ΕΝΩΣΗ ΕΠΙΣΤΗΜΟΝΙΚΟΥ ΠΡΟΣΩΠΙΚΟΥ
Γ.Ν.Α. «Ο ΕΥΑΓΓΕΛΙΣΜΟΣ» (Ε.Ε.Π.Ν.Ε.)

25ο Ετήσιο Σεμινάριο Συνεχιζόμενης Ιατρικής Εκπαίδευσης Γ.Ν.Α. «Ο Ευαγγελισμος»

Νεότερες ανοσοθεραπείες στην αντιμετώπιση των αιματολογικών κακοηθειών.

Οι βιολογικές αρχές

Σταύρος Γιγάντες
Αιματολογική και Λεμφωμάτων κλινική – ΜΜΜΟ

Δεν υπάρχει σύγκρουση συμφερόντων με τις Χορηγούς Εταιρείες:

NOVARTIS
Roche
astellas
WinMedica
Serving Health for Life
GILEAD
Advancing Therapeutics. Improving Lives.
MEDIWAY
Pfizer Oncology
SANDOZ A Novartis Division
Bristol-Myers Squibb
abbvie
Abbott
AMGEN
cardio innovation
GENESIS pharma
janssen Oncology
PHARMACEUTICAL COMPANIES OF Johnson&Johnson
Pfizer Hospital
Boehringer Ingelheim
Lilly ΦΑΡΜΑΣΕΡΒ
SERVIER
Baxter
DEMO ABEE ΒΙΟΜΗΧΑΝΙΑ ΦΑΡΜΑΚΩΝ
emergy products
AENORASIS Intuition in Healthcare
Rontis Driven by innovation
Specifar A Teva Company

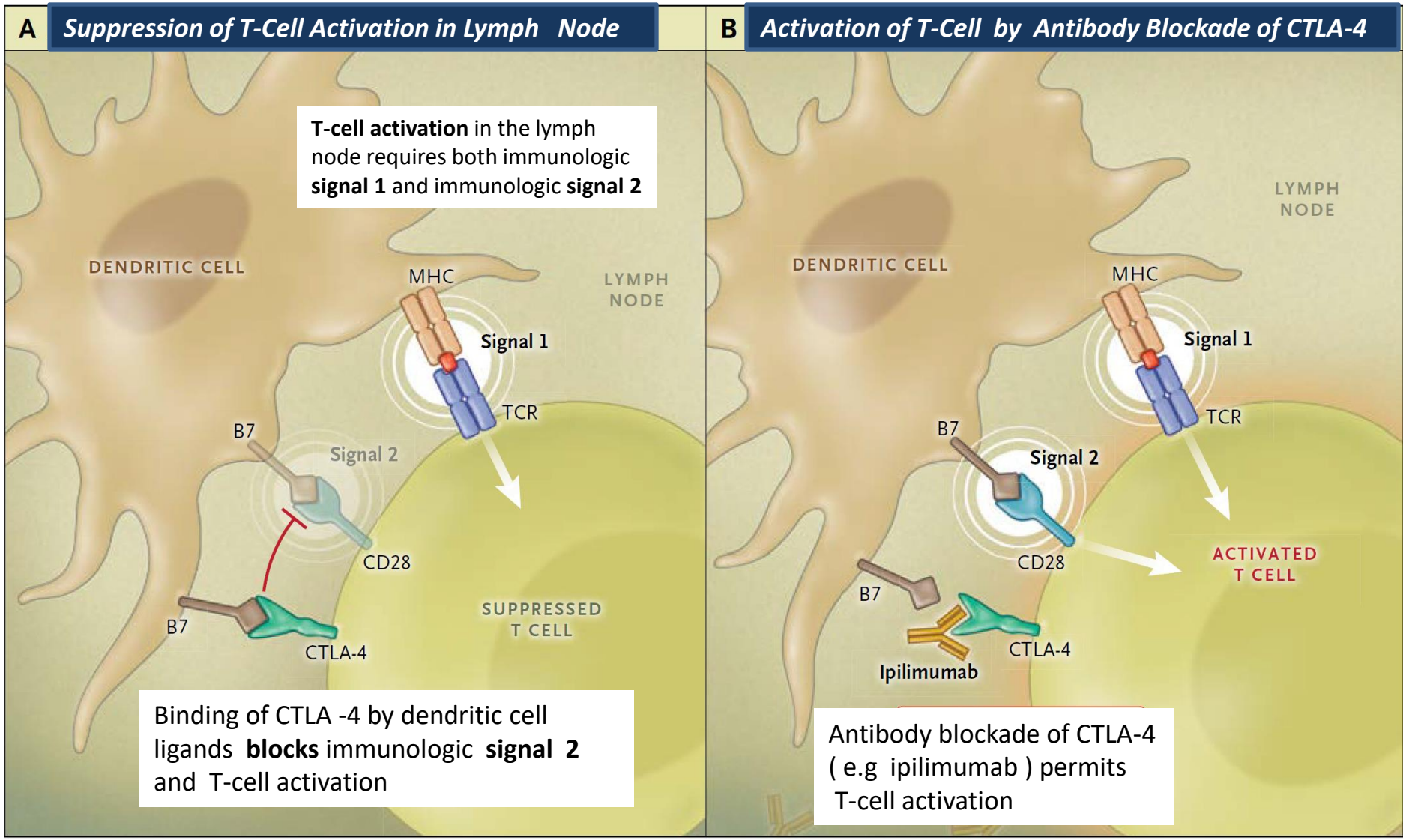
Ανοσοθεραπεία στηριζόμενη στην άρση των ανασταλτικών μηχανισμών της ενεργοποίησης των Τ-κυττάρων

Target	Gene Symbol	Aliases	Genomic Location	Exons	Protein	Binding Partners
CTLA-4	CTLA-4	CD152	2q33	4	Cytotoxic T-lymphocyte associated protein 4	B7-1 (CD 80) B7-2 (CD 86)
PD-1	PDCD1	CD279	2q37.3	6	Programmed cell death protein 1	PD-L1 (CD274) PD-L2 (CD273)
PD-L1	CD274	B7-H1 PDCD1L1	9p24	8	Programmed cell death 1 ligand 1	PD-1 (CD279) CD80
PD-L2	PDCD1LG2	CD273 B7-DC	9p24.2	7	Programmed cell death 1 ligand 2	PD-1 (CD279)
LAG-3	*LAG-3*	CD223	12p13.32	8	Lymphocyte activation gene 3 protein	MHC class II LSECtin
TIM-3	HAVCR2	CD366 KIM-3	5q33.3	7	Hepatitis A virus cellular receptor 2	Galectin-9
IDO	IDO1	INDO	8p11.21	10	Indoleamine 2,3-dioxygenase 1	n/a

CTLA- 4, cytotoxic T- lymphocyte- associated protein- 4;
IDO, indoleamine 2,3- dioxygenase 1;
LAG- 3, lymphocyte activation gene- 3;
PD- 1,programmed cell death protein- 1;
PD- L1, programmed death ligand- 1;
PD- L2, programmed death ligand- 2;
TIM-3, T cell immunoglobulin 3.

Check point inhibitors: Αναστολείς σημείων ελέγχου κυτταρικής ανοσολογικής απάντησης

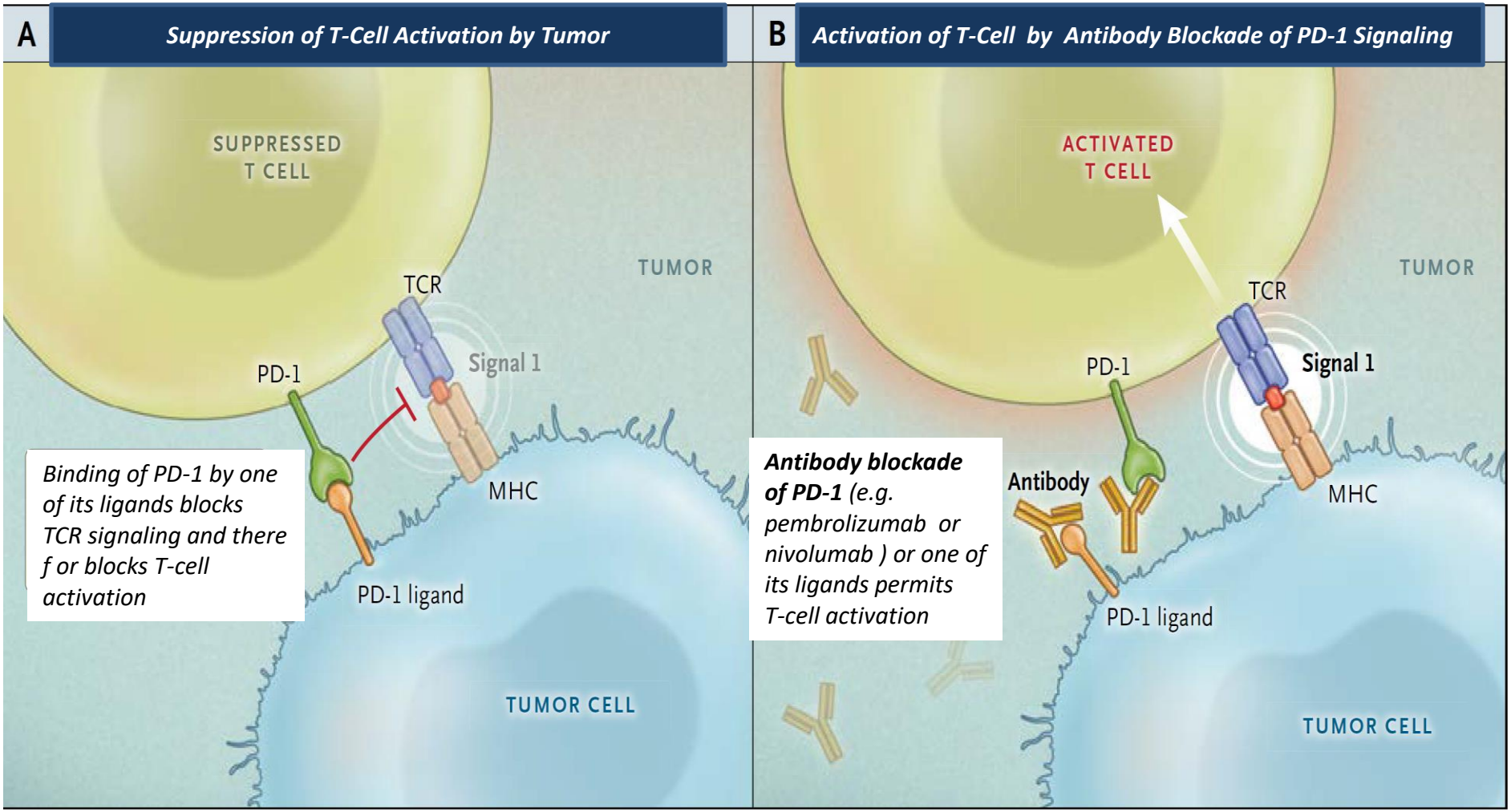
T-cell Activation in the Lymph Node.



A. Ribas : NEJM 2015

Check point inhibitors: Αναστολείς σημείων ελέγχου κυτταρικής ανοσολογικής απάντησης

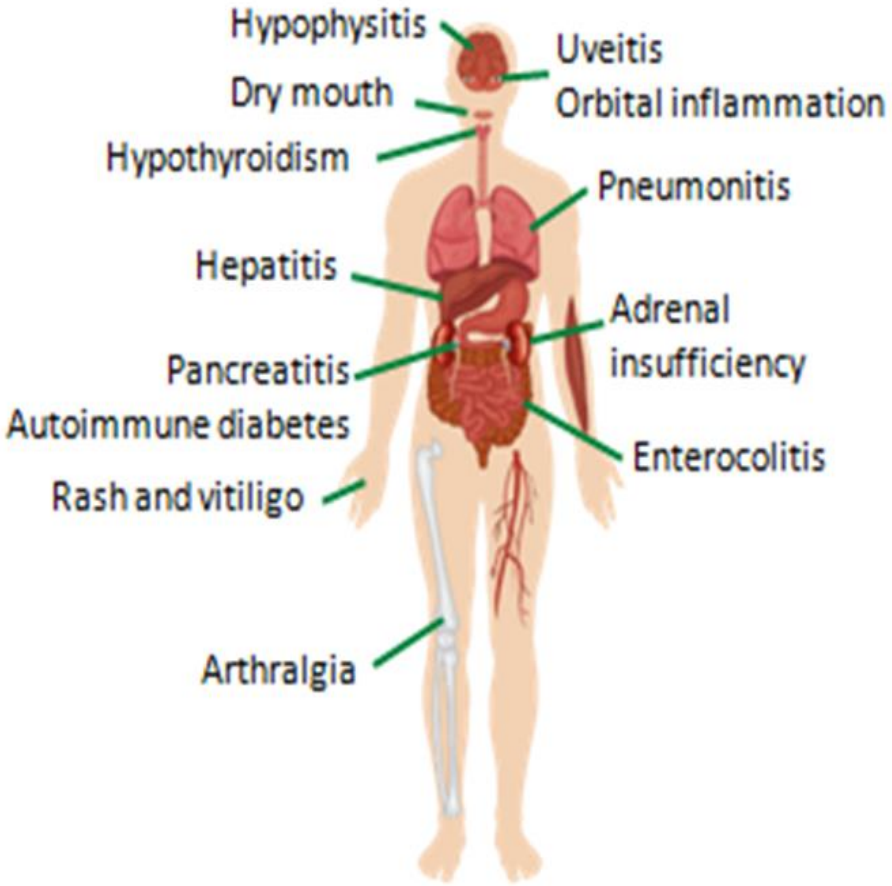
T-cell Activation in Tumor Milieu.

