA type
Phenotype
Cytotoxicity

Step 2: CTL expansion
4-7 weeks

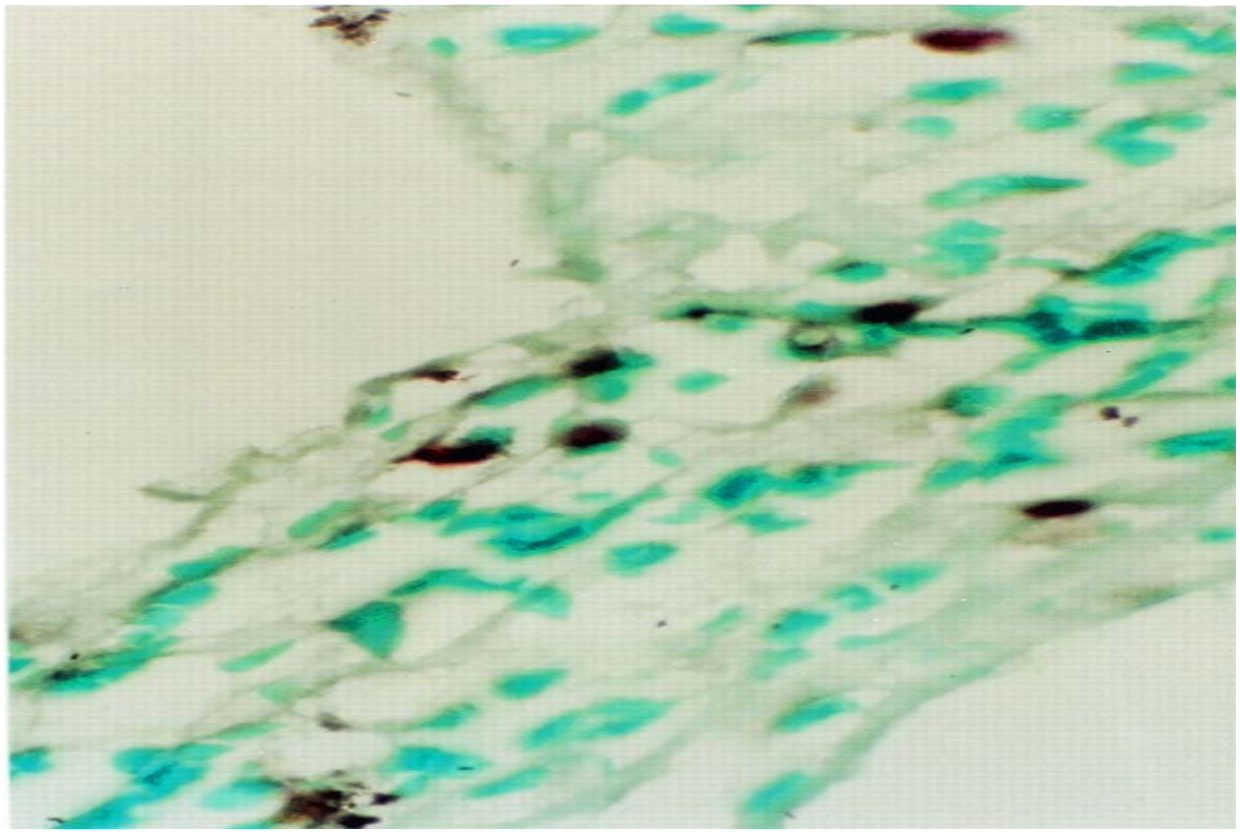
PCR for Neo shows CTL become *plentiful*



Marking Detection – CTLs *Persist*

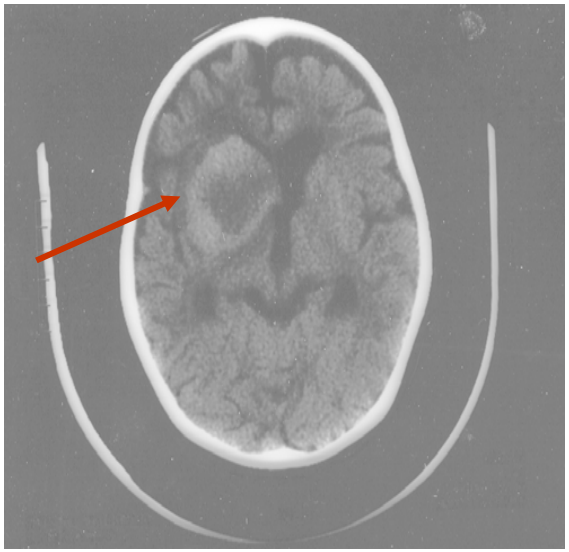


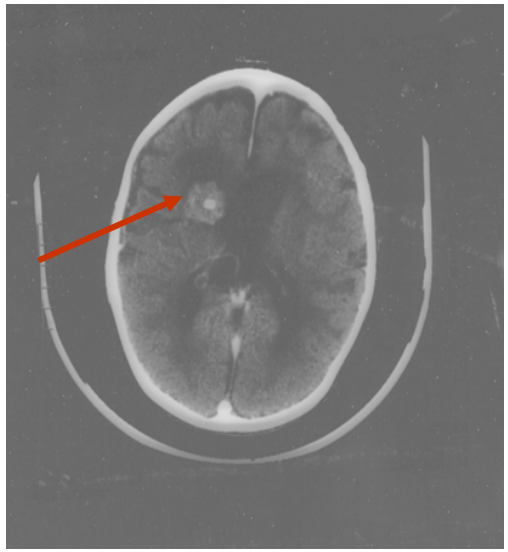
Donor-derived CTLs *Present* at tumor site



