Improving the Efficacy and Safety of G-M Virus-Specific T cells for Solid Tumors

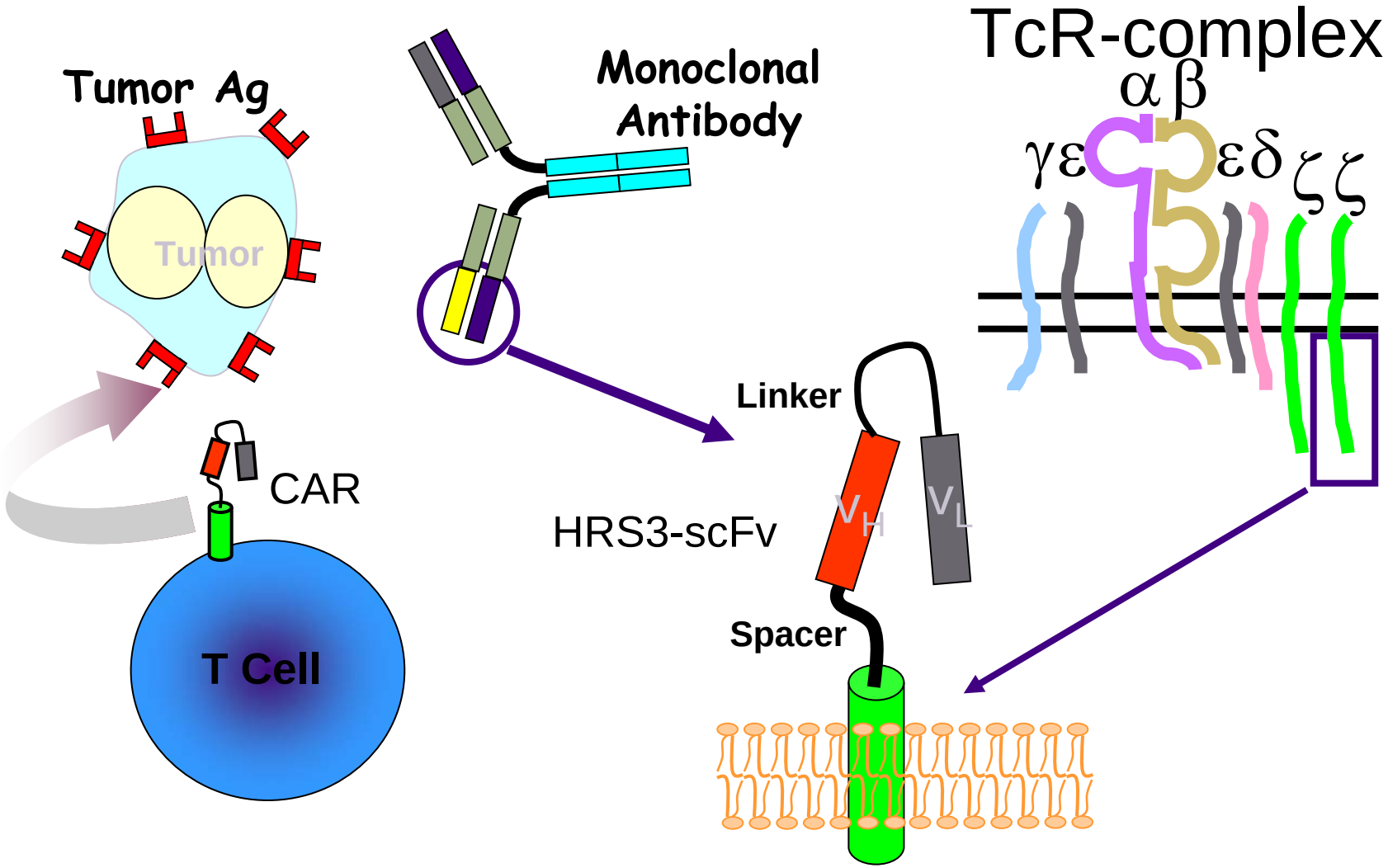
Malcolm Brenner

T lymphocytes for cancer

Specific – and (maybe) better than MAb

- Recognize internal antigens (if processed)
- Good bio-distribution - Traffic through multiple tissue planes
- Multiple effector mechanisms
- Self amplifying

Chimeric Antigen Receptor (CAR) Expression in T cells

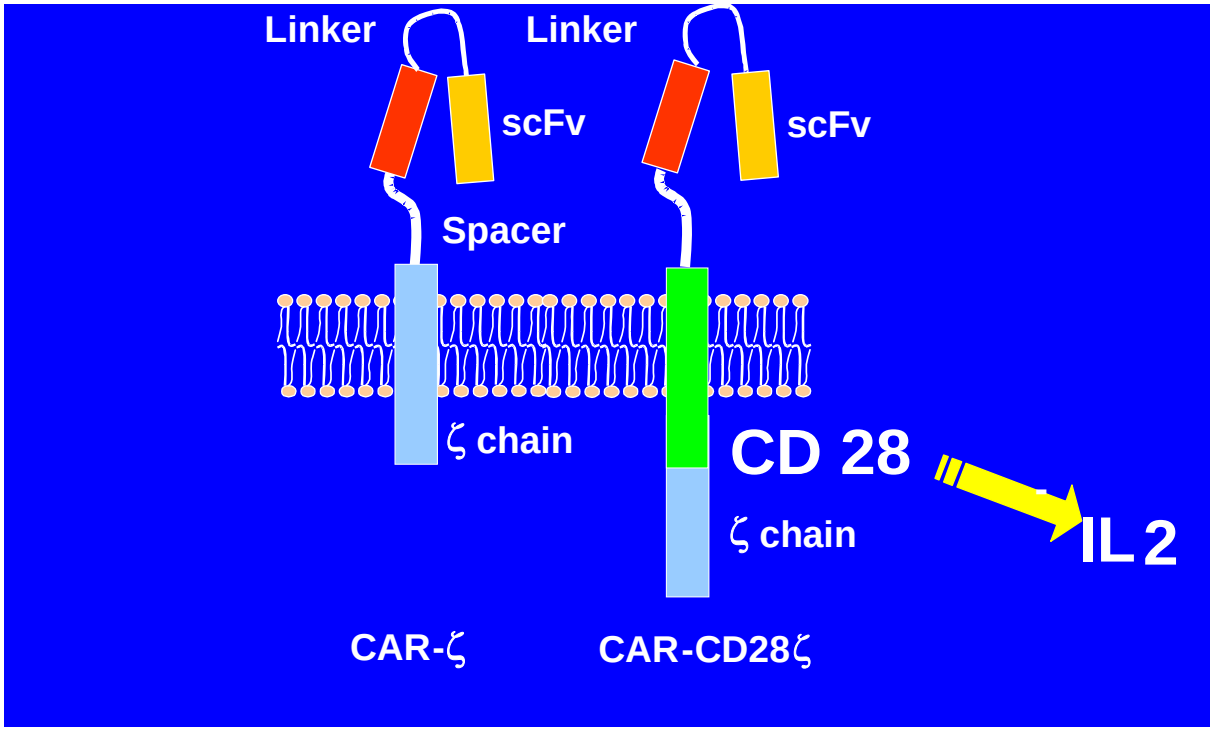


Chimeric Antigen Receptor T cells (CAR-T)

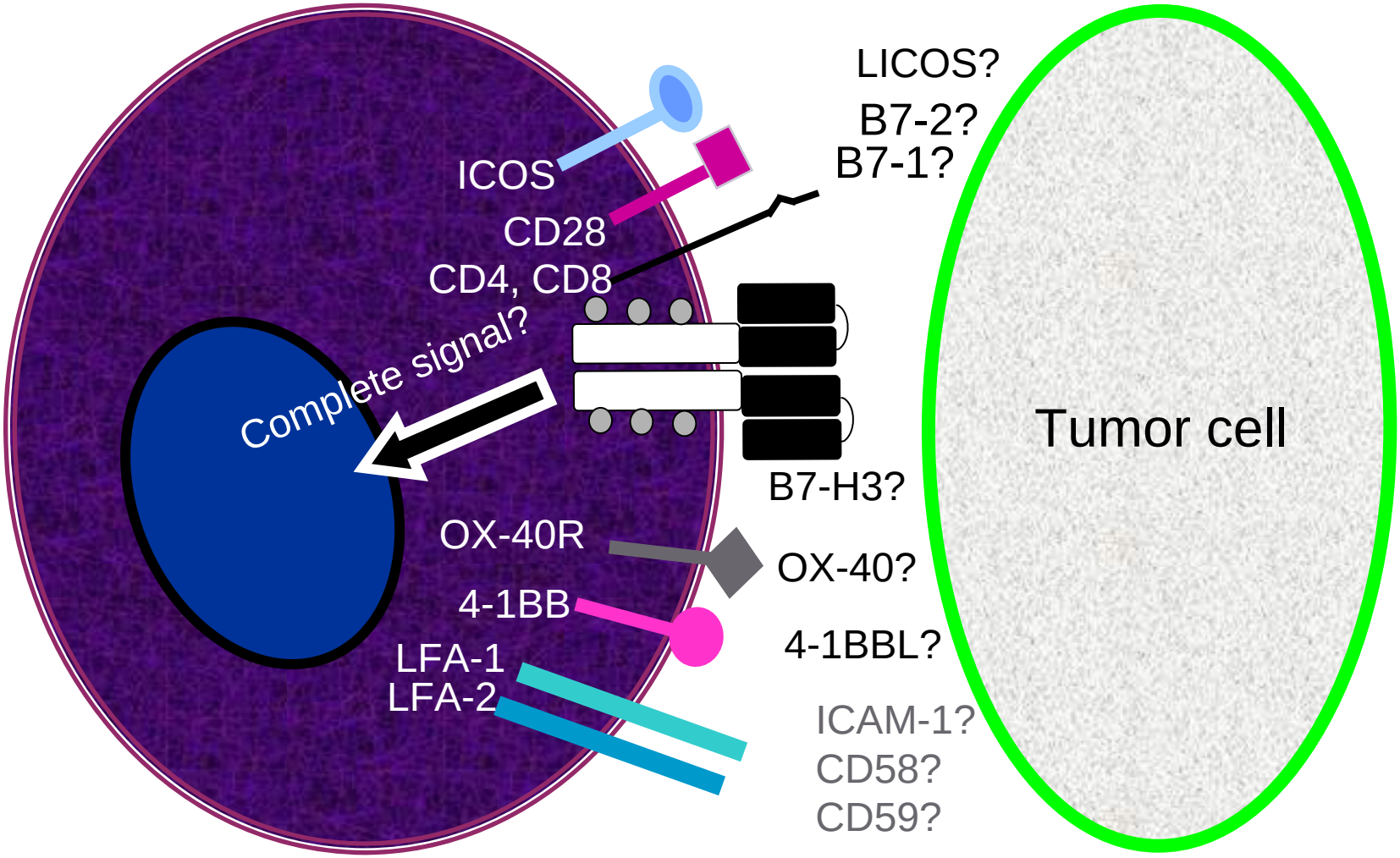
- Recognize unmodified tumor antigens in MHC unrestricted manner- bypass many tumor immune evasion strategies
- Tumor cells have other problems in presenting antigen (e.g. lack co-stimulator molecules, inhibit induction of effector phenotype)
- **Consequence – poor in vivo persistence, expansion and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

Overcoming poor co-stimulation to CAR- PTC

- Incorporate more co-stimulatory domains
  - CD28
  - CD28 and OX40
  - CD28 and 4-1BB



Chimeric receptor-mediated interaction between T cell and tumor cell



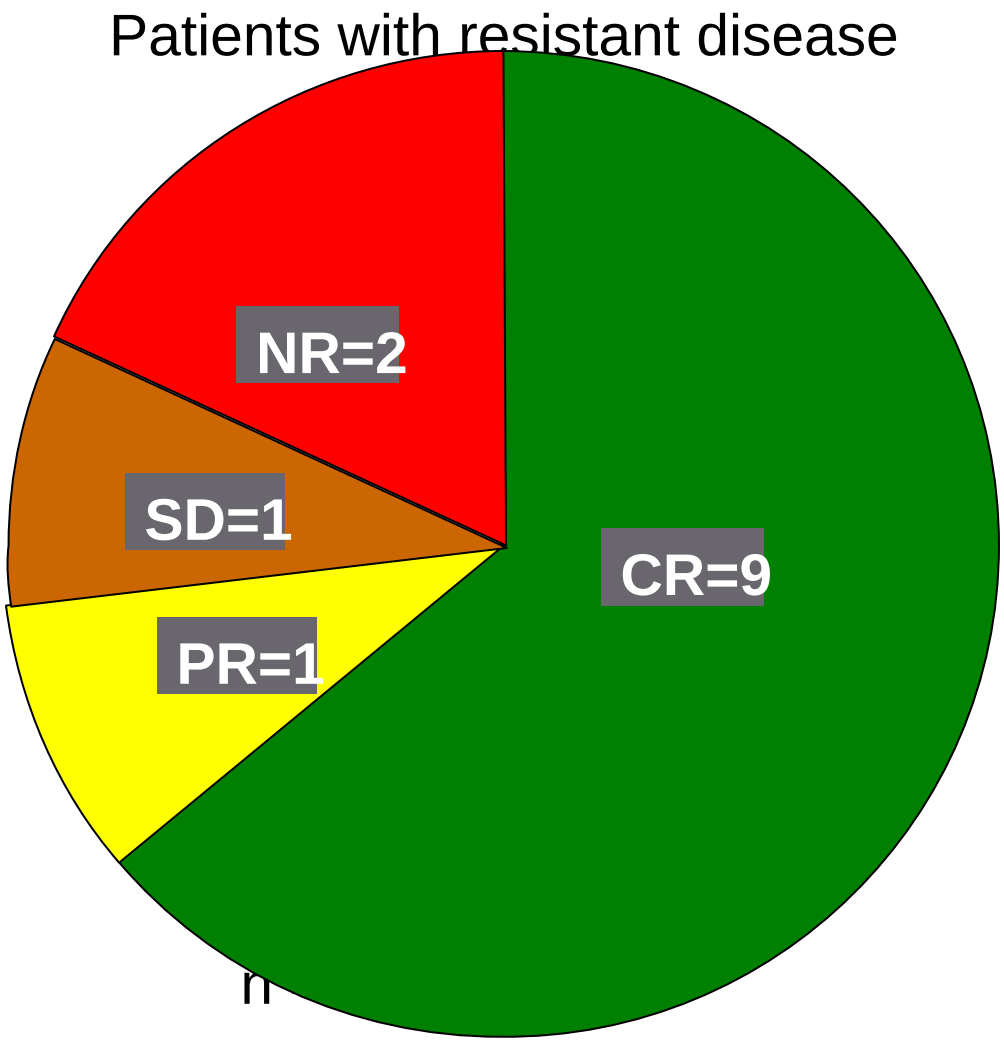
Using EBV Infected Target Cells as source of co-stimulation

