CAR-T cells: principes et mode d'action

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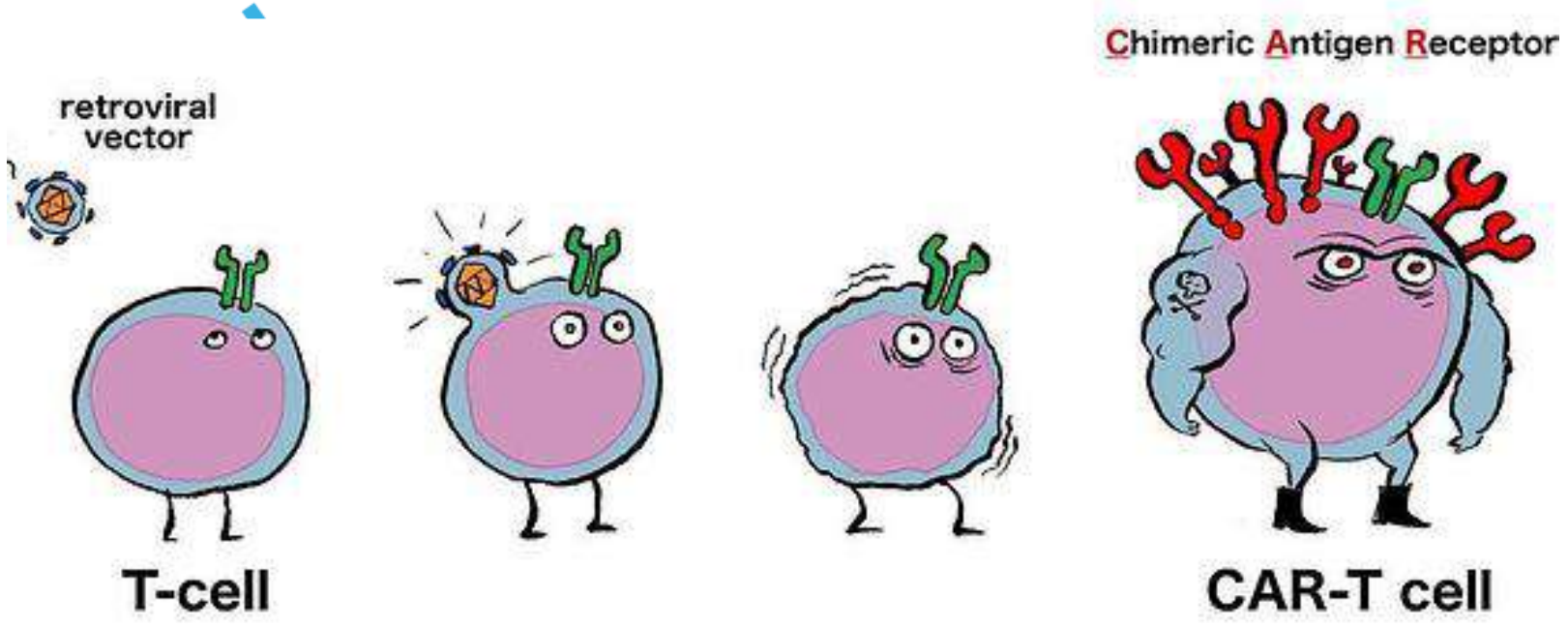
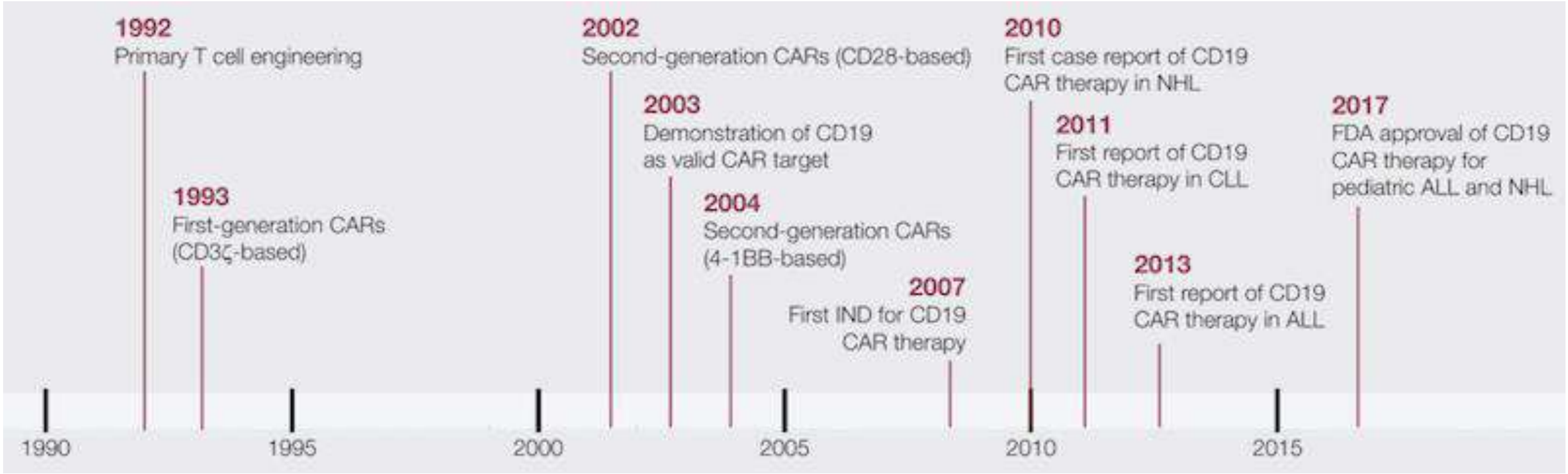
CAR T is an innovative cancer therapy which directs the patient's own immune system to target tumour cells

T CELLS ARE **ENGINEERED** TO EXPRESS CARs THAT TARGET SURFACE ANTIGENS (**e.g. CD19**)

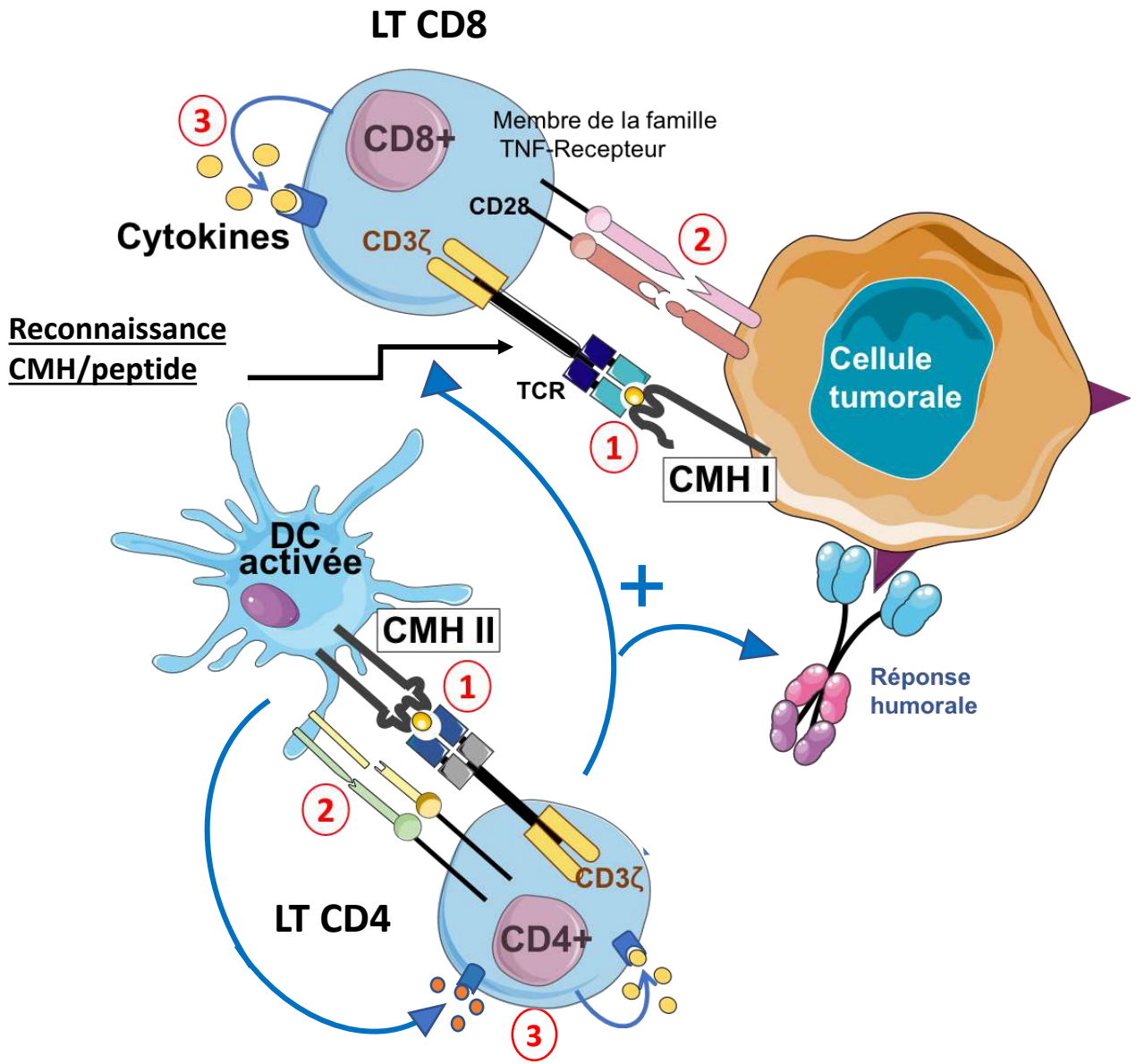


TUMOUR CELLS ARE **KILLED** AFTER CAR T CELLS BIND THEM

Les CAR-T cells: ce n'est pas si nouveau ....



Cellules impliquées dans la réponse immunitaire anti-tumorale



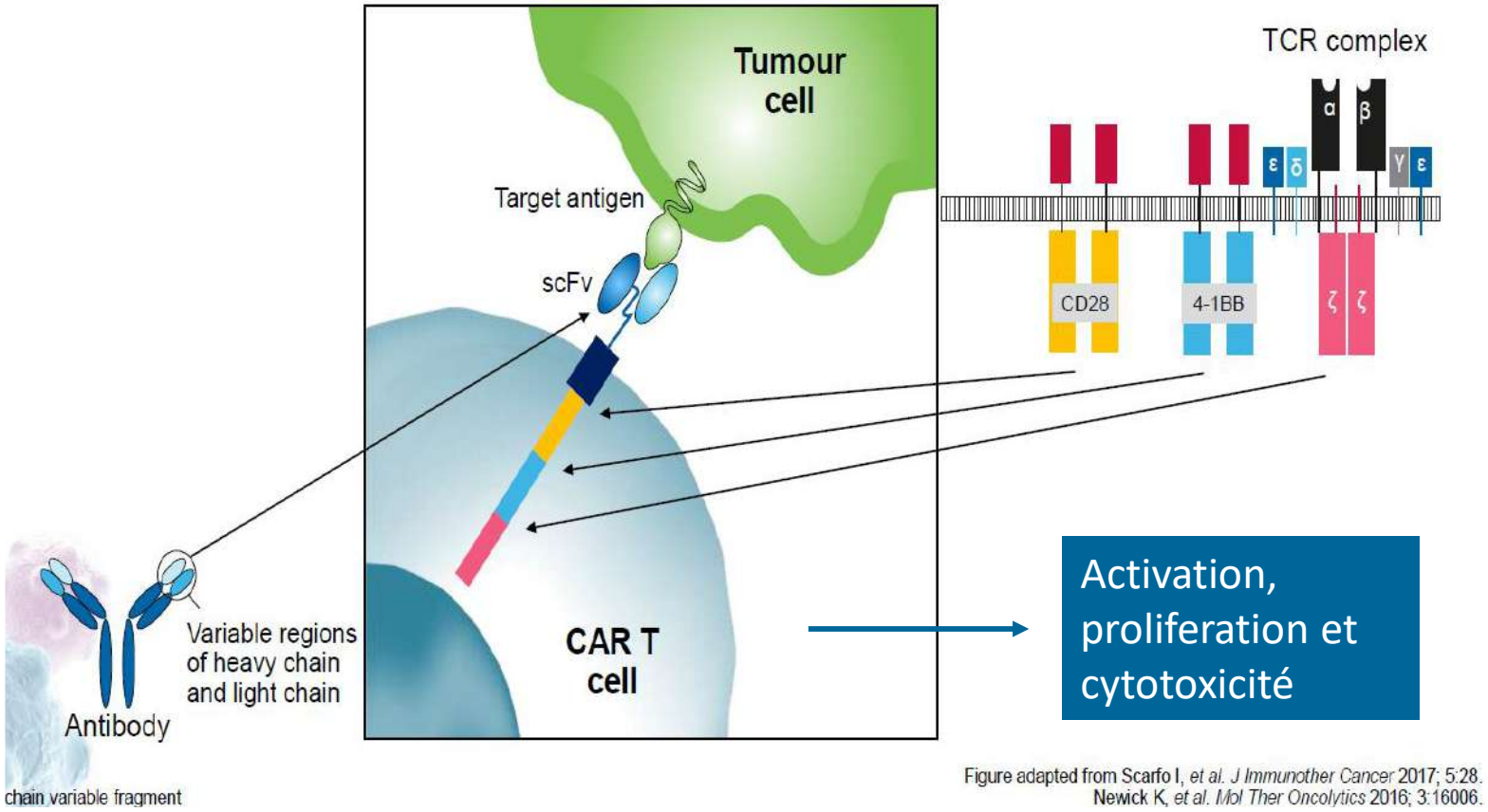
1 Reconnaissance complexe Ag/peptide
+
2 Co-stimulation
+
3 Signal cytokinique

