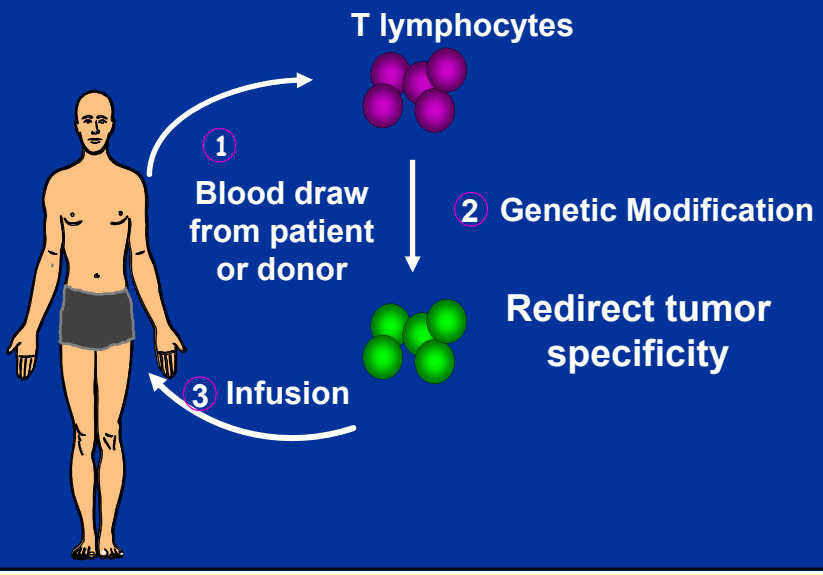
CARs in the clinic: first efficacy reports and concerns about safety

Gianpietro Dotti

Baylor College of Medicine, Houston TX, USA

Adoptive Immunotherapy of Gene Modified T Cells

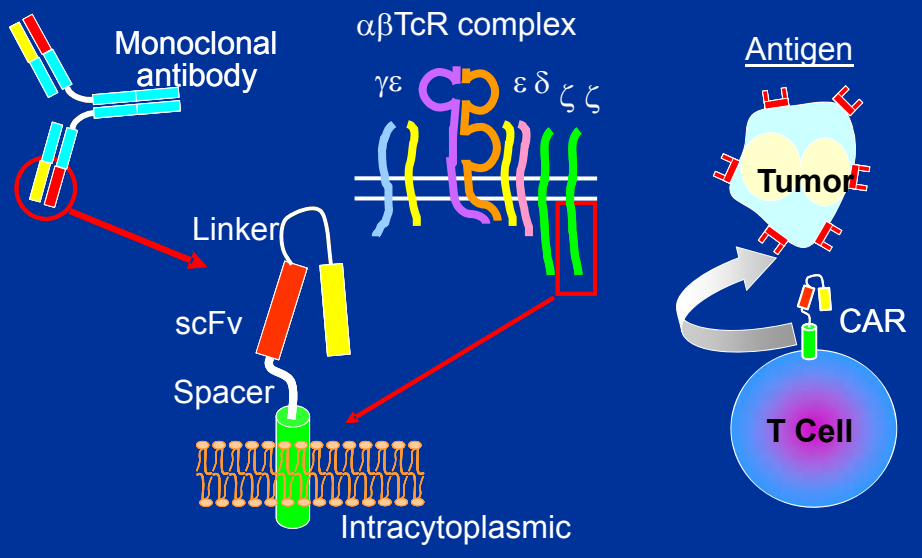


Redirect T-cell antigen specificity

- Transgenic expression of αβT-cell receptors

- Transgenic expression of Chimeric Antigen Receptors (CARs)

Gene Transfer of CARs



Eshhar Z PNAS 1993

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

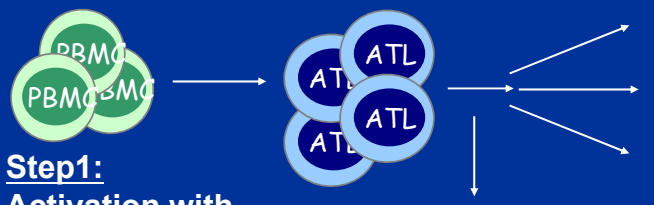
- Surface expression of the target antigen
- Potential T cell interference if the antigen is shaded

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/CD23	B cell lymphoid tumors
Light chains of Igs	B cell lymphoid tumors
GD2	Neuroblastoma Melanoma
CEA	Colon rectal carcinoma

Generation of CAR-modified Activated T Lymphocytes (ATL)

Step 2: CAR-gene transfer (DNA integration)

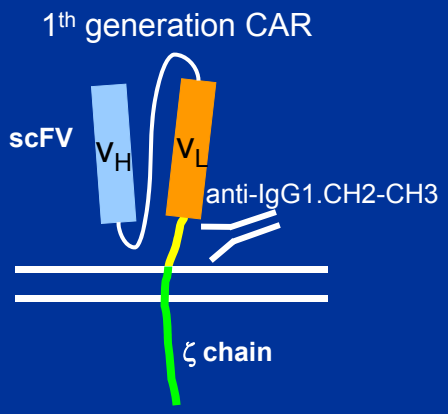


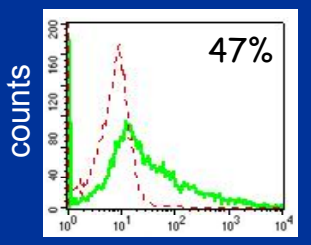
Step1:
Activation with OKT3 and IL-2

Step 3:
Expansion with IL-2

Step 4:
Sterility
Phenotype
Cytotoxicity

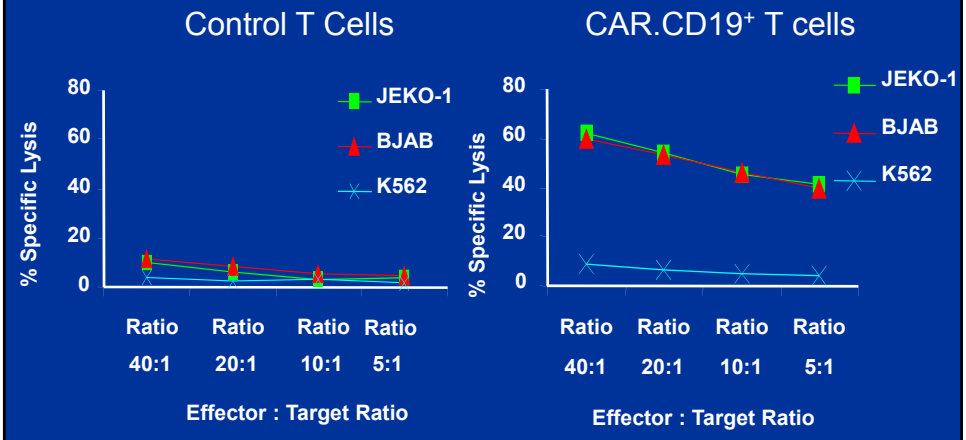
Expression of CARs by activated T lymphocytes





