Le cellule CAR T: farmaci viventi per la cura dei tumori

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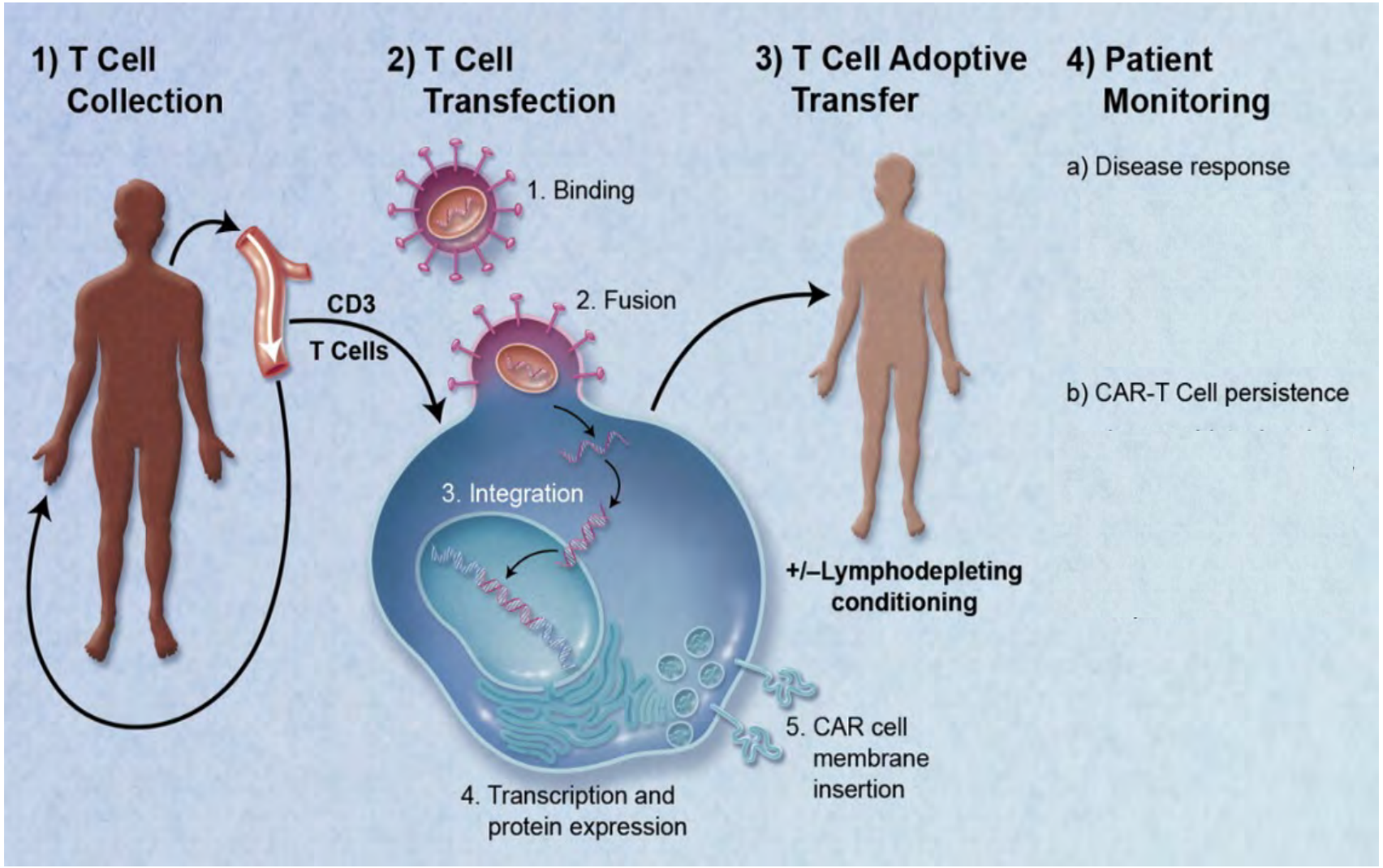
Science
Breakthrough of the Year
Cancer Immunotherapy
T cells on the attack
AAAS

CAR T Lymphocytes Targeting CD19 for patients with lymphoid malignancies



OPBG center for translational research

5,000 sqm research facility
All pediatric fields are covered by specific Labs

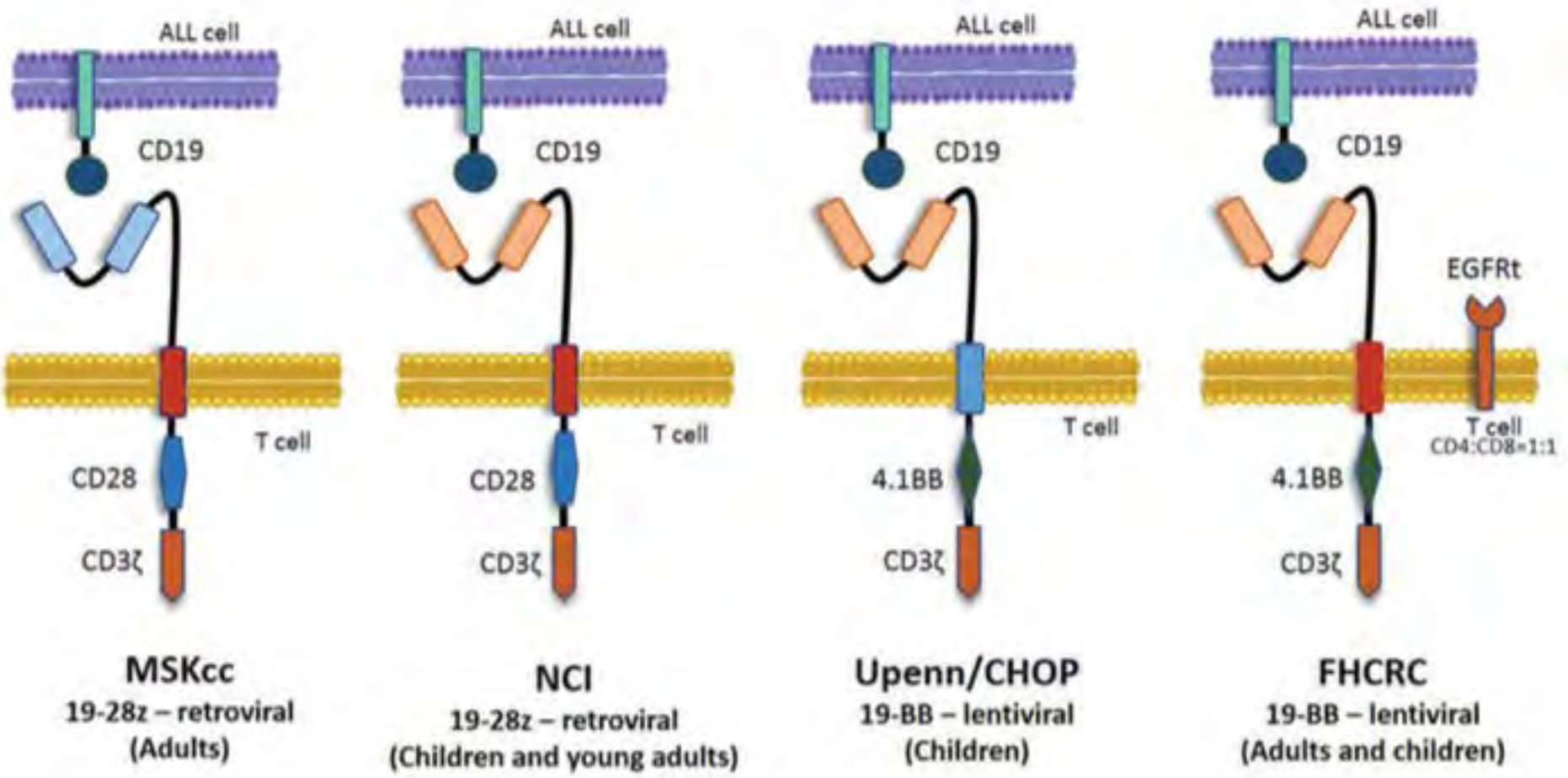
From 2014:
Cell and Gene Therapy programs for pediatric tumors

- ✓ 1,300 sqm GMP facility
- ✓ Built from 2013 to 2015
- ✓ 1 aseptic Grade A environment with Grade B for cell therapy products,
- ✓ From 3 to 8 aseptic rooms for gene therapy medicinal products
- ✓ 1 room for vectors production

AIFA Approved as GMP site in 2016 for cell therapy
AIFA Approved as GMP site in 2017 for gene therapy using viral vectors

Published constructs of 2nd generation CD19 CARs for ALL

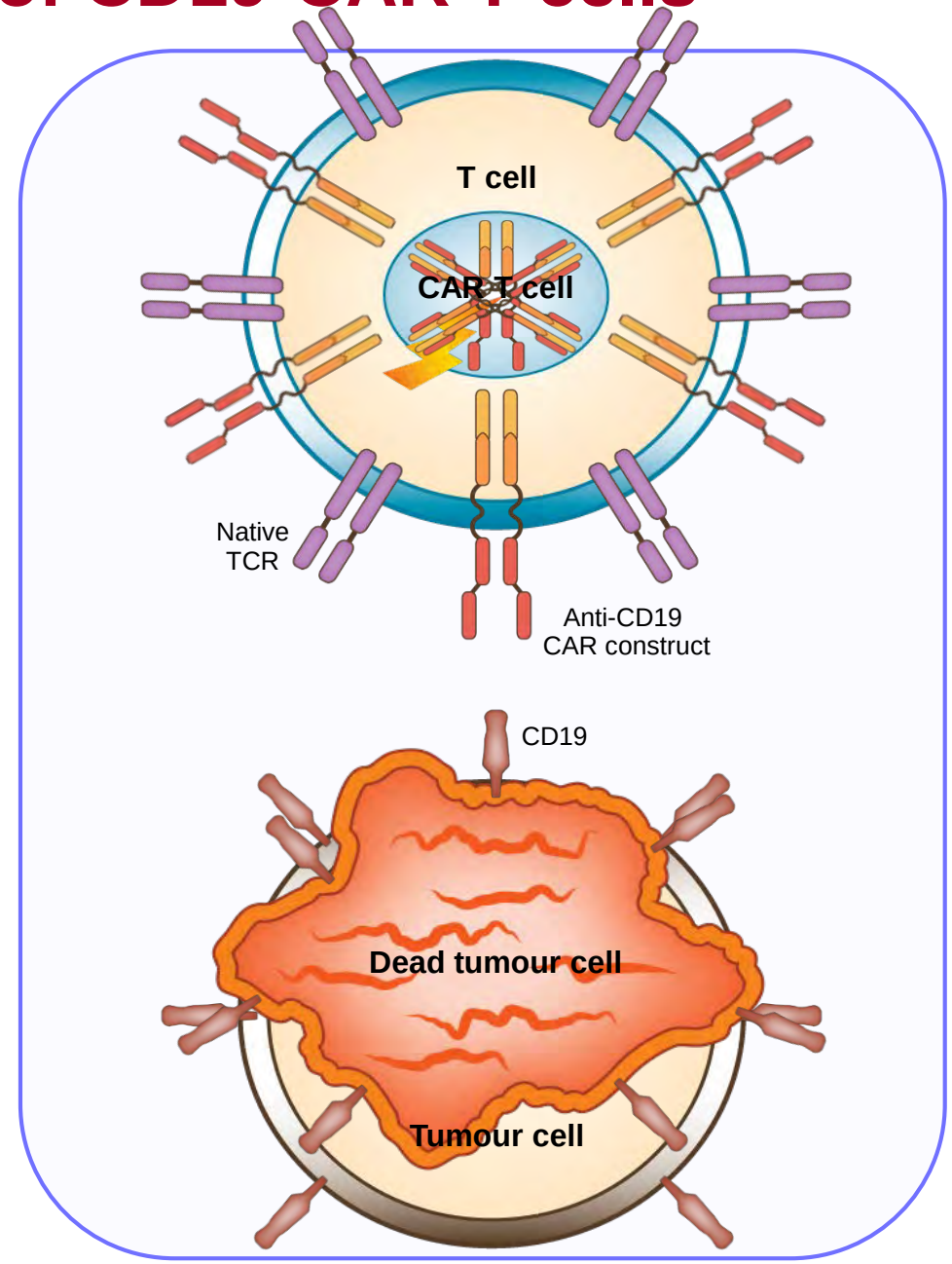
CAR design important for persistence and sustained efficacy



. Del Bufalo & Locatelli. Expert Review of Clinical Immunology 2019

Mechanism of action of CD19-CAR T cells

- Gene transfer technology is used to stably express CARs on T cells, conferring novel antigen specificity[1,2]
- CD19-CAR T cells cells can thus be directed against any cell that expresses the CD19 surface antigen
- CD19-CAR T therapy takes advantage of the cytotoxic potential of T cells, thereby killing tumour cells in an antigen-dependent manner[1,3]
- Persistent CD19-CAR T consist of both **effector (cytotoxic)** and central memory T cells[3]



1. Milone MC, et al. Mol Ther 2009;17:1453–64;
2. Hollyman D, et al. J Immunother 2009;32:169–80;
3. Kalos M, et al. Sci Transl Med 2011;3:95ra73.