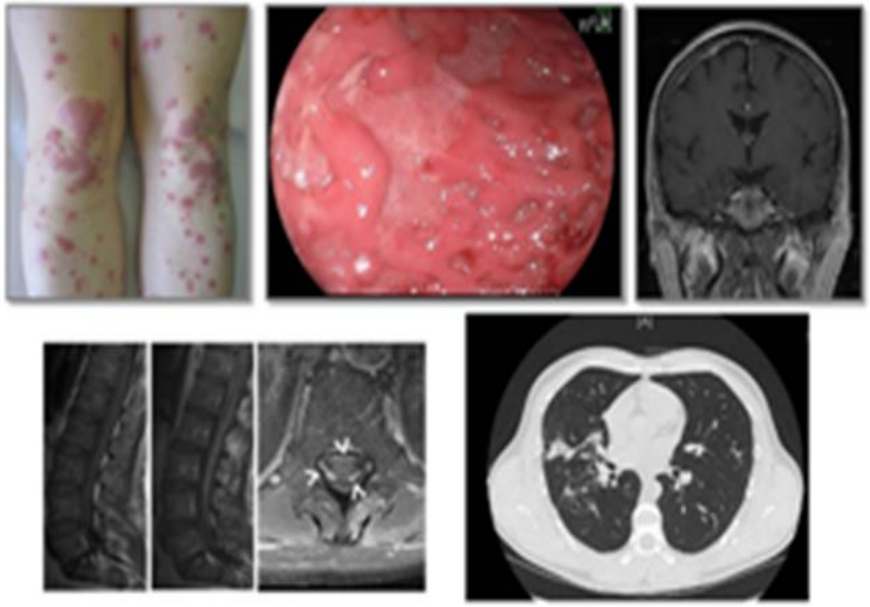


A. Ribas : NEJM 2015

Check point inhibitors: New spectrum of AEs- Immune-Related AEs

- ***Ενεργοποίηση κυττάρων ανοσολογικού συστήματος εκτός του νεοπλασματικού περιβάλλοντος***
- ***Εκδηλώσεις μιμούμενες αυτοάνοσες καταστάσεις***

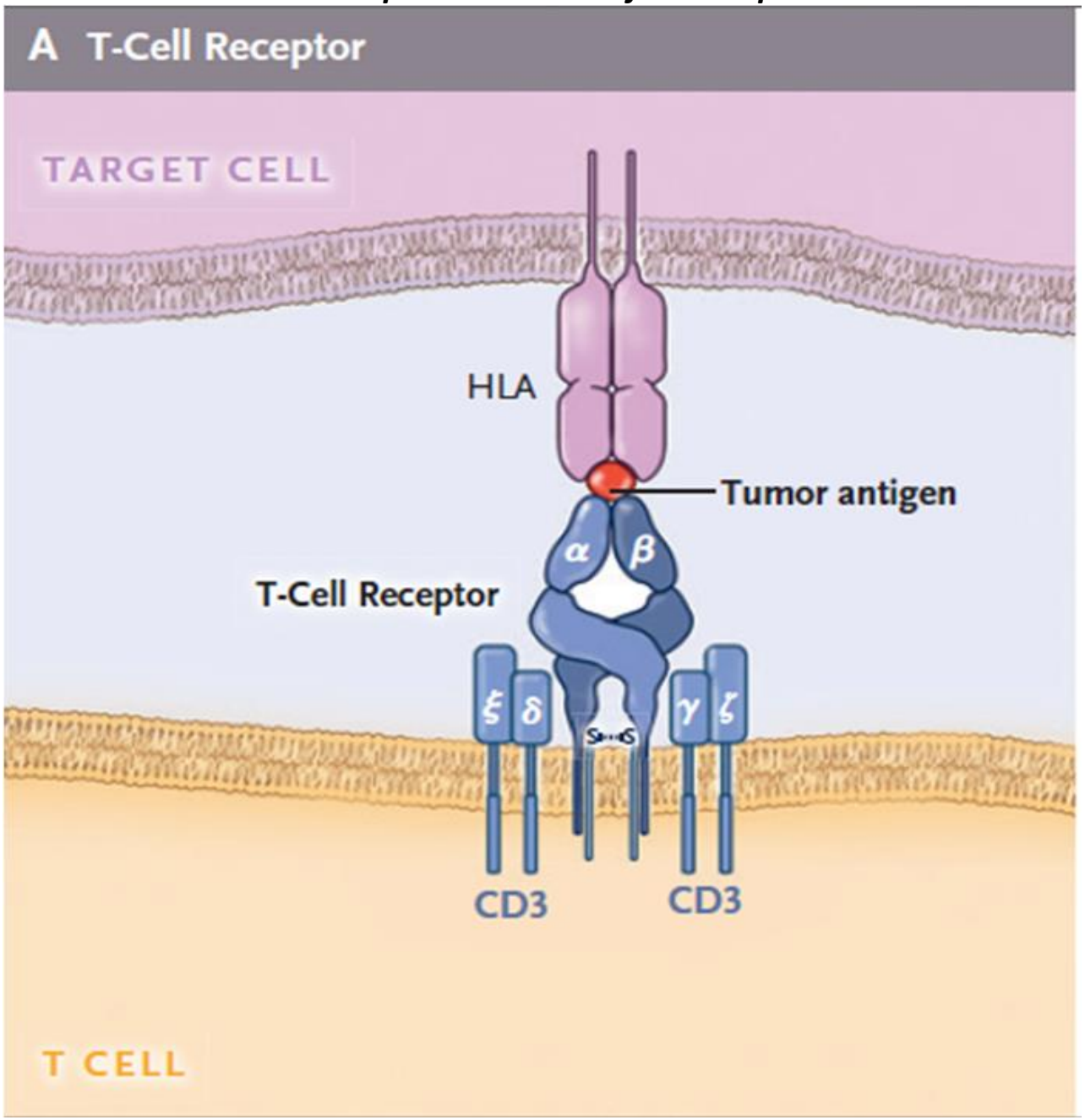




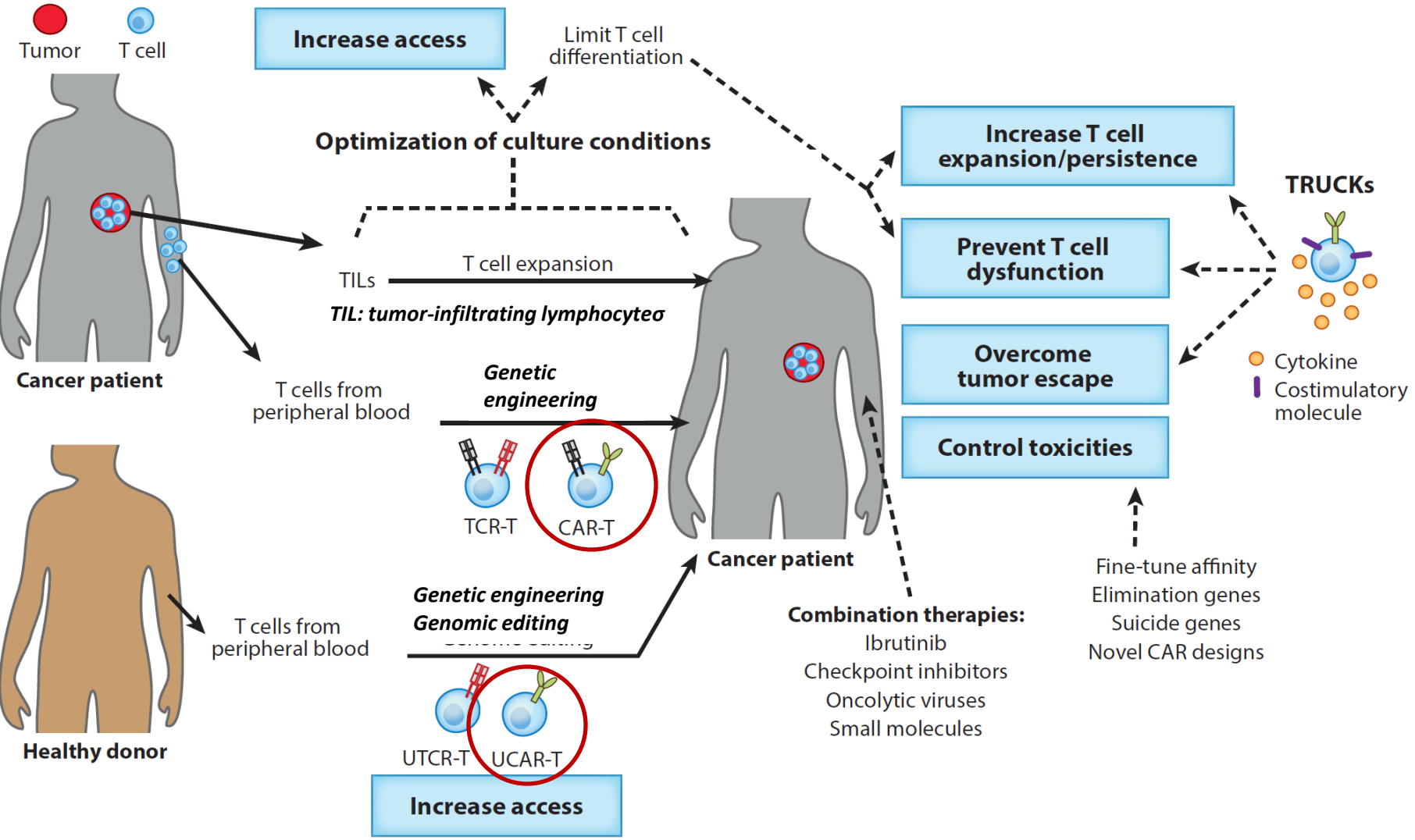
Michot. Eur J Cancer. 2016;54:139. Robert. ASCO 2017. Education session: Checkpoint inhibitor immunotherapy.
Clinical images reproduced with permission of Dr. Caroline Robert, MD, PhD.

Slide credit: clinicaloptions.c

Adoptive T cell transfer therapies



Adoptive T cell transfer therapies



CAR: chimeric antigen receptor.
TCR: T cell receptor.
TRUC: T cells redirected for universal cytokine-mediated killing.
UCAR-T: universal CAR-T cells
UTCR-T: universal TCR-T cells.

S.Guedan, M.Ruella, C.June : Annual review of immunology 2019

SPECIAL REPORT

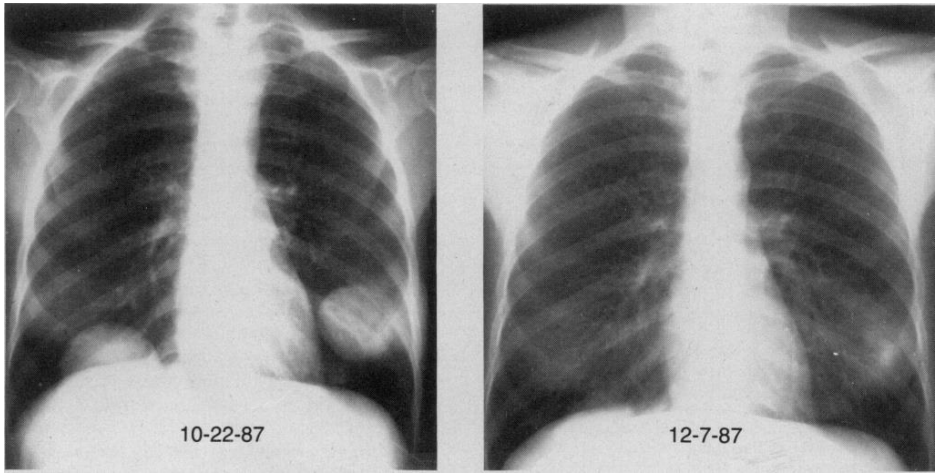
USE OF TUMOR-INFILTRATING LYMPHOCYTES AND INTERLEUKIN-2 IN THE IMMUNOTHERAPY OF PATIENTS WITH METASTATIC MELANOMA

A Preliminary Report

STEVEN A. ROSENBERG, M.D., PH.D., BEVERLY S. PACKARD, PH.D., PAUL M. AEBERSOLD, PH.D., DIANE SOLOMON, M.D., SUZANNE L. TOPALIAN, M.D., STEPHEN T. TOY, PH.D., PAUL SIMON, PH.D., MICHAEL T. LOTZE, M.D., JAMES C. YANG, M.D., CLAUDIA A. SEIPP, R.N., COLLEEN SIMPSON, R.N., CHARLES CARTER, STEVEN BOCK, M.D., DOUGLAS SCHWARTZENTRUBER, M.D., JOHN P. WEI, M.D., AND DONALD E. WHITE, M.S.

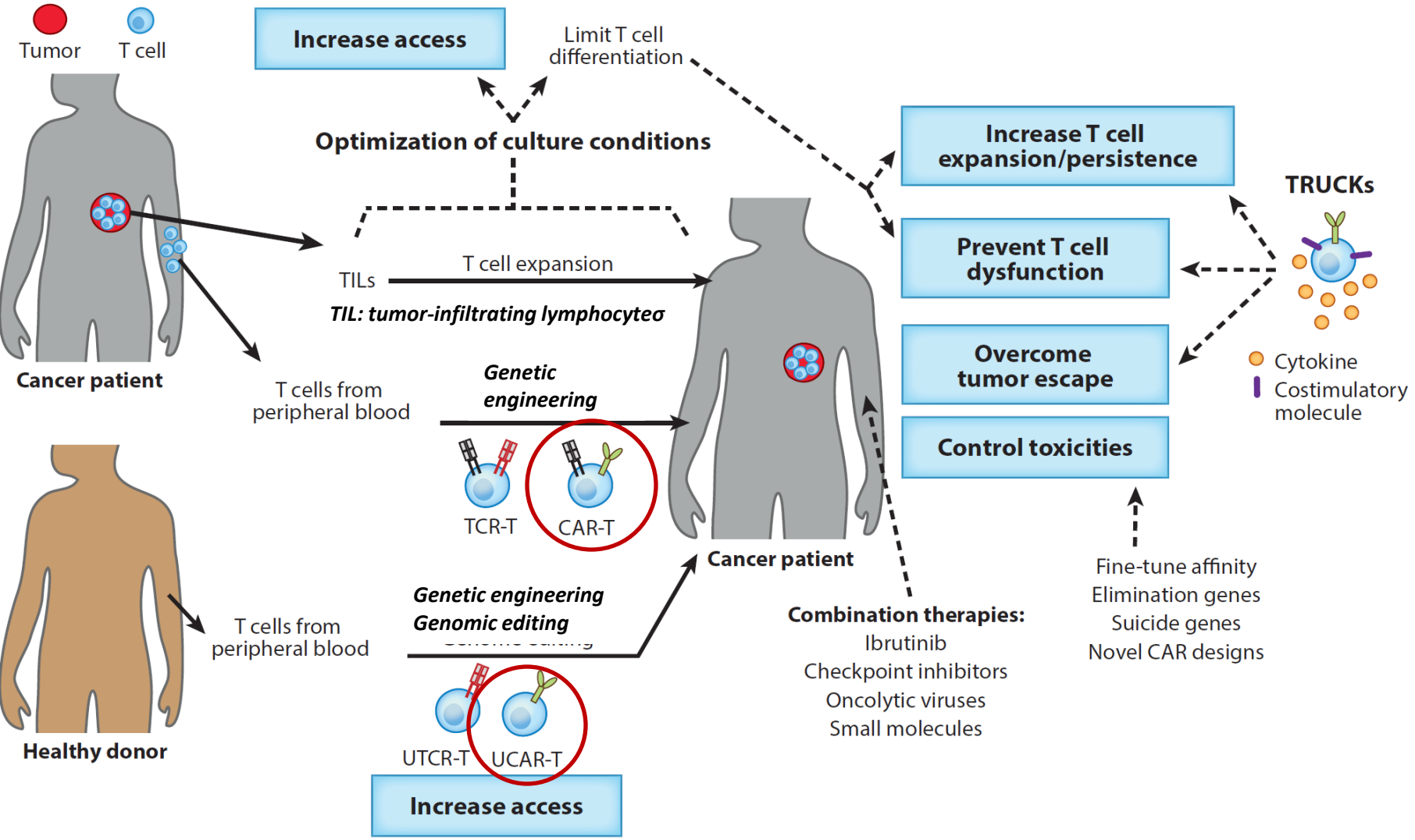
Protocol

Tumor deposits were resected, usually under local anesthesia; most resected tumors weighed between 10 and 30 g. TIL were expanded in culture for four to eight weeks, according to techniques similar to those previously described.[18] When the TIL were ready for infusion, patients first received a single intravenous dose of cyclophosphamide (25 mg per kilogram of body weight) and 36 hours later the first intravenous infusion of TIL in an intensive care unit; a maximum of 2×10^{11} cells were administered in 200 to 250 ml over a period of 30 to 60 minutes. Each patient received a total of one to seven infusions over one to two days, depending on the number of cells to be administered and the time required to harvest the cells. After the first infusion of TIL, the patients began receiving recombinant interleukin-2 (kindly supplied by the Cetus Corporation, Emeryville, Calif.) (100,000 units per kilogram, given intravenously every eight hours in 50 ml of 0.9 percent saline with 5 percent albumin).[22] Interleukin-2 was administered