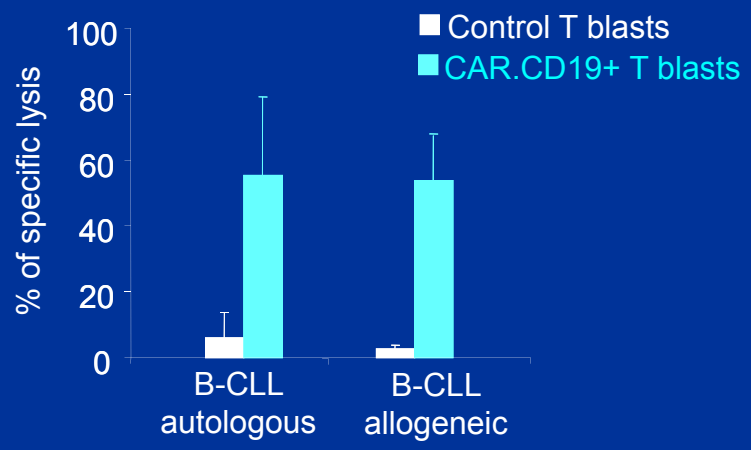
anti-IgG1.CH2CH3

Cytotoxicity of CD19 CAR⁺ T cells



JEKO-1 AND BJAB are CD19⁺ tumor cells

Cytotoxic activity of CAR-modified T cells is not MHC-restricted



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-13Rα2	1	Gliobastoma
HER2/neu	3	Osteosarcoma, lung cancer, other HER$^+$ tumors

Jena B et al Blood 2010

What are we learning from these trials?

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

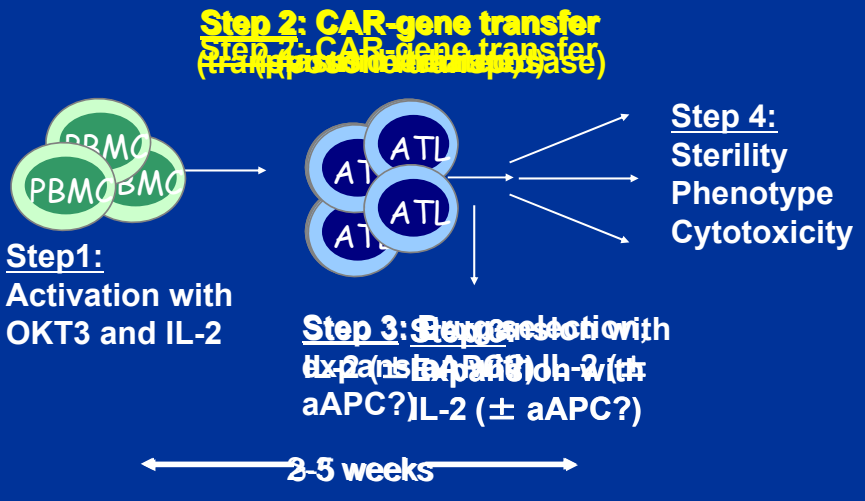
Vectors for T cell CAR expression

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

Vectors for T cell CAR expression

Vector	Speed of manufacture	Costs
Retro/Lenti	Short	high
Plasmids	Long	low
Sleeping Beauty	Intermediate	low

Generation of CAR-modified Activated T Lymphocytes (ATLs)

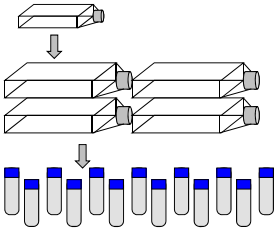


Overview of Retroviral production

Single cell clone of producer is expanded.

Multiple aliquots are cryopreserved.

Cells and supernatant are tested



Cell Bank Testing

Microbial Sterility

Bacterial / fungal Sterility

Mycoplasma testing

Endotoxin Testing

In vivo Assay for adventitious viral contaminants

In Vitro assay for adventitious viral contaminants

Mouse Antibody Production Test

Thin section electron microscopy

PCR testing for HIV, HTLV, HBV, HCV, HHV 6, HHV 7, CMV,. EBV, B19

Extended Mink PG4 S+L Focus Assay

XC plaque assay

Cell line species identification by isoenzyme electrophoresis

Vector characterization by PCR or genomic Southern

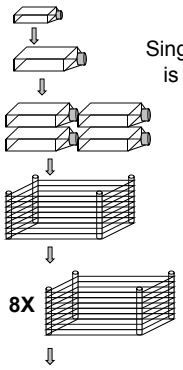
Functional testing on transduced primary T-cells

Supernatant and cryopreserved cells for archiving.

cell co-cultivation to detect GALV and ECO RCR

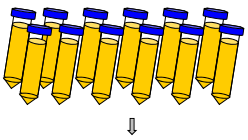
Supernatant amplification to detect GALV and ECO RCR

Overview of Retroviral production



Single aliquot from cell bank is expanded to 8 CF10s.

Supernatant is harvested, filtered, aliquoted and tested.



End-of-production cells are then harvested and tested

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Overview of Retroviral production

Overview of Retroviral production

What are we learning from these trials?