activation/proliferation des Lymphocytes T conventionnels

=

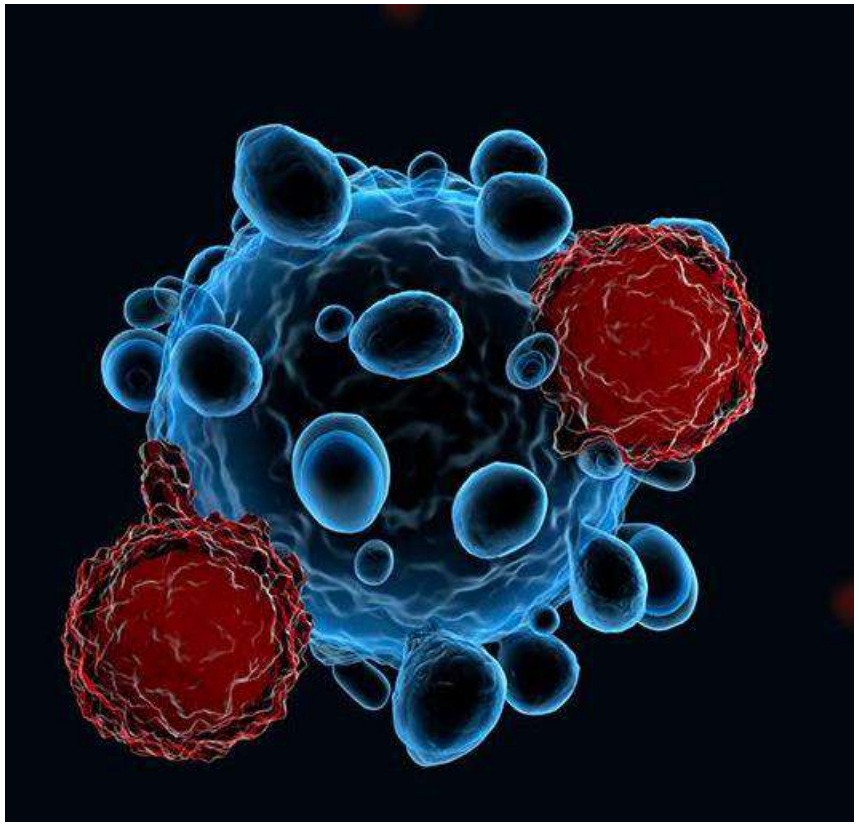
Rubio et al. Bulletin du cancer 2018

Les CAR-T cells sont armés pour mimer les signaux d'activation du lymphocyte T dès que la cellule reconnait l'antigène tumoral sur la cible

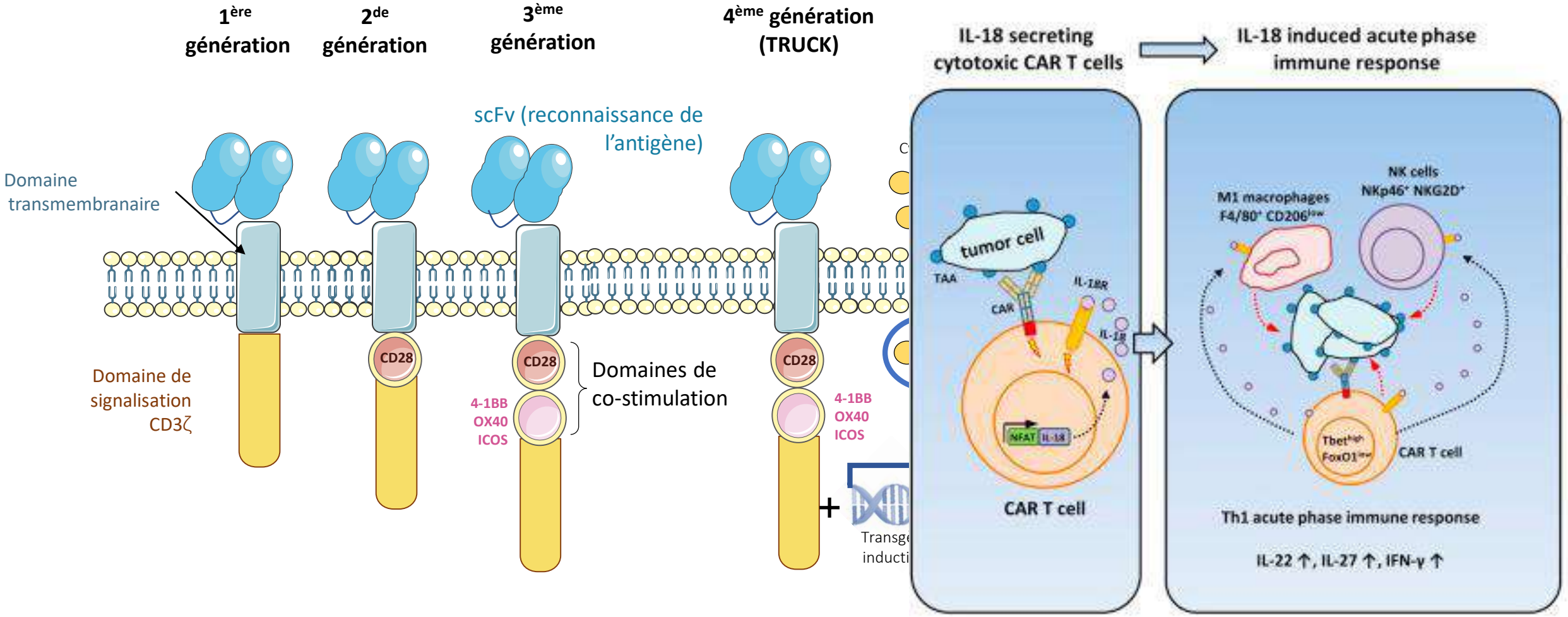


Activation, proliferation et cytotoxicité

Figure adapted from Scarfo I, *et al. J Immunother Cancer* 2017; 5:28.
Newick K, *et al. Mol Ther Oncolytics* 2016; 3:16006.