NEJM:1988

Adoptive T cell transfer therapies



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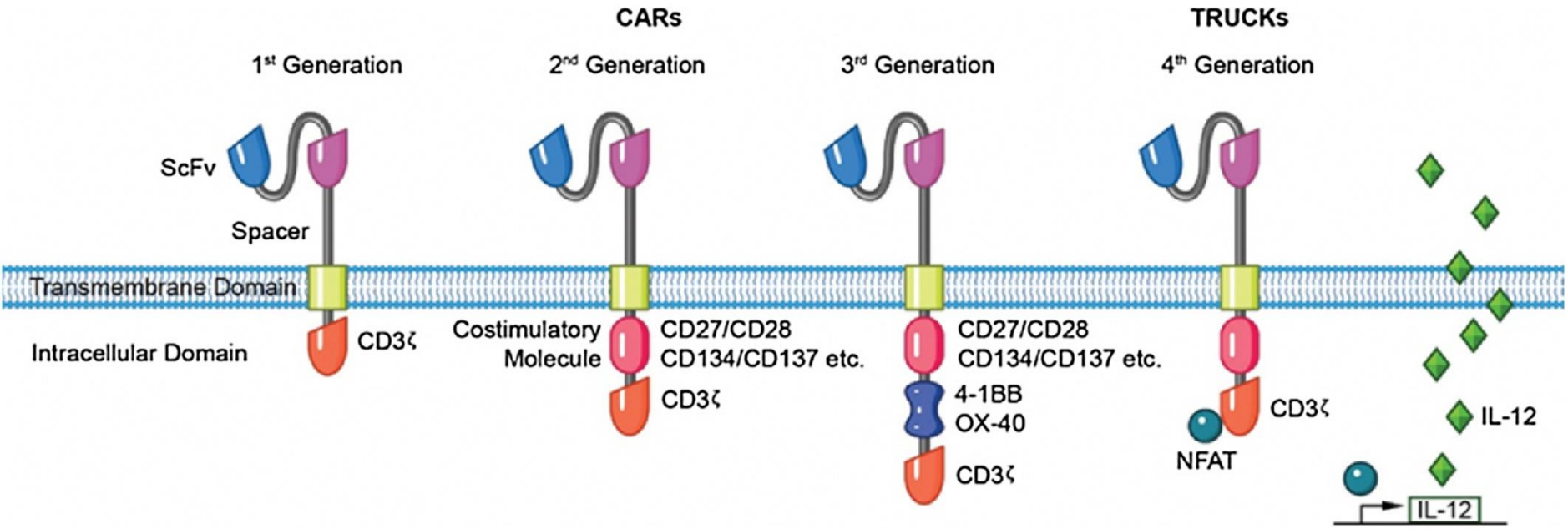
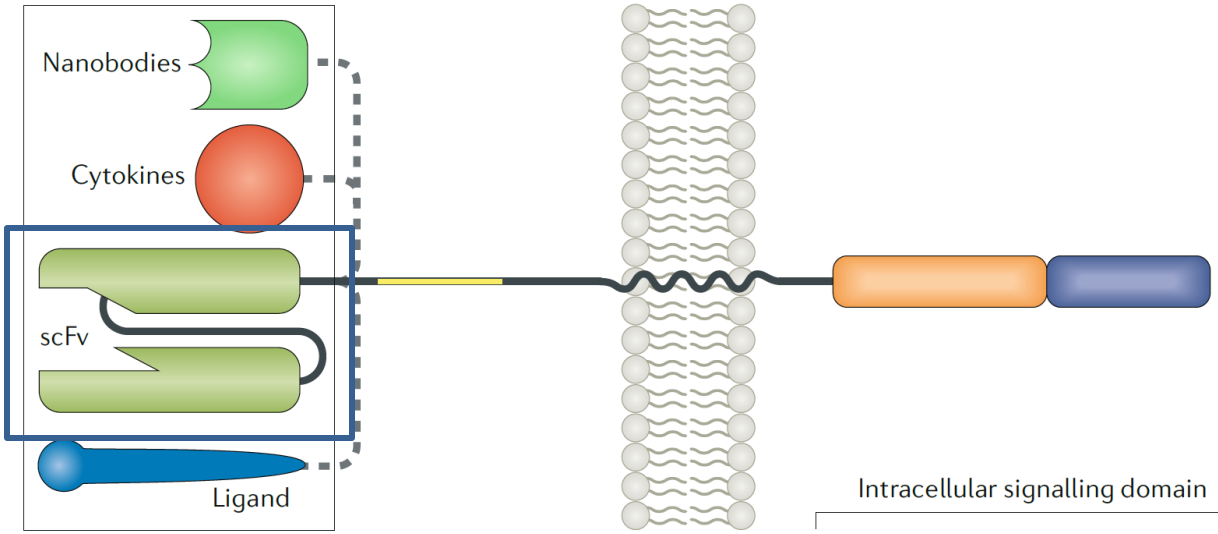
S.Guedan, M.Ruella, C.June : Annual review of immunology 2019

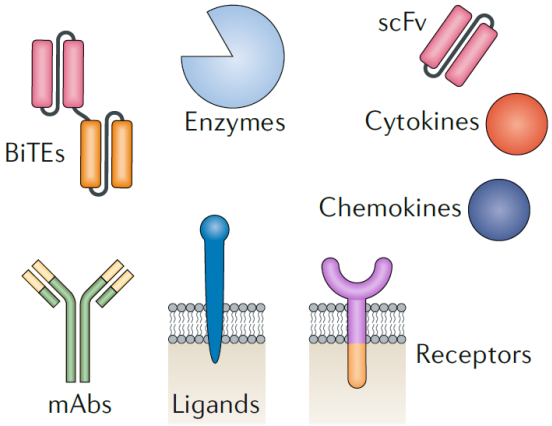

CARs
TRUCKs
1st Generation
2nd Generation
3rd Generation
4th Generation
ScFv
Spacer
Transmembrane Domain
Intracellular Domain
CD3ζ
Costimulatory Molecule
CD27/CD28
CD134/CD137 etc.
CD3ζ
CD27/CD28
CD134/CD137 etc.
4-1BB
OX-40
CD3ζ
CD3ζ
NFAT
IL-12
IL-12

Ιδανικός υποδοχέας για CART κύτταρα με μεγάλη ειδικότητα.

- Να εκφράζεται το μόριο σύνδεσης ομοιογενώς σε όλα τα κύτταρα -στόχο.
- Να εκφράζεται στην επιφάνεια του κυττάρου. Τα CART δεν αναγνωρίζουν ενδοκυττάρια αντιγόνα.
- Ιδανικά να μην εκφράζονται σε φυσιολογικά κύτταρα ή τουλάχιστον να εκφράζονται σε ‘αποδεκτό’ κυτταρικό πληθυσμό.
- Δεν θα πρέπει το αντιγόνο να διαφεύγει στη κυκλοφορία.
- Να είναι καθοριστικός για την ανάπτυξη και επιβίωση του όγκου και να μη διαφεύγει εύκολα κάτω από επιλεκτική πίεση

Σχεδιασμός CAR





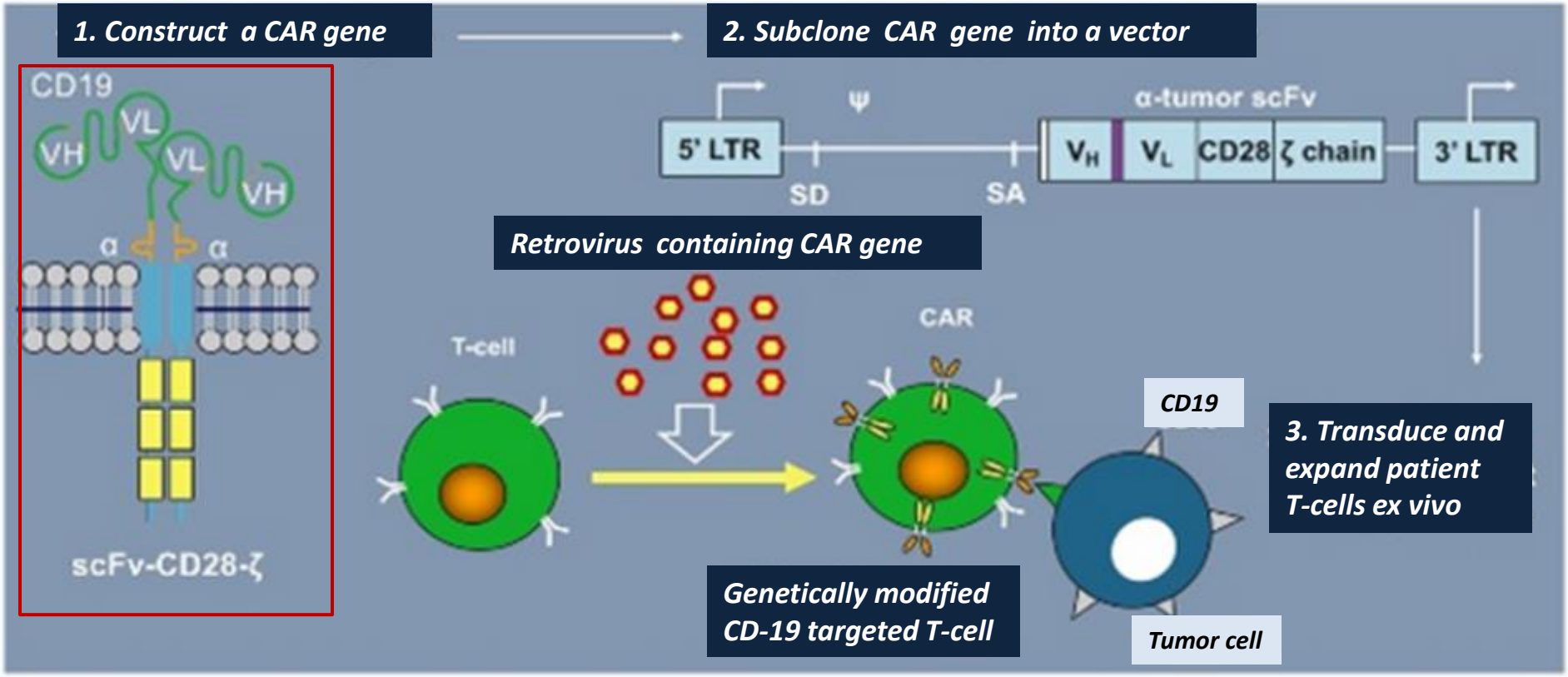
Intracellular signalling domain

Binding domain	Hinge	Transmembrane domain	Co-stimulation	Activation	Armour
• scFv • Nanobodies (camelid V_HH domains) • Cytokines • Ligands • Peptides (adnectins and DARPins)	• CD8 • CD28 • IgG1 • IgG4	• CD3ζ • CD8α • CD4 • CD28 • ICOS	• CD28 ⇄ • 4-1BB ⇄ • OX40 • ICOS • CD27 • MYD88–CD40 • KIR2DS2	• CD3ζ	• Cytokines or chemokines • Cytokine or chemokine receptors • Ligands or receptors • mAbs • BiTEs • scFv • Peptides (IL-1Ra, off-switch receptors or inducible suicide constructs) • Enzymes

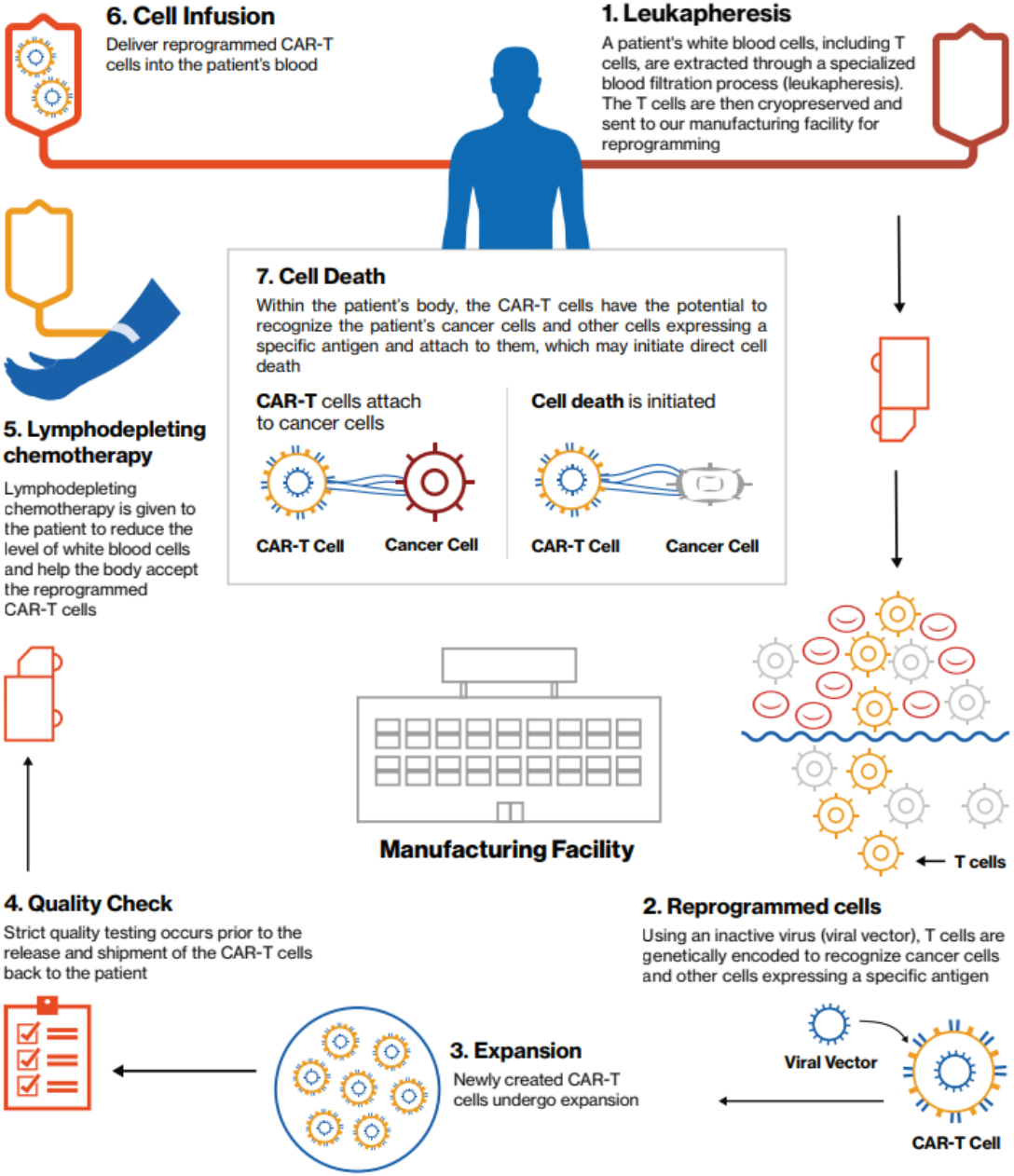
scFv: Single –chain variable fragment
DARPins: designed ankyrin repeat protein
ICOS: inducible T cell co-stimulators
KIR2DS2: killer cells immunoglobulin-like receptor 2DS2
V_HH: Heavy chain variable domain

