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CAR-T: Una nuova frontiera per l'Immunoterapia Cellulare

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- **Chimeric antigen receptor (CARs)** are proteins that incorporate: 1) an **antigen recognition domain**, 2) a **co-stimulatory domains,** and 3) a **T-cell activation domains.**
- T cells genetically modified to express CARs specifically recognize and eliminate malignant cells expressing a target antigen **(CD19, BCMA, CD30 etc)**
- **T-cell activation and proliferation** requires **both** signaling through the **TCR (signal 1)** and signaling through a **costimulatory receptor (signal 2)** (CD28, 4-1BB, OX-40)
- In the **absence of co-stimulation (signal 2),** the T-cell will either become unresponsive **(anergic)** or undergo activation-induced cell death **(AICD/apoptosis)**

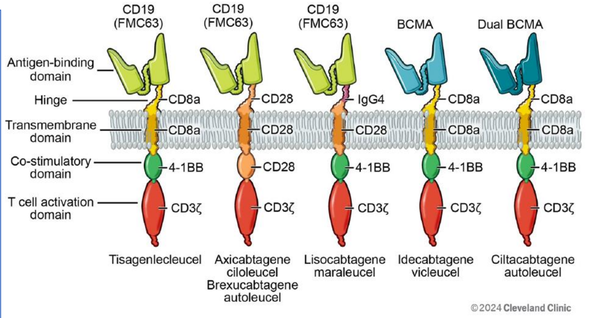

FIGURE 1 Schematic structure of the currently Food and Drug Administration-approved indications for chimeric antigen receptor T-cell therapies. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Enterprise Creative Services © 2024. All Rights Reserved. BCMA, B-cell maturation antigen.

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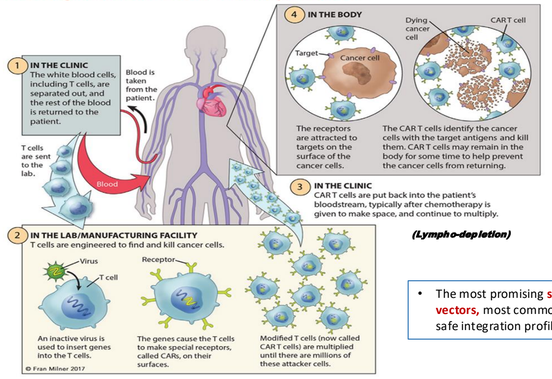
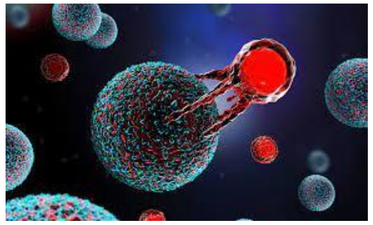
Autologous CAR T-Cell Therapy Process





- The most promising **substitutes for virus-derived vectors are transposon-derived vectors,** most commonly **sleeping beauty,** which offer great coding capability and a safe integration profile while maintaining a relatively **low production cost.**

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TABLE 1 Currently FDA-approved indications for CAR T-cell therapies, and the trials leading to their approval.

Product	Structure	Disease	Phase	Indication	Study	Results (ORR/CR)
Tisa-cel (Kymriah)	CD19-BBζ	B-ALL	2	R/R, ≤25 years	ELIANA	81%/60% (CRi 21%)
		LBCL	2	R/R after ≥2 lines of therapy	JULIET	53%/39%
		FL	2	R/R after ≥2 lines of therapy	ELARA	86%/69%
Axi-cel (Yescarta)	CD19-28ζ	LBCL	2	R/R after ≥2 lines of therapy	ZUMA-1	82%/54%
			3	Refractory to or relapsing at <12 months of first-line therapy	ZUMA-7	83%/65% (vs. 50%/32% in SOC arm[a])
			2	Refractory to or relapsing at <12 months of first-line therapy, ineligible for autoHSCT	ALYCANTE	90%/79%
		FL	2	R/R after ≥2 lines of therapy	ZUMA-5	92%/74%
Brexu-Cel (Tecartus)	CD19-28ζ	B-ALL	2	R/R, ≥18 years	ZUMA-3	71%/56% (CRi 15%)
		MCL	2	R/R	ZUMA-2	91%/68%
Liso-cel (Breyanzi)	CD19-BBζ, with a 1:1 CD4:CD8 ratio	LBCL	2	R/R after ≥2 lines of therapy	TRANSCEND NHL 001	73%/53%
			3	Refractory to or relapsing at <12 months of first-line therapy	TRANSFORM	80%/74% (vs 45%/43% in SOC arm[a])
			2	R/R and not eligible for autoHSCT	PILOT	80%/54%
		CLL		R/R after ≥2 lines of therapy[b]	TRANSCEND CLL 004	43%/18%
Ide-cel (Abecma)	BCMA-BBζ	Multiple myeloma	2	R/R after ≥4 lines of therapy[c]	KarMMa	73%/33%
Cilta-cel (Carvykti)	Dual BCMA-BBζ	Multiple myeloma	2	R/R after ≥2 lines of therapy[c]	CARTITUDE-1	97%/67%

