



AMERICAN SOCIETY OF GENE & CELL THERAPY
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Presenter Disclosure Gianpietro Dotti

The following relationships exist related to this presentation:

No Relationships to Disclose

Chimeric Antigen Receptor (CAR)- Based T Cell Therapy

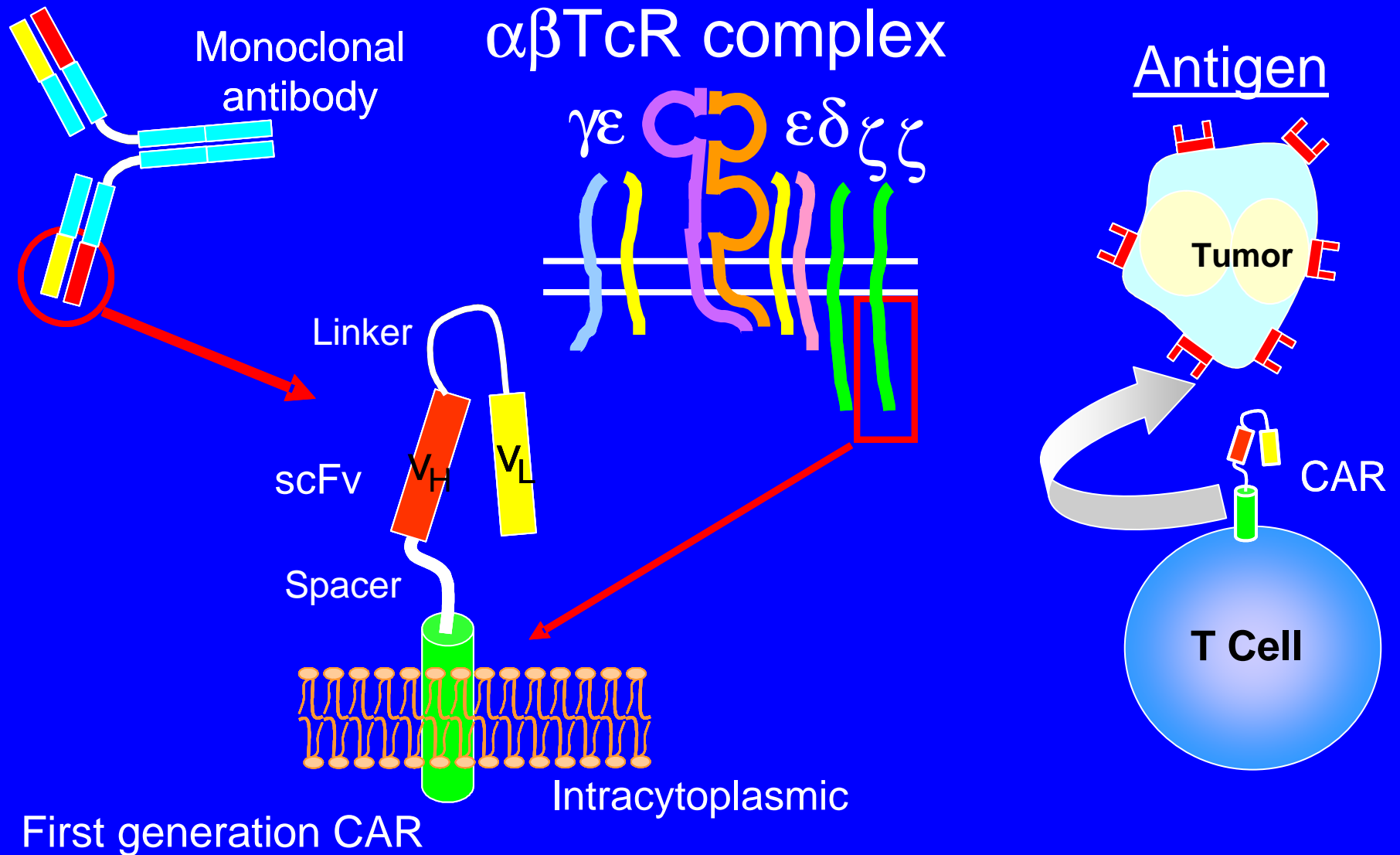
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Baylor College of Medicine
Houston, TX



Redirect T-cell antigen specificity

- Transgenic expression of $\alpha\beta$ T-cell receptors
- Transgenic expression of Chimeric Antigen Receptors (CARs)

Gene Transfer of CARs



First generation CAR

Advantages of CAR-modified T cells

- Virtually every surface antigen can be targeted
- Non-processed molecules (including carbohydrates and glycolipids) can be targeted
- Receptor specificity easily generated
- No MHC restriction

Limitations of CAR-modified T cells

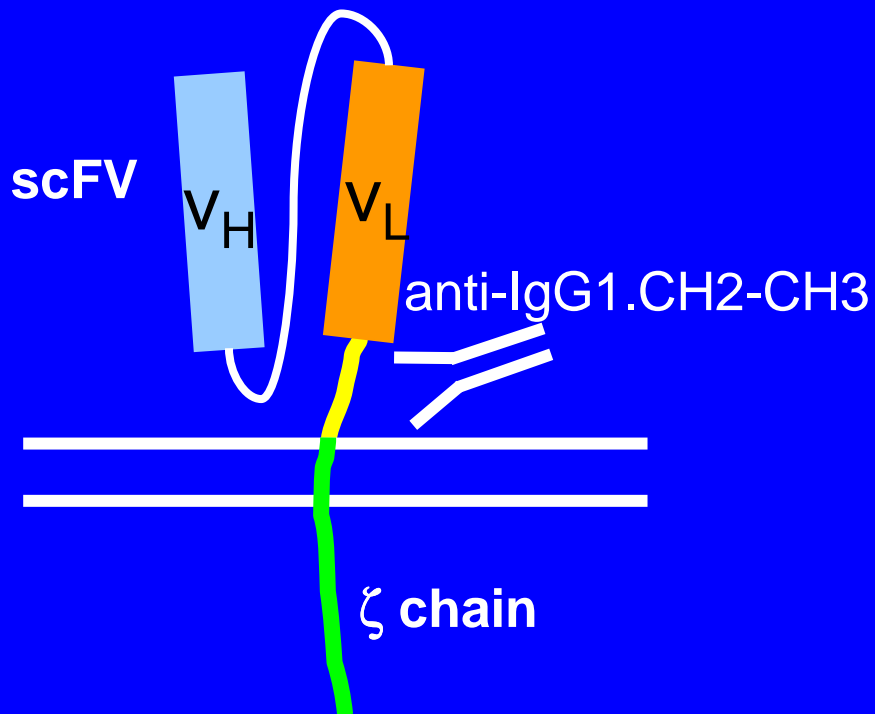
- Surface expression of the target antigen
- Potential T cell interference if the antigen is shaded
- Immunogenicity?

Chimeric Antigen Receptors

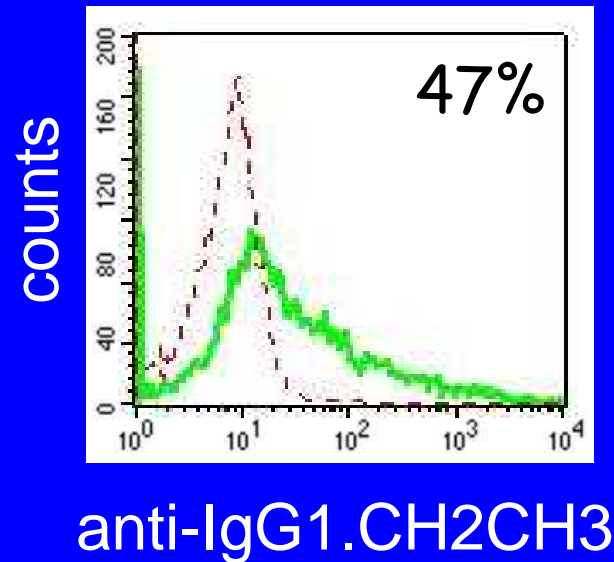
ScFv specificity	Tumor target
PMSA	Prostate cancer
HER-2	Breast, lung, brain cancers
Folate-binding protein (FBP)	Ovarian cancer
CD30	HD Lymphoma
CD19/CD20	B cell lymphoid tumors
Light chains of Igs	B cell lymphoid tumors
GD2	Neuroblastoma Melanoma
CEA	Colon rectal carcinoma

Expression of CARs by activated T lymphocytes

1th generation CAR

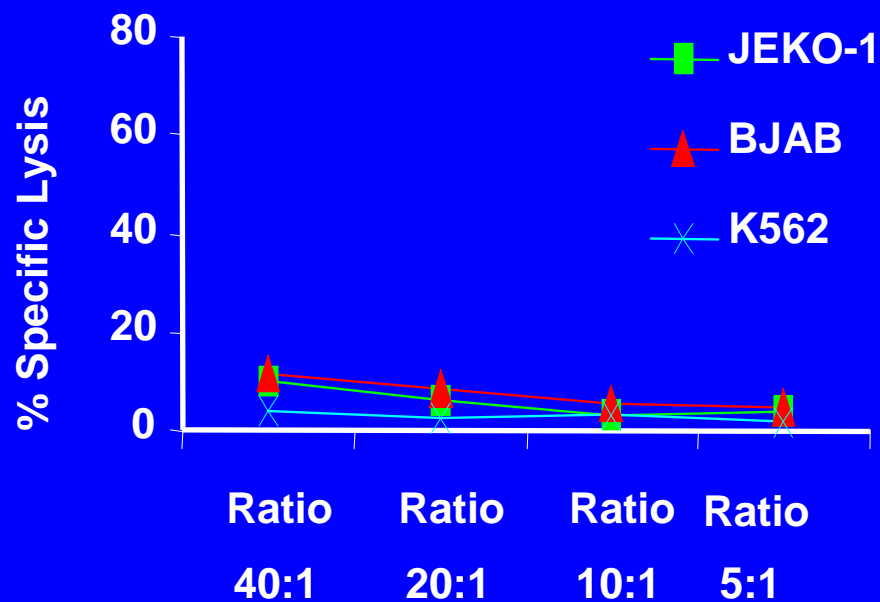


First generation CAR



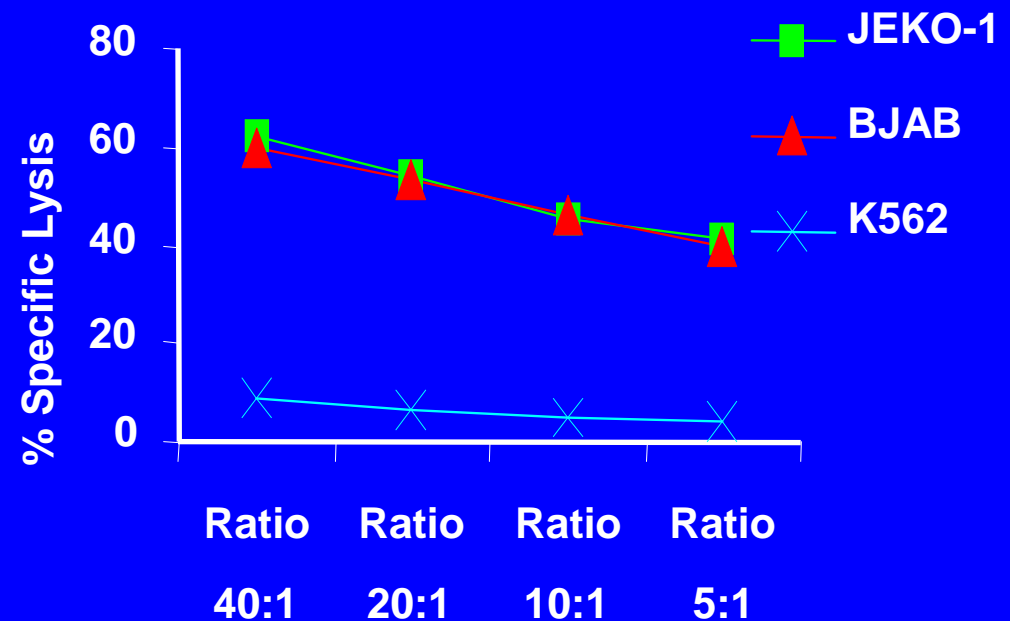
Cytotoxicity of CD19 CAR⁺ T cells

Control T Cells



Effector : Target Ratio

CAR.CD19⁺ T cells



Effector : Target Ratio

JEKO-1 AND BJAB are CD19⁺ tumor cells

Clinical trials with CAR⁺ T cells

Antigen	# of trials	Tumor
CD19	8	Hematological malignancies
CD20	2	Hematological malignancies
Kappa light chain	1	Hematological malignancies
GD2	1	Neuroblastoma
CEA	4	Adenocarcinoma
PSMA	1	Prostate cancer
CD171	1	Neuroblastoma
FR	1	Ovarian cancer
IL-13R α 2	1	Glioblastoma
HER2/neu	3	Osteosarcoma, lung cancer, other HER ⁺ tumors

What can we learn from these trials?

- Does the CAR expression vector affect the outcome?
- What co-stimulation is ideal for T cells?
- Is host lymphodepletion necessary?
- Which T cell subset should be used?
- What are the toxicities?

Vectors for T cell CAR expression

	# of trials
• Retroviral vectors	17
• Lentiviral vectors	1
• Nonviral vector gene transfer	
- plasmids	5
- transposone/transposase	
Sleeping Beauty	1#

pending approval

Vectors for T cell CAR expression

Vector	Speed of T cell manufacture	Vector cost
Retro/Lenti	Short	high
Plasmids	Long	low
Sleeping Beauty	Intermediate	low

Generation of CAR-modified Activated T Lymphocytes (ATLs)

Step 2: CAR-gene transfer
Step 2: CAR-gene transfer
(transposon insertion)



Step 1:

**Activation with
OKT3 and IL-2**



**Step 3: Expansion with
IL-2 (± aAPC?)**
**Step 3: Expansion with
IL-2 (± aAPC?)**

Step 4:
Sterility
Phenotype
Cytotoxicity

← 2-5 weeks →

What can we learn from these trials?

- Does the CAR expression vector affect the outcome?
- **What co-stimulation is ideal for T cells?**
- Is host lymphodepletion necessary?
- Which T cell subset should be used?
- What are the toxicities?