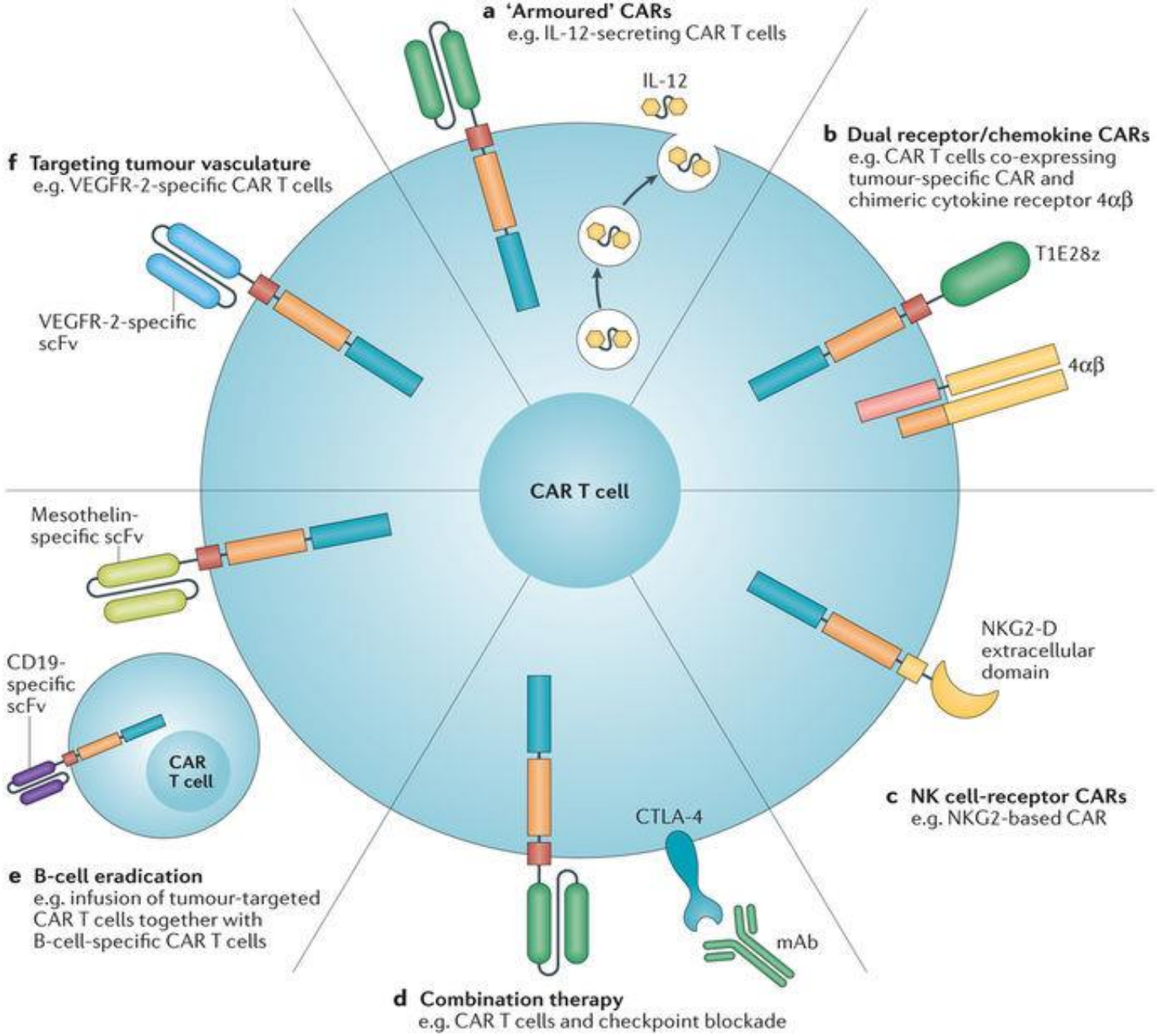
S.Rafiq et.al Nature Reviews 2019

CAR gene transduction in T-cells

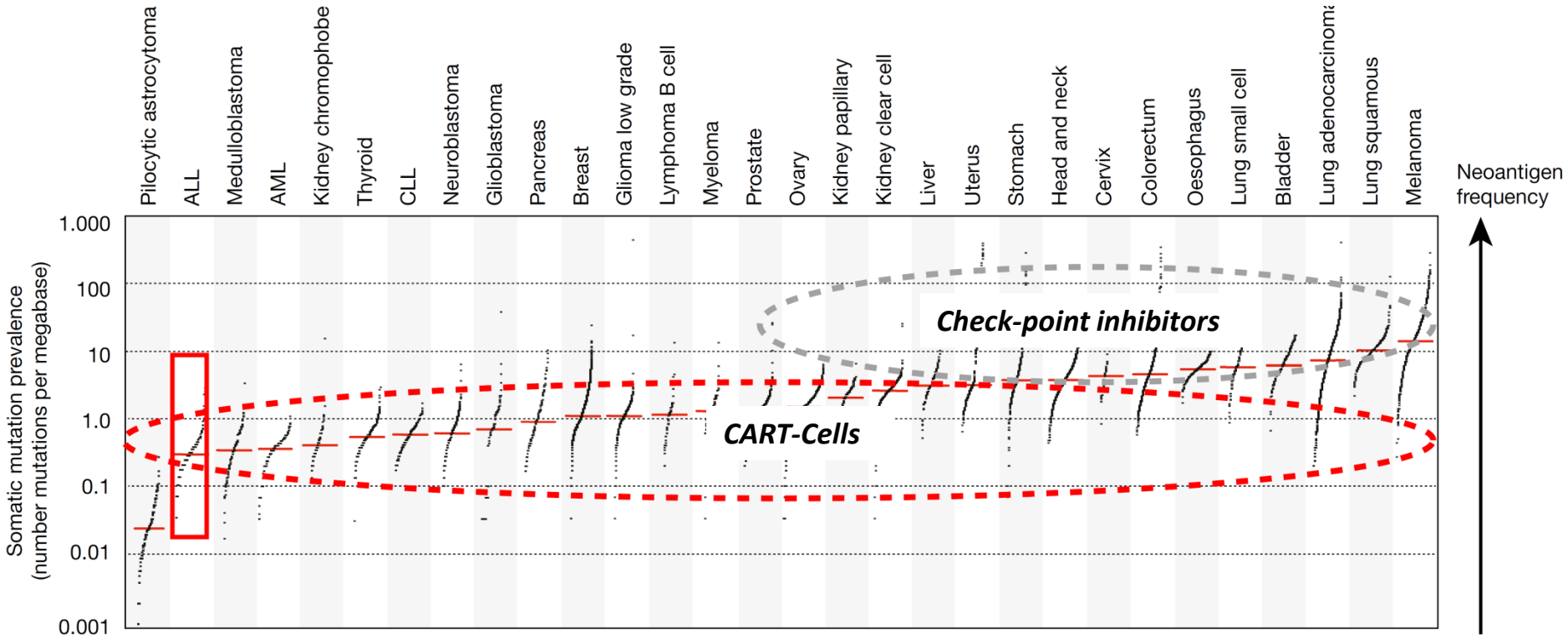


CART cells : Παρασκευή και χορήγηση

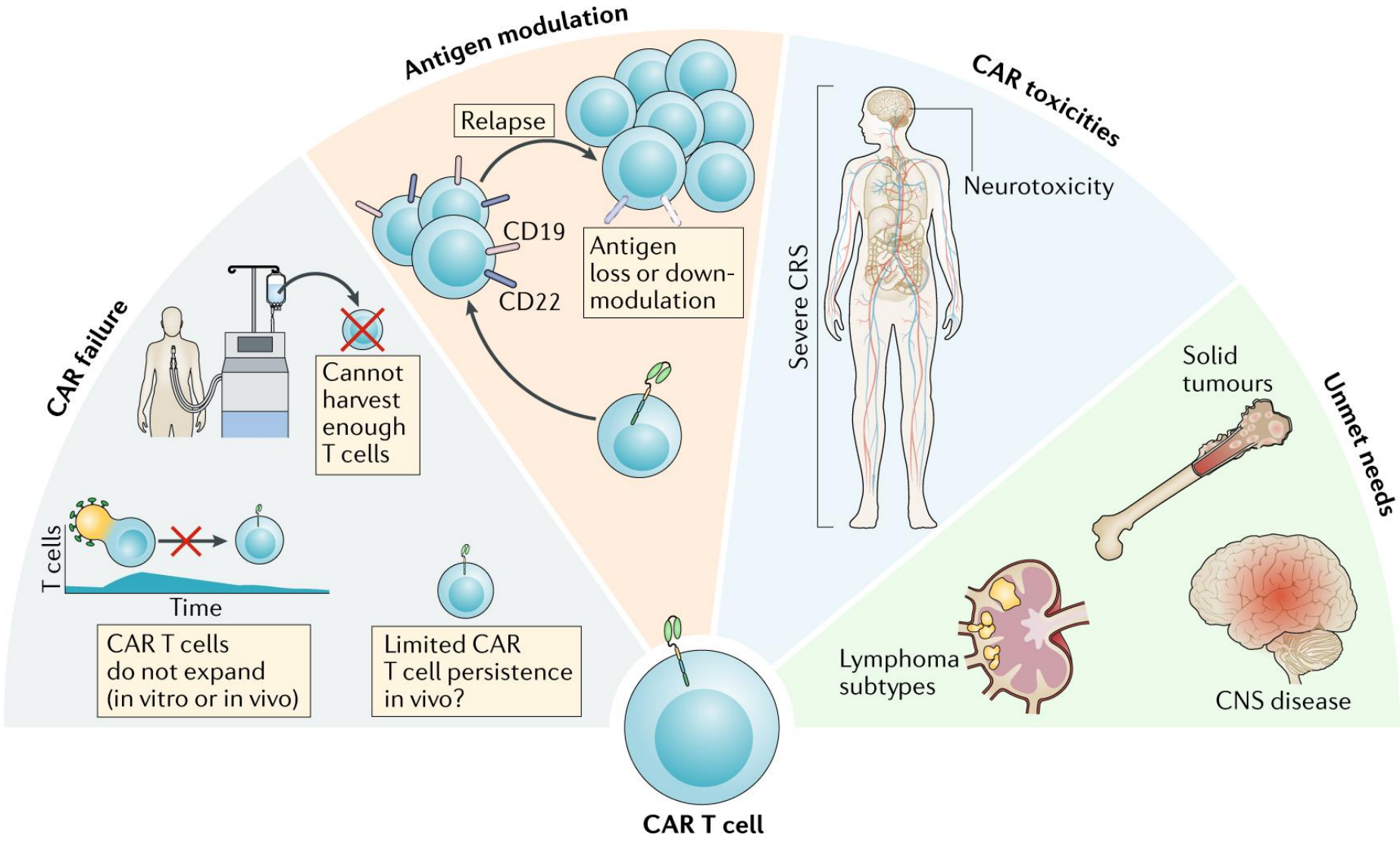



a 'Armoured' CARs
e.g. IL-12-secreting CAR T cells
IL-12
b Dual receptor/chemokine CARs
e.g. CAR T cells co-expressing tumour-specific CAR and chimeric cytokine receptor 4αβ
T1E28z
4αβ
f Targeting tumour vasculature
e.g. VEGFR-2-specific CAR T cells
VEGFR-2-specific scFv
CAR T cell
Mesothelin-specific scFv
CD19-specific scFv
CAR T cell
NKG2-D extracellular domain
c NK cell-receptor CARs
e.g. NKG2-based CAR
CTLA-4
mAb
e B-cell eradication
e.g. infusion of tumour-targeted CAR T cells together with B-cell-specific CAR T cells
d Combination therapy
e.g. CAR T cells and checkpoint blockade

CART cell θεραπεία ιδιαίτερα αποτελεσματική σε κακοήθειες με χαμηλό φορτίο μεταλλάξεων και αστοχία check-point inhibitors.