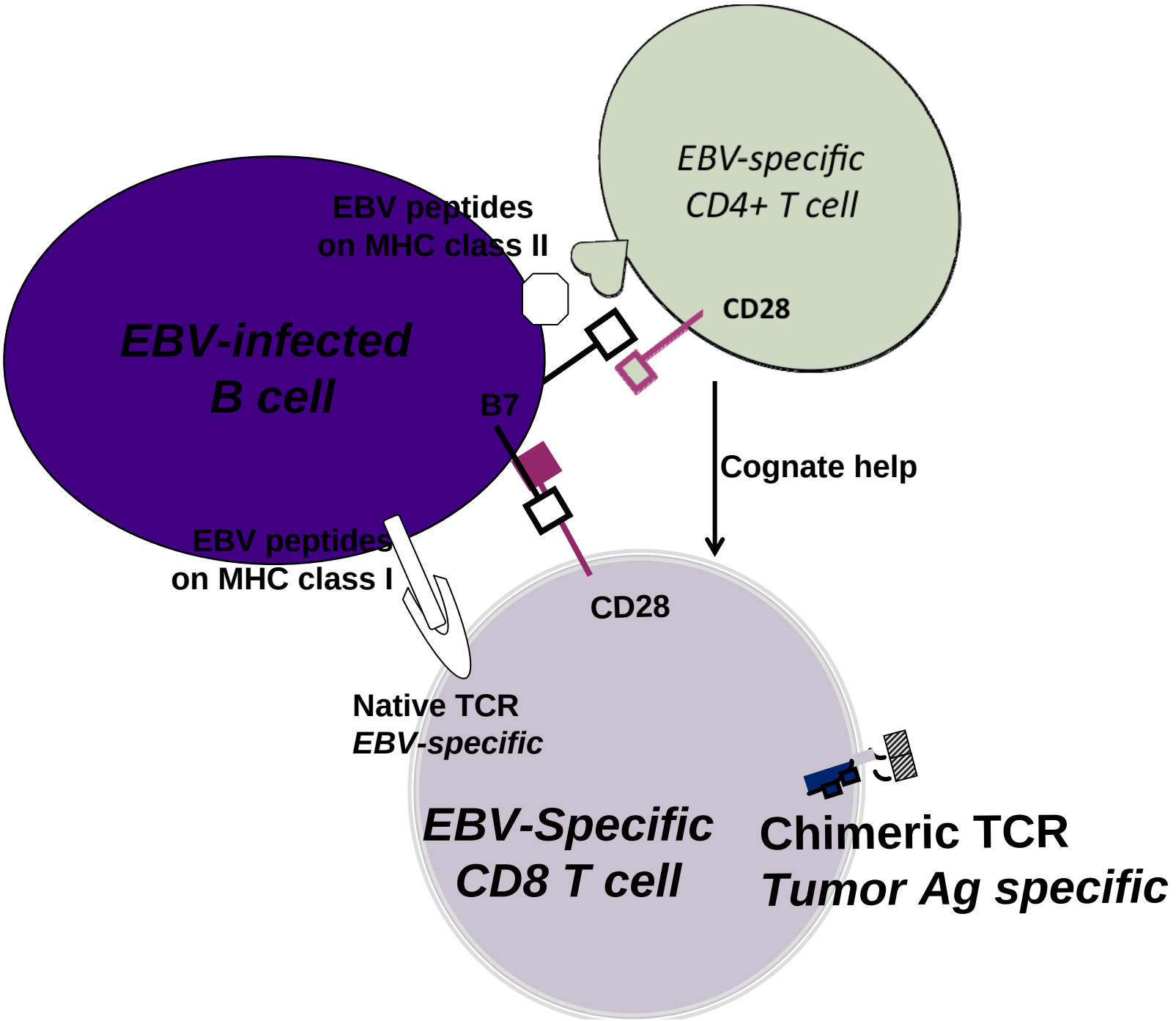
- EBV targets express all relevant co-stimulator molecules and are present lifelong
- EBV-CTL
  - Expand in vivo
  - Have effector phenotype
  - Persist long term
  - Eradicate bulky EBV+ HD/NHL, PTLD

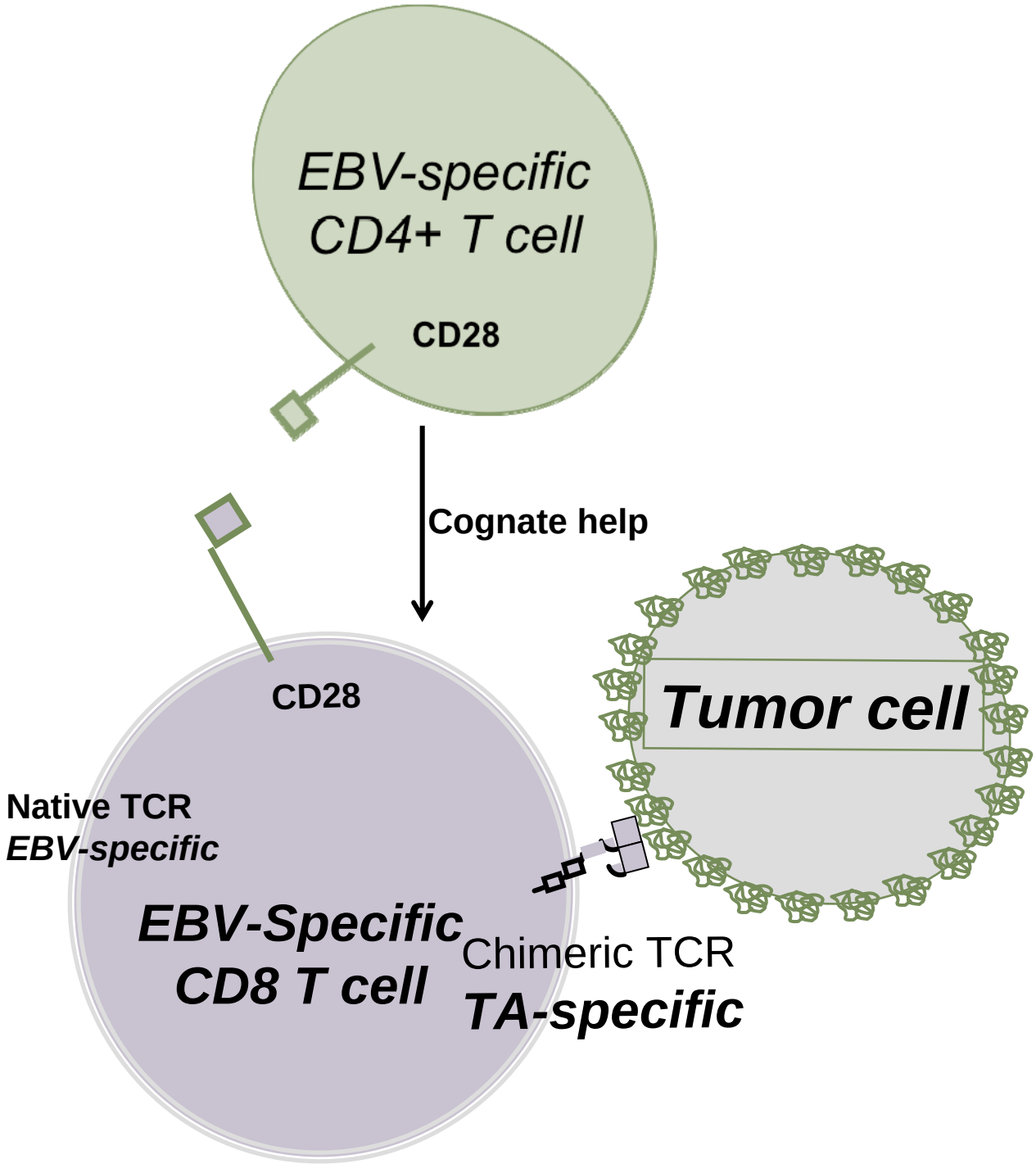
EBV CTL to treat and prevent PTLD after Transplant

- Extensive (>3 logs) in vivo expansion
- Long term (>10 years) persistence)
- No disease in >120 high risk patients receiving CTL prophylaxis versus 12% of controls
- Complete and sustained resolution of tumor in 11/13 patients with resistant lymphoma
- US Orphan drug designation granted 2007. Approval under discussion

Clinical Responses After LMP-CTL Therapy




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
CD28
Cognate help
CD28
Native TCR
EBV-specific
EBV-Specific
CD8 T cell
Tumor 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

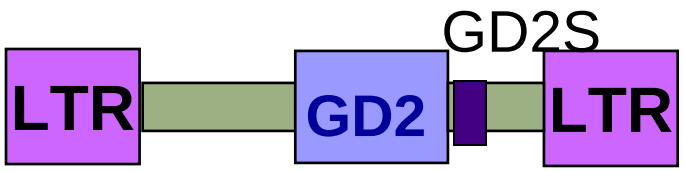
- Disialoganglioside expressed in tumors of neuroectodermal origin
- Expressed at high density on almost all neuroblastoma cells
- Poorly expressed or absent from most normal tissue
- MAb has been used with clinical responses

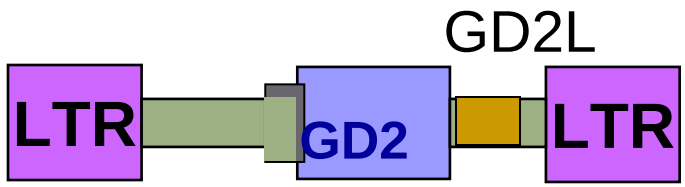
Are CAR-cytotoxic T lymphocytes (CTLs) better than CAR-activated T cells (ATC)?

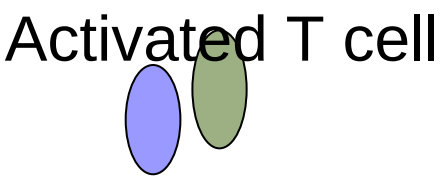
Transduce patient ATC and CTL with a vector encoding identical receptor but distinct oligonucleotide for each population.