Published studies of 2nd generation CD19 CAR-T cells for r/r ALL

Reference	Treated patients (n)	CAR vector	Response + consolidation
Maude SL, et al. N Engl J Med 2014;371:1507–17	30 (18 post-HSCT)	FMC63-41BB-ζ lentivirus	**27** CR; **22** MRD-negative **3** ➔ allogeneic HSCT
Lee DW, et al. Lancet 2015;385:517–28	20 (7 post-HSCT)	FMC63-CD28-ζ retrovirus	**13** CR + **1** CRi; **12** MRD-negative **10** ➔ allogeneic HSCT
Gardner RA, et al. Blood 2017;129:3322–31	43 (28 post-HSCT)	FMC63-41BB-ζ lentivirus	**41** CR; **41** MRD-negative **11** ➔ allogeneic HSCT
Maude SL, et al. N Engl J Med 2018;378:439–48	75 (46 post-HSCT)	FMC63-41BB-ζ lentivirus	**61** CR/CRi; **61** MRD-negative **8** ➔ allogeneic HSCT
Turtle CJ, et al. J Clin Invest 2016;126:2123–38	30 (11 post-HSCT)	FMC63-41BB-ζ lentivirus	**29** CR; **25** MRD-negative **13** ➔ allogeneic HSCT
Park JH, et al. N Engl J Med 2018;378:449–59	53 (19 post-HSCT)	SJC25C1-CD28-ζ retrovirus	**44** CR; **32** MRD-negative **17** ➔ allogeneic HSCT

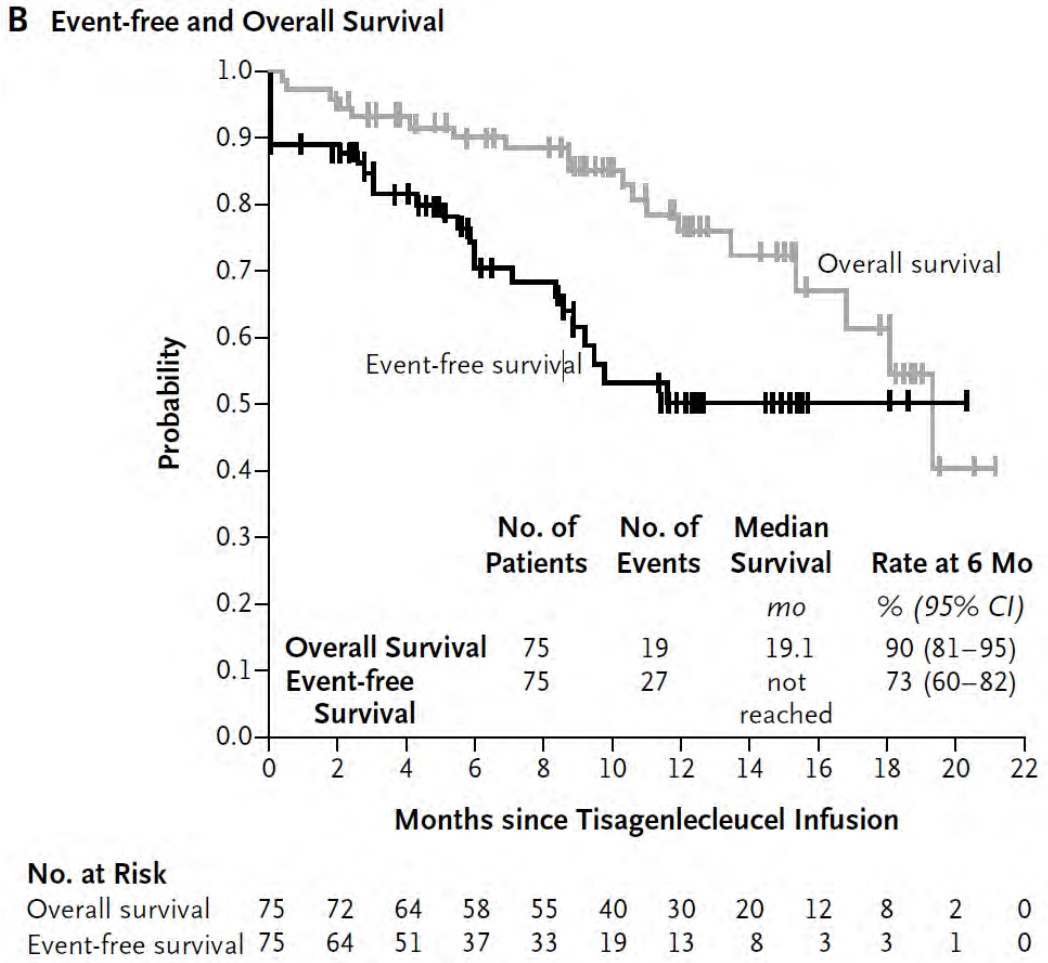
- **251 patients treated: 86% CR, 76% MRD-negative**

Summary of ELIANA study

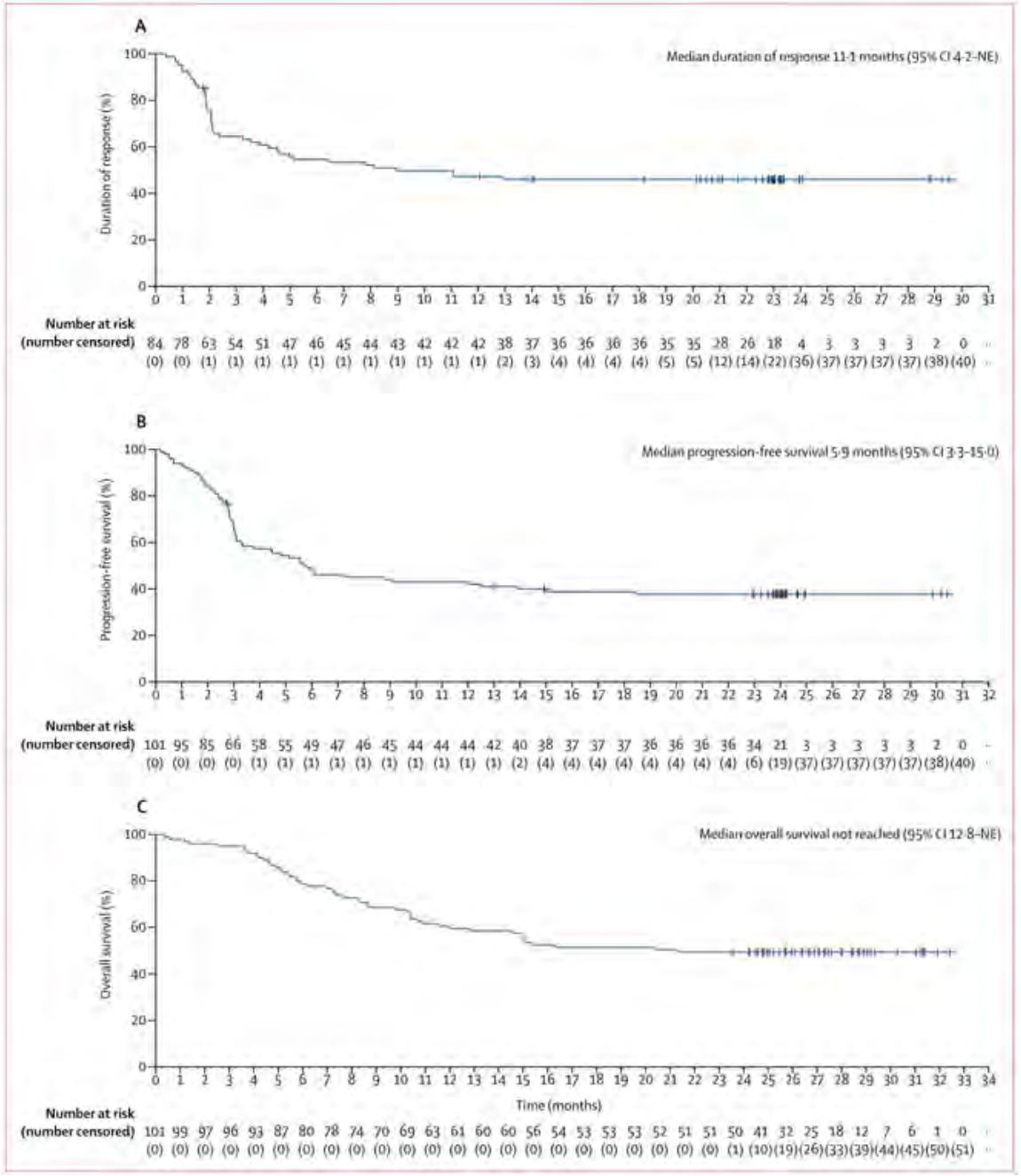
ORIGINAL ARTICLE

Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia

- 92 patients enrolled, 75 treated
- 73% Grade 3–4 AEs related to CAR-T:
- **81% → CR/CRi, all MRD negative; 66% in intention-to-treat analysis**
- 1 year EFS at 50%, no relapses after this
- **Demonstrates feasibility of delivery in multiple centres**