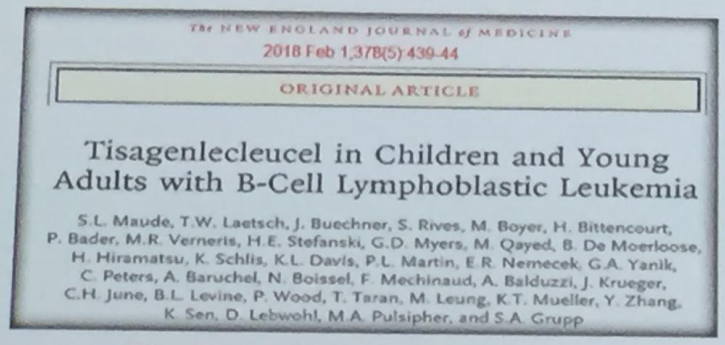


M. Sadelain, I.Riviere ,S.Riddell :Nature 2017

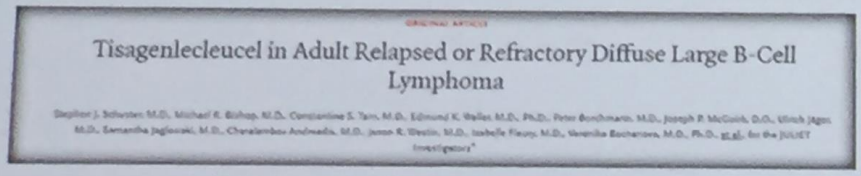

CAR failure
Antigen modulation
CAR toxicities
Unmet needs
Relapse
CD19
CD22
Antigen loss or down-modulation
Cannot harvest enough T cells
T cells
Time
CAR T cells do not expand (in vitro or in vivo)
Limited CAR T cell persistence in vivo?
Severe CRS
Neurotoxicity
Solid tumours
Lymphoma subtypes
CNS disease
CAR T cell

Two FDA Approved CAR19 Therapies in NHL; one in ALL

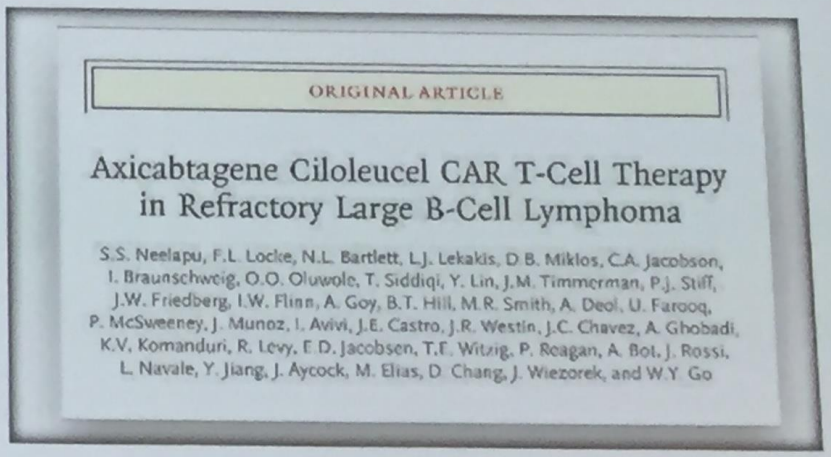
EMA approved CAR19 Kymriah in the same indication on 27 August, 2018



Tisagenlecleucel - Kymriah
Eliana ;Novartis n=75
Study Dates: April 2015 – April, 2017
Approval Date: August 30, 2017

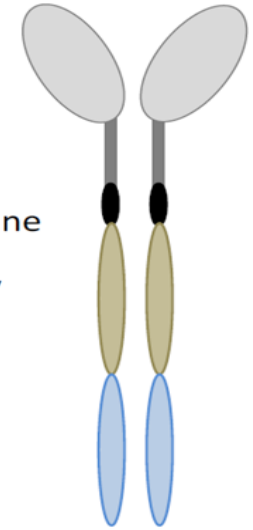


Tisagenlecleucel - Kymriah
JULIET ; Novartis n=93
Study Dates: July 2015 – December 8, 2017
Approval Date: May 1, 2018



Axicabagtene ciloleucel - Yescarta
ZUMA-1 Kite/Gilead n=101
Study Dates: November 2015 – September 2016
Approval Date: October 18, 2017

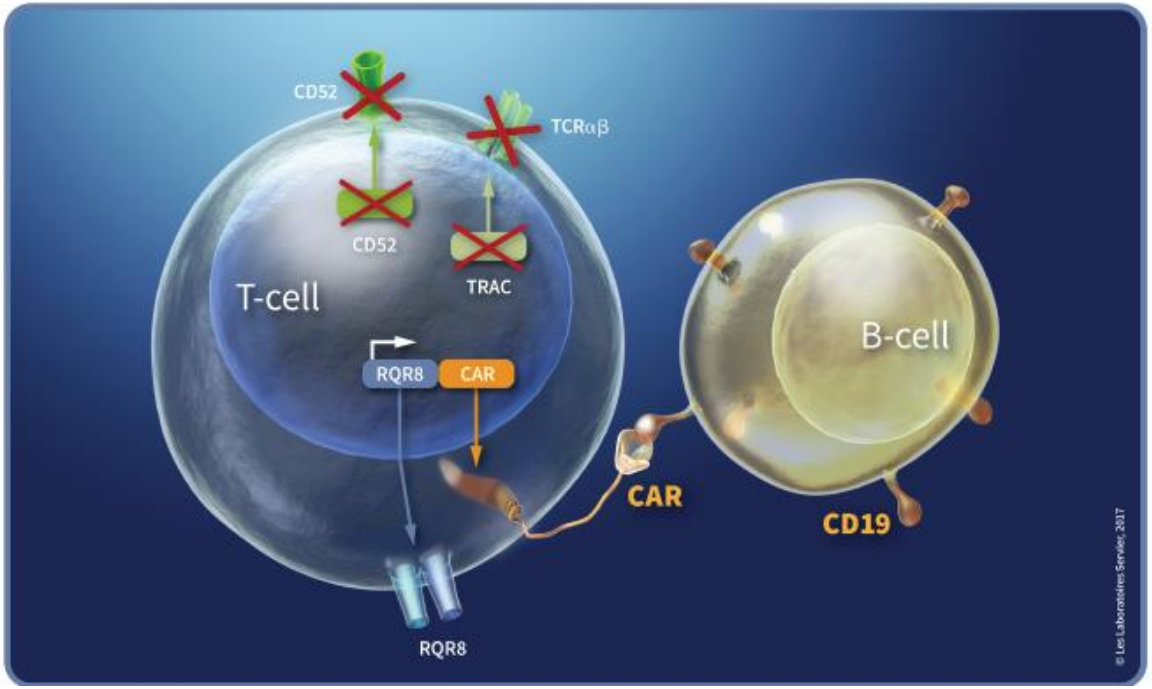
Differences in design, materials and clinical use of first approved autologous CAR T-cell products compared to other CAR T-cells (Autologous and Allogeneic) currently in clinical trials.



	Yescarta[a] Kite/Gilead	Kymriah[b] Novartis	JCAR017[c] Celgene	UCART-19[d] Cellectis/Servier	bb21217 (BCMA)[5] BlueBird/Celgene
scFv	anti-CD19 / FMC63	anti-CD19/ FMC63	anti-CD19/ FMC63	anti-CD19/ FMC63	anti-BMCA
Hinge					
Transmembrane	IgG1	CD8A	IgG4	CD8A	nk
Costimulatory domain	CD28	4-1BB	4-1BB	4-1BB	4-1BB
Signalling domain	CD3ζ	CD3ζ	CD3ζ	CD3ζ	CD3ζ
Cell population	PBMC	PBMC	$CD4^+ + CD8^+$	PBMC	PBMC
Ablation/safety module	None	None	EGFR cetuximab	RQR8 rituximab	None
Other modification	None	None	None	TCRα/CD52 ko	None
Vector	Retrovirus	Lentivirus	Lentivirus	GE/Talen	Lentivirus
T-cell activation	CD3/IL-2	CD3/CD28	nk	nk	nk
Donor	Autologous	Autologous	Autologous	Allogeneic	Autologous
Dose	$2 \times 10^6 - 2 \times 10^8$/kg $CD3^+/CAR^+$	0.2×10^6- 2.5×10^8/kg $CD3^+/CAR^+$	5×10^7 or 1×10^8 cells (total)	6×10^6 * CAR^+ cells total	$50 - 800 \times 10^6$ CAR^+ T cells total

UCART19 (CD19CAR/RQR8+_TCRαβ-_T-cells)

Allogeneic, universal, adoptive T-cell therapy targeting CD19+ malignancies



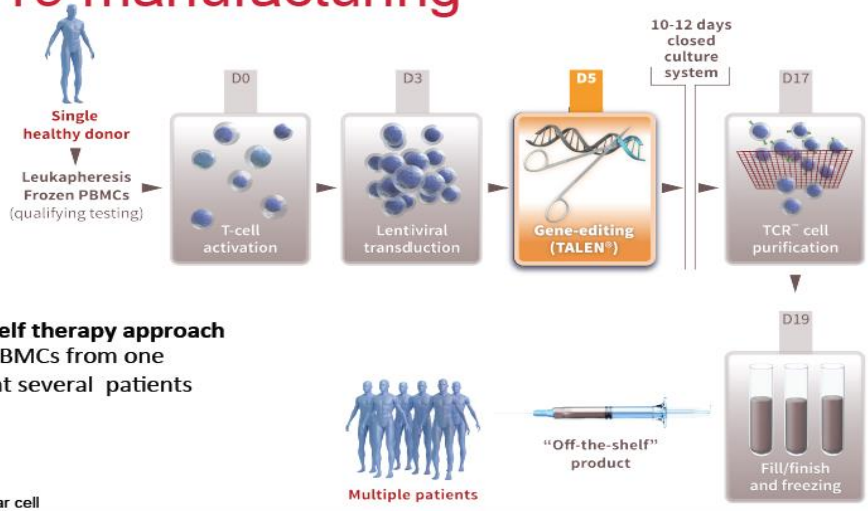
Transgene expression using lentiviral transduction

- **CAR**: anti-CD19 scFv and CD3ζ + 4-1BB
- **RQR8** (= CD20 mimotope): safety switch

Gene knock-out using TALEN® technology

- **TRAC KO**: to prevent TCR mediated recognition of patient's HLA antigens
- **CD52 KO**: to permit alemtuzumab use in lymphodepletion

UCART19 manufacturing



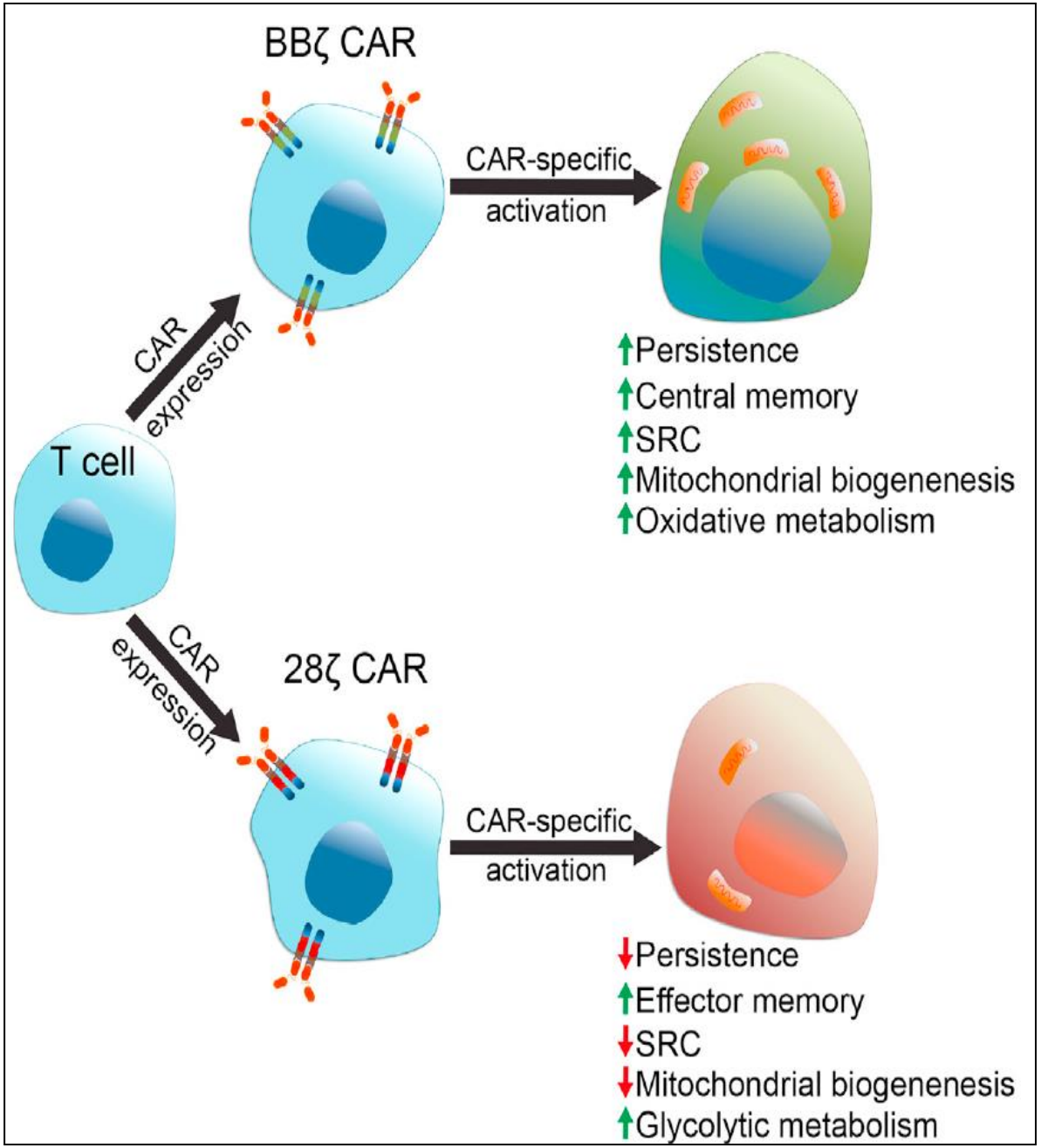
Ready-to-use, off-the-shelf therapy approach

➢ Advantage of using PBMCs from one healthy donor to treat several patients

TRAC: T cell receptor alpha constant

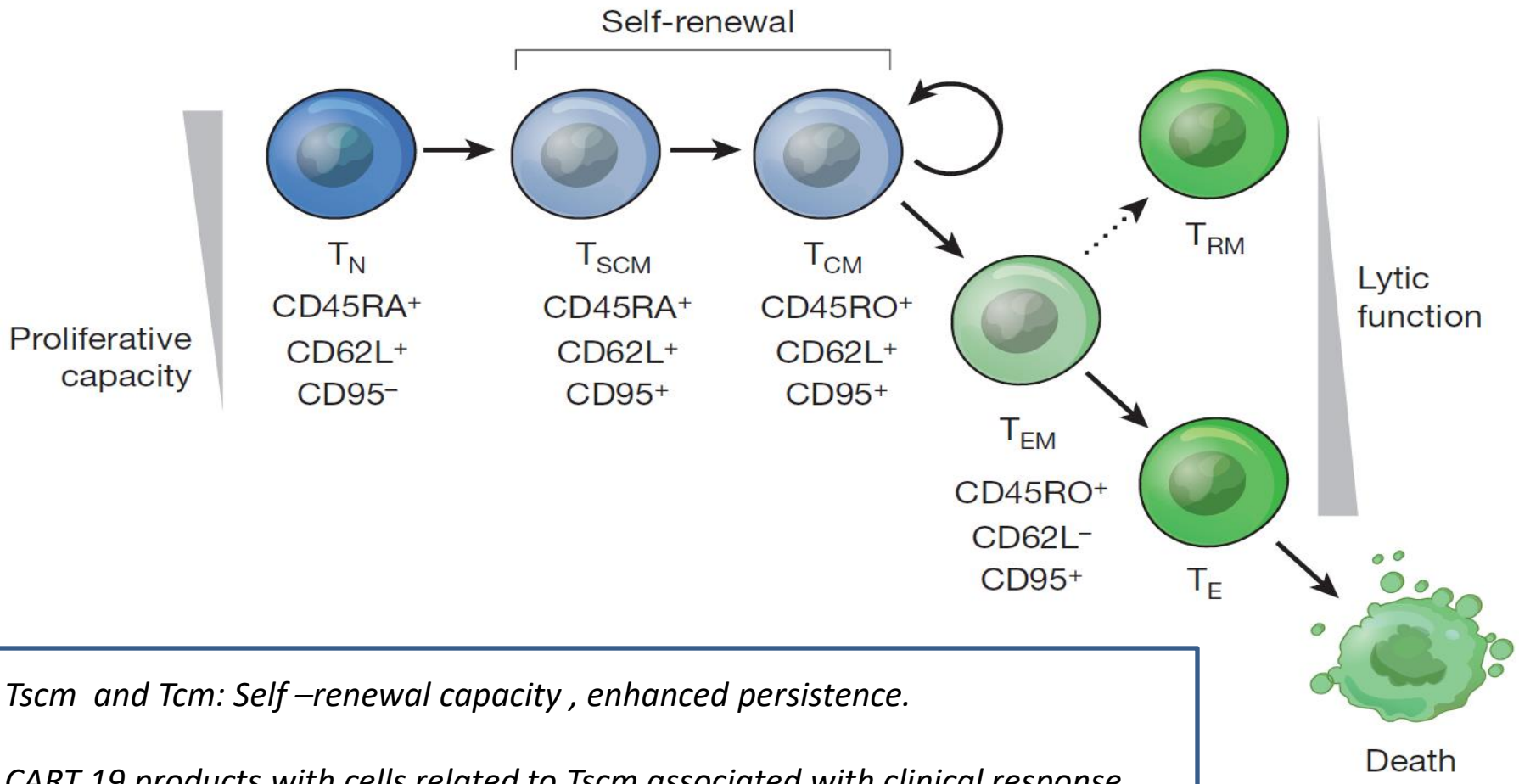
PBMC = peripheral blood mononuclear cell

CART cells: Διαφορές στα συνδιεγερτικά μόρια ➔ Διαφορετική βιολογική συμπεριφορά.