Marked CTL by in situ PCR at tumor site

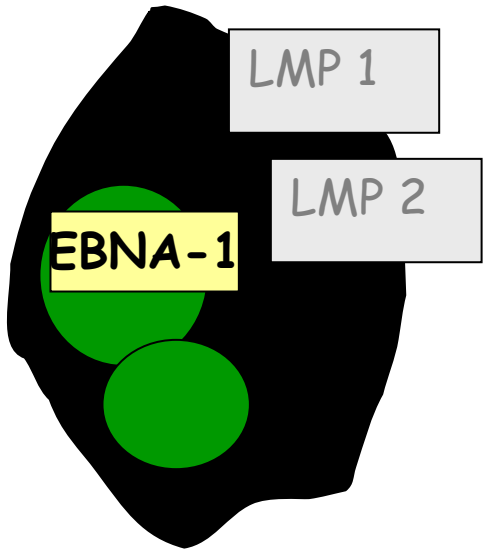
CTLs for EBV PTLD





7/8 CR in patients with bulky disease – Orphan Drug Designation in 2007

Improving CTL Therapy – Attack Targets that are Present



Type 2
Hodgkin's disease
Nasopharyngeal carcinoma

Increasing LMP2 tetramer-positive cells using Ad-LMP2 vector

LMP2 tetramers

EBNA3C tetramer

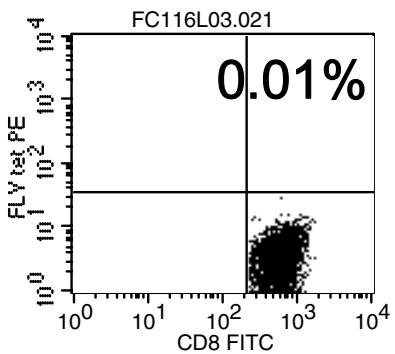
FLY

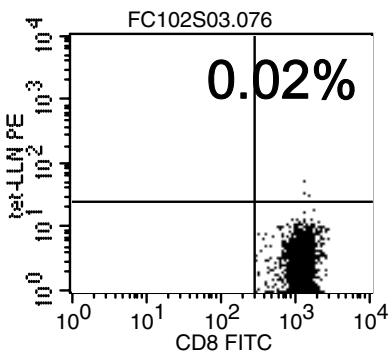
LLW

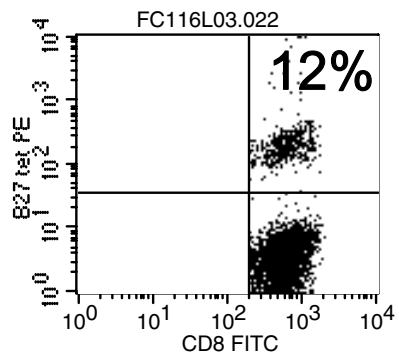
RRI

LCL

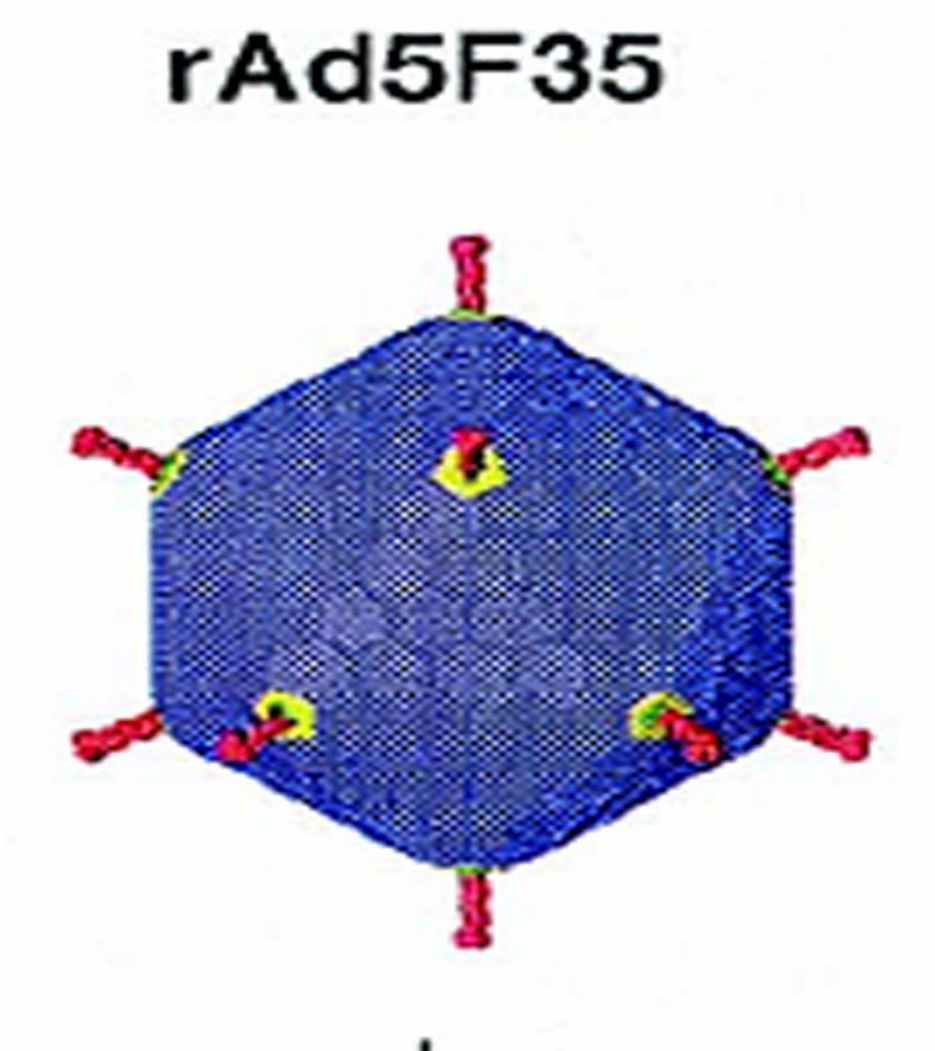
CTL





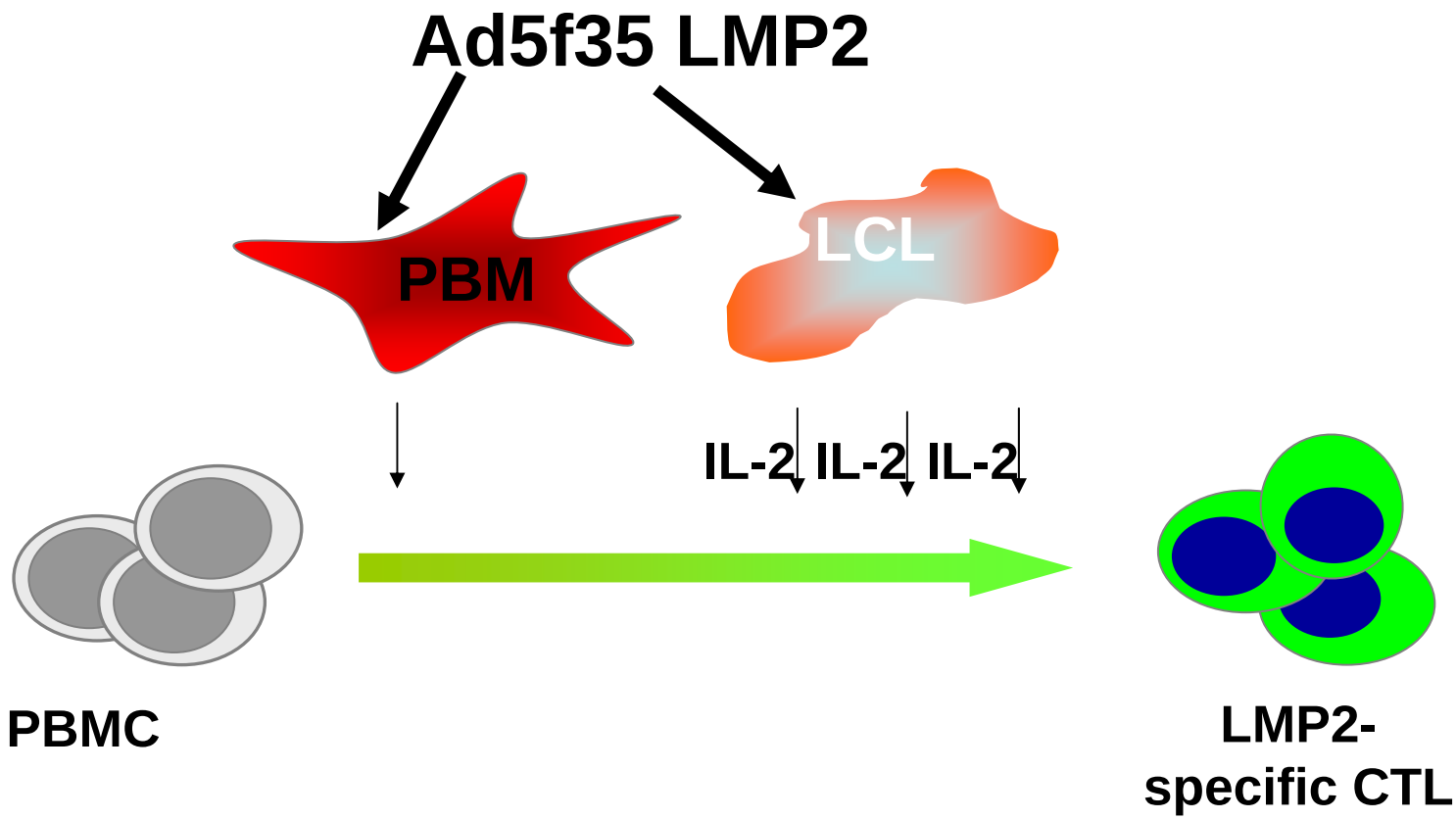


Recombinant Ad5f35 with LMP2



Chimeric Ad5F35 LMP2

Over-expression and innate immune response make a weak antigen strong



Bollard et al, J Immunother 2004

Increasing LMP2 tetramer-positive cells using Ad-LMP2 vector

LMP2 tetramers

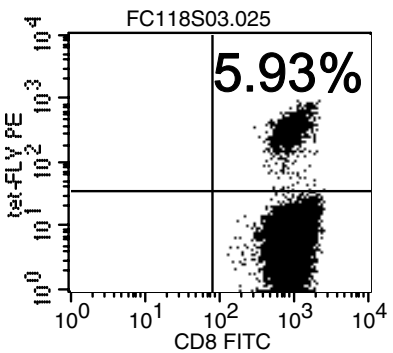