Adoptive transfer T cells expressing “first generation” CARs

- Approach feasible
- Lack of significant anti-tumor effects
- Limited persistence of CAR-modified T cells

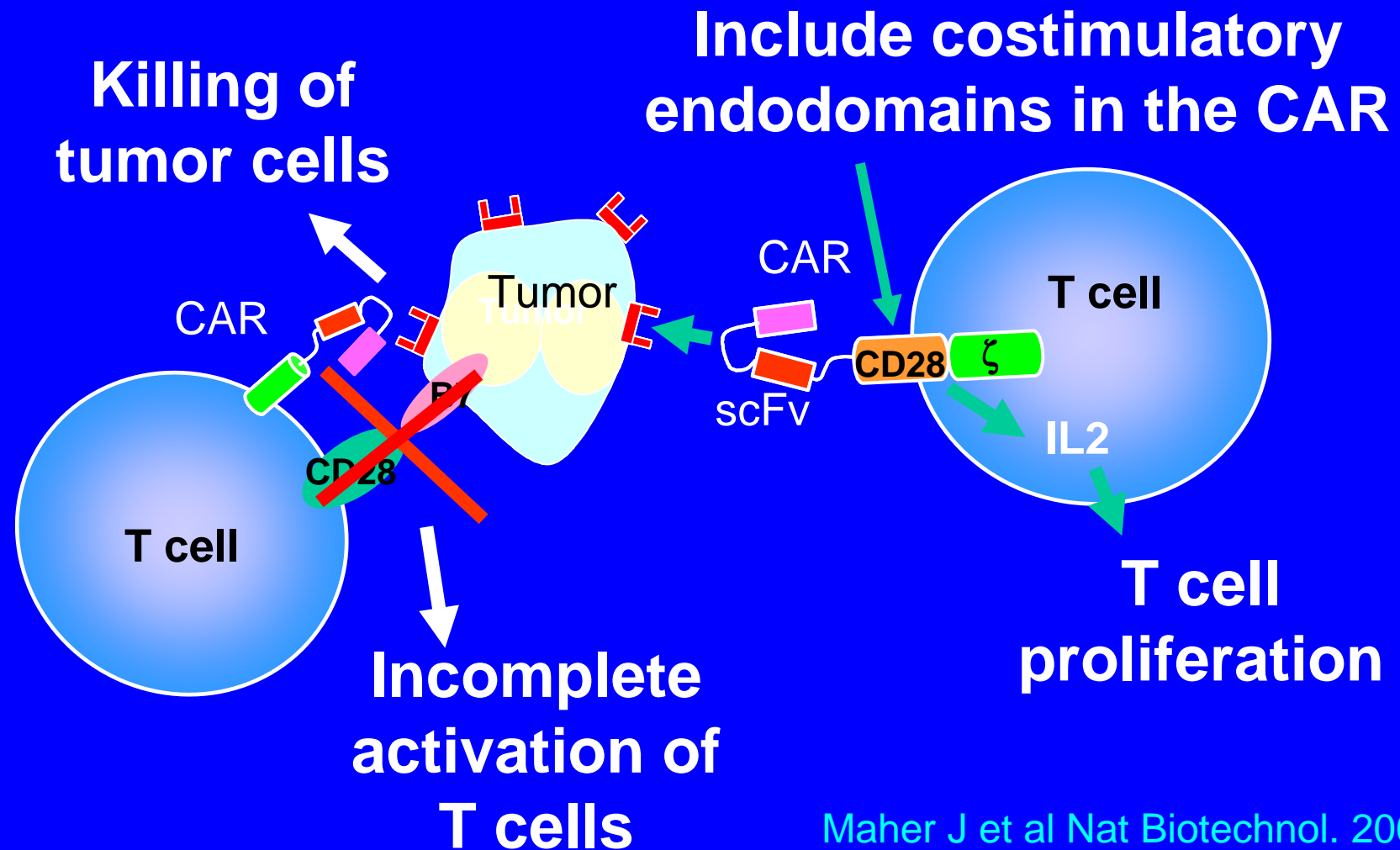
**Incomplete activation after
engagement of the CAR?**

Till et al Blood. 2008;112:2261-71

Park et al. Mol Ther. 2007;15:825-33

Kershaw et al Clin Cancer Res. 2006;12:6106-15

“Second generation” CAR to improve T cells activation



Incorporation of co-stimulatory endodomains in CARs

1th generation CAR

scFv-FcεRI



scFvζ



2th generation CAR

scFv-CD28ζ



scFv-Zap70



scFv-Syk



scFv-4-1BBζ



3th generation CAR

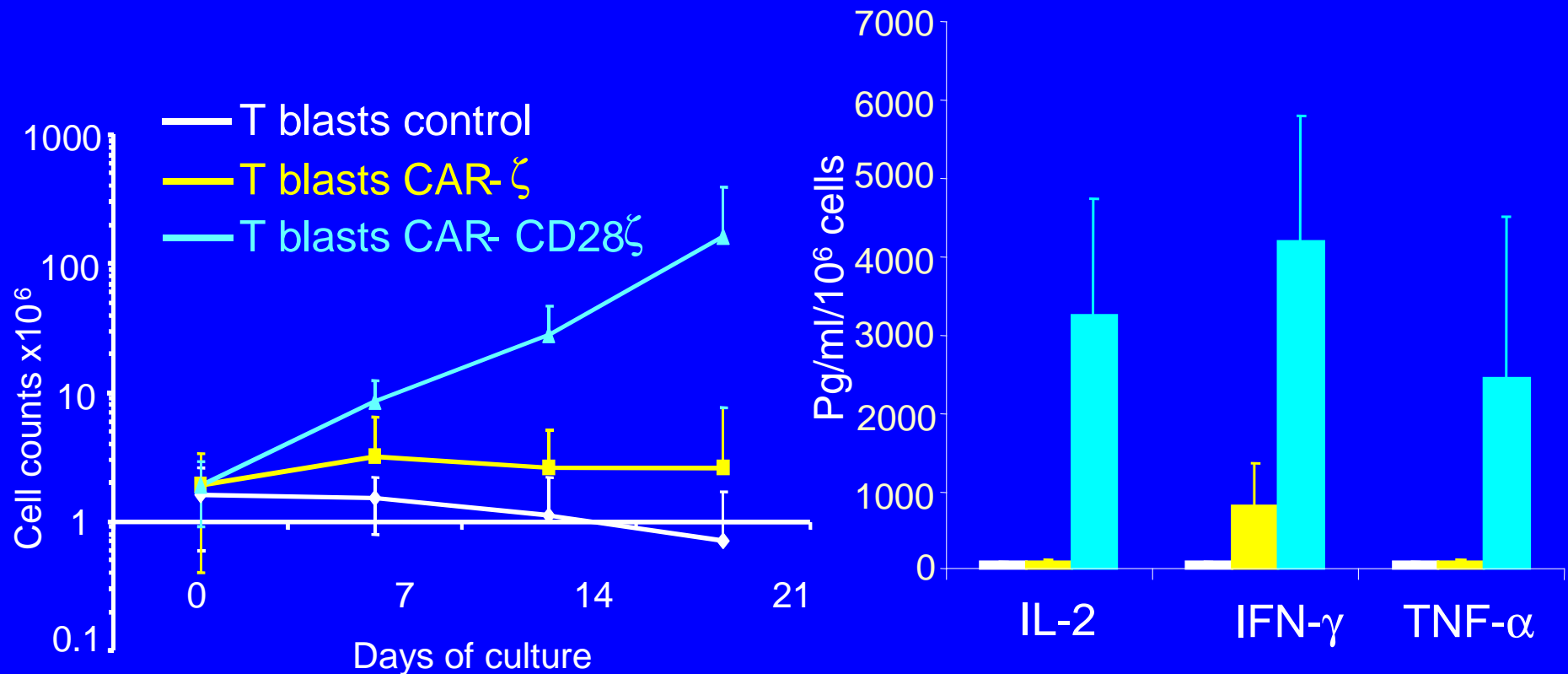
scFv-CD28-OX40ζ



scFv-CD28-4-1BBζ



CD28 induces T-cell expansion after antigen stimulation



Clinical trials with CAR⁺ T cells

Antigen	Trial	Costim. Endodomain
CD19	NCT00586391	1 th vs. 2 nd gen. CAR with CD28
CD19	NCT01029366	1 th vs. 2 nd gen. CAR with 4-1BB
CD19	NCT00466531	2 nd gen. CAR with CD28
CD20	NCT00621452	3 rd gen CAR with CD28 and 4-1BB
CEA	NCT00673829	2 nd gen. CAR with CD28
CEA	NCT00673322	2 nd gen. CAR with CD28
HER2/neu	NCT00924287	3 rd gen CAR with CD28 and 4-1BB
HER2/neu	NCT00924287	2 nd gen CAR with CD28

CD19 Chimeric Antigen Receptor expressing T lymphocytes in B-cell NHL and CLL (CRETI-NH)

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

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Study 1 of 1 for search of: **NCT00586391**
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CD19 Chimeric Receptor Expressing T Lymphocytes In B-Cell Non Hodgkin's Lymphoma And Chronic Lymphocytic Leukemia (CRETI-NH)

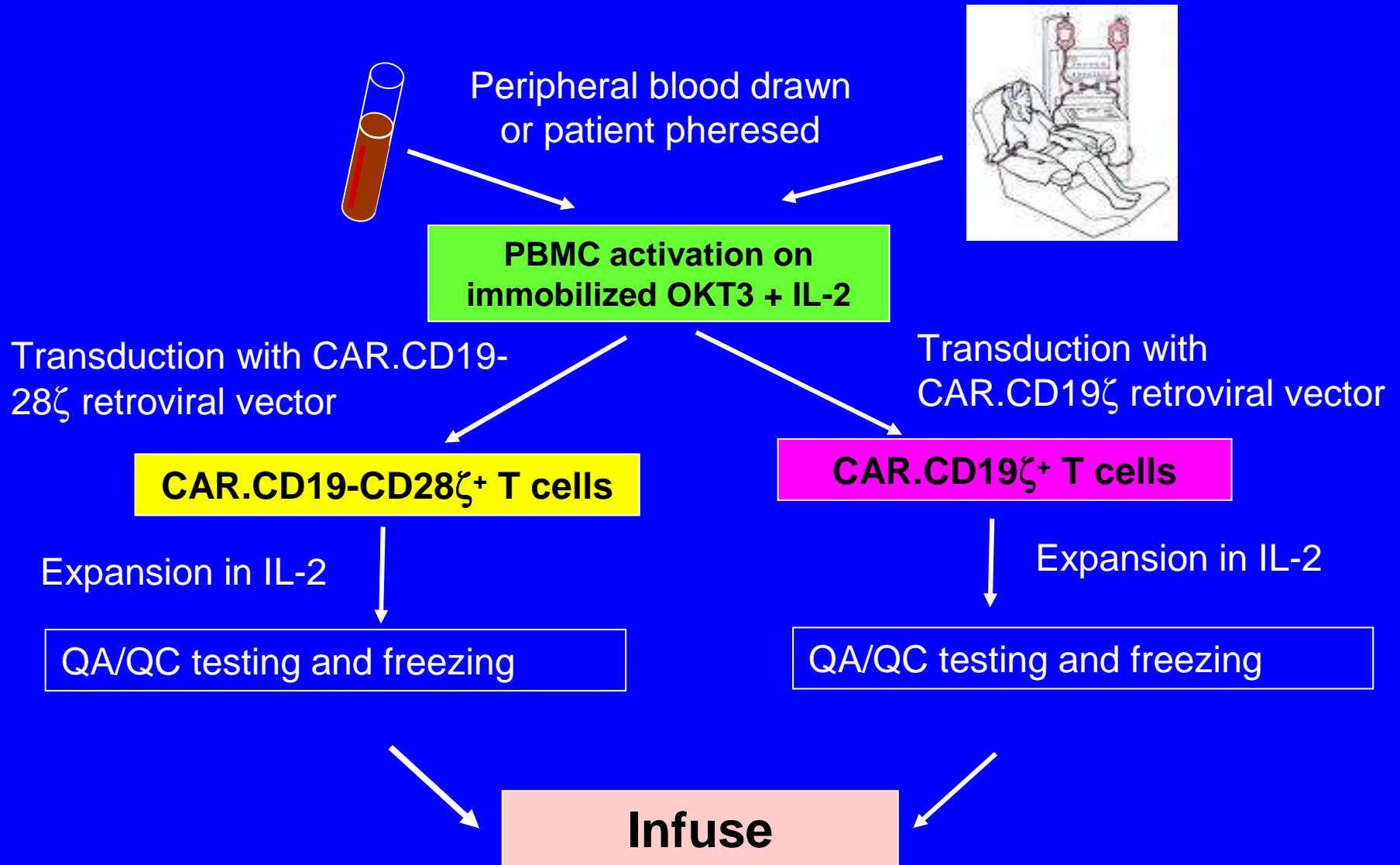
This study is currently recruiting participants.
Verified by Baylor College of Medicine, March 2010

First Received: December 21, 2007 Last Updated: April 21, 2010 [History of Changes](#)

Sponsor:	Baylor College of Medicine
Information provided by:	Baylor College of Medicine
ClinicalTrials.gov Identifier:	NCT00586391

Session Info: Simultaneous Oral Abstract Sessions: Cancer-Immunotherapy T Cell Studies (2:00 PM-4:00 PM)

Generation of CAR⁺ T cells



CRETI-NH: NCT00586391

-Eligibility criteria

- Refractory/recurrent B-CLL or B-NHL, or newly diagnosed unable to receive/complete standard therapy
- Life expectancy \geq 12 wks

-Outcome Measures

- Safety
- Survival and function of CAR T cells in vivo
Compare 1st vs 2nd generation CAR
- Anti-tumor activity

