4TH INTERNATIONAL CONFERENCE

TRANSLATIONAL RESEARCH IN ONCOLOGY

CAR T CELLS: CHIMERA OR REALITY?

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Bambino Gesù
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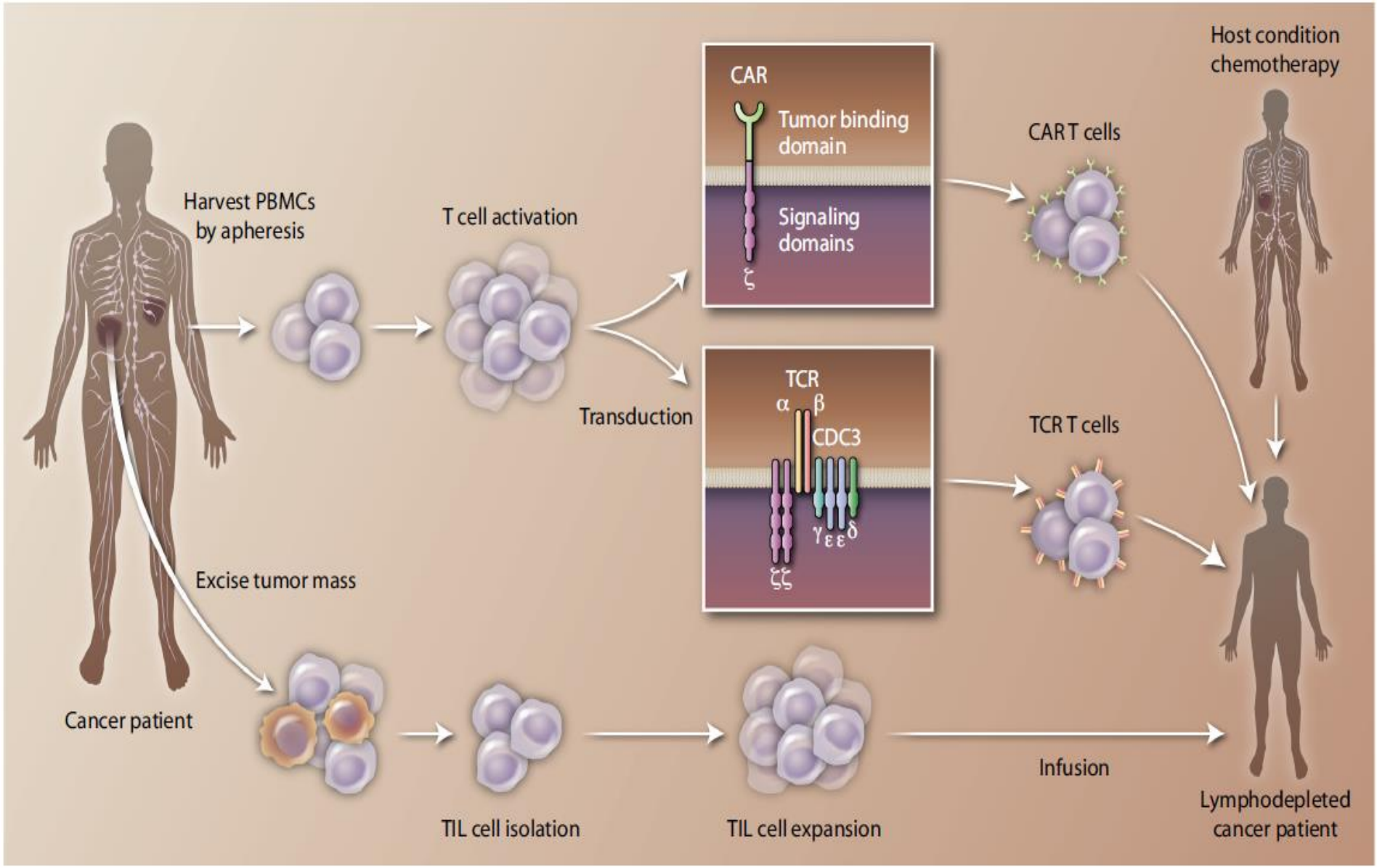
Science
Breakthrough of the Year
Cancer Immunotherapy
T cells on the attack
AAAS

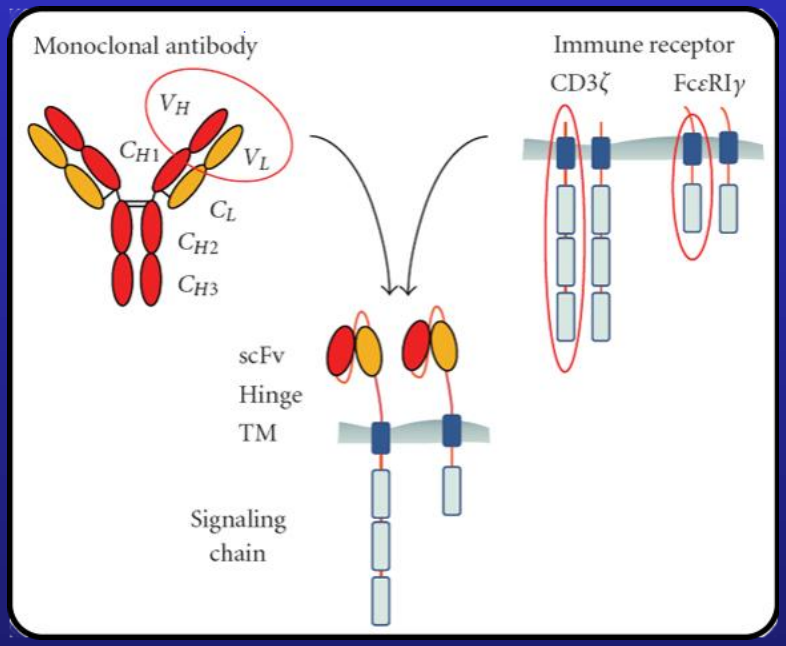
From antibodies to adoptive cell therapy

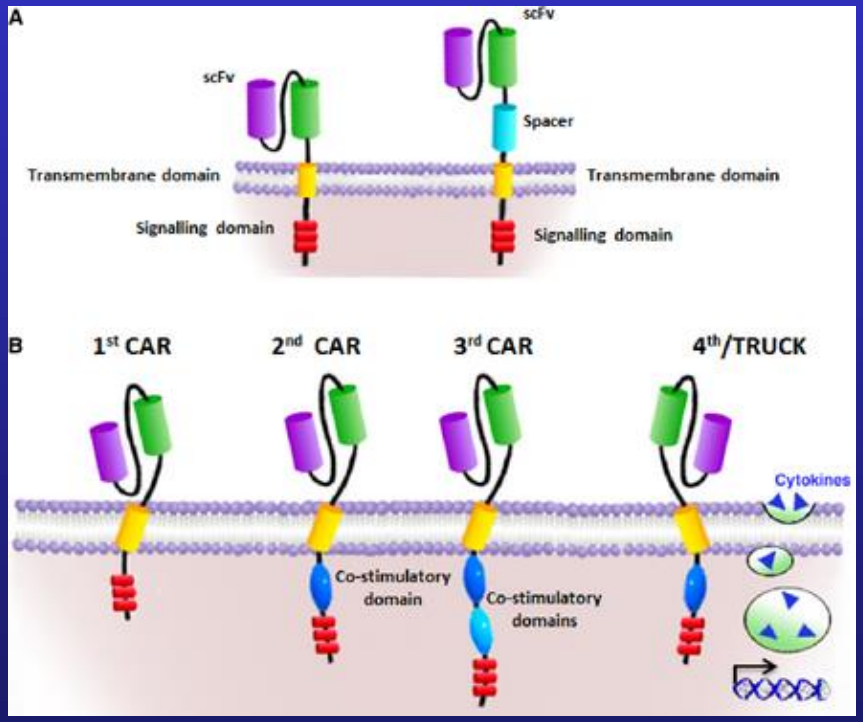


Adoptive T cell therapy projects at OPBG

- Evaluation of potential tumor-specific antigens;
- Cloning of specific T Cell Receptors (TCR, HLA-restricted);
- Cloning of specific Chimeric Antigen Receptors (CAR, HLA-unrestriceted);
- Production of clinical grade products;
- Conduction of Clinical Trials.