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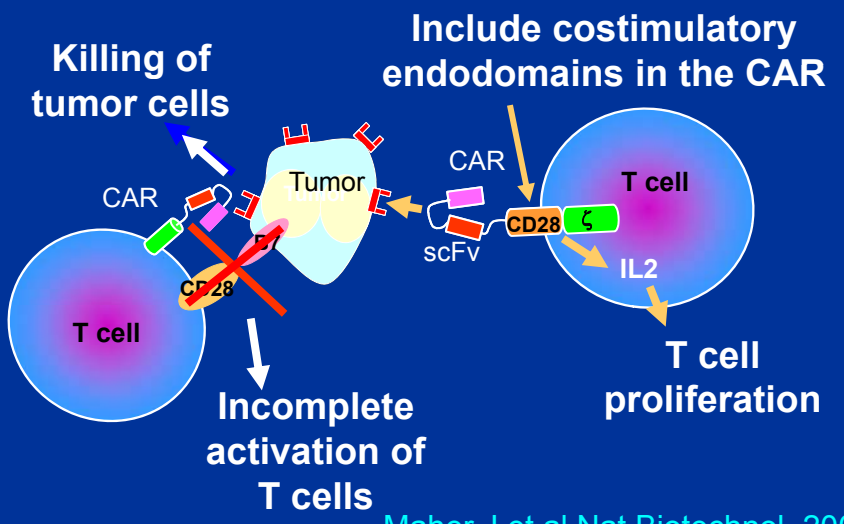
- Which co-stimulation is ideal for T cells?
- Which T cell subset should be used?
- What are the toxicities?

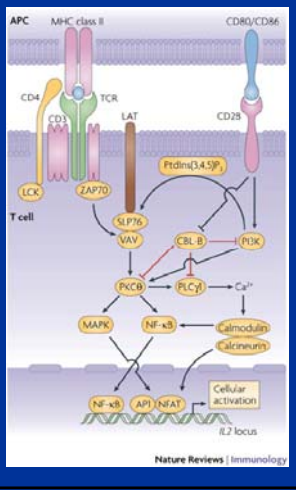
"Second generation" CAR to improve T cells activation



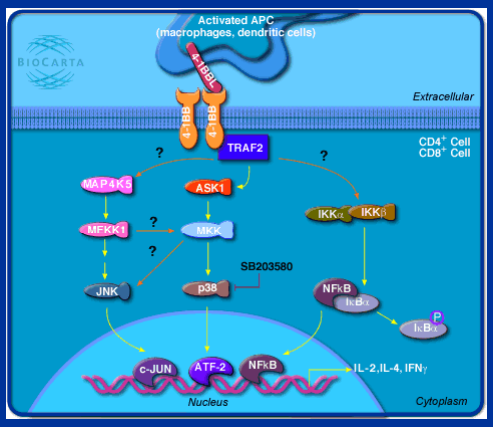
Maher J et al Nat Biotechnol. 2002

Rational for exploring alternative costimulatory endodomains

"Early (CD28/PtdIns) costi."



"Late (4-1BB/TRAF) costi."



Incorporation of co-stimulatory endodomains in CARs

1th generation CAR

scFv-FcεRI — ScFv | spacer | FcεRI

scFvζ — ScFv | spacer | Zeta

2th generation CAR

scFv-CD28ζ — ScFv | spacer | CD28 | Zeta

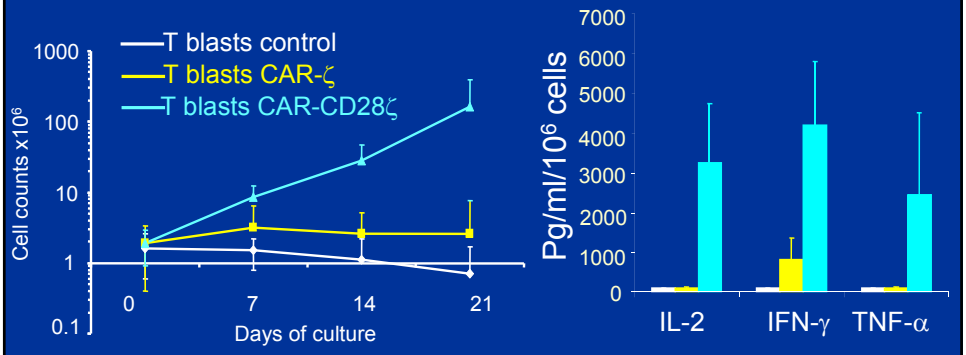
scFv-4-1BBζ — ScFv | spacer | 4-1BB | Zeta

3th generation CAR

scFv-CD28-OX40ζ — ScFv | spacer | CD28 | OX40 | Zeta

scFv-CD28-4-1BBζ — ScFv | spacer | CD28 | 4-1BB | Zeta

CD28 induces T-cell expansion after antigen stimulation



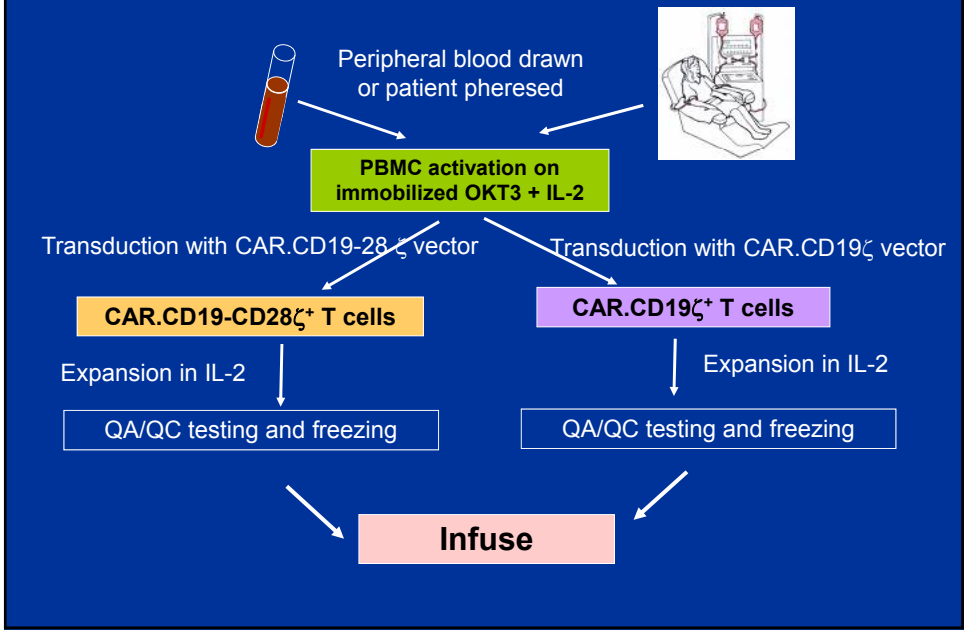
Vera et al, Blood 2006;108(12):3890-7

Are CAR-CD28ζ⁺ T cells superior to CARζ⁺ T cells?