O.Kawalekar et.al Immunity 2016

Cell sources for T cell engineering



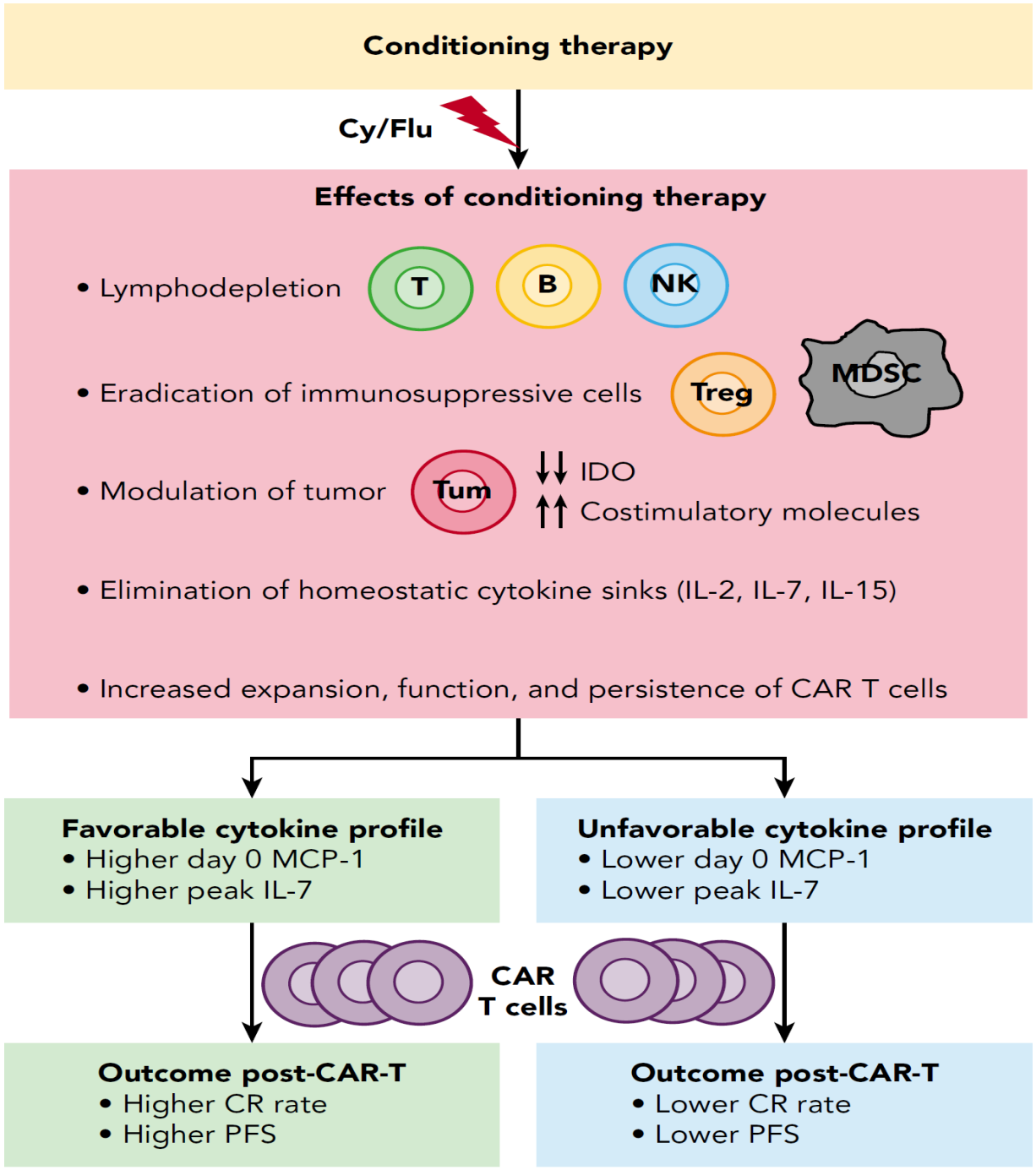
Tscm and Tcm: Self –renewal capacity , enhanced persistence.

CART 19 products with cells related to Tscm associated with clinical response.

Presence of Tscm cells associated with CART 19 expansion.

Increased efficacy of CART 19 cells manufactured from Tcm (preclinical).

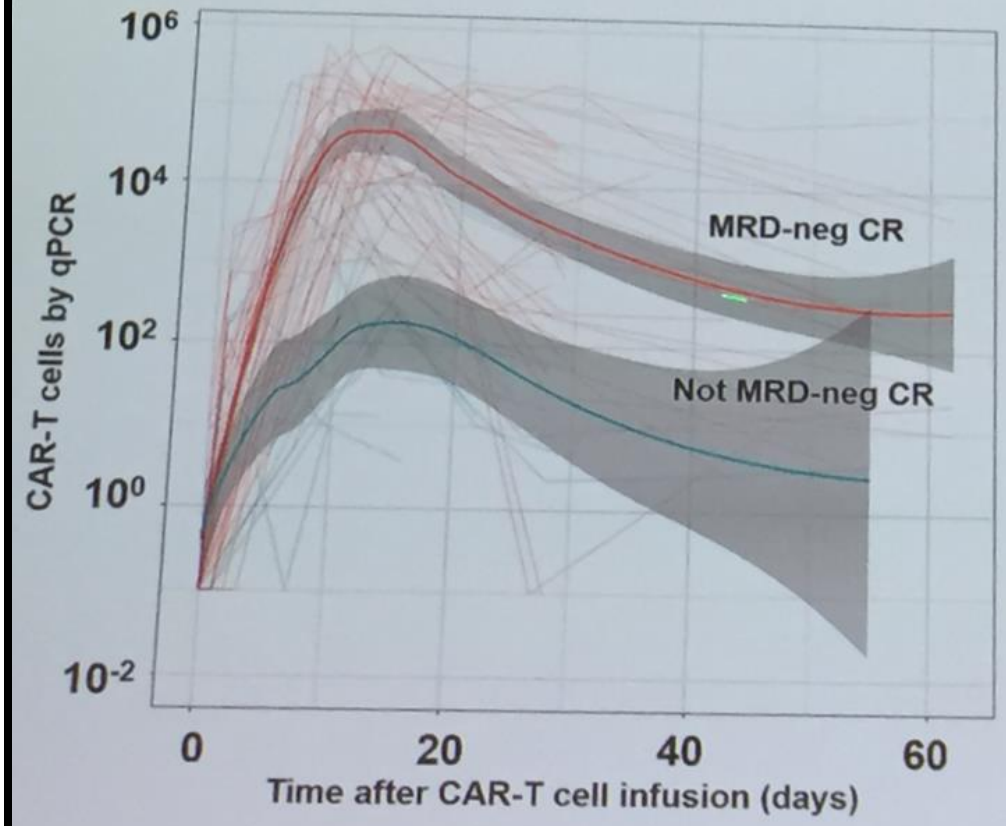
Επίδραση του σχήματος προετοιμασίας στην αποτελεσματικότητα των CART cells

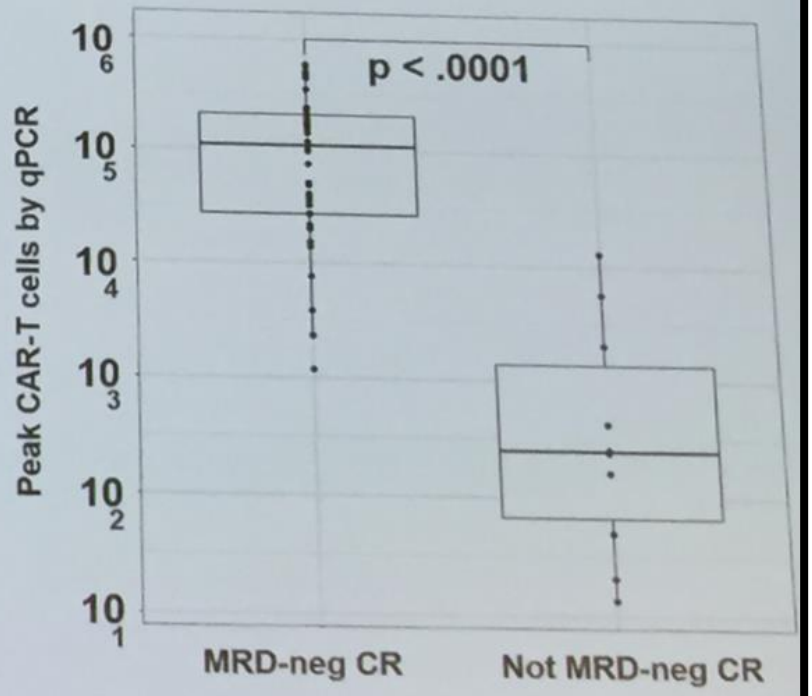


MPC-1: monocyte chemoattractant protein-1
IDO: indoleamine 2,3 -dioxygenase

Sattva Neelapu . Blood 2019
Hirayama AV. et.al . Blood 2019

Robust *in vivo* CAR-T cell expansion is associated with achievement of MRD-negative CR





Hay et al., Blood 2019 and Abstract #7005, ASCO 2018.
HAY KA et al. Blood 2019 :blood-2018-11-883710

Κλινική ανταπόκριση στη θεραπεία με CD19 /CART-cells

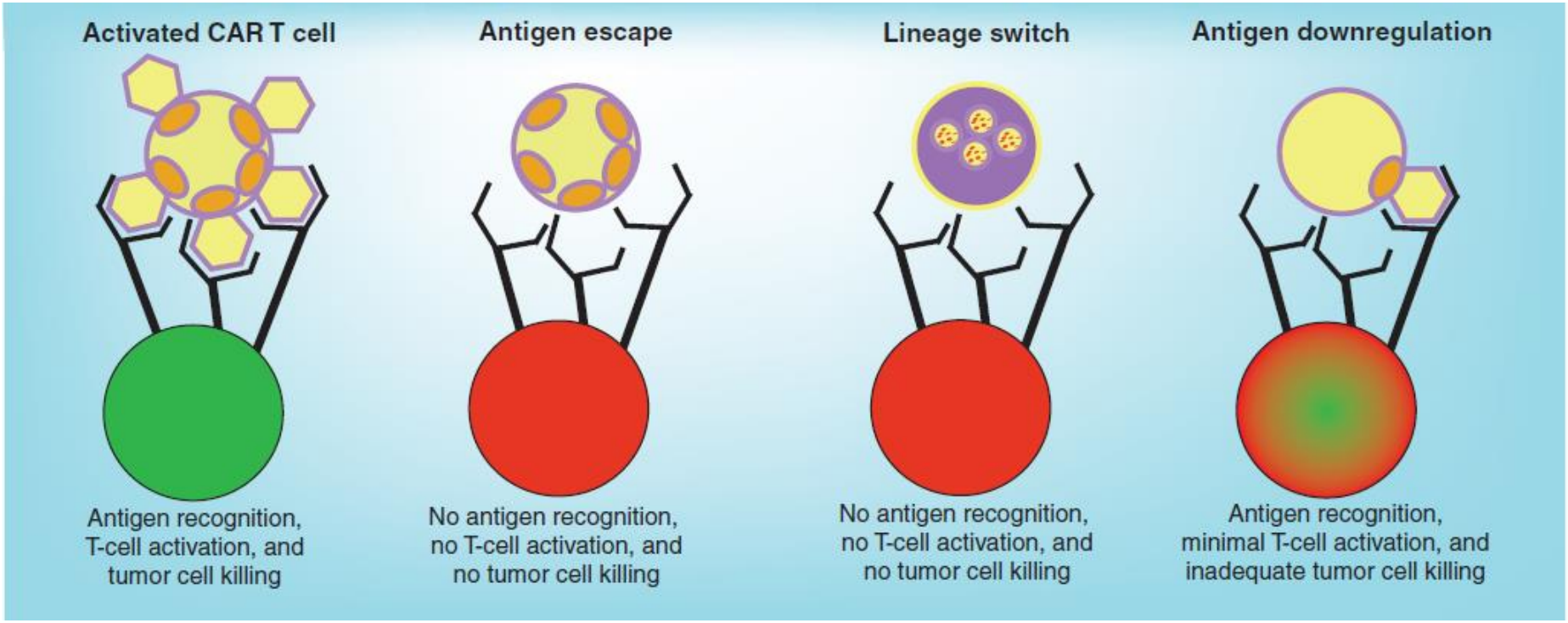
Study	Disease	CAR	V	N	Cond.	T cells	CR rate
Davila, 2014 (ref. 72)	ALL (Ad.)	CD28	γRV	16	CY	Auto	88%
Maude, 2014 (ref. 73)	ALL (Paed.)	4-1BB	LV	25	CF	Auto	90%
Lee, 2015 (ref. 74)	ALL (Paed.)	CD28	γRV	21	CY	Auto	68%
Turtle, 2016 (ref. 75)	ALL (Ad.)	4-1BB	LV	29	CY/CF	1:14/8	93%
Qasim, 2017 (ref. 150)	ALL (Paed.)	4-1BB	LV	2	IC	Allo	100%
Kochenderfer, 2015 (ref. 71)	NHL/CLL	CD28	γRV	15	CF	Auto	53%
Kochenderfer, 2016 (ref. 137)	B-mix	CD28	γRV	20	IC	Allo	30%
Turtle, 2016 (ref. 132)	NHL	4-1BB	LV	32	CY/CF	1:14/8	79%