Chimeric Antigen Receptor





mAbs vs CARs

mAbs	CARs
Transient effect	Persistence for the prolonged lifetime of the cell
Limited tissue bio-distribution	Active penetration of solid tissues
Requirement for high expression of the target molecule	Ability to recognize tumor cell subsets with low antigen density
	Multiple lytic activities following target recognition

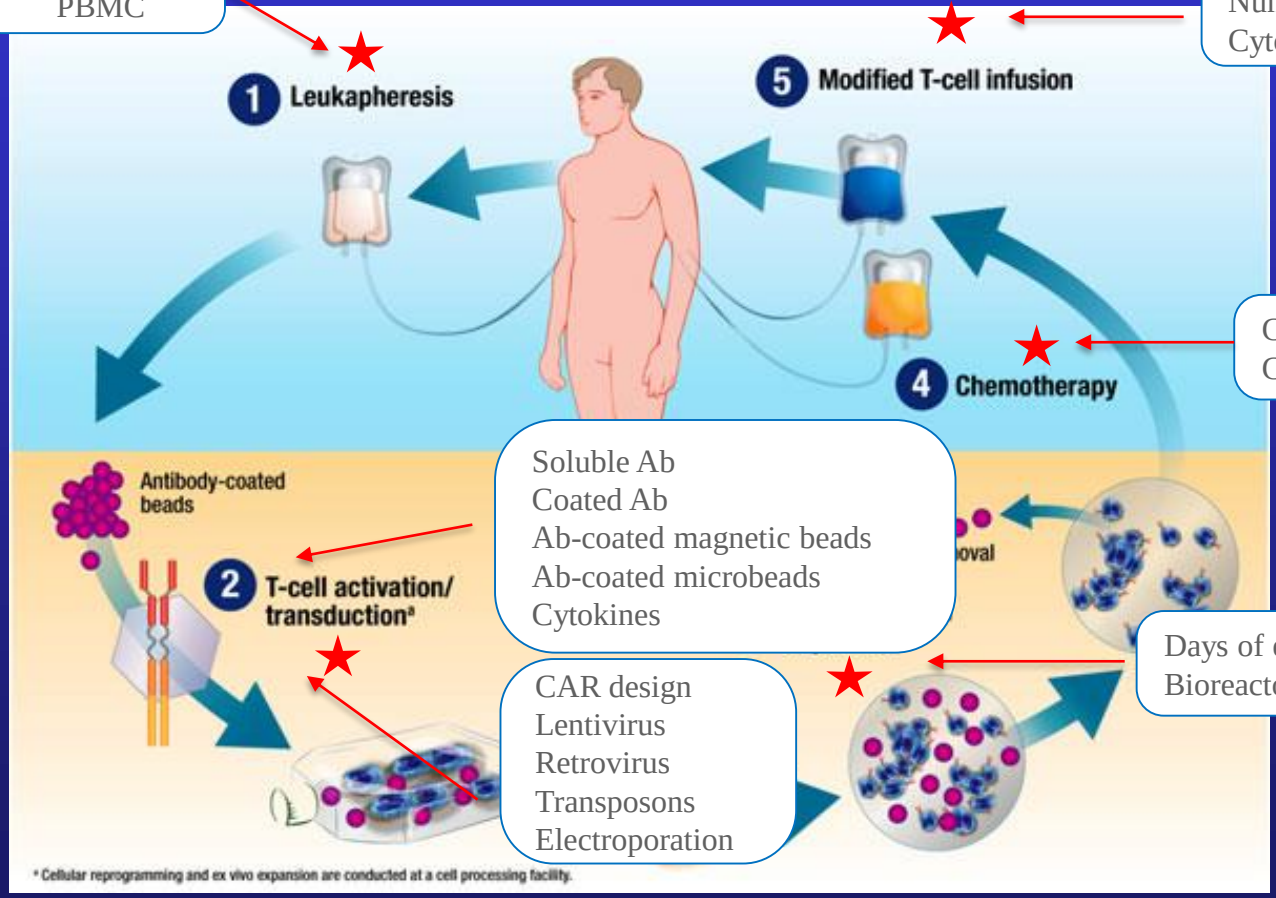
Toxicity

- Cytokine Release Syndrome (CRS)
  - Severity related to disease burden
  - Reversed with anti-IL6 therapy
  - Severe CRS mirrors HLH/MAS
- Tumor Lysis Syndrome
  - Not a prominent feature, but may be with high WBC
- Neurotoxicity
  - Seen in several CD19 immunotherapy trials: NCI, CHOP/UPENN, MSKCC, Blinatumomab
  - Fatal events have been recently reported
- Chronic B cell aplasia requiring IgG replacement

Example of approach to CAR T cell production

Fresh/frozen PBMC

Number of infused CAR+ T cells
Cytokine support Y/N



Conditioning chemotherapy Y/N
Chemotherapy sensitive disease Y/N

Soluble Ab
Coated Ab
Ab-coated magnetic beads
Ab-coated microbeads
Cytokines

Days of expansion
Bioreactors Y/N

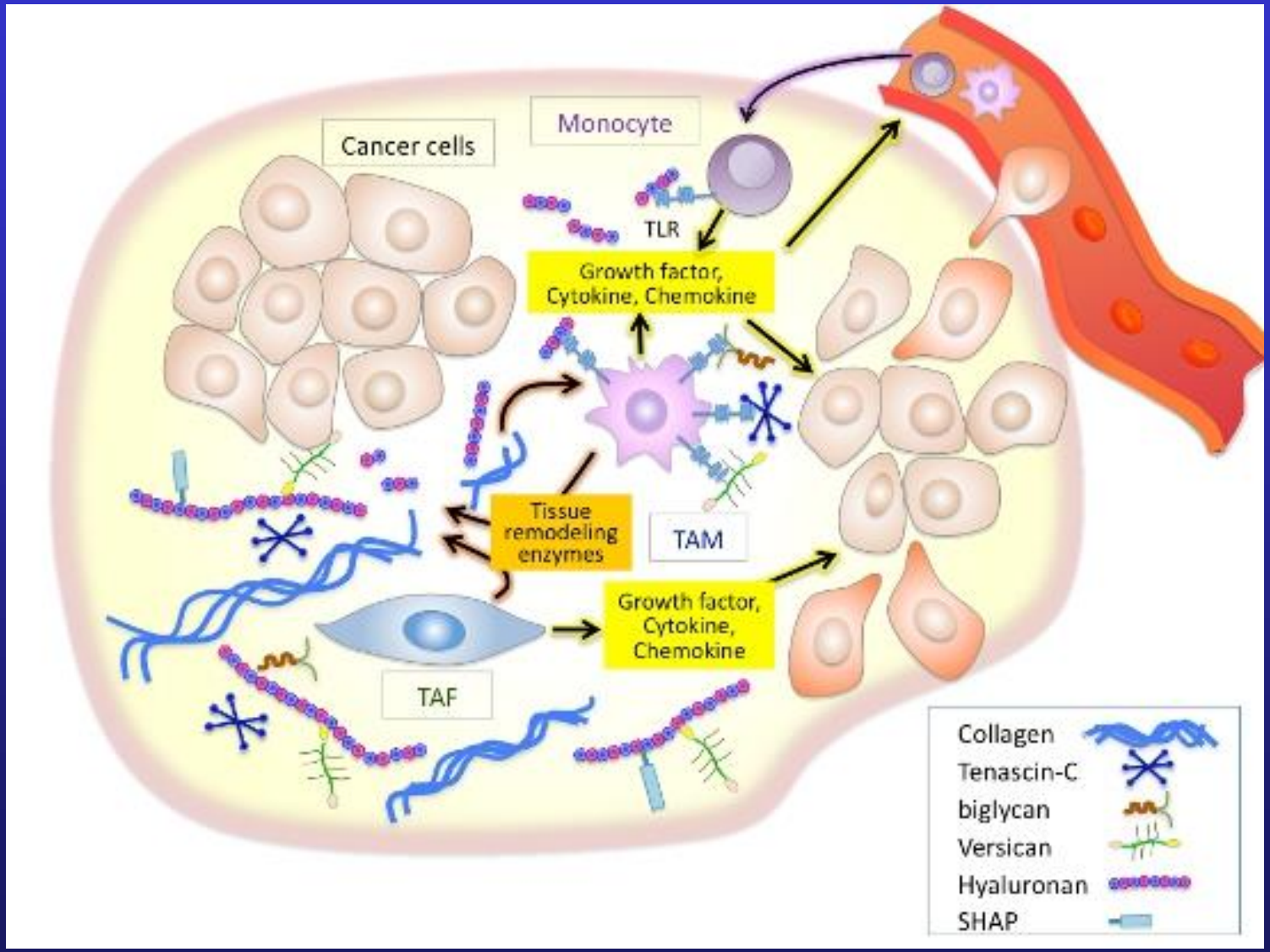
CAR design
Lentivirus
Retrovirus
Transposons
Electroporation

★ Major differences in the production/therapy steps for each trial

Ab, antibody; PBMC, peripheral blood mononuclear cells

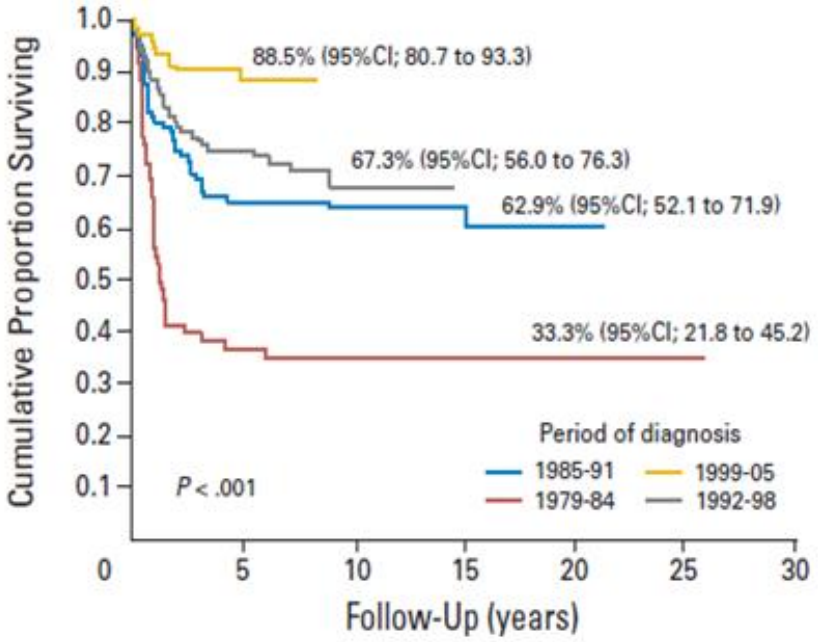
Adapted from http://global.onclive.com/publications/contemporary-oncology/2014/august-2014/chimeric-antigen-receptor-car-t-cell-immunotherapy-for-leukemia-and-beyond. Accessed April 2016