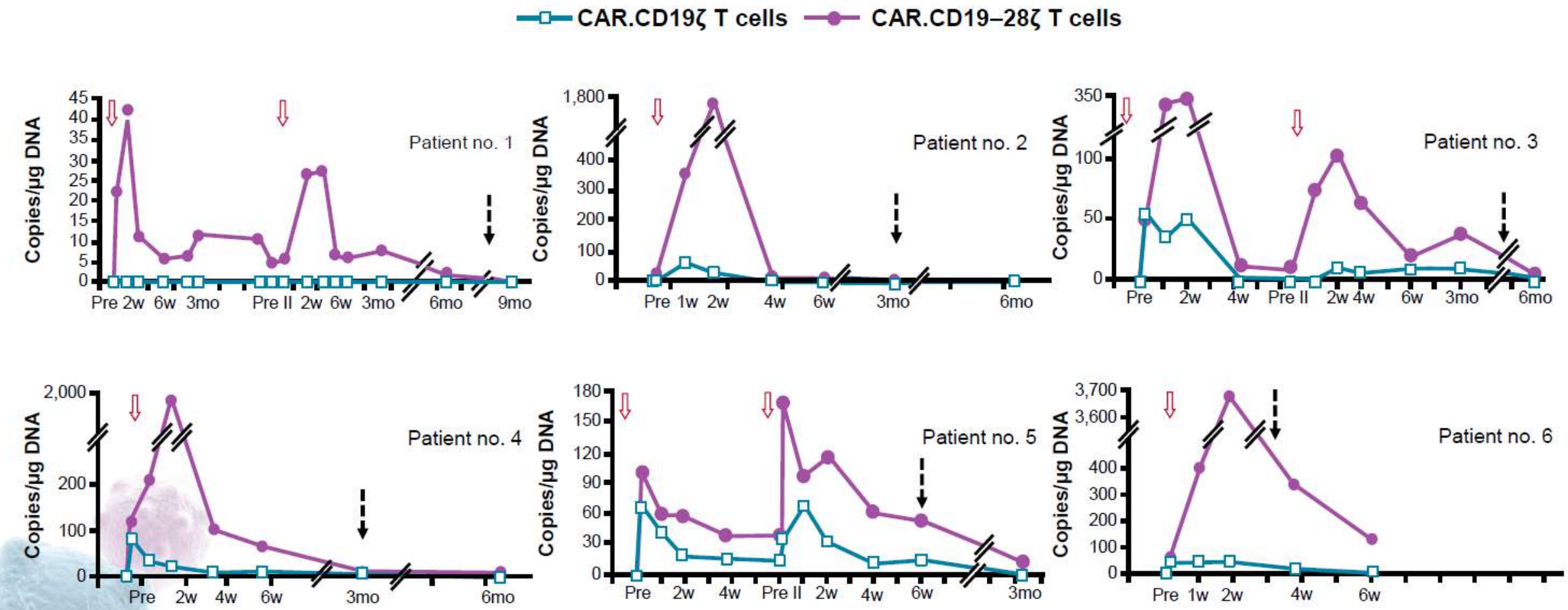


Différentes générations de CAR-T cells ont été développées



Rubio et al. Bulletin du cancer 2018

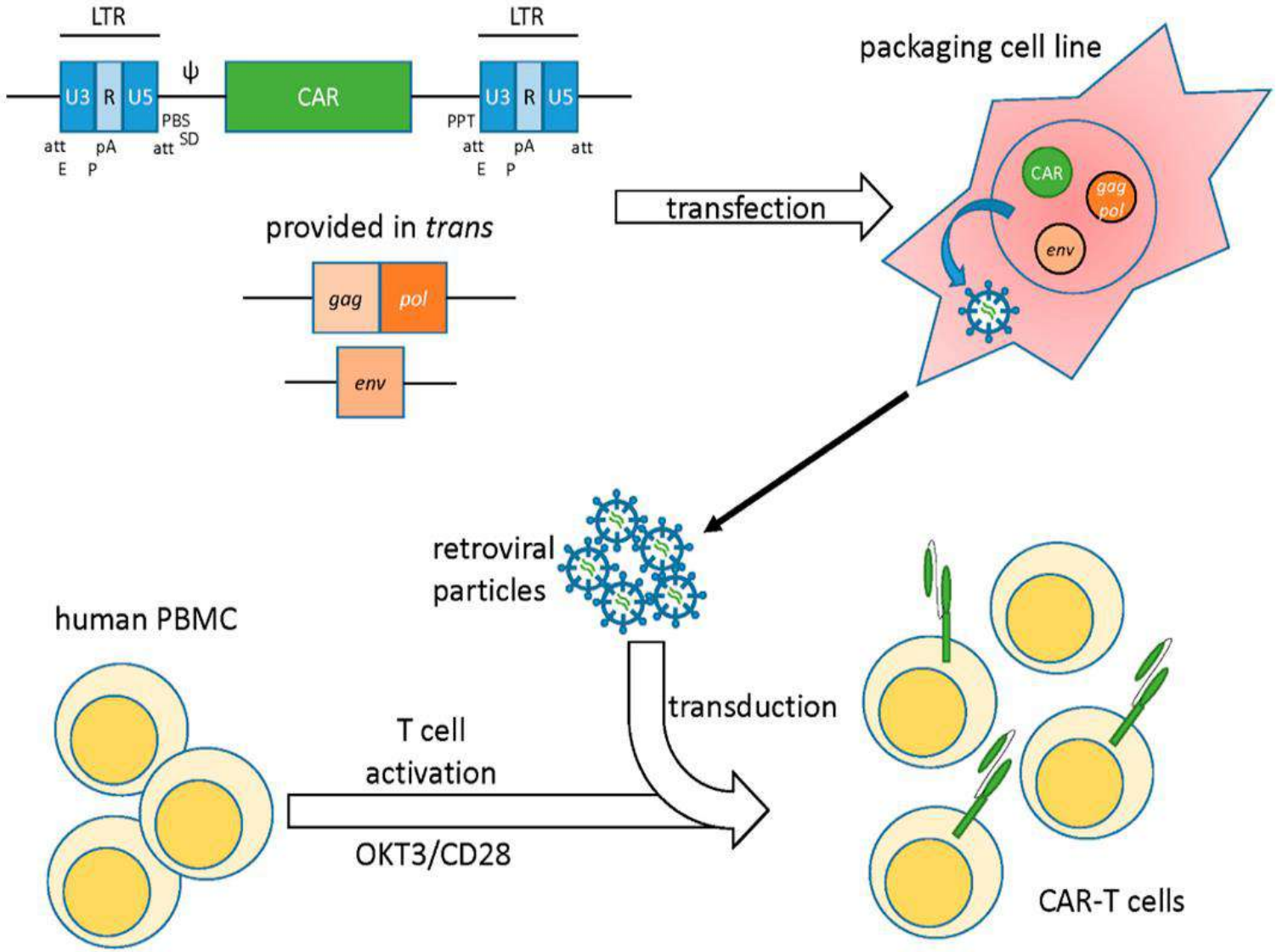
Les CAR de 2eme génération (CD3ζ + CD28) permettent une meilleure expansion in vivo des CAR-T cells par rapport aux CAR de 1ere génération



Open arrows: time of T-cell infusion
Dashed arrows: time when chemotherapy was initiated for disease progression

Savoldo B, *et al. J Clin Invest* 2011; 121:1822–1826.

Comment produit on des CAR-T cells au laboratoire ?



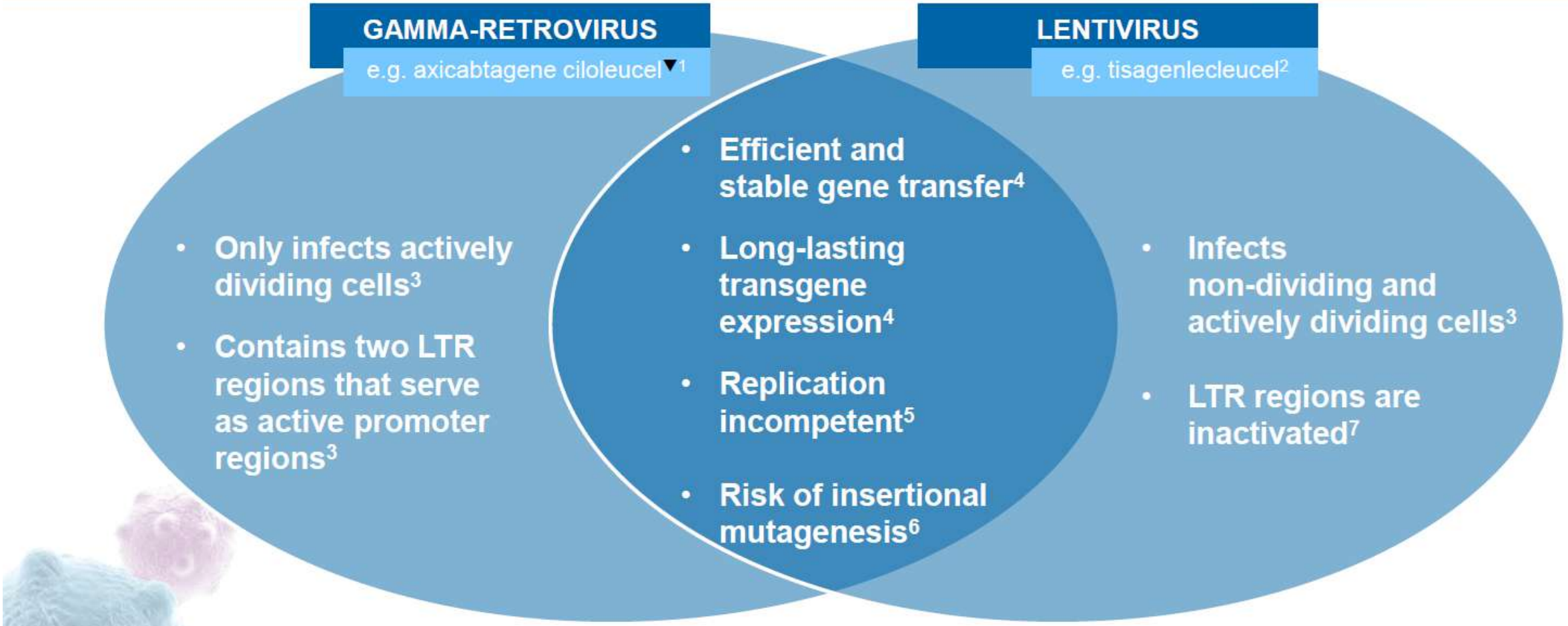
Caractéristiques différentes des 2 médicaments CAR-T ayant l'AMM

	Axicabtagene ciloleucel	Tisagenlecleucel
	YESCARTA-GILEAD	KYMRIAH-NOVARTIS
Anti-CD19 scFv	VL, FMC63, VH	VL, FMC63, VH
Hinge		
Transmembrane	CD28	CD8α
Costimulatory domain	CD28	4-1BB
Essential activation domain	CD3ζ	CD3ζ
Gene transfer	Gamma-retrovirus	Lentivirus
Starting cells	PBMCs	PBMCs

PBMC: peripheral blood mononuclear cell

Figure adapted from van der Stegen SJ, *et al. Nat Rev Drug Discov* 2015; 14:499–509.
Axicabtagene ciloleucel SmPC (Jan 2019; available at www.ema.europa.eu).
Tisagenlecleucel SmPC (Sep 2018; available at www.ema.europa.eu).

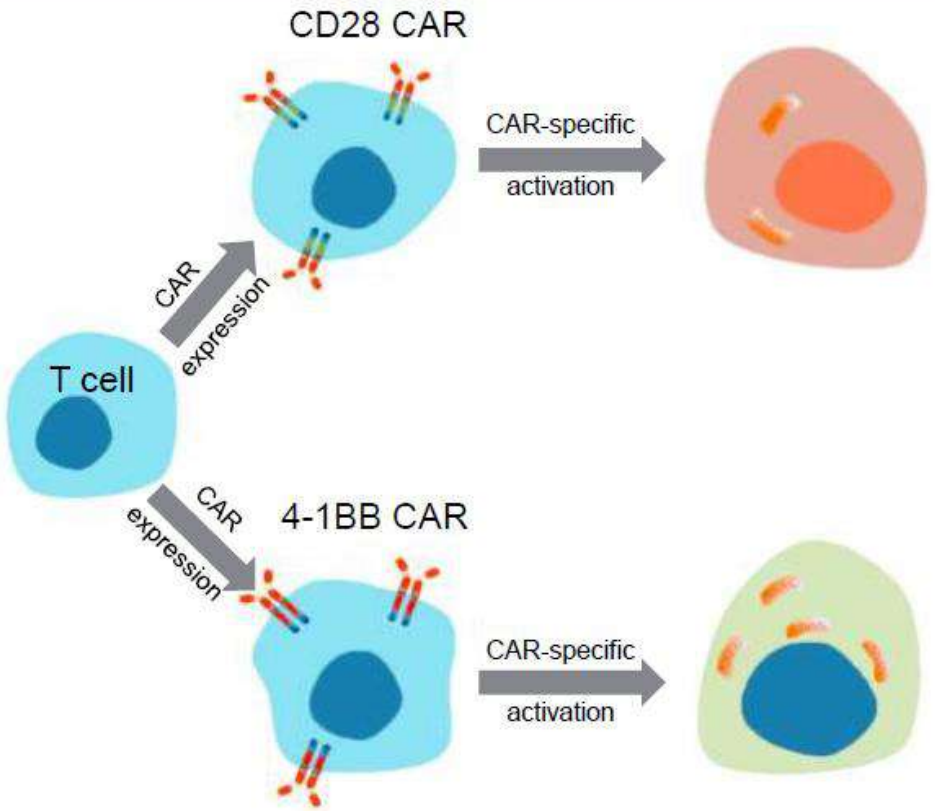
Caractéristiques des différents virus utilisés pour générer des CAR-T cells



LTR: long terminal repeat

1. Axicabtagene ciloleucel SmPC (Jan 2019; available at www.ema.europa.eu). 2. Tisagenlecleucel SmPC (Sep 2018; available at www.ema.europa.eu). 3. Demeulemeester J, *et al. Bioessays* 2015; 37:1202–1214. 4. Yi Y, *et al. Curr Gene Ther* 2011; 11:218–228. 5. Chen X & Gonçalves MA. *Mol Ther* 2016; 24:447–457. 6. Zhang C, *et al. Biomark Res* 2017; 5:22. 7. Wu C & Dunbar CE. *Front Med* 2011; 5:356–371.

In vitro, les CAR CD28 prolifèrent plus vite mais moins longtemps que les CAR 4-1BB



- ↑ IL-2 production
- ↑ Consistent PI3K pathway activation
- ↓ Persistence
- ↑ Effector memory
- ↓ SRC
- ↓ Mitochondrial biogenesis
- ↑ Glycolytic metabolism

- ↑ Tonic signalling
- ↑ Persistence
- ↑ Central memory
- ↑ SRC
- ↑ Mitochondrial biogenesis
- ↑ Oxidative metabolism