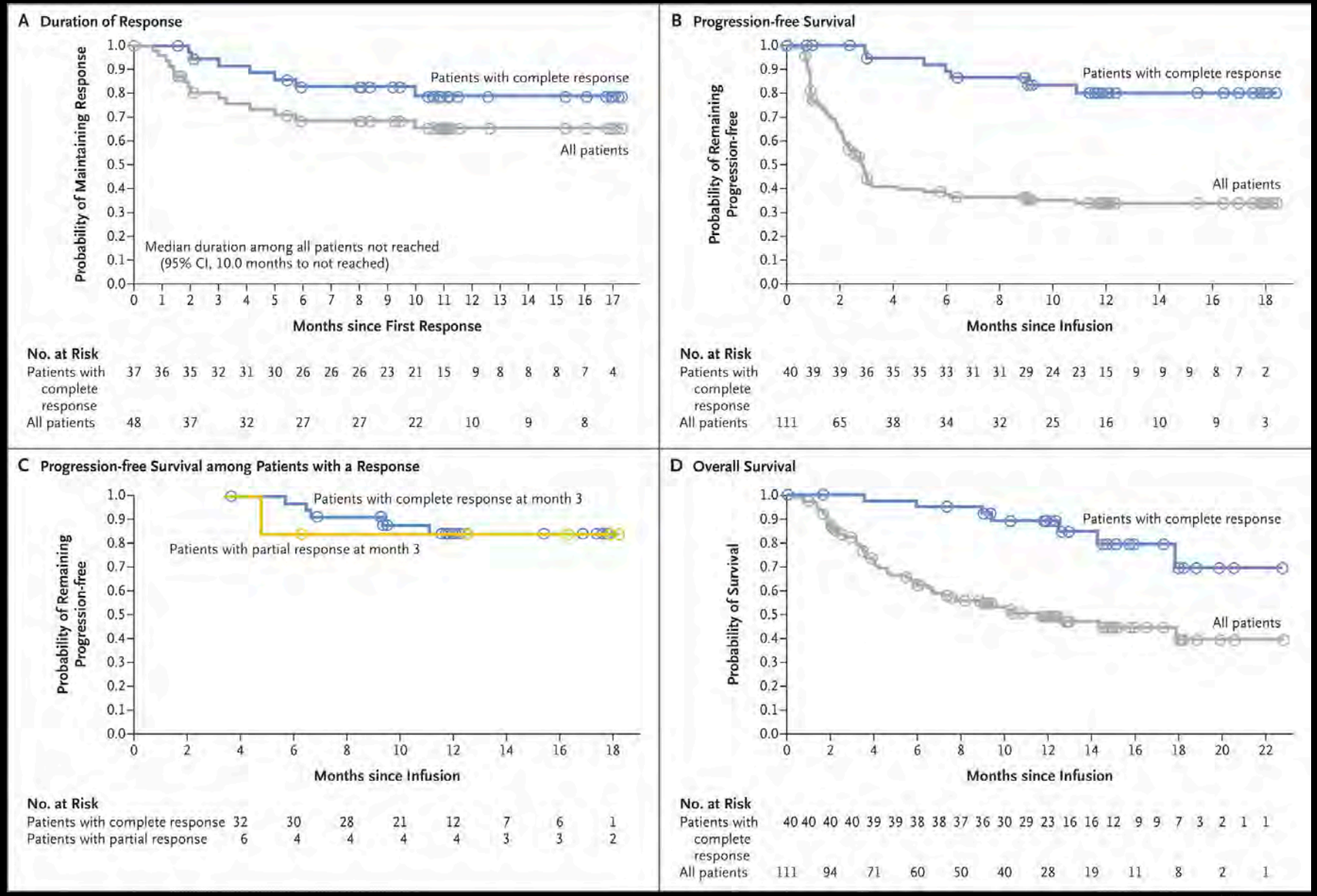


Maude SL, et al. N Engl J Med 2018;378:439–48;



Locke FL, et al. Lancet Oncol. 2019 Jan; 20(1): 31–42.

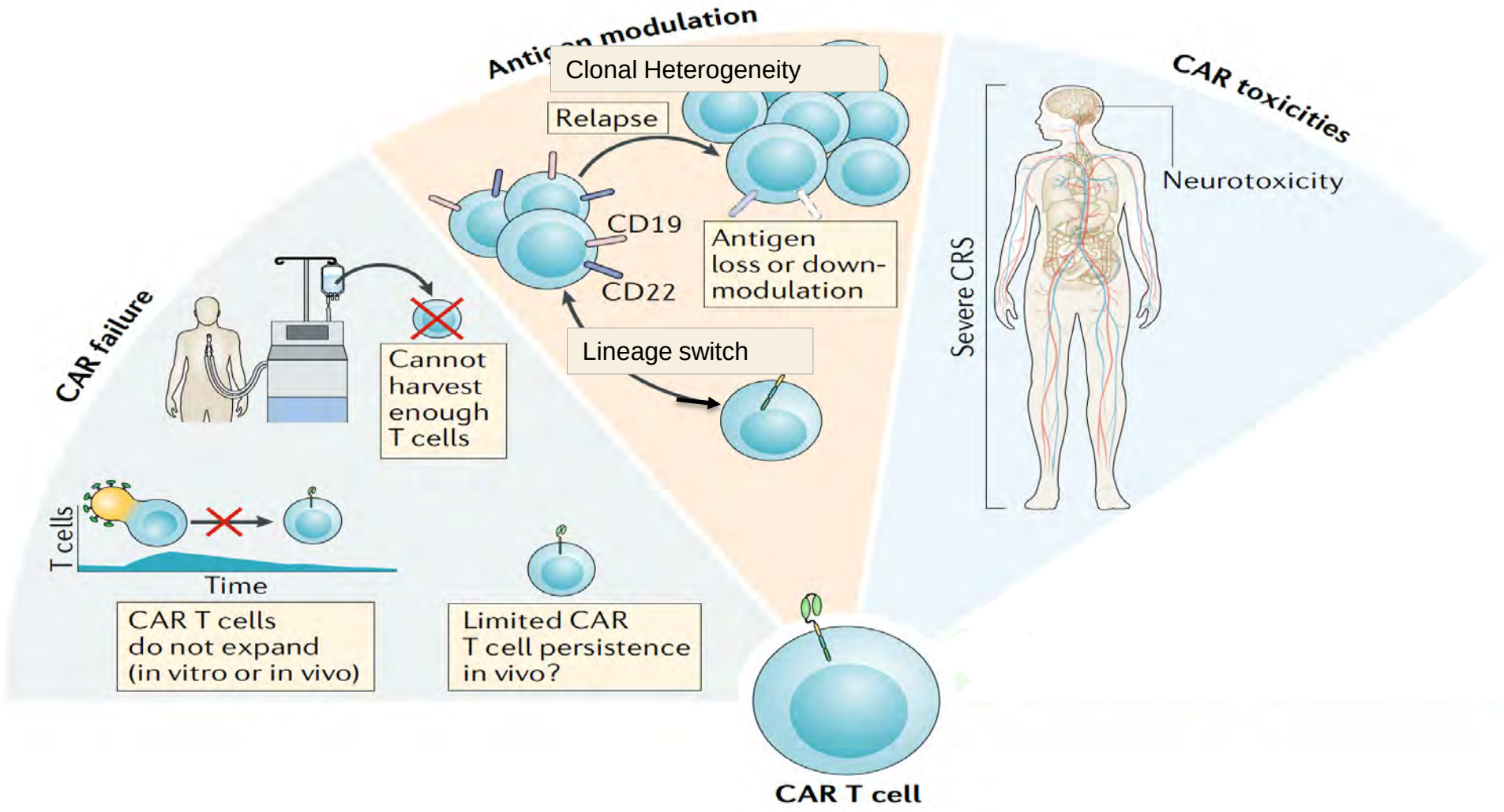
Duration of Response, Progression-free Survival, and Overall Survival.



Schuster SJ et al. N Engl J Med 2019;380:45-56

The NEW ENGLAND JOURNAL of MEDICINE

Current Limitations of CAR T Cells



A. Wayne, adapted from Shah & Fry, Nat Rev Clin Oncol 2019

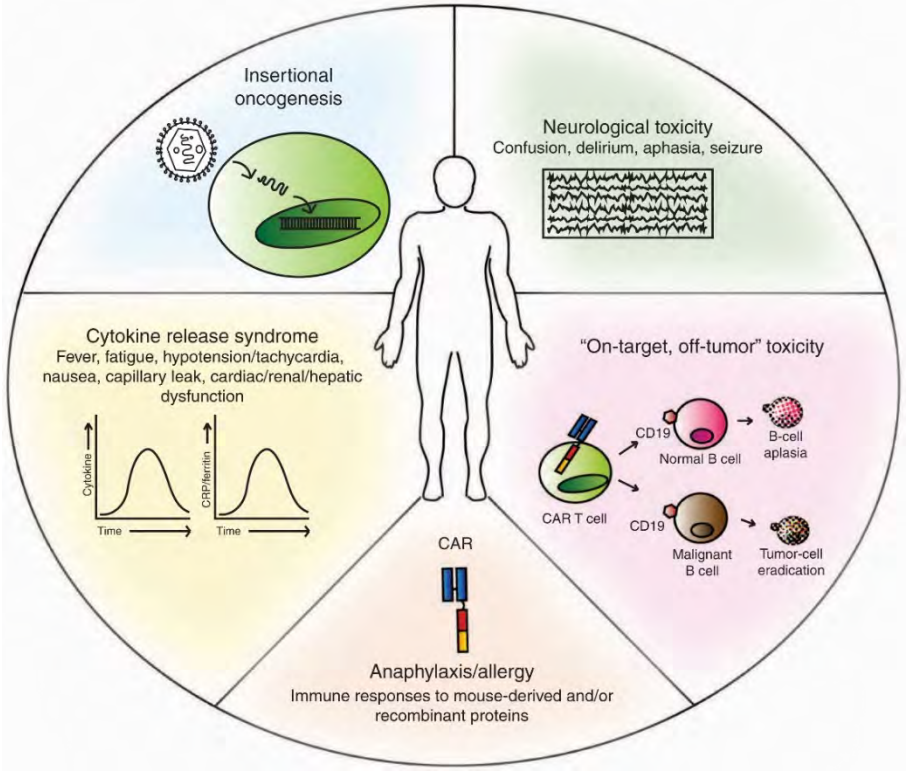
Peculiar toxicities associated with CD19.CAR-T cell

“On-target, off-tumor” toxicities

- B cell-aplasia

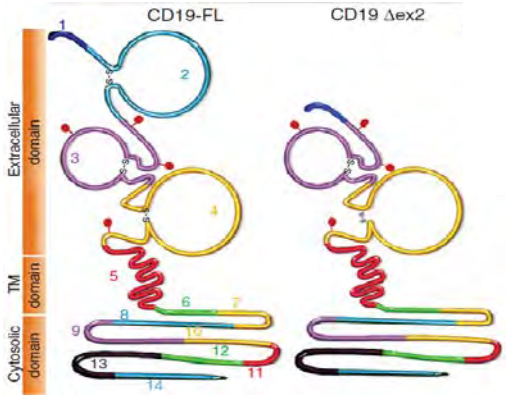
Non-antigen specific toxicities

- Cytokine release syndrome (CRS)
- Neurotoxicity
- HLH



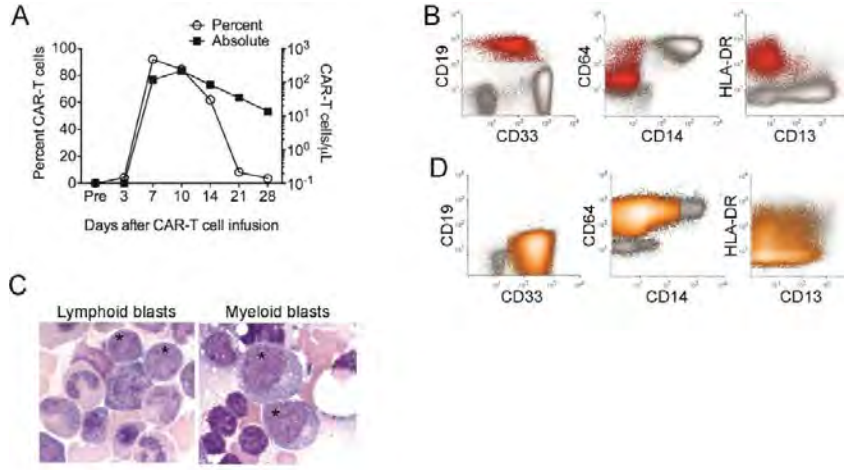
Mechanisms of leukemia escape after CAR T cell therapy

Tumor evasion systems in BCP-ALL: CD19-negative relapses



➢ Loss of CAR-recognized epitope as a result of alternative exon splicing forms of the CD19 gene where exon 2 was lost **(Sotillo *et al.*, *Cancer Discov*, 2015)**;