Treatment plan

- Three dose levels
- Single dose
- Modified continual reassessment method

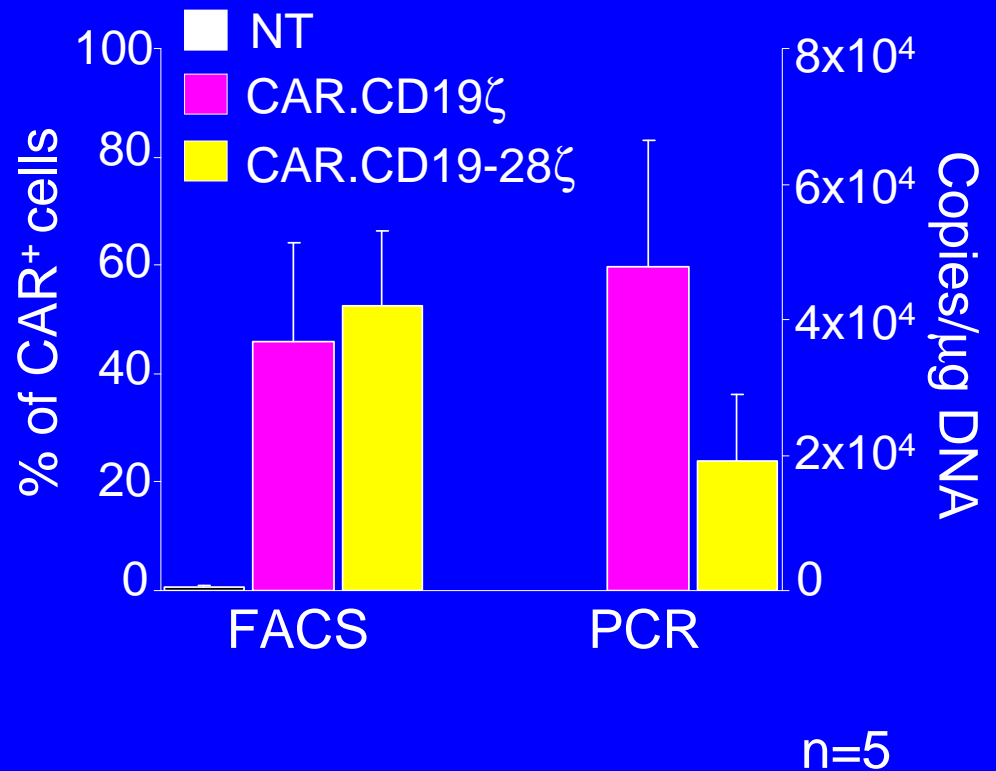
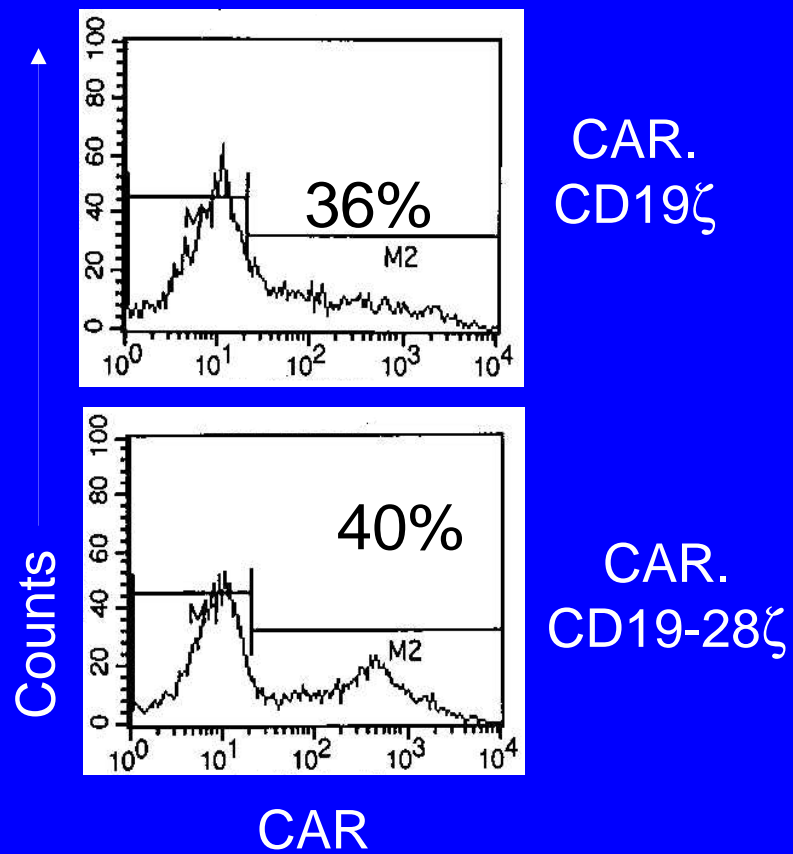
Dose level 1: 2×10^7 cells/m² of each product

Dose level 2: 1×10^8 cells/m² of each product

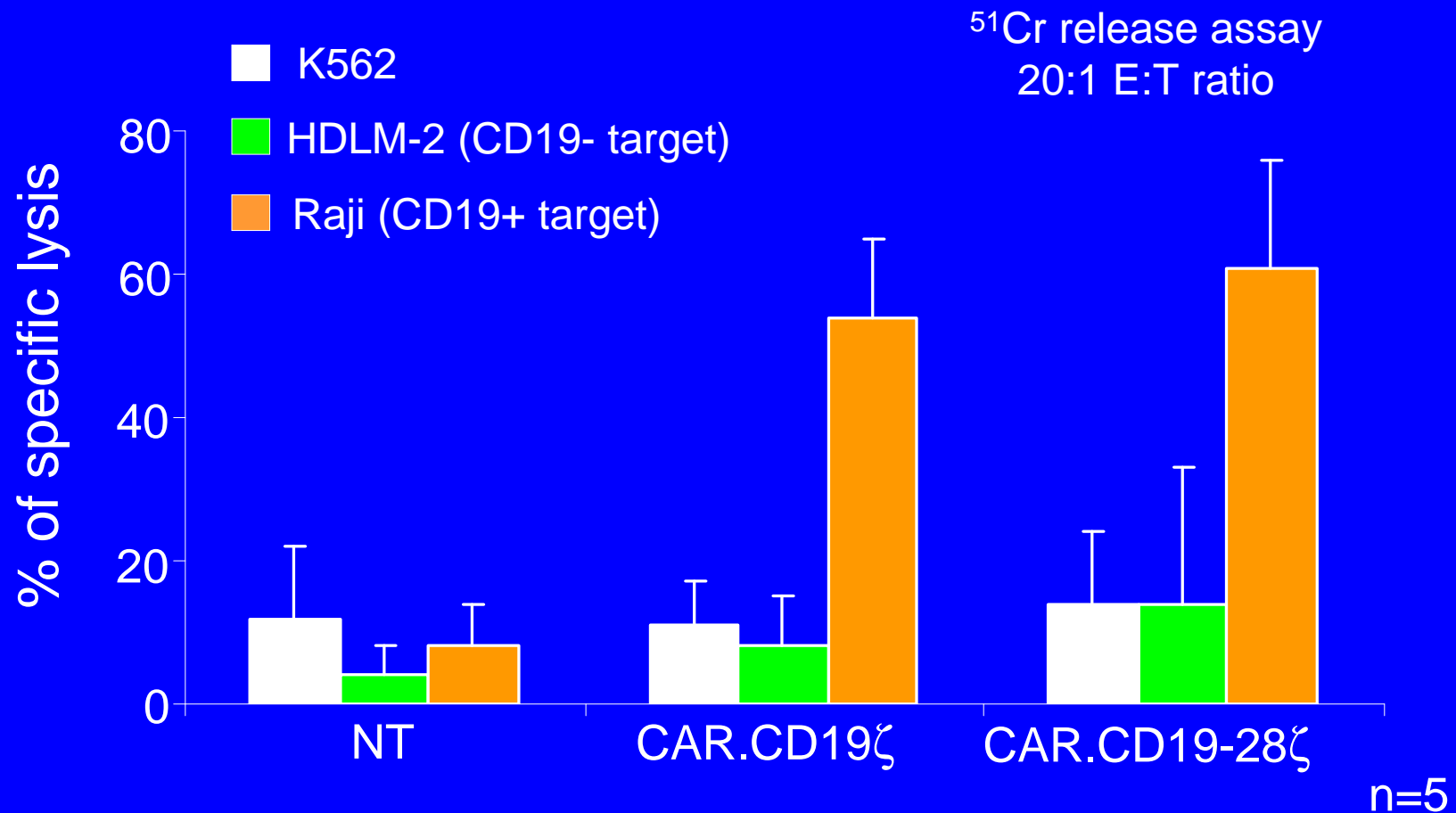
Dose level 3: 2×10^8 cells/m² of each product

- Second infusion if stable disease/PR

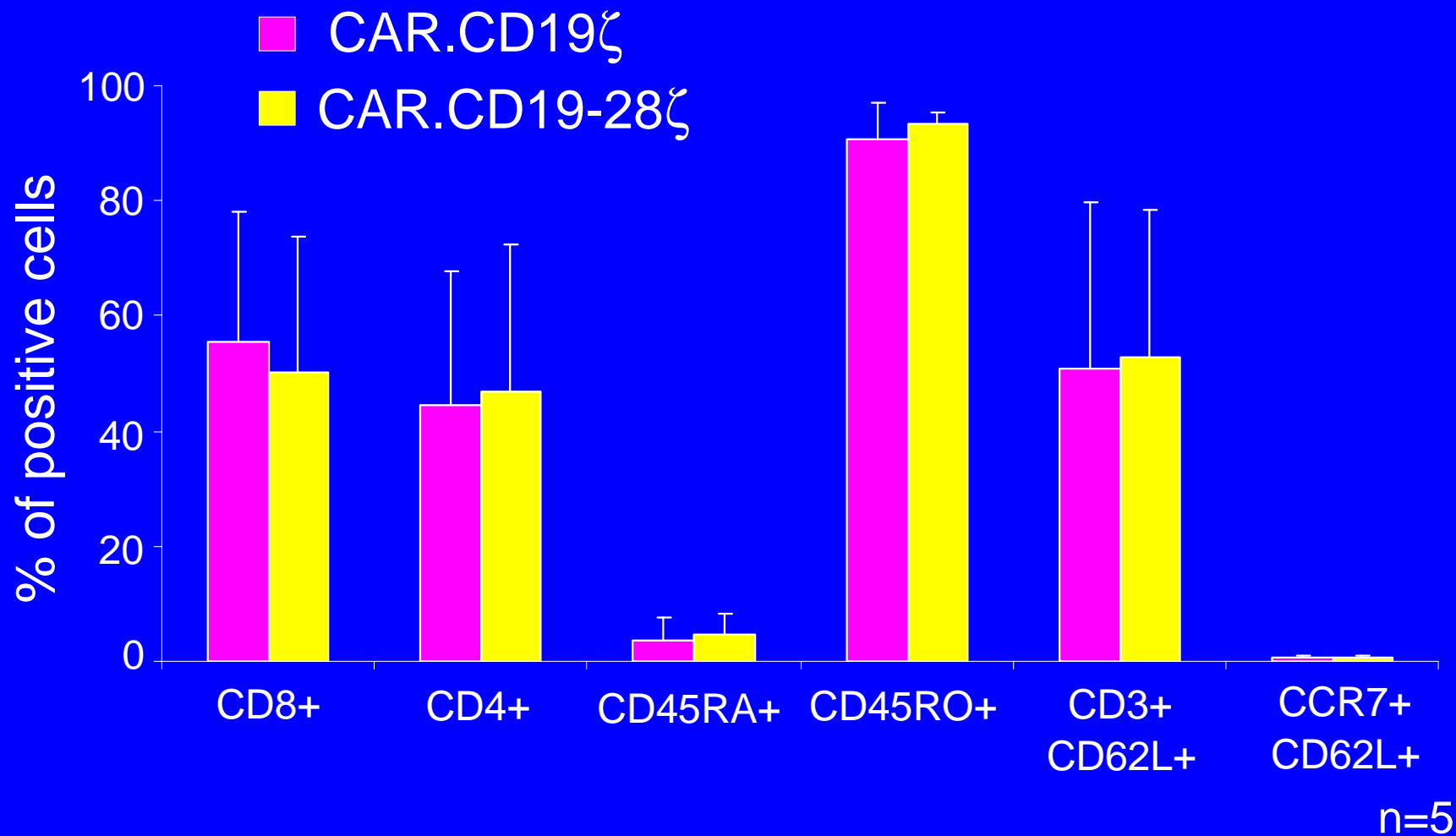
Comparable transduction of CAR.CD19 ζ^+ and CAR.CD19-28 ζ^+



Equal *ex vivo* killing by CAR.CD19 ζ^+ and CAR.CD19-28 ζ^+ T cells



Identical Phenotype of CAR.CD19 ζ^+ and CAR.CD19-28 ζ^+ T cells



Objective #1

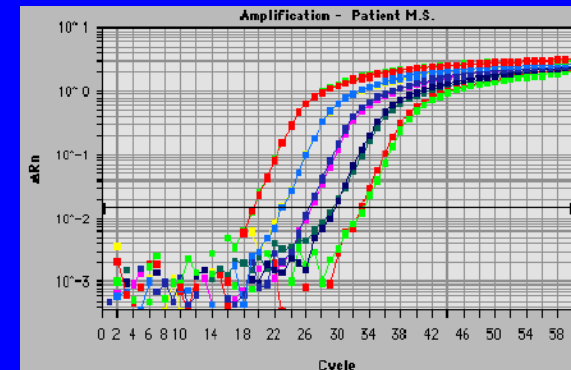
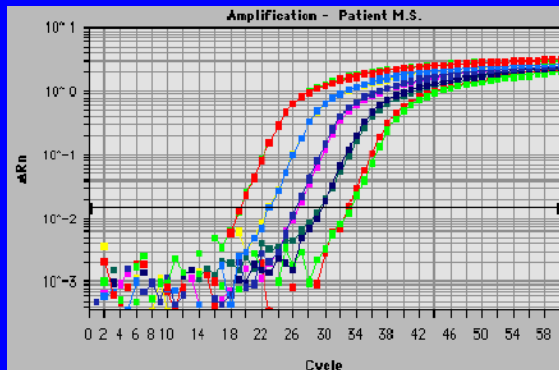
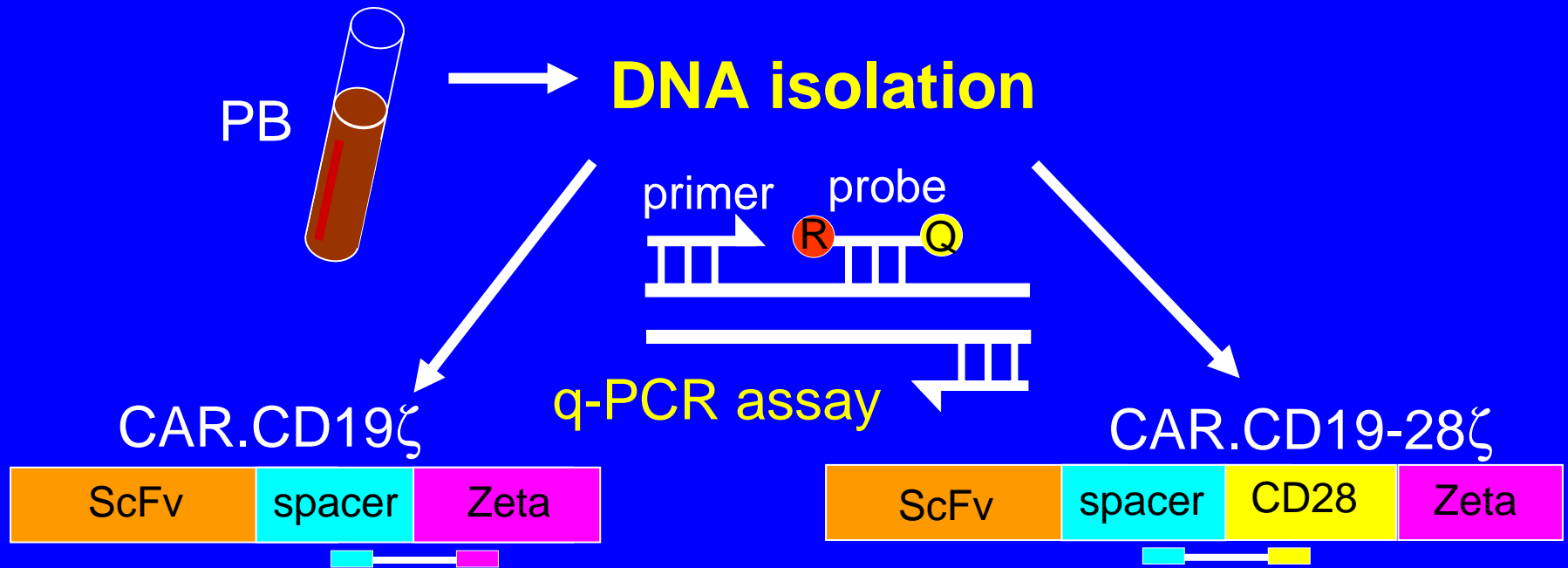
Is transfer of CAR.CD19 ζ^+ and CAR.CD19 - 28 ζ^+ T cells safe?