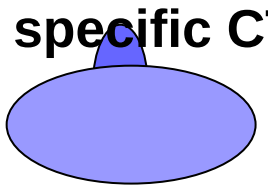
Vectors in Clinical Study

Patient One

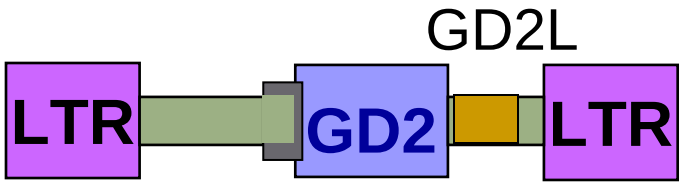


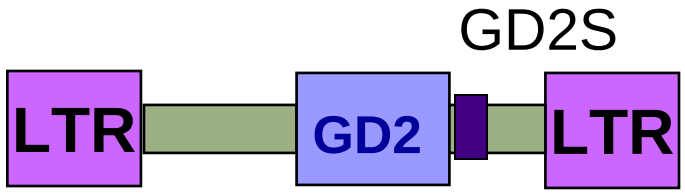




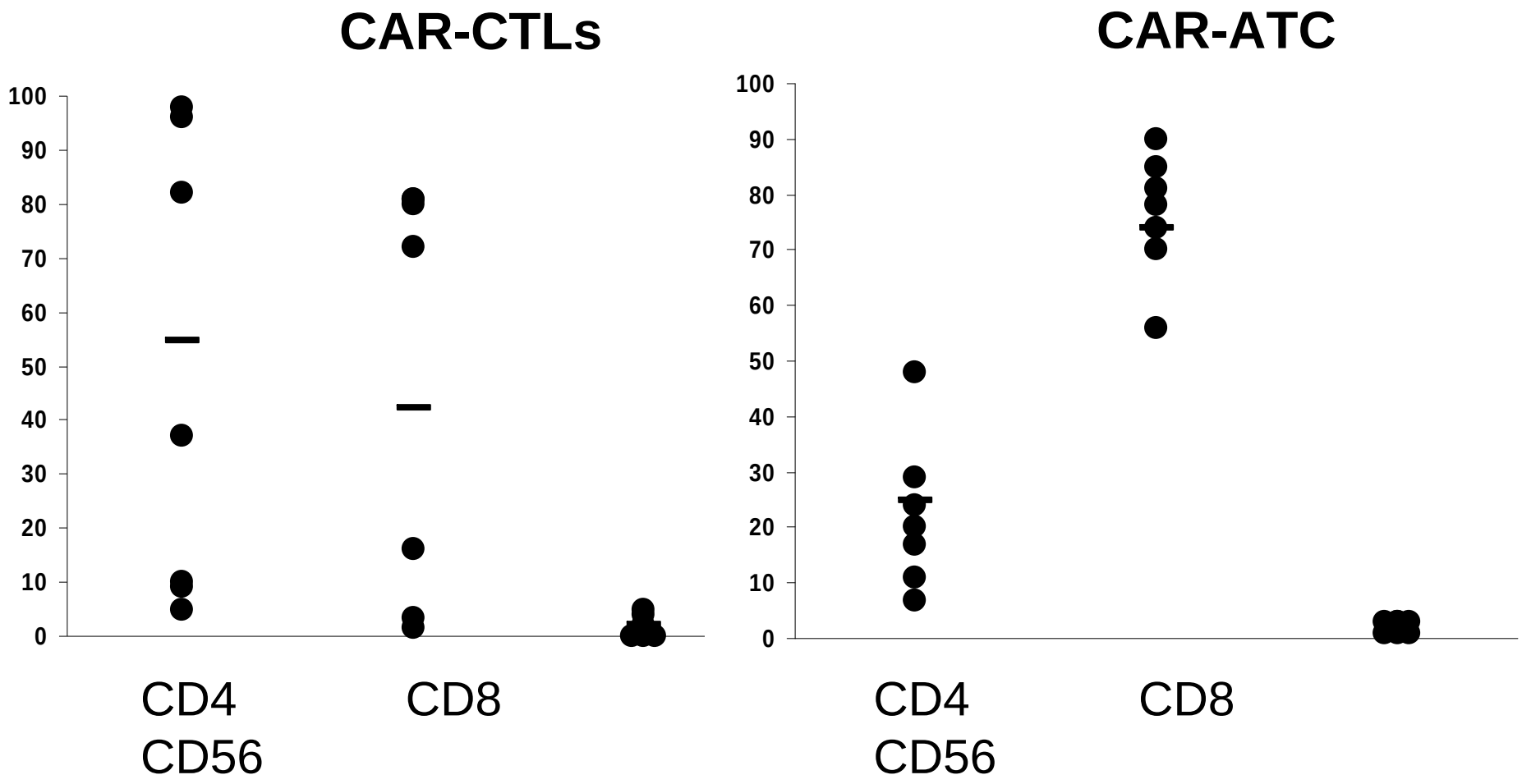


Patient Two





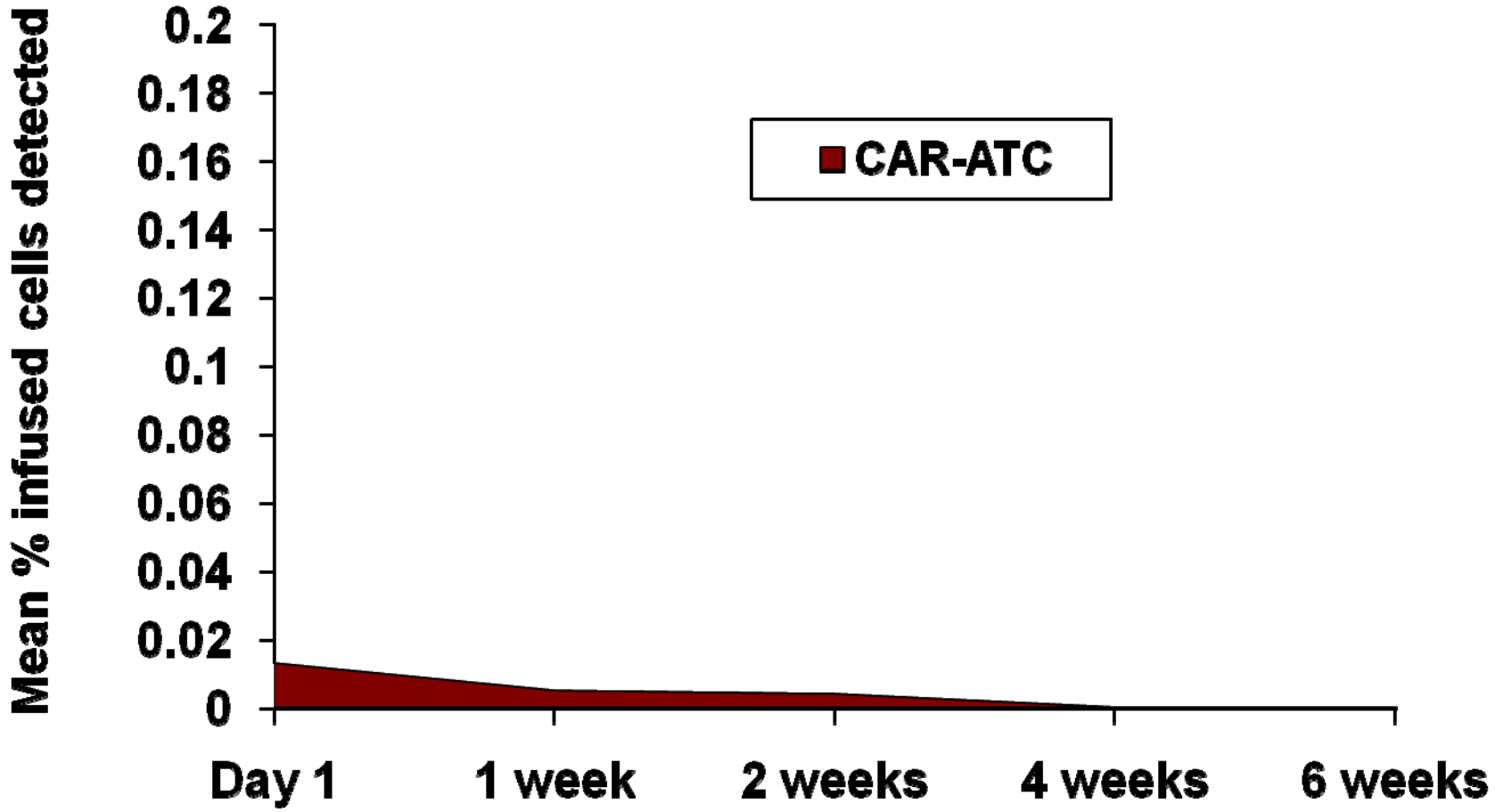
Phenotype of cell product



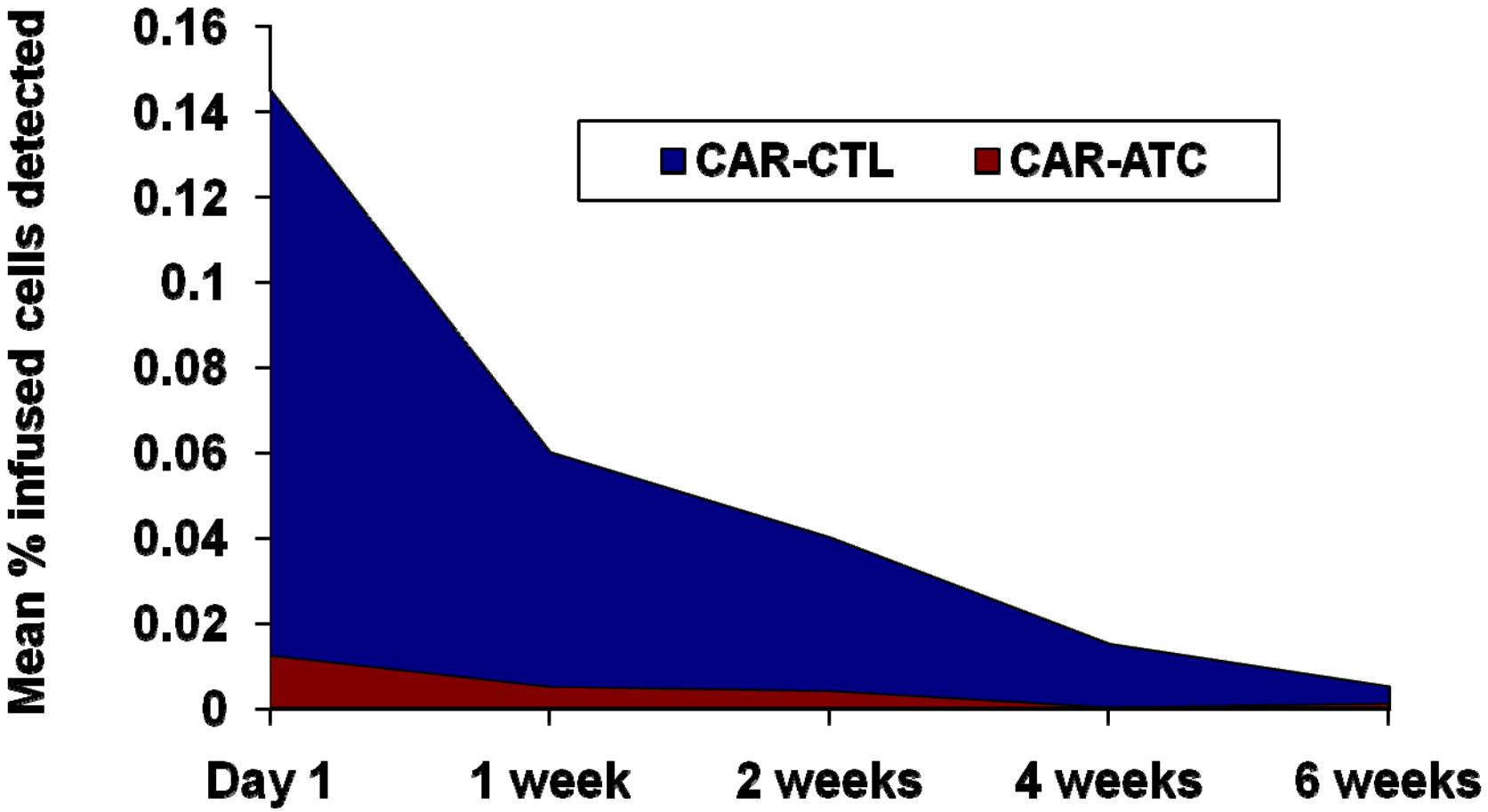
What should CAR-CTL do?

- Persist longer at higher levels than CAR-activated T cells (ATC)

Percent gene modified EBV CTL or ATC in PBMNC

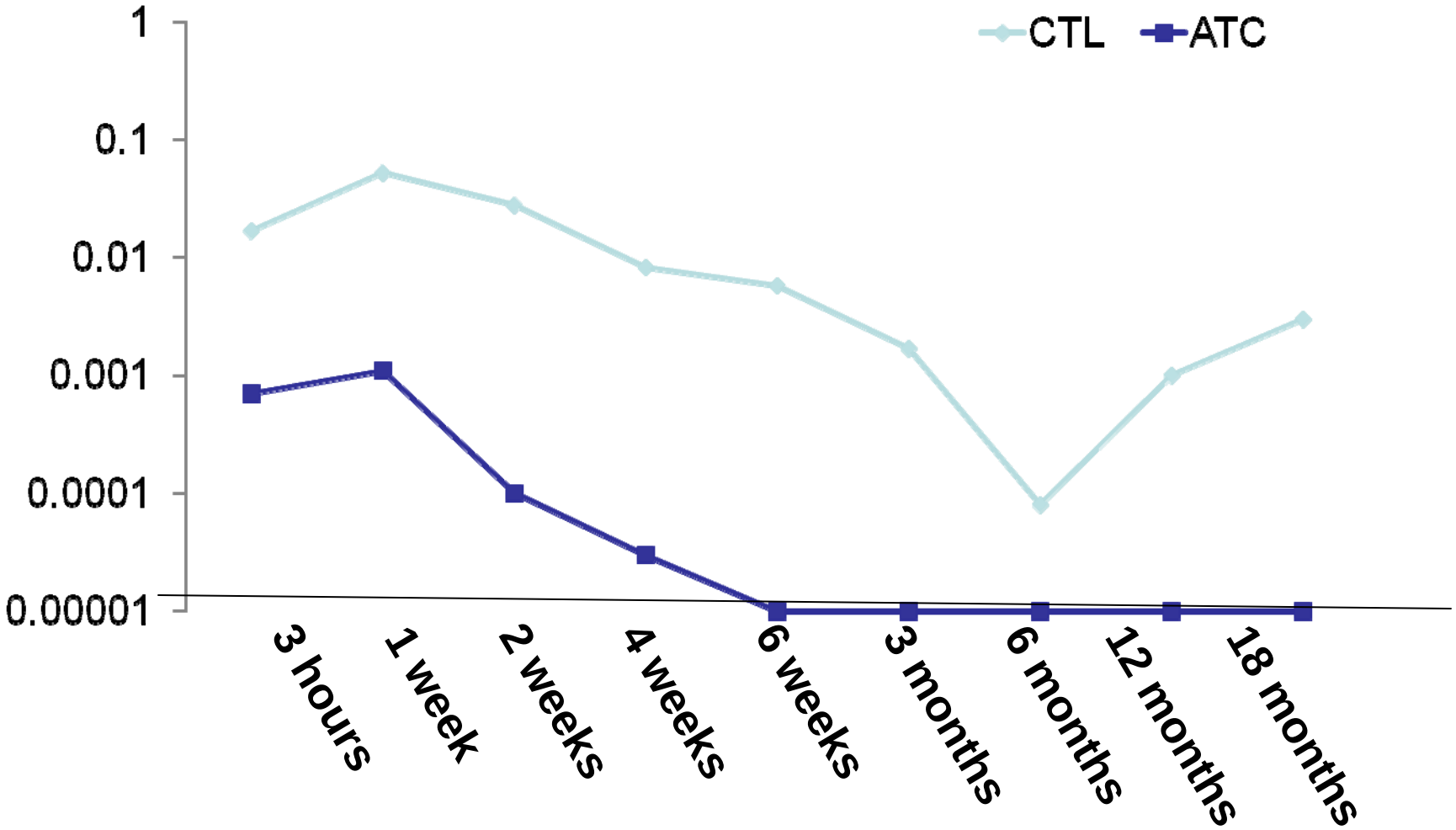


What do we want for CAR T cells?



Persistence of ATC versus CTL

9 year old with relapsed neuroblastoma
Remains in CR 18 months post T cell infusions



Clinical Responses

- 5/10 patients with active relapsed/resistant disease had tumor response/regression
- 3 Complete remissions (2 sustained >4yrs, >12 Months)

Increasing Value of CAR-CTLs

- Increase Range of Solid Tumors Treated
  - Her2Neu+

    Medulloblastoma; Glioma; Non-Small Cell Lung Cancer

Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing ERBB2

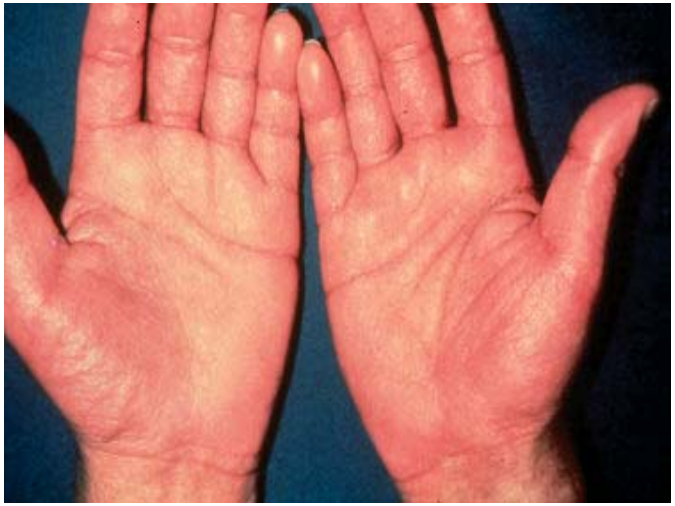
Richard A Morgan, James C Yang, Mio Kitano, Mark E Dudley, Carolyn M Laurencot and Steven A Rosenberg

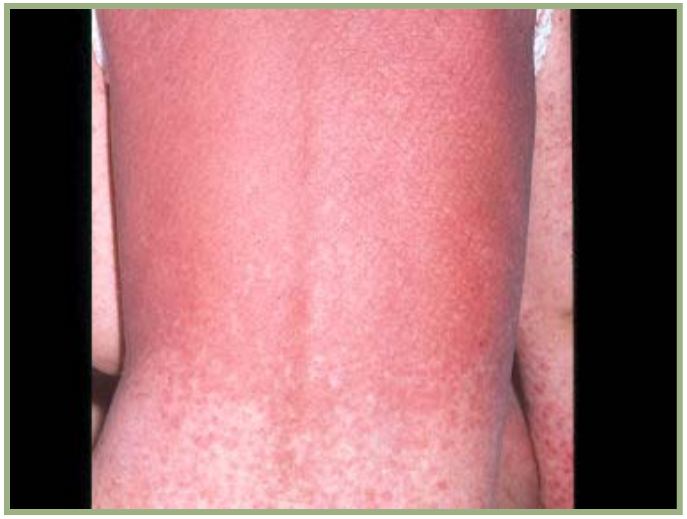
Molecular Therapy 18, 843-851
(April 2010)

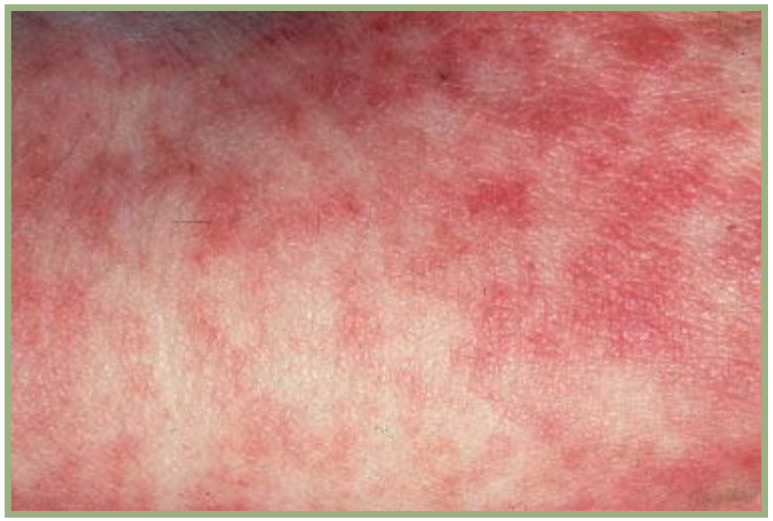
Small molecule/MAb toxicities generally improve with time