Abbreviations: autoHSCT, autologous haematopoietic stem cell transplantation; B-ALL, B-cell acute lymphoblastic leukaemia; BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; CR, complete remission; CRi, complete remission with incomplete haematological recovery; FDA, Food and Drug Administration; FL, follicular lymphoma; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; ORR, overall response rate; R/R, relapsed/refractory; SOC, standard of care.
[a]SOC arm: immunochemotherapy + autoHSCT.
[b]Including a Bruton's tyrosine kinase (BTK) inhibitor and a B-cell lymphoma 2 (BCL-2) inhibitor.
[c]Including an immunomodulatory agent, a proteasome inhibitor and an anti-CD38 monoclonal antibody.

Gregoire et al, Br J Haematol 2024

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Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study

Stephen J Schuster, Constantine S Tam, Peter Borchmann, Nina Worel, Joseph P McGuirk, Harald Holte, Edmund K Waller, Samantha Jaglowski, Michael R Bishop, Lloyd E Damon, Stephen Ronan Foley, Jason R Westin, Isabelle Fleury, P Joy Ho, Stephan Mielke, Takanori Teshima, Murali Janakiram, Jing-Mei Hsu, Koji Izutsu, Marie José Kersten, Monalisa Ghosh, Nina Wagner-Johnston, Koji Kato, Paolo Corradini, Marcela Martinez-Prieto, Xia Han, Ranjan Tiwari, Gilles Salles, Richard T Maziarz

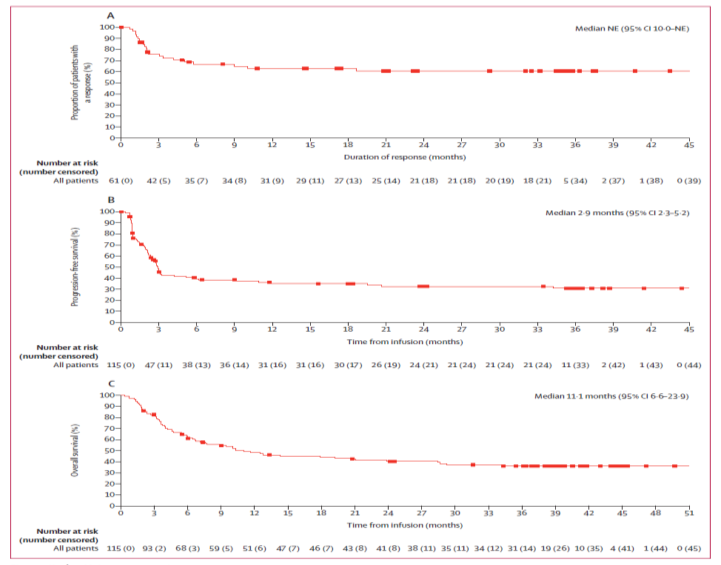

Figure 1: Kaplan-Meier outcome estimates
(A) Duration of response. (B) Progression-free survival. (C) Overall survival. NE=not estimable.