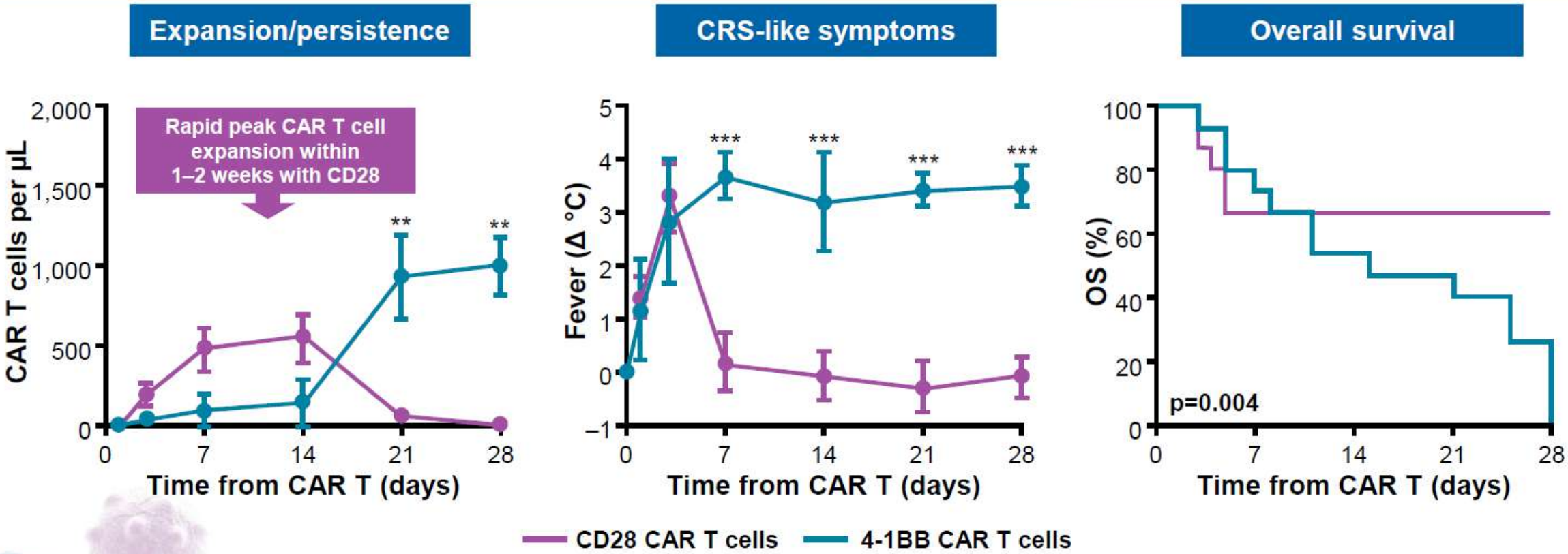
- Both CD28 and 4-1BB CARs augment cytokine secretion relative to first-generation CARs[1]
  - More rapidly for CD28 compared with 4-1BB
- *In vitro* studies do not address the complex interactions in the tumour tissue[2]

THERE IS CURRENTLY NO STANDARDISED MEASUREMENT OF CAR T-CELL PERSISTENCE *IN VIVO*[3], SO CROSS-STUDY COMPARISONS CANNOT BE MADE

IL: interleukin; SRC: spare respiratory capacity

Figure adapted from Kawalekar OU, *et al. Immunity* 2016; 44:380–390. 1. van der Stegen SJ, *et al. Nat Rev Drug Discov* 2015; 14:499–509. 2. Abken H, *et al. Immunity* 2016; 4:214–216. 3. Park JH, *et al. Blood* 2016; 127:3312–3320.

In vivo chez la souris, les CAR CD28 prolifèrent plus vite mais persistent moins longtemps que les CAR 4-1BB

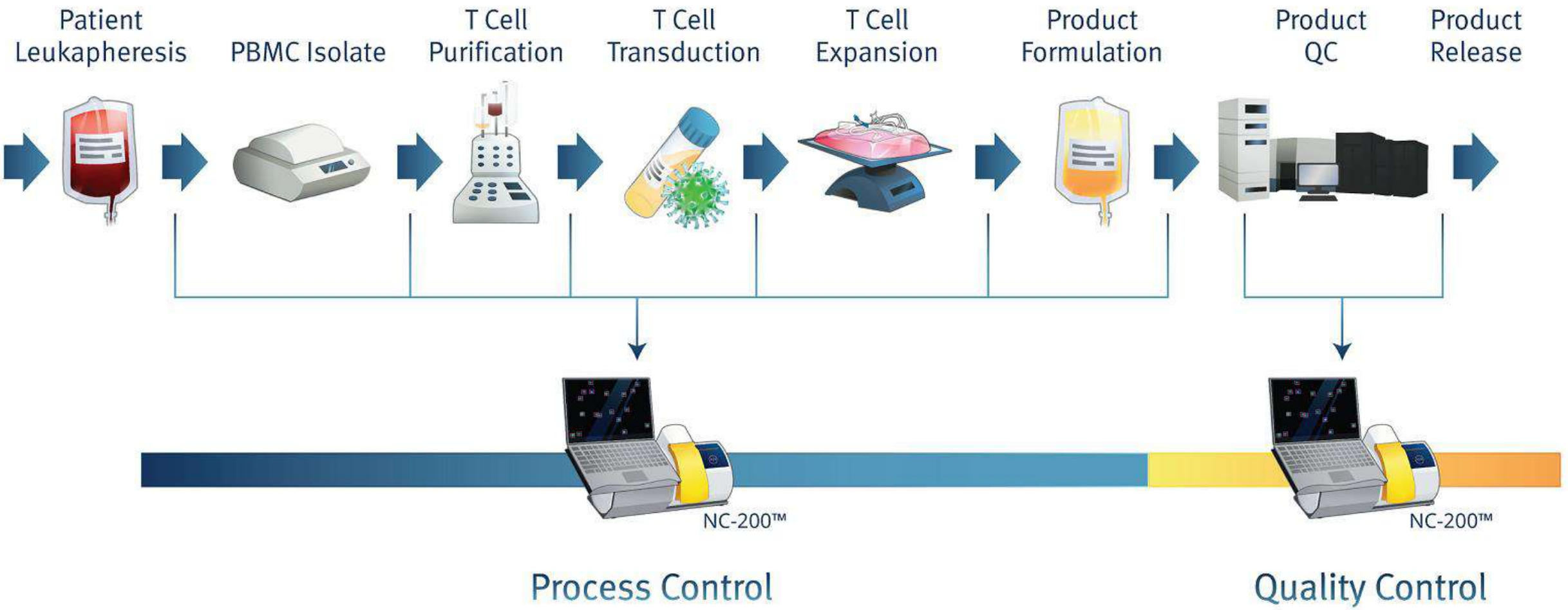


EXPANSION AND PERSISTENCE MAY NOT BE PREDICTIVE OF OVERALL SURVIVAL; THE NUMBER OF CAR T CELLS INFILTRATING LYMPHOMA MASSES COULD BE A MORE IMPORTANT INDICATOR[2]

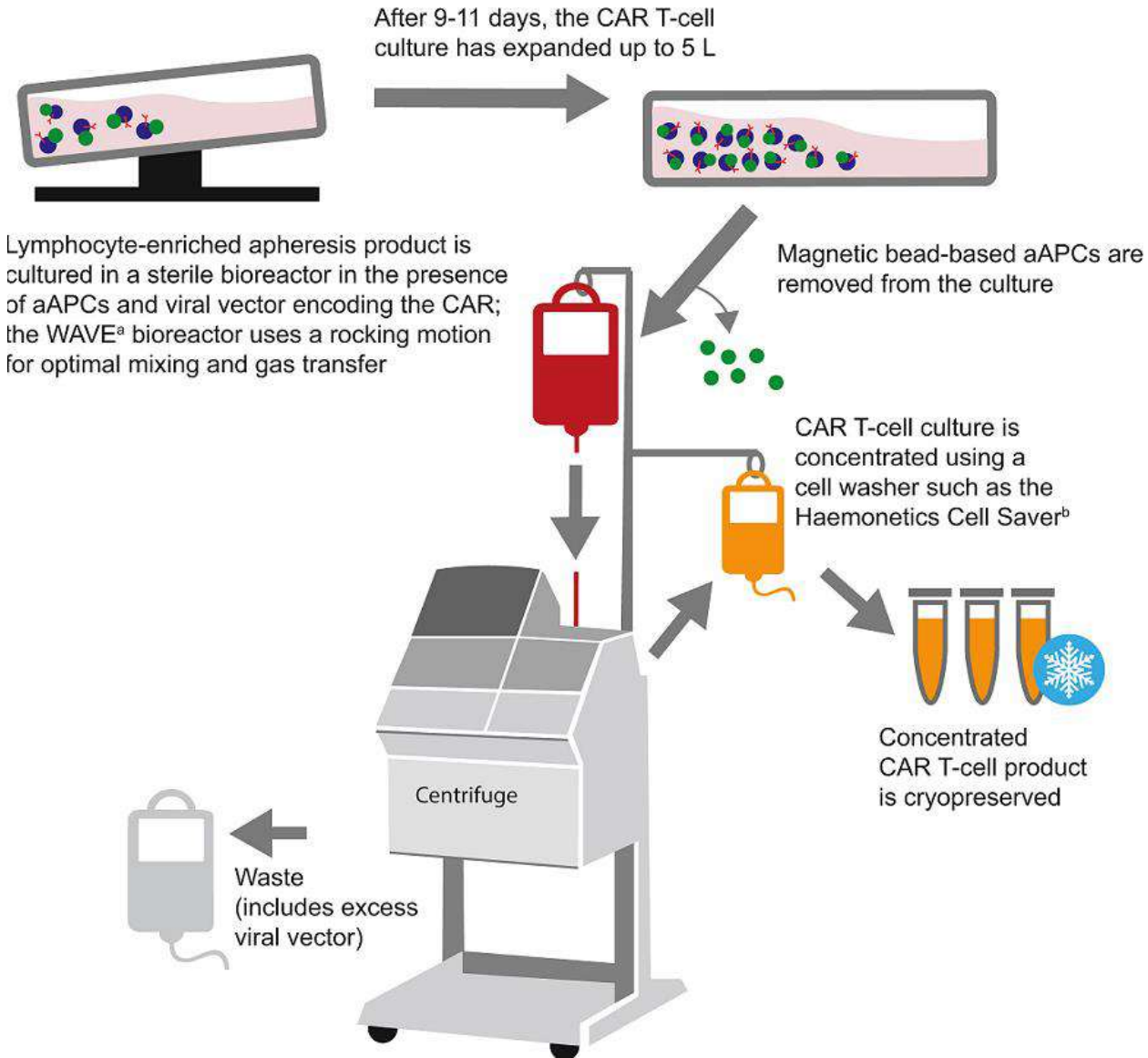
** p<0.01; *** p<0.001. HuSGM3 mice with high leukaemia burden were infused with nHuSGM3 T cells transduced with either a CD44v6.28z CAR (n=10 from two independent experiments) or a CD44v6.BBz CAR (n=10)
CRS: cytokine release syndrome; OS: overall survival