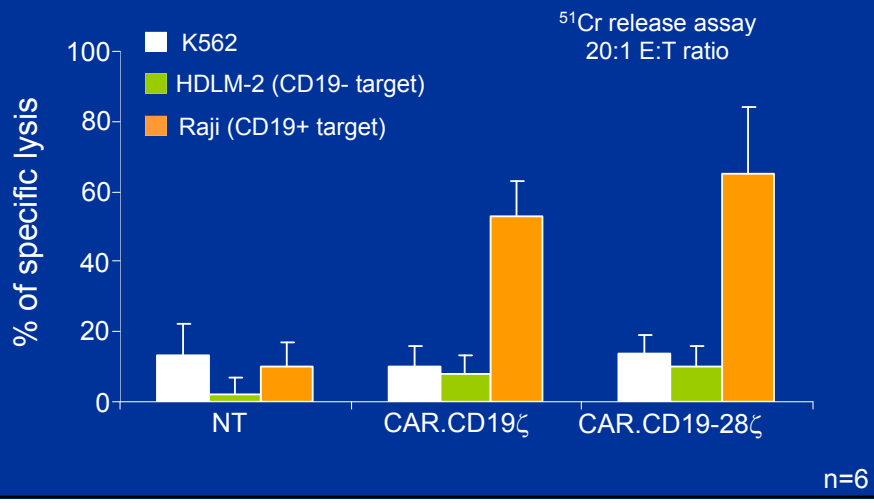
- Prepare two autologous activated T cell lines expressing **CAR.CD19ζ** or **CAR.CD19-28ζ** for each patient
- Infuse both T cell populations
- Track each T cell population *in vivo*

Savoldo et al, JCI 2011

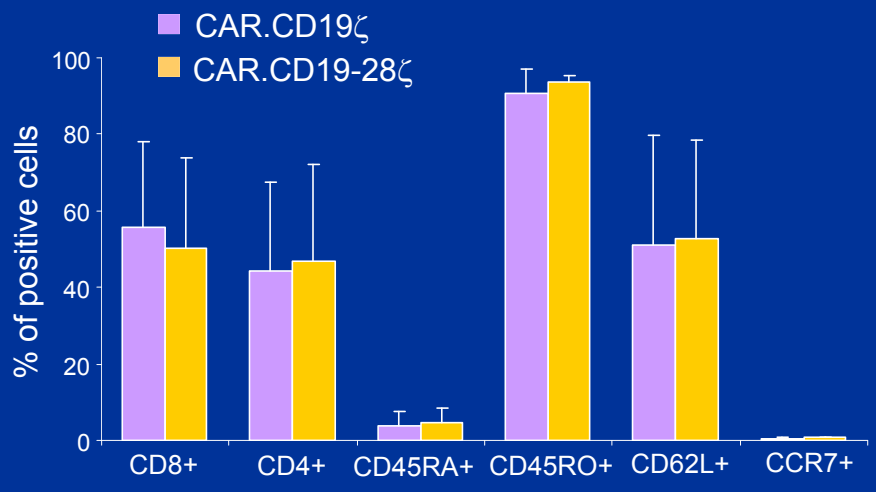
Generation of CAR$^+$ T cells



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



CAR.CD19^{+} and CAR.CD19-28^{+} T cells have comparable immunophenotypes



n=6

Treatment plan

-Three dose levels

-Single dose

- Modified continual reassessment method

Dose level 1: 2x10^{7} cells/m^{2} of each product

Dose level 2: 1x10^{8} cells/m^{2} of each product

Dose level 3: 2x10^{8} cells/m^{2} of each product

-Second infusion if stable disease/PR

Follow up studies

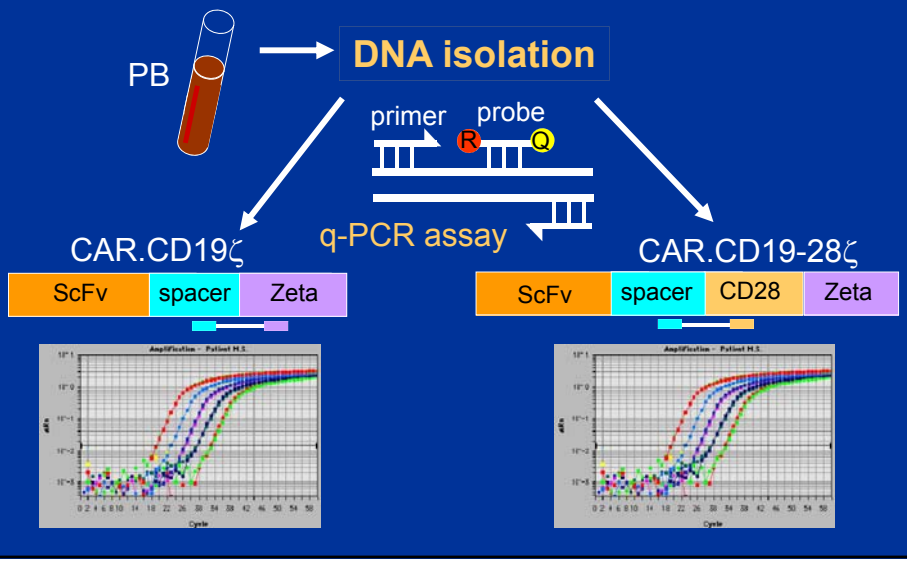
- Q-PCR for transgene in PBMC at multiple times after infusion

