ΟΔΟΣ ή ΛΕΩΦΟΡΟΣ ΚΥΤΤΑΡΙΚΩΝ ΘΕΡΑΠΕΙΩΝ

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Κυτταρική Θεραπεία

Κάθε θεραπευτική εφαρμογή με κύτταρα (αυτόλογα ή αλλογενή) μετά από *ex vivo* χειρισμούς

Χειρισμός: καλλιέργεια ή/και γενετική αλλαγή

Μεταμόσχευση κυττάρων

Χωρίς *ex vivo* χειρισμούς (πχ Μεταμόσχευση αιμοπ. κυττάρων)

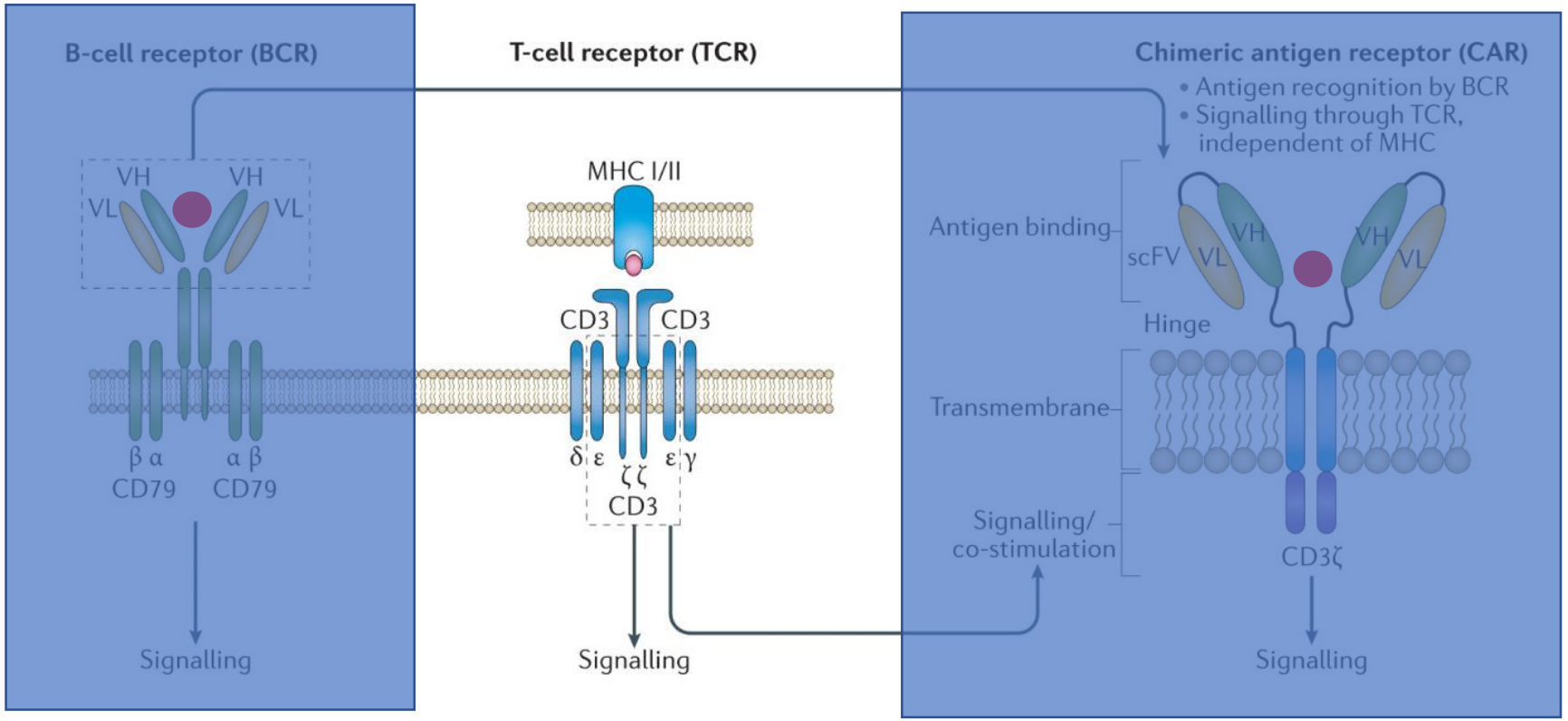
Οδός ή Λεωφόρος: εξαρτάται από το πλήθος των εφαρμογών

Γιατί Κυτταρικές Θεραπείες Σήμερα ?

1. Υπάρχει 30+ εμπειρία με μεταμοσχεύσεις κυττάρων (αιμοποιητικών)
2. Υπάρχει 10+ εμπειρία με μεταμοσχεύσεις γενετικά τροποποιημένων αιμοποιητικών κυττάρων
3. Συλλογική εμπειρία τεχνογνωσίας σε άσηπτες καλλιέργειες
4. Συλλογική εμπειρία τεχνογνωσίας σε ασφαλείς γενετικούς χειρισμούς
5. Τεκμηριωμένο θεραπευτικό όφελος
6. Εμπέδωση κανόνων λειτουργίας / αδειοδότησης
7. Είσοδος της φαρμακοβιομηχανίας στο χώρο

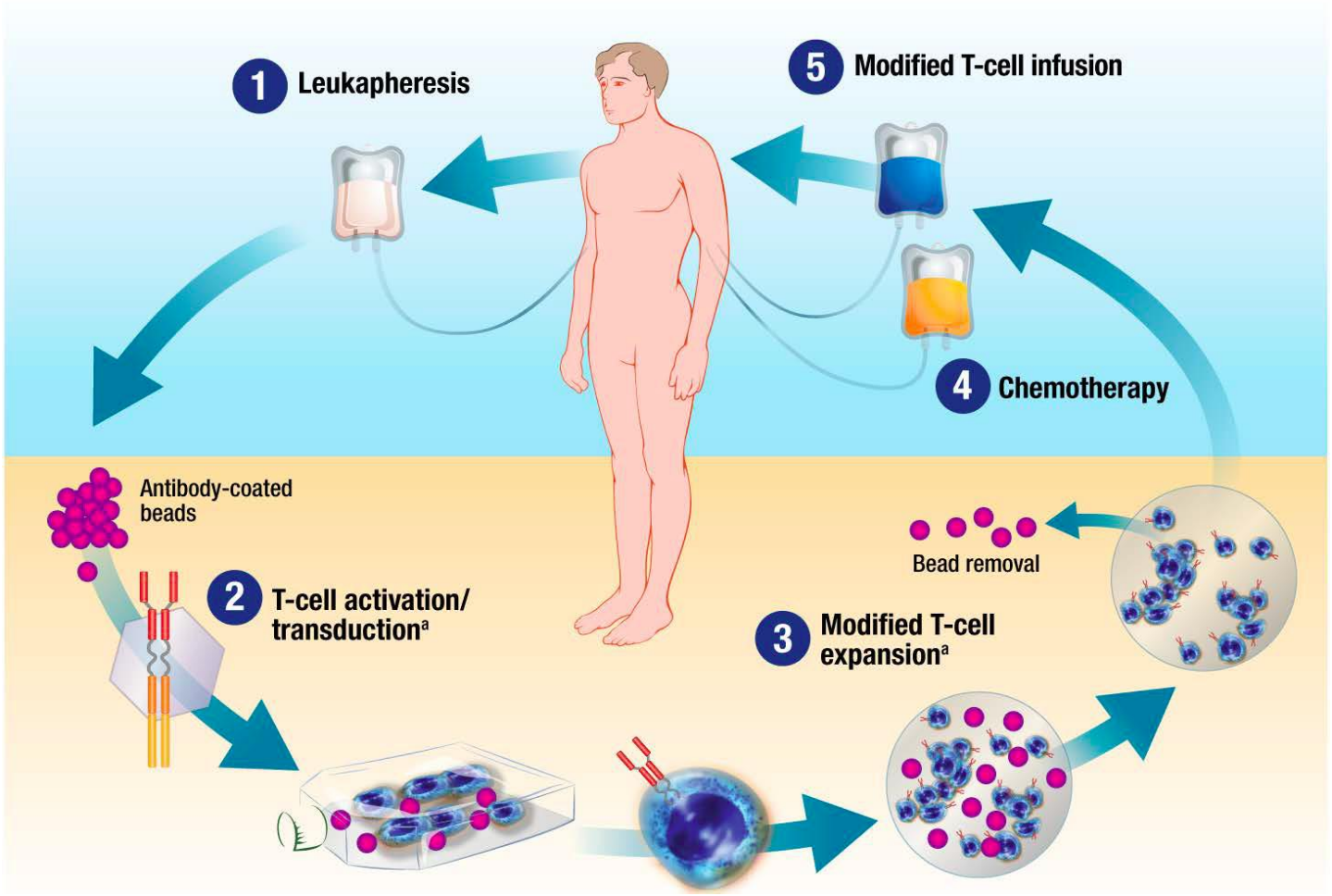
What can a Chimeric Antigen Receptor (CAR)-T cell do for you?