So far YES -> no acute or long-term toxicities at 1st and 2nd dose levels

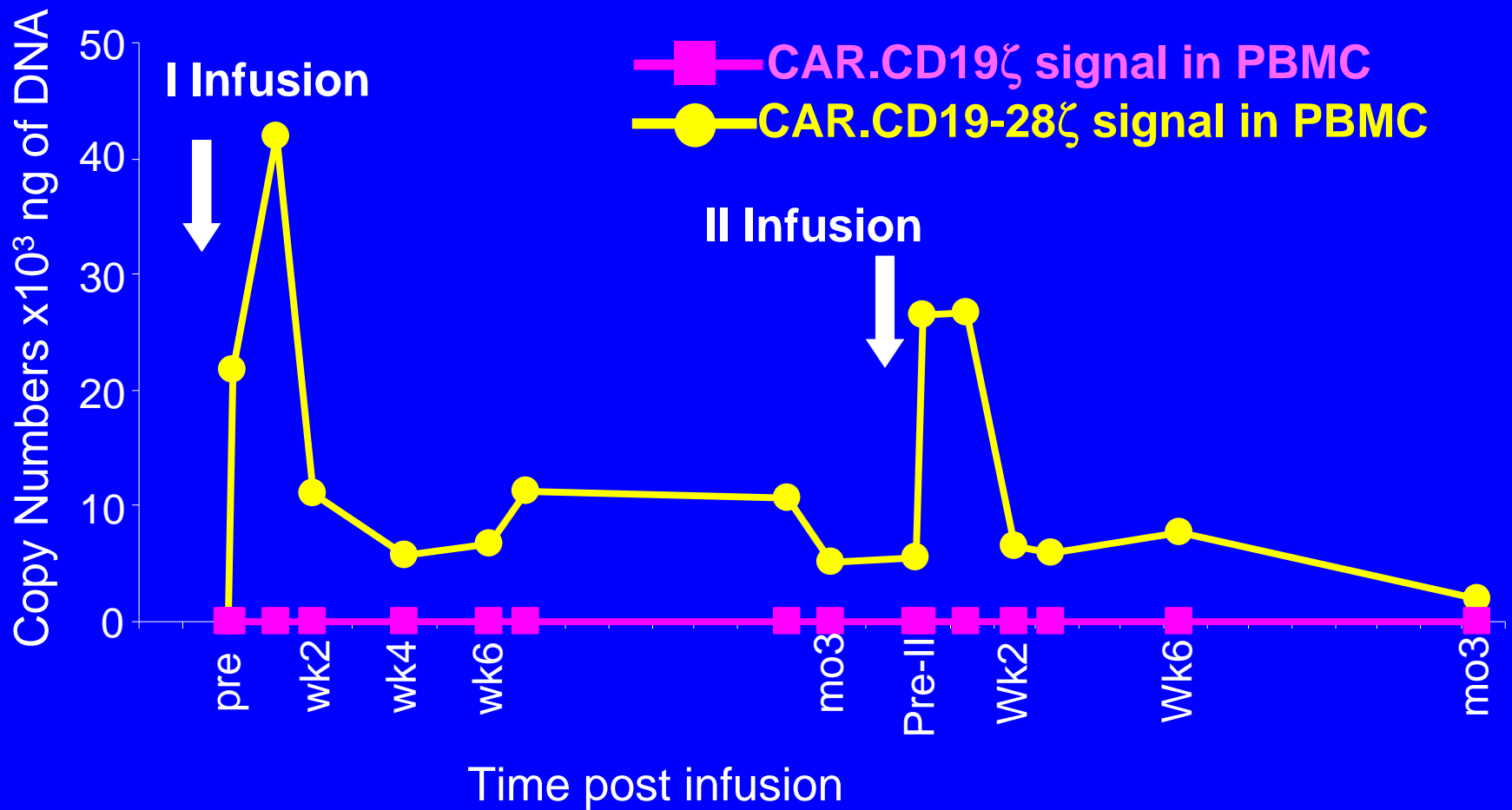
Other objectives

- Do CAR.CD19-28 ζ ⁺ T cells expand and persist better than CAR.CD19 ζ ⁺ T cells?
- Are there anti-tumor effects?

Monitoring expansion and persistence

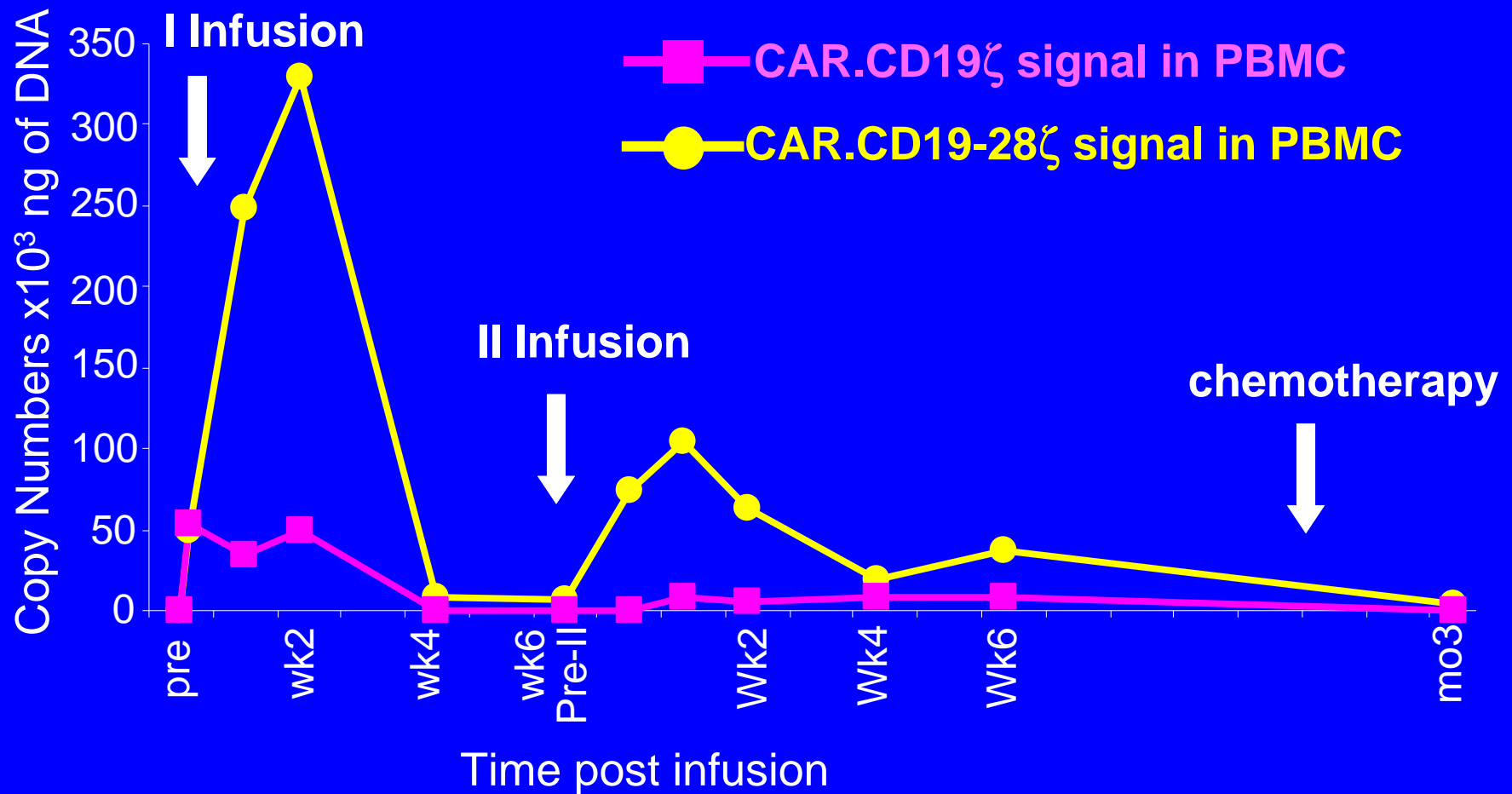


CAR.CD19-28 ζ ⁺ T cells have greater *in vivo* expansion and persistence



Pt #1, dose level 1

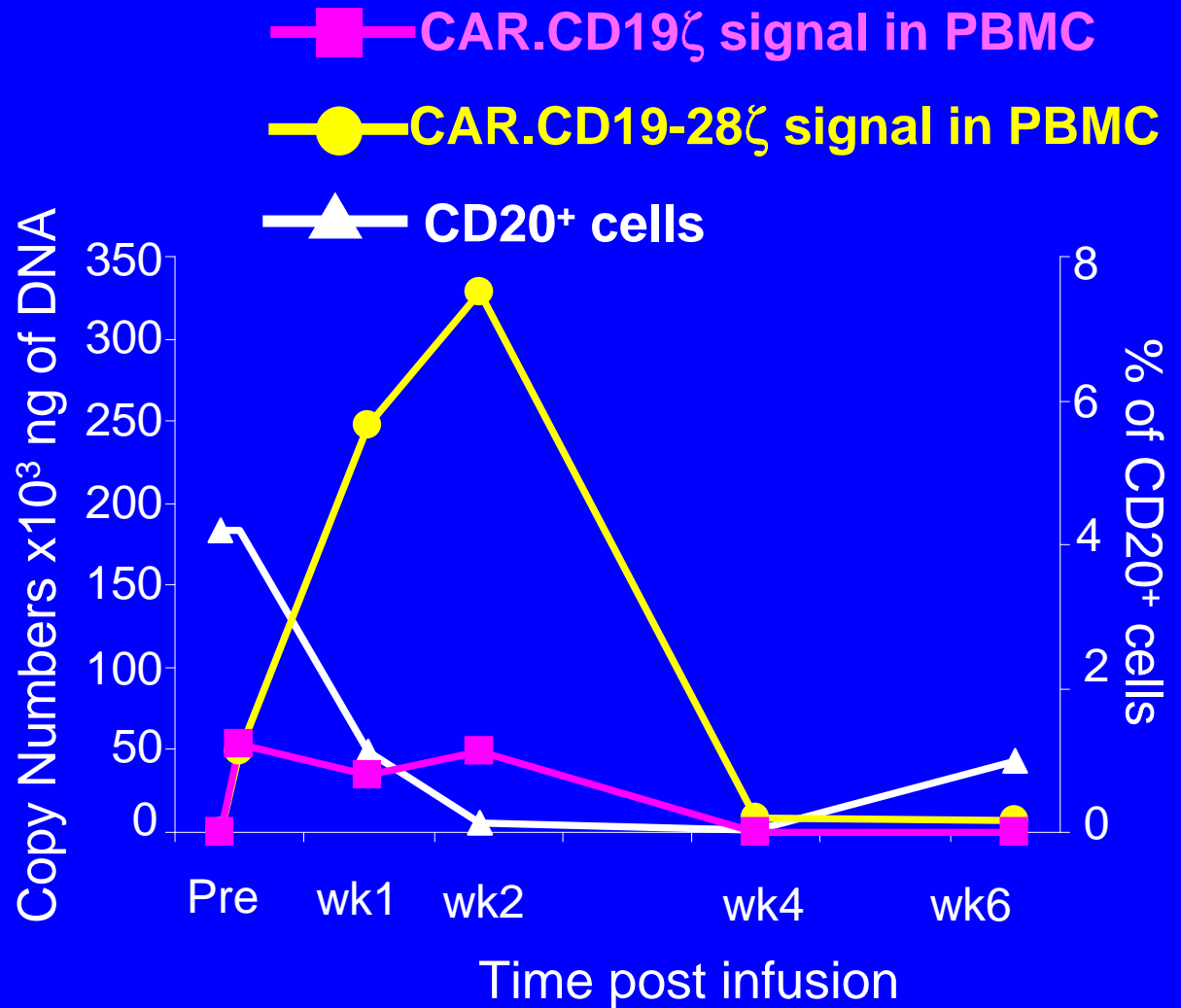
CAR.CD19-28 ζ ⁺ T cells have greater *in vivo* expansion and persistence



Pt #3, dose level 2

Effects on circulating B cells

	B cells pre vs 6 wks post
Pt #1	NS
Pt #2	<0.1%
Pt #3	4% vs 1%
Pt #4	<0.1%



Conclusions

- CAR.CD19⁺ T cells safe at the dose levels so far tested
- CAR.CD19-28 ζ ⁺ T cells
 - Expand better
 - Persist longerthan CAR.CD19 ζ ⁺ T cells
- Clinical responses are limited
- Are other co-stimulatory endodomains superior to CD28?