- CD19+ B-cells in peripheral blood

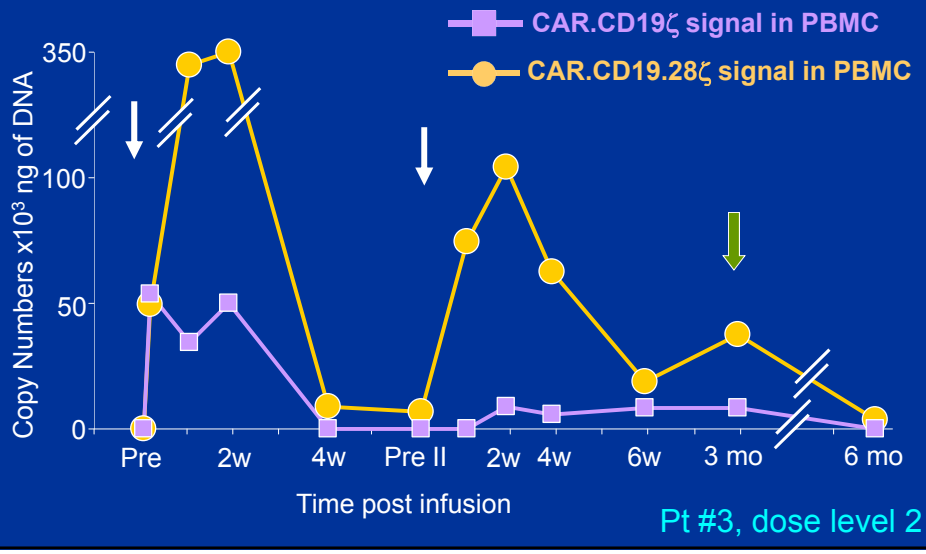
- Imaging (PET or CT scan) at 6 weeks

T cell infusions were well tolerated with no dose limited toxicity

Monitoring expansion and persistence of each product



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



Pt #3, dose level 2

Critical points

There is a consensus that CAR-modified T cells need the incorporation of costimulatory endodomains

Open questions

- Which is the best costimulatory endodomain?
- Do we need 3rd generation CARs "early and late" costimulation?
- Are preclinical models really predictive of efficacy and safety of 2nd vs. 3rd generation CARs?

We are planning to carefully compare second vs. third generation in lymphoma patients

What can we learn from these trials?

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

Which T cell subset?

- Polyclonal activated T lymphocytes
- Virus-specific T cells
- Central memory T cells
- Natural Killer cells
- Gamma/delta T cells
- NKT cells

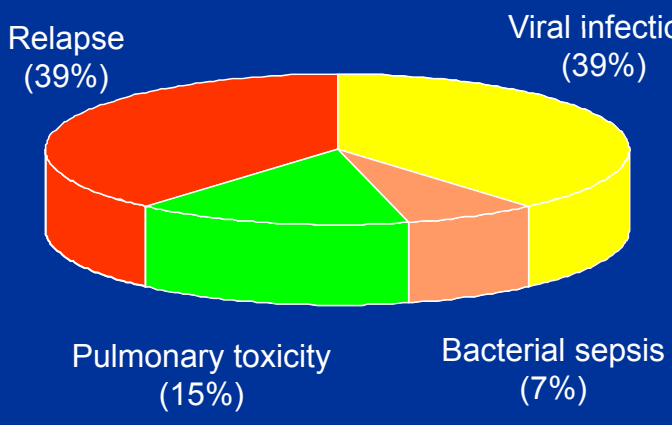
Multivirus-specific CTLs after HSCT-protective and efficacious *in vivo*

- Infused to 35 patients HSCT
- no evidence of GvHD

- Cleared
  - 9 of 9 EBV reactivations
  - 7 of 8 CMV reactivations
  - 8 of 8 adenovirus reactivations