EBNA3C tetramer

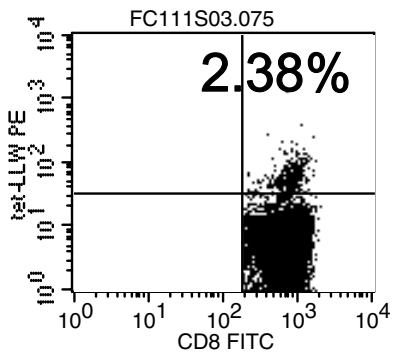
FLY

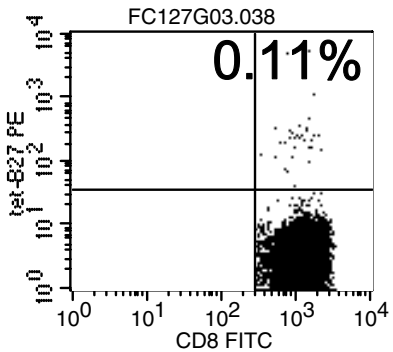
LLW

RRI

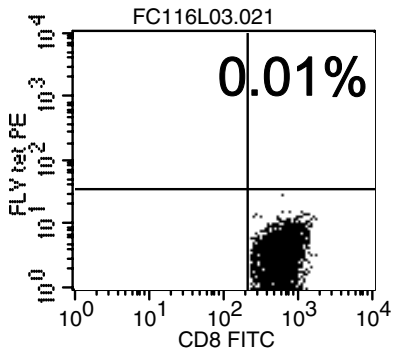
LMP2 CTL

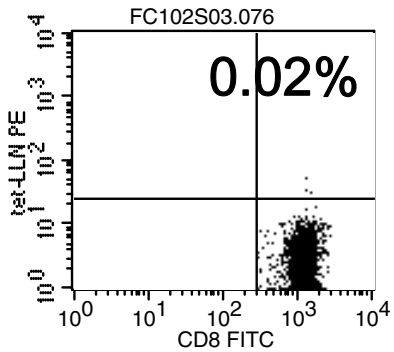


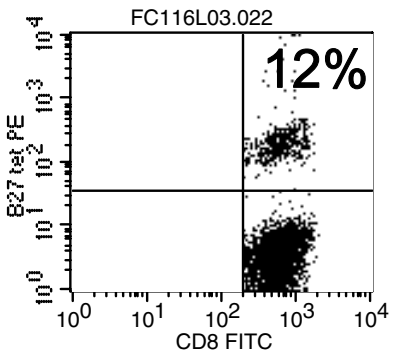




LCL CTL



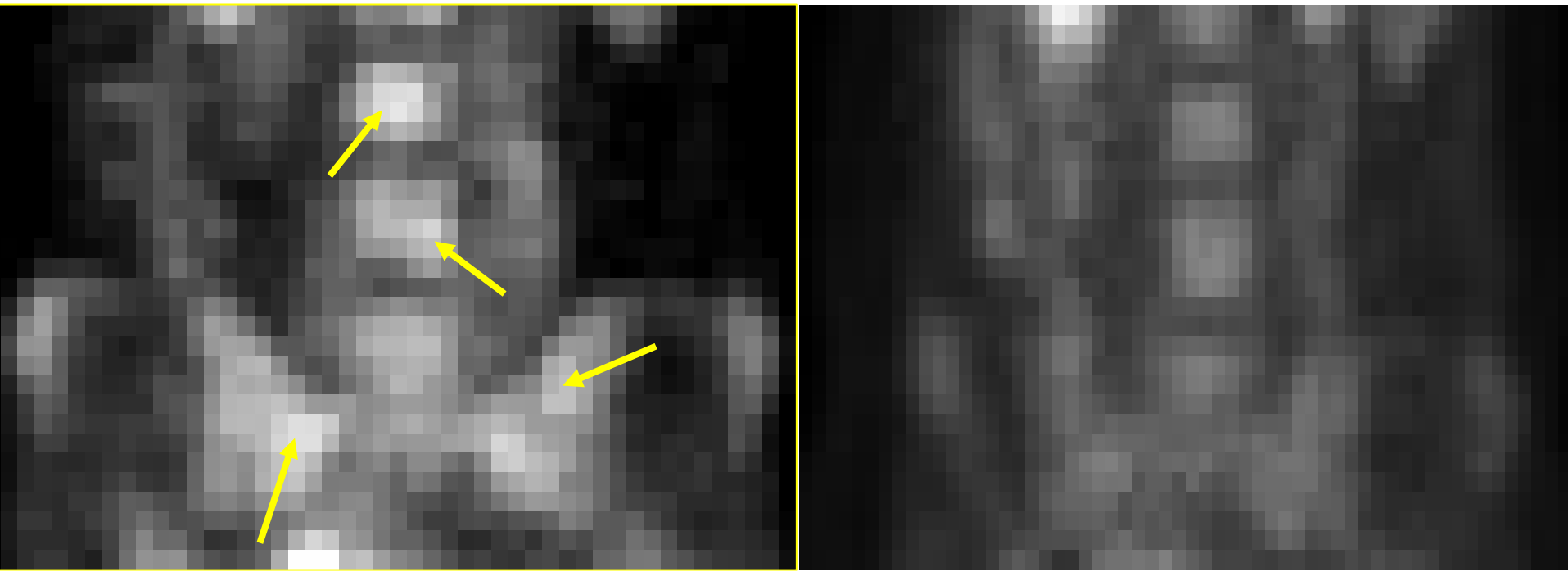




Resolution of Bony Lesions In HD

Pre CTL

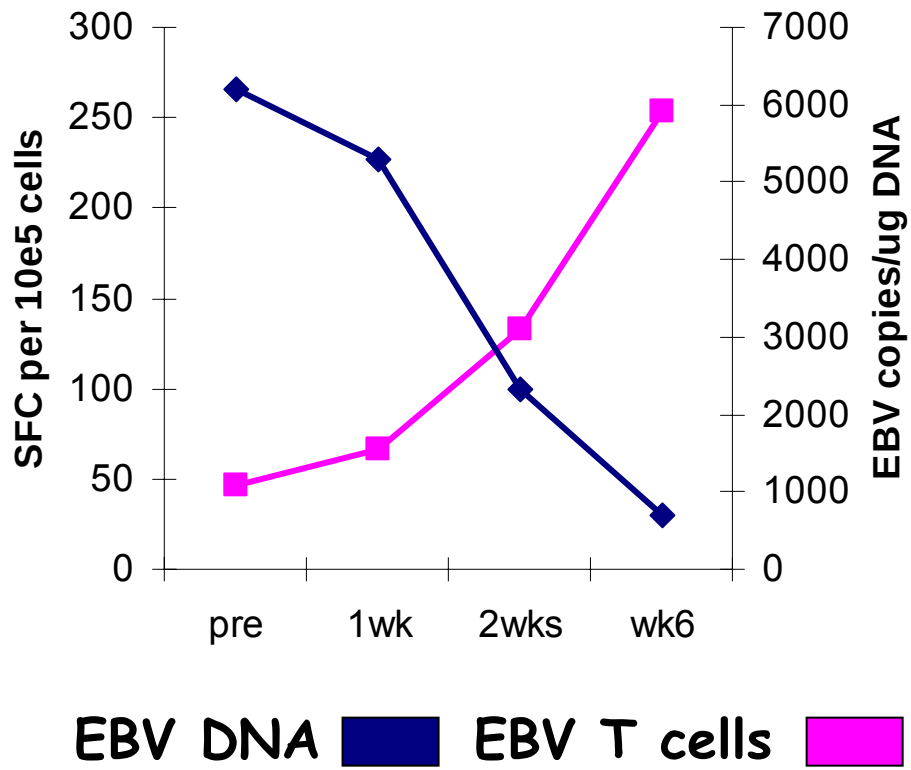
3mth Post CTL



Complete Radiological Response EBV+ve NK-T NHL

Pre CTL

Post CTL

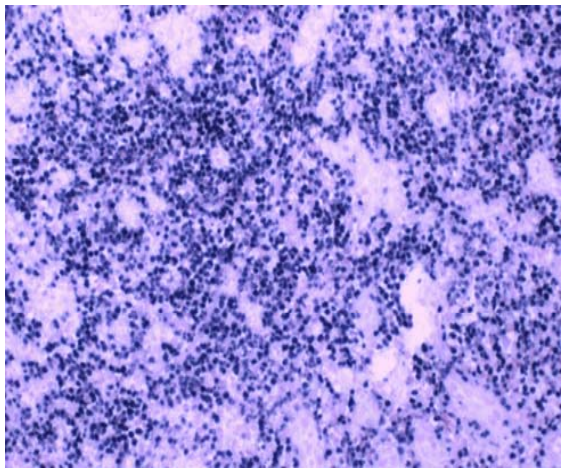


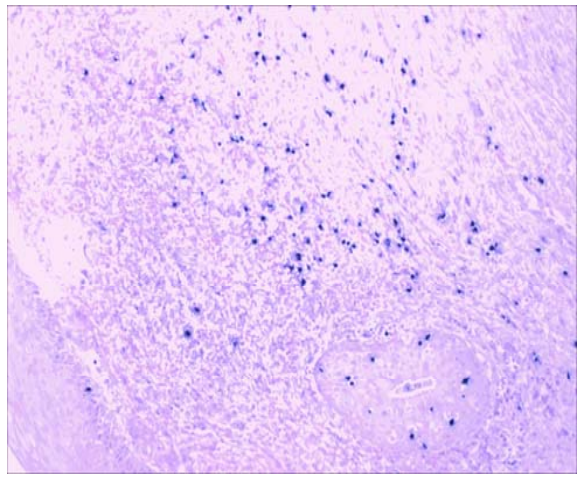
Immunohistochemistry
Left Carytenoid

Pre CTL

Post CTL

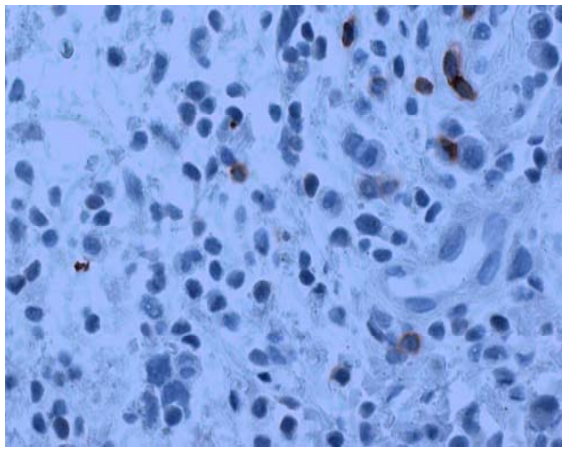
EBER

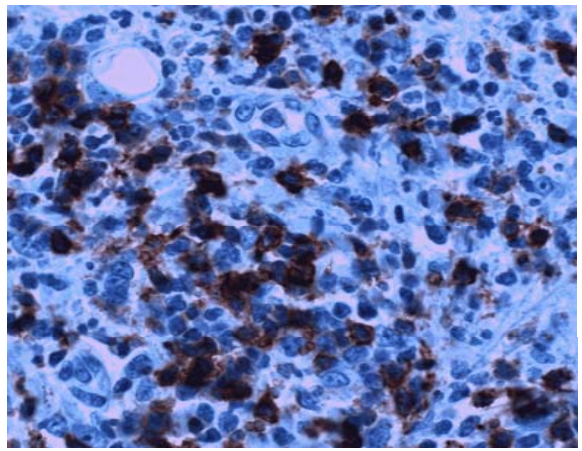




10x

CD4

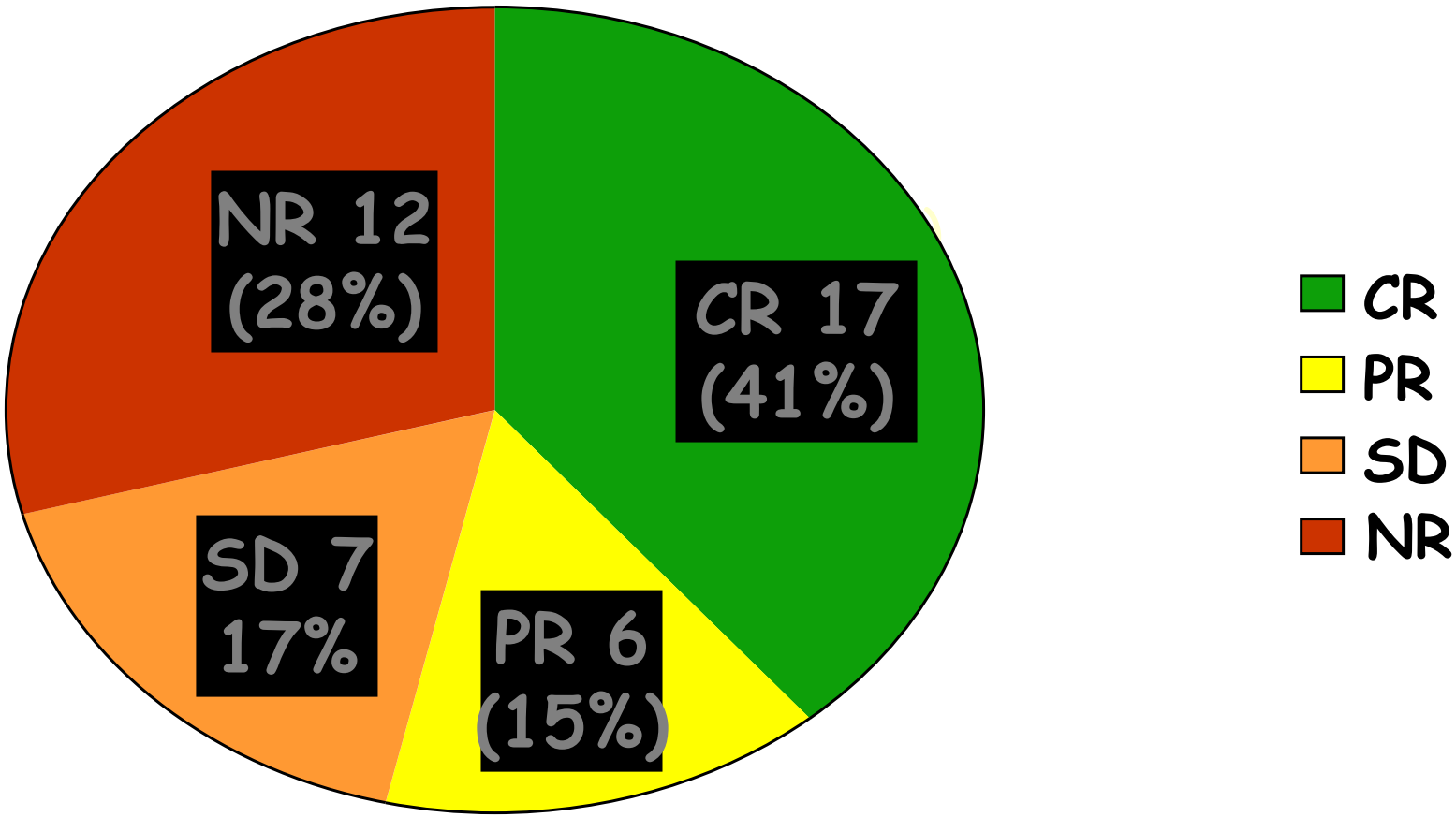




40x

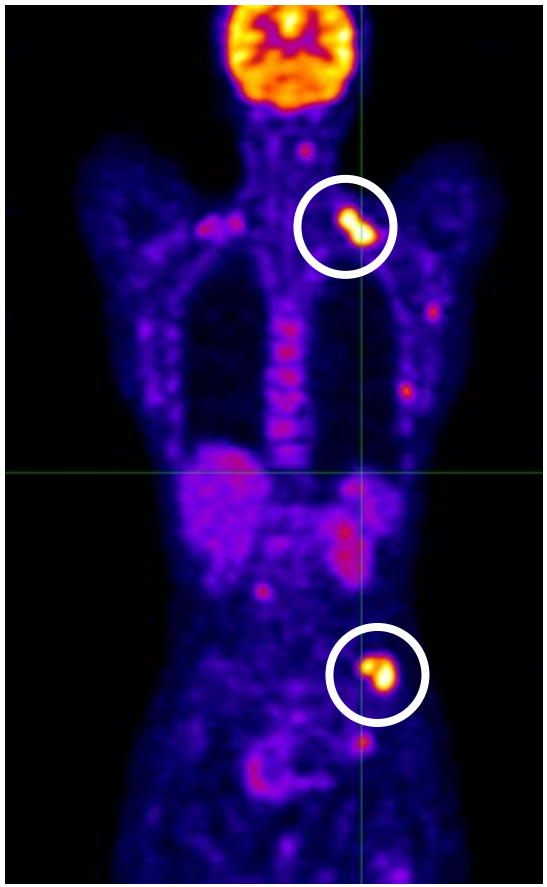
CTL Studies targeting EBV antigens in EBV+ve lymphoma