Lancet Oncol 2021; 22: 1403–15
Published Online September 10, 2021
https://doi.org/10.1016/S1470-2045(21)00375-2

See Comment page 1347

Lymphoma Program, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA (S J Schuster MD); Peter MacCallum Cancer Center, Royal Melbourne Hospital and University of Melbourne, Melbourne, VIC, Australia (C S Tam MD); Clinic I for Internal Medicine, University Hospital of Cologne, Cologne, Germany (P Borchmann MD); Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria (N Worel MD); Department of Internal Medicine, The University of Kansas Health System, Kansas City, KS, USA (J P McGuirk DO); Department of Oncology, Oslo University Hospital, Oslo, Norway (H Holte MD); Bone Marrow and Stem Cell Transplant Center, Emory University Winship Cancer Institute, Atlanta, GA, USA (E K Waller MD); Blood and Marrow Transplant Program,

Comparison of 2-year outcomes with CAR T cells (ZUMA-1) vs salvage chemotherapy in refractory large B-cell lymphoma

Sattva S. Neelapu,[1] Frederick L. Locke,[2] Nancy L. Bartlett,[3] Lazaros J. Lekakis,[4] Patrick M. Reagan,[5] David B. Miklos,[6] Caron A. Jacobson,[7] Ira Braunschweig,[8] Olalekan O. Oluwole,[9] Tanya Siddiqi,[10] Yi Lin,[11] Michael Crump,[12] John Kuruvilla,[13] Eric Van Den Neste,[14] Umar Farooq,[15] Lynn Navale,[16] Venita DePuy,[17] Jenny J. Kim,[16] and Christian Gisselbrecht[18]

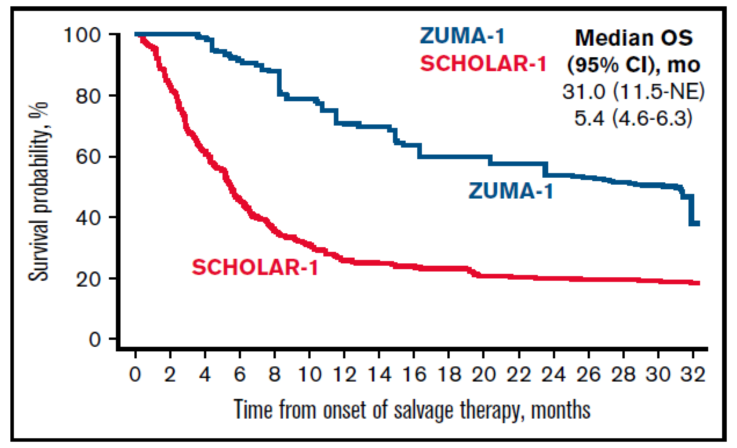

Figure 2. Comparison of confounder-adjusted OS. To control for confounding, the treatment-specific survival functions were obtained using augmented inverse-probability weighted complete-case estimators[24] on the primary common support data set for survival (ZUMA-1, N = 81; SCHOLAR-1, N = 331). mo, month; NE, not estimable.

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Real-life CAR-T in Italy for NHLs

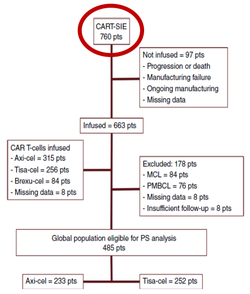

Figure 1. Patient flow diagram. MCL, mantle cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; pts, patients.

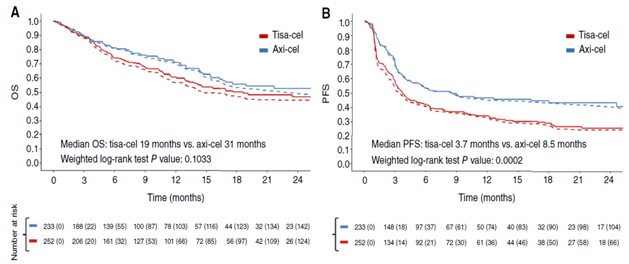

Figure 2. Survival from infusion of tisa-cel vs. axi-cel before (continuous line) and after (dotted lines) PS weighting. **A**, OS. **B**, PFS.