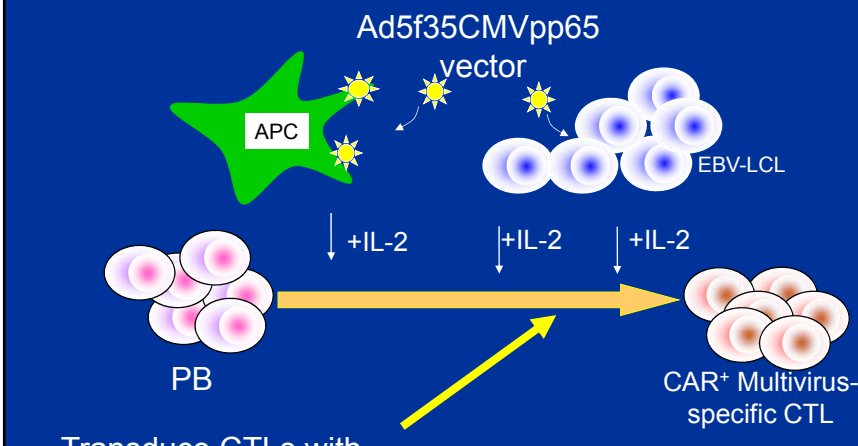
Nat Med. 2006;12(10):1160-1166

Critical problems: viral infections and relapse post-alternative donor SCT



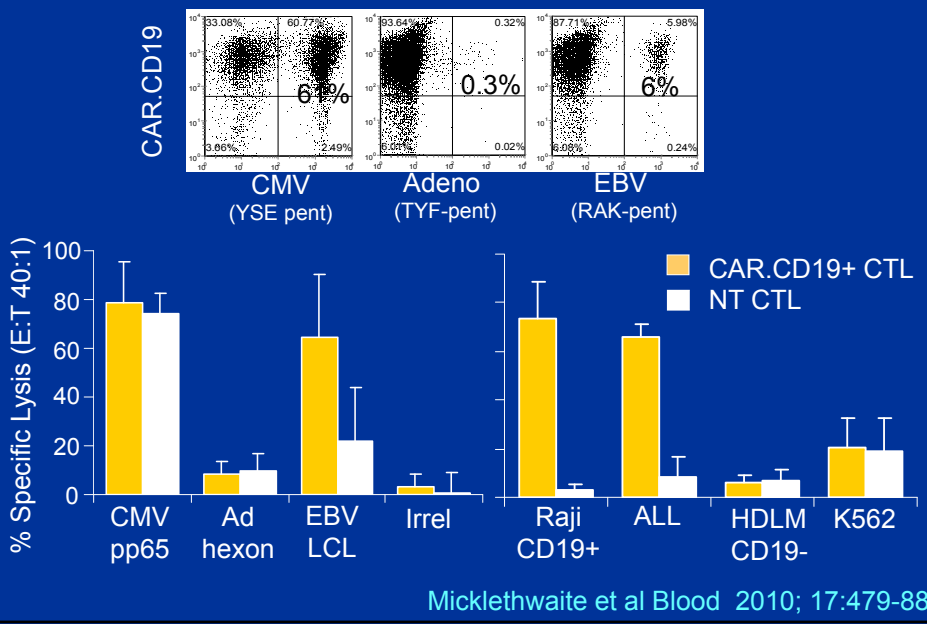
Alana Kennedy-Nasser, BBMT 2008

Generation of CAR.CD19^{+} multivirus-specific CTLs



Micklethwaite et al Blood 2010; 17:479-88

CAR.CD19 Multi Virus-specific CTLs



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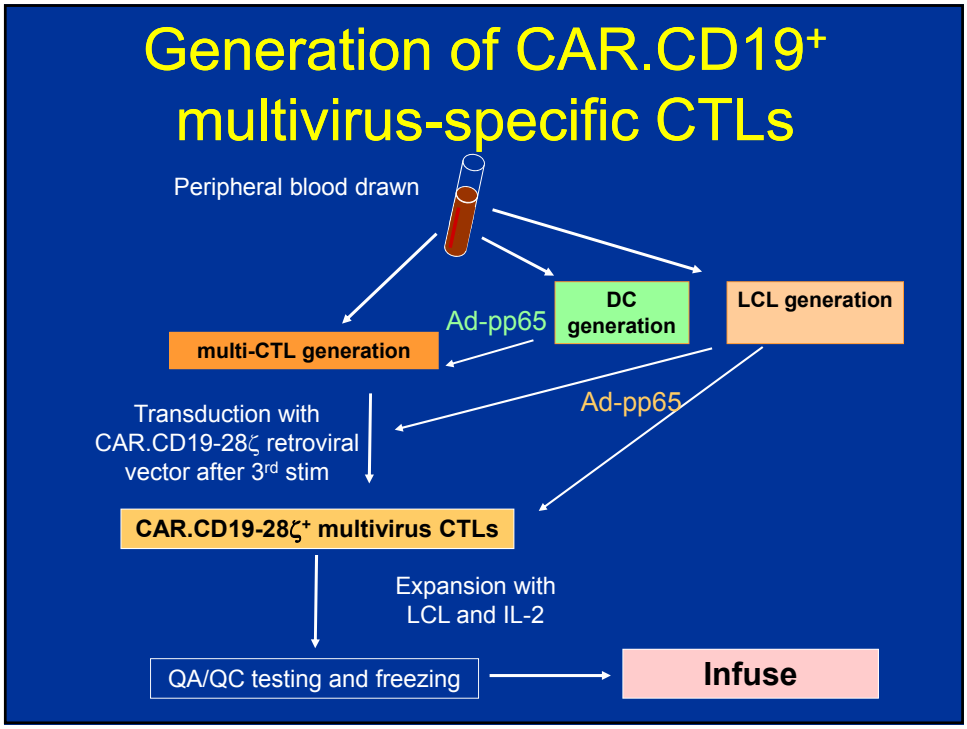
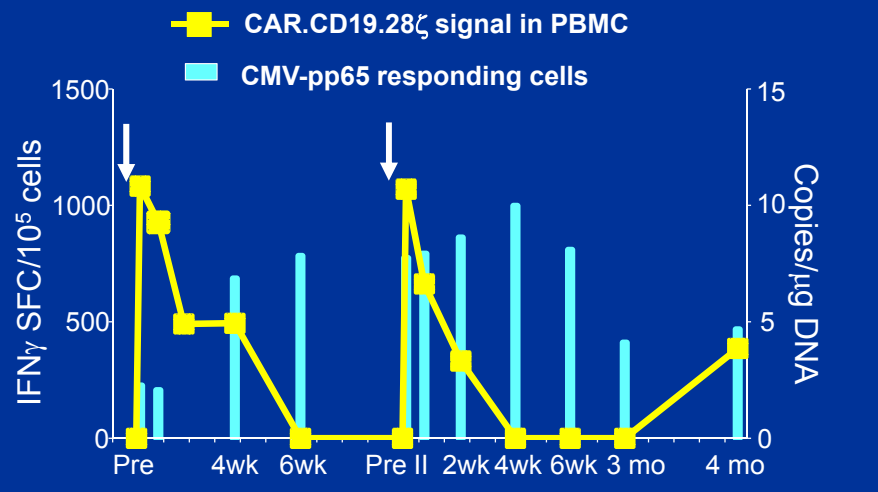
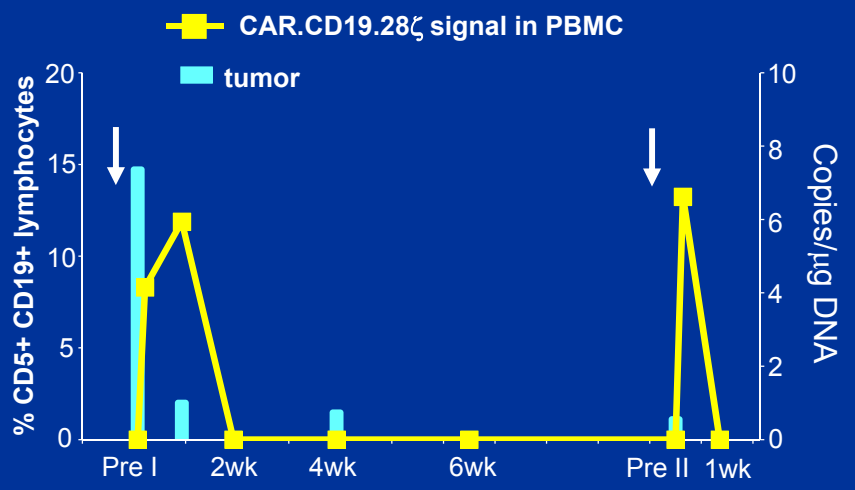


Pt 1: viral immune reconstitution and detection of CTLs



No viral infections

Pt 2: antitumor response and detection of CTLs



What can we learn from these trials?