42 Patients with Active Rel.Disease

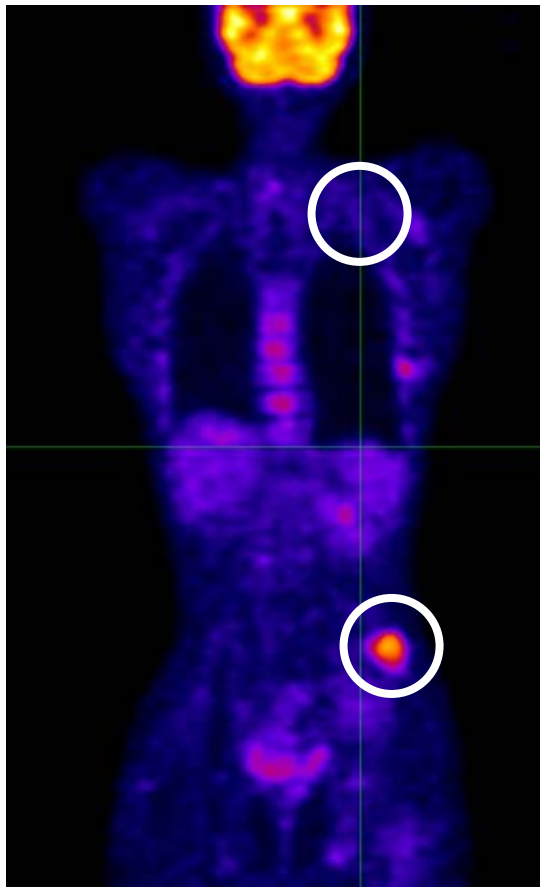


NPC Clinical Response post EBV-CTL: Reduction of FDG uptake in metastases

Pre CTL

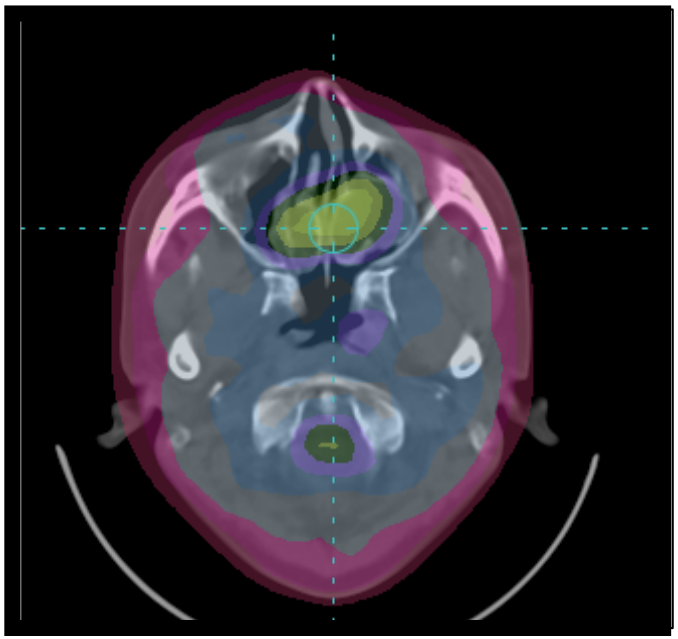


Post CTL

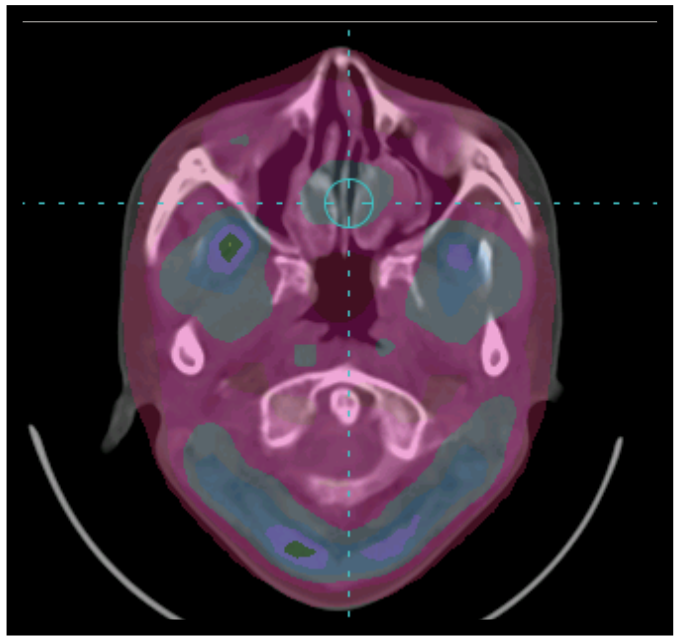


Complete Remission of Refractory NPC

Pre-CTL



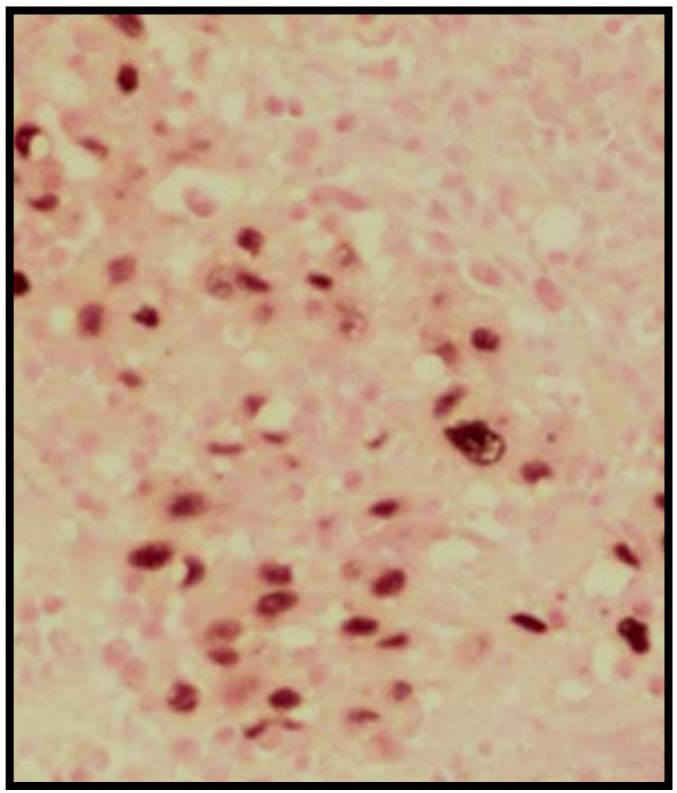
Post-CTL



Absent uptake of F-18 fluorodeoxyglucose (FDG) 8 weeks post CD45 MAbs and EBV-CTL infusion

Complete Remission of Refractory NPC

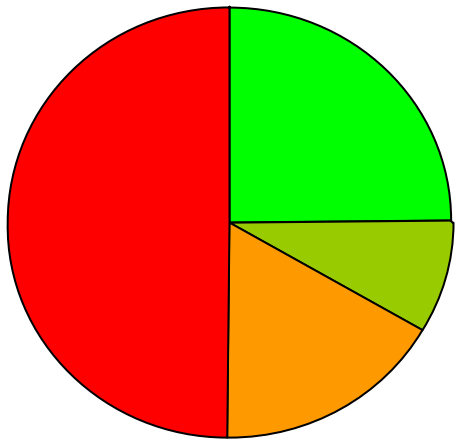
Pre CTL: EBV pos



Post CTL: EBV neg

Conclusions

- Anti-tumor activity seen in 12/24 patients with active NPC treated with EBV-CTL



No Response

Complete Response (6)

Partial Response (2)

Stable Disease (4)

Broadening the Applicability of EBV-CTLs

- Manufacturing is robust (98% success rate in >200 clinical lines)
- "Exportable" concept

O'Reilly; MSKK
Lucas; UAB
Wang; HMS
Commoli; Pavia
Khanna; QIMR, Brisbane
Volk; Charite, Berlin
Amrolia: ICH/GOS, London
Crawford; Univ. Edinburgh

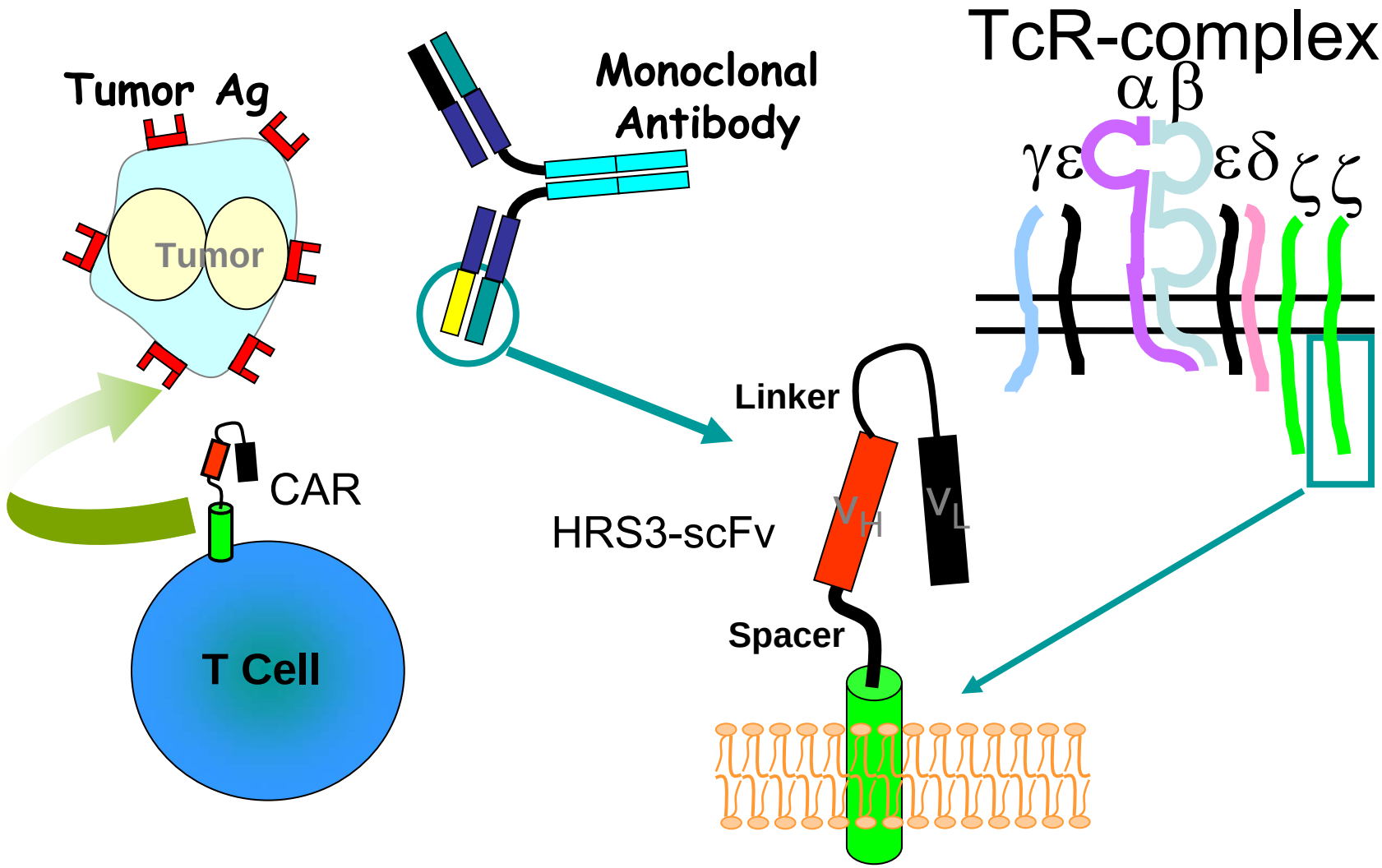
Broadening the Applicability of EBV-CTLs

- Manufacturing is robust (98% success rate in >200 clinical lines)
- "Exportable Concept"
- Accelerate and simplify production –

  Was >10wks: Now <10 days

- Increase range of diseases to be treated

Chimeric Antigen Receptor (CAR) Expression in T cells



Chimeric Primary T cells (CAR-PTC)

- Recognize unmodified tumor antigens in MHC unrestricted manner- bypass many tumor immune evasion strategies

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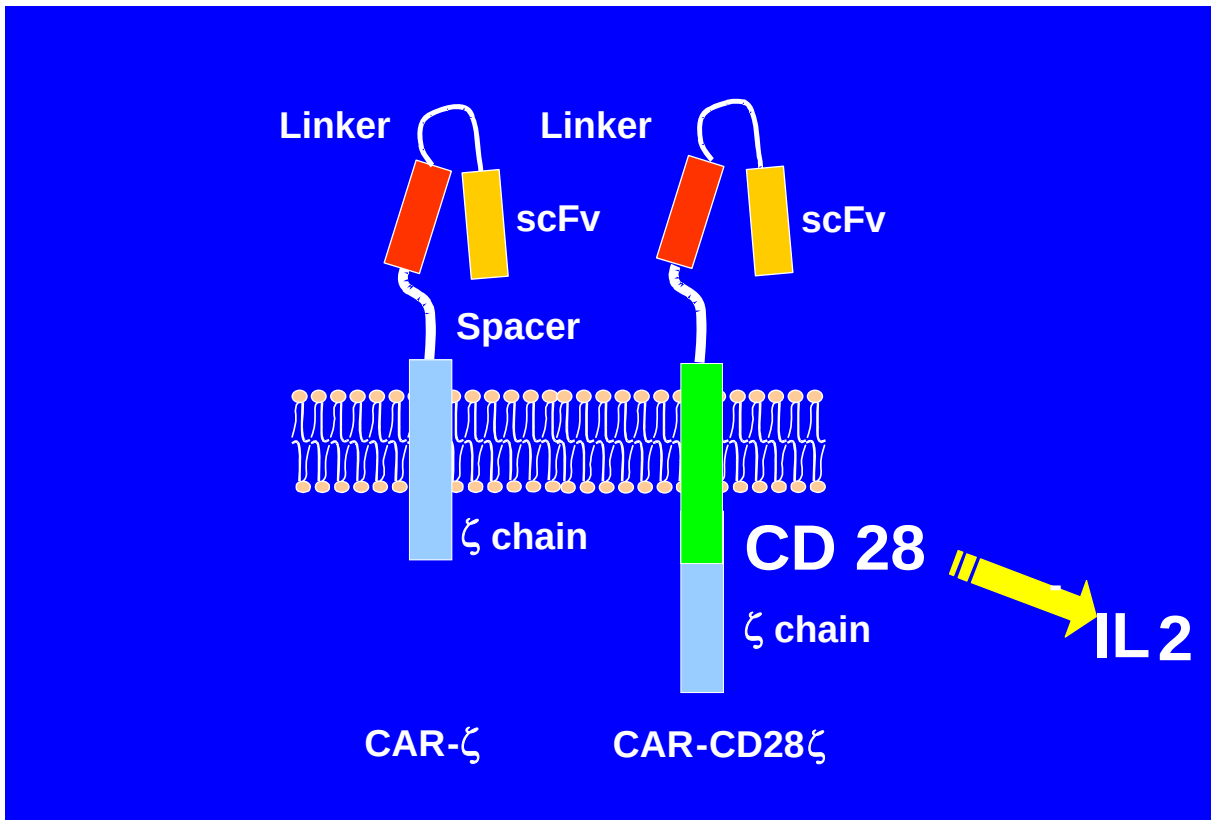
- Recognize unmodified tumor antigens in MHC unrestricted manner- bypass many tumor immune evasion strategies
- Tumor cells have other problems in presenting antigen (e.g. lack co-stimulator molecules, inhibit induction of effector phenotype)
- May be expressing receptor in Treg
- Consequence – poor in vivo persistence, expansion and function