NEXT CHALLENGES: CAR T cell in solid tumors

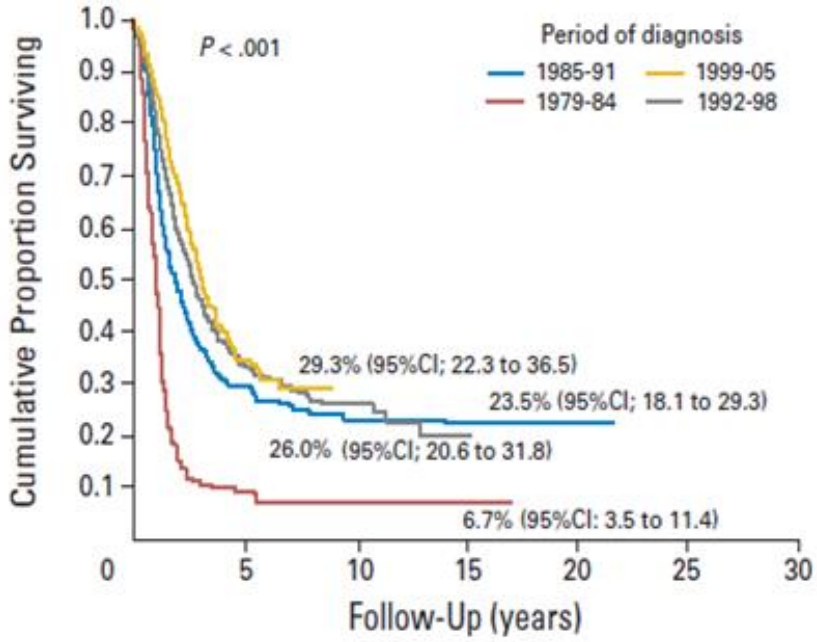


Neuroblastoma

Localized Neuroblastoma



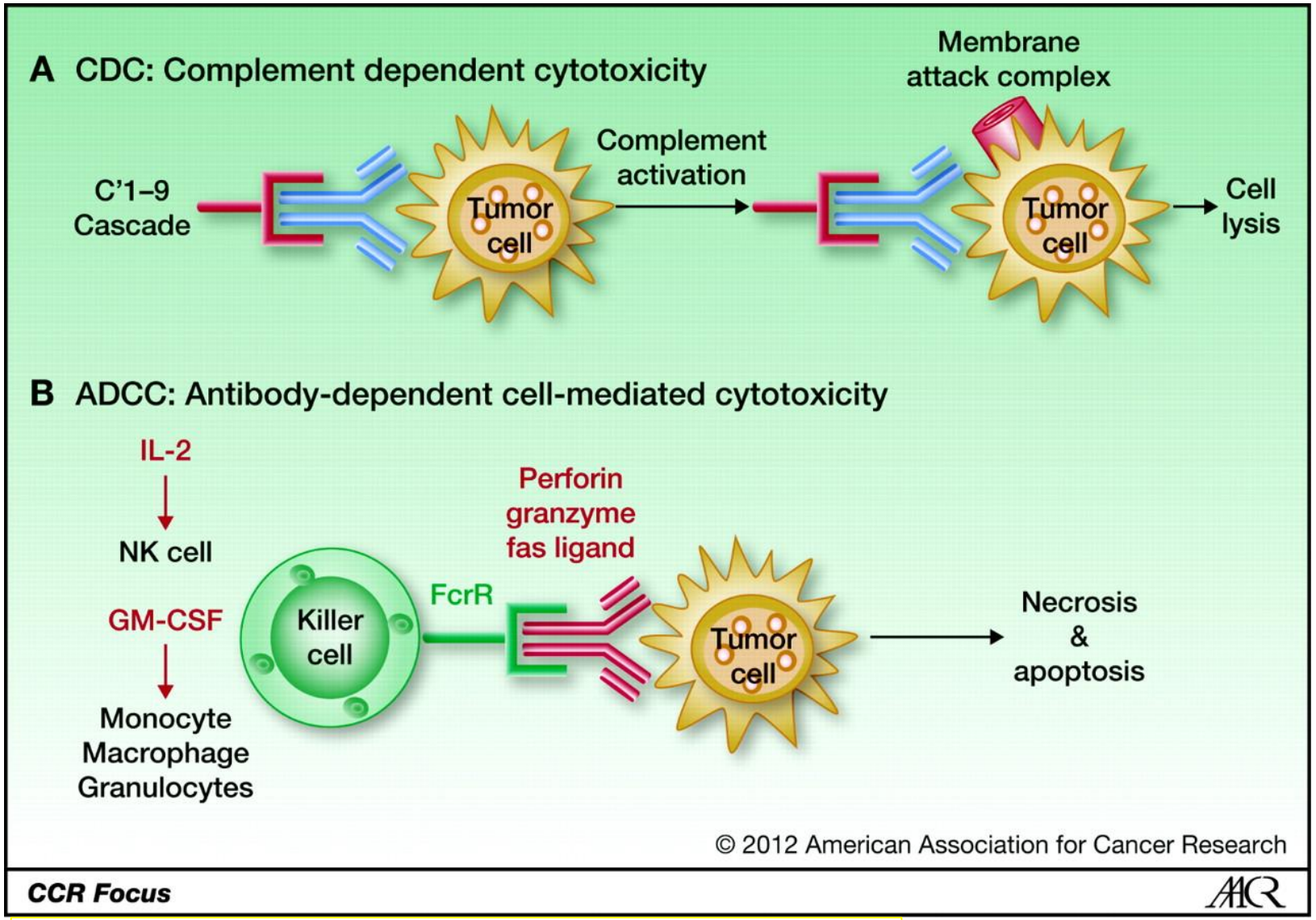
Disseminated Neuroblastoma



Abbreviations: GN, ganglioneuroma; GNB, ganglioneuroblastoma.

Zage PE et al. new aspect of neuroblastoma treatment: ASPHO 2011 symposium review. Pediatric blood & cance 20012.