A CAR-T can harness the cytotoxic power of CD8+
T-cells without HLA restriction



Nat Rev Clin Oncol. 2016 January ; 13(1): 25–40.

CAR-T cell Therapy requires *ex vivo* Selection, Transduction and Expansion steps



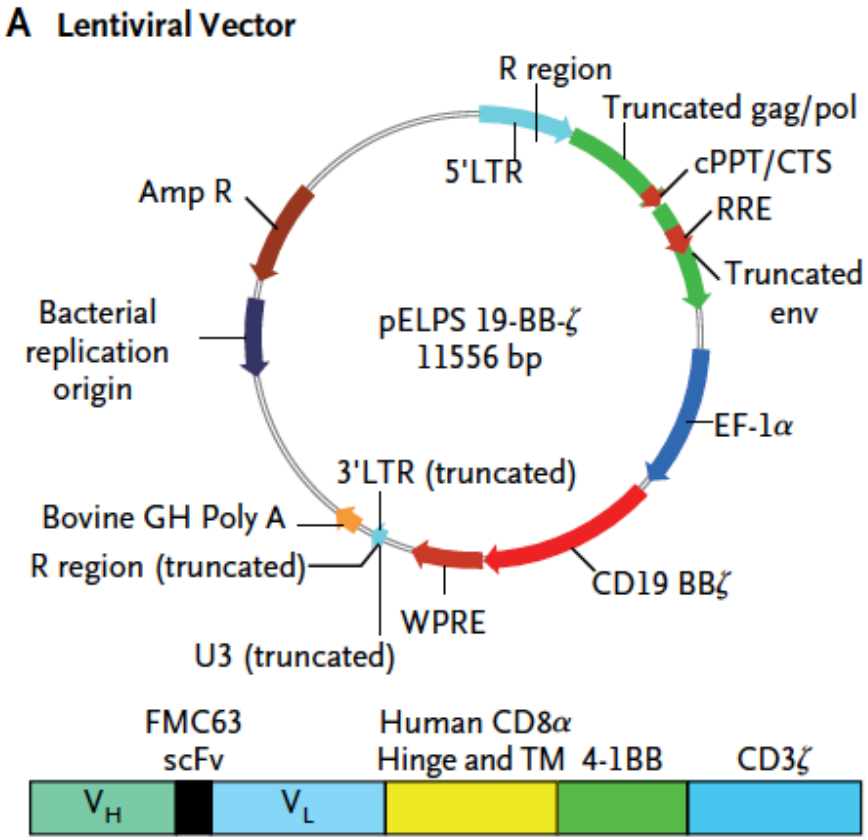
Similar to ASCT but with 7-10 days of ex vivo manipulations

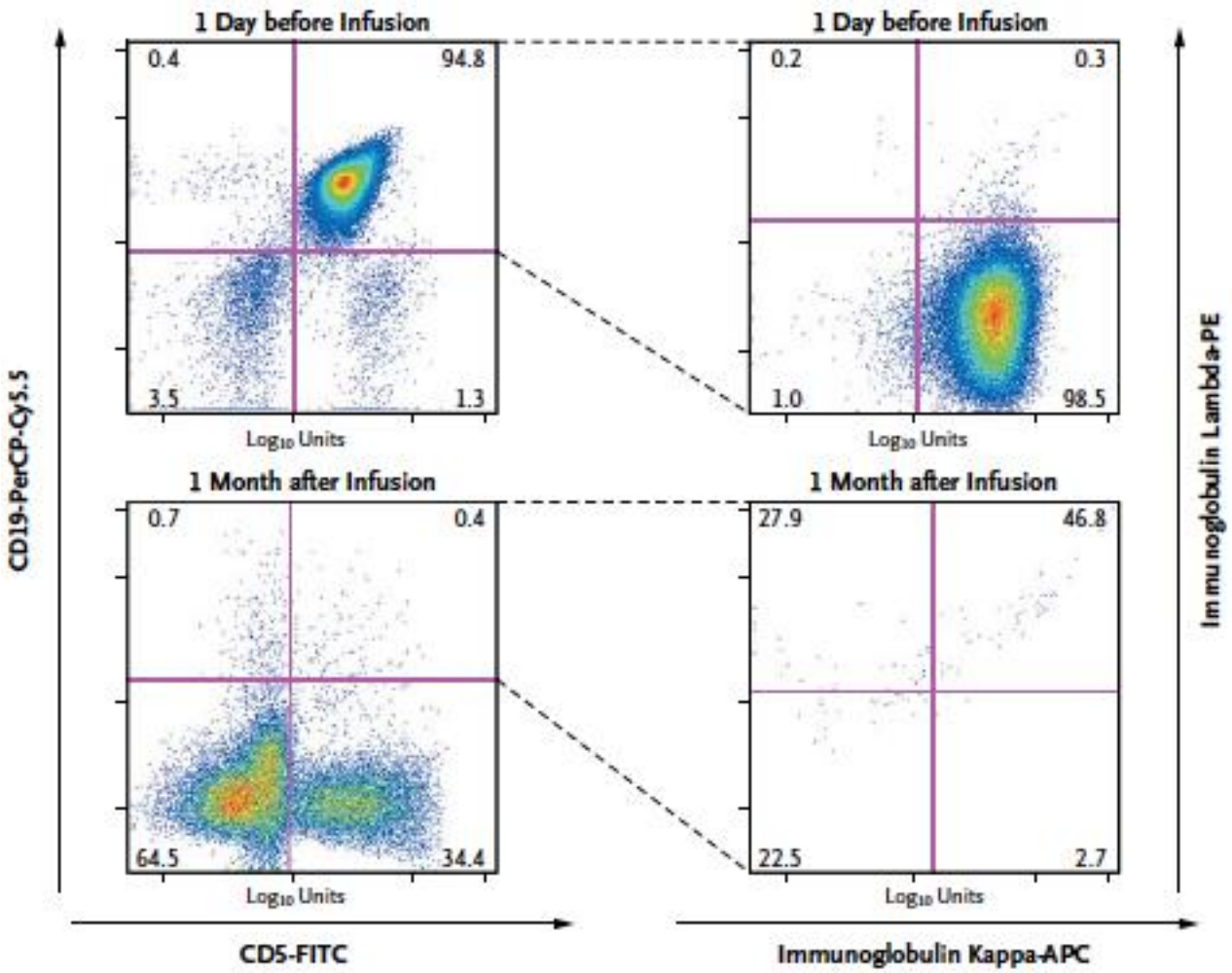
The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia

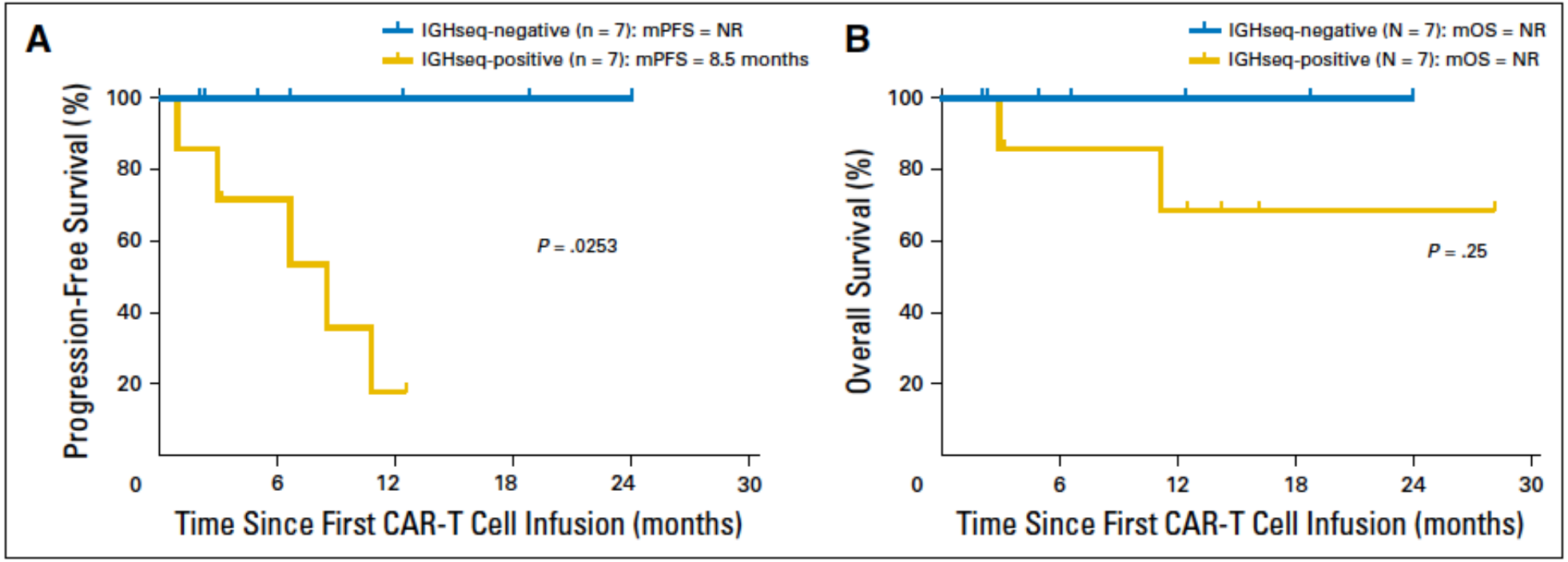
David L. Porter, M.D., Bruce L. Levine, Ph.D., Michael Kalos, Ph.D., Adam Bagg, M.D., and Carl H. June, M.D.