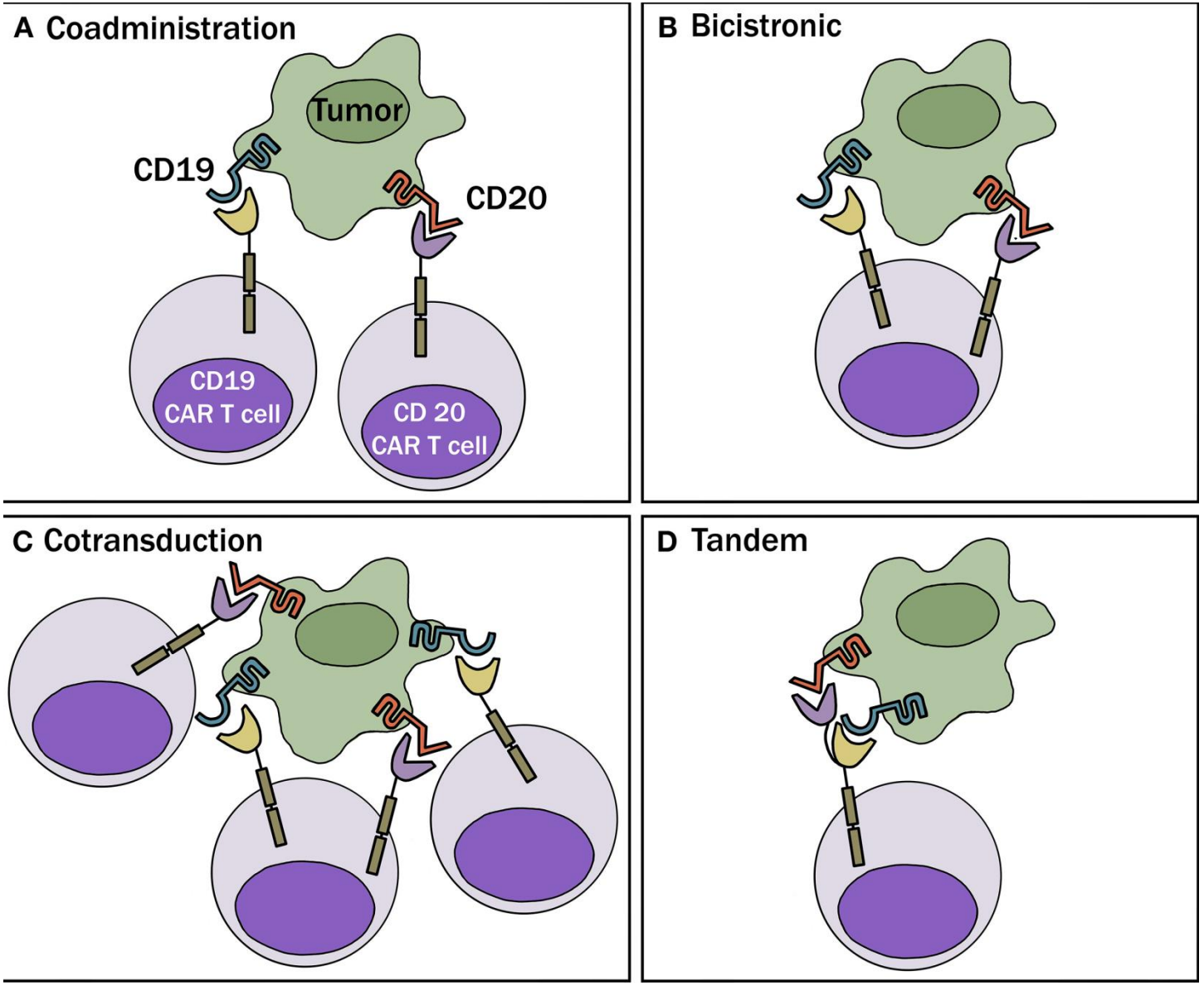
M. Sadelain, I.Riviere ,S.Riddell :Nature 2017

ALL relapse: Antigen escape in CD19/CART cells clinical trials

Trial	Population	CD19 CAR construct	Relapse rate	CD19-negative relapse rate
Children's Hospital of Philadelphia phase I	Pediatric	FMC63-4-1BB-ζ	36% (20/55)	24% (13/55)
Novartis phase II (ELIANA)	Pediatric	FMC63-4-1BB-ζ	33% (20/61)	25% (15/61)
Seattle Children's Research Institute phase I	Pediatric	FMC63-4-1BB-ζ	45% (18/40)	18% (7/40)
NCI phase I	Pediatric	FMC63-CD28-ζ	29% (8/28)	18% (5/28)
Memorial Sloan Kettering phase I	Adult	SJ25C1-CD28-ζ	57% (25/44)	9% (4/44)
Fred Hutchinson Cancer Center phase I	Adult	FMC63-4-1BB-ζ	31% (9/29)	7% (2/29)

Απώλεια αντιγονικού στόχου

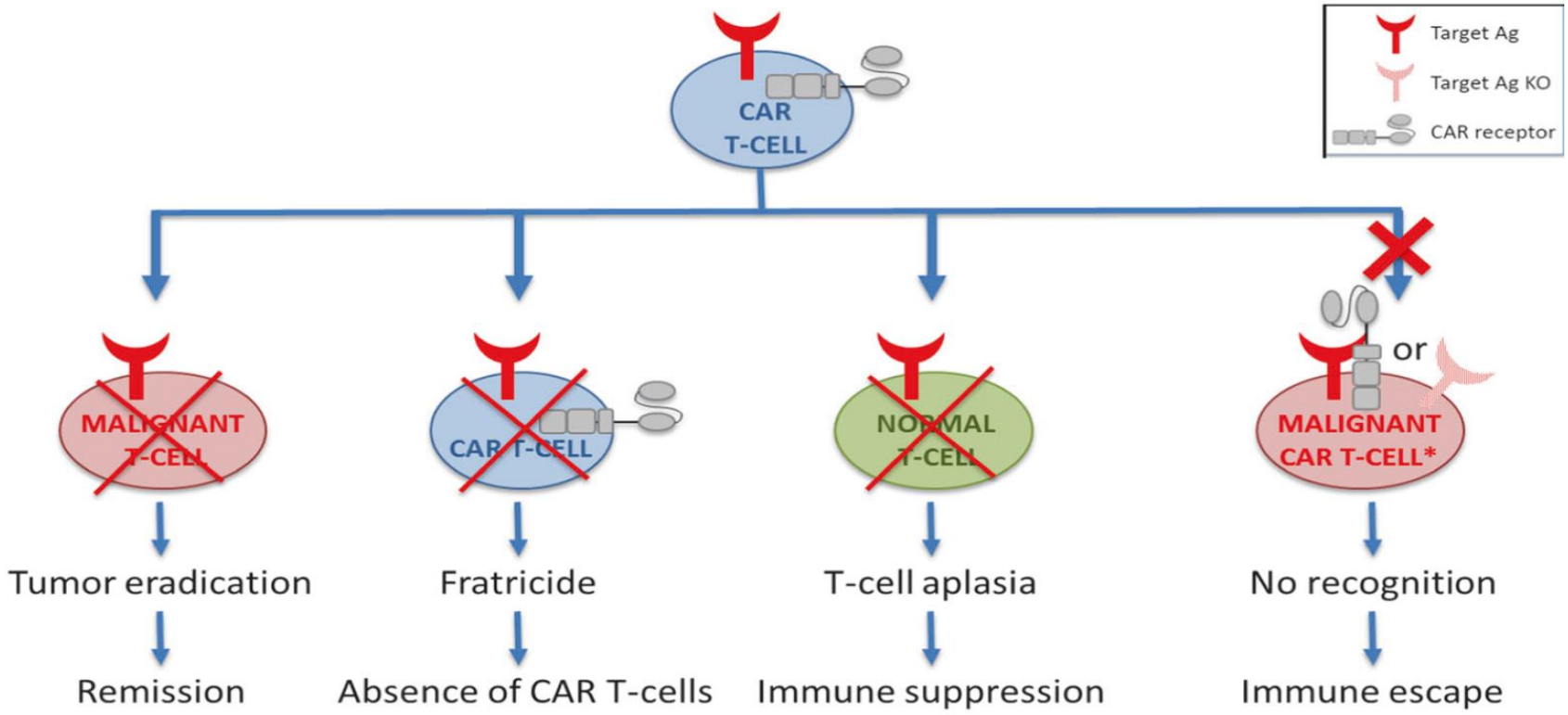




A) Coadministration—involves production of two separate CAR-T cell products infused together or sequentially
B) Bicistronic vector—allows expression of 2 different CARs on the same cell.
C) Cotransduction—encode 2 CAR constructs via transduction with multiple vectors. With this process, one will also obtain cells that express each CAR alone
(D) Tandem—encode 2 CARs on same chimeric protein using a single vector.

CAR T κύτταρα εναντίον Τ- κακοηθειών:

- *Σοβαροί περιορισμοί οι κοινοί αντιγονικοί στόχοι και οι παρόμοιες λειτουργικές ιδιότητες.*
- *Αδυναμία διάκρισης μεταξύ **θεραπευτικών**, **φυσιολογικών** και **κακοήθων Τ κυττάρων.***
- ***Επιμόλυνση του CART προϊόντος** με κακοήθη κύτταρα **(CAR tumor T-cells).***



- **Selective targeting T- cell subsets**
- **Gene editing preventing expression of target T-cell Ag**
- **Non T- CAR cell (CAR-NK cell)**

- **Selective targeting T- cell subsets**
- **Safety switch**
- **Short-lived CAR cells (alloCART-cell, CAR-NK)**
- **Allo-HSCT**

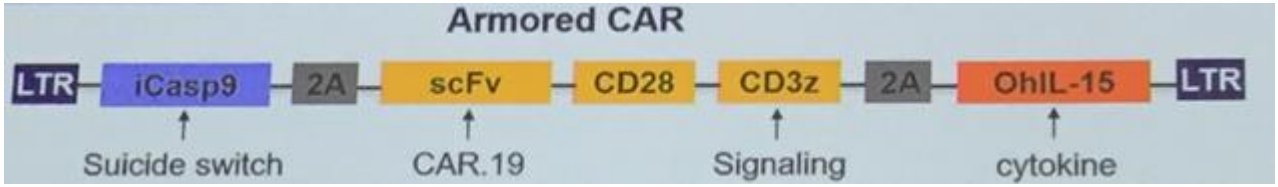
- **Allogeneic CART-cells**
- **Non -T CAR cells (CAR- NK cells)**
- **Allo-HSCT ?**

M.Alcantara et.al Leukemia 2018

Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

Enli Liu, M.D., David Marin, M.D., Pinaki Banerjee, Ph.D., Homer A. Macapinlac, M.D., Philip Thompson, M.B., B.S., Rafet Basar, M.D., Lucila Nassif Kerbauy, M.D., Bethany Overman, B.S.N., Peter Thall, Ph.D., Mecit Kaplan, M.S., Vandana Nandivada, M.S., Indresh Kaur, Ph.D., Ana Nunez Cortes, M.D., Kai Cao, M.D., May Daher, M.D., Chitra Hosing, M.D., Evan N. Cohen, Ph.D., Partow Kebriaei, M.D., Rohtesh Mehta, M.D., Sattva Neelapu, M.D., Yago Nieto, M.D., Ph.D., Michael Wang, M.D., William Wierda, M.D., Ph.D., Michael Keating, M.D., Richard Champlin, M.D., Elizabeth J. Shpall, M.D., and Katayoun Rezvani, M.D., Ph.D.

iC9/CAR19/IL15 CAR-NK cells

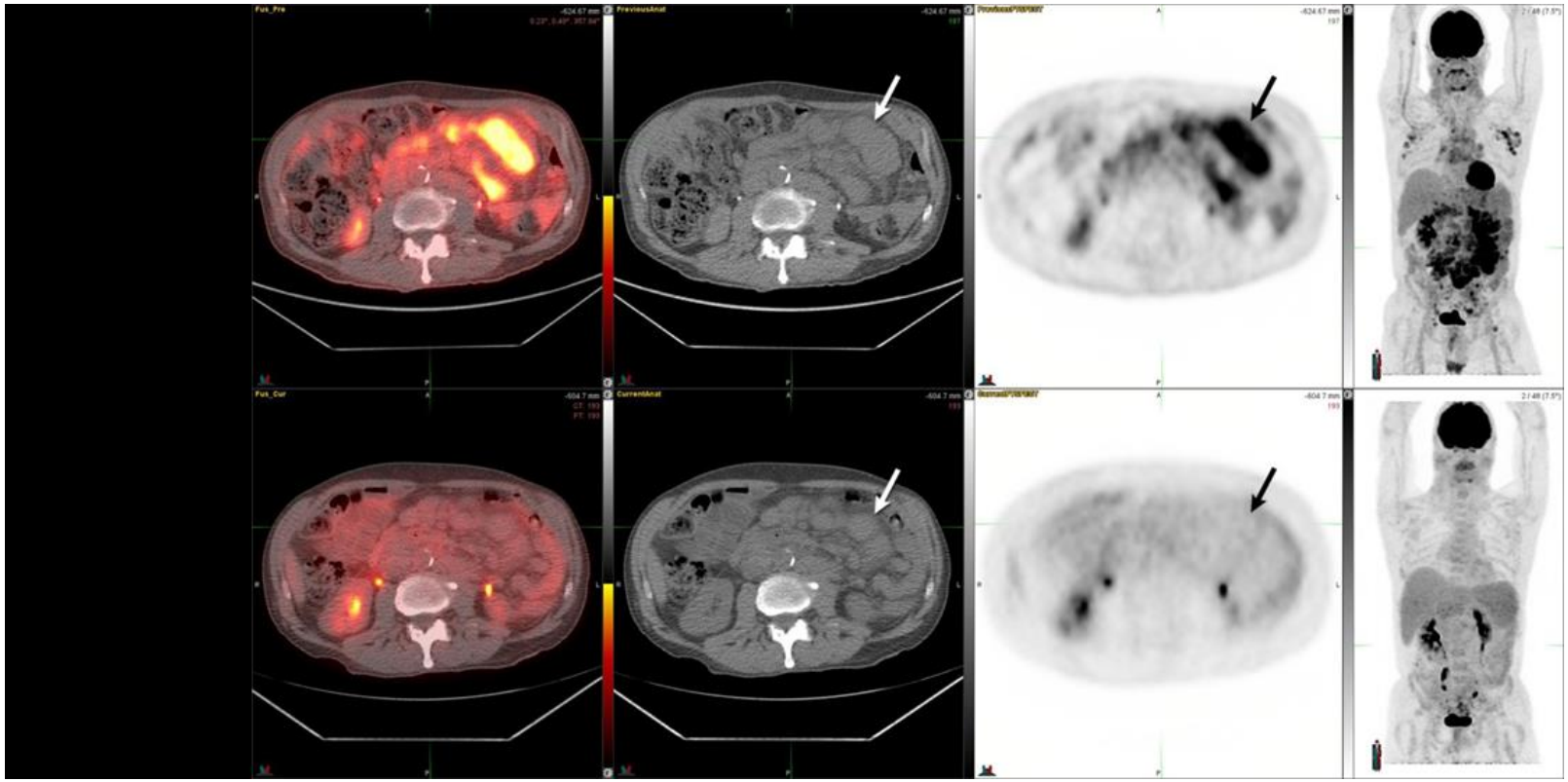


The administration of CAR-NK cells ***was not associated with the development of cytokine release syndrome, neurotoxicity,*** or graft-versus-host disease, and there was no increase in the levels of inflammatory cytokines, including interleukin-6, over baseline. The maximum tolerated dose was not reached. Of the 11 patients who were treated, ***8 (73%) had a response***; of these patients, 7 (4 with lymphoma and 3 with CLL) had a complete remission, and 1 had remission of the Richter's transformation component but had persistent CLL. Responses were rapid and seen within 30 days after infusion at all dose levels. ***The infused CAR-NK cells expanded and persisted at low levels for at least 12 months.***