Stella et al, Blood Cancer Discovery 2024

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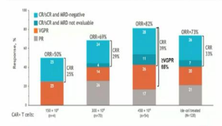
Ide-cel approval: the KarMMa trial

FDA approved in 2021
EMA approved in 2021

Second generation CAR-T cell, anti-BCMA murine scFv, 4-1BB costimulatory domain

KarMMa, phase 2 study (N = 128)		
Median prior lines: 6 (3–16)	84% of patients were triple-class refractory	Bridging possible Flu-Cy lymphodepletion

EU: At least 3 prior therapies, including an IMiD, a PI, and an anti-CD38 antibody, with disease progression on the last therapy



AE,* n (%)	Ide-Cel-Treated (N=128)	
	Any Grade	Grade ≥3
Hematologic		
Neutropenia	117 (91)	114 (89)
Anemia	89 (70)	77 (60)
Thrombocytopenia	81 (63)	67 (52)
CRS	107 (84)	7 (5)
Neurotoxicity	23 (18)	4 (3)

Munshi N, et al. *NEJM* 2021

Cilta-cel approval: the CARTITUDE-1 trial

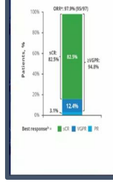
FDA approved in 2022
EMA approved in 2022

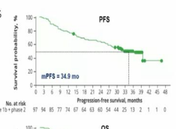
Second generation CAR-T cell, 2 anti-BCMA camelid VHH single domains, 4-1BB costimulatory domain

CARTITUDE-1, phase 2 study (N = 97)		
Median prior lines: 6 (3–18)	88% of patients were triple-class refractory	Bridging possible Flu-Cy lymphodepletion

EU: At least three prior therapies, including an IMiD, a PI, and an anti-CD38 antibody, with disease progression on the last therapy.

12 mos sustained MRD rate: 53%
PFS @ 30 mos: 75%





AE, n (%)	Cilta-cel-Treated (N=97)	
	Any Grade	Grade ≥3
Hematologic		
Neutropenia	93 (96)	92 (95)
Anemia	79 (81)	66 (68)
Thrombocytopenia	77 (80)	58 (60)
CRS	92 (95)	8 (5)
Neurotoxicity	20 (21)	10 (10)

Berdeja J, et al. *Lancet* 2022;
Lin Y, et al. ASCO 2023

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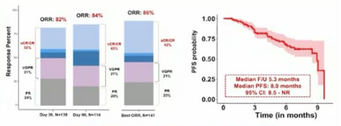
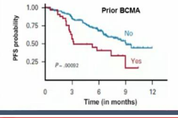
Real-world data (US consortium)

Ide-cel, n= 159



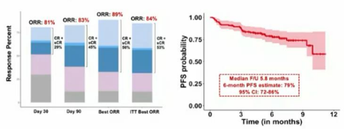
77% of patients (N=150) would have been ineligible for participation in the KarMMa trial

KarMMa-1 exclusion criteria at leukapheresis	No. (%)
Organ failure (renal, cardiac, hepatic)	60 (31)
Prior anti-BCMA therapy	43 (22)
Platelets < 50,000/µL	42 (21)
Hemoglobin < 8g/dL	33 (17)
ECOG PS ≥ 2	33 (17)
ANC < 1000/uL	29 (15)
PCL, POEMS, amyloidosis, non-secretory myeloma	26 (13)



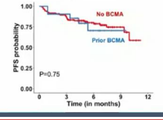
Hansen et al. ASCO 2022 & *JCO* 2023

Cilta-cel, n= 143



97% of patients (N=81) would have been ineligible for participation in the CARTITUDE-1 trial

CARTITUDE-1 exclusion criteria at leukapheresis	No. (%)
Cytopenias	24 (17)
Oligosecretory/Non-secretory	23 (16)
Organ dysfunction	17 (12)
Prior anti-BCMA	17 (12)
ECOG PS ≥ 2	14 (10)
Plasma Cell Leukemia	10 (7)
History of CNS Pathology	9 (6)
Amyloid	4 (3)