Overcoming poor costimulation to CAR- PTC

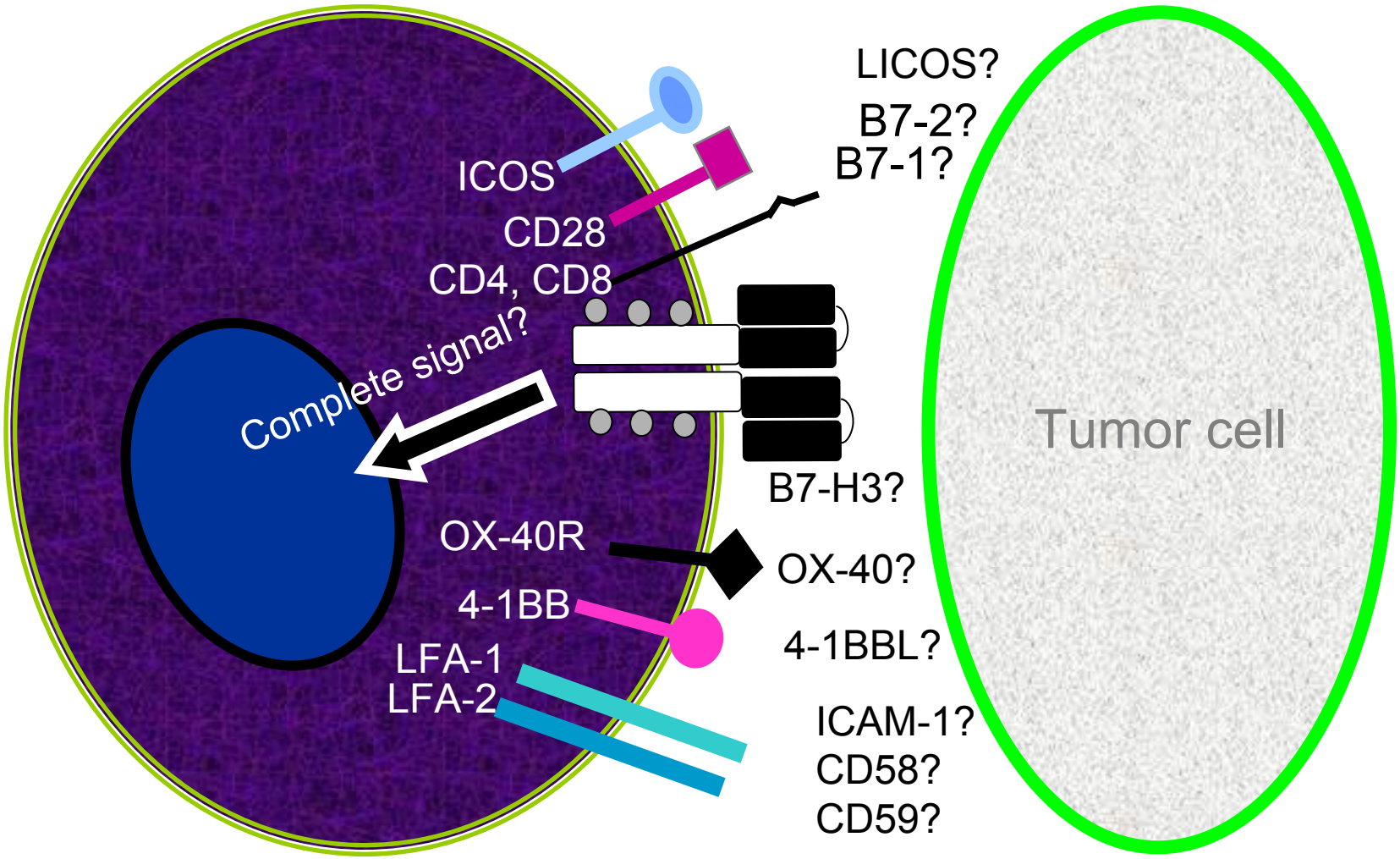
- Incorporate more co-stimulatory domains

  CD28 (Maher et al, Nat Biotech 2002; Finney et al, J Immunol 2004; Dotti et al Blood 2006)

  CD28 and OX40 (Pule et al. Mol Therapy 2005)

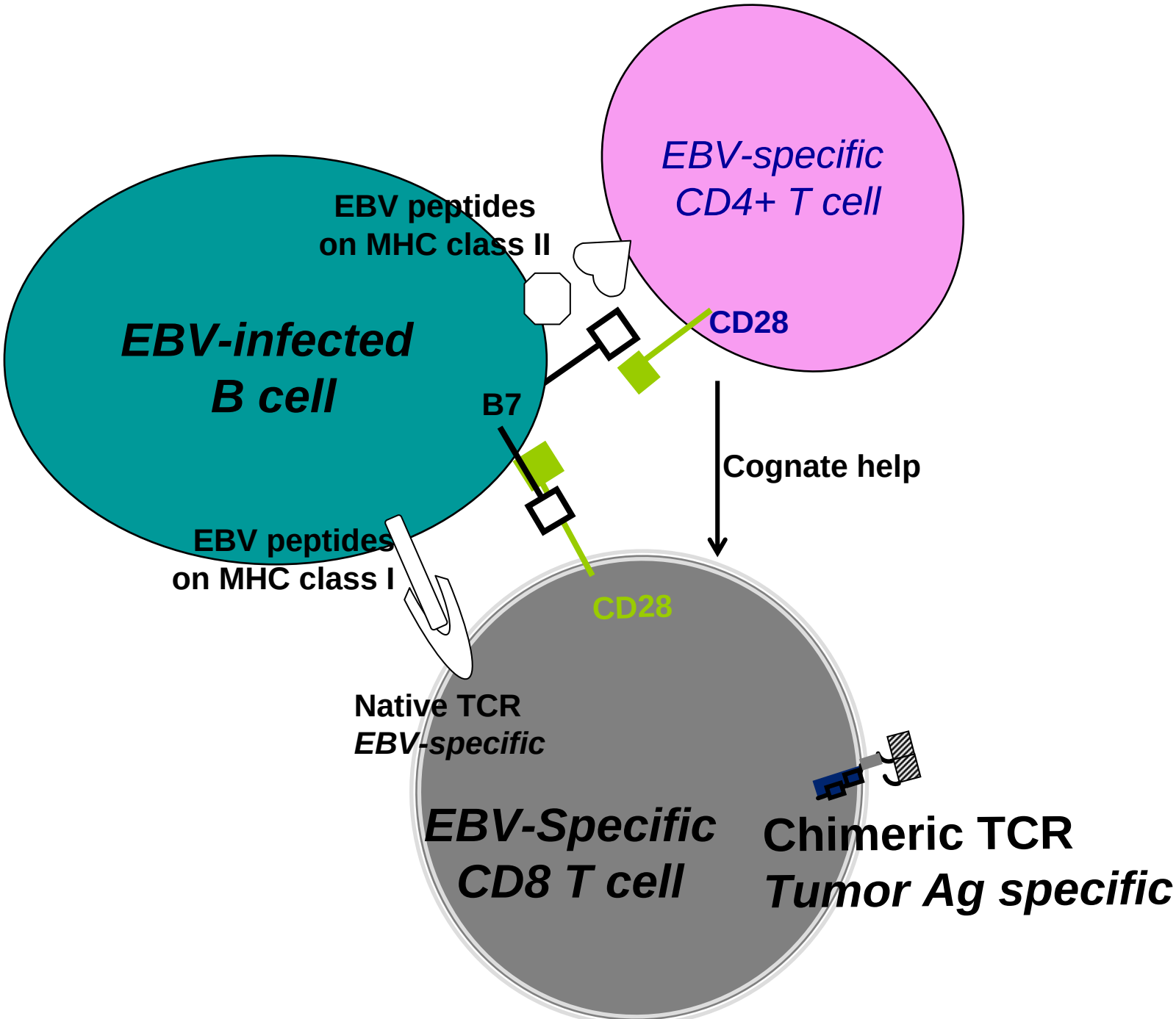


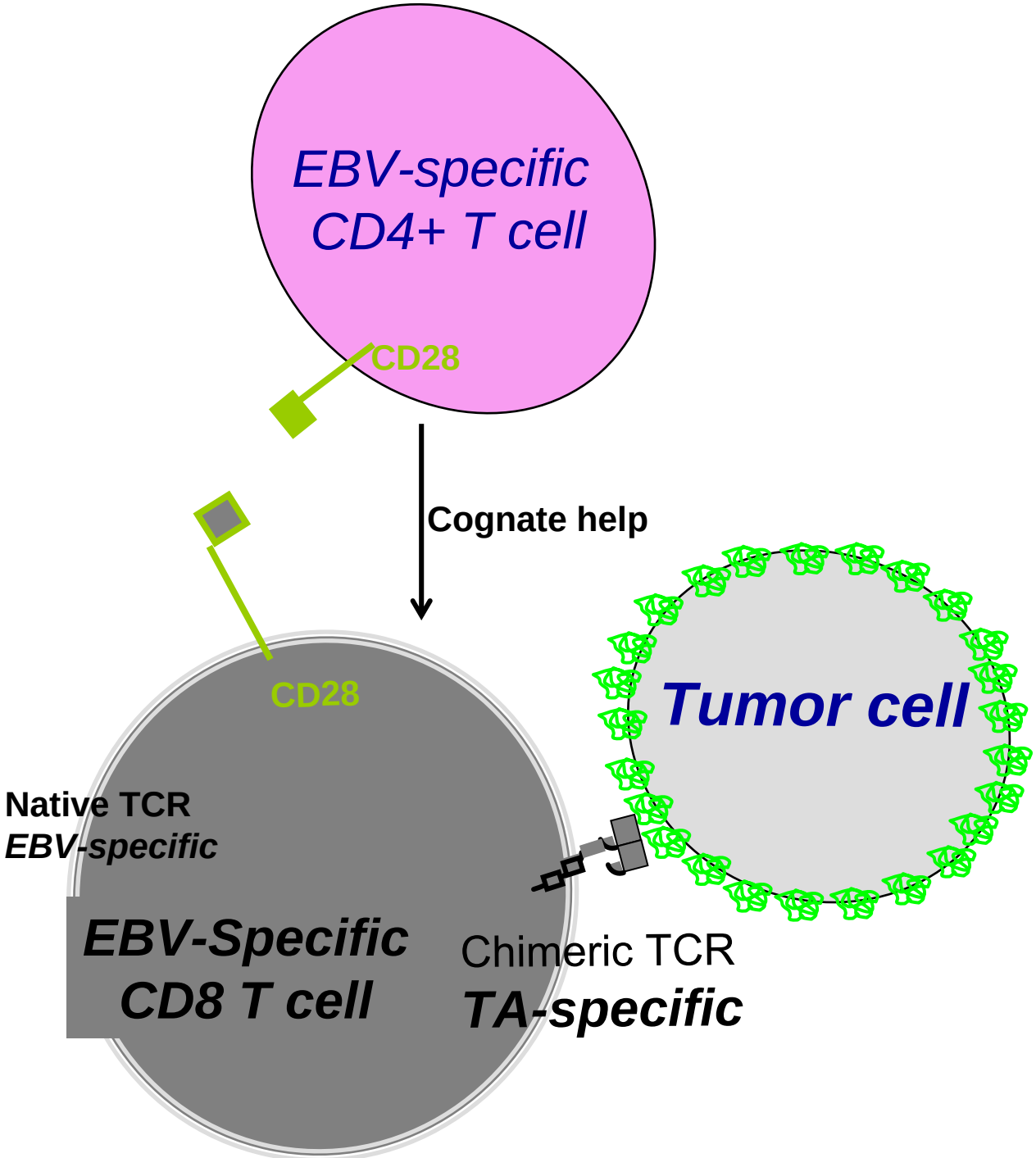
Chimeric receptor-mediated interaction between T cell and tumor cell



Using EBV Infected Target Cells as source of co-stimulation

- EBV targets express all relevant co-stimulator molecules and are present lifelong
- EBV-CTL
  - Expand in vivo
  - Have effector phenotype
  - Persist long term
  - Eradicate bulky tumors


EBV-specific
CD4+ T cell
EBV peptides
on MHC class II
EBV-infected
B cell
CD28
B7
Cognate help
EBV peptides
on MHC class I
CD28
Native TCR
EBV-specific
EBV-Specific
CD8 T cell
Chimeric TCR
Tumor Ag specific


EBV-specific
CD4+ T cell
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Native TCR
EBV-specific
Tumor cell
EBV-Specific
CD8 T cell
Chimeric TCR
TA-specific