Mechanism of GD2 antibody-targeted destruction of neuroblastoma by CDC and ADCC



Matthay K K et al. Clin Cancer Res 2012;18:2740-2753

The NEW ENGLAND JOURNAL of MEDICINE

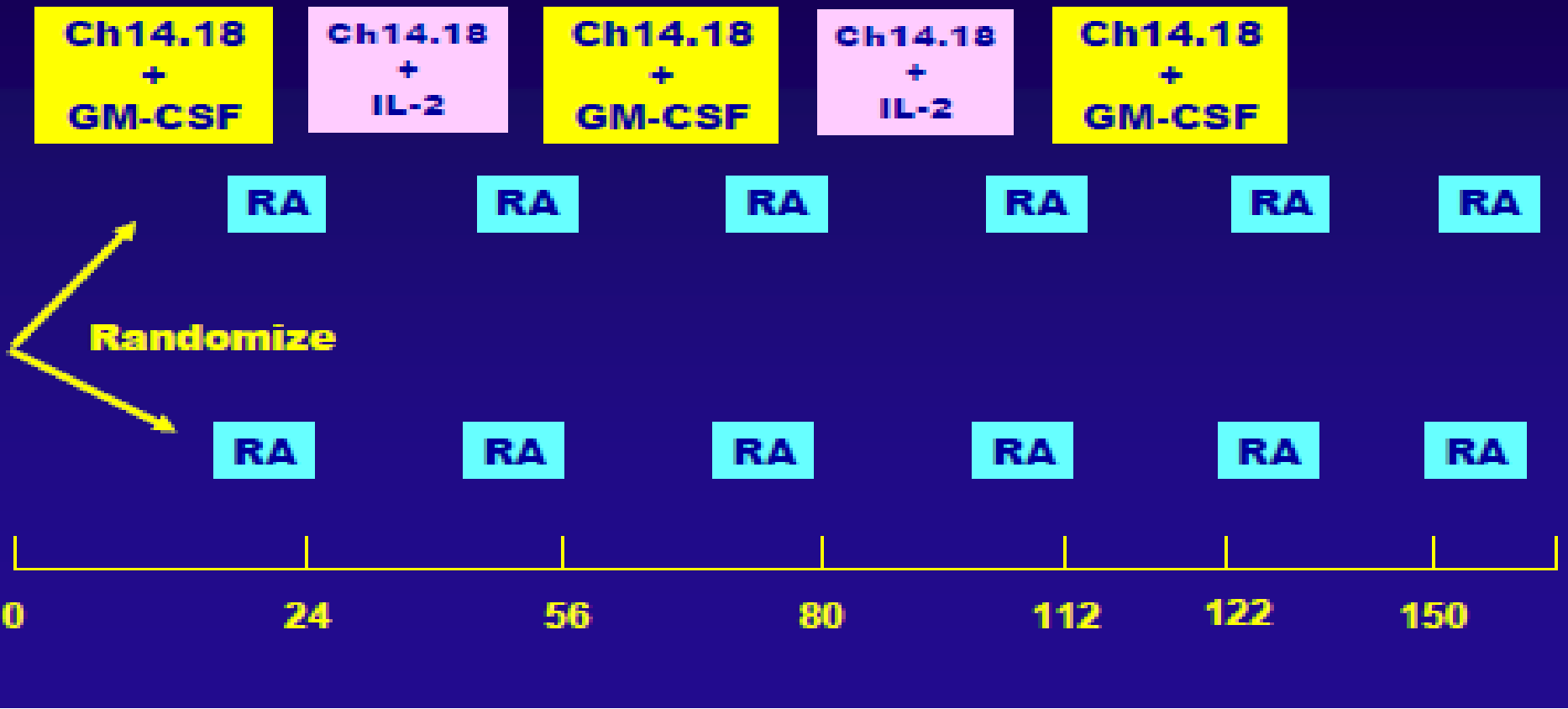
ORIGINAL ARTICLE

Anti-GD2 Antibody with GM-CSF, Interleukin-2, and Isotretinoin for Neuroblastoma

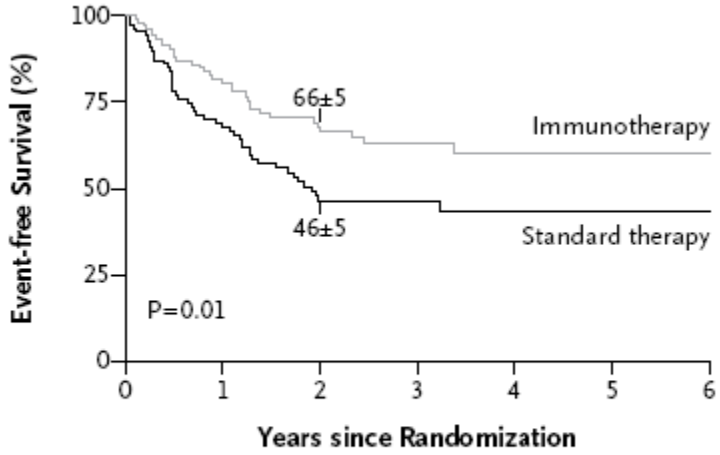
Alice L. Yu, M.D., Ph.D., Andrew L. Gilman, M.D., M. Fevzi Ozkaynak, M.D., Wendy B. London, Ph.D., Susan G. Kreissman, M.D., Helen X. Chen, M.D., Malcolm Smith, M.D., Ph.D., Barry Anderson, M.D., Judith G. Villablanca, M.D., Katherine K. Matthay, M.D., Hiro Shimada, M.D., Stephan A. Grupp, M.D., Ph.D., Robert Seeger, M.D., C. Patrick Reynolds, M.D., Ph.D., Allen Buxton, M.S., Ralph A. Reisfeld, Ph.D., Steven D. Gillies, Ph.D., Susan L. Cohn, M.D., John M. Maris, M.D., and Paul M. Sondel, M.D., Ph.D., for the Children's Oncology Group

ANBL0032: A COG Study

13-cis-Retinoic Acid ± Ch14.18 with cytokines post Stem Cell Transplant



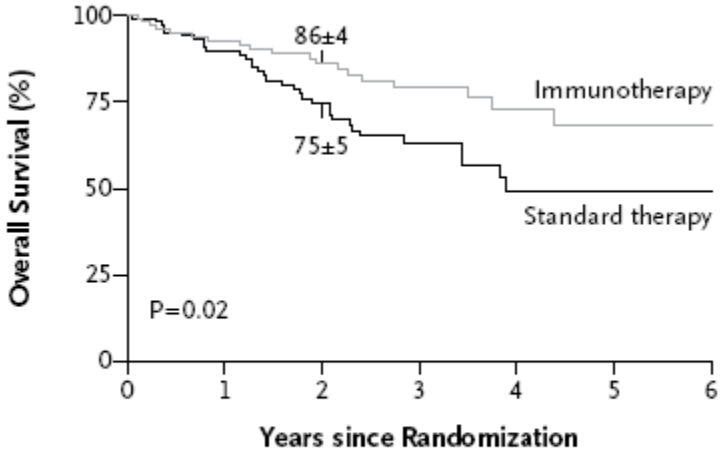
A Event-free Survival



No. at Risk

Immunotherapy	113	69	47	29	15	9	3
Standard therapy	113	59	32	20	10	8	1

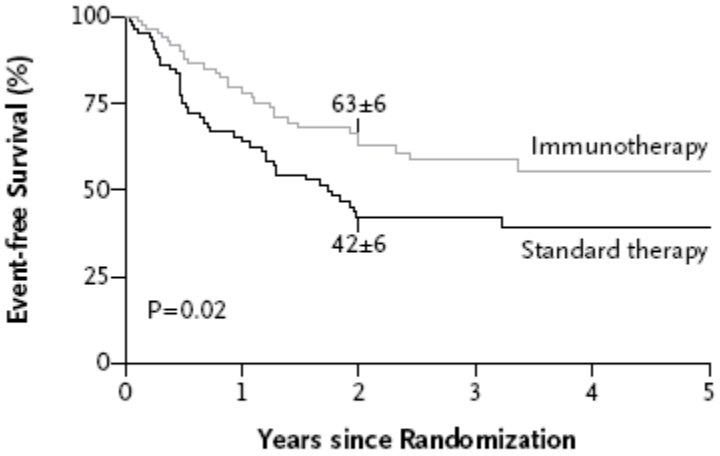
B Overall Survival



No. at Risk

Immunotherapy	113	77	59	37	20	10	3
Standard therapy	113	79	51	26	12	9	1

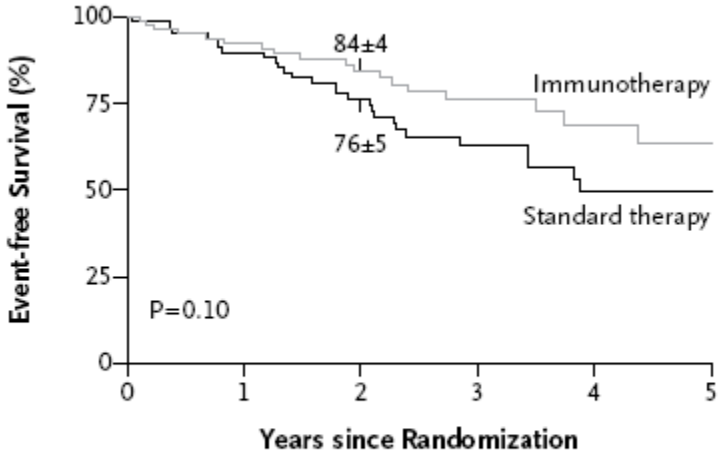
C Event-free Survival for ≥1-Yr-Olds with Stage 4 Disease



No. at Risk

Immunotherapy	89	56	37	22	11	7
Standard therapy	90	46	26	19	10	8

D Overall Survival for ≥1-Yr-Olds with Stage 4 Disease



No. at Risk

Immunotherapy	89	64	49	30	16	8
Standard therapy	90	65	45	25	12	9

Yu AL, et al. N Engl J Med 2010

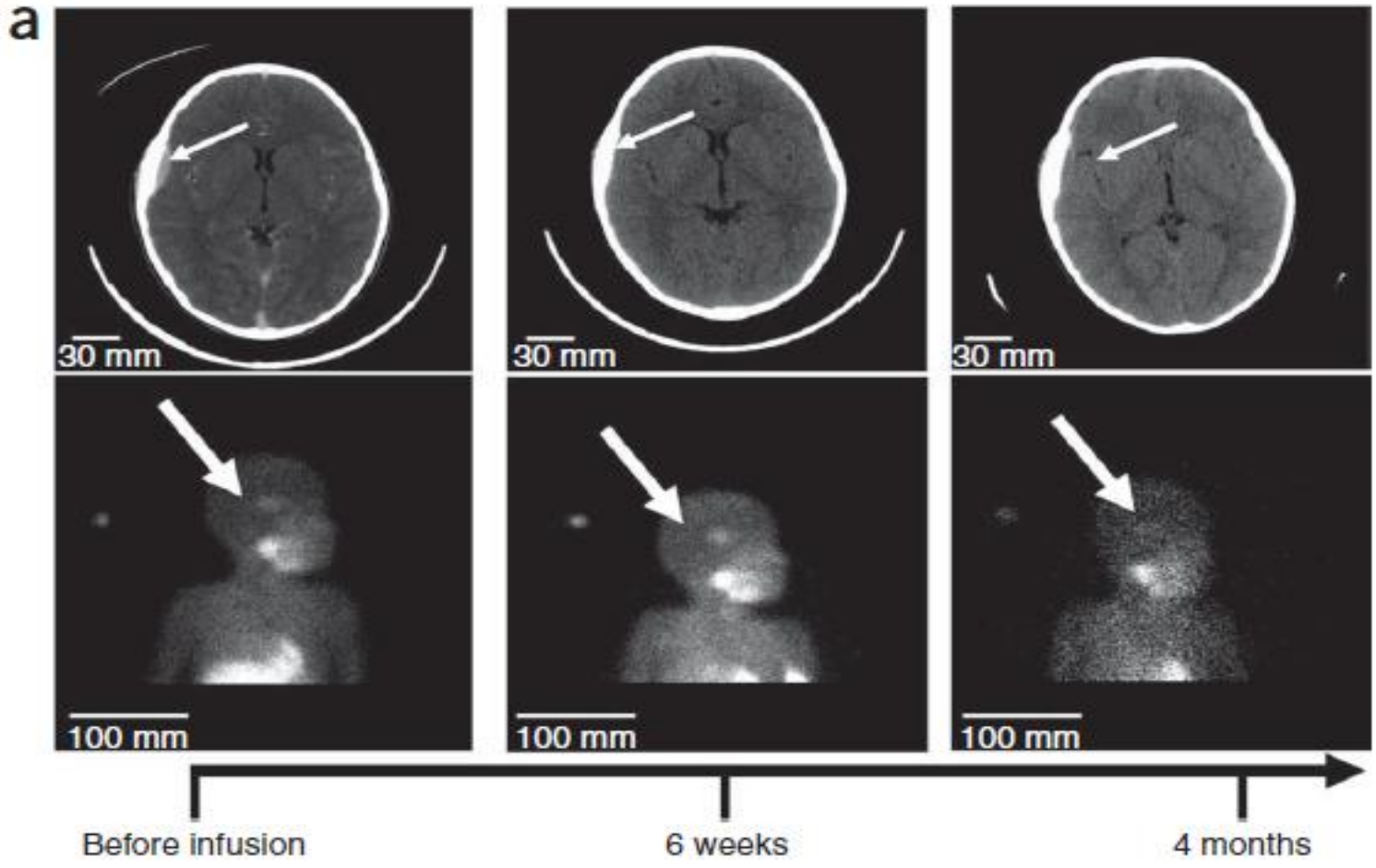
Baylor College of Medicine (BCM) Phase I trial (NCT00085930) – Study design

- 19 pts with High-Risk Neuroblastoma, relapsed/refractory or after initial therapy
- Partial in vivo lymphodepletion (unconjugated rat anti-hCD45)
- First generation CAR-ATCs + CAR-CTLs administered at 3 dose levels:
  - $1{,}2 \times 10^7$ cells/m^2 ($0{,}4 \times 10^6$ cells/Kg)
  - 5×10^7 cells/m^2 (1.7×10^6 cells/Kg)
  - 1×10^8 cells/m^2 (3.3×10^6 cells/Kg)

Safety data

No severe or dose-limiting toxicities have been identified.
Three patients had grade 1 to 3 localized pain (2 at a site of biopsy-proven tumor necrosis and 1 in her lower leg at a site with no evidence of active disease).