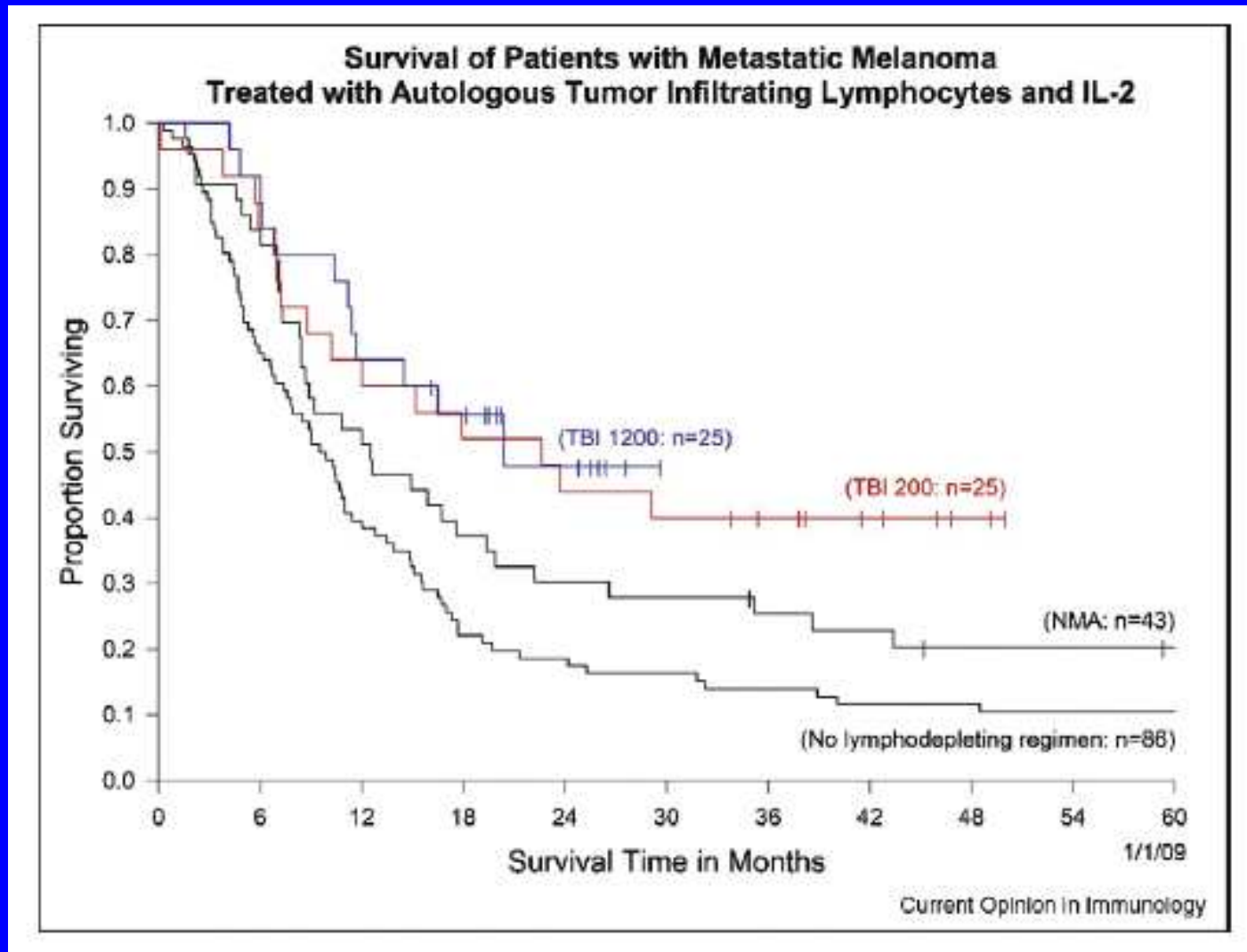
What we will learn from these trials?

- Does the CAR expression vector affect outcome?
- What co-stimulation is ideal for T cells?
- **Is host lymphodepletion necessary?**
- Which T cell subset should be used?
- What are the toxicities?

Host lymphodepletion

- Depletion of suppressive CD4⁺CD25⁺ T-regulatory lymphocytes
- Elimination of cellular "sinks" for homeostatic cytokines such as IL-7 and IL-15
- Enhance APC activation and availability?

Adoptive transfer of TIL in melanoma



Clinical trials with CAR⁺ T cells

Antigen	Trial	Lymphodepletion
K-light chain	NCT00881920	2 nd gen. CAR with CD28 + Cytosan
CD19	NCT00466531	2 nd gen. CAR with CD28 + Cytosan
CD19	NCT00924326	2 nd gen CAR with CD28 + non-myeloablative conditioning regimen
HER2/neu	NCT00924287	3 rd gen CAR with CD28 and 4-1BB + non-myeloablative conditioning regimen

Clinical trials with CAR⁺ T cells and host lymphodepletion

Prolonged Regression of Lymphoma and Elimination of CD19⁺ Cells in a Patient That Received Anti-CD19 Chimeric Antigen Receptor-Transduced T Cells

Authors: J. N. Kochenderfer et al.

Marked Regression of Adenopathy Following Infusion of Autologous T Cells Genetically Targeted to the CD19 Antigen in a Patient with Bulky CLL

Authors: Renier J. Brentjens et al.

Session Info: Simultaneous Oral Abstract Sessions:
Cancer – Immunotherapy T Cell Studies (2:00 PM-4:00 PM)

What can we learn from these trials?

- Does the CAR expression vector affect the outcome?
- What co-stimulation is ideal for T cells?
- Is host lymphodepletion necessary?
- Which T cell subset should be used?
- What are the toxicities?

Which T cell subset?

- Polyclonal activated T lymphocytes
- Central memory T cells (CD62L+ cells)
Berger C et al J Clin Invest. 2008;118:294-305
- Virus-specific cytotoxic T lymphocytes
- Natural Killer cells
- $\gamma\delta$ T cells

Platform For Manufacturing T_{CM}⁻ Derived Bispecific T-cell Grafts

21-28 Days



1. Depletion: CD4, CD45RA
2. Enrichment: CD62L-biotin/
Anti-biotin Microbeads

Lenti
Transduction



Expansion
in IL2/IL15



CD3xCD28
Dynal Bead
Activation

Jensen M et al.

Which T cell subset?

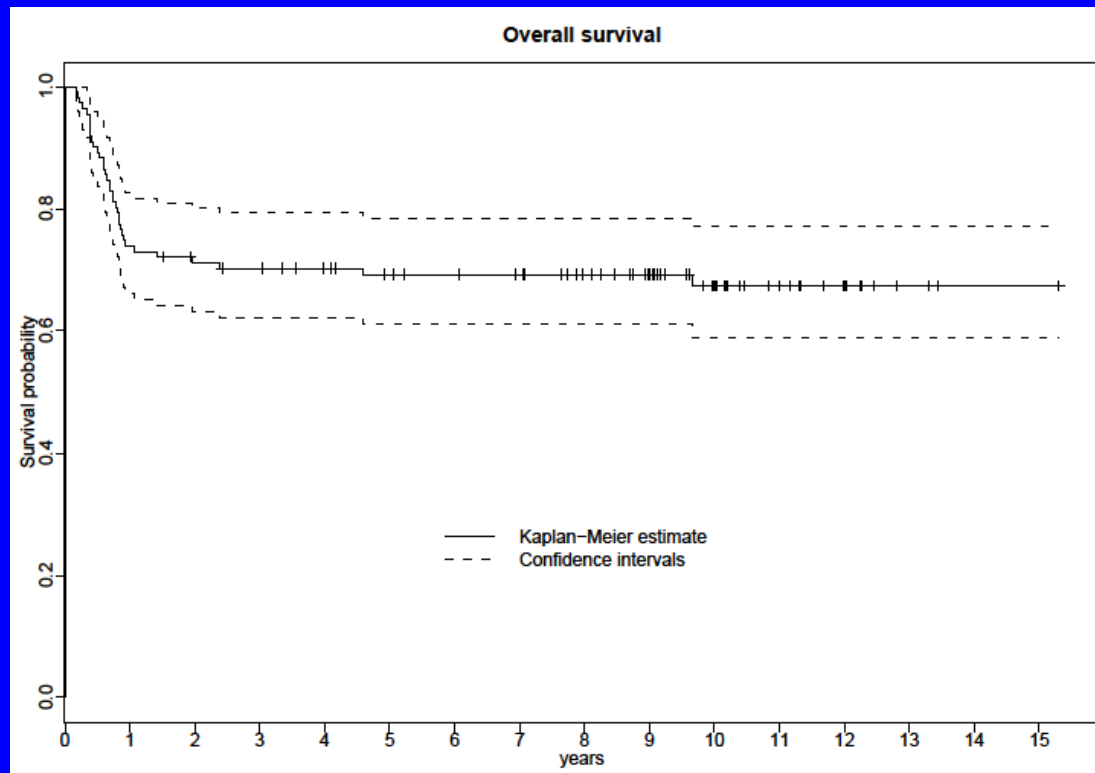
- Polyclonal activated T lymphocytes
- Central memory T cells (CD62L+ cells)
Berger C et al J Clin Invest. 2008;118:294-305
- **Virus-specific cytotoxic T lymphocytes**
- Natural Killer cells
- $\gamma\delta$ T cells

What do we want from CAR-T cells?