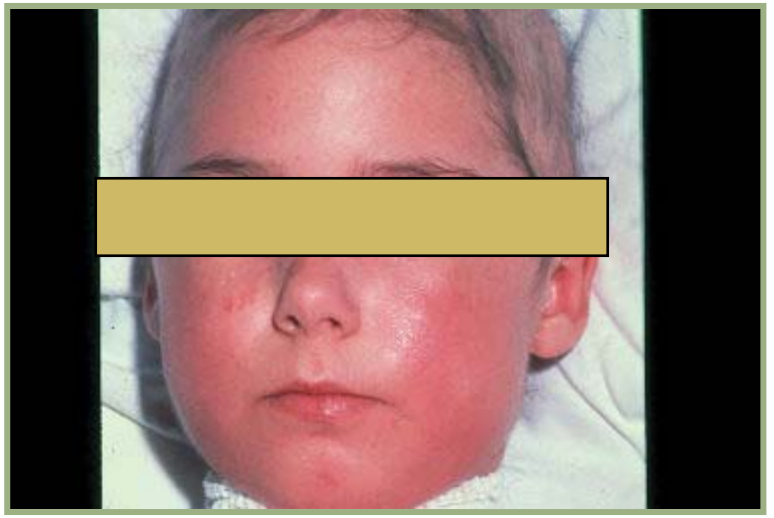
Toxicities from cells persist and worsen

Acute GVHD skin

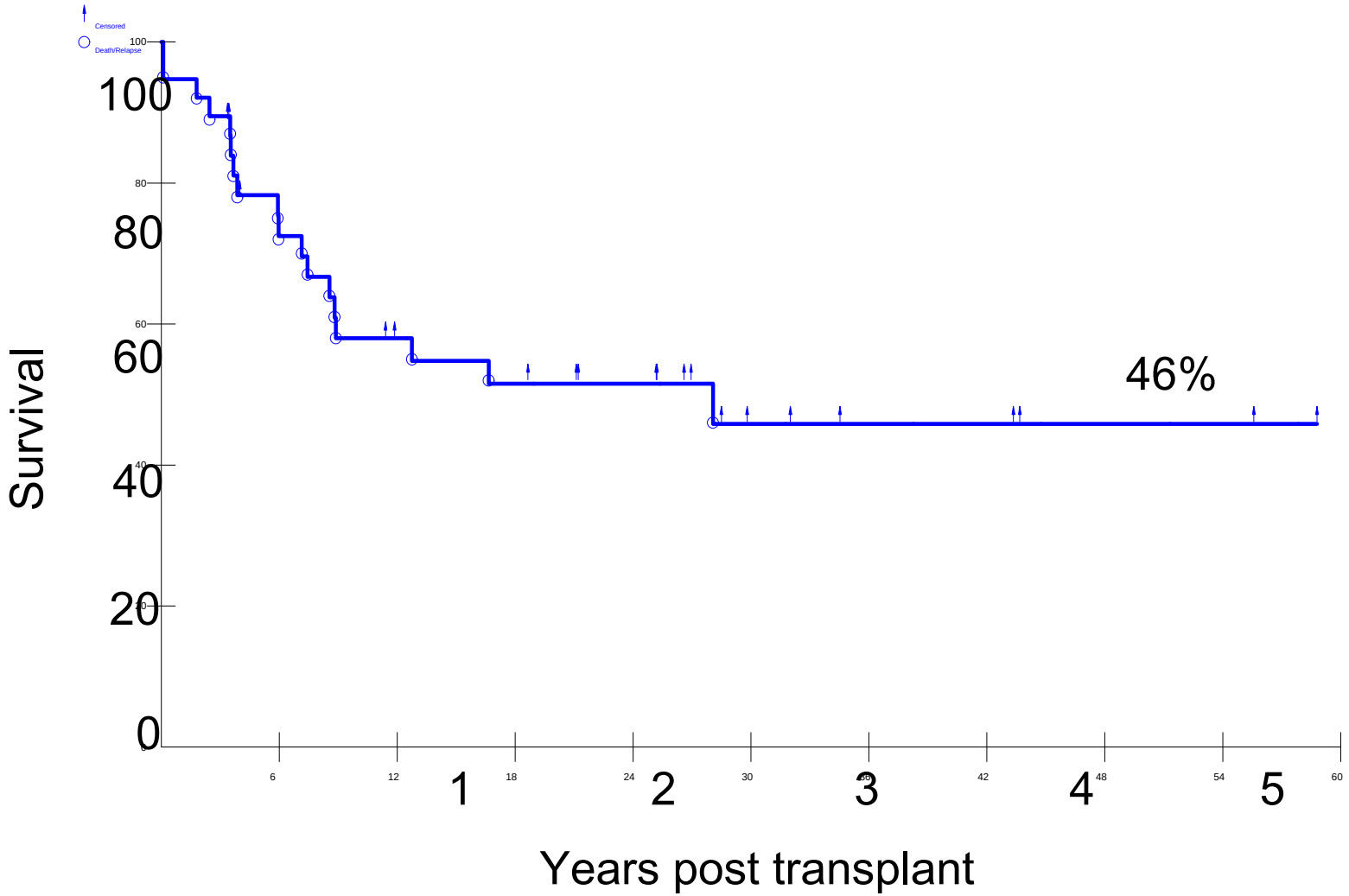








CD34 Selected Haploidentical Transplants (BCM 1999-2004)

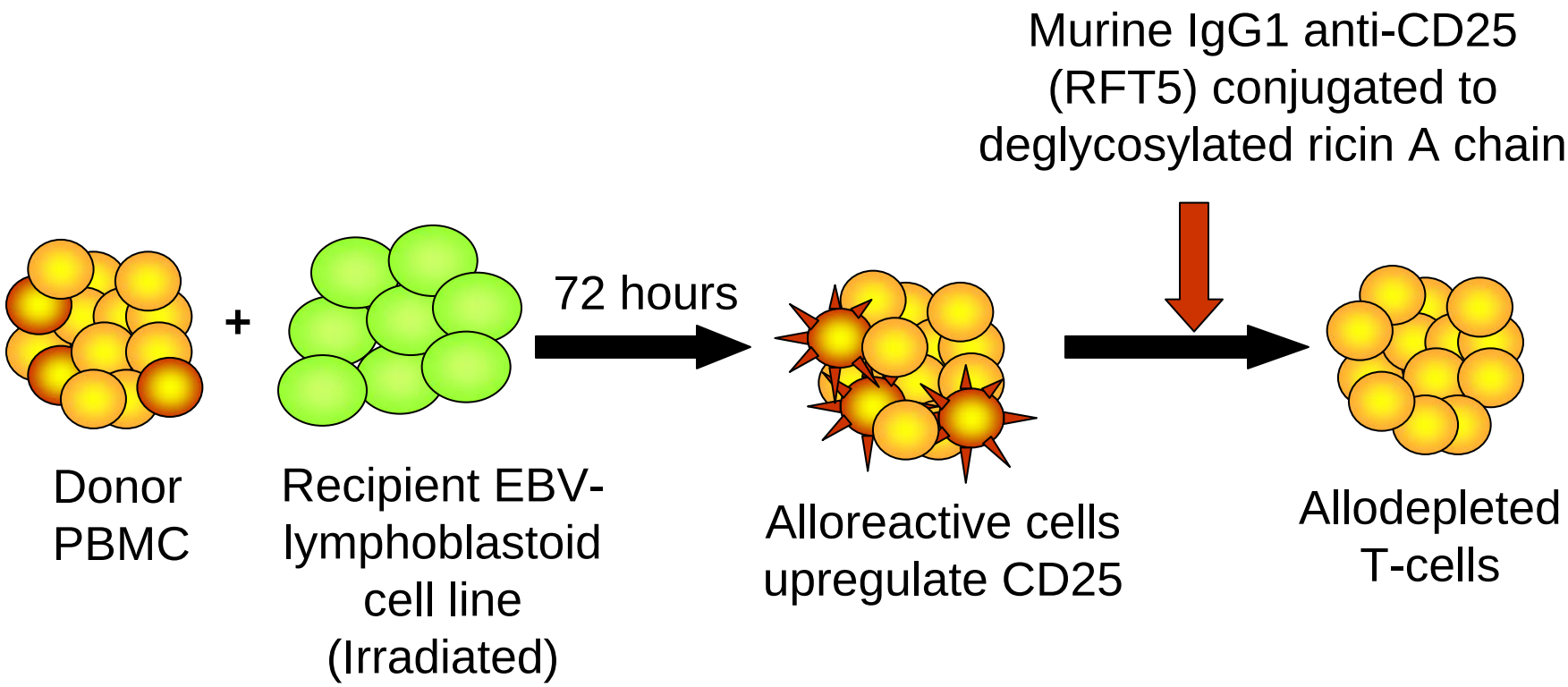


Causes of Failure

- Regimen related mortality 5%
- Relapse 21%
- Infection 21%

Slow immune recovery due to T cell depletion

Selective Depletion Of Alloreactive Cells



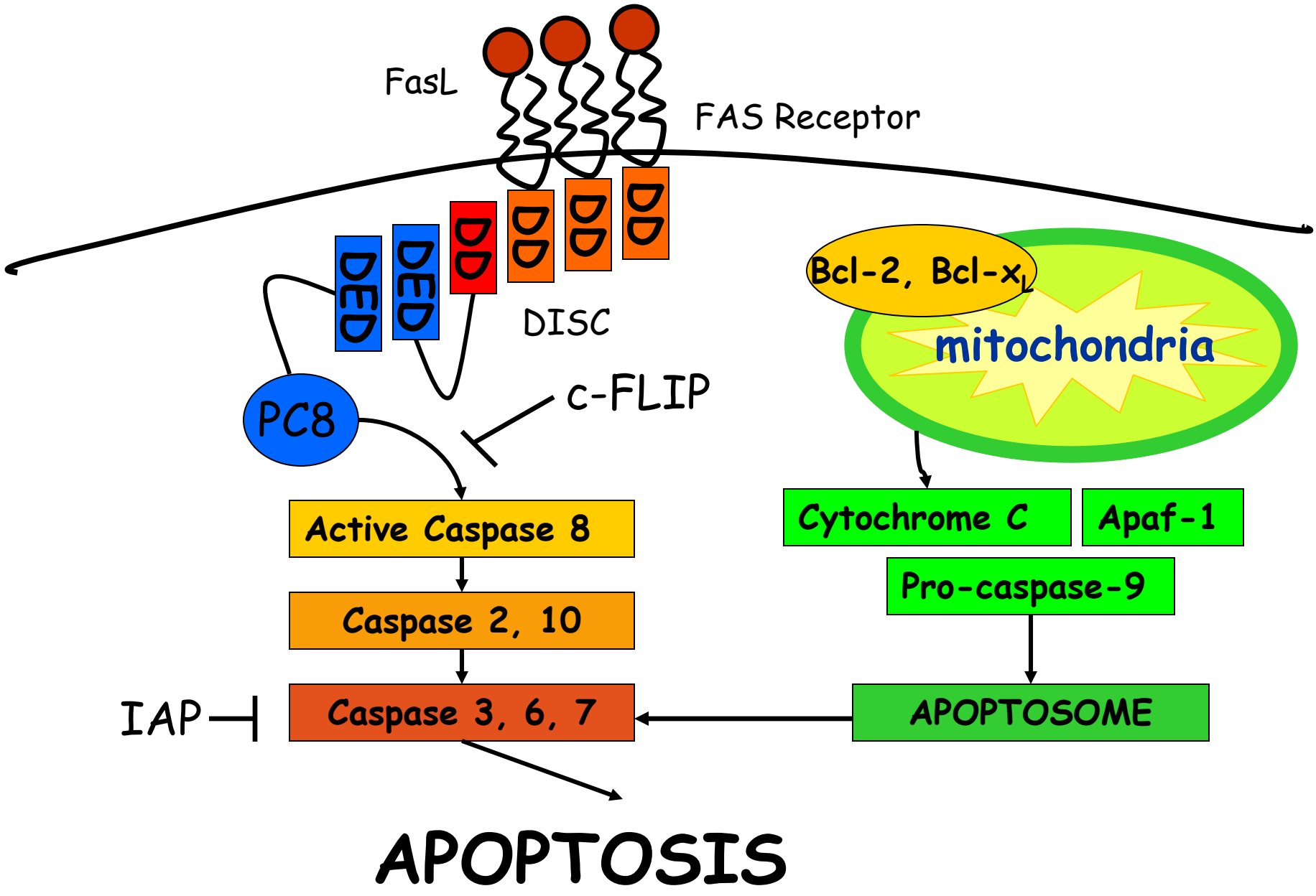
Suicide Gene Therapy to Control Toxicities from Cell Therapy

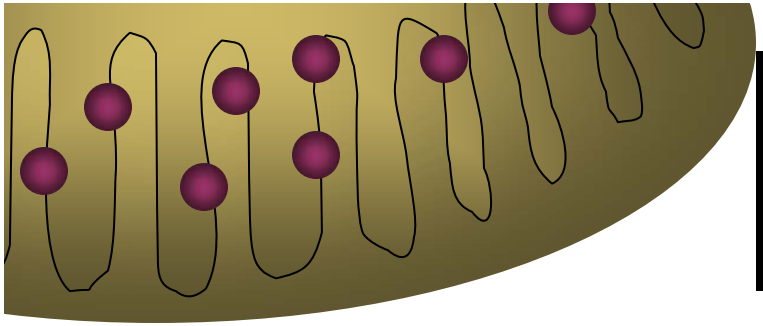
- Herpes Simplex Thymidine Kinase most tested suicide gene
- Phosphorylates pro-drug (e.g Ganciclovir) to triphosphonucleoside
- Inhibits DNA polymerase/Host cell DNA synthesis

Suicide Gene Characteristics

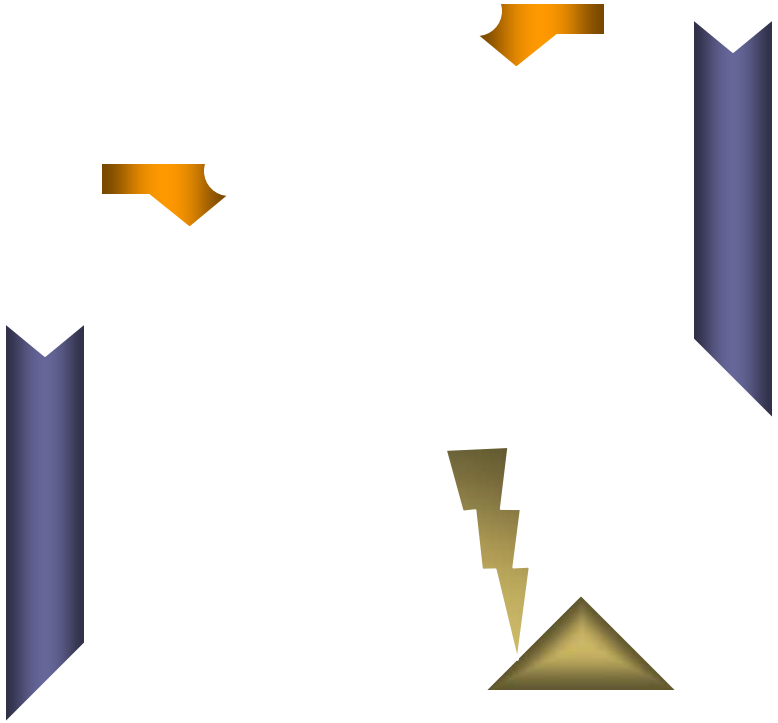
Herpes Simplex Virus Thymidine Kinase (HSVtk) works, but has disadvantages:

	HSVtk
Source	Foreign →Immunogenic
Activating drug	Ganciclovir. Widely used to treat CMV.
Mechanism	Inhibits DNA synthesis – slow killing even of dividing cells


FasL
FAS Receptor
DD
DD
DD
DD
DED
DED
DISC
PC8
c-FLIP
Bcl-2, Bcl-xL
mitochondria
Active Caspase 8
Caspase 2, 10
Caspase 3, 6, 7
IAP
Cytochrome C
Apaf-1
Pro-caspase-9
APOPTOSOME
APOPTOSIS



Caspase 9



Cytochrome C

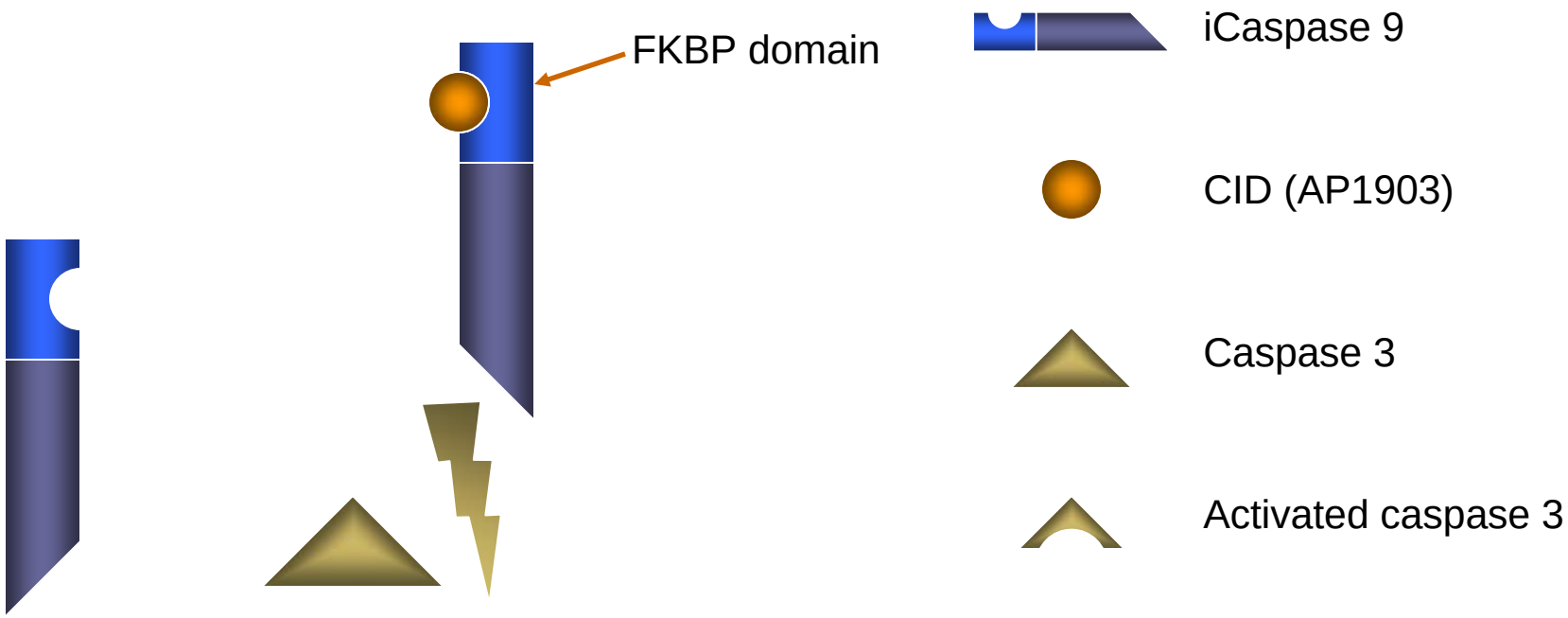
Apaf-1

Caspase 9

Caspase 3

Activated caspase 3

Inducible caspase 9

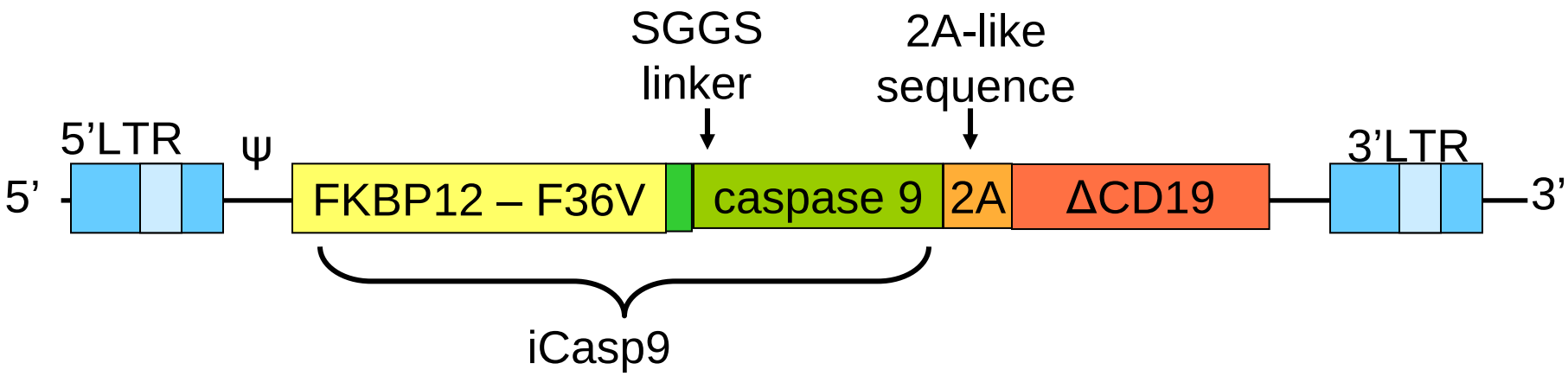


Suicide gene

	HSVtk	iCasp9
Source	Foreign →Immunogenic	Human derived → less immunogenic
Activating drug	Ganciclovir. Widely used to treat CMV.	Non therapeutic small molecule
Mechanism	Dividing Cells (DNA synthesis)	All cells by apoptotic pathway. Rapid killing.

Retroviral vector

SFG.iCasp9.2A.ΔCD19



CASPALLO: Eligibility criteria

- Haploidentical stem cell transplantation
- Hematologic malignancy
- Lymphoproliferative disorders
  - HLH, FLH, VAHS, SCAEBV, XLP

CASPALLO: Study objectives

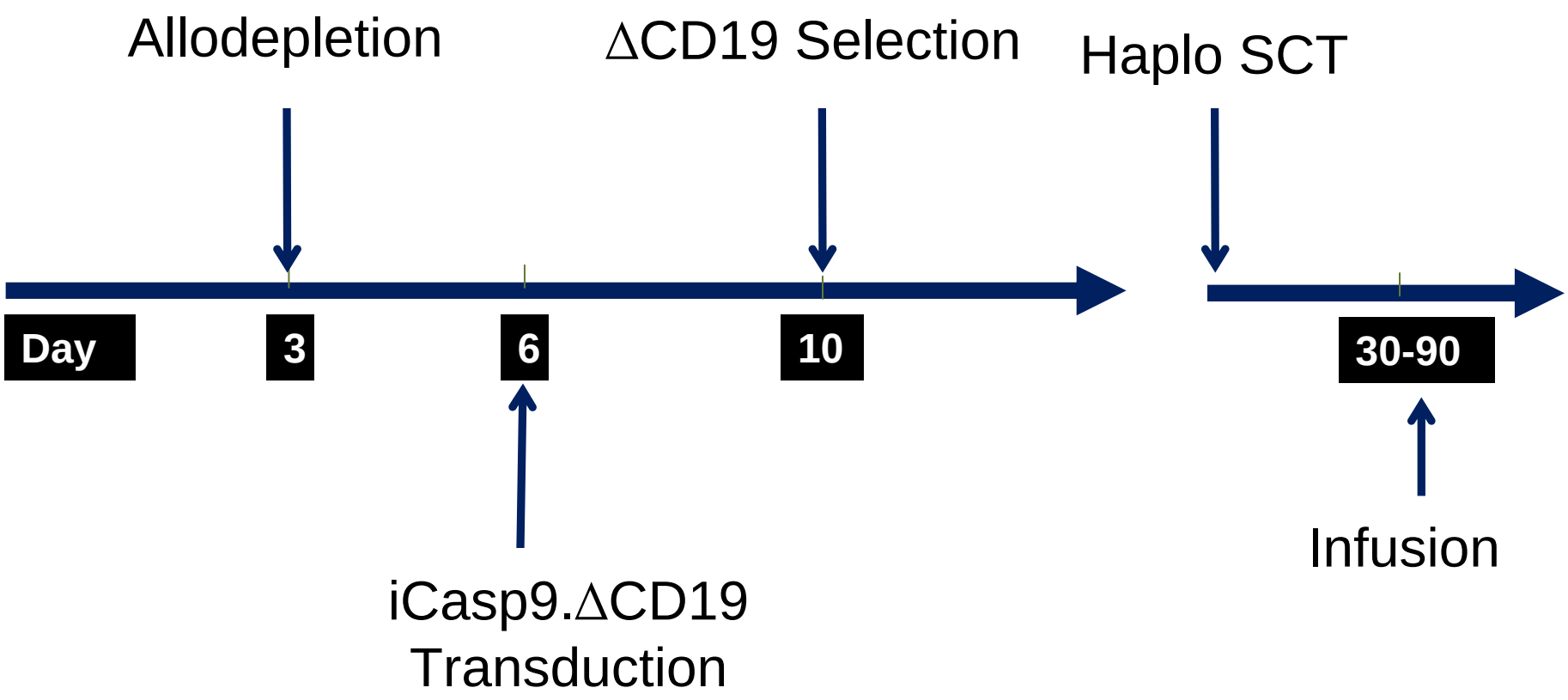
PRIMARY

- Highest dose of allodepleted donor T cells with grade III-IV acute GvHD rate ≤ 25%

  (Range 10e6 to 5 x 10e7/kg)

- Biological and clinical effects of administration of AP1903

CASPALLO: Protocol overview



Use icasp9 for Additional Cell Types

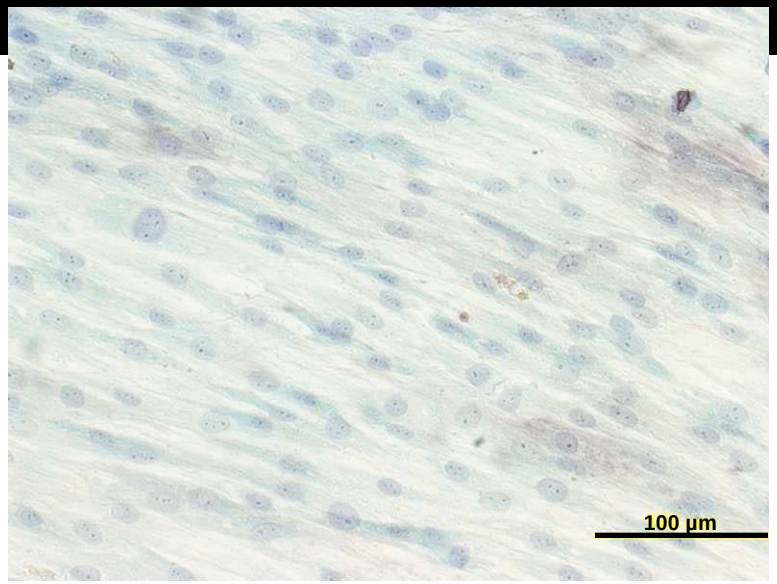
- Post mitotic

  e.g. progeny of mesenchymal stromal cells

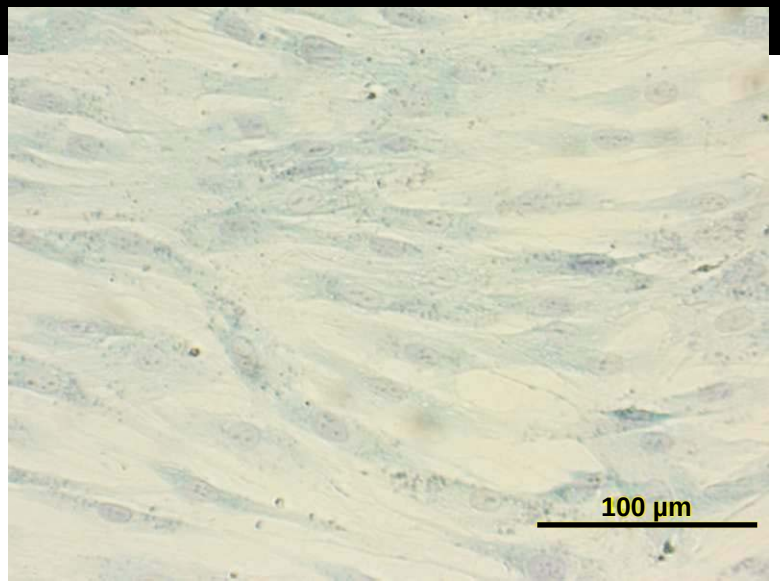
Extend Dimerizer to Mesenchymal Stromal Cells

- Site-directed delivery
  - Injury repair
    - Cartilage? (Black *et al.*, Vet Ther 2007)
    - Myocardium? (Chen *et al.*, Chin Med J 2004)
    - Spinal cord? (Moviglia *et al.*, Cytotherapy 2006)
- Systemic delivery
  - Congenital deficiencies
    - Osteogenesis imperfecta (Horwitz *et al.*, Nat Med 1999)
  - Injury repair
    - Stroke? (Bang *et al.*, Ann Neurol 2005)
    - GvHD