1. Norelli M, *et al. Nat Med* 2018; 24:739–748.
2. Kochenderfer JN, *et al. J Clin Oncol* 2015; 33:540–549.

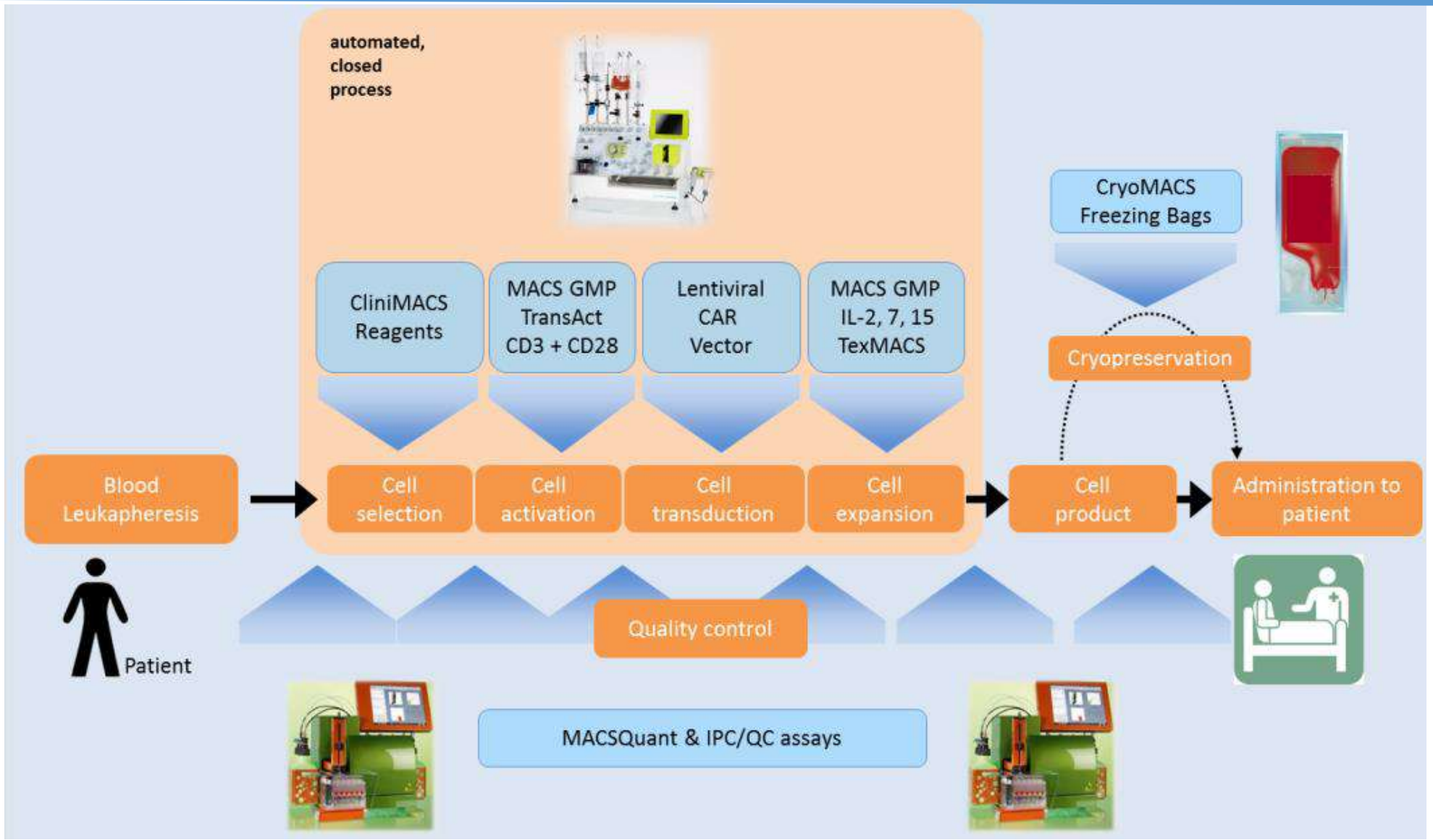
Circuit de production long et complexe



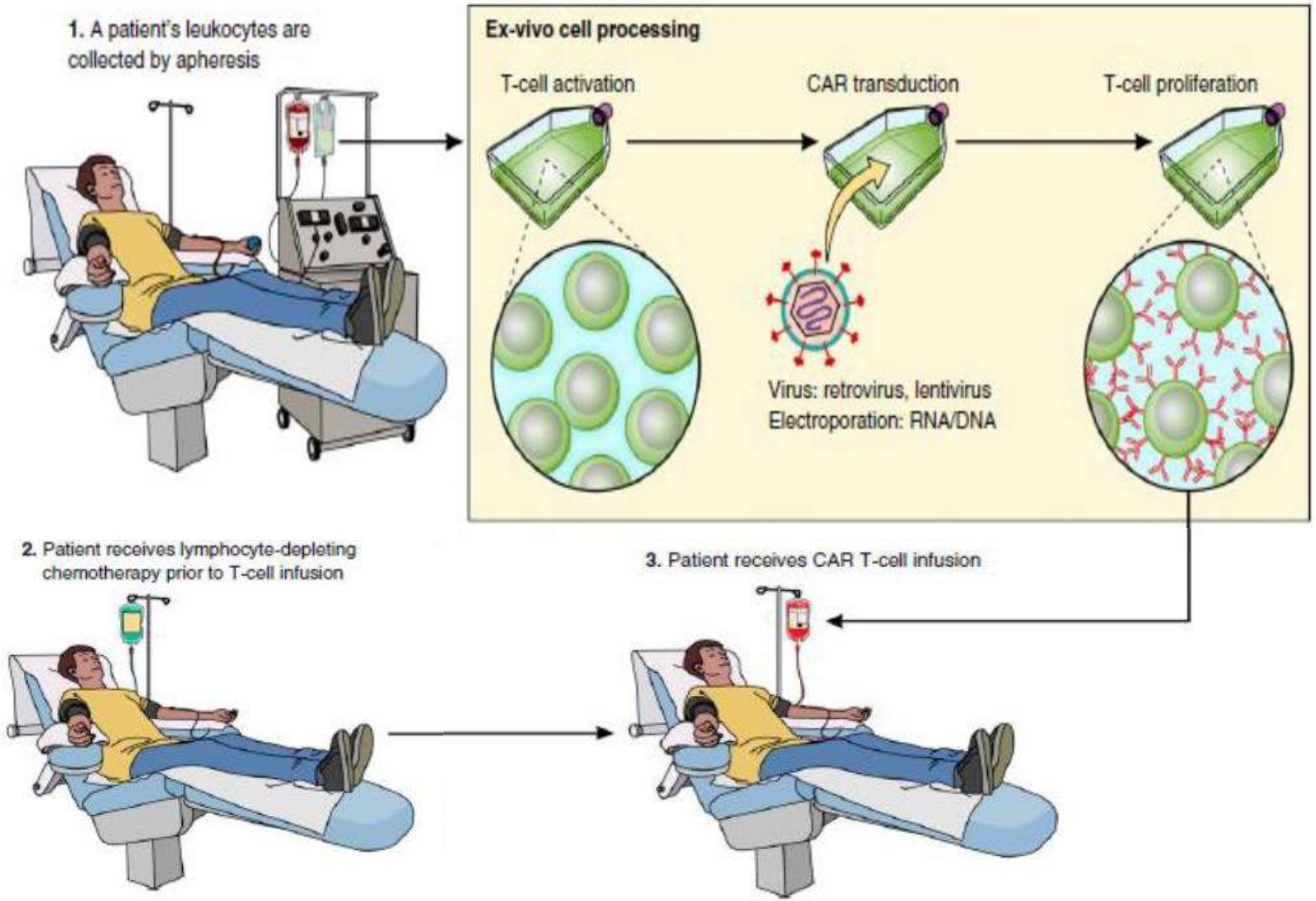
Exemple de production en bioréacteur de CAR-T cells



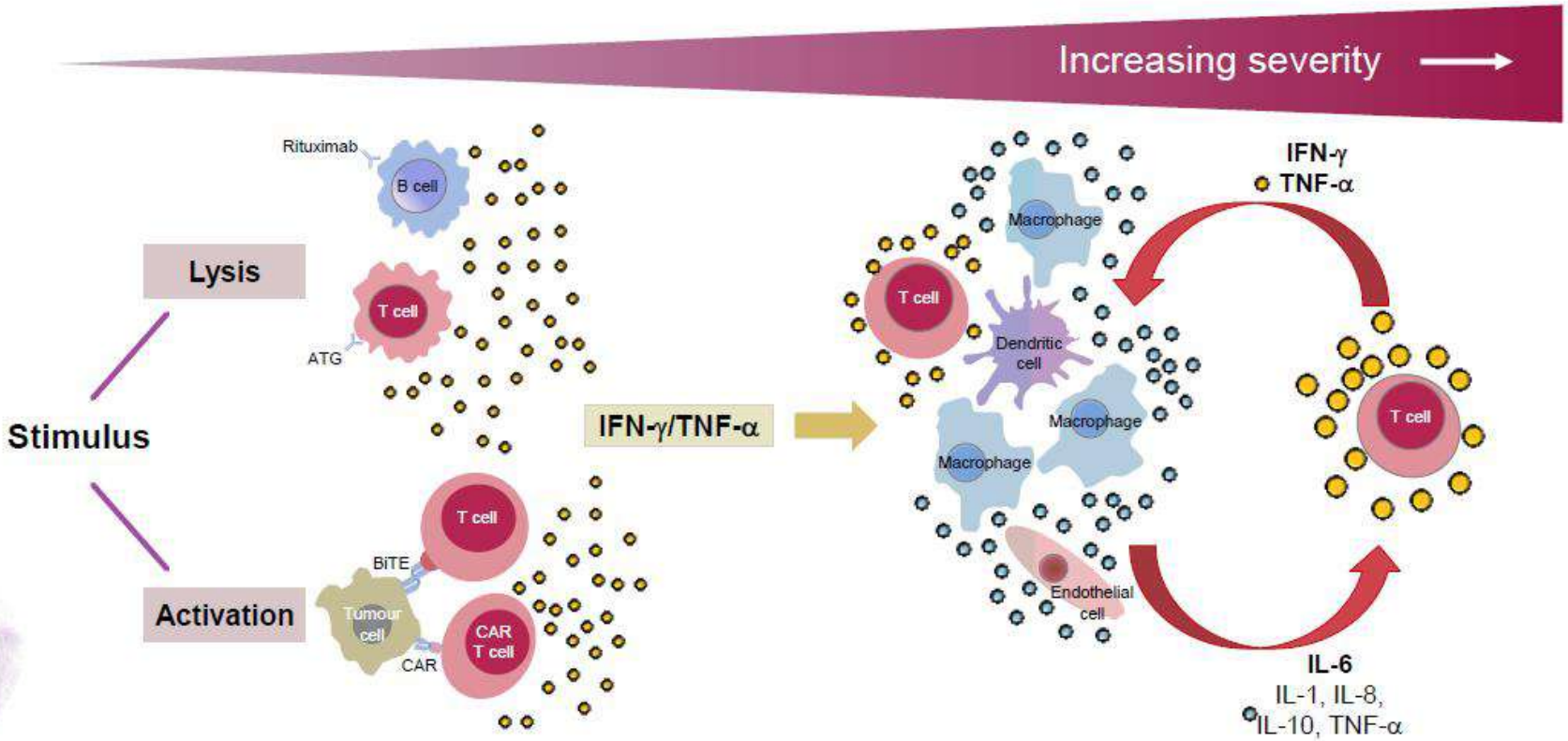
Exemple de production automatisée de CAR-T cells



Parcours patient « CAR-T »

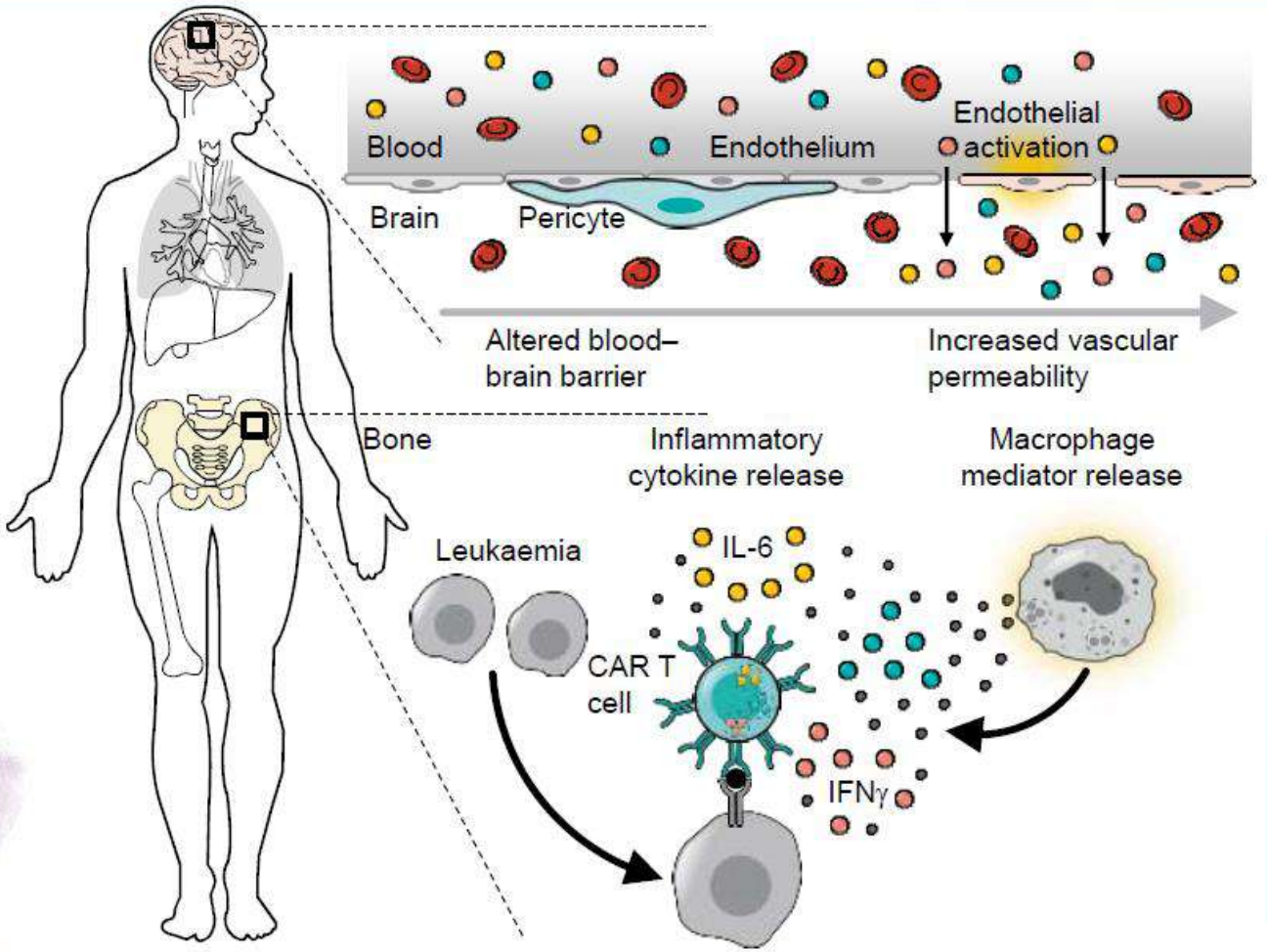


Complication des immunothérapies induisant la lyse ou l'activation rapide et massive de lymphocytes: le syndrome de relargage cytokinique (CRS)



Shimabukuro-Vornhagen A, *et al. J Immunother Cancer* 2018; 6:56.