Louis C. et al, Blood 2001



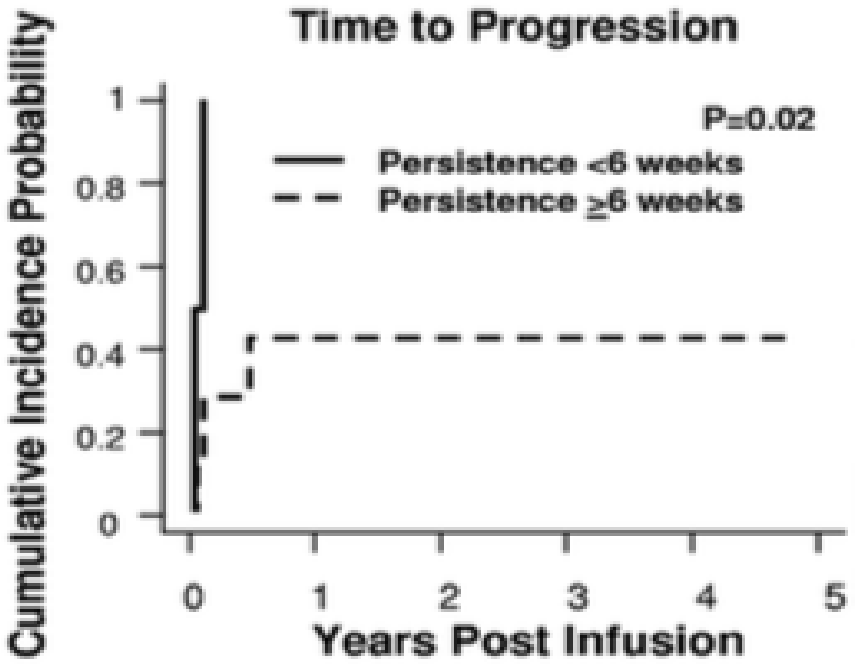
Pule M, et al. Nature Medicine 2008

BCM Phase I trial (NCT00085930) – Results (1)

CAGT no.	Age, y	Sex	Stage at diagnosis	Dose level	Disease burden at CTL infusion	Response at 6 weeks	Best response	GD2 T cells last detected, weeks after infusion	Clinical outcome
1662	9	Male	IV	1	NED	NED	NED	24	NED 11 mo after infusion
1738	5	Male	IV	1	NED	NED	NED	1	NED 10 mo after infusion
1705	4	Male	IV	1	NED	NED	NED	4	NED 10 mo after infusion
1632	20	Female	IIa	1	Relapsed, NED	NED	NED	2	AWD 13 mo after infusion
1629	7	Male	IV	1	Relapsed, NED	NED	NED	12	AWD 7 mo after infusion
1571	4	Female	IV	1	Relapsed, bone lesion	PD	PD	4	DOD 4 mo after infusion
1290	9	Female	IV	1	Relapsed, bone lesion	CR	CR	72	CR 1 yr 9 mo after infusion
1144	4	Female	IV	1	Refractory, bone lesion	PR	CR	192	CR 4 y 10 mo after infusion
1040	10	Male	IV	1	Relapsed, bulky	PD	PD	6	DOD 10 mo after infusion
717	11	Male	IV	1	Relapsed, bulky	PD	PD	1	DOD 2 mo after infusion
1151	10	Female	IV	2	Relapsed, NED	NED	NED	2	DOD 3 y after infusion
1089	4	Female	IV	2	Relapsed, NED	NED	NED	96	NED 3 y 3 mo after infusion
1035	15	Female	IV	2	Relapsed, bone marrow	CR	CR	6	DOD 6 mo after infusion
1117	9	Female	IV	2	Relapsed, bulky	PD	PD	28	DOD 10 mo after infusion
1208	3	Male	IV	2	Relapsed, bulky	SD	SD	12	DOD 6 mo after infusion
1253	9	Female	III	2	Relapsed, bulky	Tumor necrosis	Tumor necrosis	4	DOD 14 mo after infusion
1353	7	Male	IV	3	Relapsed, NED	NED	NED	12	DOD 2 y 7 mo after infusion
1237	4	Female	IV	3	Relapsed, bulky	Tumor necrosis	Tumor necrosis	2	DOD 2 mo after infusion
1361	7	Male	IV	3	Relapsed, bulky	SD	PR	72	AWD 2 y 8 mo after infusion

Louis C. et al, Blood 2001

BCM Phase I trial (NCT00085930) – Results (2)

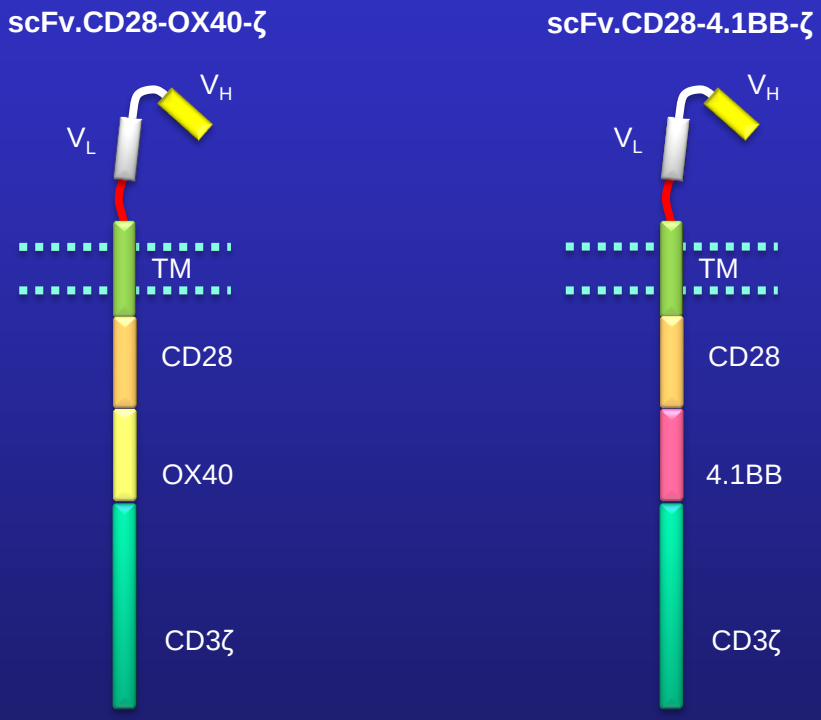


→ Improving CAR T cells persistence is mandatory:

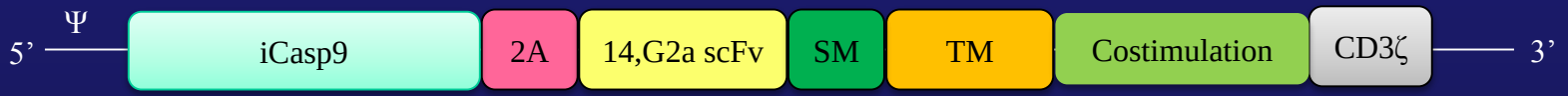
- Lymphodepletion
- 2nd and 3rd generation CAR constructs

Louis C. et al, Blood 2001

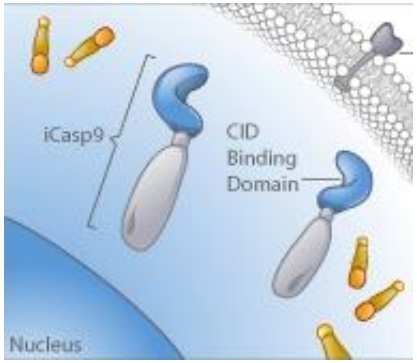
Third Generation CARs Targeting GD2 (14.G2a)



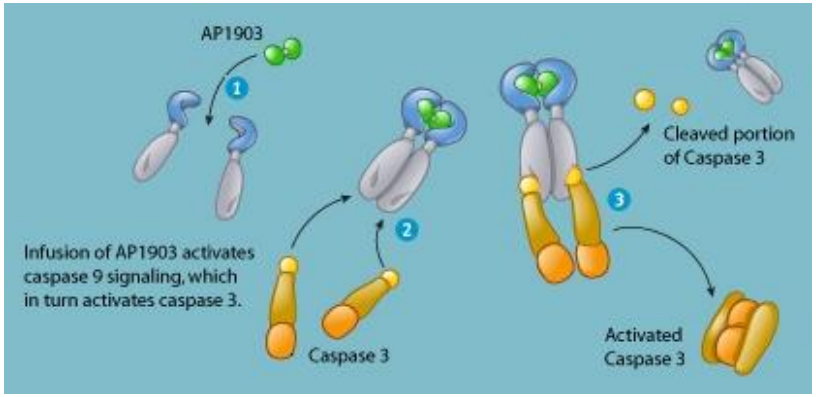
Which is the optimal co-stimulation for adoptive T cell therapy in neuroblastoma?