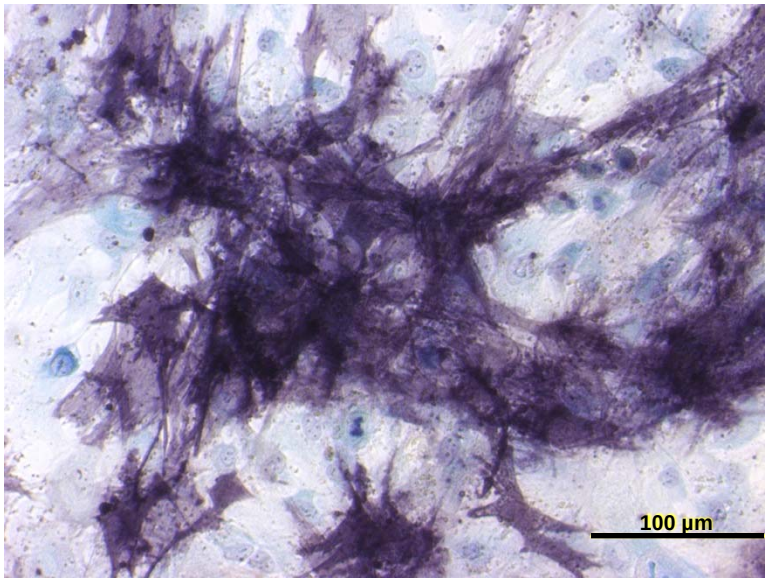
MSC differentiation



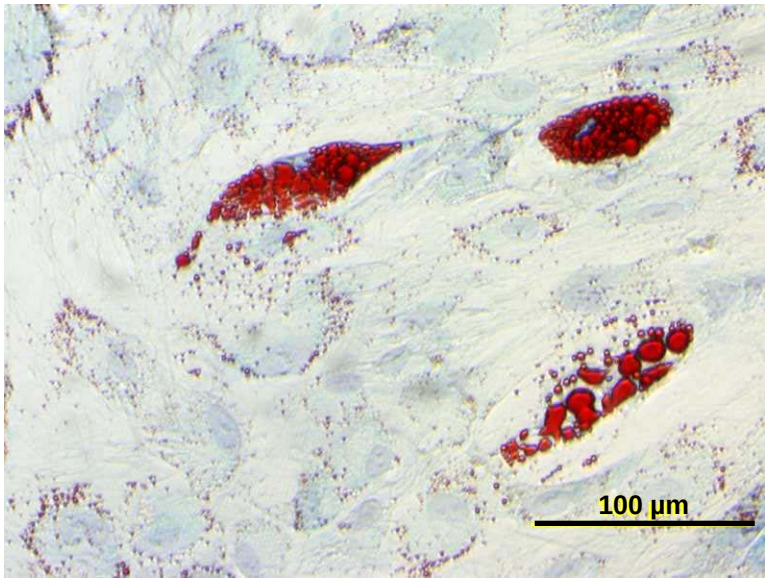
Expansion medium (alk phos/methylene blue)



Expansion medium (oil red/methylene blue)

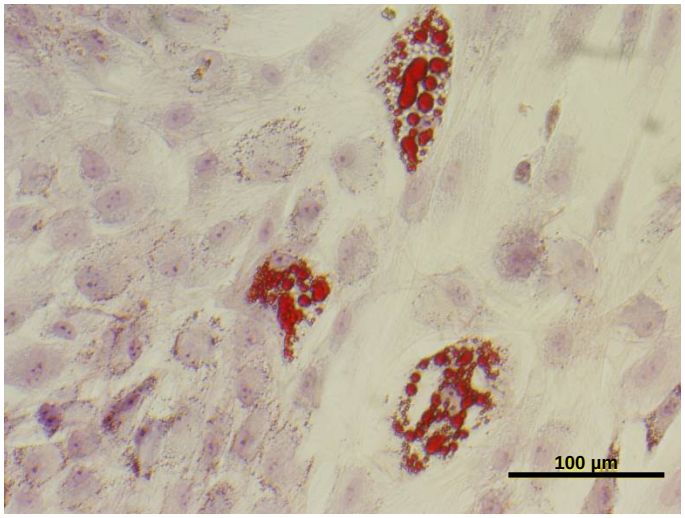


Osteodiff medium (alk phos/methylene blue)

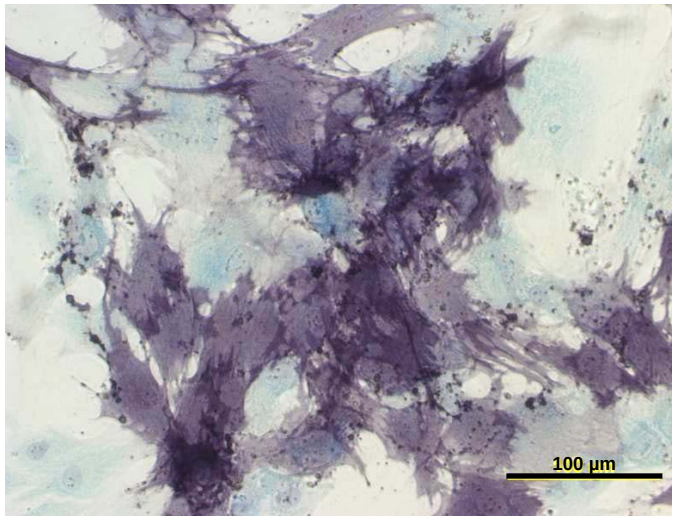


Adipodiff medium (oil red/methylene blue)

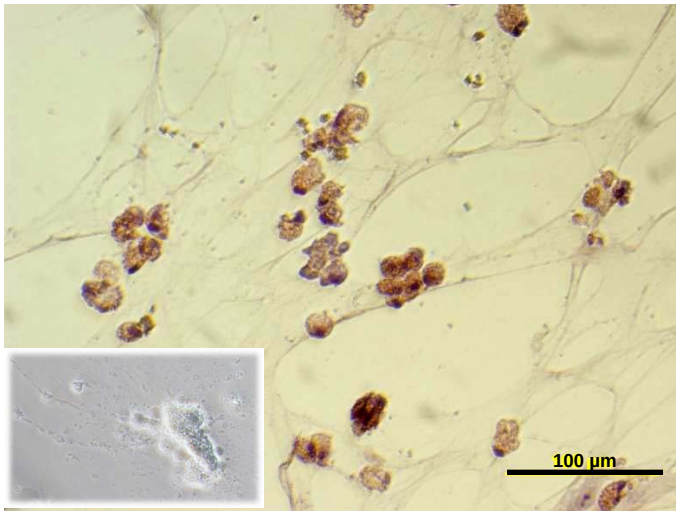
iCasp9-MSC are multipotent and killed by exposure to CID



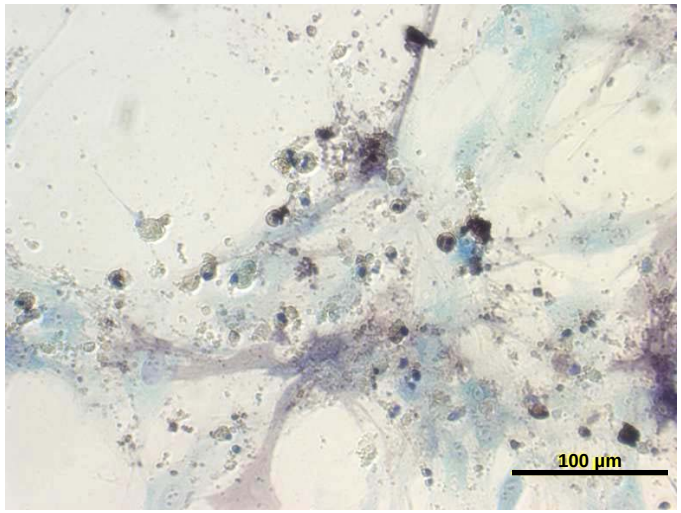
Adipodiff medium (oil red/eosin/azure)



Osteodiff medium (alk phos/methylene blue)

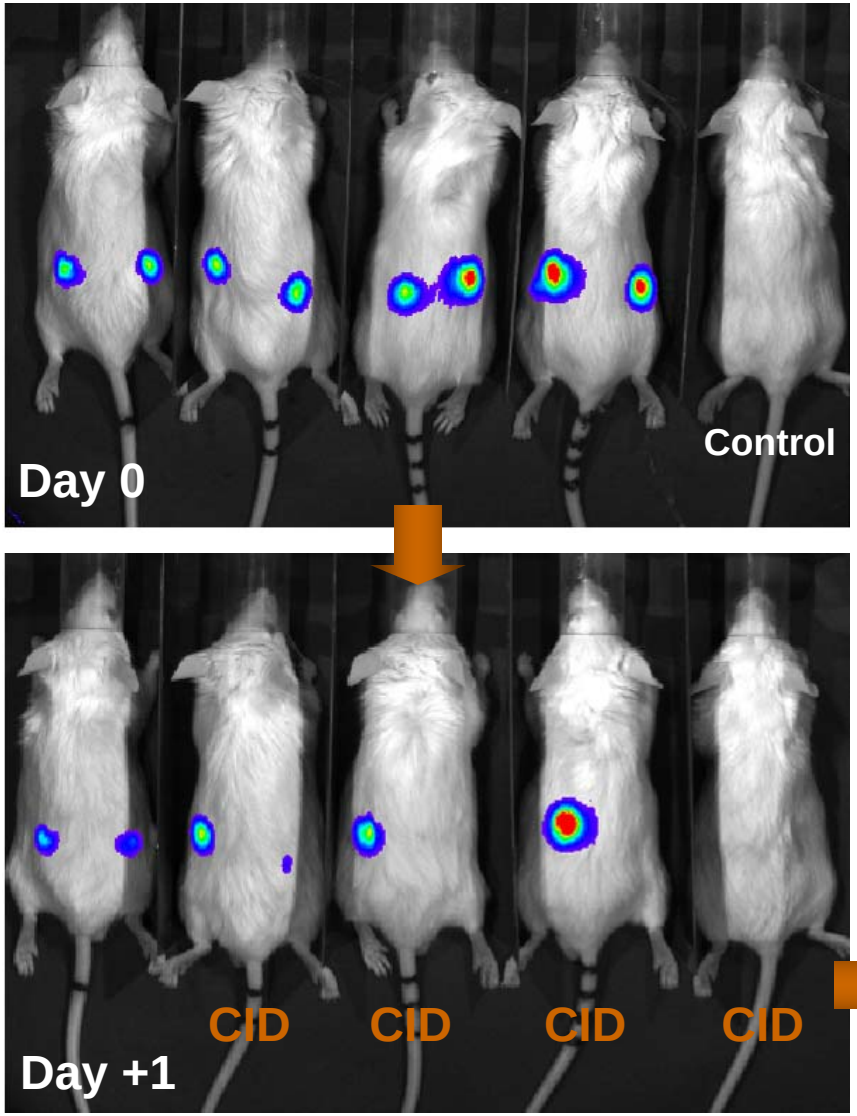


Adipodiff medium + CID



Osteodiff medium + CID

In vivo delivery



- Left flank: MSC only
- Right: MSC/iCasp9

50 µg CID q24h × 2 on day 0/+1

Use icasp9 for Additional Cell Types

- Post mitotic

  e.g. progeny of mesenchymal stromal cells
- Prevent neoplasia

  hESC

  iPS

Summary

- EBV-specific cytotoxic T lymphocytes (CTLs) can be modified to express CAR against solid tumors
- CAR-CTLs can survive long term and produce CR in neuroblastoma even in absence of lymphoablation - ?added benefit
- Extending approach beyond neuroblastoma
- Safety may be enhanced by fast acting suicide gene icasp9

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GLP Laboratory

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Rong Cai
Jijiu Tong

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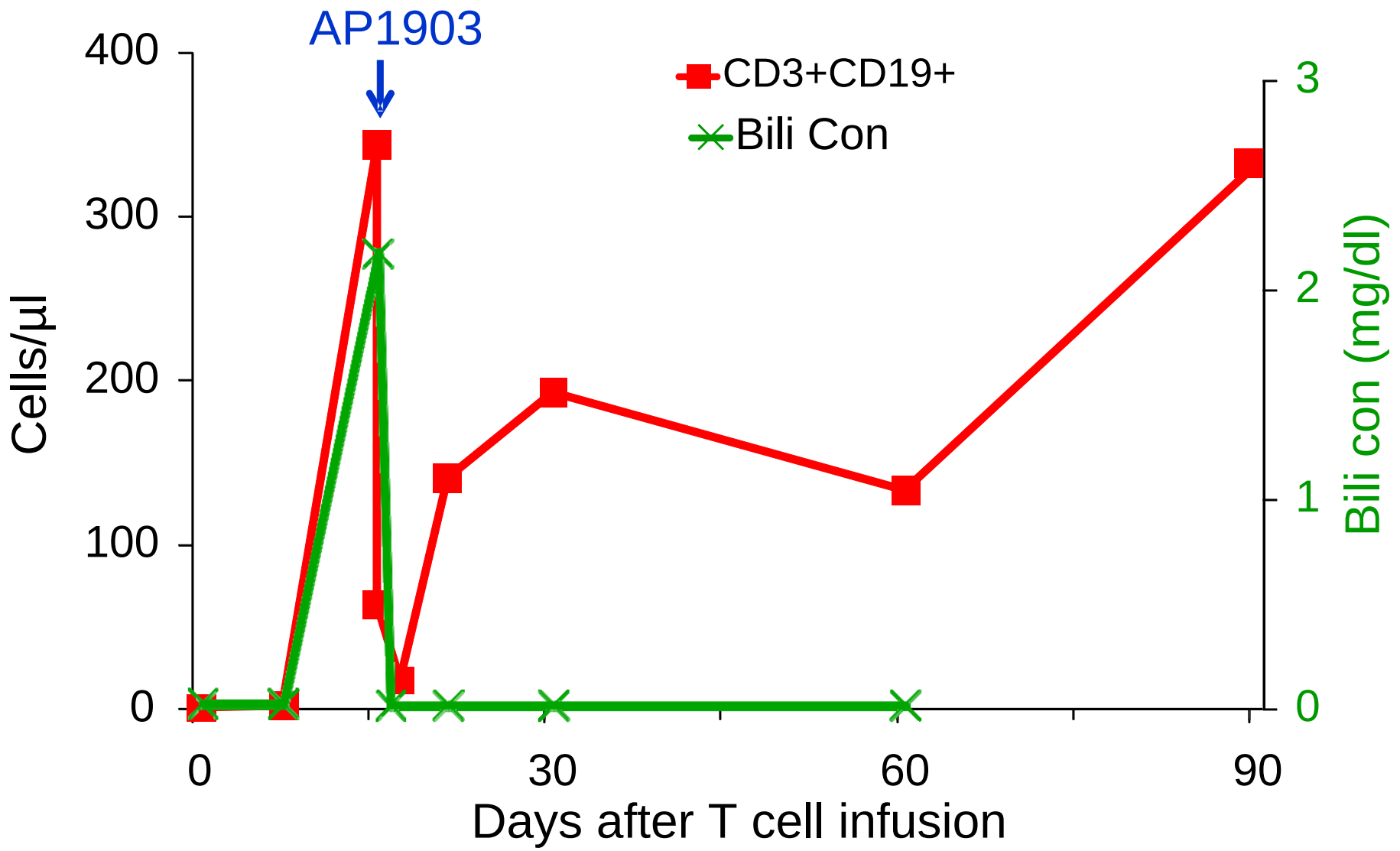
CASPALLO: Fate of residual iCasp9 T cells