- Plentiful
- Persistent
- Migrate at tumor
- Produce tumor regression

.....the same as from Epstein-Barr-Virus-specific CTLs

EBV-specific CTLs prevent and treat EBV-related diseases after HSCT



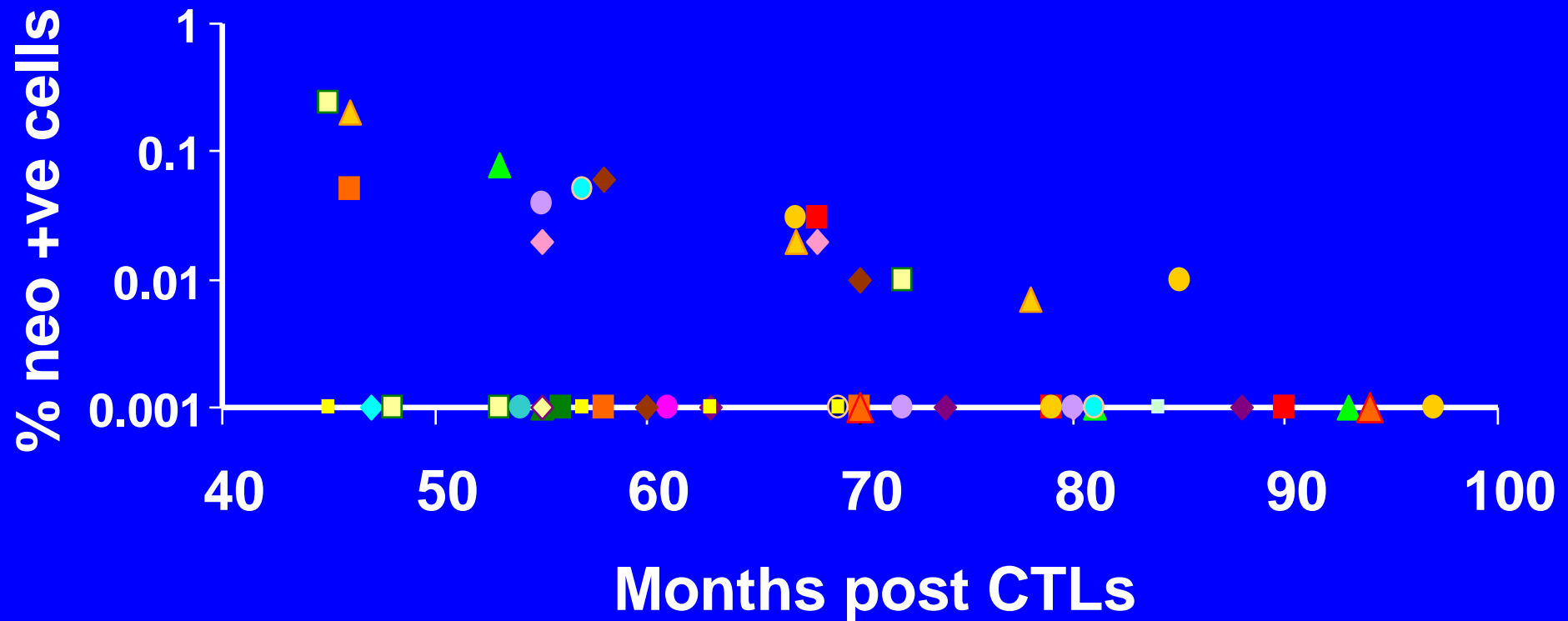
Diagnosis of PTLD



Two months after CTL infusion

Heslop et al Blood. 2010;115:925-35

Long-term detection of EBV-CTLs marked with *Neo* gene

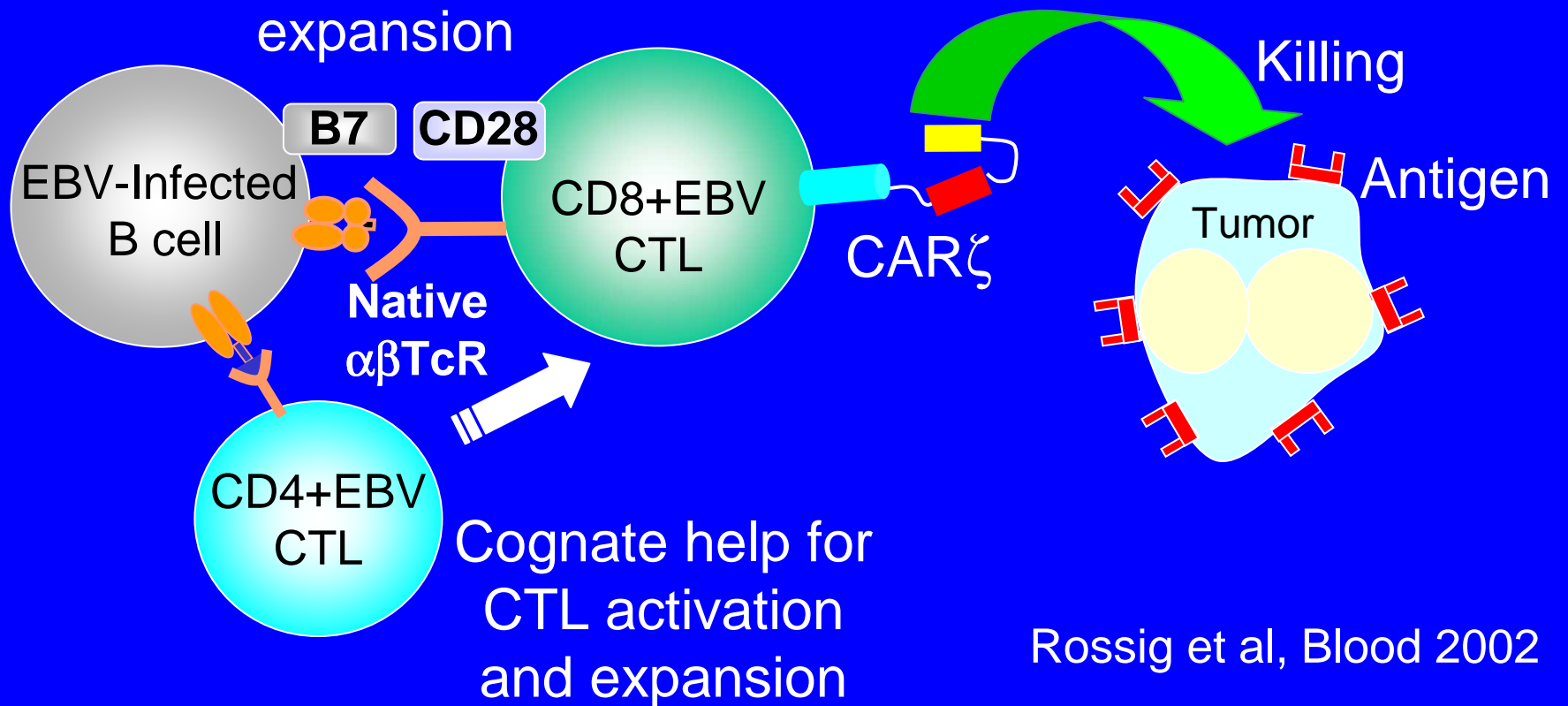


Real Time PCR in PBM

Heslop et al. Nat Med 1996, and Blood 2010

“Dual specific” EBV-CTLs

Co-stimulation for CTL
activation and
expansion



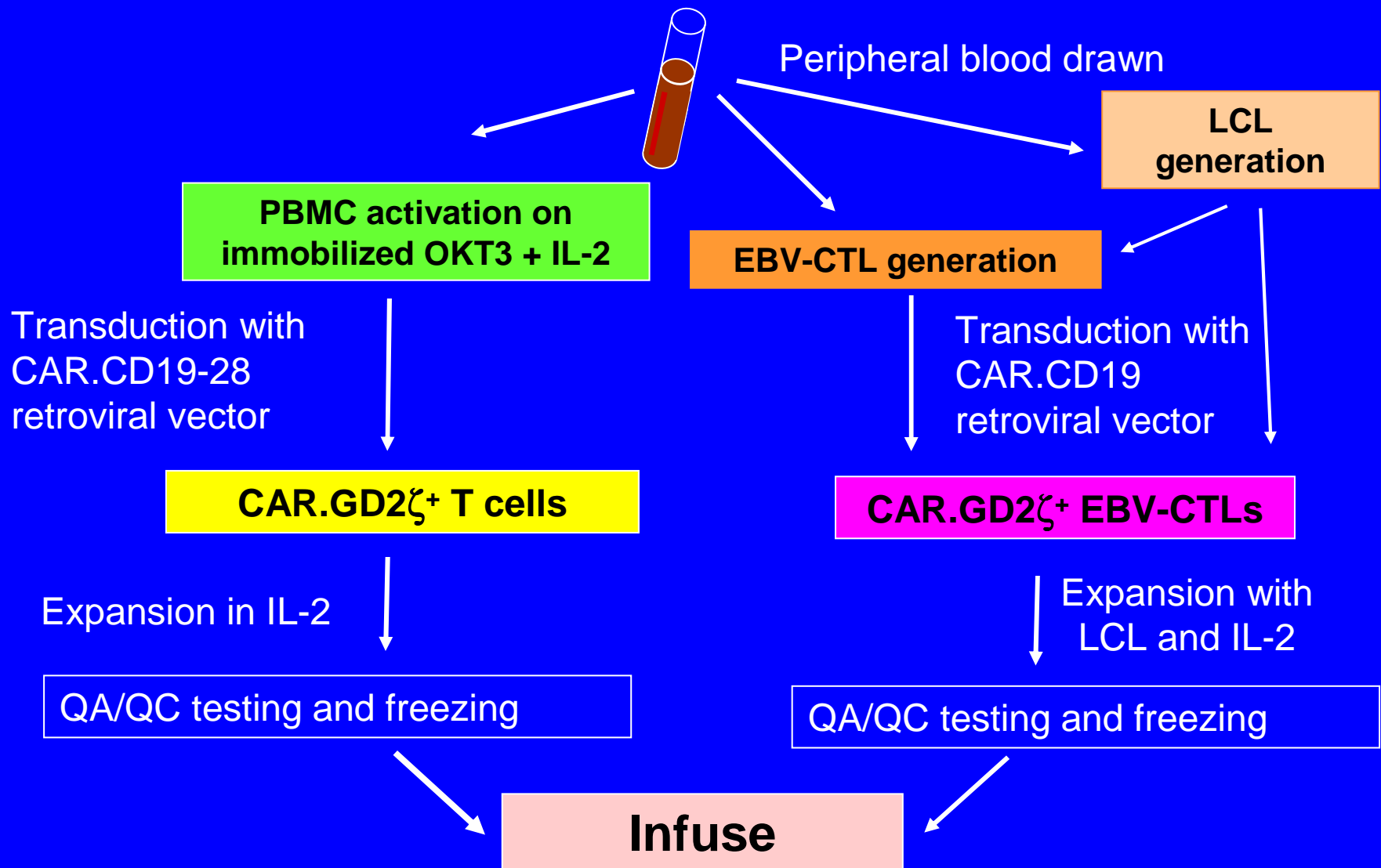
Rossig et al, Blood 2002

Savoldo et al, Blood 2007

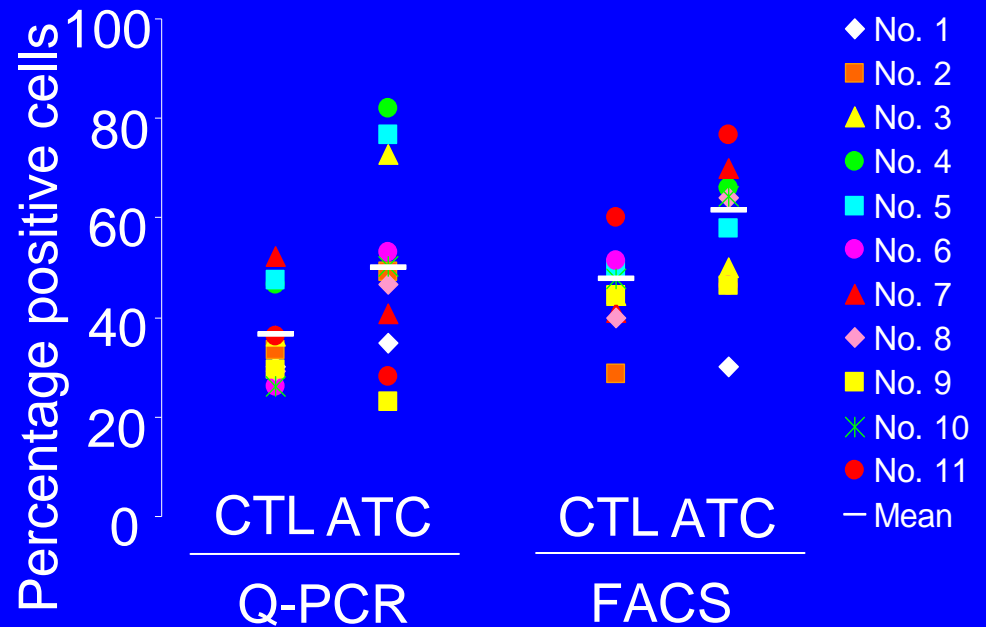
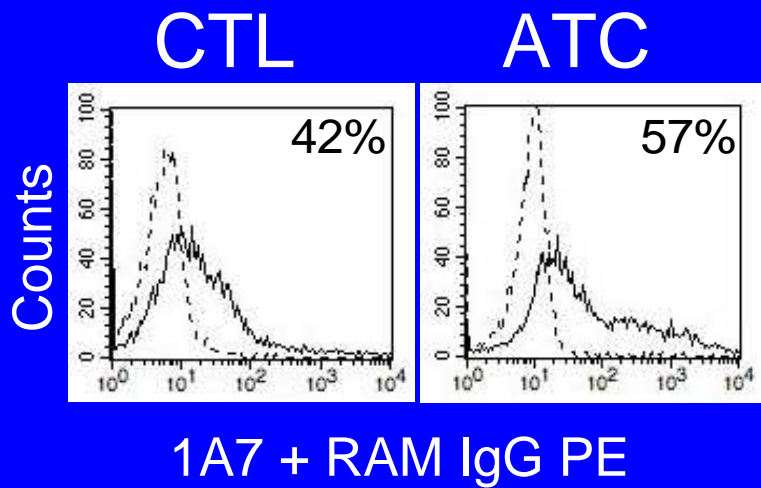
Phase I study with CAR-redirected T cells in neuroblastoma

- Prepare autologous EBV-CTL and activated T cells (ATC) lines for each patient
- Transduce the cells with 1st generation CAR targeting GD₂
- Infuse both T cell populations
- Track each T cell population in vivo

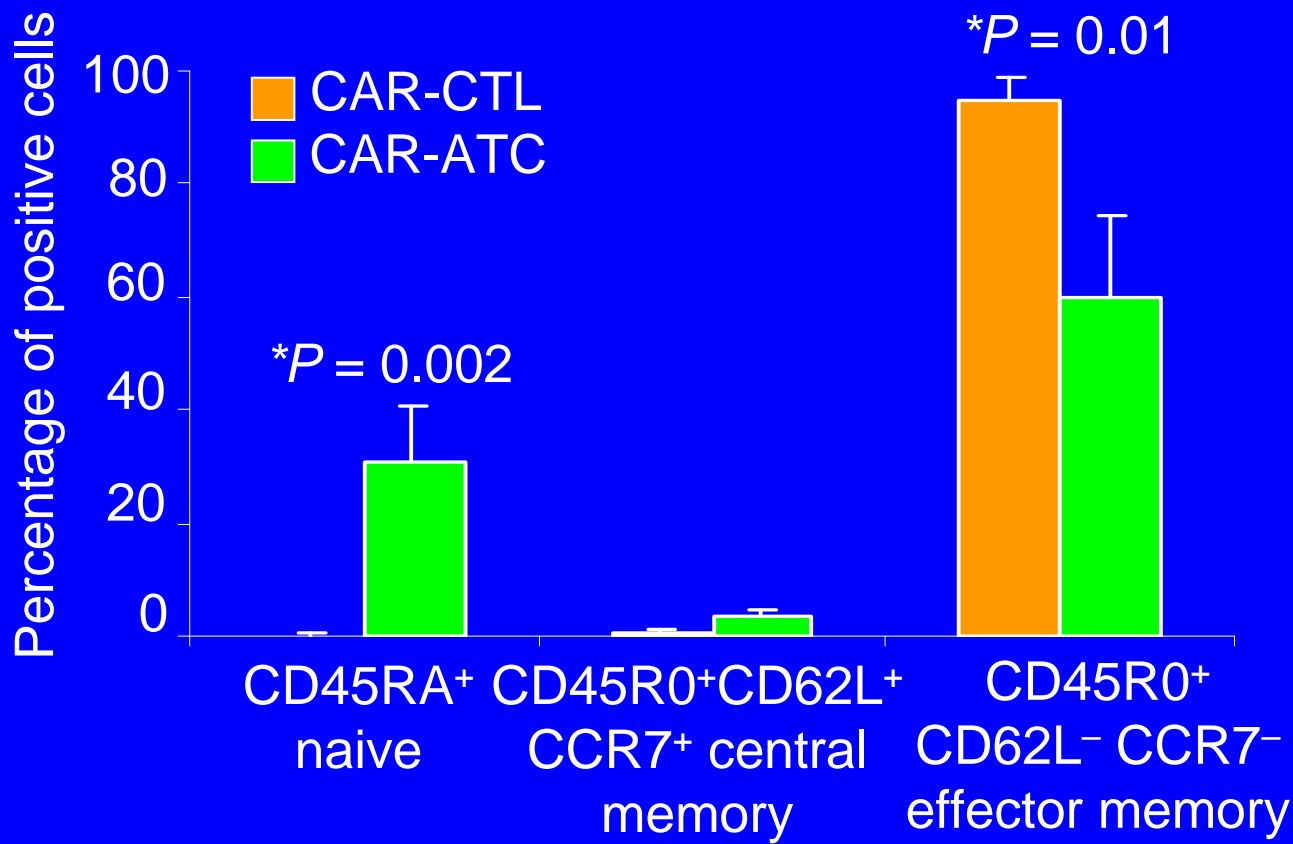
Generation of CAR⁺ T cells and CAR⁺ EBV-specific CTLs