NEJM 365;8 august 25, 2011

PFS and OS in CLL patients who cleared disease from bone marrow at 4 weeks after CAR-T cell infusion by FCM and had no detectable IGH copies



CAR T Timeline

1989
Double chain CAR
Gross et al

1993
SINGLE chain CAR
AntiTumor CAR (Her2)
Eshhar et al

1996
CoStimul
CARs

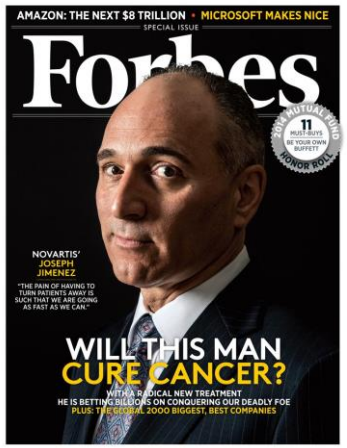
2000-06
Anti Solid tumor CAR
Ovarian/RCC

2010-14
Clinical Trials
ALL & CLL

Emily WHITEHEAD

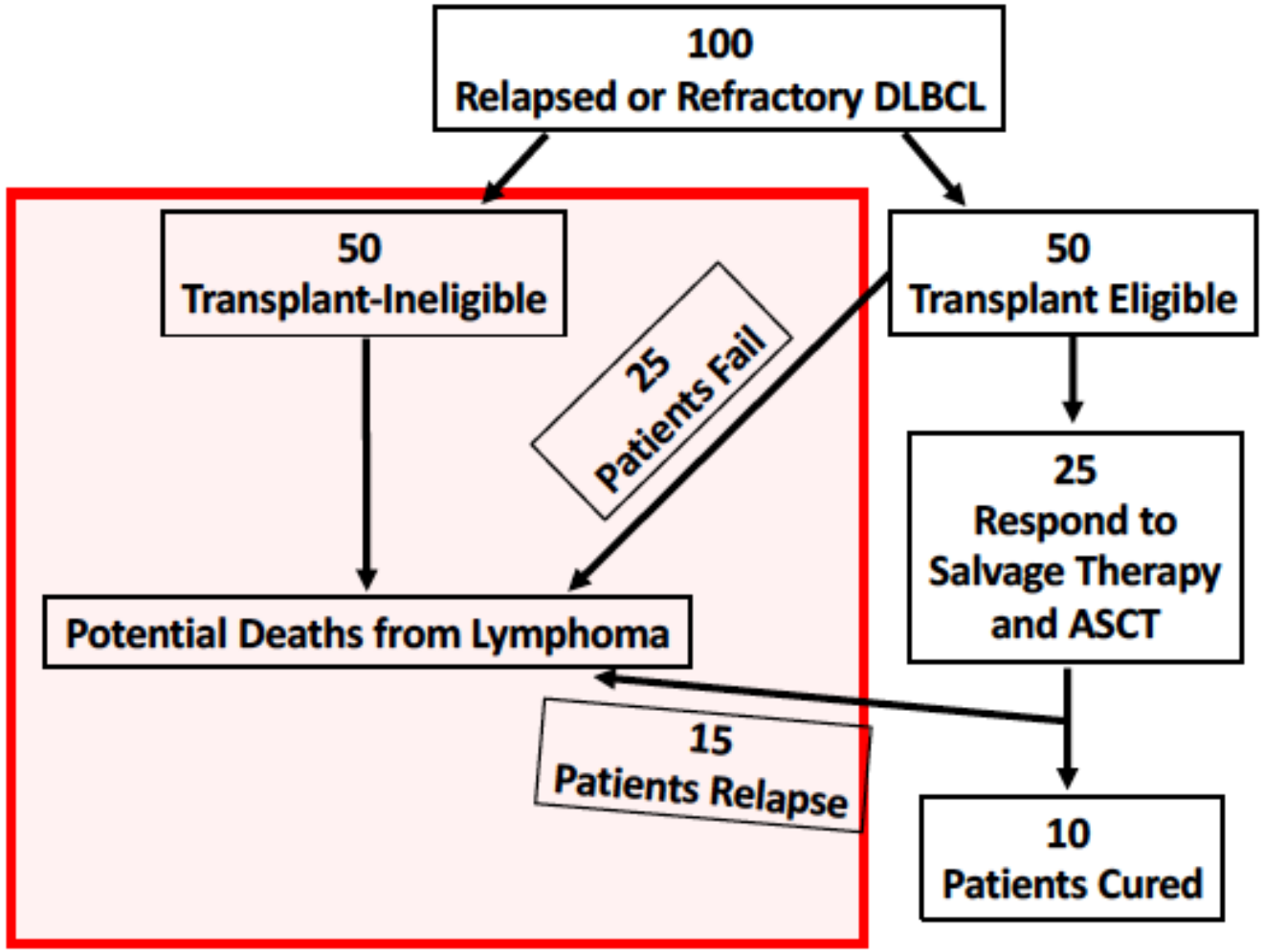
2011

2014 Media Frenzy



2016
GILEAD aqu KITE
11.2 billion

2018
CELGENE aqu JUNO
9 bn



Ελλάδα

± 100 ανά έτος

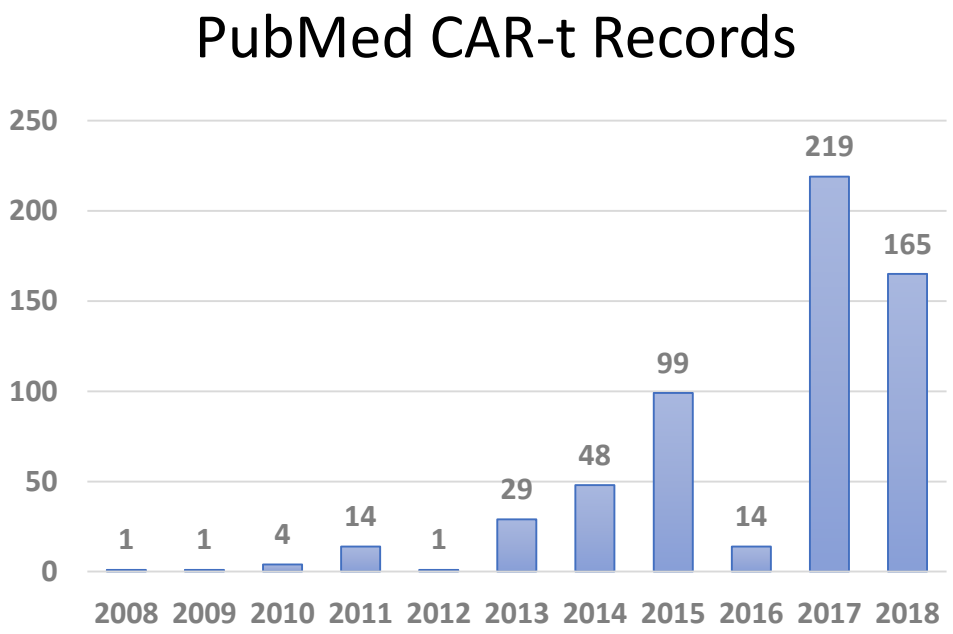
Selected phase 1/2 trials of autologous CD19-targeted CAR T cells for patients with R/R NHL