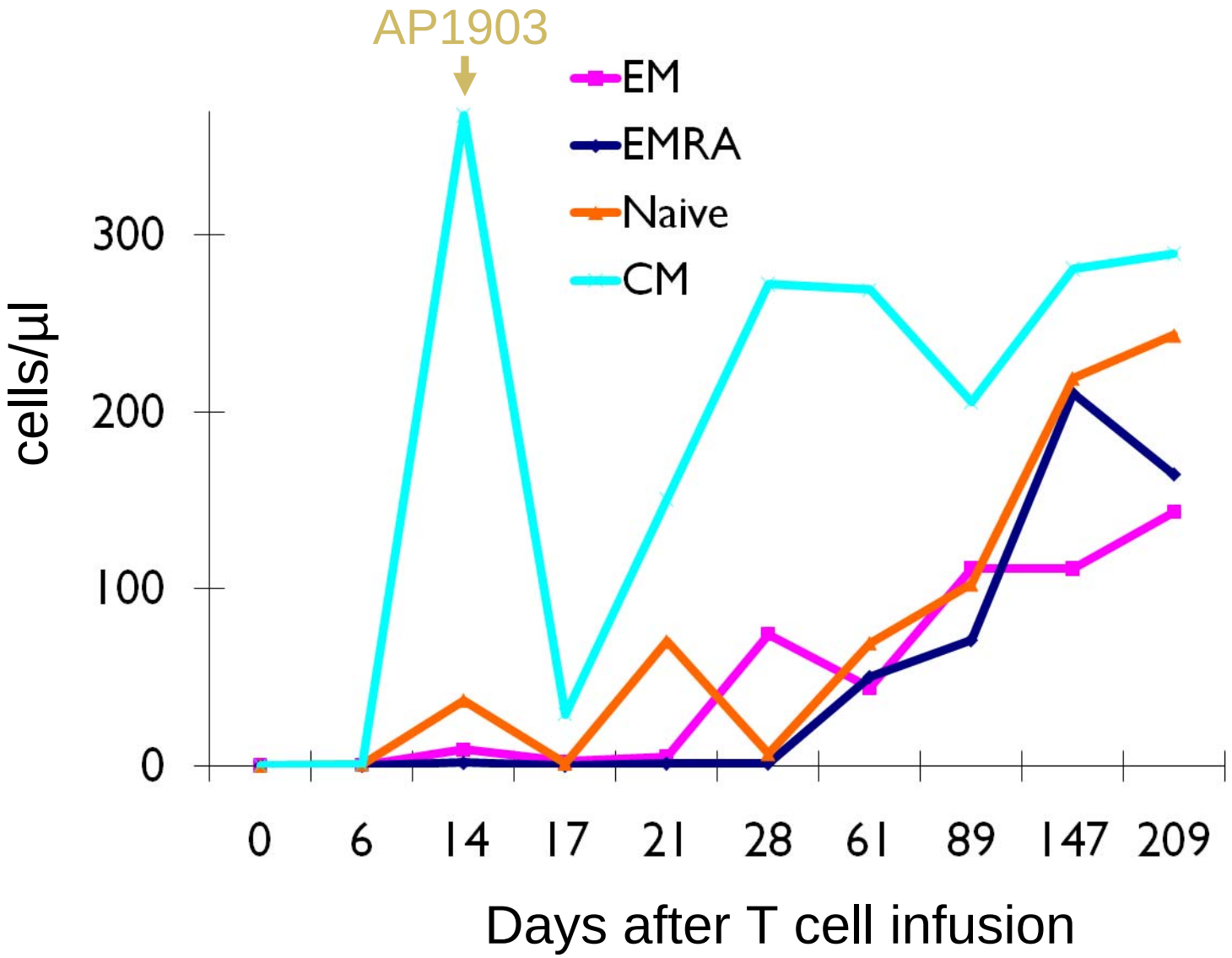
QUESTION #4:

- Do residual iCasp9 T cells re-expand without causing GvHD?

Residual iCasp9 T cells re-expand after AP1903 without GvHD (pt 1)



Naïve, CM, EM reconstitution after infusion (pt 1)

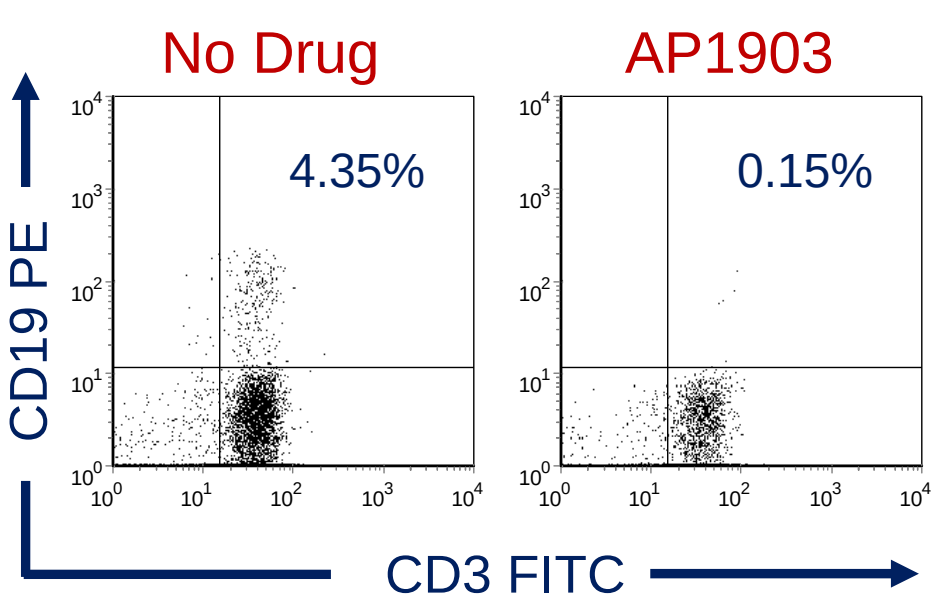


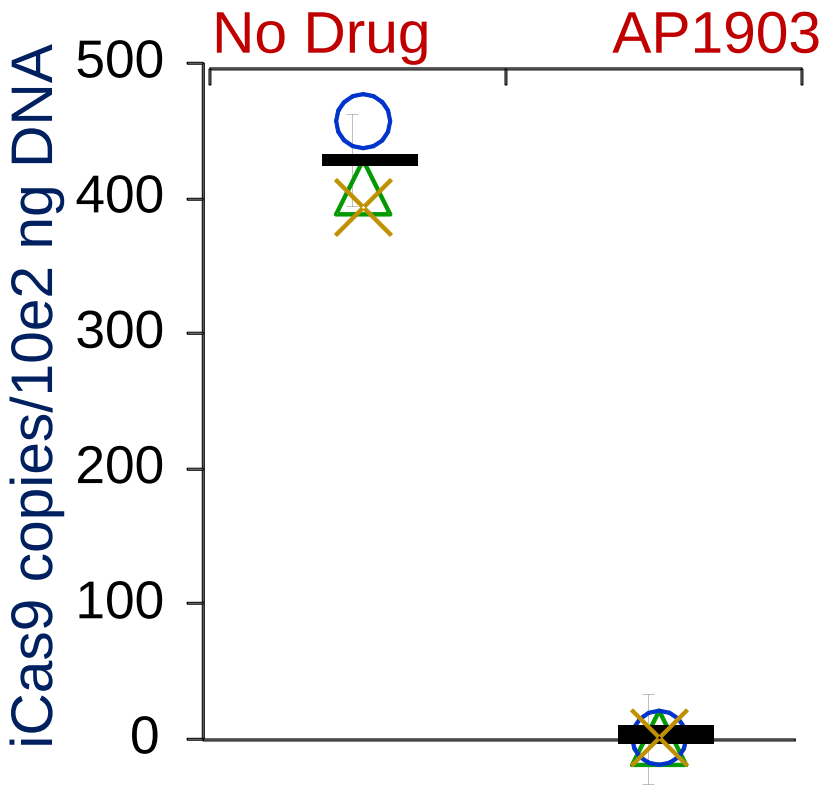
CASPALLO: Assessing continued responsiveness of infused T cells to AP1903

QUESTION #5:

- Do residual surviving iCasp9 T cells retain response to AP1903 long-term after infusion?

iCasp9 T cells remain sensitive to dimerizer >6 months after infusion



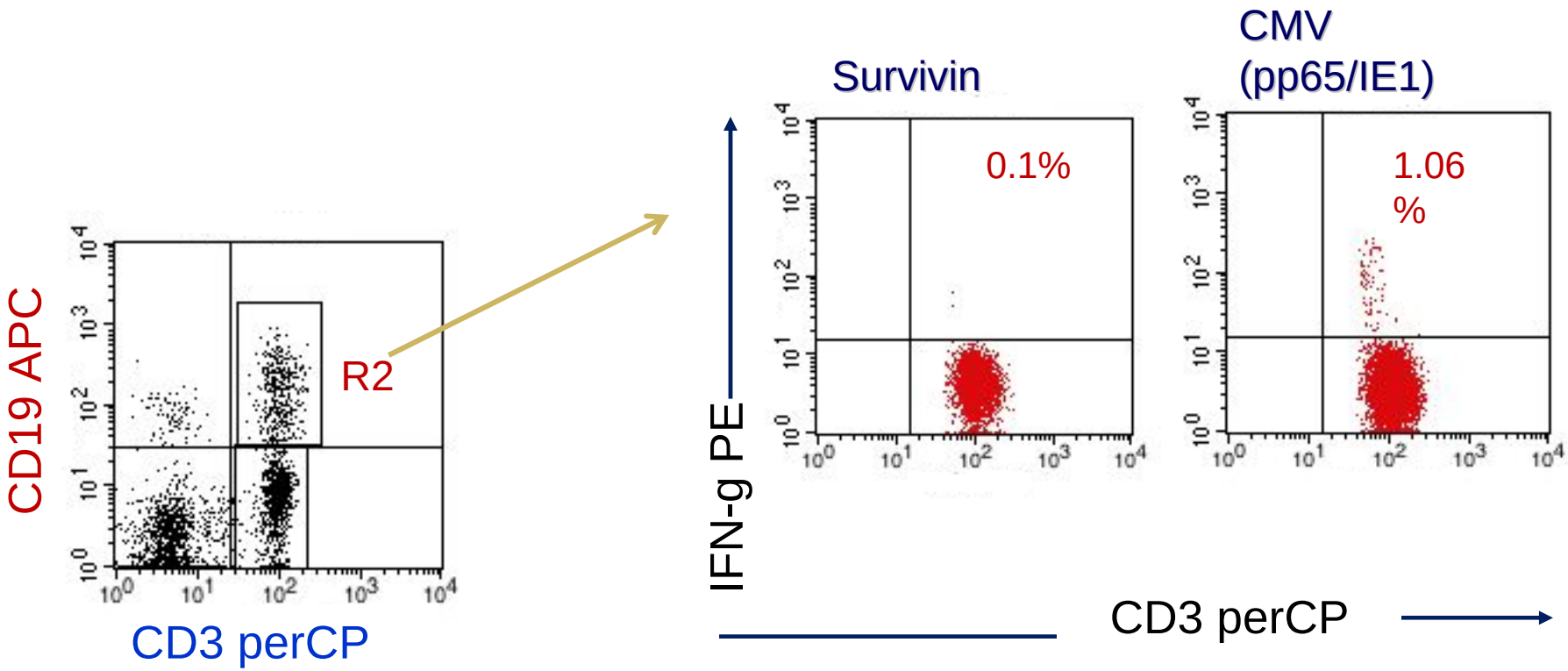


CASPALLO: Antiviral reconstitution

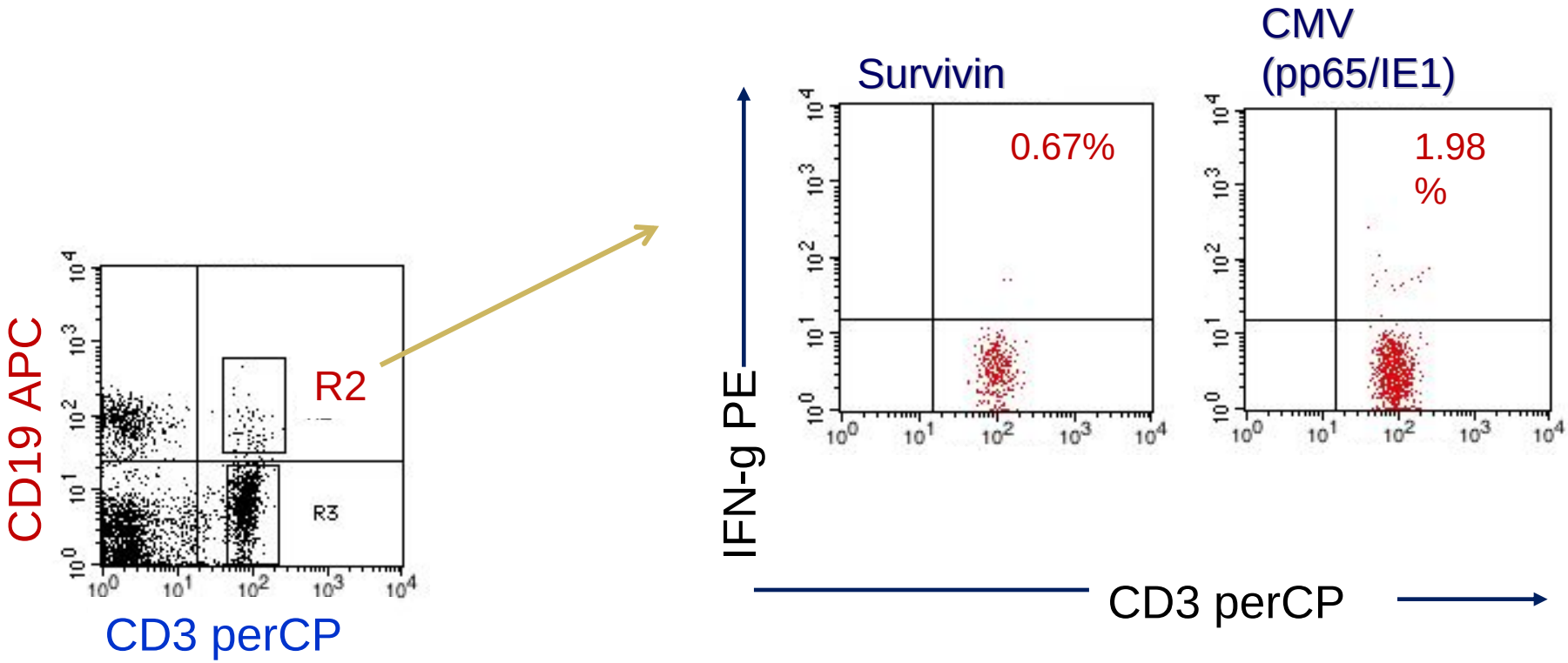
QUESTION #6:

- Do iCasp9 T cells contribute to reconstitution of antiviral immunity?