Principales complications des CAR-T cells: CRS et neurotoxicité



NEUROLOGICAL EVENTS

- Headache
- Confusion
- Hallucinations
- Delirium
- Aphasia
- Paresis
- Seizures
- Cerebral oedema
- Intracranial haemorrhage

CYTOKINE RELEASE SYNDROME

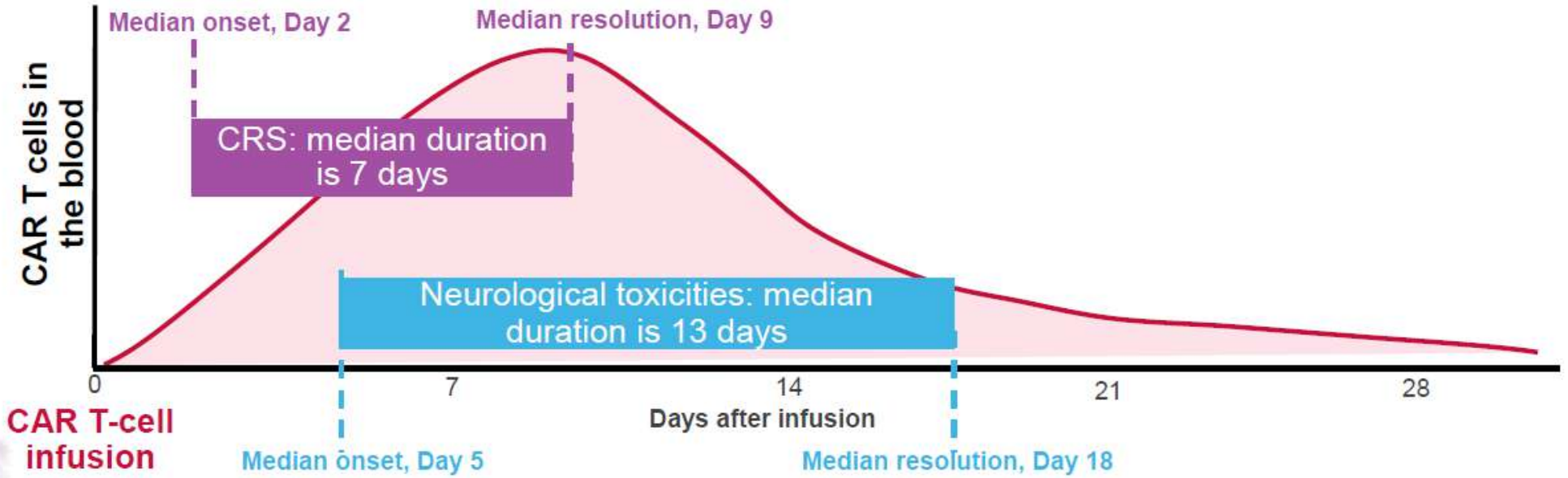
- Pyrexia
- Hypotension
- Arrhythmia
- Capillary leak syndrome
- Coagulopathy
- HLH/MAS

HLH/MAS: haemophagocytic lymphohistiocytosis/macrophage-activation syndrome

Figure adapted from June CH, *et al. Science* 2018; 359:1361–1365.

Cinétique d'apparition du CRS et de la neurotoxicité après administartion des CAR-T cells: dans les 15 jours, surveillance en soins intensifs +/- passage en réanimation

CRS coincides with maximal T-cell expansion[1]
CRS may occur within minutes but more typically within days[1,2]

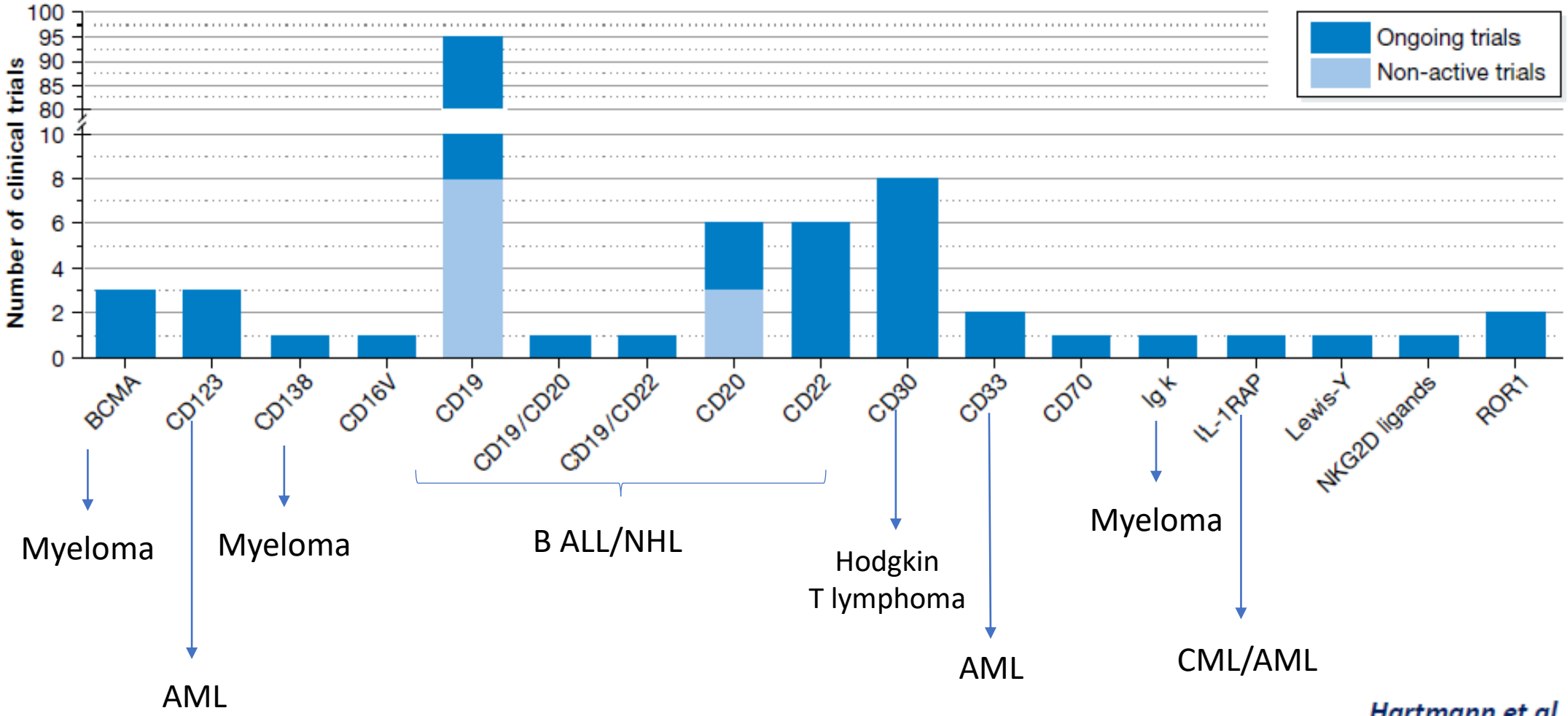


Neurological toxicities are generally reversible in most patients; rare cases of long-term symptoms[1]

CRS: cytokine release syndrome

1. Lee DW, *et al. Blood* 2014;124:188–195. 2. Axicabtagene ciloleucel SmPC (Jan 2019; available at www.ema.europa.eu).

Autres antigènes ciblés par les CAR-T en hématologie



Hartmann et al., EMBO 2017

CAR-T: efficacité à long terme, risque d'échappement ?

Tumor Antigen Escape from CAR T-cell Therapy

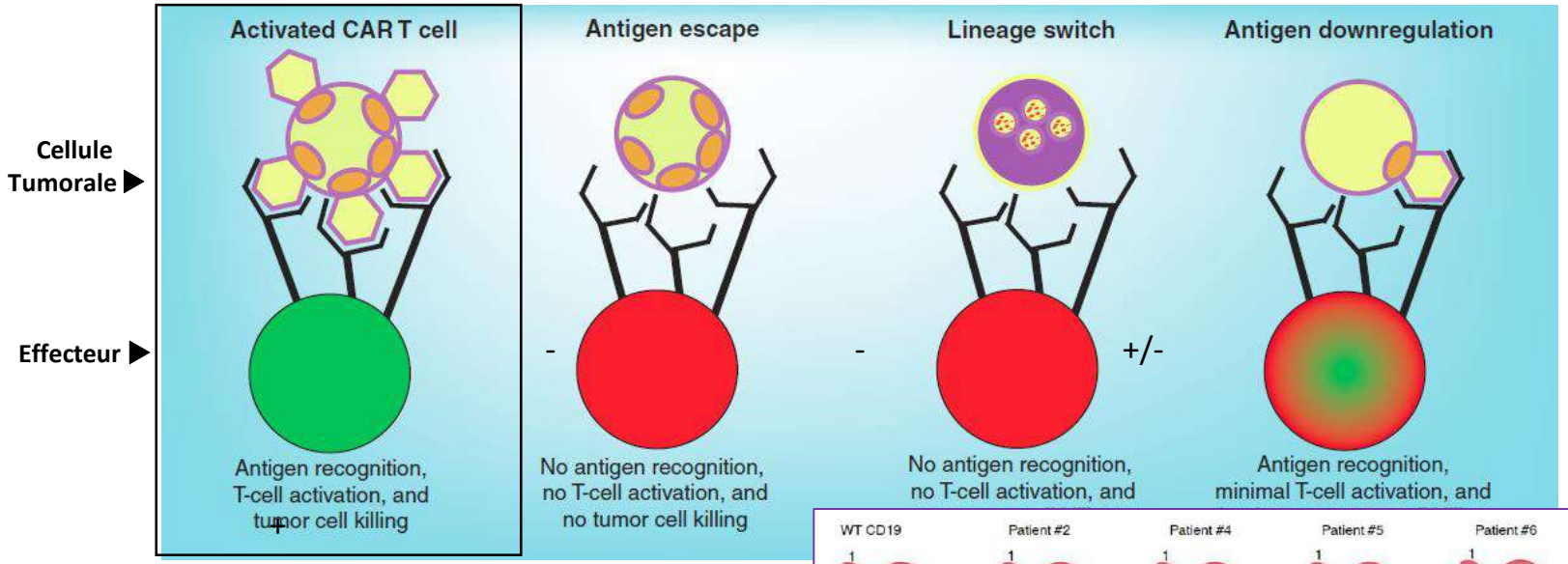
Robbie G. Majzner[1] and Crystal L. Mackall[1,2,3]

Cancer Discov; 8(10); 1–8. ©2018 AACR.