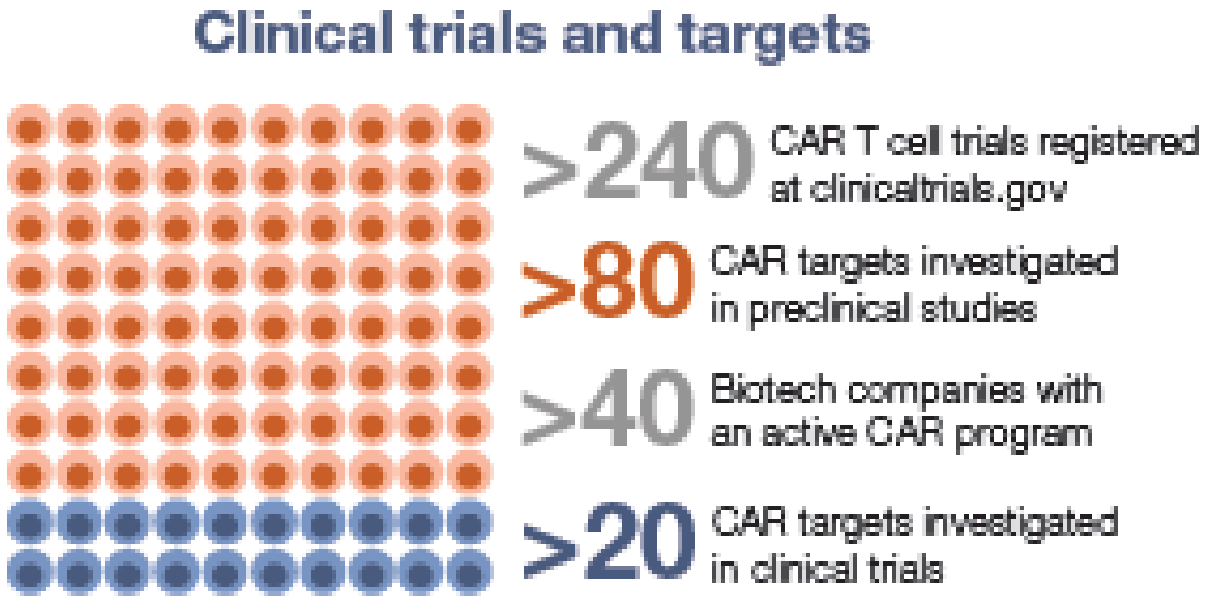
Reference	No. of patients treated	CAR construct	CAR T-cell dose or dose range	ORR, %/ CR rate, %
21	DLBCL (13), TFL (4), FL (2), PMBL (2), MCL (1)	CD19scFv/CD28/CD3ζ	1×10^6/kg to 6×10^6/kg	73/55
[redacted]				
42	DLBCL (11), TFL (10), FL (5), MCL (4)	CD19scFv/4-1BB/CD3ζ	2×10^5/kg to 2×10^7/kg (CD4:CD8, 1:1)	63/33
43	DLBCL (14), FL (14)	CD19scFv/4-1BB/CD3ζ	2×10^5/kg to 2×10^7/kg	64/57
10	DLBCL (77), PMBCL (8), TFL (16)	CD19scFv/CD28/CD3ζ	2×10^6/kg	82/54

ORR 70%
CR 50%

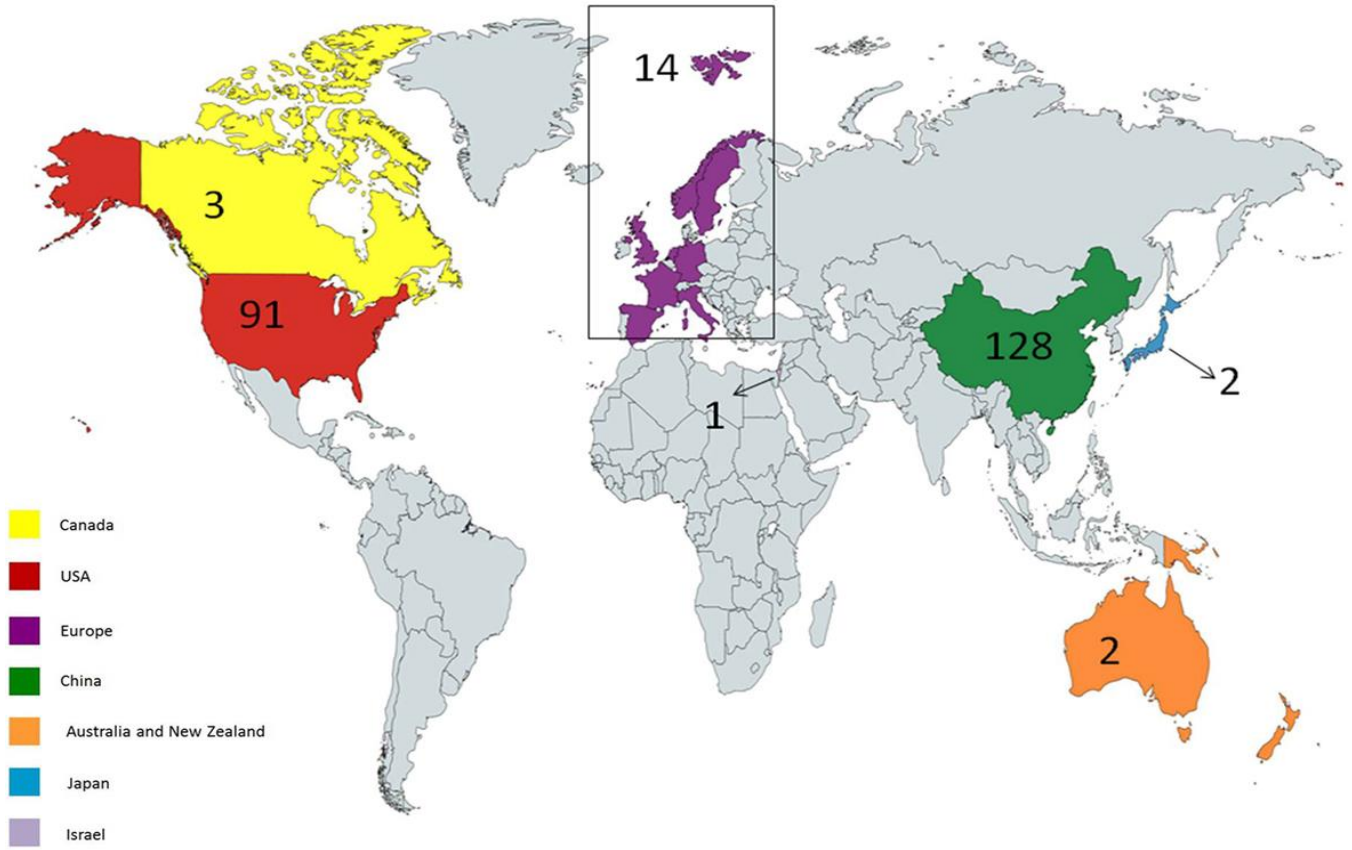
BLOOD 14 JUNE 2018 | VOLUME 131, NUMBER 24 2623