➢ Altered trafficking of CD19 protein to the cell membrane of blast cells **(Braig *et al.*, Blood, 2016)**

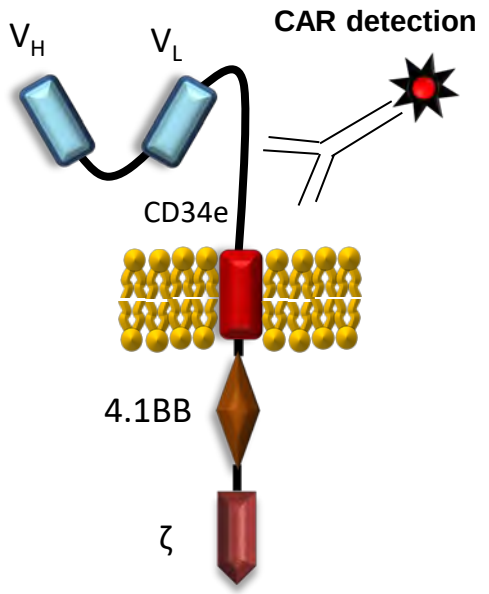


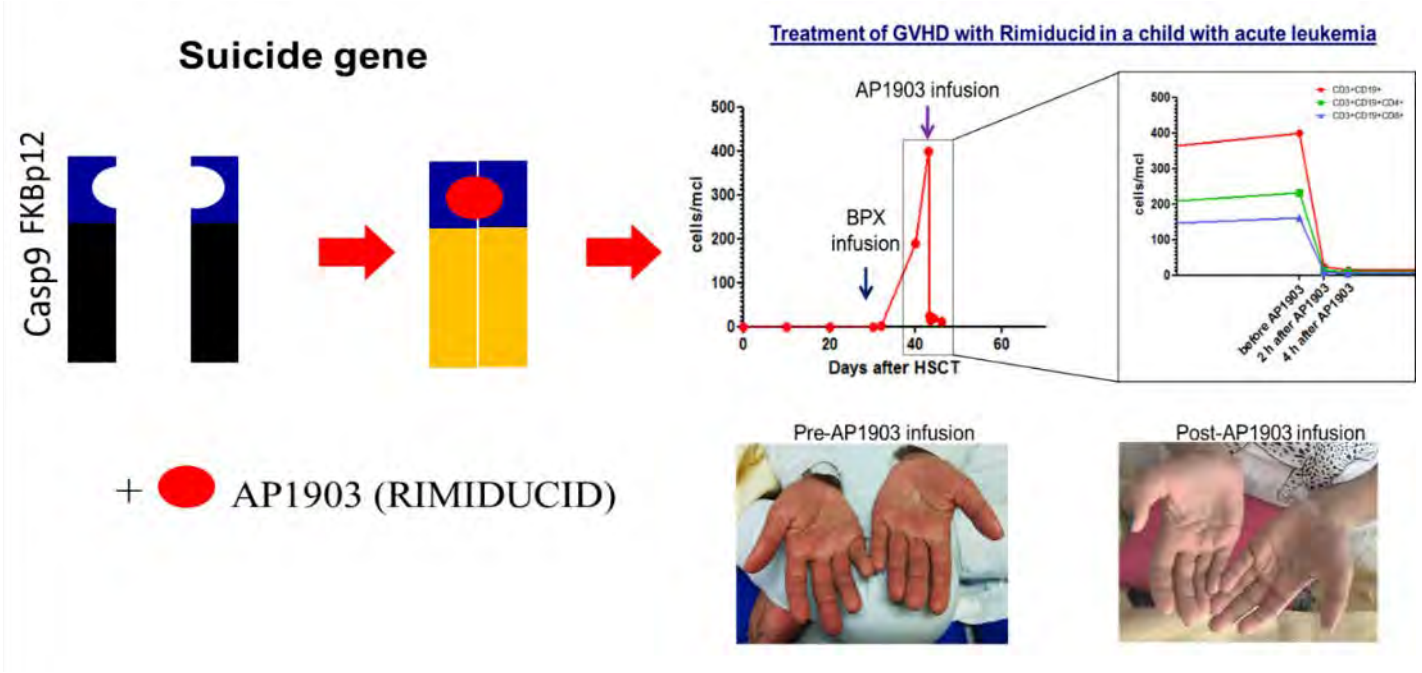
➢Myeloid switch and loss of CD19 in patients with mixed-phenotype leukemia and MLL rearrangement **(Gardner *et al.*, *Blood*, 2016)**;

➢Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell **(Ruella M et al Nature Med, 2018)**

The contribution of academic institutions
The OPBG Model
Second Generation CAR Targeting CD19

CAR.CD19-4.1BB-ζ





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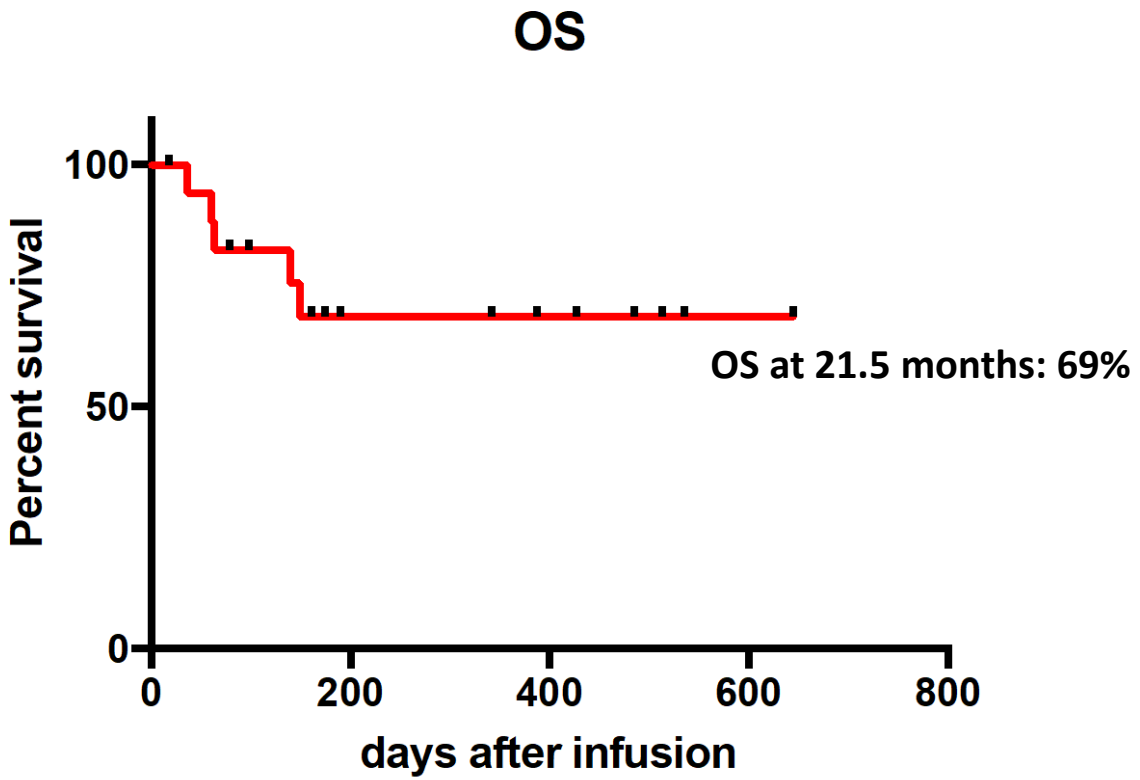
Quintarelli C, et al. Mol. Ther. 2016

The contribution of academic institutions
The OPBG Model

- **Fourteen out of the 17 (82%) patients with Bcp-ALL infused obtained CR with MRD negativity after DP infusion**



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What are the new challenges for CAR-T cell therapy?

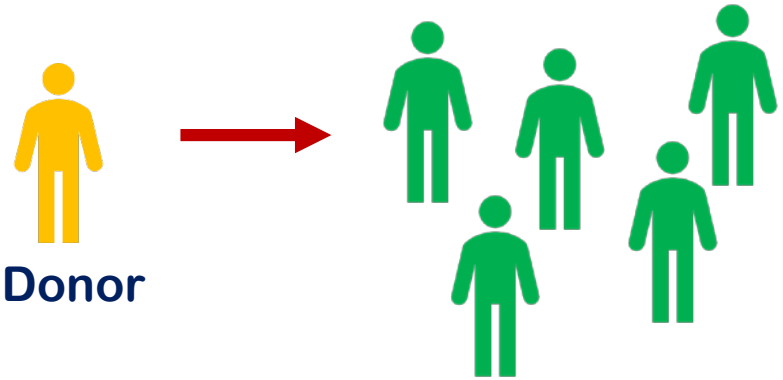
- Increasing the number/capacity of manufacturing sites
- Testing new indications and/or new targets
- Improving efficacy
- Reducing toxicity
- Increasing innovation and sustainability