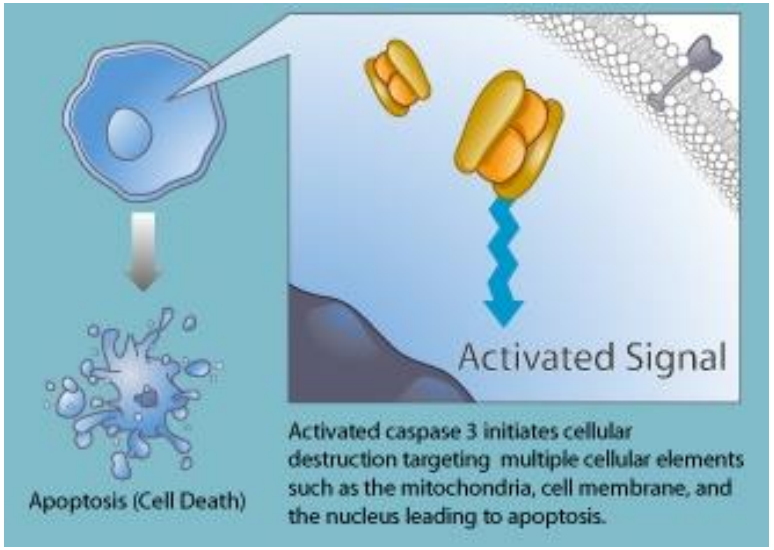


iC9 suicide gene



iCasp9 = FKBP12v36 + ΔCaspase9
*All human sequence





BP-004 Study
Clinical trial started on September 2014 in OPBG

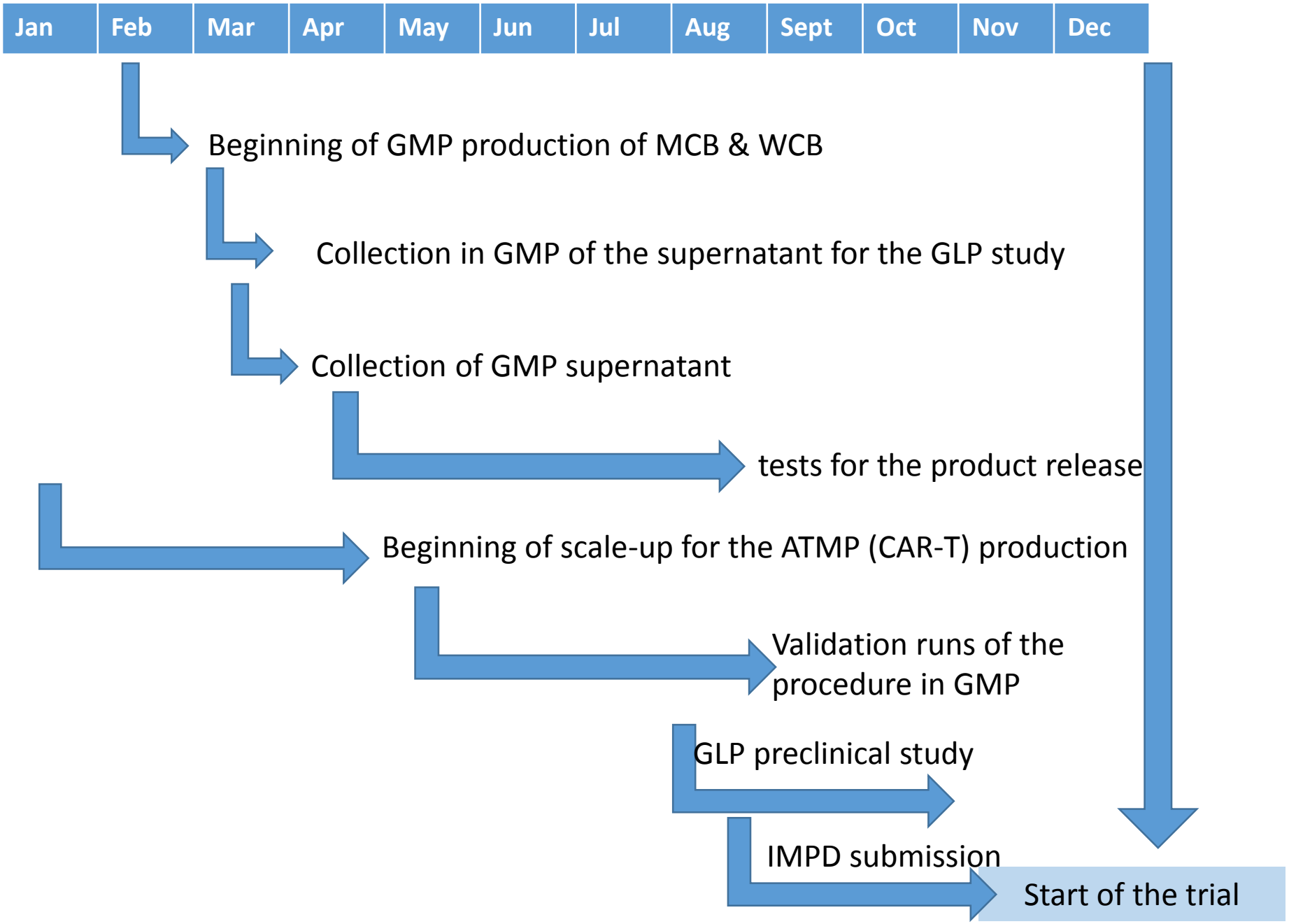
Phase I/II Study of BPX-501 T Cells from an HLA-partially Matched Family Donor After Negative Selection of TCR αβ+T Cells in Pediatric Patients With Hematological (malignant and non-malignant) Disorders

ClinicalTrials.gov identifier: NCT02065869
EUDRACT number: 2014-000584-41

Safety profile

- ✓ **Cytokine-dependent expansion**
- ✓ **Vector copy Number Analysis**
- ✓ **Polyclonality**
- ✓ **Cytogenetic Analysis**
- ✓ **Molecular Cytogenetic Analysis (CGH Array)**
- ✓ **Telomer elongation**

2016 timeline for starting treatment of patient



Ongoing clinical trials

Antigen	Cancers	Gene transfer	CAR construct	Lymphodepletion	Dose levels	Phase/ID	Sponsor
GD2	Sarcoma Neuroblastoma Melanoma	Retrovirus	3rd generation + iC9	Cyclo (1800 mg/m^2/day x 2 days)	DL1: 1 x 10^5/Kg DL2: 1 x 10^6/Kg DL3: 3 x 10^6/Kg DL4: 1 x 10^7/kg	I/NCT02107963	NCI
GD2	Neuroblastoma	Lentivirus	Not reported (4th generation)	Flu (25 mg/m^2 days -4, -3, -2) + Cyclo (300 mg/m^2, days -4, -3, -2)	Not reported	II/NCT02765243	Zhujiang Hospital
GD2	GD2-positive sarcoma	Retrovirus	3rd generation + iC9 (VZV-Tcells)	None (VZV vaccine boosting)	DL1: 1 x 10^6/m^2 DL2: 1.5 x 10^7/m^2 DL8: 1 x 10^8/m^2	I/NCT01953900	BCM
GD2	Neuroblastoma	Retrovirus	3rd generation + iC9	Cyclo (500 mg/m^2 x 2 days) + Flu (30 mg/m^2 x 3 days) + Pembrolizumab (2 mg/kg on days -1 and +21)	DL1: 1 x 10^8/m^2 DL2: 1.5 x 10^8/m^2 DL3: 2 x 10^8/m^2	I/NCT01822652	BCM
GD2	Neuroblastoma	Not reported	Not reported	Cohort I: none Cohort II: Cyclo (300 mg/m2, days -4 to -1) Cohort III: Cyclo (300 mg/m2, days -4 to -1) + Flu (25 mg/m^2 days -5 to -1)	DL1: 1 x 10^7/m^2 DL2: 1 x 10^8/m^2	I/NCT02761915	Cancer Research UK
GD2	Neuroblastoma	Retrovirus	3rd generation + iC9 (NK T cells)	Cyclo (500 mg/m^2 x 2 days) + Flu (30 mg/m^2 x 3 days)	DL1: 3 x 10^6/m^2 DL2: 1 x 10^7/m^2 DL3: 3 x 10^7/m^2 DL4: 1 x 10^8/m^2	I/NCT02439788	BCM

How can we improve patients outcome ?

- One of the characteristics of the ideal tumor-specific T cell is the ability to infiltrate tumor environment.