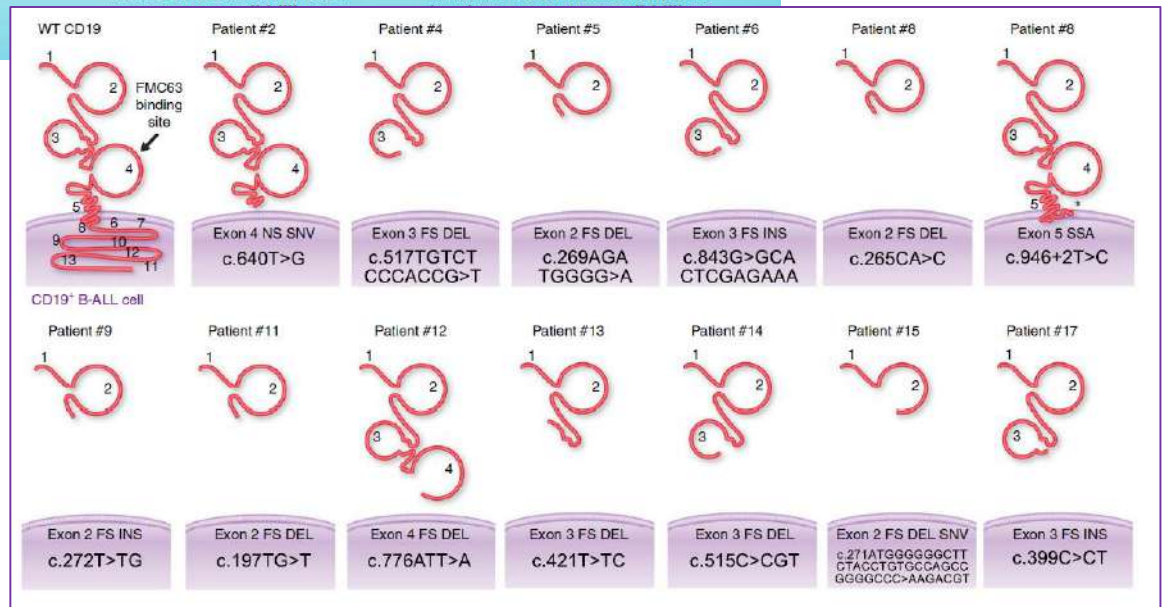
Table 1. A summary of antigen escape in CD19 CAR trials for ALL

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)

CAR-T: mécanismes d'échappement



Perte d'expression de l'épitope de CD19 reconnu par le CAR-T

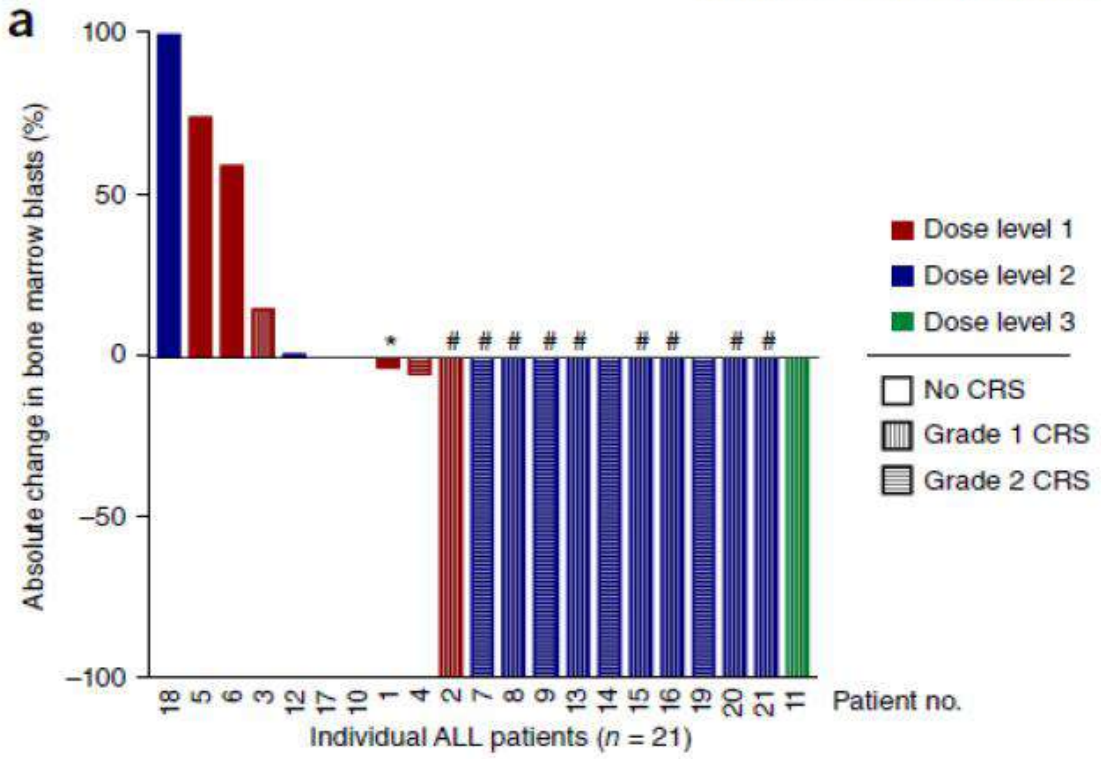


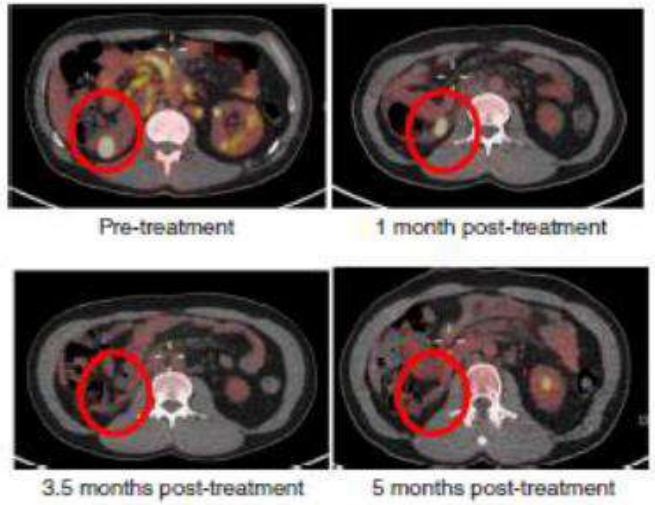
Orlando et al, Nat Med 2018

CAR-T anti-CD22 ou Dual/tandem CAR-T

Phase I évaluant un CAR CD22 chez 21 patients (adultes & enfants)
dont 17 précédemment traités avec une immunothérapie CD19 (15 CAR CD19)

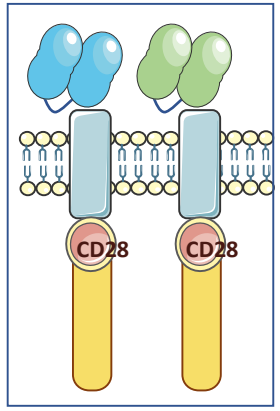
CR : 12/21 patients (57%)
CRS : 16/21 patients (76%)
Rechute : 8/21 patients (38%)