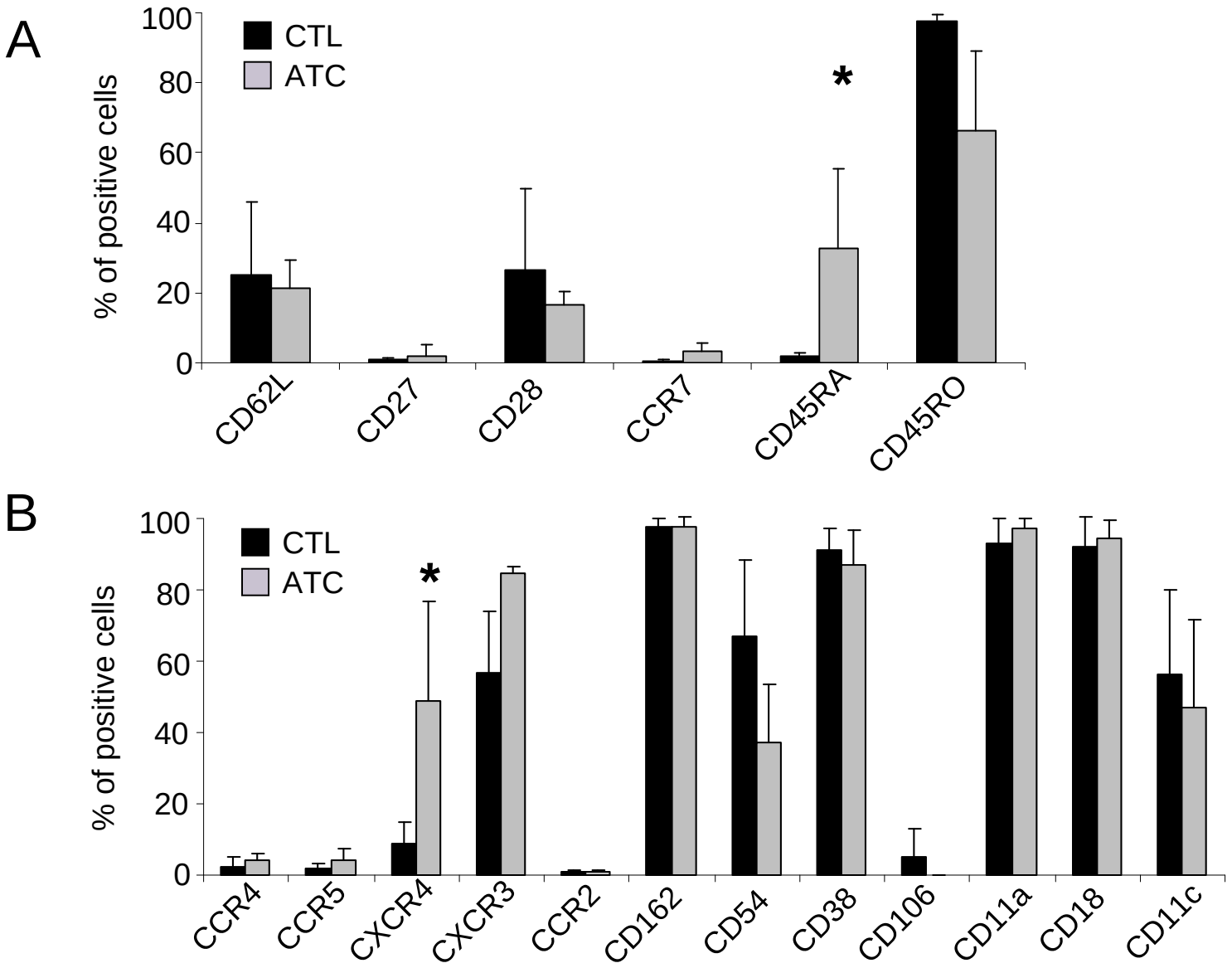
CMV specific response from patient #1 PBMC 6 days pre AP1903 administration



CMV specific CD3+CD19+T cells 7 days after AP1903



Phenotype of Transduced ATC and CTL



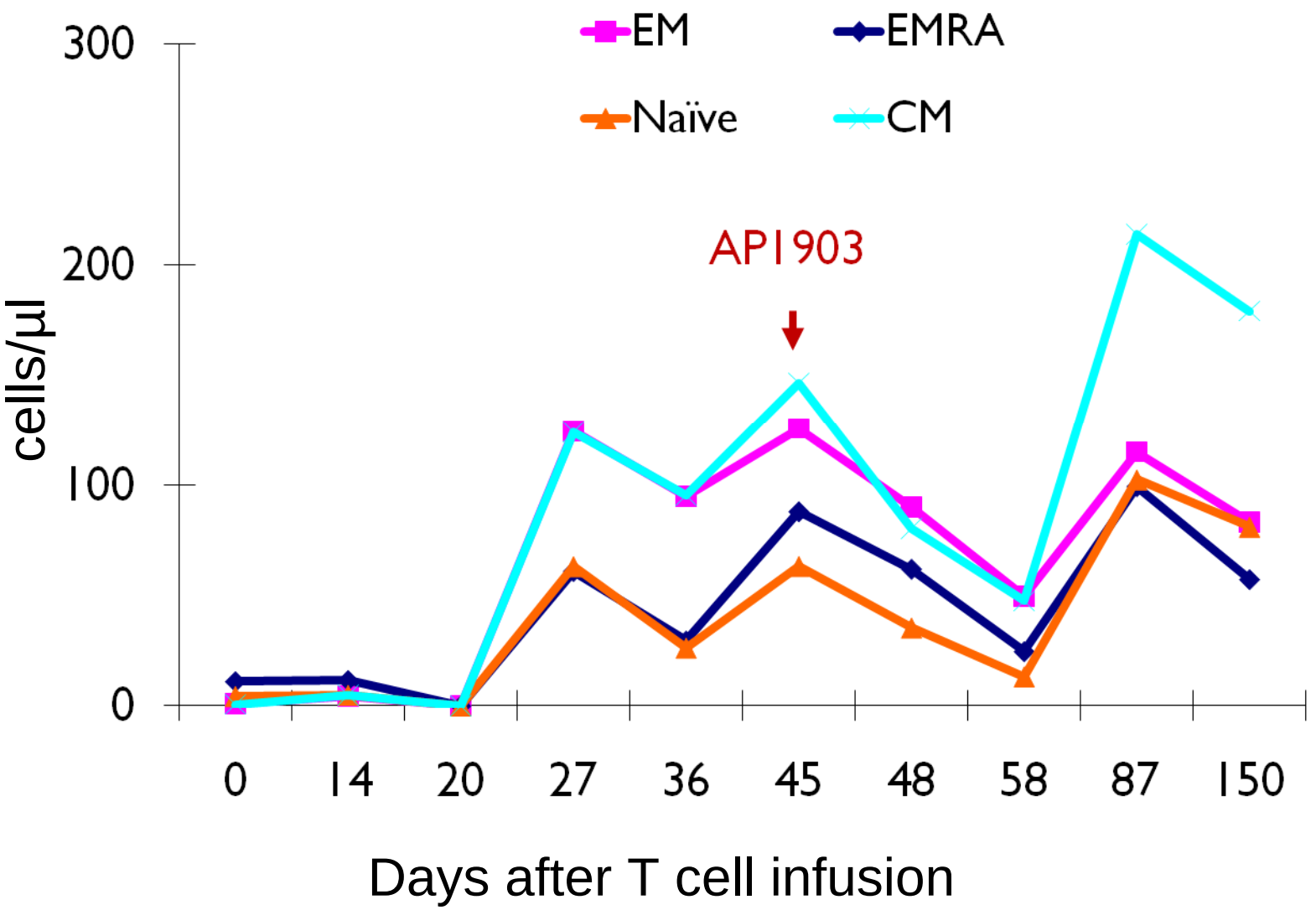
CASPALLO: patients on study

Pt (dose level)	SCT -last f/u (days)	Disease status at last f/u
1 (1)	219	CR
2 (1)	167	CR
3 (2)	170	CR

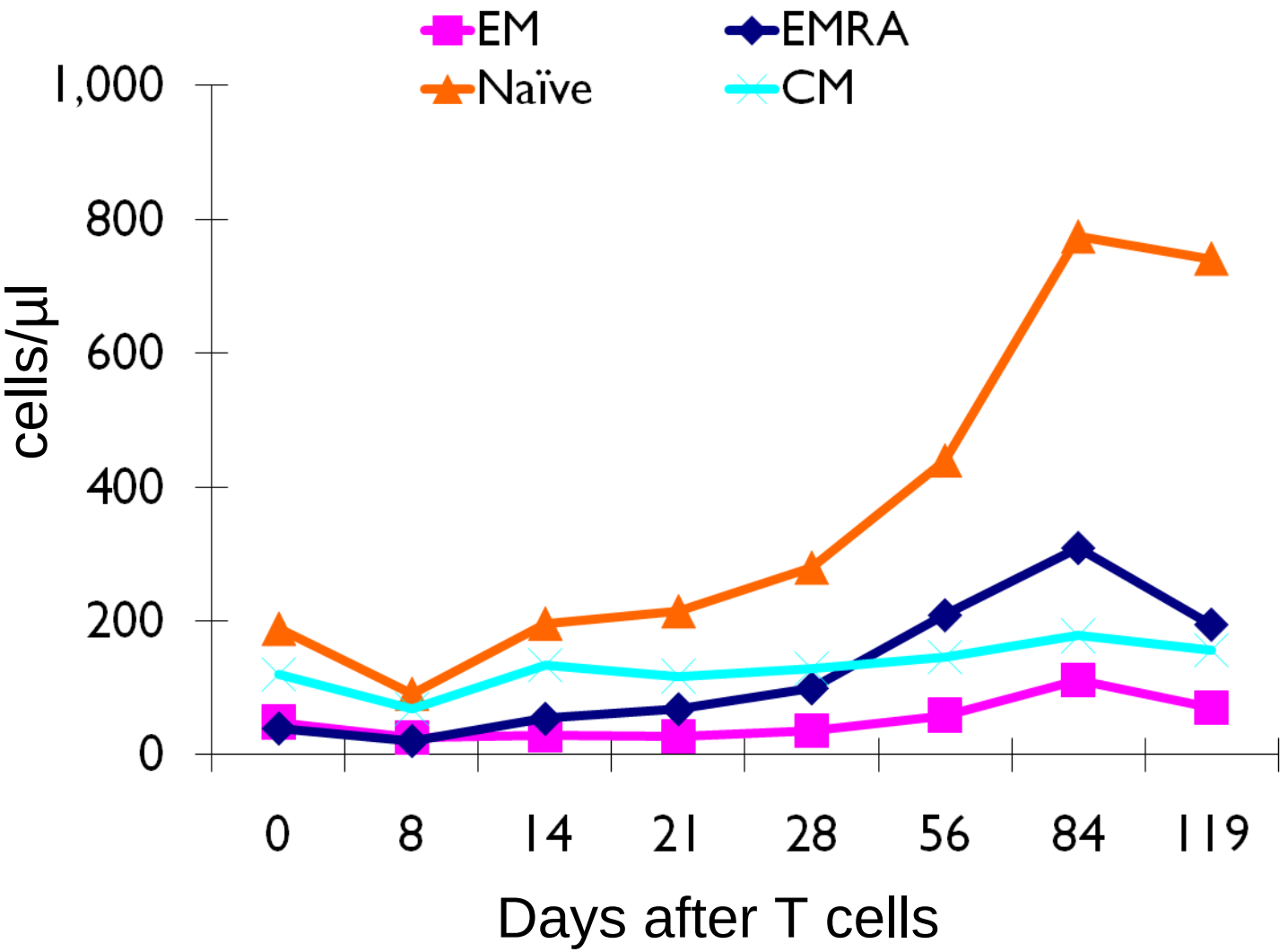
CASPALLO: patients on study

Pt (dose level)	Sex/ Age (Y)	Dx	Status at SCT	SCT-infusion (days)
1 (1)	M (3)	MDS/ AML	CR2	63
2 (1)	F (17)	B-ALL	CR2	80/111
3 (2)	M (8)	T-ALL	CR1 (PIF)	109

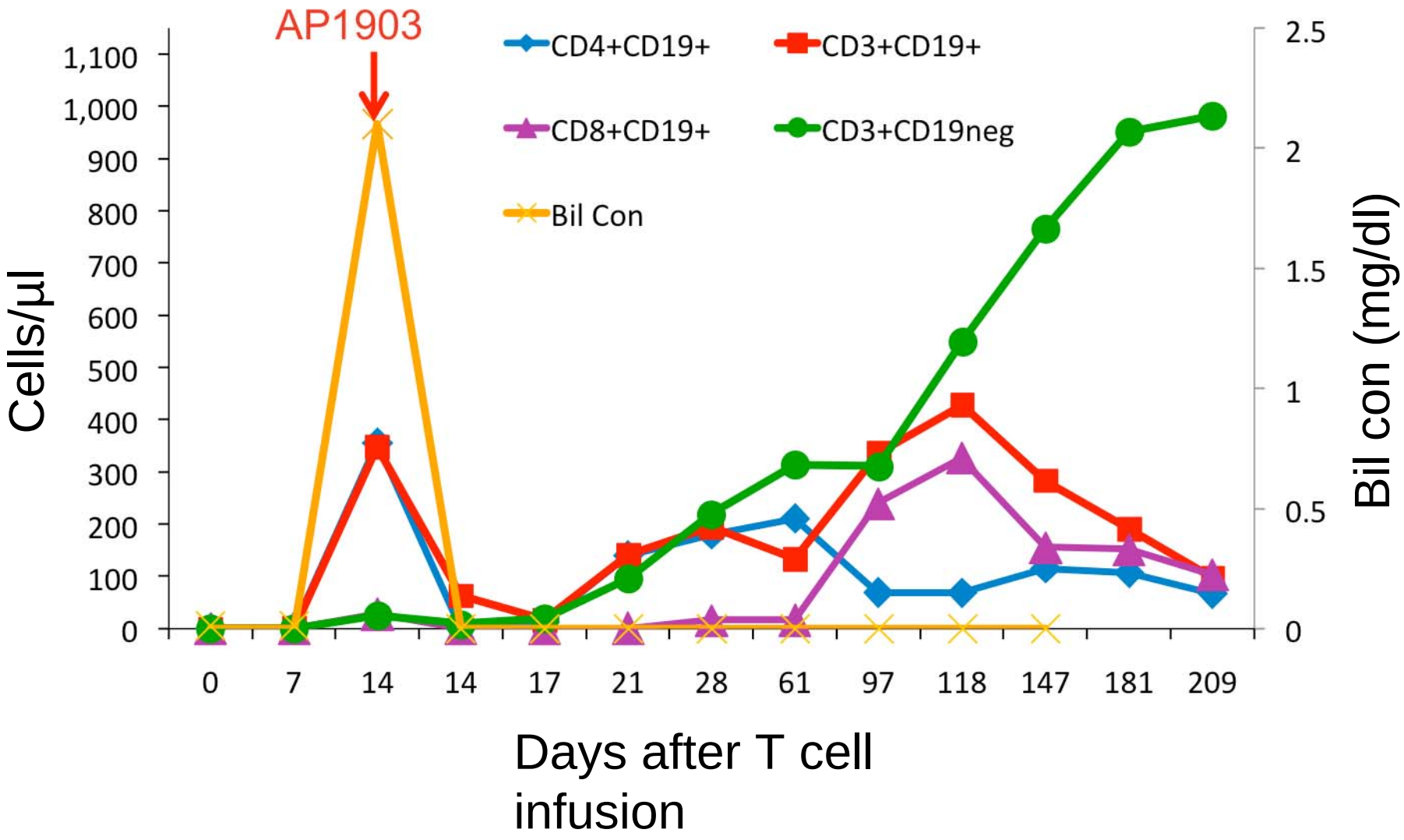
Naïve, CM, EM reconstitution after infusion (pt 2)



Naïve, CM, EM reconstitution after infusion (pt 3)



CASPALLO: Immune-reconstitution (pt 1)



CASPALLO: Immune-reconstitution(Pt 2)