Hansen et al. ASCO 2023

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CAR-T in earlier phases of disease (2/4 - 1/3 prior LoT) as compared to SoC

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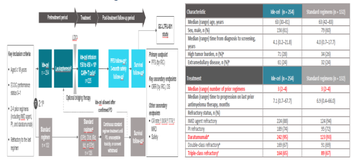
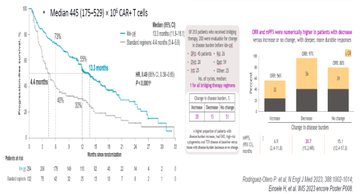
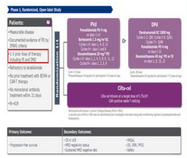
KarMMa-3: design, baseline characteristics and PFS

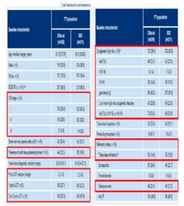




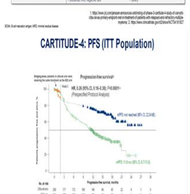
Rodriguez-Otero P. *et al. N Engl J Med* 2023; 388:1002-1014.
Estrada N. *et al. BIG 2023 encore Poster P008.*

CARTITUDE-4: study design

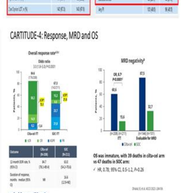




CARTITUDE-4: PFS (ITT Population)



CARTITUDE-4: Response, MRD and OS



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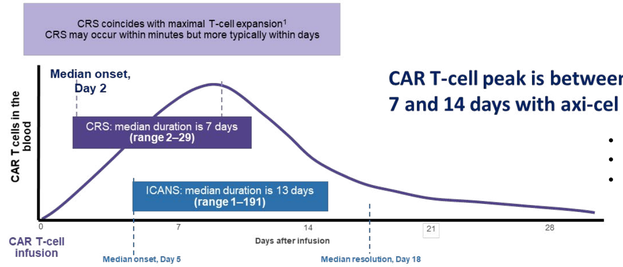
Clinical trials have established the timing and duration of acute adverse events



CAR T-cell peak is between 7 and 14 days with axi-cel

- *Pro-inflammatory cytokines*
- *Activated T- and myeloid cells*
- *Endothelial activation → blood–brain barrier disruption*

While CRS and ICANS are acute AEs, other AEs (NEs, cytopenias, hypogammaglobulinemia and infections) may be also present later (>Day 30 post infusion) and can be long-lasting.

AE: adverse event; CAR: chimeric antigen receptor; CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome); NEs: neurological event

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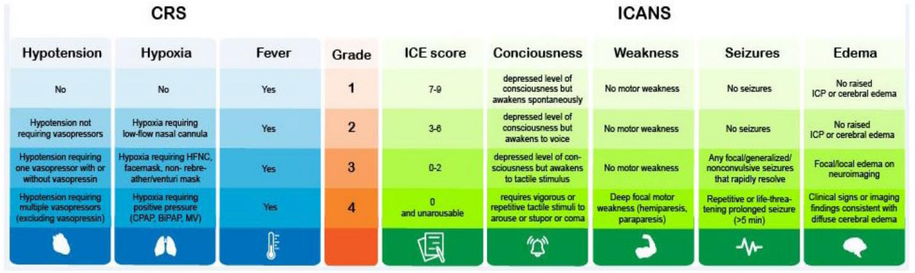

CRS				ICANS				
Hypotension	Hypoxia	Fever	Grade	ICE score	Conciousness	Weakness	Seizures	Edema
No	No	Yes	1	7-9	depressed level of consciousness but awakens spontaneously	No motor weakness	No seizures	No raised ICP or cerebral edema
Hypotension not requiring vasopressors	Hypoxia requiring low-flow nasal cannula	Yes	2	3-6	depressed level of consciousness but awakens to voice	No motor weakness	No seizures	No raised ICP or cerebral edema
Hypotension requiring one vasopressor with or without vasopressin	Hypoxia requiring HFNC, facemask, non- rebre-ather/venturi mask	Yes	3	0-2	depressed level of con-sciousness but awakens to tactile stimulus	No motor weakness	Any focal/generalized/ nonconvulsive seizures that rapidly resolve	Focal/local edema on neuroimaging
Hypotension requiring multiple vasopressors (excluding vasopressin)	Hypoxia requiring positive pressure (CPAP, BiPAP, MV)	Yes	4	0 and unarousable	requires vigorous or repetitive tactile stimuli to arouse or stupor or coma	Deep focal motor weakness (hemiparesis, paraparesis)	Repetitive or life-threa-tening prolonged seizure (>5 min)	Clinical signs or imaging findings consistent with diffuse cerebral edema