iC9/CAR19/IL15 CAR-NK cells

Pre therapy

Day +29 post CART-NK cells



Male 61 yrs CLL ➔ Richter transformation

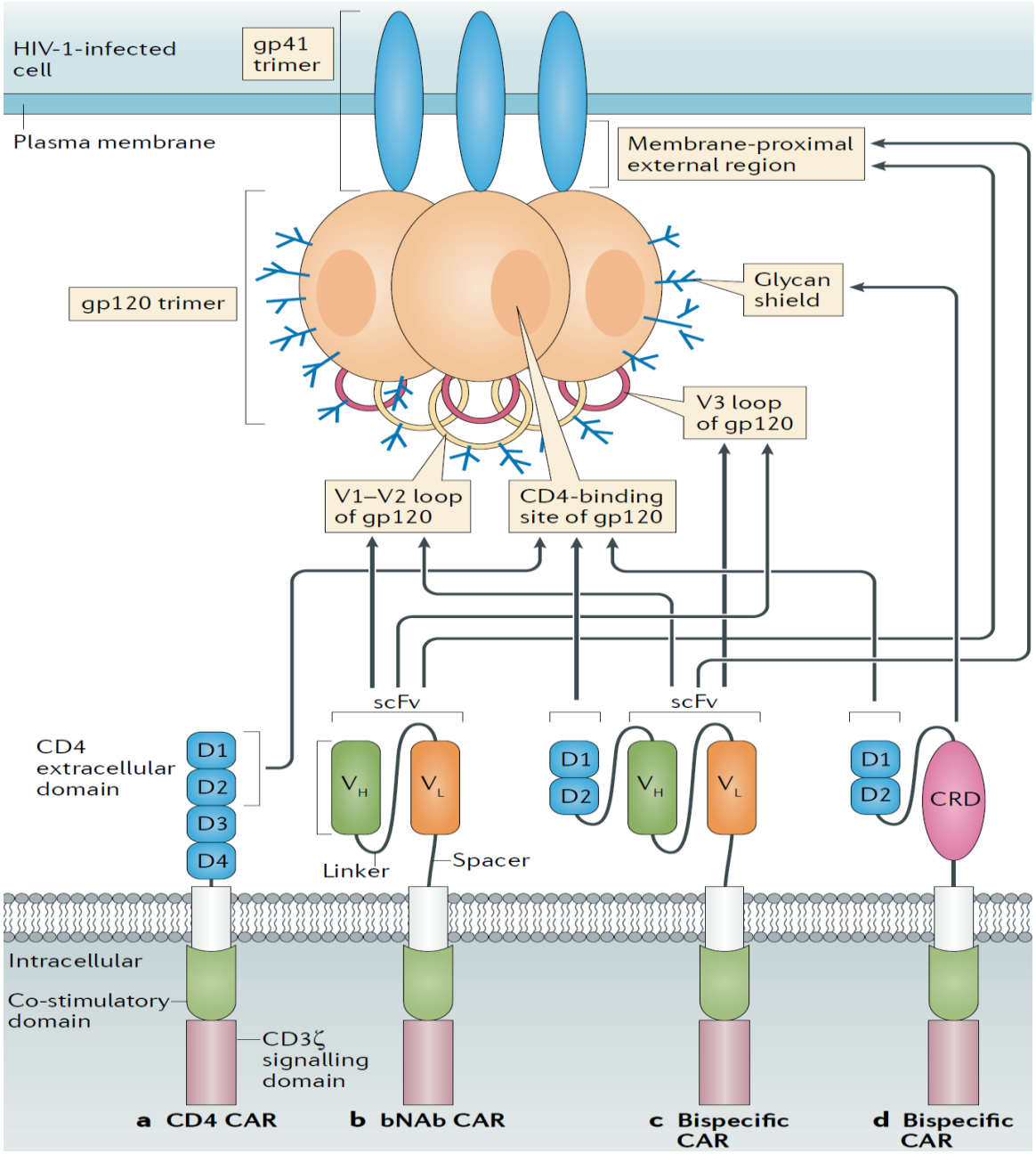
E. Liu et.al NEJM 2020

CAR-NK θεραπεία

NK cells	***T cells***
Συστατικό φυσικής ανοσίας	Συστατικό υιοθετούμενης ανοσίας
CD56+CD3-	CD3+CD4+ /CD3+CD8+
Διαφοροποίηση στον μυελό	Διαφοροποιείται στο θύμο
Δεν απαιτείται αντιγονική προεργασία	Απαιτείται αντιγονική διέγερση
Κυρίως στη περιφέρεια	Εμφανίζουν αντιγονική ειδικότητα
Όχι / ελάχιστος κίνδυνος GVHD	*Αλλογενή Τ κύτταρα επάγουν GVHD*
Η αναγνώριση γίνεται μέσω σύνθετου δικτύου υποδοχέων	*Η αναγνώριση γίνεται μέσω TCR υποδοχέα*

CD19+CAR NK	***CART***
Αλλογενές προϊόν *Άμεσα διαθέσιμο* *Δυνητικά χαμηλότερου κόστους* *1 UCB = >100 δόσεις*	*Αυτόλογο προϊόν* *Χρονοβόρα παρασκευή* *Υψηλό κόστος* *1 ασθενής = 1 δόση*
Απουσία ή ελάχιστο GVHD *Τουλάχιστον από αρχικές μελέτες, χαμηλή τοξικότητα*	*Αλλογενές: Κίνδυνος GVHD* *Σοβαρή τοξικότητα: Σύνδρομο κυτταροκινών , Νευροτοξικότητα* *~50% απαιτεί νοσηλεία ΜΕΘ*

Extracellular antigen recognition domains of chimeric antigen receptors (CARs): *specificity for HIV-1 by targeting different regions of the HIV envelope protein (Env).*



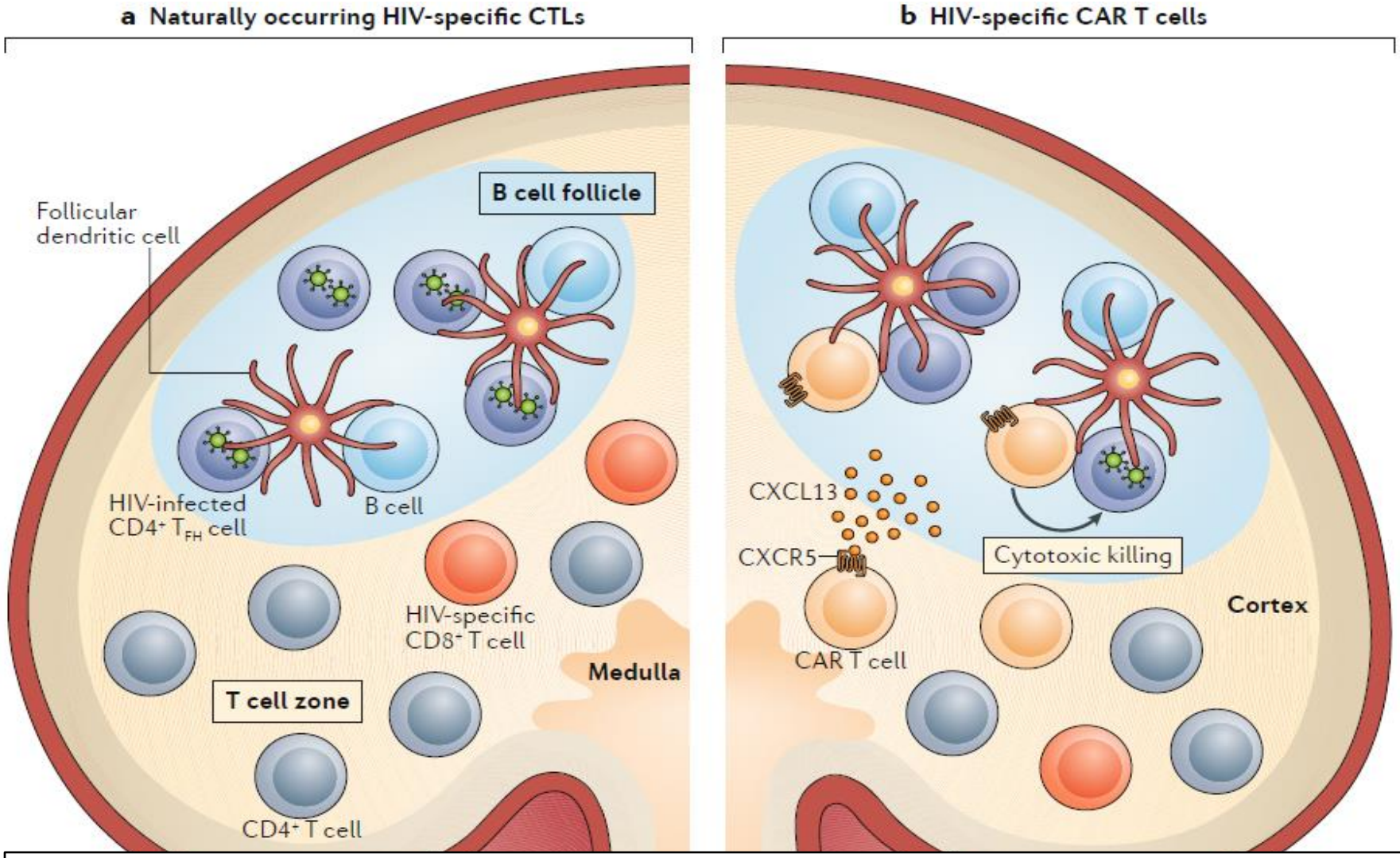
a | The full- length extracellular domain .

b | CARs containing broadly neutralizing antibody (bNAb): (e.g Ad VRCO1 and PG9)

c,d | Bispecific CARs : Dual specificity for HIV .

- CD4–gp120 interaction + scFv to an alternative region in Env.
- CD4–gp120 interaction + carbohydrate recognition domain (CRD) of a C- type lectin to glycan motifs on Env.

C.Maldini et.al Nature review immunology 2018



Engineering CAR T cells to traffic to B cell follicles.

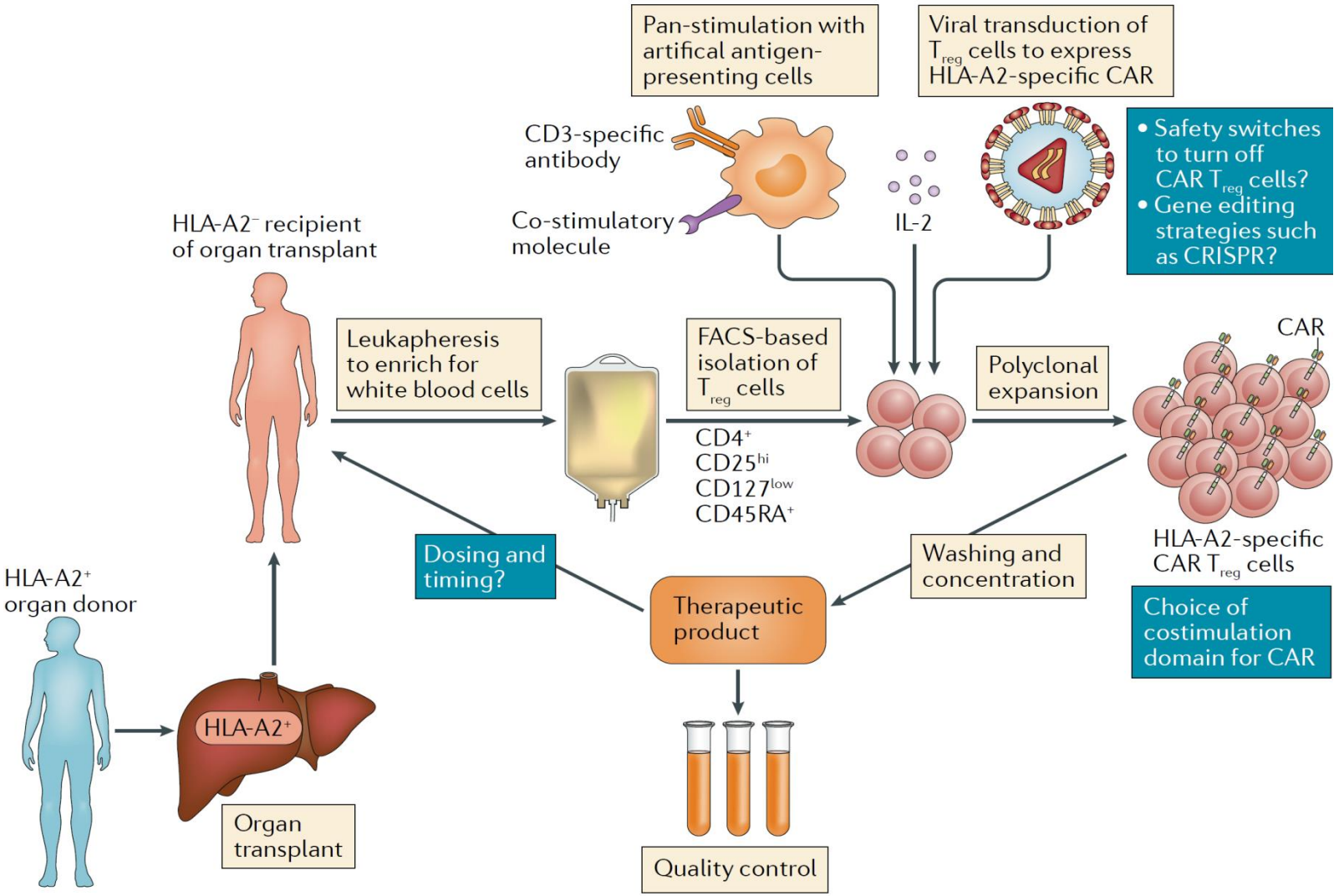
a | Naturally occurring HIV- specific CD8+ cytotoxic T lymphocytes (CTLs) are present in the extrafollicular region of a lymph node. CTLs fail to access the B cell follicle because they lack expression of the follicular homing receptor CXC- chemokine receptor 5 (CXCR5), which can mediate chemotaxis along a CXC- chemokine ligand 13 (CXCL13) concentration gradient.

b | CXCR5 gene engineering could enable *HIV- specific CAR T cells to enter into the B cell follicle.*

C.Maldini et.al Nature review immunology 2018

Towards the first clinical trial ***of CAR- expressing regulatory T cell therapy***

Allospecific Treg cells: Chimeric antigen receptor (CAR)-expressing regulatory T (Treg) cells that recognize HLA molecules.
Ιδανικό σενάριο: Πρόκληση ανοσολογικής ανοχής σε μεταμόσχευση με HLA διαφορά συμβατότητας.



FACS, fluorescence-activated cell sorting.