The Exponential Growth of the CAR T field circa 2018

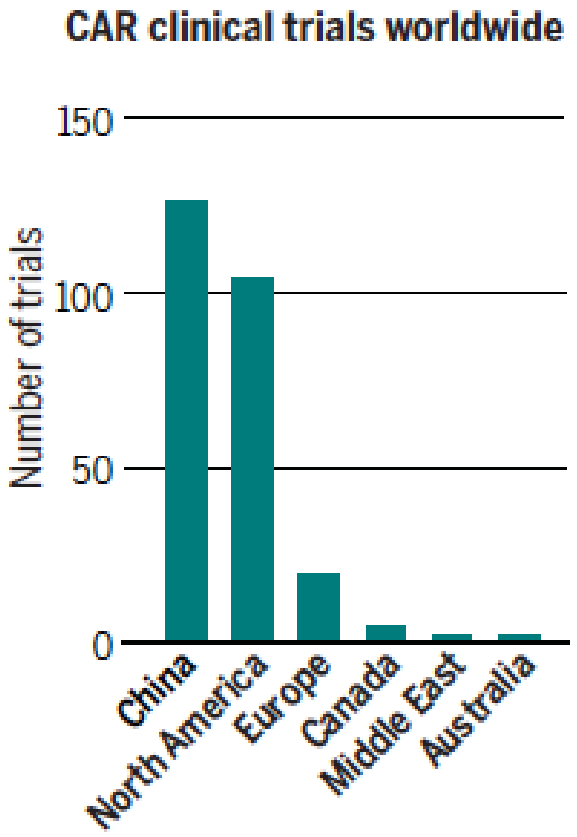


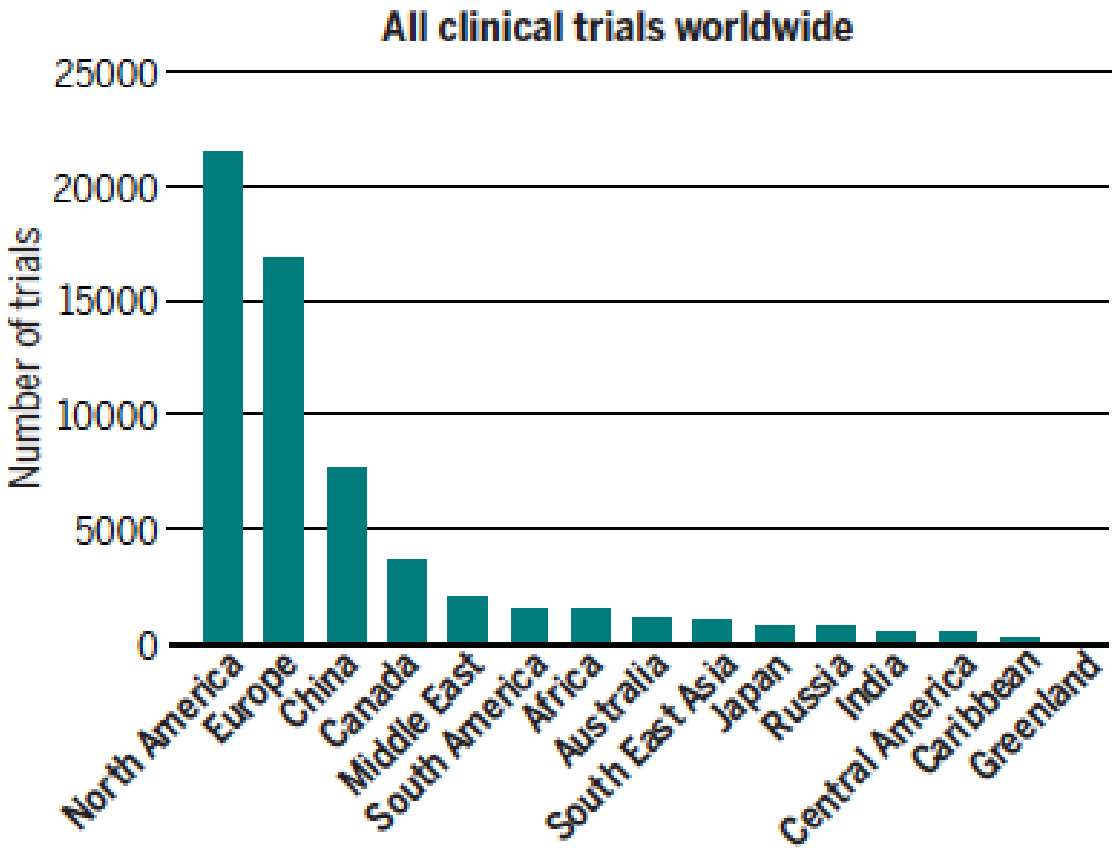


2018: CAR-T cell trials world-wide



CHINA is the leader in advanced biological therapies





	CTL019 * tisagenlecleucel		KTE-C19 ** axicabtagene ciloleucel		JCAR017 *** lisocabtagene maraleucel	
Disease state	r/r DLBCL	r/r tFL	r/r DLBCL	r/r tFL/PMBCL	r/r DLBCL	r/r tFL
Response evaluable pts, n	89	22	77	24	53	20
Follow-up, median	14 months		15.4 months		8 months	
Efficacy	n = 111		n = 101		N = 73	
ORR / CR	52% / 40% [w/in 3 mo]		82% / 54% [best]		59% / 45% [at 3 mo]	
% PFS for CR @ 12 mos	78.5%		79%		[88% 3 mo-CR in CR @ 6 mo]	
DOR (CR + PR; median)	not reached		11.1 months		not reached	
DOR (CR; median)	not reached		not reached		not reached	
Safety	n = 111		n = 101		n = 73	
CRS	22% grade 3/4*		13% grade ≥ 3**		1% grade ≥ 3**	
Neurotoxicity	12% grade 3/4		28% grade > 3		15% grade 3/4	

Company	NOVARTIS	GILEAD
USD	373K (NHL) 475K (ALL)	373K

Utilizing a Foamy viral vector for engineering safer CD19CAR-T cells

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[1] Cell and Gene Therapy Lab, BRFAA, Athens, Greece, [2] Division of Hematology, Medical School, University of Thessaly Larisa University Hospital, Larisa, Greece , [3]Ludwig Institute for Cancer Research at the University of Lausanne, Switzerland, [4] Departement d'oncologie, CHUV, Lausanne, Switzerland.

INTRODUCTION

Adoptive immunotherapy using Chimeric Antigen Receptors (CARs) expressing T-cells targeting CD19 is regarded as one of the most compelling breakthroughs in cancer treatment in recent years. These advances in the field of Immuno-oncology has led to unprecedented clinical outcomes for several cancer patients and to the subsequent approval of CD19 CAR based cell therapies for the treatment of relapsed/refractory hematologic malignancies (1). In the clinic, gene delivery of CD19 CARs in patient derived T-cells, has been achieved predominantly by the use of gamma-retroviral or lentiviral vectors. Despite their vast use in the field, these vectors however possess potential threat for patients due to the random integration events in the human genome and the possibility of mutagenesis (2,3). To address the safety issues in the delivery of CAR-T cell therapies, we employed a spumaviral vector, namely Foamy Virus (FV) that has been credited with having safer properties for the host genome in gene therapy (4,5). In this study we investigate the use of FV vectors for engineering safer CD19-CAR-T cells.

METHODS

- **Gene Cloning:** We used an Foamy Viral (FV) vector to clone our CD19CAR cassette (6) under the control of a human EF-1alpha promoter (FV-CD19CAR).
- **CAR-T-cell engineering** : Peripheral blood was obtained from normal donors and PBMCs were isolated with Lymphoprep (STEMCELL technologies) with standard protocols. T-cells were subsequently isolated from PBMCs using magnetic beads (Miltenyi). FV virus was produced in 293T cells using established protocols (7) and then used to transduce our isolated T-cells. Afterwards, cells were expanded for 1-2 weeks in tissue culture, under cytokine stimulation (IL5/IL17) before functional testing.
- **Reverse-Transcriptase PCR (RT-PCR)**: RNA was extracted from FV-CD19CAR-T cells and the CD19CAR expression was verified by RT-PCR.
- **Flow cytometry** : We demonstrate the transduction of T-cells using a GFP expressing vector (namely FV-GFP) by flow cytometry (iQue, Sartorius). Also functional evaluation of CAR-T cells was performed in co-incubation assays with relevant CD19 expressing Target cells (Raji) in Killing assays.
- **Incucyte Killing assay** : Effector cells (E, CAR-T cells) and Target cells (T, Raji) were co-incubated at different E:T ratios (5:1 or 10:1) in 96-well plates for 3 days. CytotoxRED (Cf 250 nM) was added in each well and the total dead cells (red dots) were scanned with a 10X lens in the incucyte instrument (Sartorius).
- Cell cytotoxicity was measured as the amount of fluorescent RED dye captured from the systems detector. IncuCyte® integrated analysis software was used to quantify the fluorescent signal and minimize the background fluorescence. Different parameters (either Total Red Object area, or red Intensity or Total RED counts) were used to visualize the cell cytotoxicity.
- **Elisa** : The detection of secreted IL-2 from effector cells in co-incubation assays (4h) was performed with a commercially available kit (Biolegend).

RESULTS

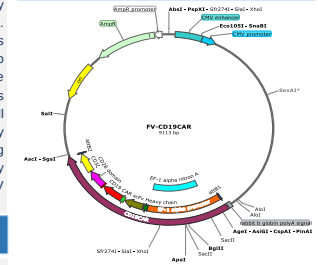

Figure 1. Map of the Foamy Viral vector FV-CD19CAR. The backbone of a basic Foamy viral vector in which the CD19CAR cassette was cloned with Gateway cloning (attB1/B2 flanking sequences indicate the cloning sites). The CD19CAR represents a 2nd generation chimeric antigen receptor (CAR) and is comprised of the heavy and light chains of the scFV against human CD19 along with the intracellular signaling domains of the CD3z and CD28 genes. The insert is under the control of the EF-1 apha human promoter.

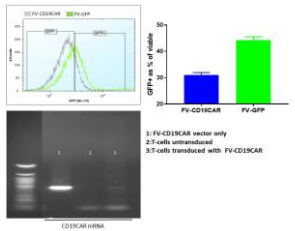

Figure 2. Upper panel, Flow cytometric analysis demonstrates the transduction of T-cells with a GFP expressing FV vector (FV-GFP). Histogram (left) and a representative graph (left) of GFP expression. Lower panel, RT-PCR illustrates the expression of the CD19-CAR mRNA in T-cells transduced with the FV-CD19CAR vector (lane 3).

RESULTS

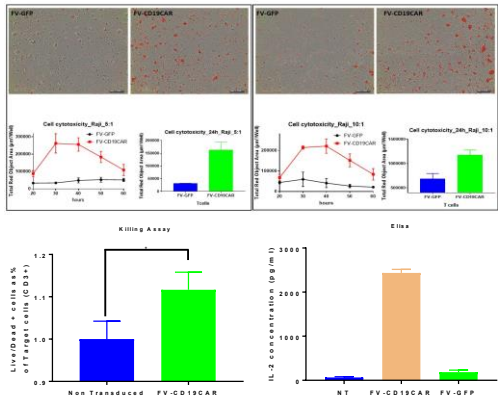

Figure 3. Functional testing. Upper panel, Killing assay (Incucyte) of FV-CD19CAR-T cells in co-incubation assays with Raji (CD19+). Top, photo indicates increased cell killing against Raji (red dots, cytotoxRED staining) of FV-CD19CAR effector cells compared to the control FV-GFP transduced T-cells. Bottom, the grpahs illustrate the killing capacity of the CAR-T cells at different cell ratios and timepoints. Bottom panel. On the left a flow cytometric based killing assay points out the increase in target dead cells (as determined here by the staining of Live/Dead-FR dye, Thermofisher) in co-incubations of target cells (Raji) with either FV-CD19CAR-T or the non-transdcuced T-cells (NT). On the right, the graph represents the marked increase in IL-2 release as detected by Elisa in co-incubations (4h) of Raji cells with CAR-T cells.