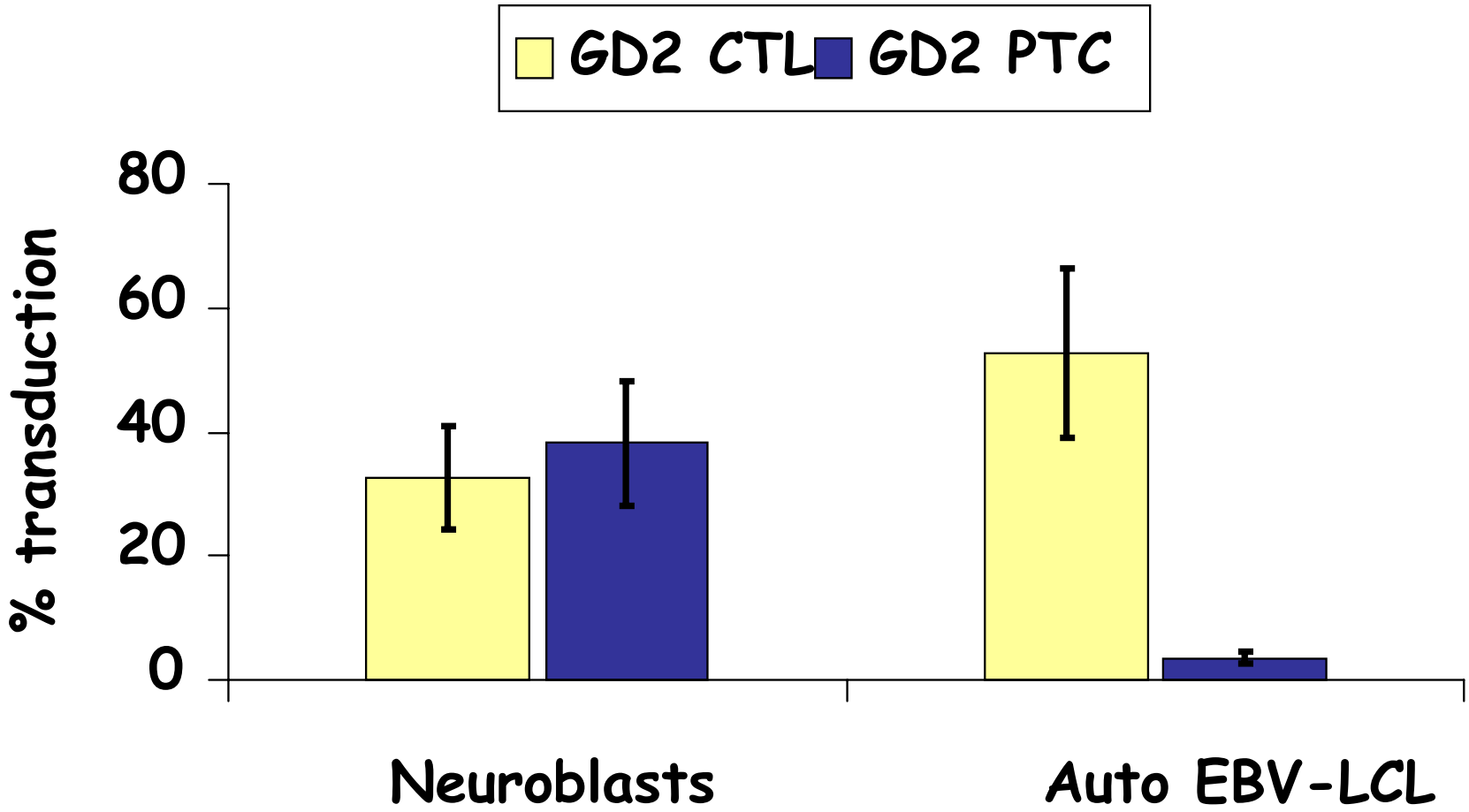
Neuroblastoma

- Commonest extracranial solid tumor of childhood
- May respond to intensive therapies
- High relapse risk in advanced disease
- Neural crest tumor and expresses many developmental antigens
- Lack MHC molecules – problem for CTL

Neuroblastoma Target antigen: GD2

- Disialoganglioside expressed in tumors of neuroectodermal origin
- Expressed at high density on almost all neuroblastoma cells
- Poorly expressed or absent from most normal tissue
- MAb has been used with clinical responses

Killing of Neuroblastoma and Autologous LCL by PTC/CTL

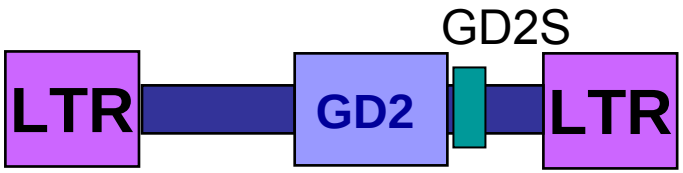


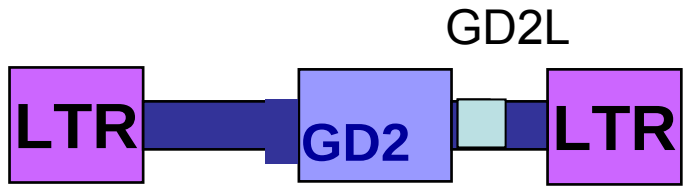
Are CAR-CTL better than CAR-PTC in neuroblastoma patients?

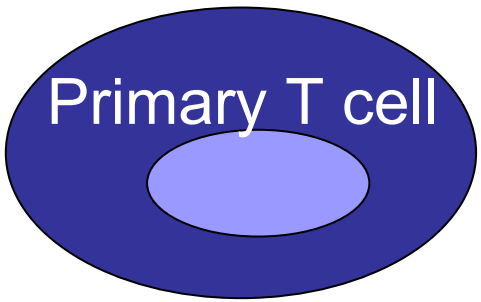
Transduce patient PTC and CTL with a vector encoding identical receptor but distinct oligonucleotide for each population.

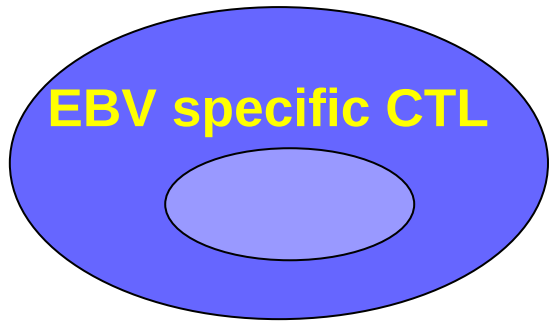
Vectors in Clinical Study

Patient One

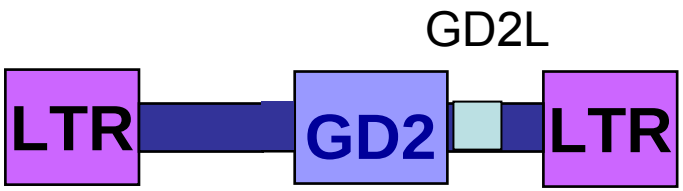


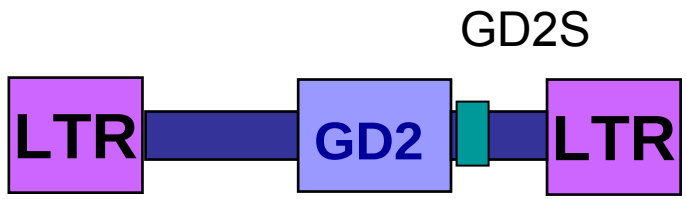






Patient Two





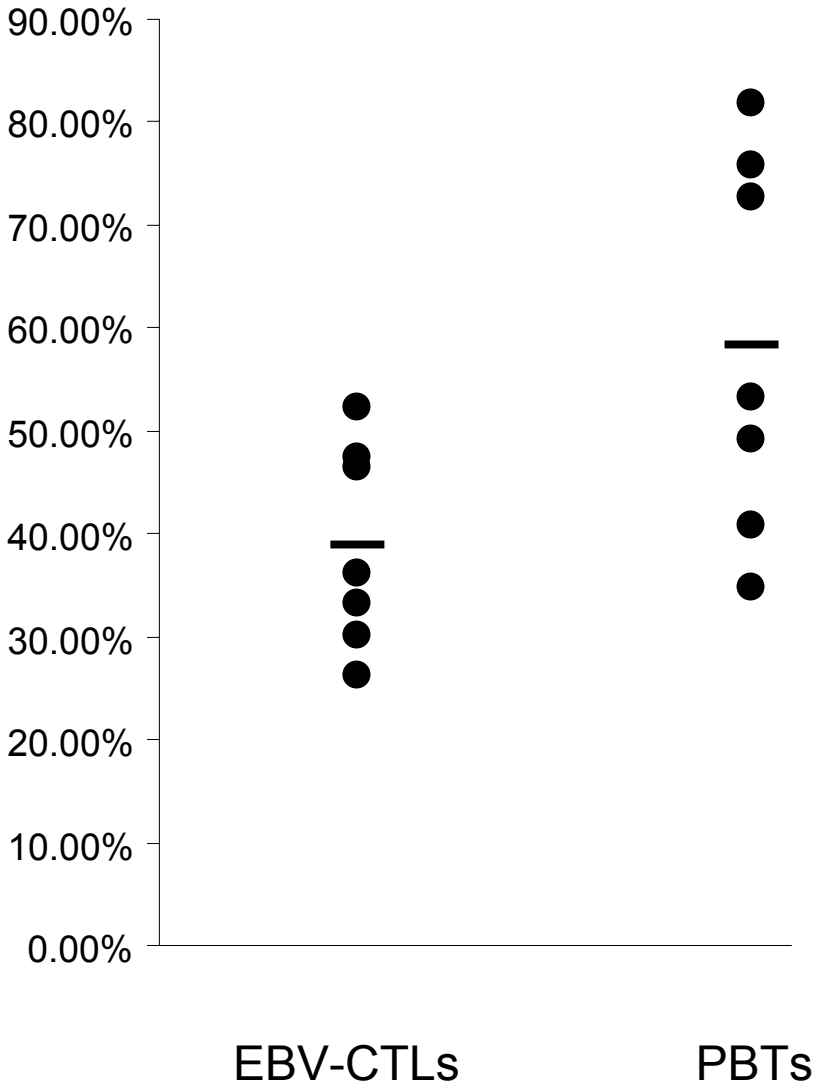
Phase I Dose Escalation Study

- Relapsed/Refractory or incompletely treated NB patients
- Evaluate safety of GD2 redirected T-cells (T-GD2)/EBV CTL (CTL-GD2)
- Compare persistence of CTL-GD2 and T-GD2
- Evaluate clinical outcome

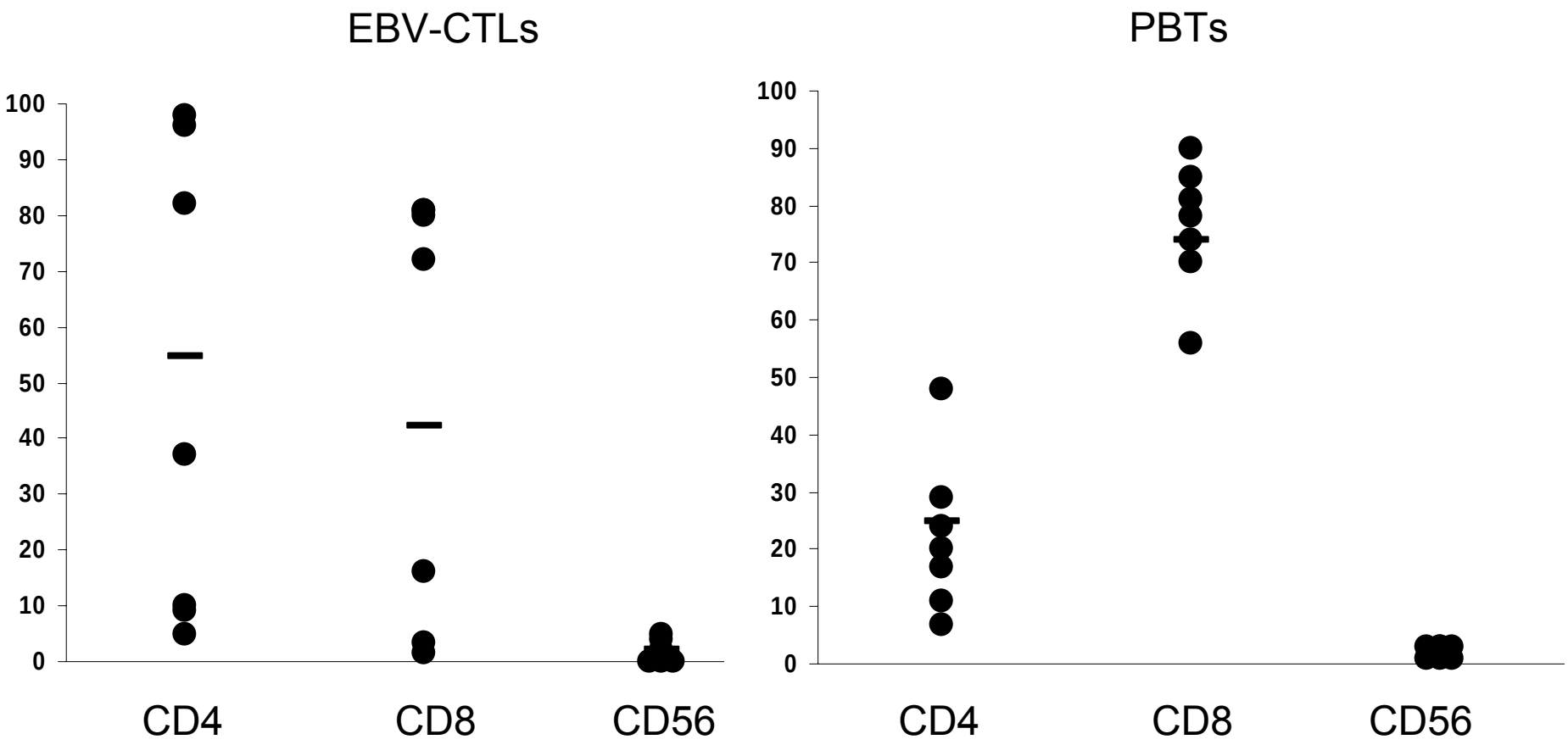
Patient Details

- 11 Patients with relapsed disease
- Age 3yrs - 15 yrs (Median 10yrs)
- 3 Received dose level 1(10^7)
- 6 received dose level 2 (5×10^7)
- 2 received dose level 3 (10^8)