Advantages of NK cells for CAR therapy

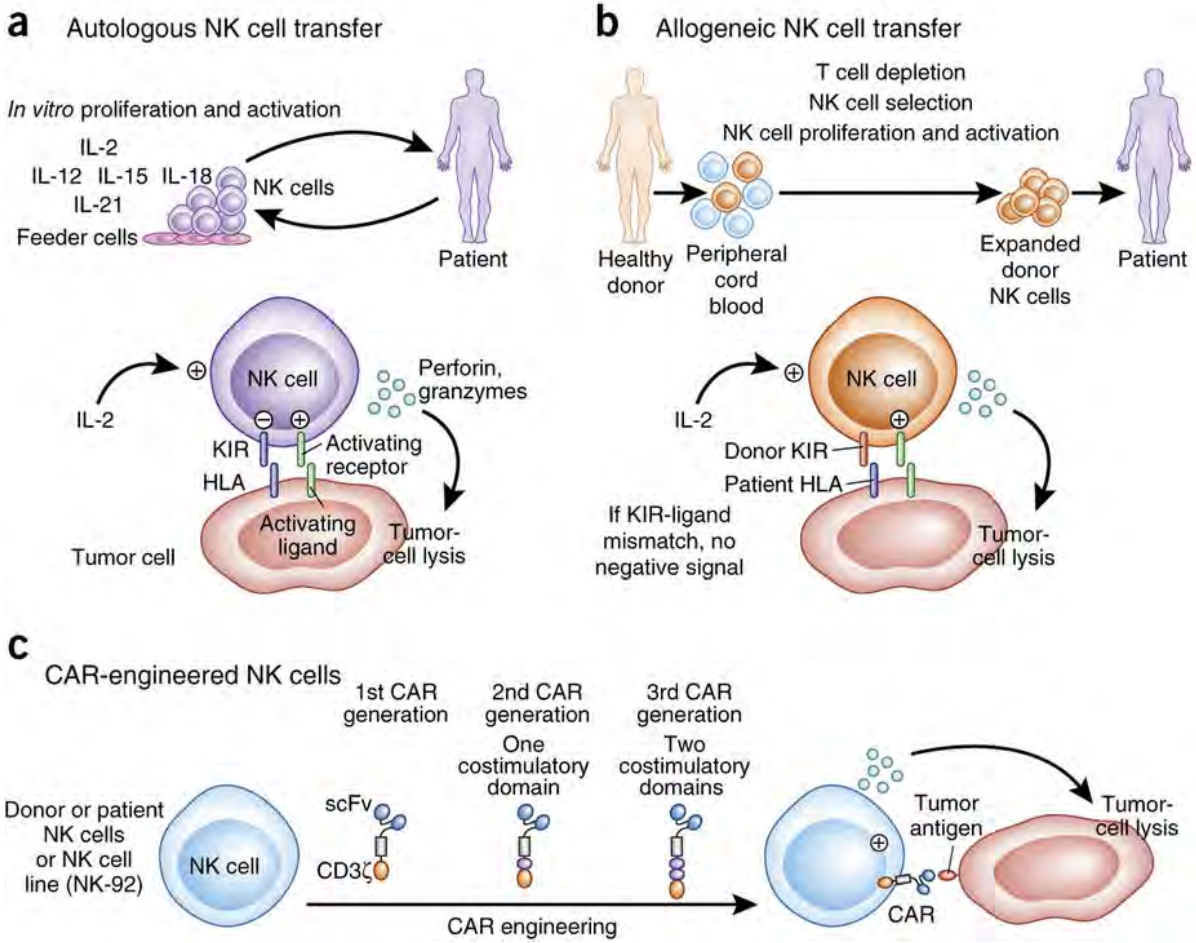
CAR-NK CD19

- **Allogenic Product**

  - ✓ ‘Off the shelf’
  - ✓ Potential low cost

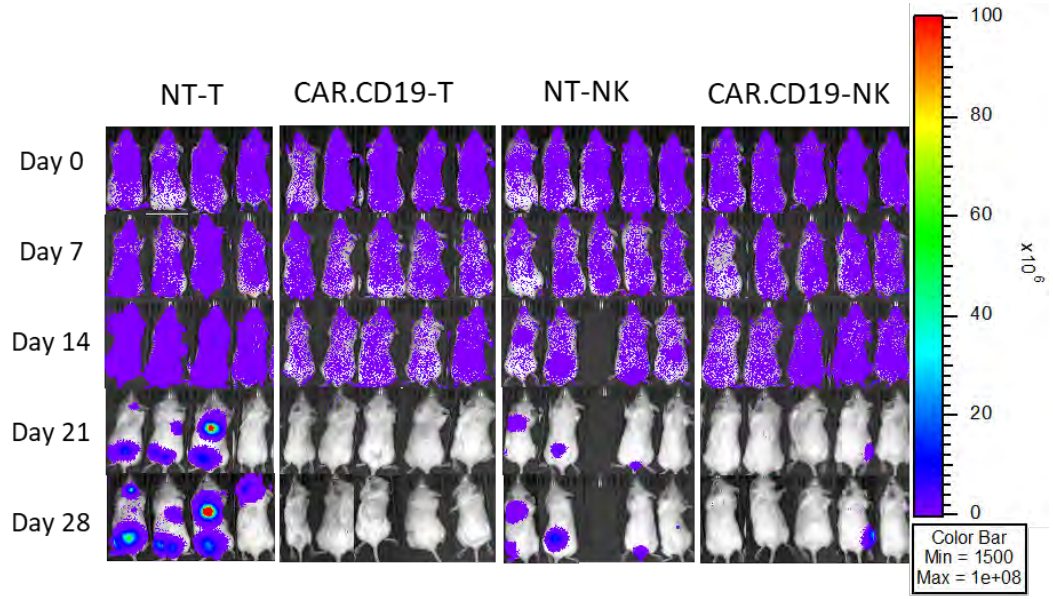


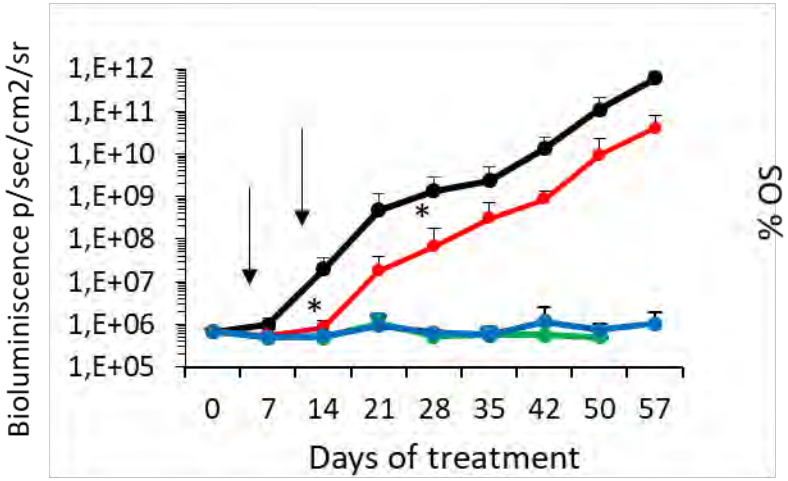
- Low/Absent GVHD

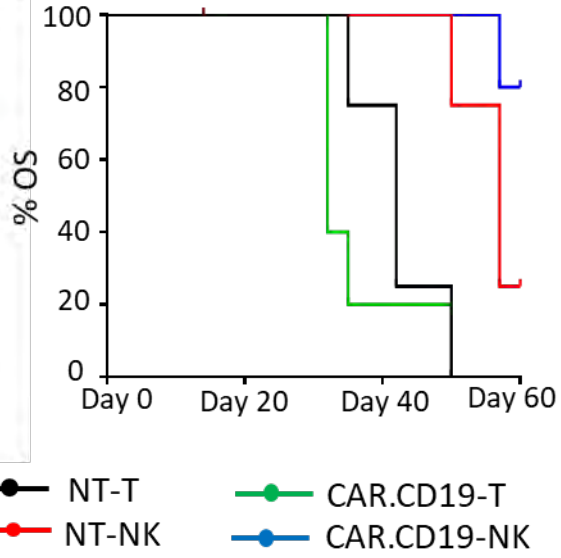


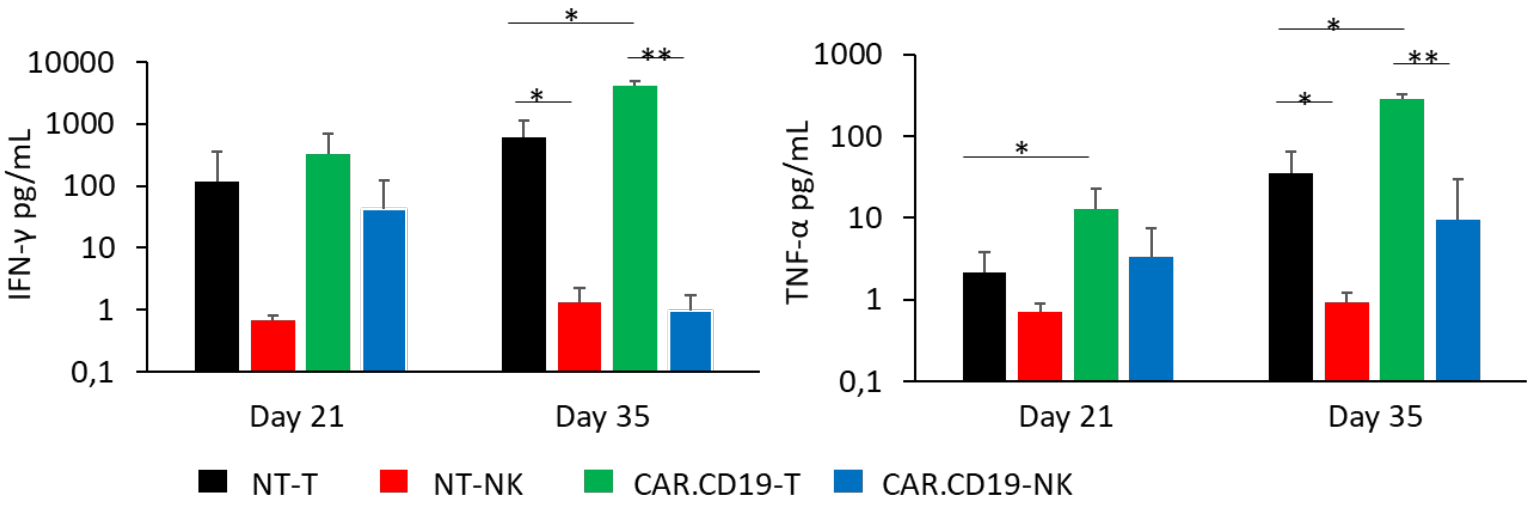
Camille Guillerey *et al.* Nature Immunology 2016

CAR NK cells are equally effective but less toxic than CAR T cells







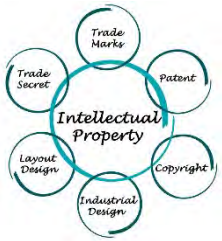


	p value
NT-T vs NT-NK	0,018
NT-T vs CAR.CD19-T	n.s.
NT-NK vs CAR.CD19-NK	n.s.
CAR.CD19-T vs CAR.CD19-NK	0,001
NT-T vs CAR.CD19-NK	0,003
NT-NK vs CAR.CD19-T	0,01

Quintarelli et al. Leukemia (2020)

Progetto CAR T Italia

Trasferimento tecnologico



IOV;
Partecipanti:
Molmed, OSR, IEO,
Rete AMOre

Ottimizzazione processo

WP1

WP6

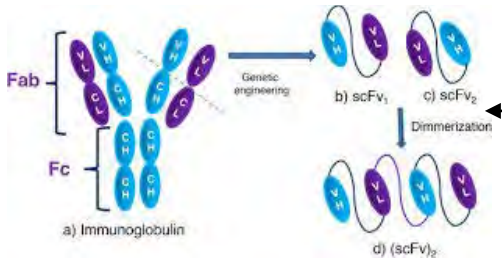
OPBG;
Molmed, San Gerardo

Ottimizzazione della sicurezza

WP5

OSR;
OPBG, INT, AMORe
Humanitas

Identificazione nuovi target



WP2

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Ospedale Pediatrico

Coordinamento

WP4

Potenziamento
dei CAR T

Meldola;
OSR, OPBG, IRE, Rete AMORe
(Pascale, Bari, Rionero), San
Gerardo, CRO

Sviluppo
pre-clinico

WP3

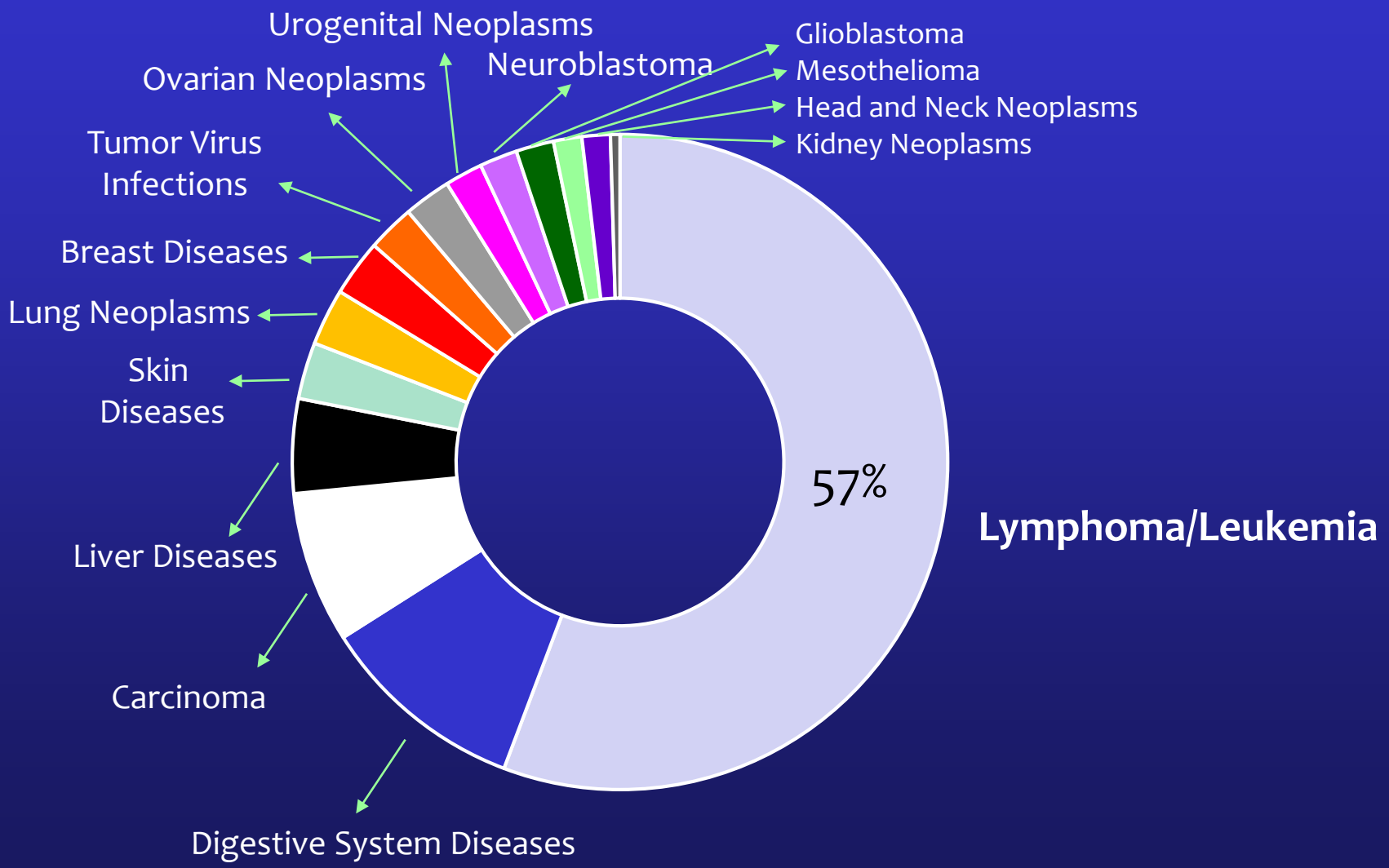
Candiolo;
Gemelli, San Gerardo, Meldola, OPBG,
IEO, IOV, INT