CONCLUSIONS

- We demonstrate the transduction of T-cells using FV-GFP vectors
- FV-CD19CAR-T cell activation was confirmed by a marked increase in cytokine levels in co-incubation assays.
- The effector function of FV-CD19CAR-T is not compromised and the cells can elicit effective anti-tumor in vitro response against tumor cell lines expressing the CD19 antigen.
- Overall, our results indicate the feasibility of engineering safer CD19CART cells that retain their activation and in vitro tumor cell killing properties and could potentially be used as alternative vehicle for gene delivery.

LITERATURE CITED

1. June, C.H. et al N Engl J Med, 2018
2. Haceinn-Bey-Abina et al, Science, 2003
3. Fischer, A.S et al, Gene, 2013
4. Trobridge G.D et al, PNAS, 2006
5. Hocum J.D et al, Sci Rep, 2016
6. Korchenderfer J.N et al, J. Immunother, 2009
7. Trobridge G. et al, Mehtods Enzymol, 2002

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RESULTS

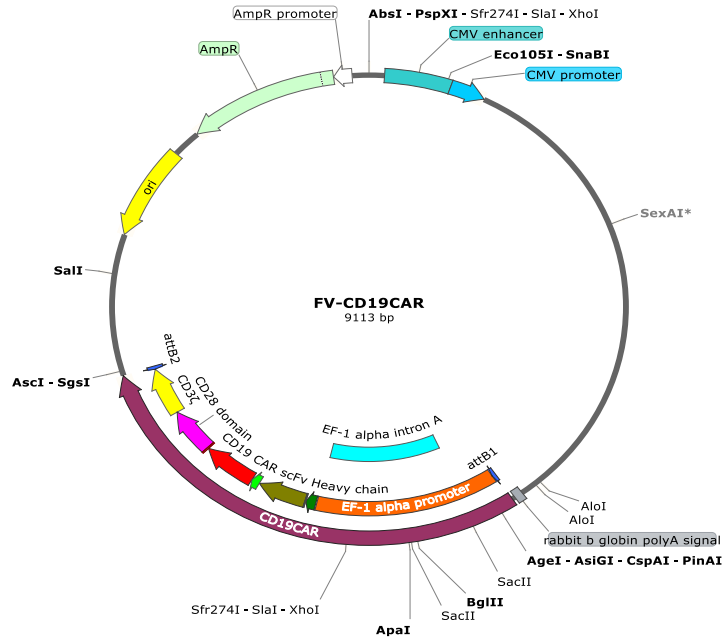
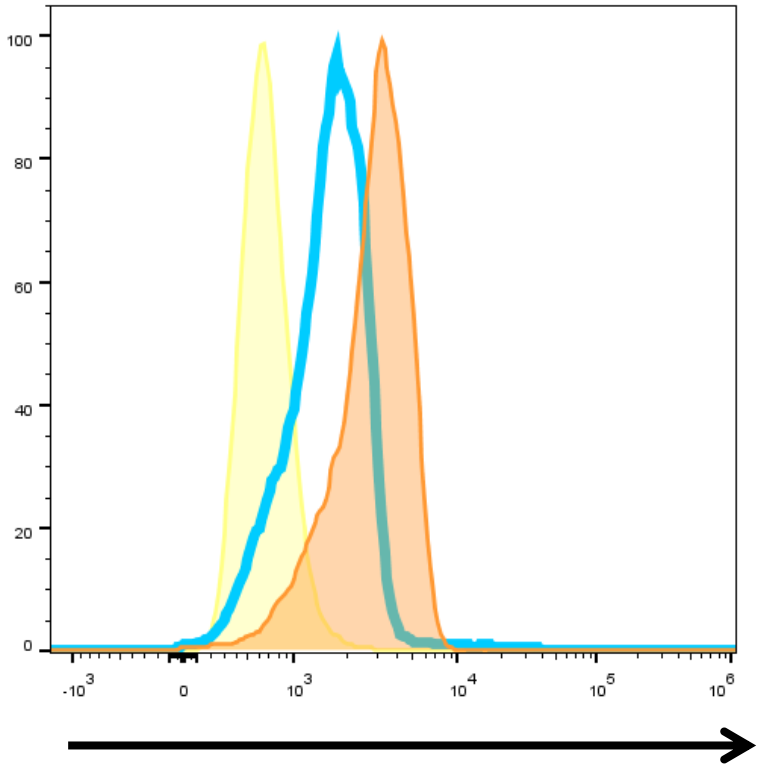


Figure 1. Map of the Foamy Viral vector FV-CD19CAR. The backbone of a basic Foamy viral vector in which the CD19CAR cassette was cloned with Gateway cloning (attB1/B2 flanking sequences indicate the cloning sites). The CD19CAR represents a 2nd generation chimeric antigen receptor (CAR) and is comprised of the heavy and light chains of the scFV against human CD19 along with the intracellular signaling domains of the CD3z and CD28 genes. The insert is under the control of the EF-1 apha human promoter.