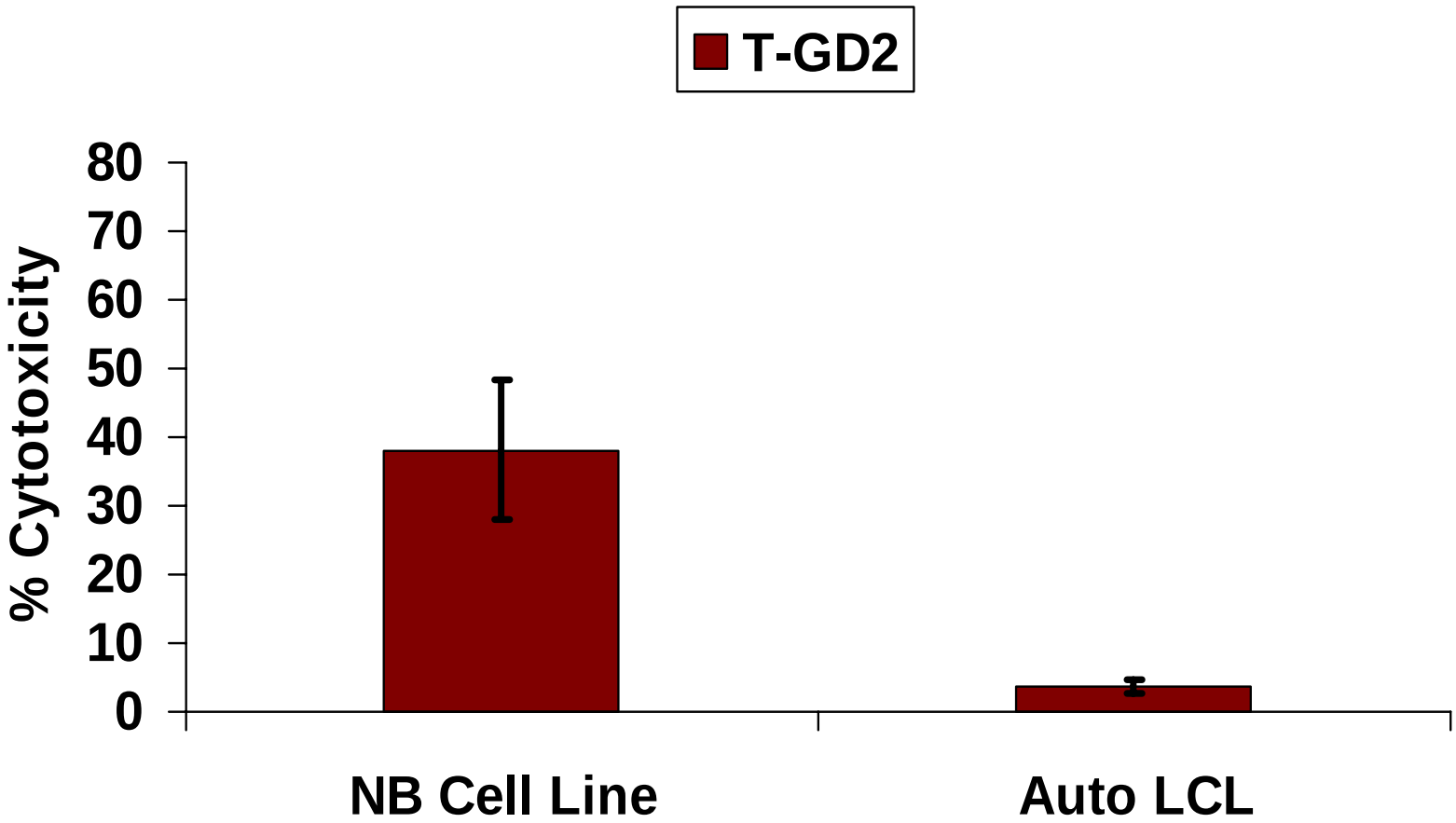
Clinical Product Transduction Efficiency