FIGURE 4. Grading for Cytokine Release Syndrome (CRS) and Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). BiPAP indicates bilevel positive airway pressure; CPAP, continuous positive airway pressure; HFNC, high-flow nasal cannula; ICE score, immune effector cell-associated encephalopathy score; ICP, intracranial pressure; MV, mechanical ventilation.

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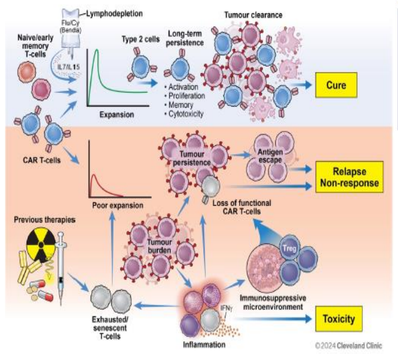

FIGURE 2 Major determinants of response and failure after CAR T-cell therapy. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Enterprise Creative Services © 2024. All Rights Reserved. CAR, chimeric antigen receptor; Flu/Cy, fludarabine/cyclophosphamide; IFN, interferon; IL, interleukin.

TABLE 3 Factors associated with resistance to CAR T-cell therapy.

T cells	• Poor in vivo expansion following infusion	Systemic factors	• Systemic inflammation (IL6, ferritin, CRP)
	• Low proportion of early memory or SCM T-cells		• Circulating M-MDSCs
	• High proportion of senescent $CD8^+$ T-cells	Therapy-related factors	• Higher number of previous lines of therapy
	• High proportion of exhausted $CD8^+$ T-cells		• Recent exposure to bendamustine
	• High proportion of Treg		• Previous exposure to blinatumomab in ALL?
	• Low proportion of type 2 cells		• Previous exposure to other BCMA-directed therapies in multiple myeloma?
	• High expression of genes involved in IFN response and type 1 differentiation		• Absence of response to bridging therapy?
Tumour-related factors	Tumour burden		• Suboptimal lymphodepletion
	• Lymphoma		
	○ High tumour volume		
	○ High circulating tumour DNA		
	○ Number of extranodal sites		
	• ALL: high percentage of BM blasts		
	• Multiple myeloma: high BM infiltration, high serum BCMA		
	Tumour genetic alterations		
	• *TP53* genomic alterations		
	• DNA copy number alterations		
	• Loss of the FAS death receptor[38]		
	• Mutation of *MYC*, *BCL2* or *CDKN2A*		
	Tumour inflammation/immunosuppressive microenvironment		
	• Tumour IFN signature		
	• Tumour expression of T-cell inhibitory ligands (including PD-L1 and PD-L2)		
	• Intra-tumoural regulatory T cells		
	• Higher stromal and immunosuppressive signature (including hypoxia)		

Gregoire et al, Br J Haematol 2024

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How to improve CAR-T activity and reduce toxicity?

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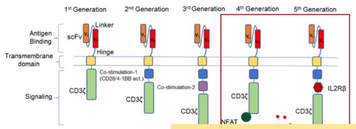


Figure 1. Structure of the generations of chimeric antige... intracellular signal component CD3ζ, one more costimula... another costimulatory molecule is added to the third gener... second generation can activate a downstream transcriptio... The fifth generation of CARs based also on the second gen... from IL2Rβ.