Gemelli;
IEO, IBB-CNR, OPBG, Candiolo, Casa
Sollievo, INT, CNR

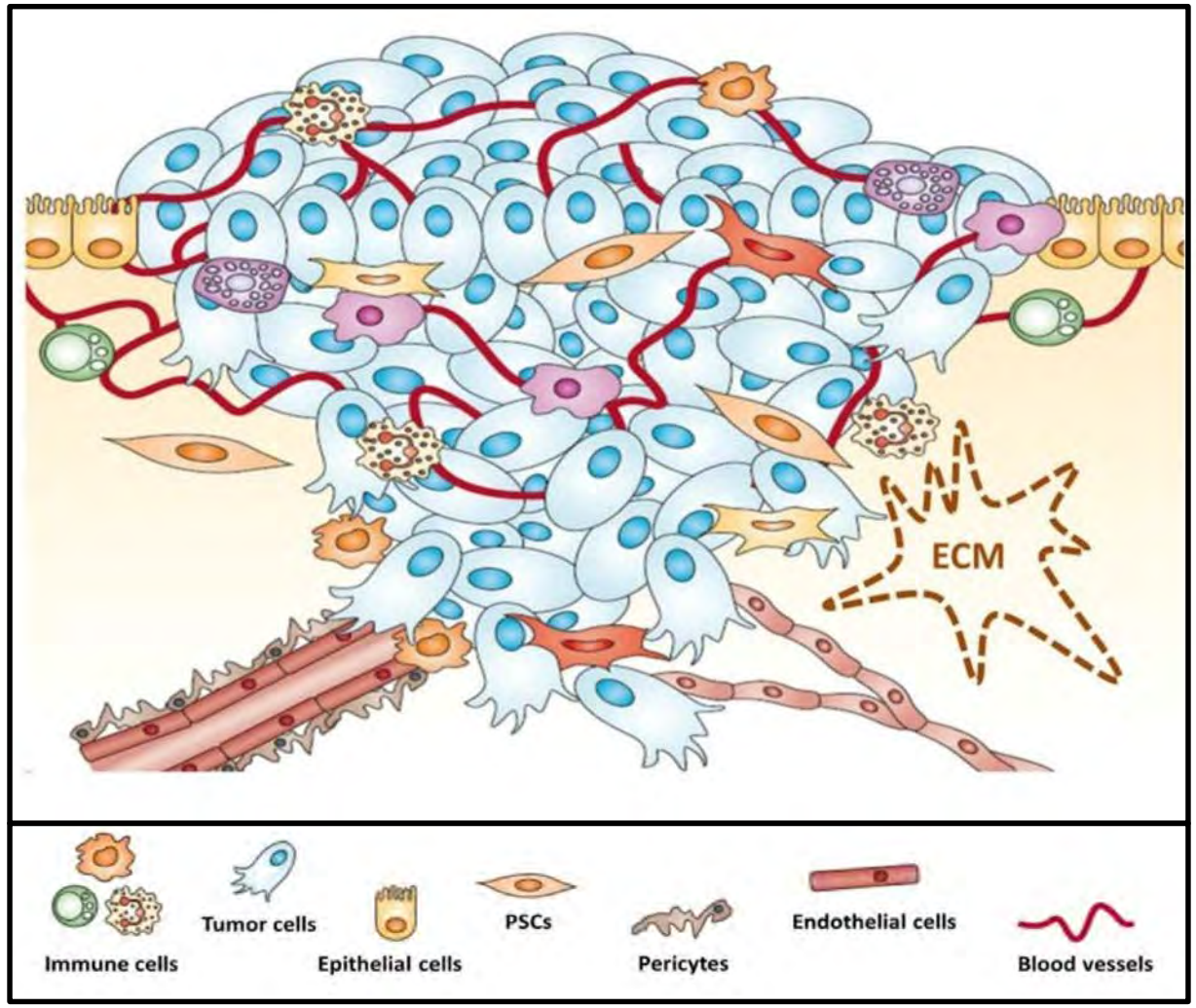
ALLEANZA
CONTRO
IL CANCRO

Ministero della Salute

DISEASE DISTRIBUTION OF ACTIVE CLINICAL TRIALS ON CAR T CELLS



The great challenges of solid tumours



1) Identification of suitable target antigens, selectively or preferentially expressed by tumor cells

2) Escape from the immune-depotentiating activity of tumor microenvironment

3) Penetration of CAR T cells in the tumor mass

4) Survival into the hypoxic tumor environment

5) Long-term persistence of CAR T cells

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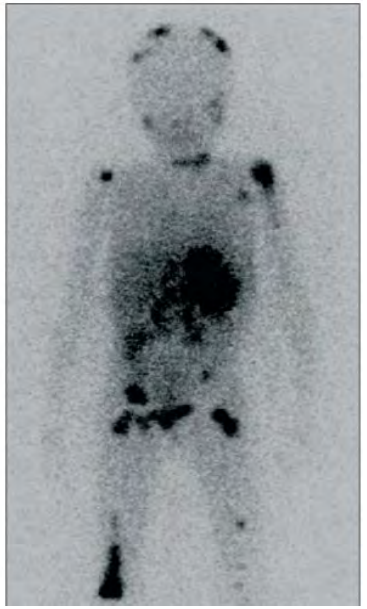
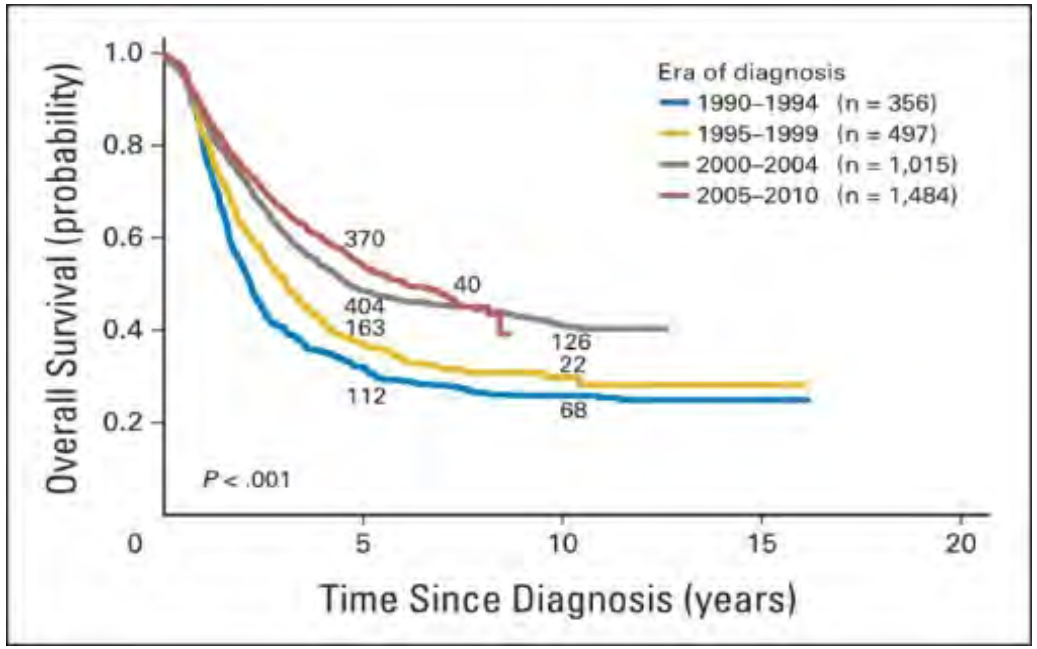
Neuroblastoma (NB)



1. The most common malignant extracranical solid tumor of childhood
2. Derived from the sympathetic nervous system
3. Present as an abdominal mass originating from the adrenal gland, but the neck, chest and pelvis are other common sites of origin
4. Metastatic evolution is frequently observed

The prognosis of High-Risk Neuroblastoma remains poor



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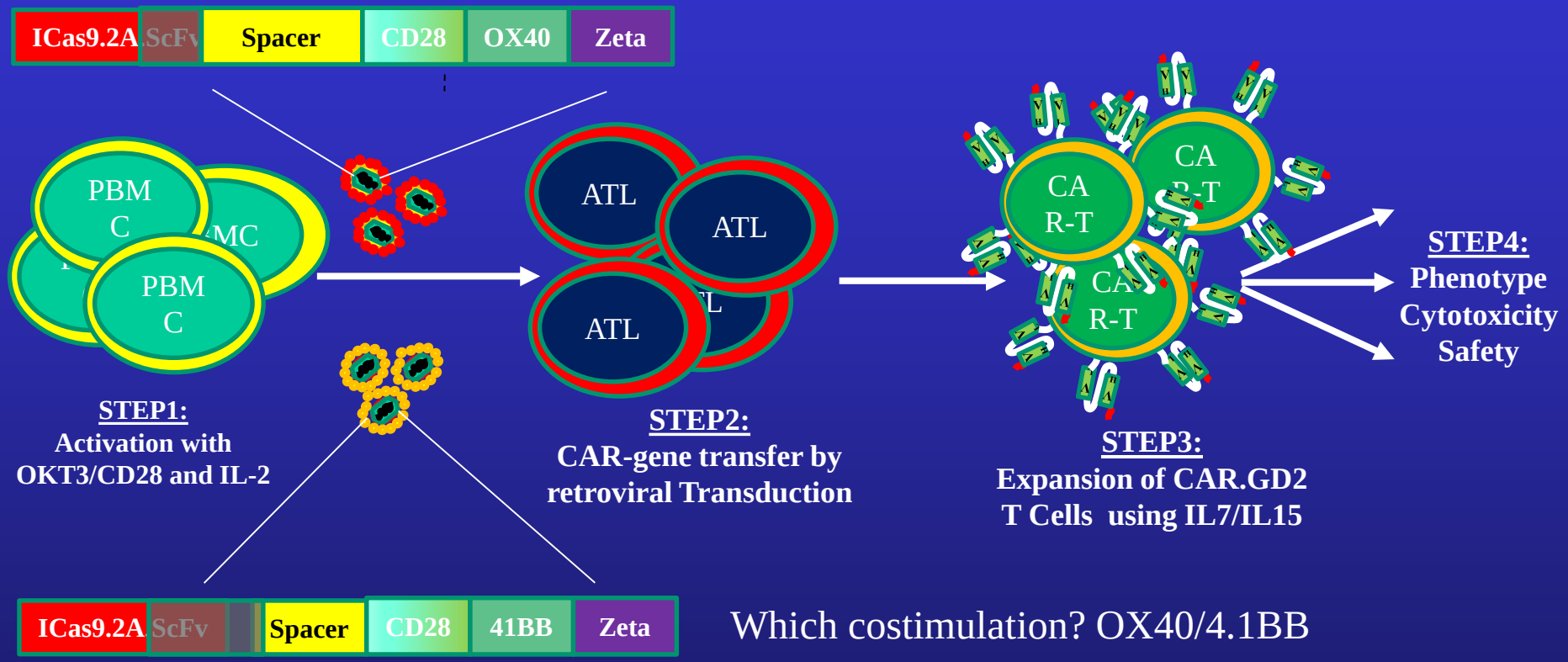
Pinto et al. J Clin Oncol 2015