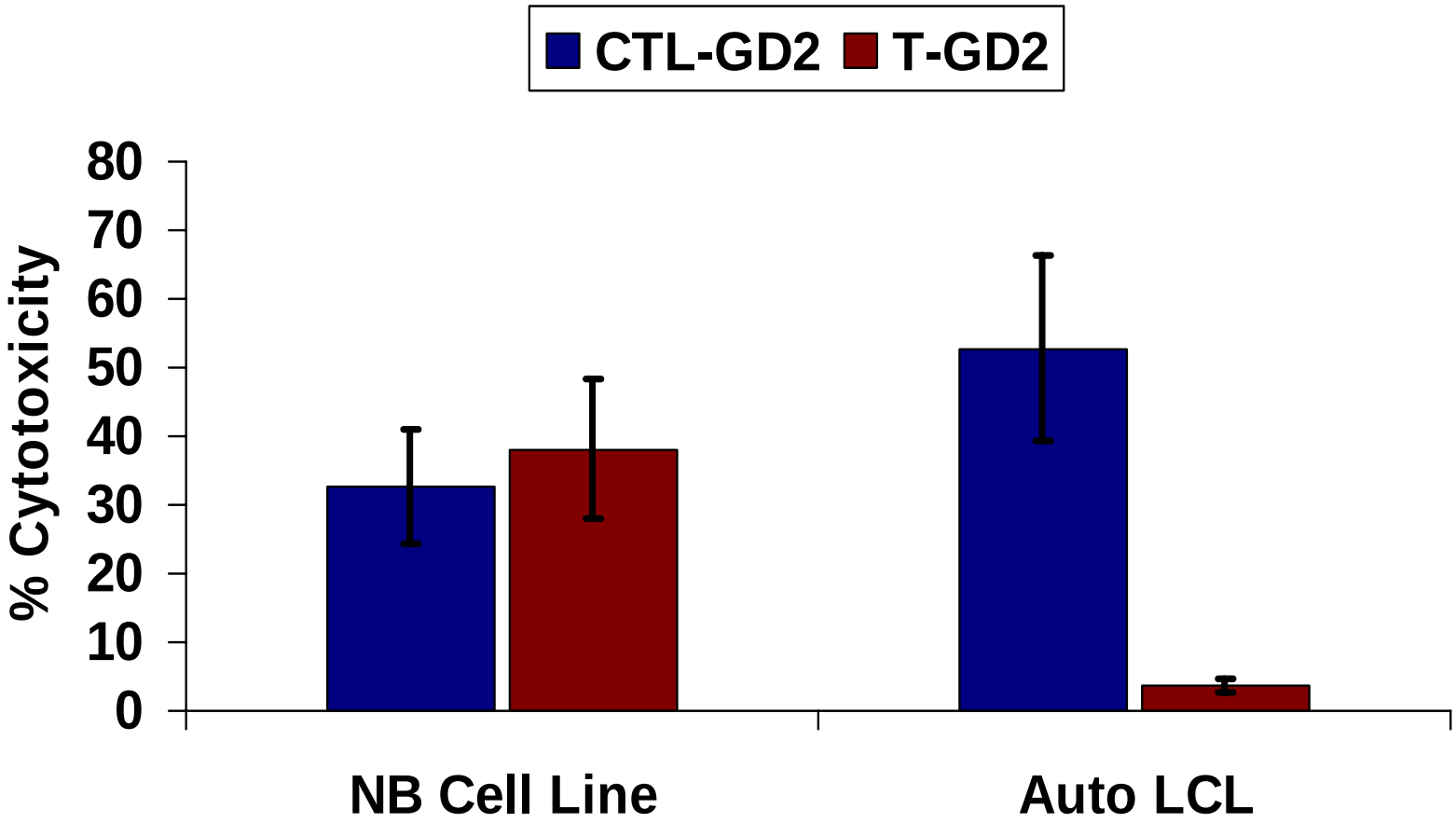
Phenotype of cell product



T-GD2 Cells Kill Neuroblastoma In-Vitro; No Killing Of Autologous LCL



CTL-GD2 Kill Both Neuroblastoma And Autologous LCL



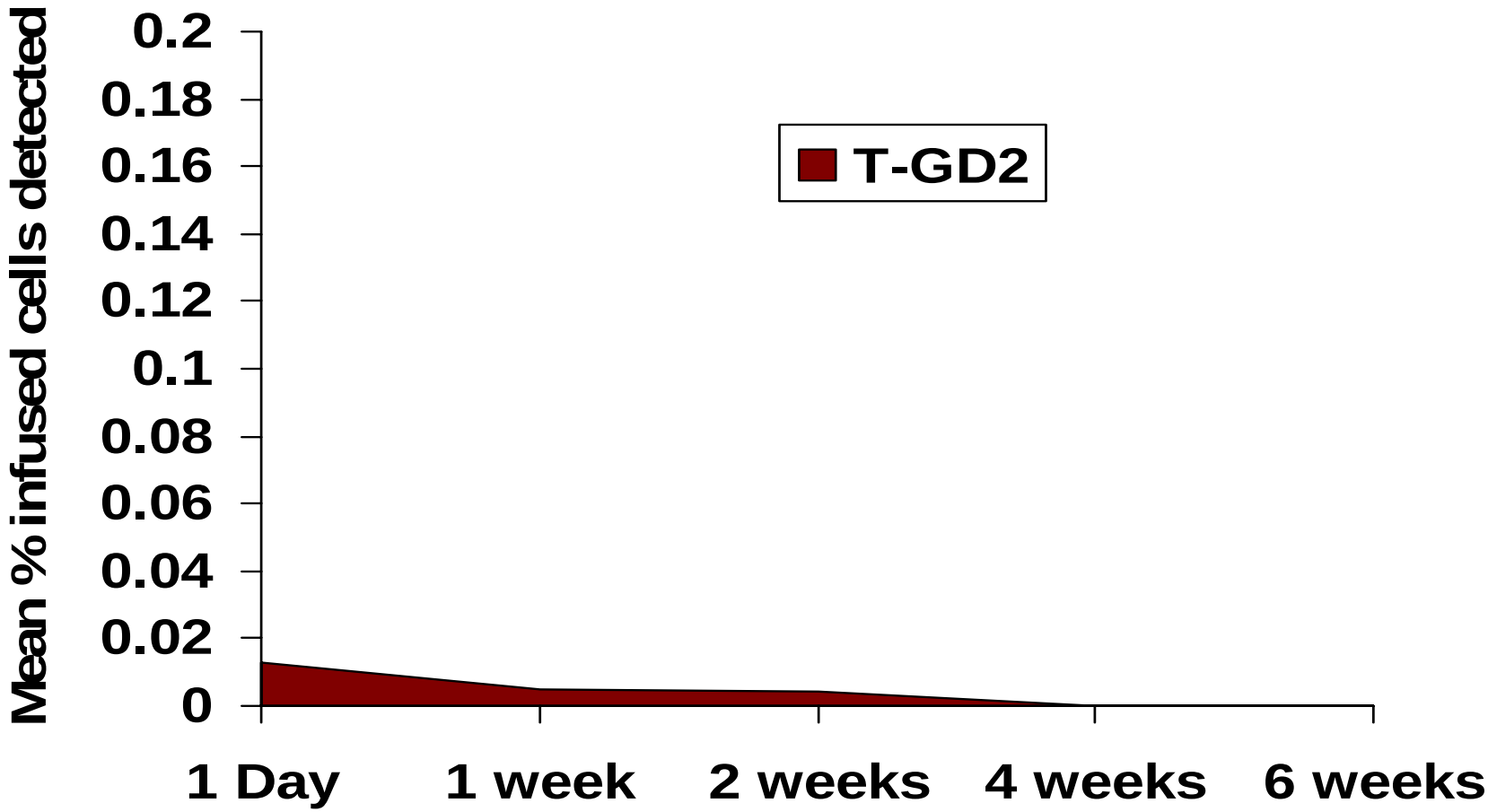
Safety of Infusions

No severe adverse effects attributable to study agent

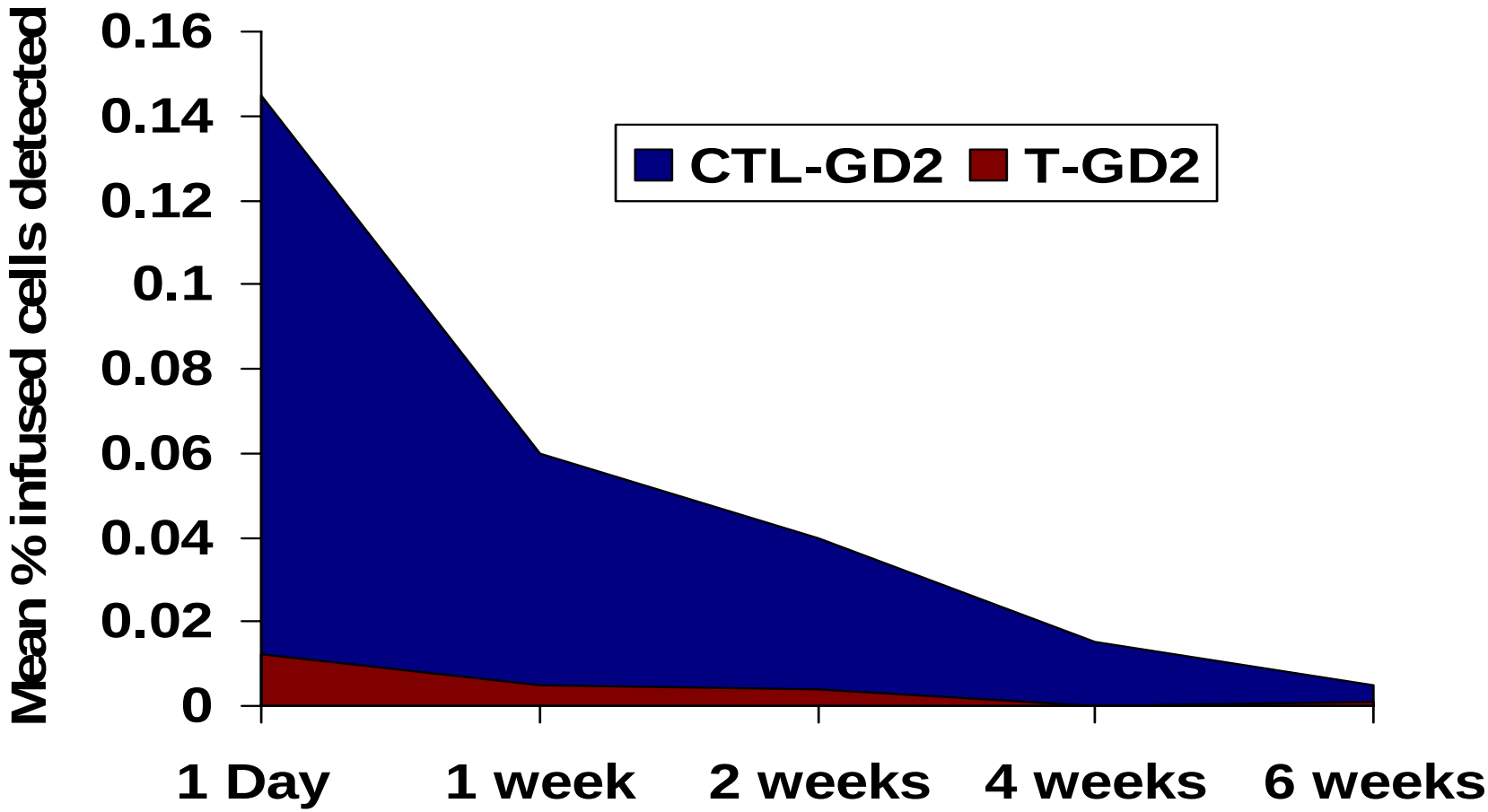
What should CAR-EBV CTL do?

- Persist longer at higher levels than CAR-Primary T cells (PTC)

Percent Gene Modified EBV CTL or Primary T cells in PBMNC



Percent gene modified EBV CTL or Primary T Cells in MNC



Successful T Cell Therapy of Cancer

Minimal Requirements

Effector Cells need to be

- Plentiful (Proliferate)
- Persistent
- Present in tumor

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Increase persistence

- Depletion of lymphocytes enhances homeostatic proliferation of transferred cells
- Autografting is standard of care for high risk Neuroblastoma
- Give modified CTL after autograft

Successful T Cell Therapy of Cancer

Minimal Requirements

Effector Cells need to be

- Plentiful (Proliferate)
- Persistent
- Present in tumor

Increase Persistence

- TGFβ secreted by many tumors including HD and neuroblastoma
- Transduction of Dominant Negative receptor blocks TGFβR trimer formation and downregulation of CTL in vitro/vivo
- Clinical trial of DNR approved and awaiting final vector release

Successful T Cell Therapy of Cancer

Minimal Requirements

Effector Cells need to be

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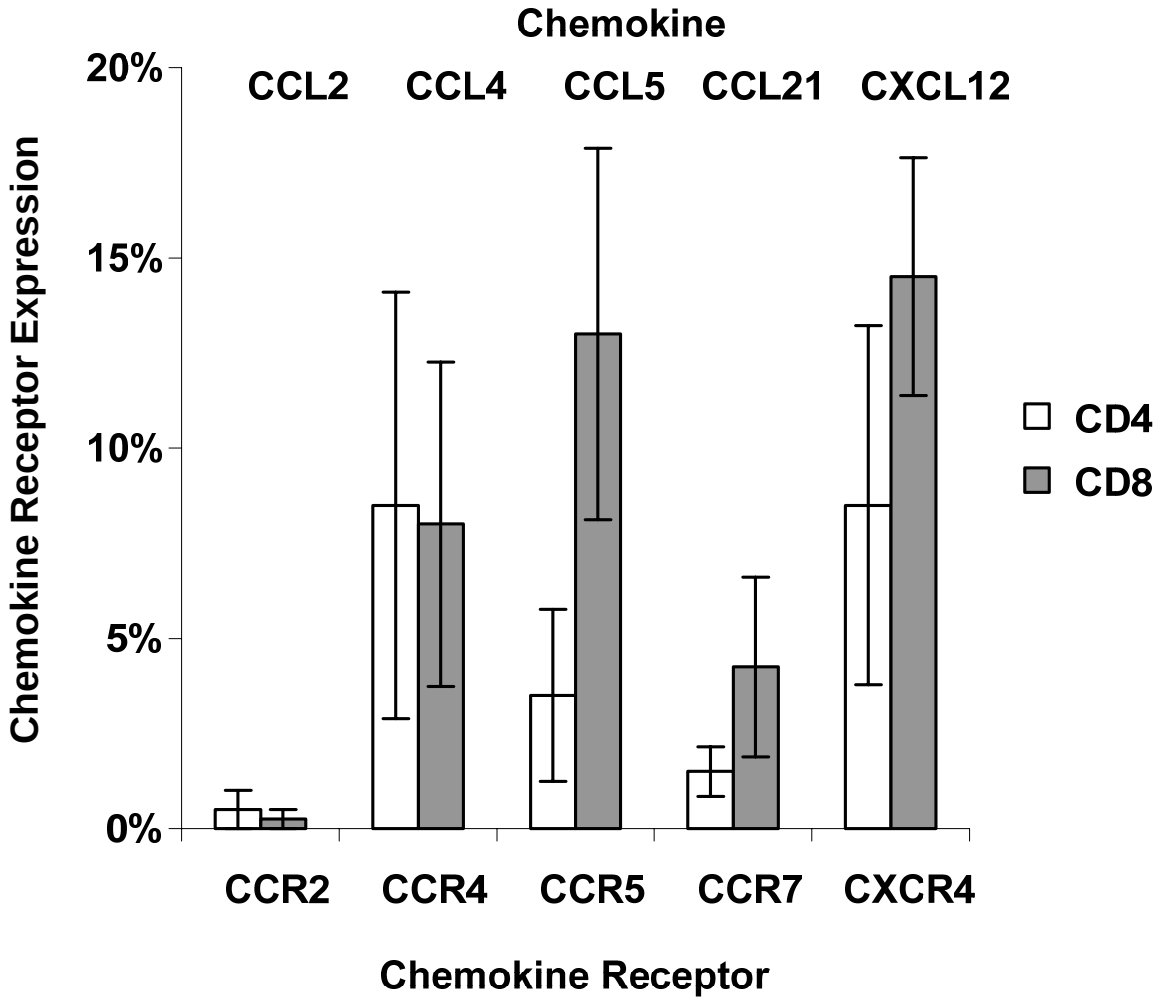
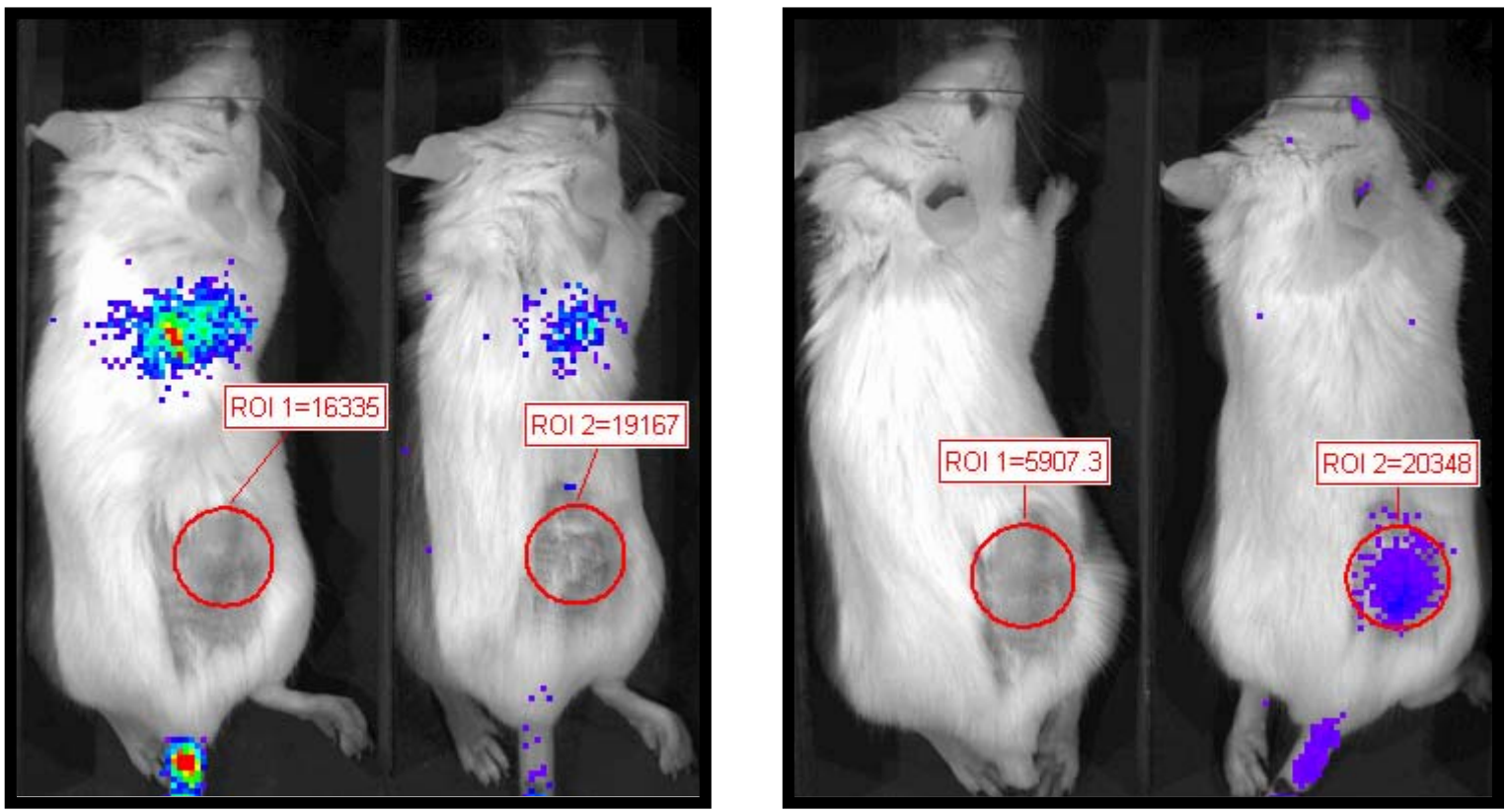
Expression of Chemokine Receptors on EBV-Specific CTL



CCR2b-T Cells Homing



Summary
Gene Transfer to retarget CTLs

- Retroviral gene marking confirms EBV-CTL's effective against post-transplant lymphoma.
- Adviral vectors enhance specificity of CTL for weak tumor antigens – HD and NPC
- CAR gene transfer allows CTL to effectively bear alternative anti-tumor specificities- Solid tumors
- Further engineering should enhance clinical efficacy

Immunotherapy

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