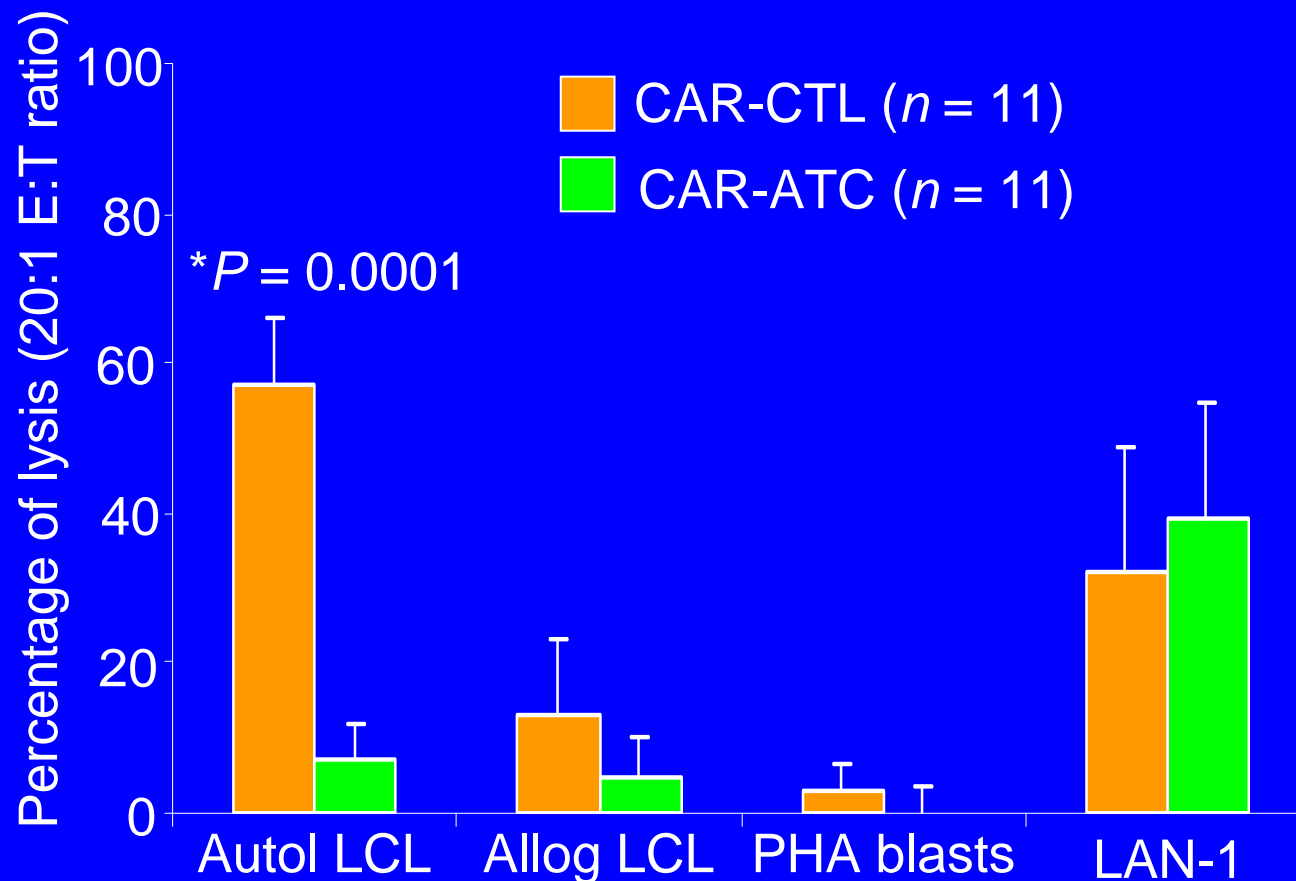
Transduction efficiency of infused EBV-CTLs and ATC



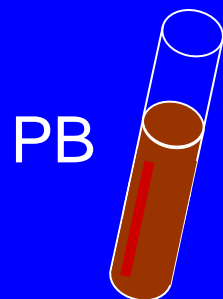
Memory phenotype of CAR⁺ ATC versus CAR⁺ CTLs



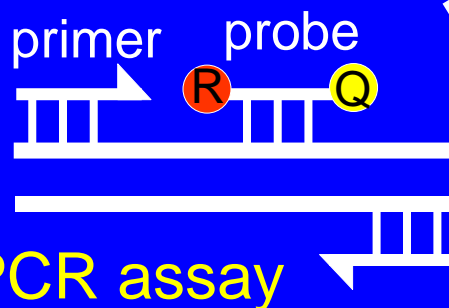
EBV-CTLs remain EBV-specific via $\alpha\beta$ TCRs and target GD2⁺ tumors through CAR



Monitoring expansion and persistence of ATC vs. CTLs



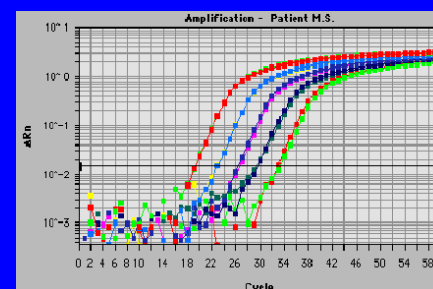
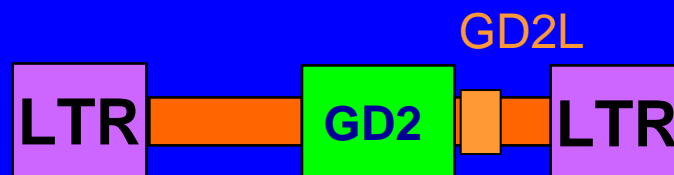
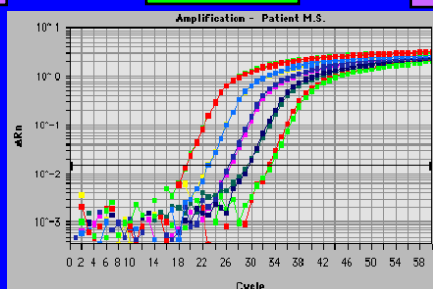
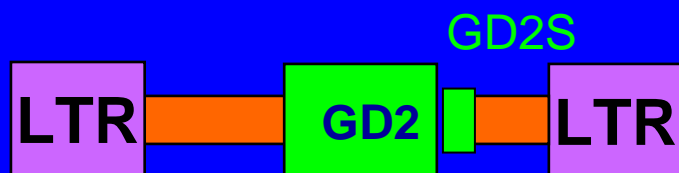
DNA isolation



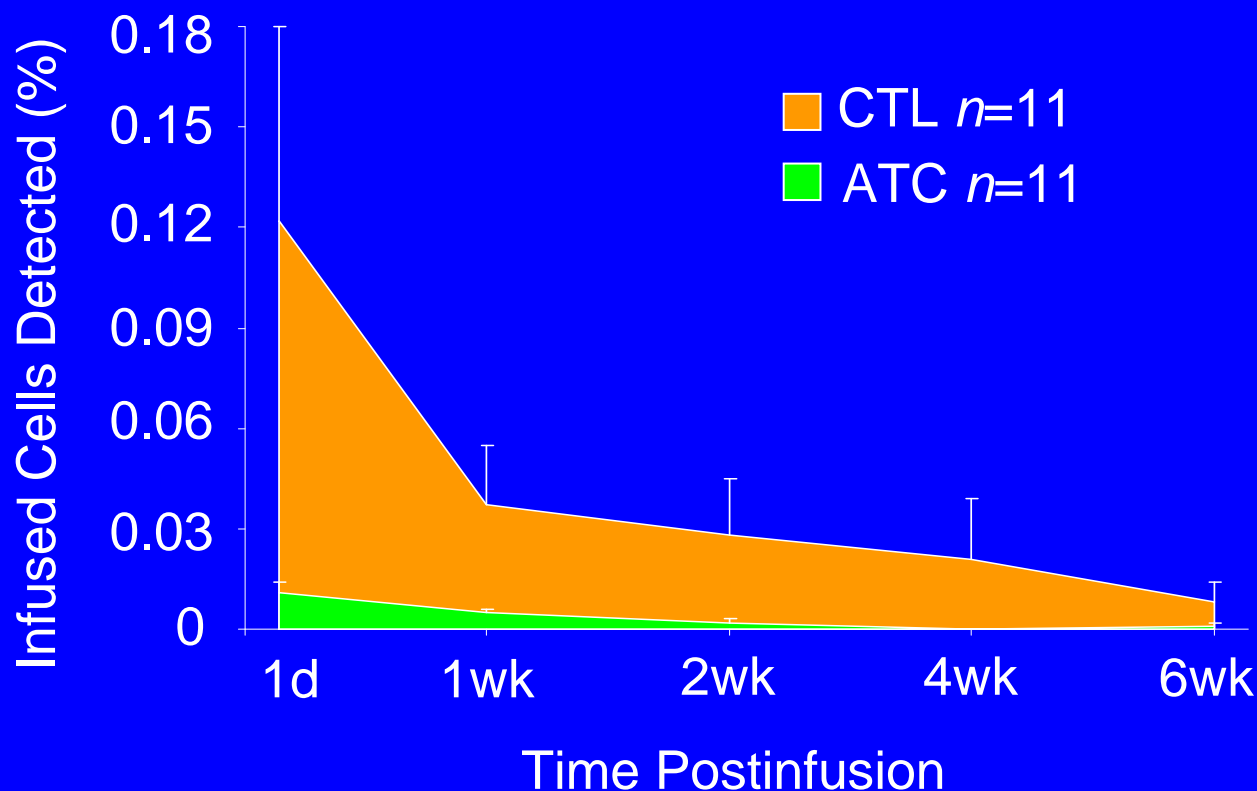
q-PCR assay

CAR.GD2 from ATC

CAR.GD2 from CTLs



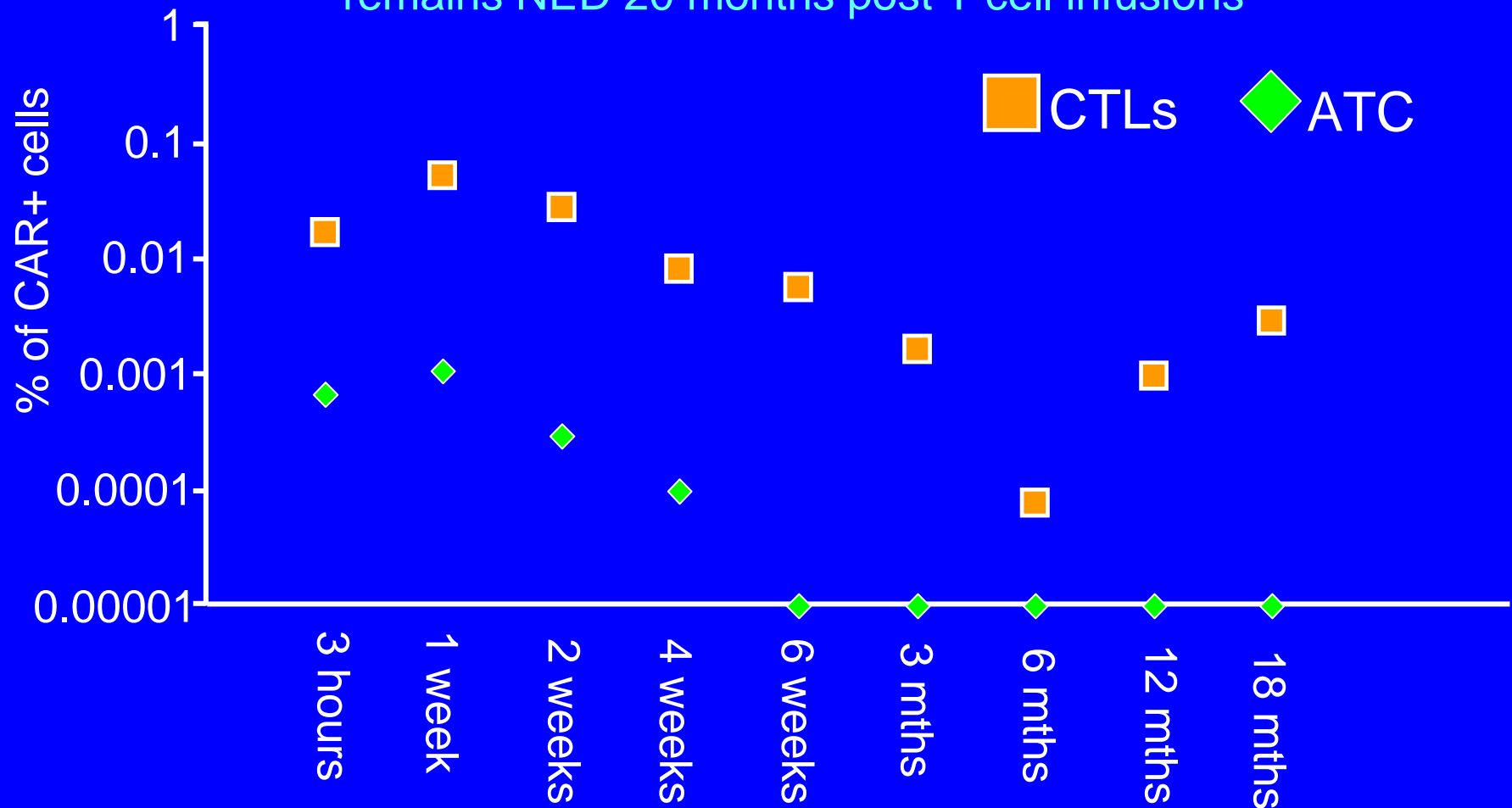
CAR⁺ CTLs have longer persistence *in vivo* than CAR⁺ ATCs



Quantification of Q-PCR signal in blood samples

Long term persistence of CAR⁺ CTLs vs. CAR⁺ ATL

9 year old with relapsed neuroblastoma remains NED 20 months post T cell infusions



Clinical Responses

- 19 patients treated
- 8/9 patients with high risk disease treated in remission remain in remission (>6 to >48months)
- 5/10 patients with active relapsed/resistant disease had tumor response/regression
- 2 complete remission (>48 months, >9 months)

CAR⁺ EBV-CTLs vs. CAR⁺ ATC

- Administration of CAR⁺ T cells targeting GD2 was safe
- CAR-GD2 EBV-CTLs had greater persistence and retained functionality
- Objective clinical responses are achieved
- Are CAR-modified EBV-CTLs superior to polyclonal activated T cells expressing 2nd or 3rd generation CAR?

Study of T-cells or EBV Specific CTLs for Advanced B-Cell Non Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia (ATECRAB)

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

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Study 1 of 1 for search of: atecrab
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Study of the T-cells or EBV Specific CTLs for Advanced B-Cell Non Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia (ATECRAB)

This study is currently recruiting participants.
Verified by Baylor College of Medicine, March 2010

First Received: July 1, 2008 Last Updated: April 21, 2010 [History of Changes](#)

Sponsor:	Baylor College of Medicine
Collaborators:	Texas Children's Hospital The Methodist Hospital System
Information provided by:	Baylor College of Medicine
ClinicalTrials.gov Identifier:	NCT00709033

What can we learn from these trials?