Pre-clinical validation of efficacy and safety of GD2-CAR T cell

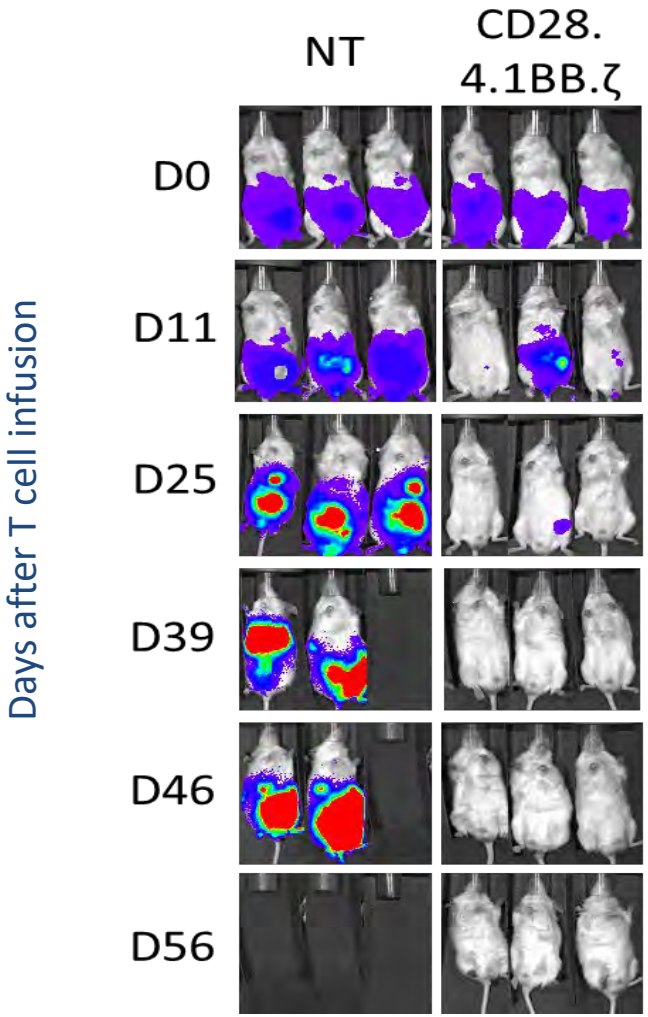


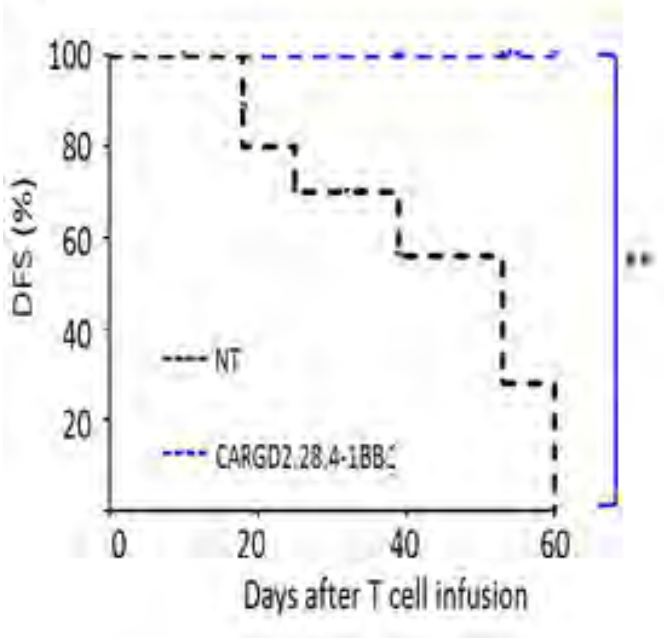
Which costimulation? OX40/4.1BB

- Cytoxicity (Efficient Tumor regression)
- Cytokine Production
- Persistence
- Safety