293T Transfected with Foamy Virus CAR-T19.2 or Lenti Virus CAR-T19.2

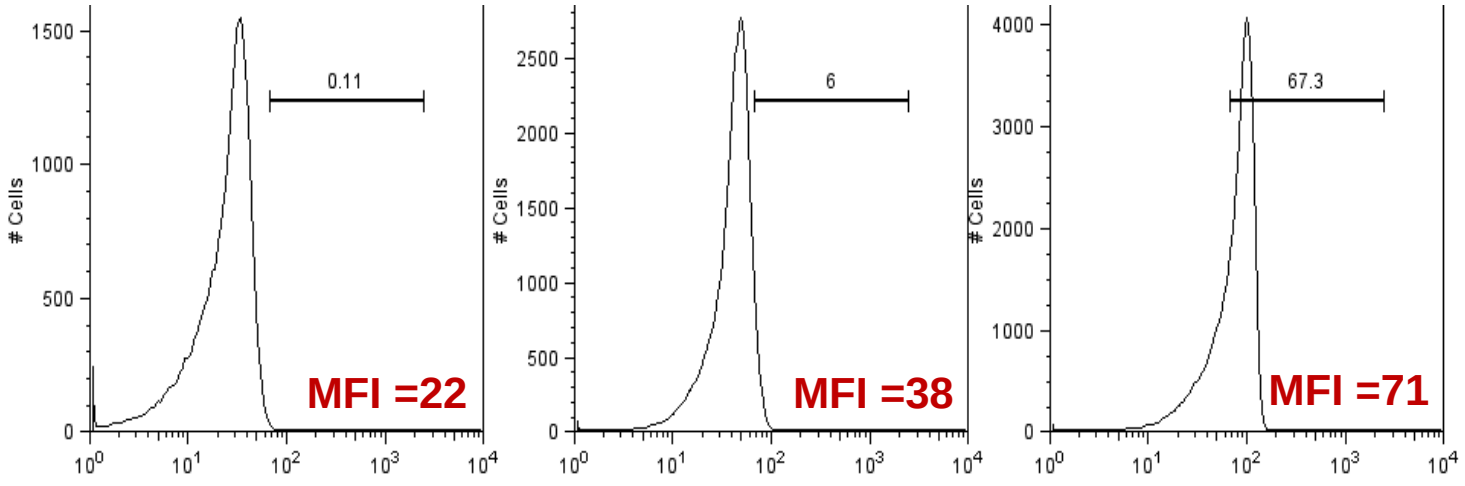


Control stained MFI=700

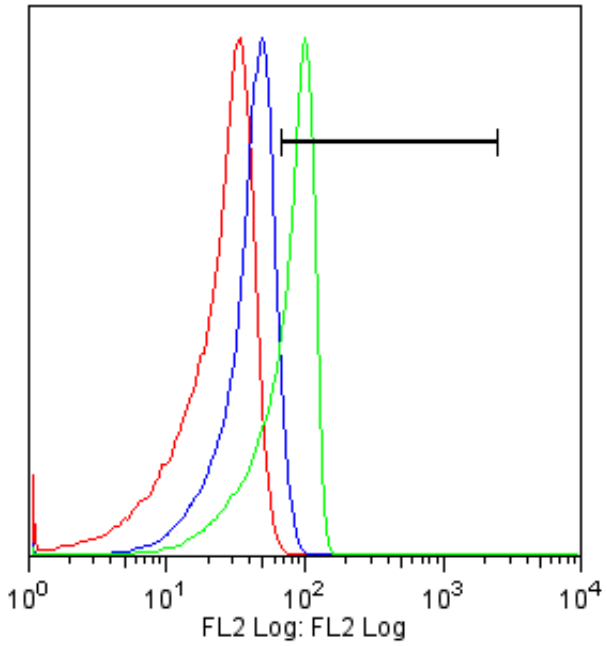
LV CART19.2 MFI=1743

FV CART19.2 MFI=3050

293 Cells Transduced with anti CD19 FV-CART Vectors: New CART design expresses nearly double levels of transgene



Control **Original vector** **NEW vector**

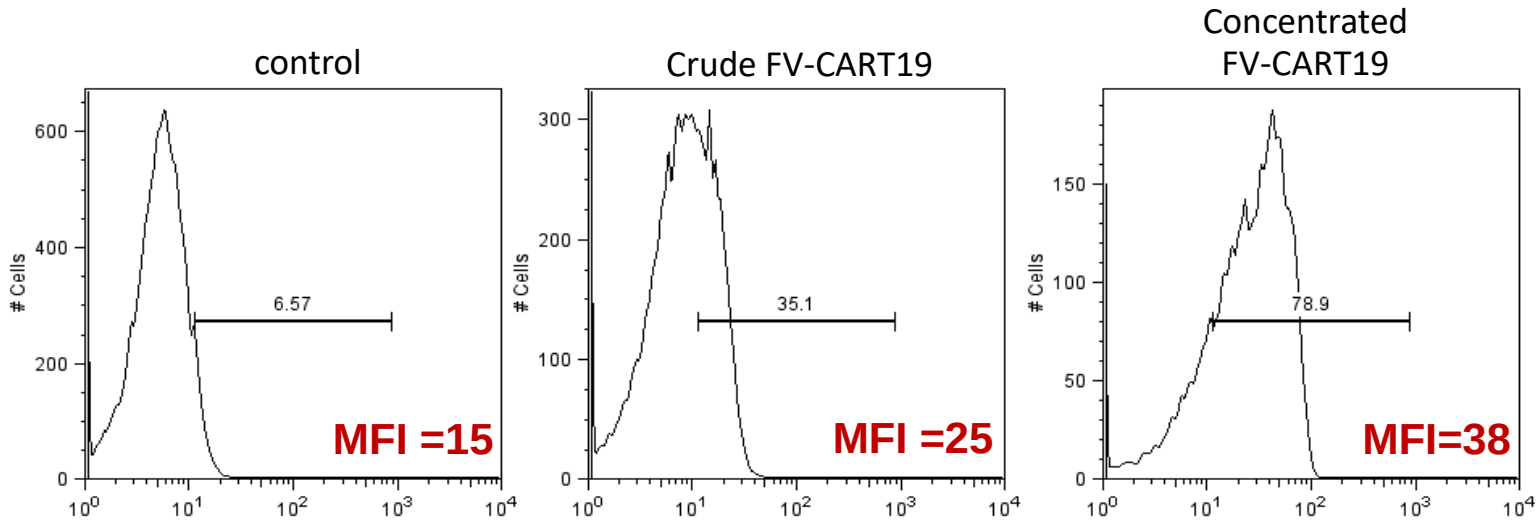


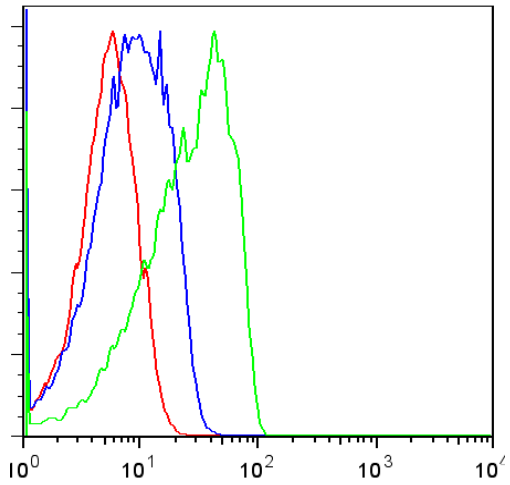
Control stained

Original CART

New CART

Transduction of CB derived T-cells with pΔΦ.E1.CART19



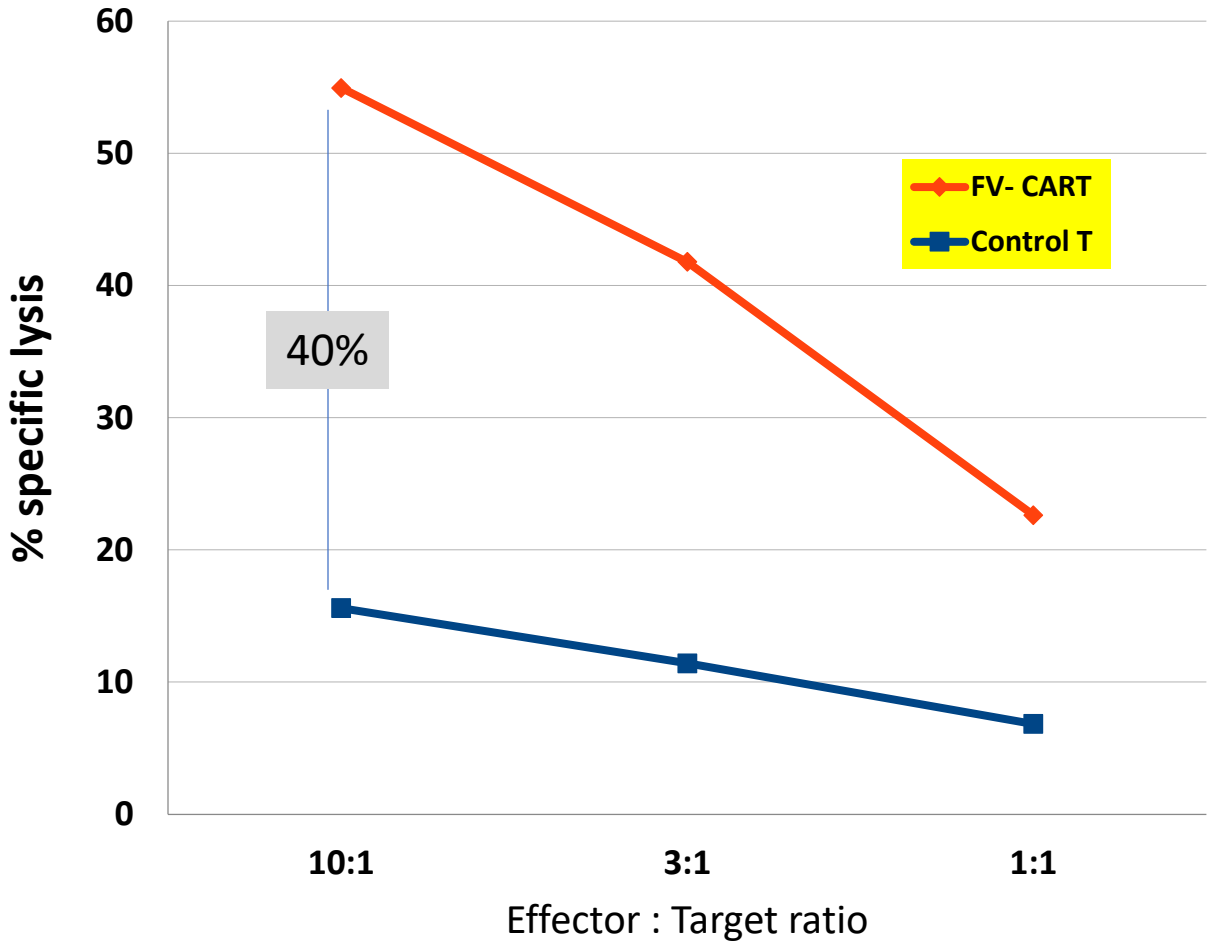


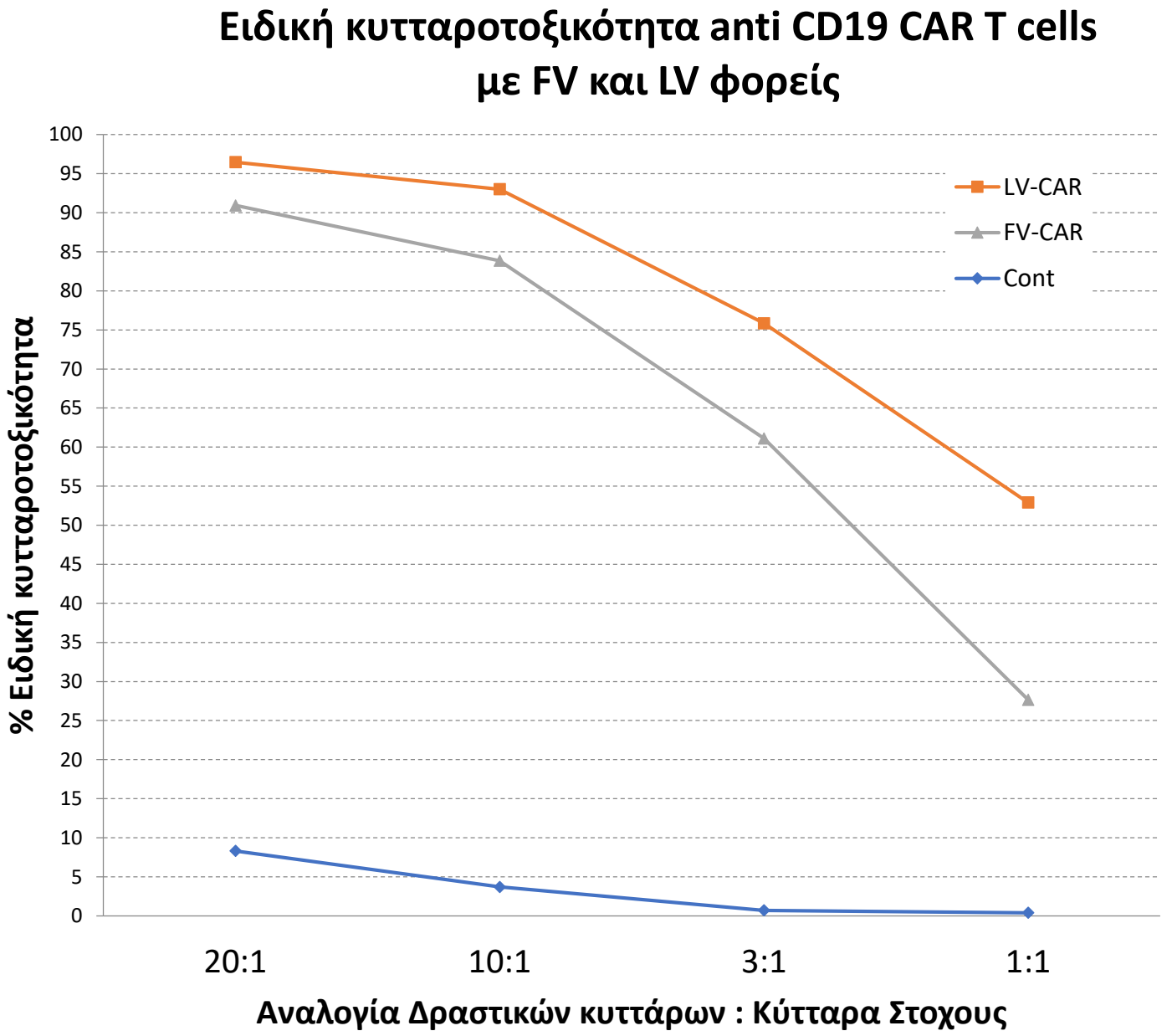
Control stained

Crude FV-CART19

Concentrated FV-CART19

% Specific Lysis of Daudi cells by FV-CART19 Transduced T cells




Ειδική κυτταροτοξικότητα anti CD19 CAR T cells
με FV και LV φορείς
% Ειδική κυτταροτοξικότητα
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
LV-CAR
FV-CAR
Cont
20:1
10:1
3:1
1:1
Αναλογία Δραστικών κυττάρων : Κύτταρα Στοχους

CAR T cells in Solid Tumors Have so far Limited Efficiency

1. Antigen Presentation
2. CAR T invasion in tumor mass

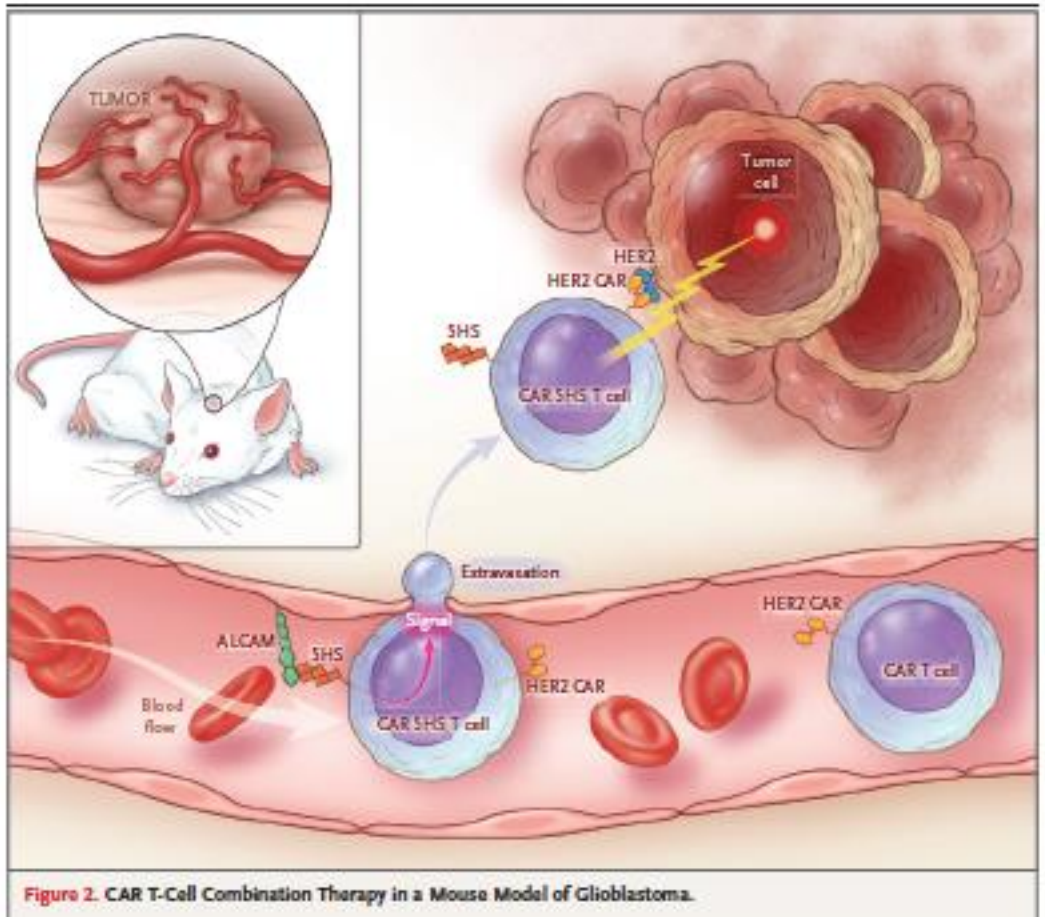


Figure 2. CAR T-Cell Combination Therapy in a Mouse Model of Glioblastoma.