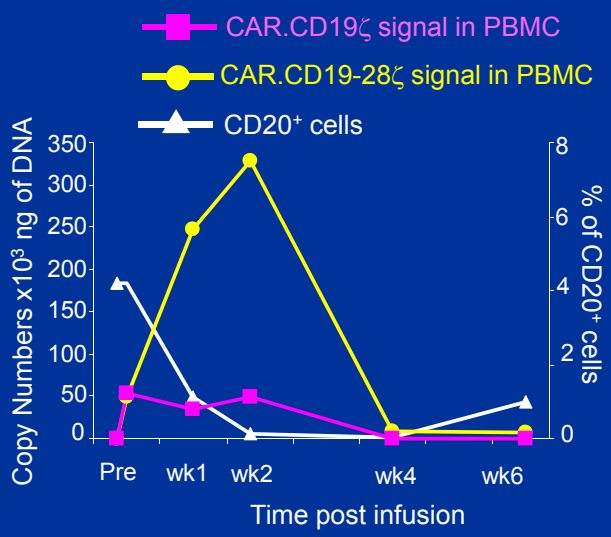
- Does the CAR expression vector affect the outcome?
- What co-stimulation is ideal for T cells?
- Is host lymphodepletion necessary?
- Which T cell subset should be used?
- What are the toxicities?

Toxicities

- On target toxicity
- On target but out of organ toxicity
- Massive T cell expansion/cytokine storm

On target toxicity (CD19-CAR)

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

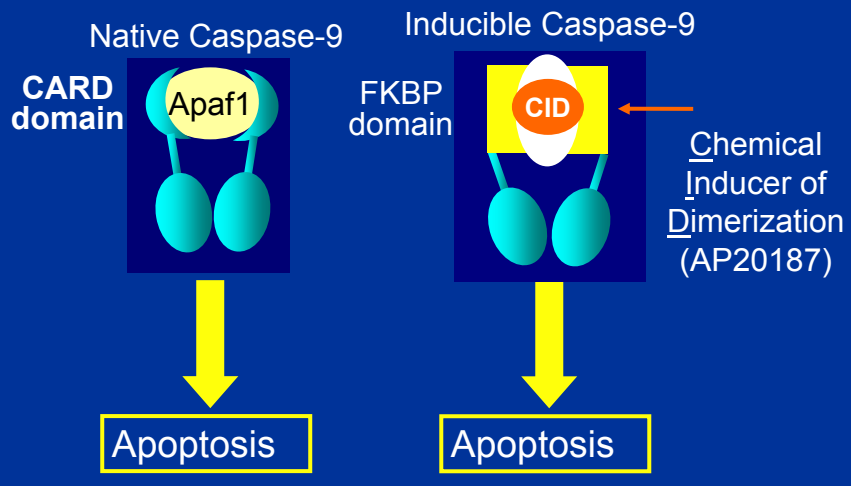


Toxicities

- On target toxicity
- On target but out of organ toxicity
  Her2 toxicity?
- Massive T cell expansion/cytokine storm
  4-1BB costimulation toxicity?

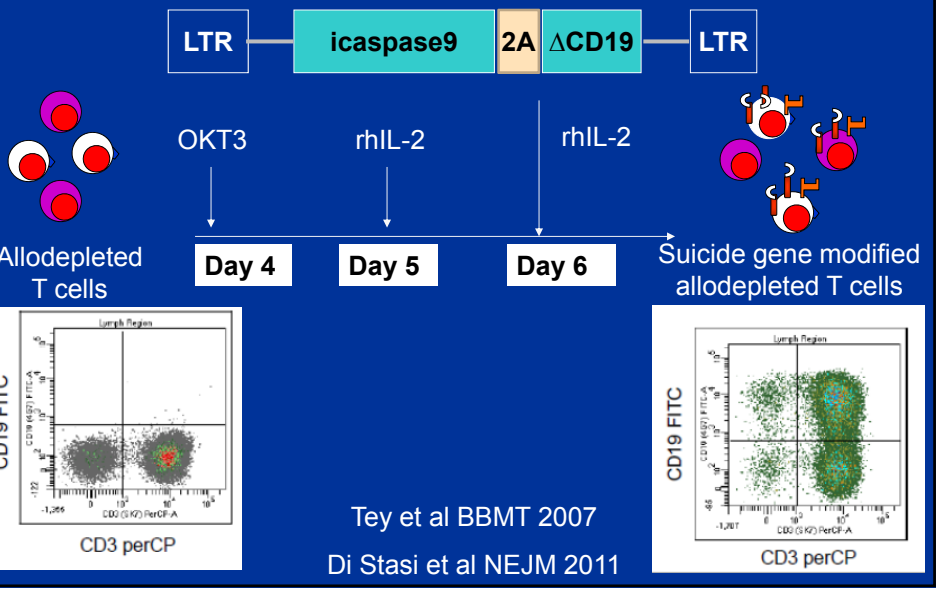
Do we need a suicide gene?

Inducible Caspase-9 suicide gene



Straathof et al. Blood 2005

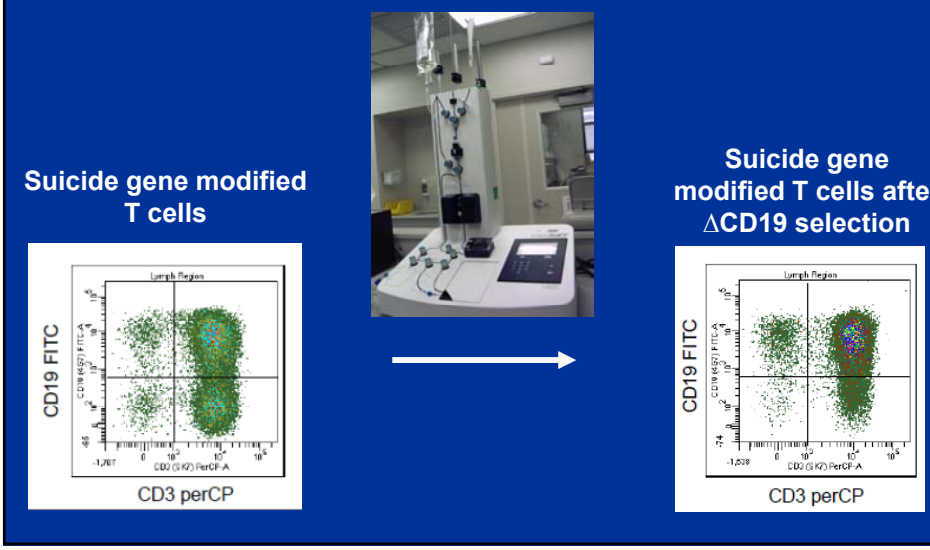
Validation of the suicide gene in a Phase I clinical trial