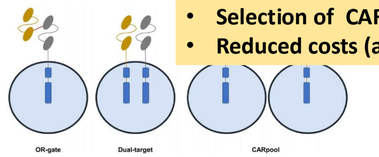
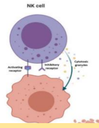
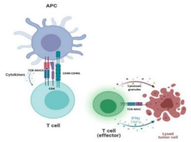


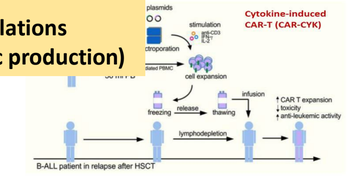
Figure 2. Overview of current multi-targeted chimeric antigen receptor T-cell approaches. Multi-targeting may be a mean to improve efficacy of CAR-T. Three major methods could be exploited: OR-gate (tandem) CAR-T: consist of the expression of two different CAR on the same T cell; dual-target CAR-T: consist of encoding two different target specific single-chain variable fragment antibodies on same CAR protein using a single vector; CARpool: involves production of two separate single target CAR-T products infused together or sequentially.





T cells. On the left, NK cells are killing a tumor cell, as inhibitory receptors ... NK cells secrete granulocytes such as perforin and granzyme in order to kill ... rimed for activation by APC and cytokine stimulation. An effector T cell kills ... other cytokines into the tumor microenvironment.

- Selection of patients
- Shortened CAR-T production time
- Off-the-shelf
- Selection of CAR-T populations
- Reduced costs (academic production)



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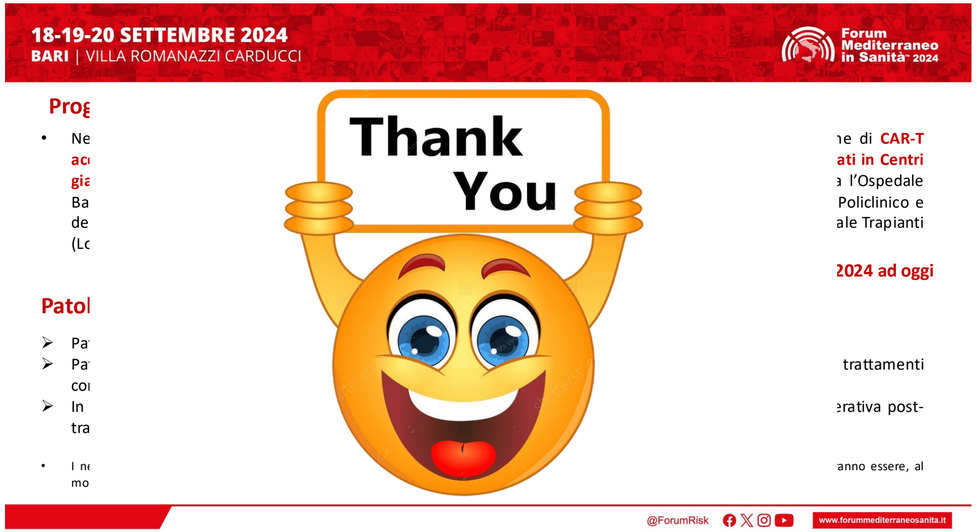

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- Messa a disposizione del pubblico, in un sistema di reti telematiche, mediante connessioni di qualsiasi genere, di un'opera dell'ingegno protetta, o di parte di essa (art. 171, legge n.633/1941 comma 1 lett. a) bis)
- Reati di cui al punto precedente commessi su opere altrui non destinate alla pubblicazione qualora ne risulti offeso l'onore o la reputazione (art. 171, legge n.633/1941 comma 3)
- Abusiva duplicazione, per trarne profitto, di programmi per elaboratore; importazione, distribuzione, vendita o detenzione a scopo commerciale o imprenditoriale o concessione in locazione di programmi contenuti in supporti non contrassegnati dalla SIAE; predisposizione di mezzi per rimuovere o eludere i dispositivi di protezione di programmi per elaboratori (art. 171-bis legge n.633/1941 comma 1)
- Riproduzione, trasferimento su altro supporto, distribuzione, comunicazione, presentazione o dimostrazione in pubblico, del contenuto di una banca dati; estrazione o reimpiego della banca dati; distribuzione, vendita o concessione in locazione di banche di dati (art. 171-bis legge n.633/1941 comma 2)
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