- Does the CAR expression vector affect the outcome?
- What co-stimulation is ideal for T cells?
- Is host lymphodepletion necessary?
- Which T cell subset should be used?
- **What are the toxicities?**

“On target” toxicity

CD19- or CD20-specific CAR will target leukemic and normal B lymphocytes

Carbonic anhydrase IX (CAIX)-specific CAR induces cholangitis

Lamers et al J Clin Oncol 2006

Serious adverse events

Case Report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2

Morgan et al Mol Ther 2010;18:843-51

Treatment of Chronic Lymphocytic Leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial

Brentjens et al Mol Ther 2010;18:666-8

Conclusions

CAR-based T cell therapy is feasible

Clinical trials currently ongoing will define

Safety

Appropriate gene transfer approach

Appropriate co-stimulation

Appropriate host preparation

Various human malignancies may benefit this approach

Acknowledgements

Post doc and fellows

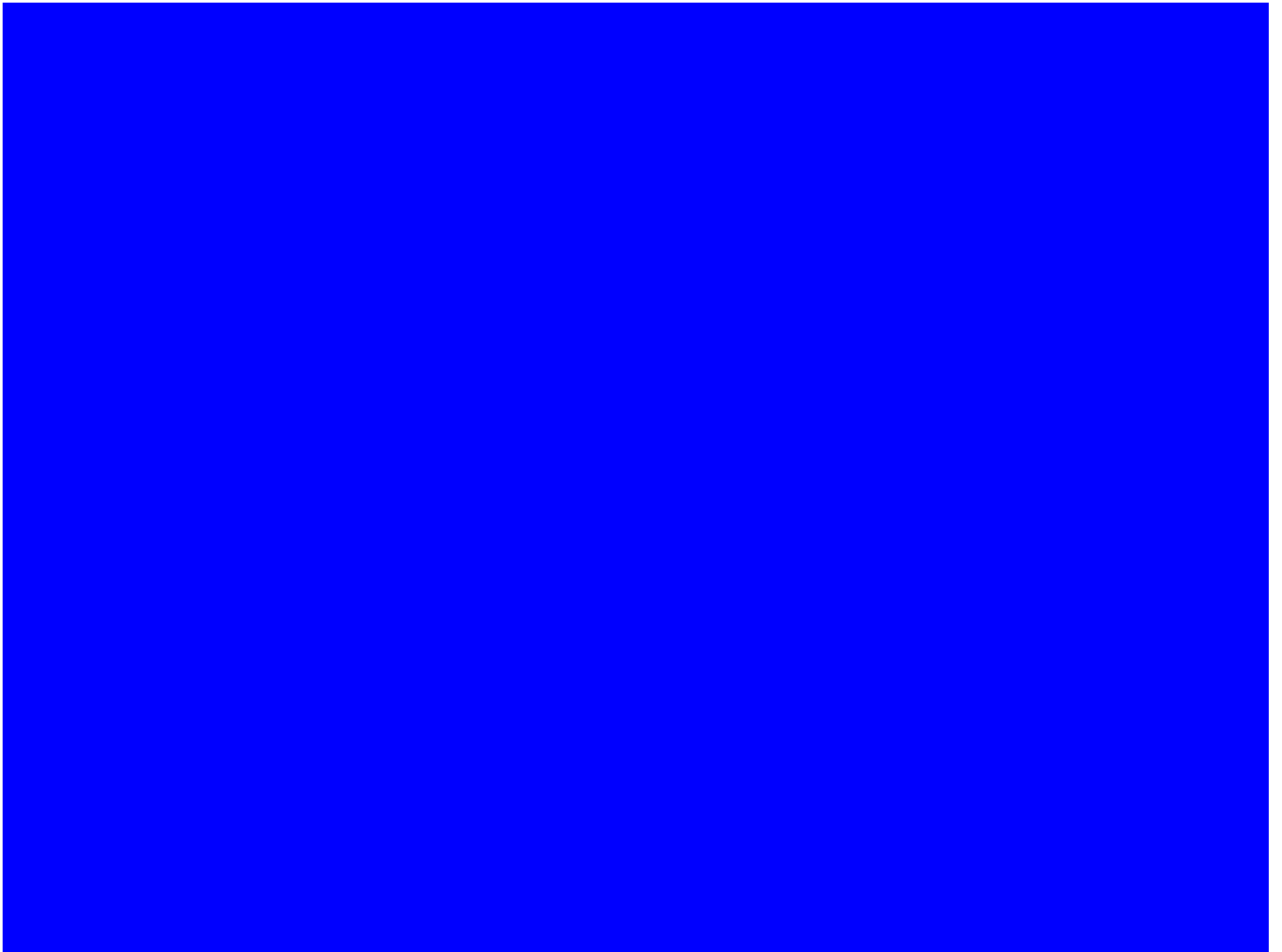
Juan Vera
Concetta Quintarelli
Antonio Di Stasi
Valentina Hoyos
Greta Giordano
Martin Pule
Claudia Rossig
Kenneth Micklethwaite
Biagio De Angelis

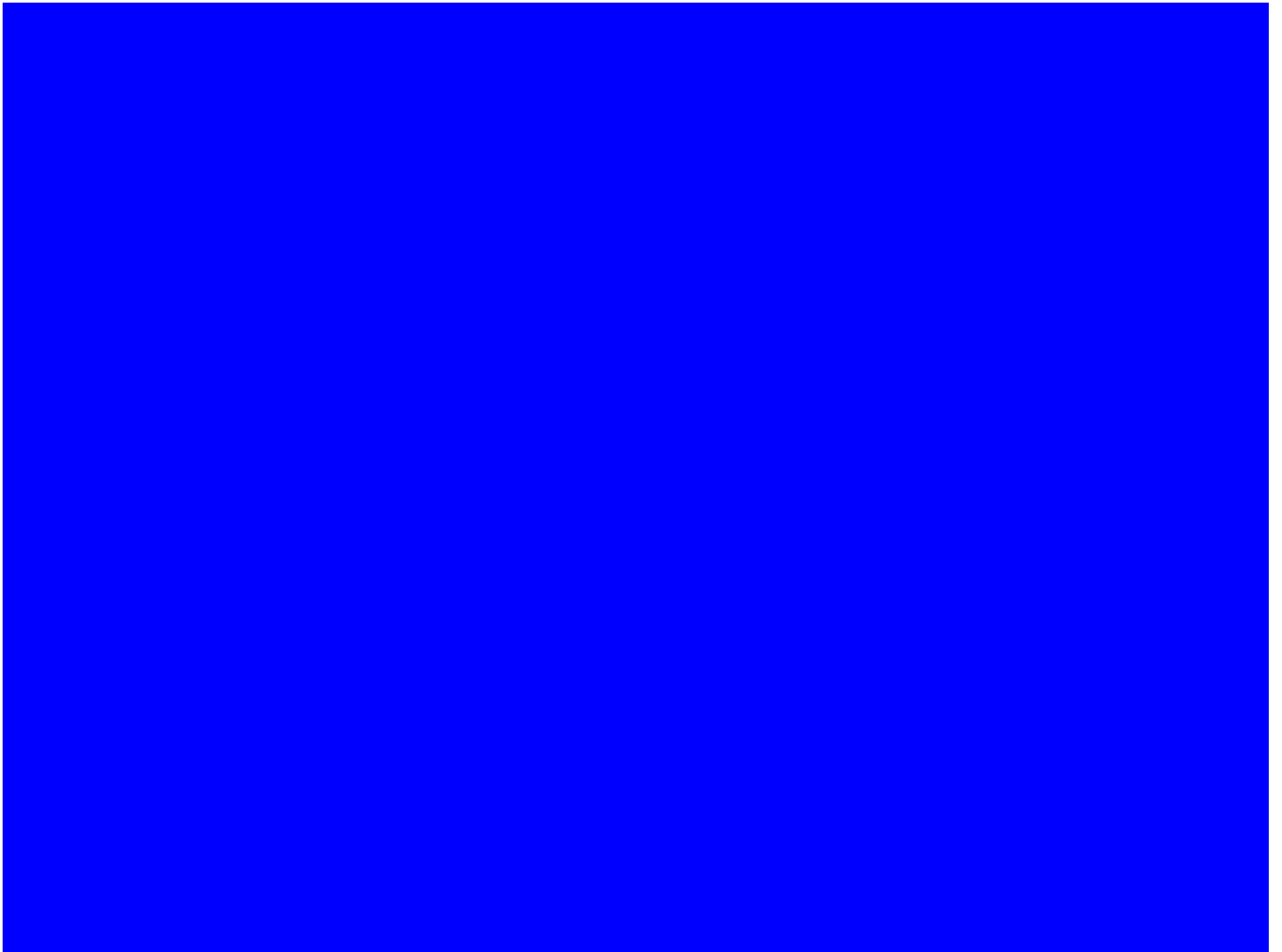
CAGT

Malcolm Brenner
Cliona Rooney
Helen Heslop
Robert Krance
Adrian Gee
Barbara Savoldo
Aaron Foster
Catherine Bollard
Steven Gottschalk

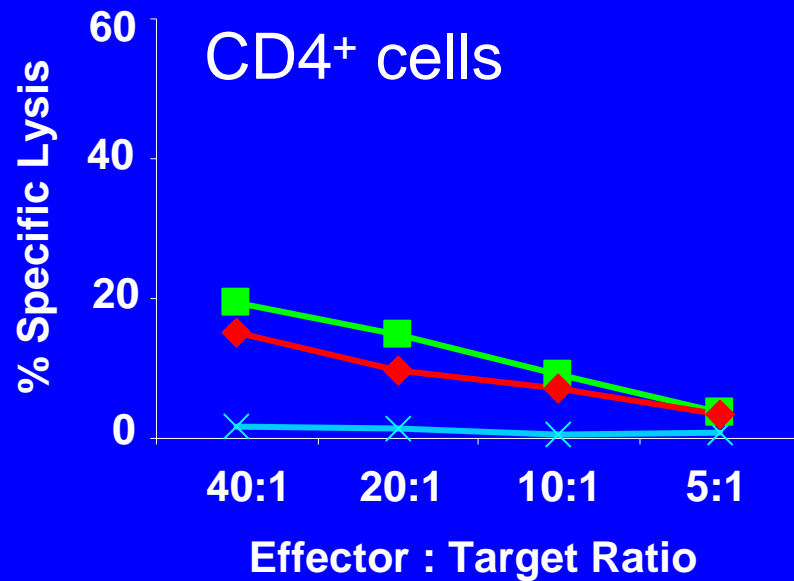
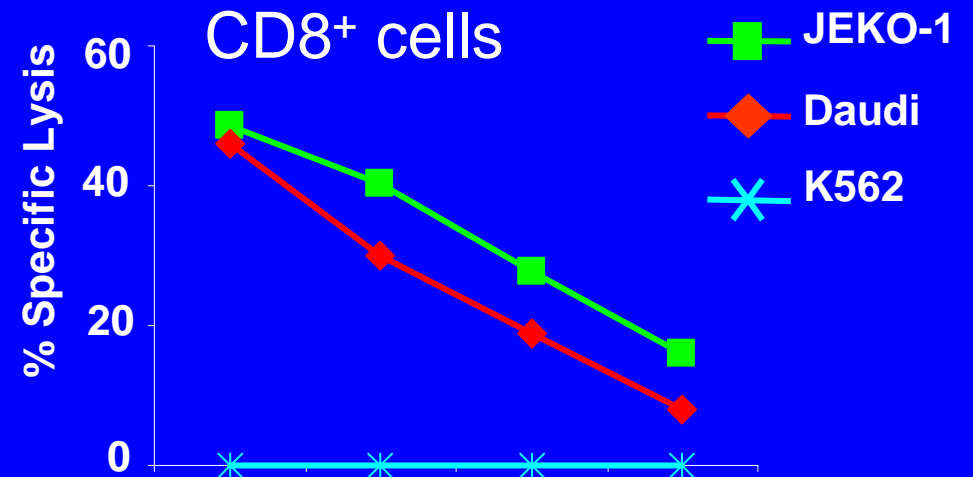
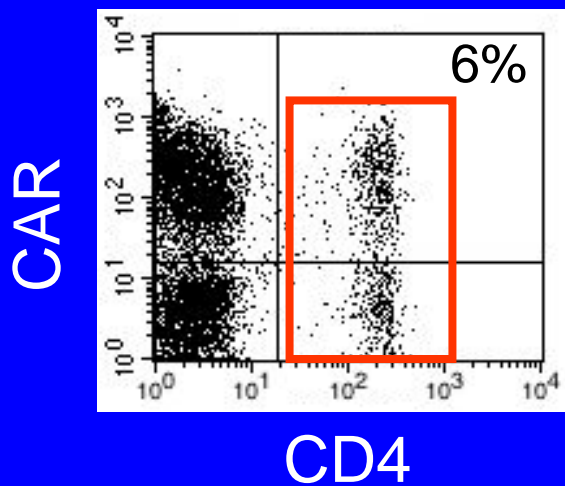
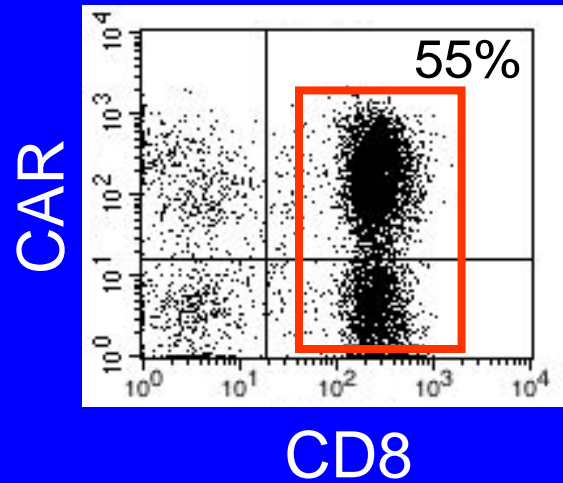
CAGT

Luis Crystal
Ann Leen
Zhuyong Mei
Bambi Grilley
Tessie Lopez
Eric Yvon
Enli Liu





Cytotoxic activity of CAR⁺ T cells



CAR targeting CD19

Anti-tumor activity: clinical outcome

	Cell Dose	F/U (mo)	Clinical Response
Pt #1	I = $2 \times 10^7 / \text{m}^2$ II = $2 \times 10^7 / \text{m}^2$ (4mo post I)	12	Stable disease 10 mo; progressed 6 mo post II infusion
Pt #2	I = $2 \times 10^7 / \text{m}^2$	9	Progressive disease 2 ½ mo post I infusion
Pt #3	I = $1 \times 10^8 / \text{m}^2$ II = $0.5 \times 10^8 / \text{m}^2$ (2 mo post I)	4	Stable disease 4 mo; progressed 2 ½ mo post II infusion
Pt #4	I = $1 \times 10^8 / \text{m}^2$	3	Progressive disease 2 mo post I infusion

Patient details

Gender/ Age (yrs)	Diagnosis	Previous Therapy	Disease at enrollment
M/53	B-CLL (4/2004)	Rituxan, Fludarabin Cytosan	PET: persisting malignancy
M/58	IVB DLBCL (2/2004)	R-CHOP x 8, XRT FCR x 6, ICE x 2, cisplatin, cytarabine	PET: neck residual disease
M/47	IVB DLBCL (3/2007)	R-CHOPx6, R-ESHAP x4, R-ICEx2, R-IGEV, TTR, Rituxan, Hyper CVADx2	PET: diffuse retroperitoneal lymphadenopathy
M/57	IIA DLBCL (1/2009)	R-CHOP x 4, R-ESHAP, HSCT (8/2009)	PET: 2 residual neck LNs