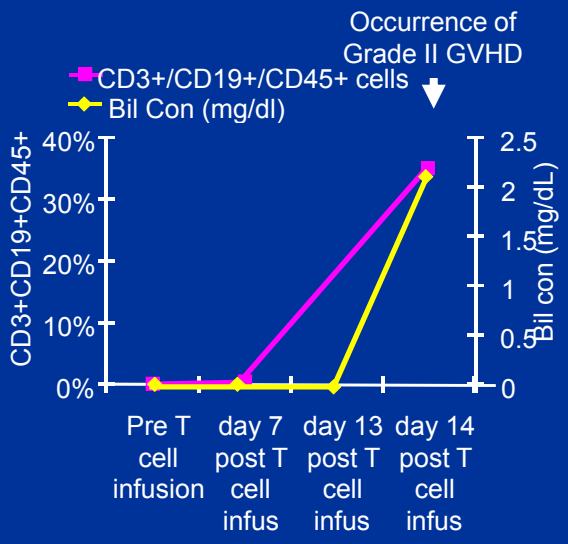
Tey et al BBMT 2007

Di Stasi et al NEJM 2011

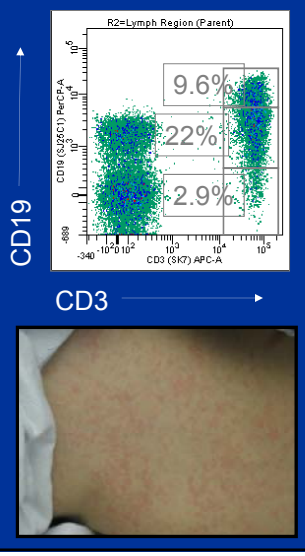
ΔCD19 iCasp9 Enrichment by Clinimacs



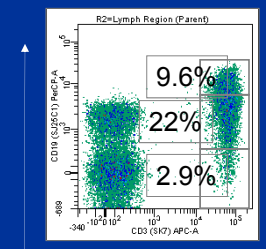
T Cell Depletion after CID



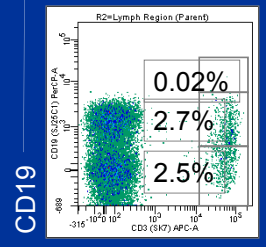
Peripheral Blood
14d post T cell infusion



T Cell Depletion after CID and skin GVD improvement



Pre CID infusion



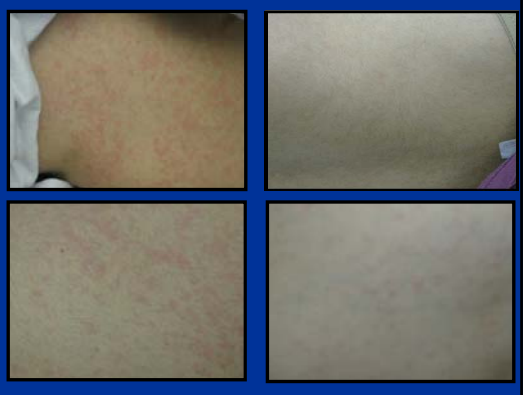
2 days after CID infusion

CD19

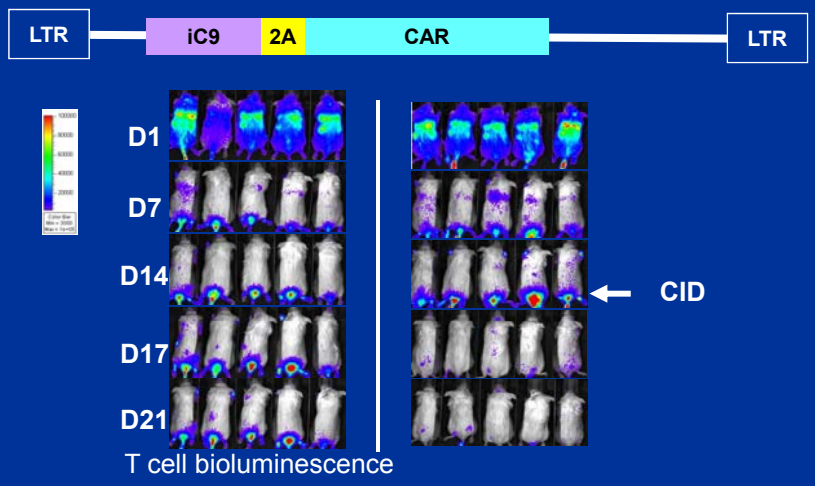
CD3

Pre AP1903

2d Post AP1903



Activation of the Caspase9 suicide gene eliminates CAR T cells *in vivo*



Hoyos et al. Leukemia 2010

Conclusions

- Adoptive transfer of CAR modified T cells is a realistic therapeutic opportunity
- T cell costimulation is required
- Profound lymphodepletion may not be required
- Other T cell subsets should be explored in specific clinical contexts
- Toxicity remains a concern

Acknowledgements

Malcolm Brenner
Cliona Rooney
Helen Heslop

Barbara Savoldo
Carlos Ramos
Catherine Bollard
Ann Leen
Stephan Gottschalk

Leonind Metelitsa

Postdocs
Russel Cruz
Kenneth Micklethwaite
Valentina Hoyos

Clinical Research
Bambi Grilley
Alicia Brown
Florence Noel
Yu-Feng Lin
Vicky Torrano

Statistical analysis
Hao Liu

GMP Laboratory
Adrian Gee
Oumar Diouf
Zhang Huimin
Joyce Ku
Weili Liu
Pallavi Mahopatra

Enli Liu
Debbie Lyon
Zhuyong Mei

Funding: PO1, Leukemia and Lymphoma Society Specialized Center of Research, Leukemia and Lymphoma Society translational Research grants, Doris Duke Distinguished Clinical Scientist Award, Doris Duke Developmental Award, NIH NGVL, R01 from NCI, CLL Global Research Foundation, DOD, GCRC