Quintarelli C, et al. Oncoimmunology 2018

IN VIVO ACTIVITY OF IIICAR.GD2 T CELLS



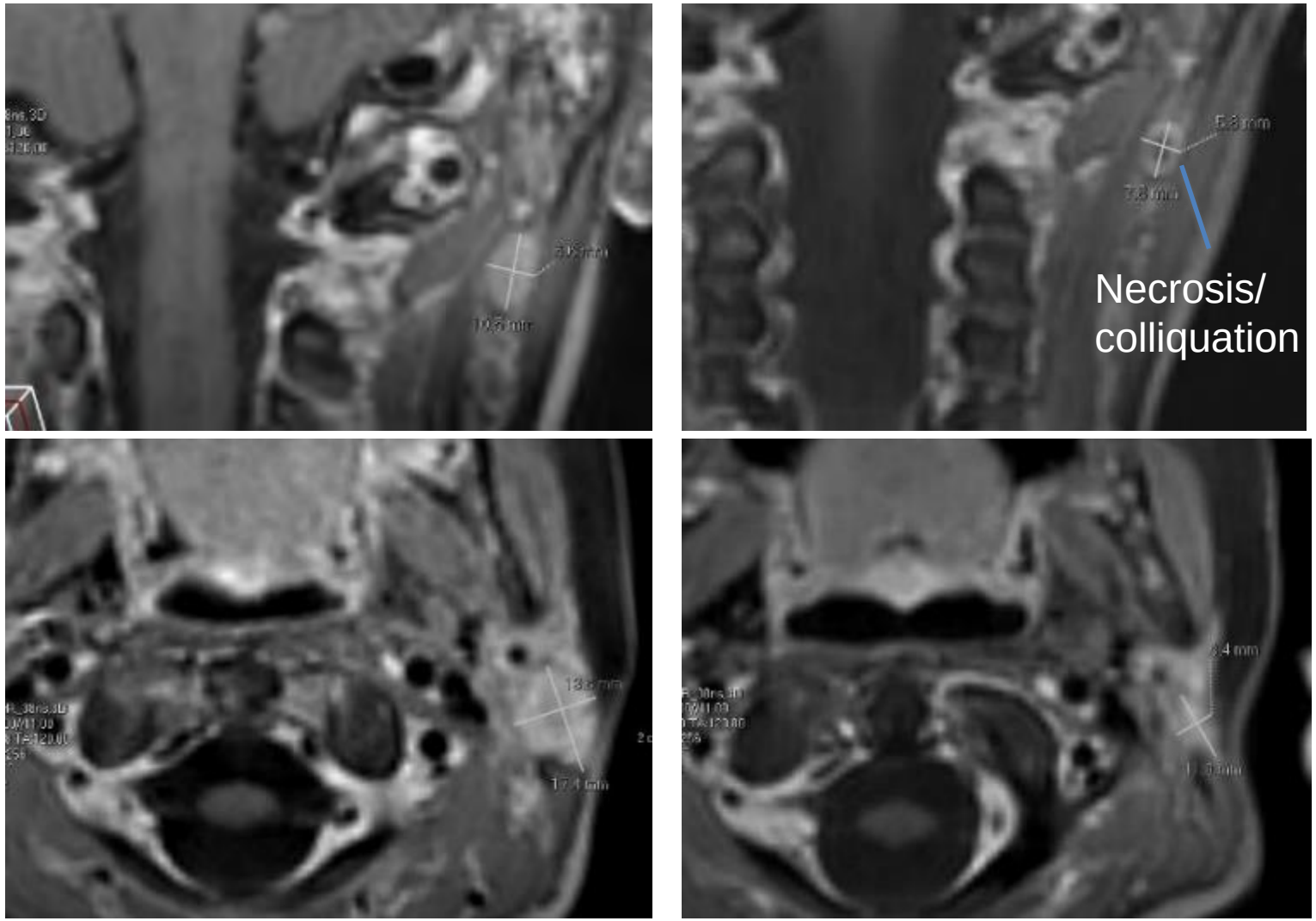


Quintarelli C, et al. Oncoimmmunology 2018

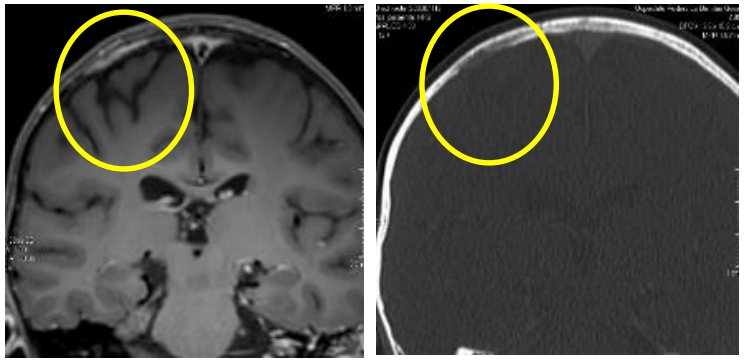
GD2-OPBG-002: disease evaluation MRI

Pre-CAR

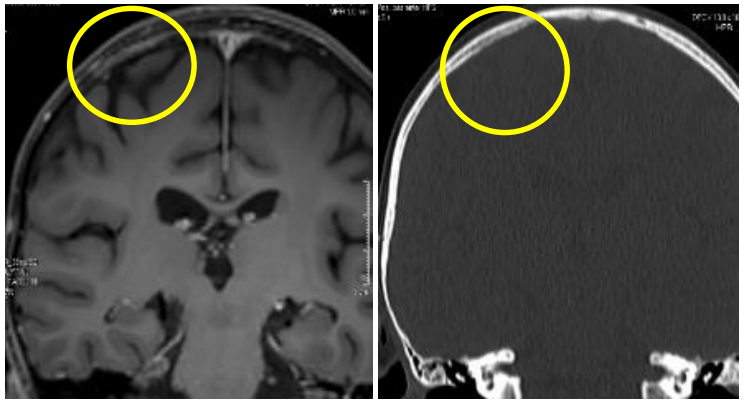
Week +6



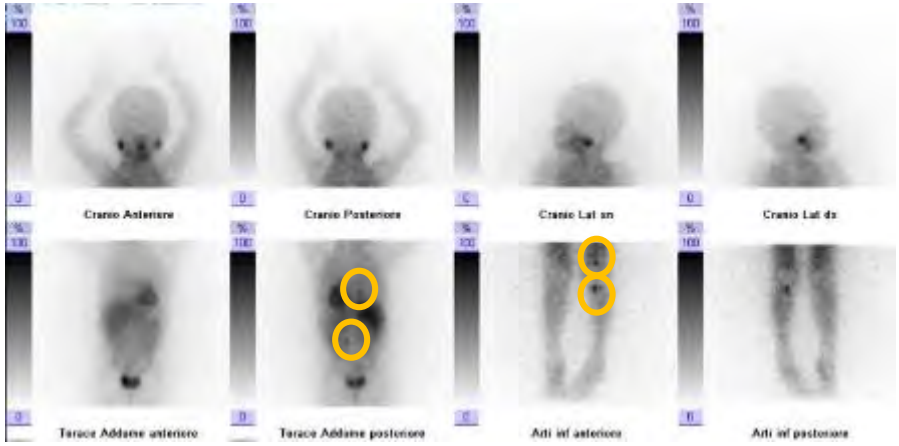
Pre-CAR



Week +6

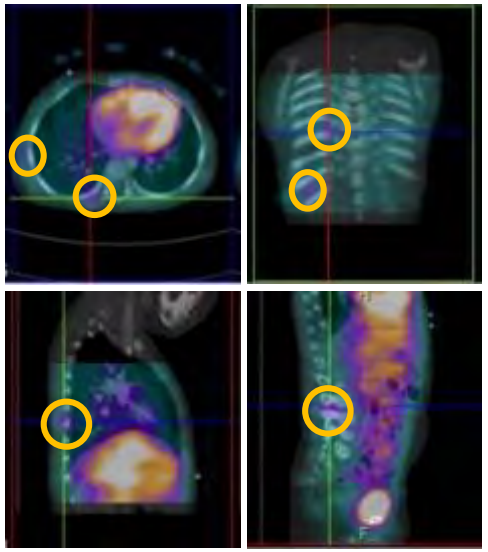


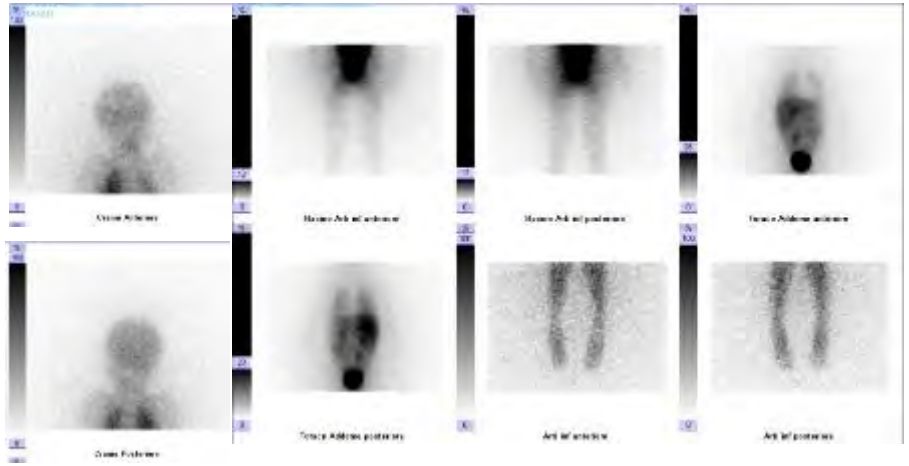
GD2-OPBG-005: disease evaluation MIBG-scintigraphy



Pre-CAR

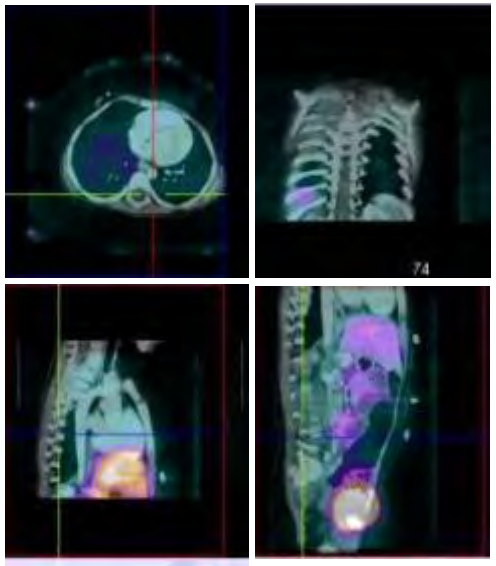
(multiple bone lesions)





Week +6 and 12

(CR)



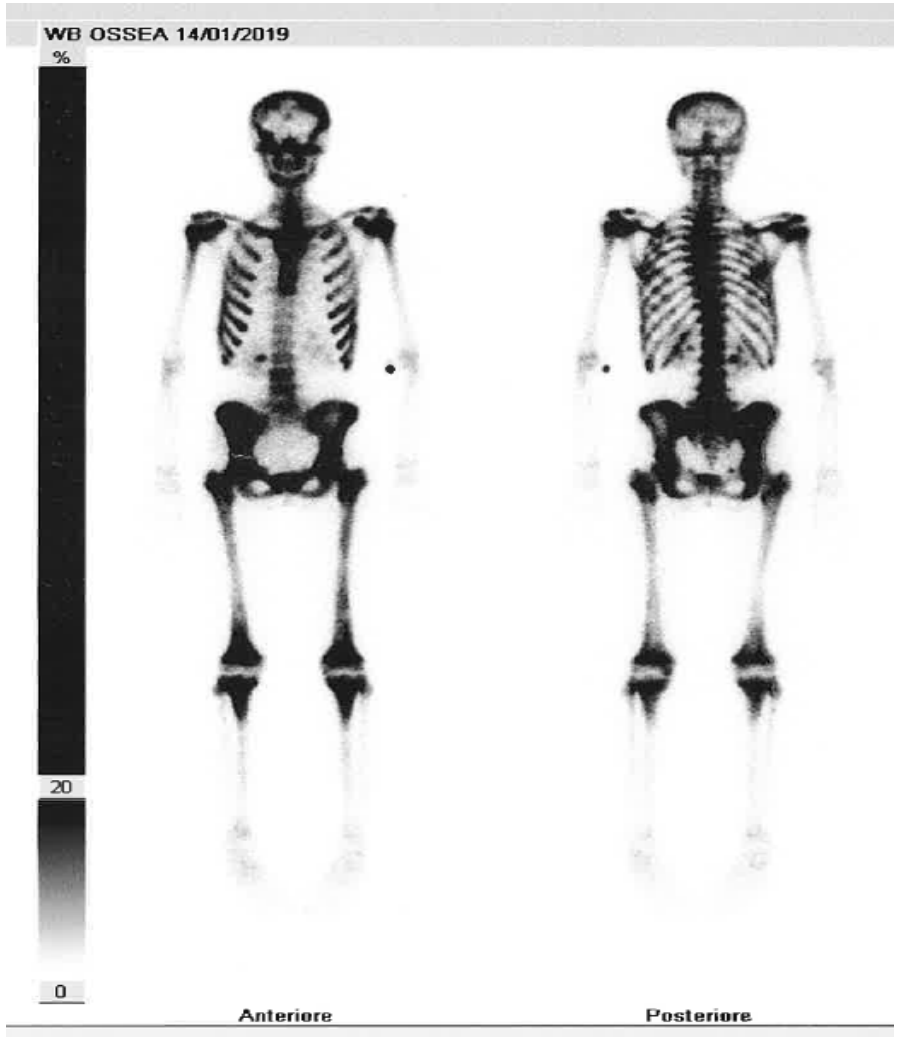
Clinical trial

Phase II study of anti-GD2 Chimeric Antigen Receptor-Expressing T cells in pediatric patients affected by relapsed/refractory GD2+ NEOPLASM

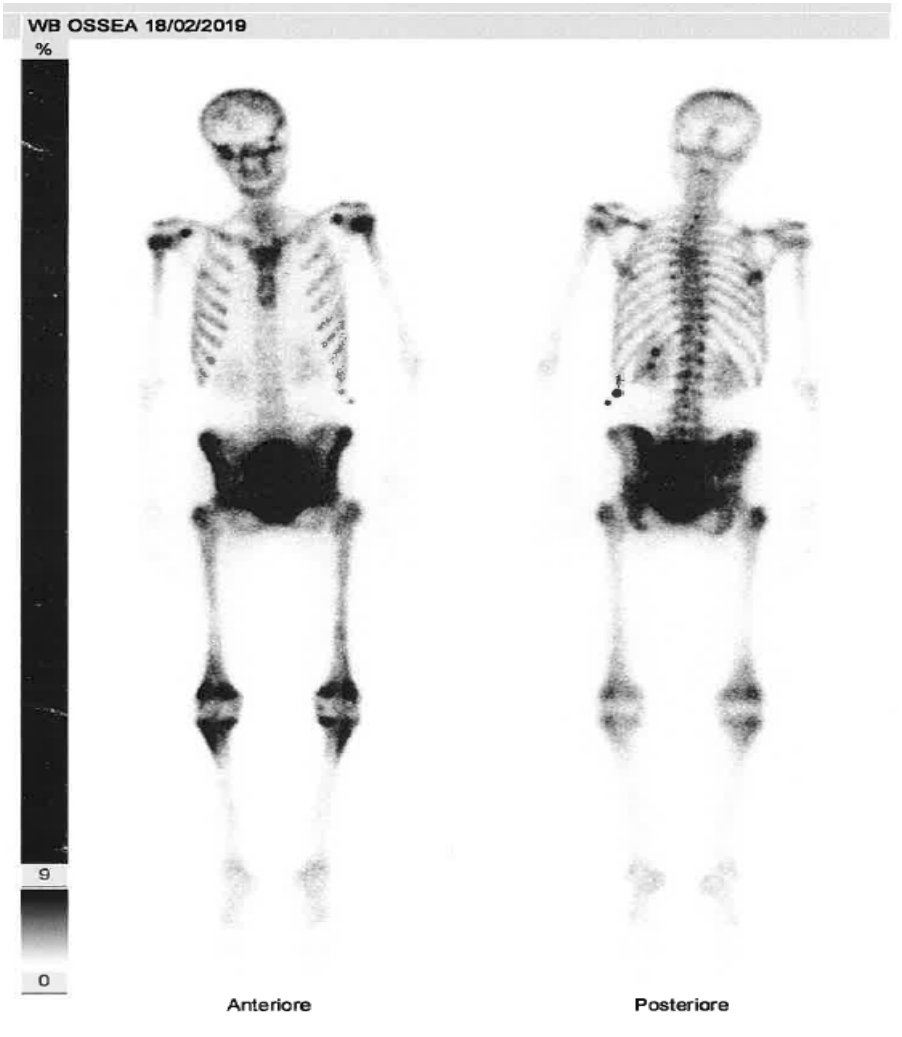
PI: Prof. Franco Locatelli

GD2-OPBG-012: disease evaluation Ewing' Sarcoma

Pre-CAR T infusion



1month post-CAR T infusion



Future directions for improving treatment efficacy

- ✓**Combinatorial treatment with checkpoint inhibitors unleashing both T and NK cells**
- ✓**Repeated infusions of GD2-CAR T cells**
- ✓**Combinatorial treatment to reduce tumor microenvironment immune-suppression**
- ✓**Heparanase/metalloproteases-armed CAR T cells (bulky disease)**
- ✓**New constructs targeting other Ags (also in the perspective of dual-CAR?)**

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OSPEDALE PEDIATRICO

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

ACKNOWLEDGEMENTS

Department of Hematology/ Oncology
Clinical Unit

Gene and Cell Therapy Unit
Concetta Quintarelli, Francesca Del Bufalo, Mara Vinci, Ignazio Caruana, Biagio De Angelis, Enrico Velardi

GMP Facility
Franca Fassio
Monica Gunetti
Stefano Iacovelli
All Team!

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ALLEANZA CONTRO IL CANCRO

Cell Manipulation Unit
Giuseppina Li Pira
Simone Biagini

CAGT (BCM, Houston)
Malcolm K. Brenner
Cliona M. Rooney
Helen E. Heslop

REPVBBLICA ITALIANA
Ministero della Salute

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REGIONE LAZIO

Immunology Research Area
Lorenzo Moretta
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AGENZIA ITALIANA DEL FARMACO

Fondazione Italiana ONLUS
per la Lotta al Neuroblastoma