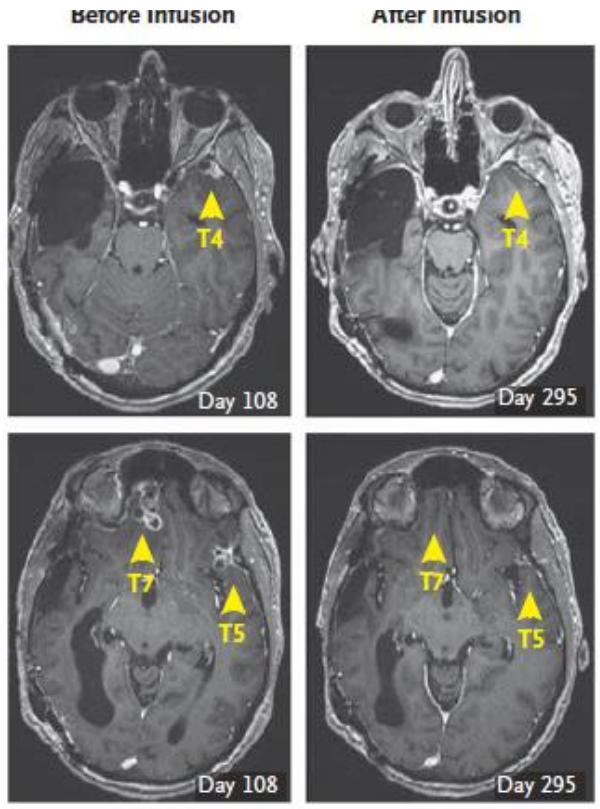
The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

2016

Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy

Regression of Recurrent Multifocal Glioblastoma, Including Spinal Metastases, after Intraventricular Delivery of IL13Rα2-Targeted CAR T Cells.



Relative Questions / Issues

1. Which health care system is going to pay 300-400K/patient dose?

Non-Affordable

2. Could it be produced nationally as a limited license / hospital use?

YES (Hospital Clinic, IDIBAPS - Hematology C/ Rossello 149-153 Barcelona Barcelona 08036, Spain)

3. Does the Market price reflect the real cost-of-goods?

NO: estimated at around 30-40K/batch

4. Manufacturing

Central (NOVARTIS/GILEAD) vs Point-of-care manufacture

CENTRAL: Logistics, Risk, Monopolies

PointOfCare: Modular, tech diffusion, added value, minimal invest

The case of Miltenyi's PRODIGY

Integrated Closed System for Blood Cell Manipulations

1. Isolate T cells from apheresis
2. Expand in closed bags
3. Transduce with viral stock
4. Further expansion
5. Final product (7-10 days)

Οδός ή Λεωφόρος: εξαρτάται από το πλήθος των εφαρμογών

1. CAR T cells
2. Virus Specific T cells
3. Off the shelf products
4. Cell banks