- To invade tumor environment T cells must digest :
  - Sub-endothelial basement membrane (SBM)
  - Extracellular matrix (ECM)

- Major components of SBM and ECM are Heparan sulphate proteoglycans (HSPGs)

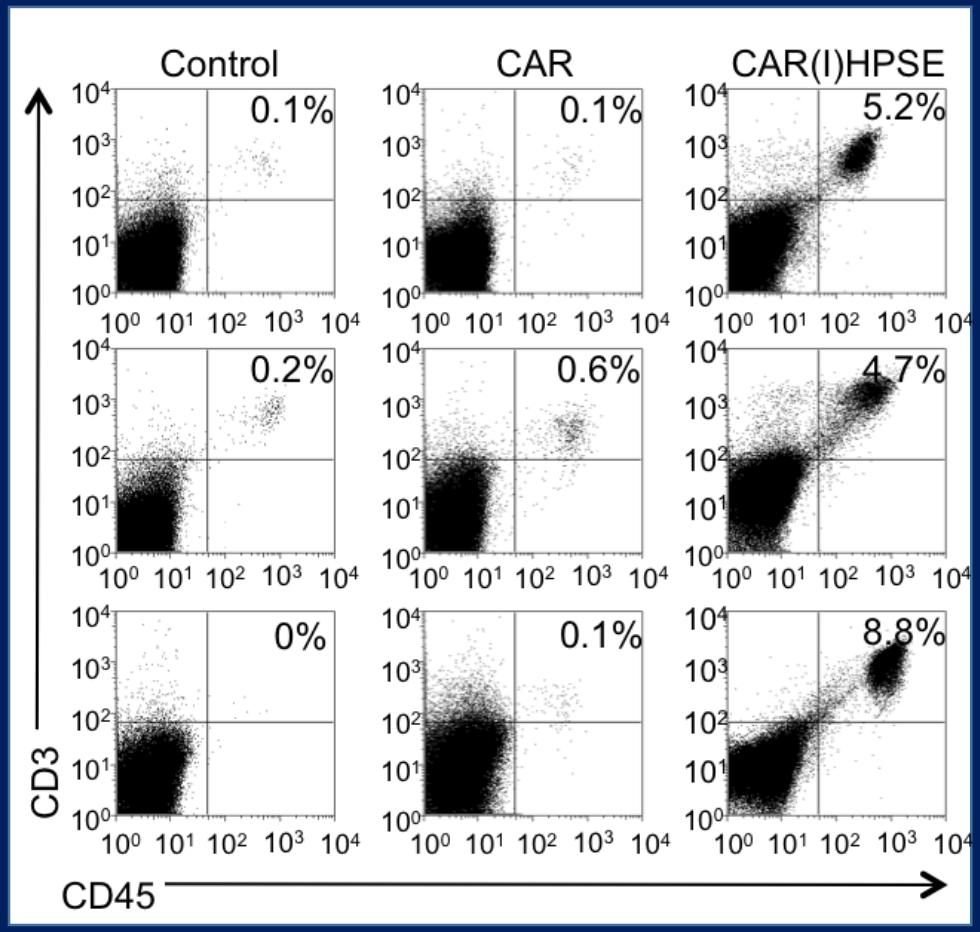
- T cells must release the enzyme heparanase to degrade HSPGs

Caruana I, et al. Nature Medicine, April 2015

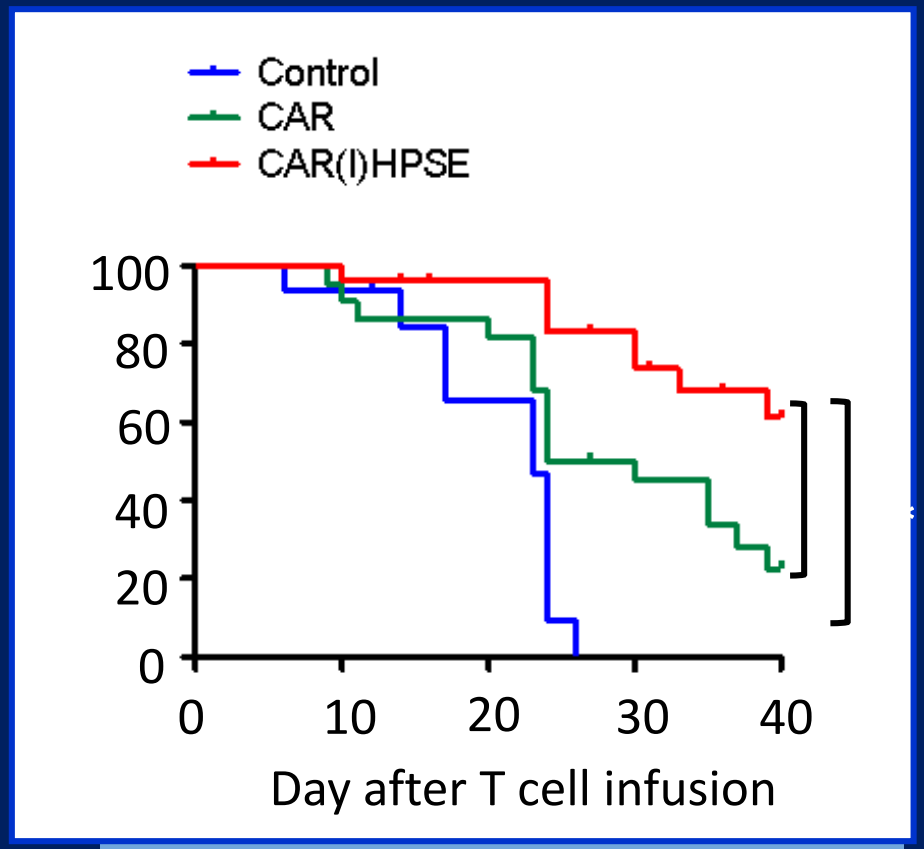
HPSE and CAR-GD2 co-expression improve overall survival in NB xenograft mouse models (I)

NOG/$\gamma_c^{-/-}$ 

CHLA255 - IP T cells - IP



Day 10 after T cell infusion



Tumor free at day 40:

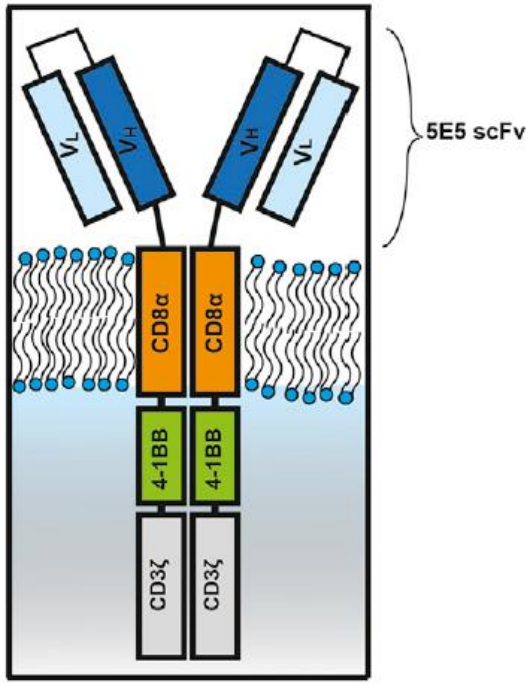
CAR: 6/22

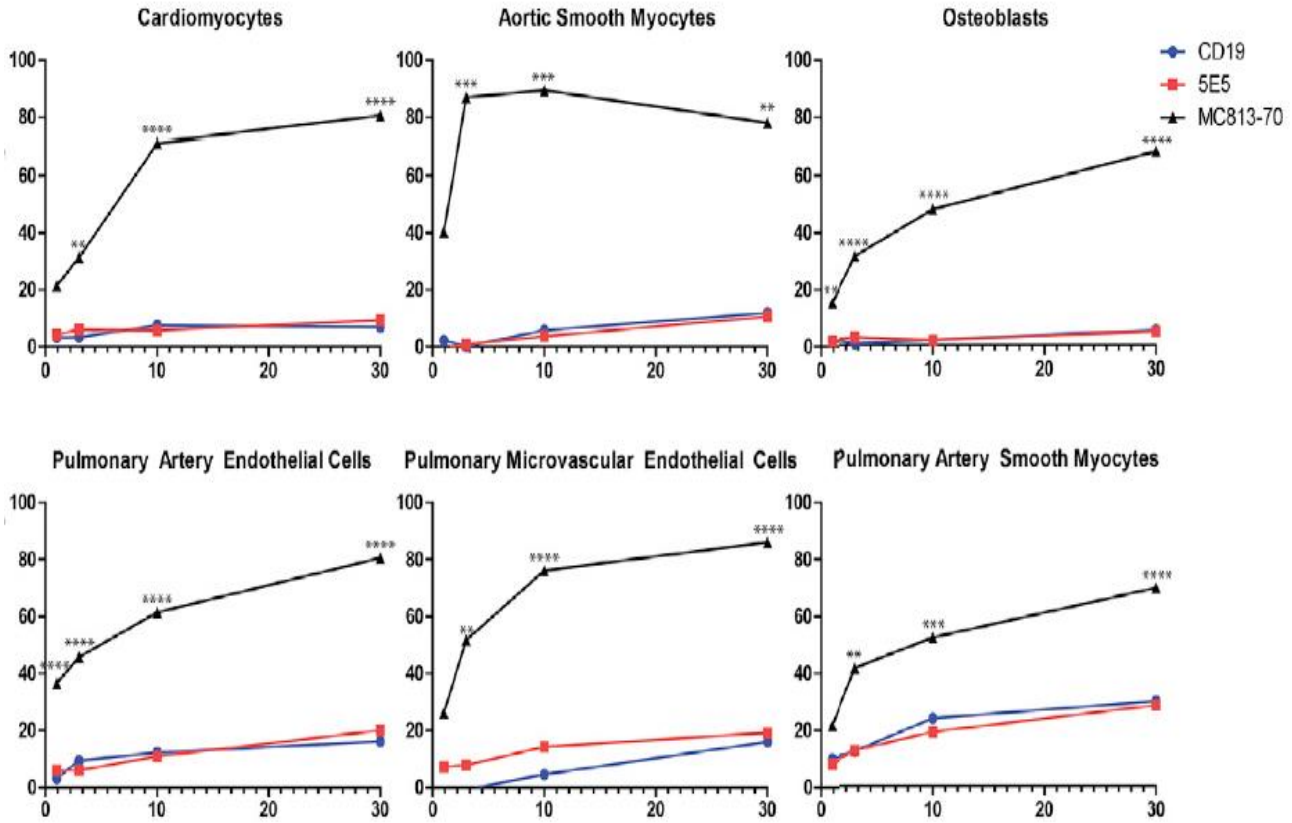
CAR(I)HPSE: 18/26

p=0.008

Immunity

Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma

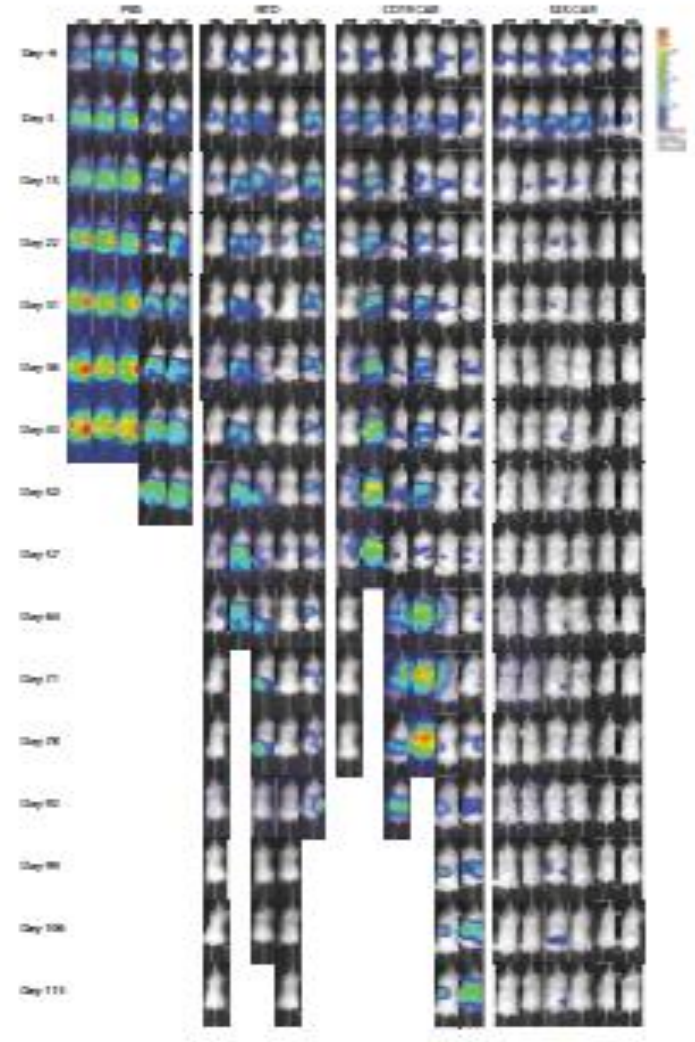


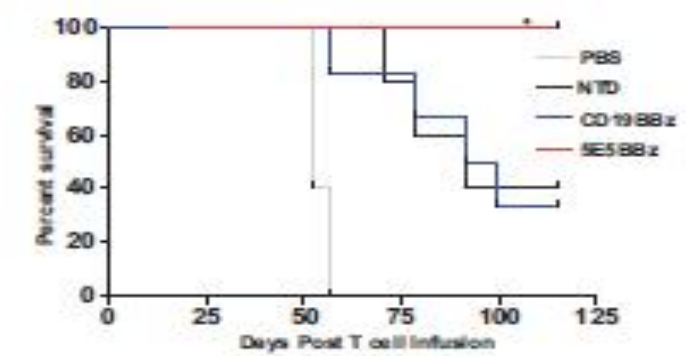


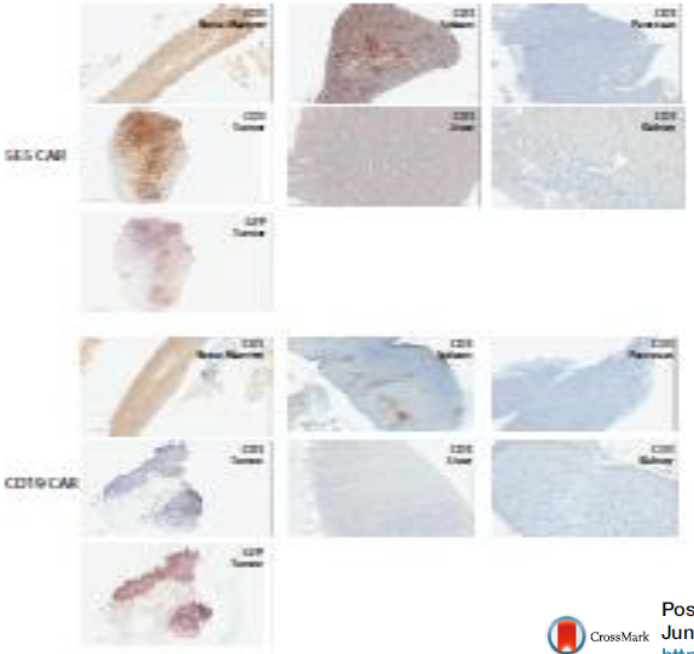
Posey et al., 2016, Immunity *44*, 1444–1454
June 21, 2016 © 2016 Elsevier Inc.
http://dx.doi.org/10.1016/j.immuni.2016.05.014

Immunity

Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma

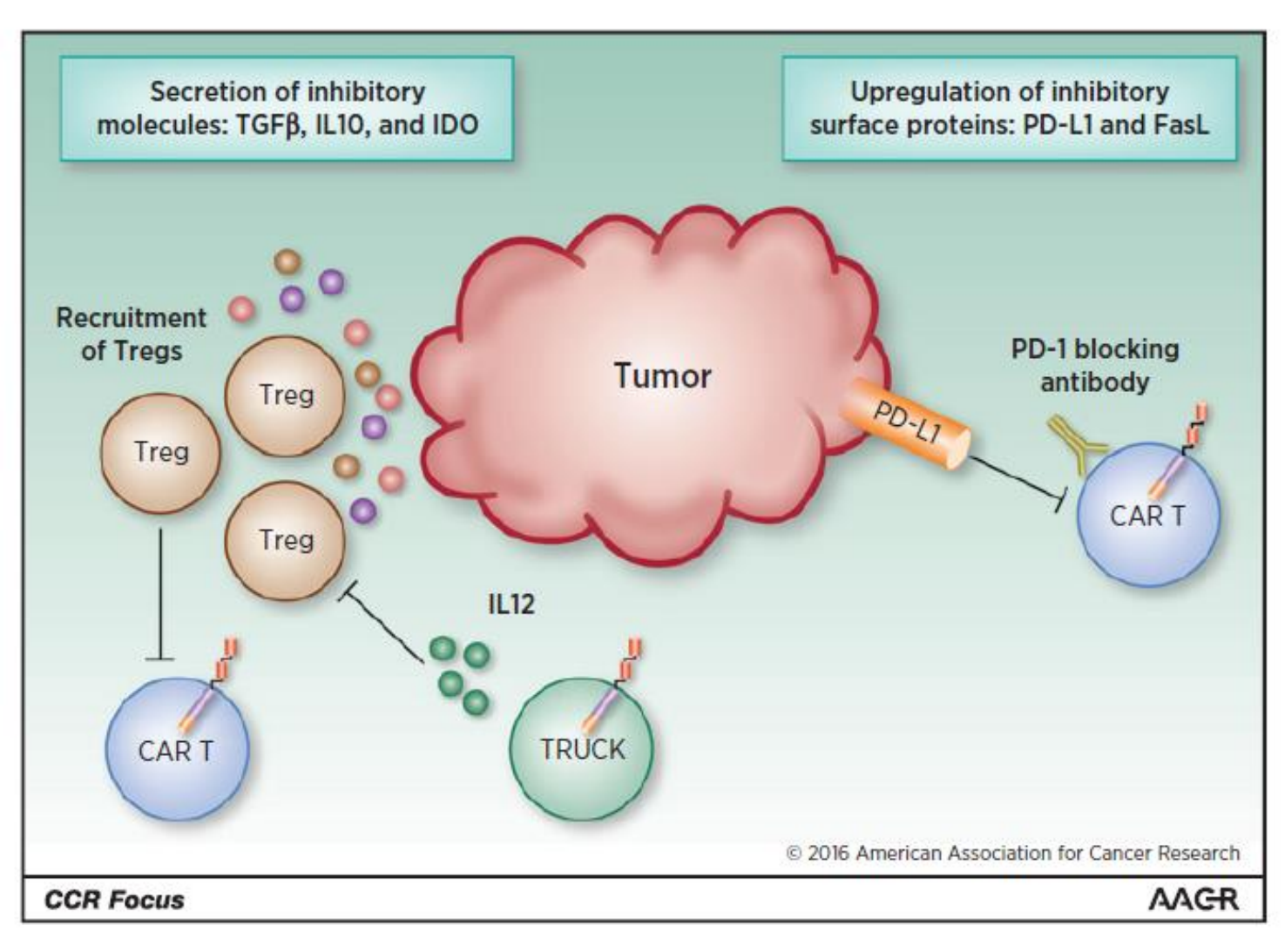






Posey et al., 2016, Immunity *44*, 1444–1454
June 21, 2016 © 2016 Elsevier Inc.
http://dx.doi.org/10.1016/j.immuni.2016.05.014

Factors influencing CAR T-cell activity



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Marco Dieci
Andrea La Sala
Carla Paganin

Now, this is not the end. It is not even the beginning of the end, but it is, perhaps, the end of the beginning…….