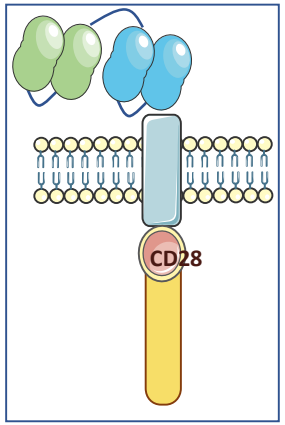


Fry et al., Nat Med 2017

Dual-CAR

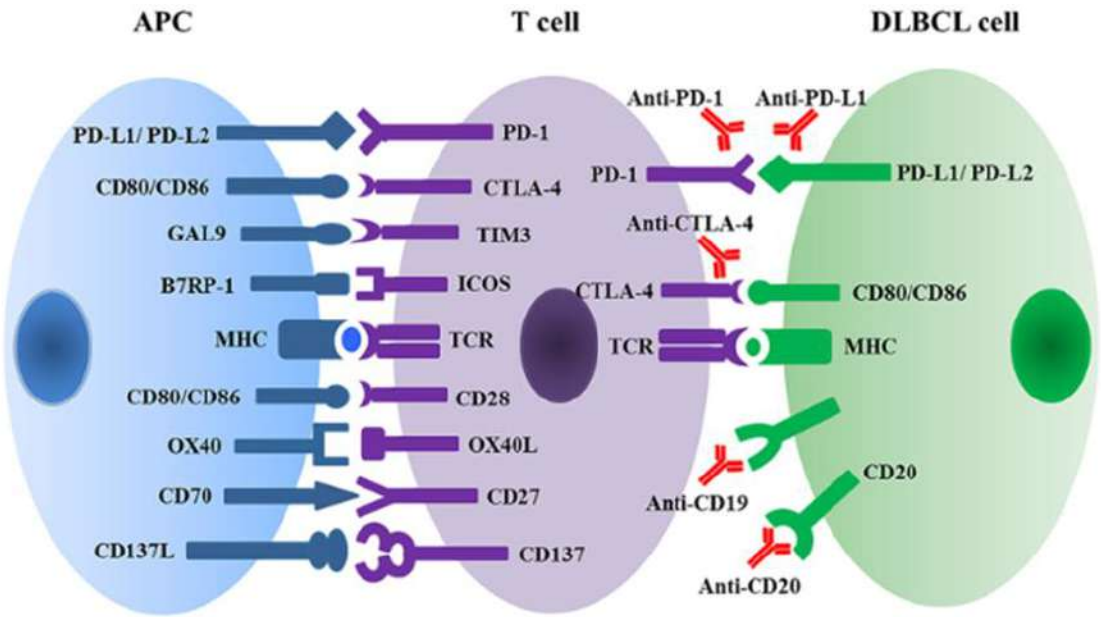


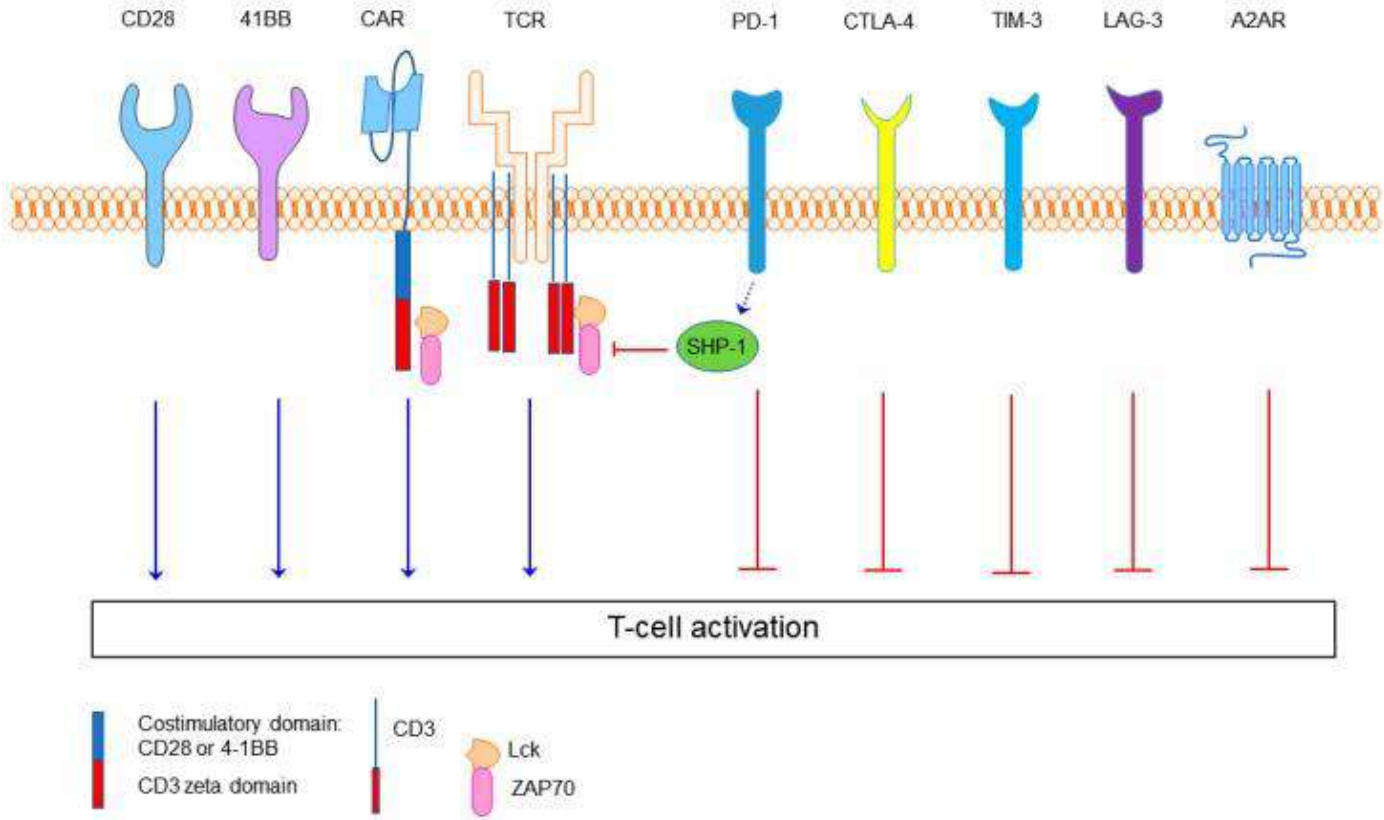
CAR en tandem



CD19/CD22
ou
CD19/CD20

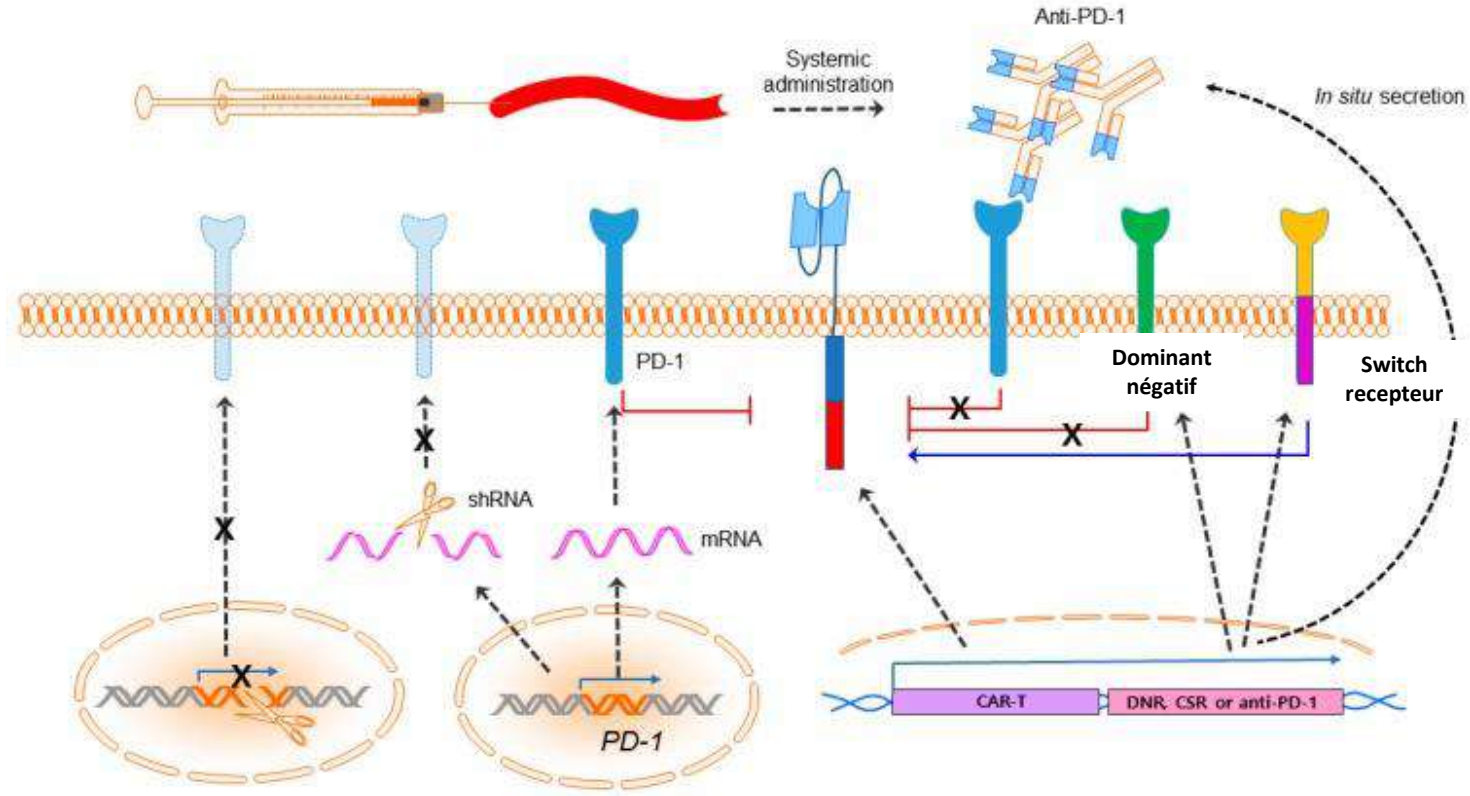
Role de l'exhaustion dans l'inactivation des CAR-T





Zhang; Frontiers in Oncology 2018

Approches permettant de by passer l'exhaustion des CAR-T



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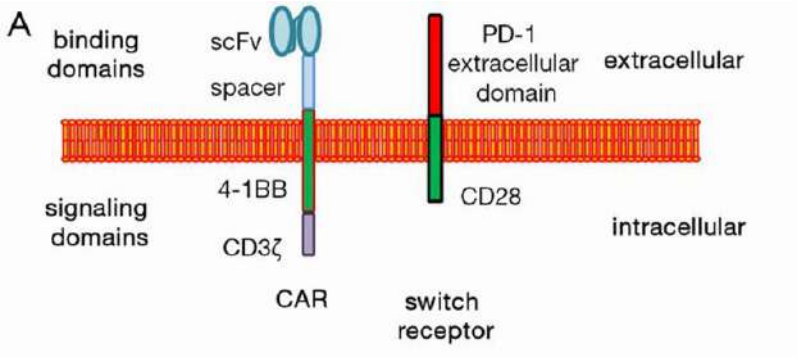
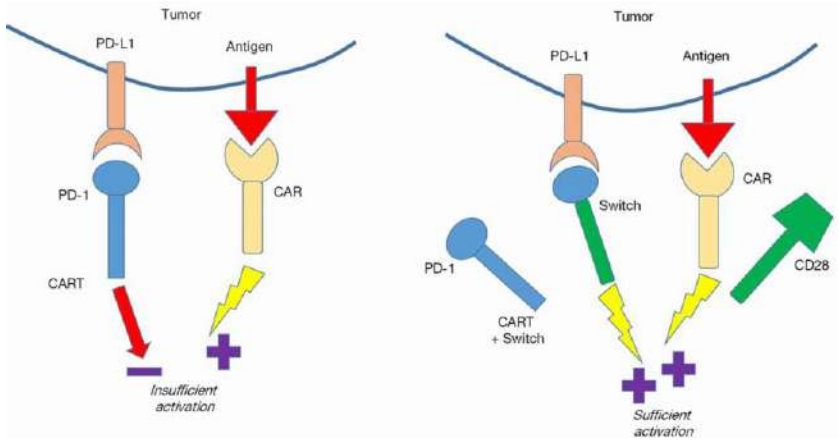




Yoon; Int J Mol Sci 2018

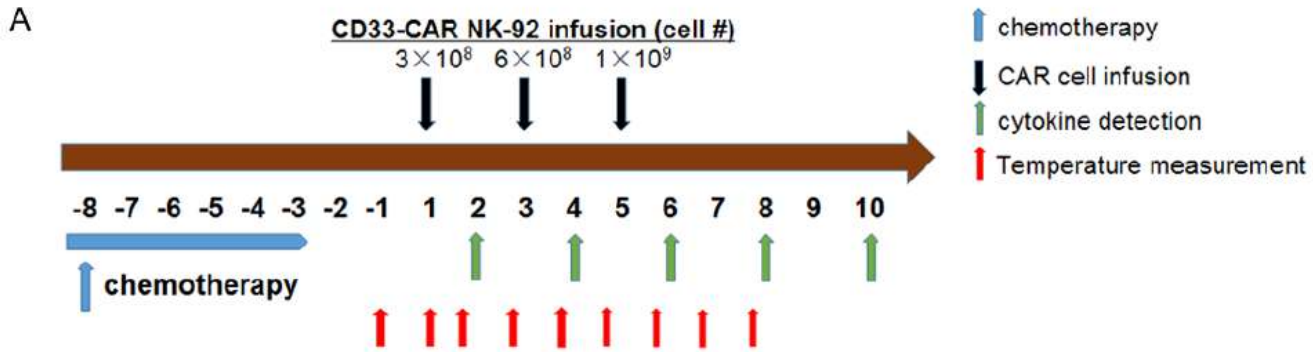
Rafik S; Nature biot 2018

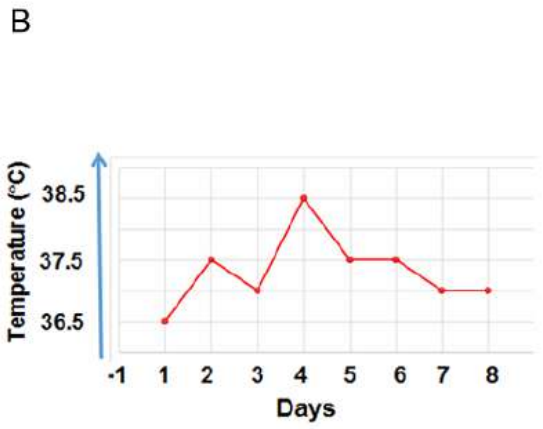
Etudes cliniques associant une approche anti-PD1 et CAR-T cells

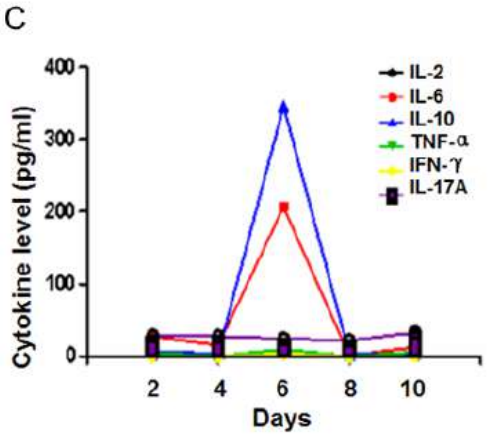
Open clinical trials exploring the role of immune-checkpoint blockade with CAR-T cell therapy.

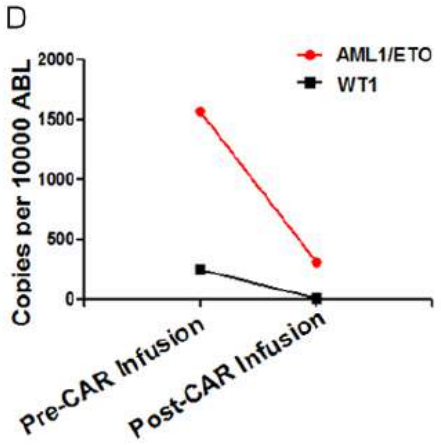
Clinical Trials.gov Identifier (ref.)	CAR-T (Trial Nickname)	Blockade of PD-1 Axis	Target Disease	Sponsor
NCT02706405	JCAR014	Durvalumab; on D28 + up to 10 doses, every 4 weeks or on D-1 and -28 (and additional dose allowed)	DLBCL, double-hit lymphoma, PMBL, tDLBCL	Fred Hutchinson Cancer Research Center
NCT03310619	JCAR017 (PLATFORM)	Durvalumab; Dose/schedule: NA	Aggressive B-NHL	Celgene
NCT02926833 [48]	KTE-C19 (ZUMA-6)	Atezolizumab; 4 doses every 3 weeks from D1, D14 or D21 in each cohort	DLBCL	Kite pharma
NCT03287817	CD19/22 CAR-T (ALEXANDER)	Pembrolizumab as consolidation Dose/schedule: NA	DLBCL	Autolus limited
NCT02650999 *	CTL019	Pembrolizumab; every 3 weeks up to 18 doses	$CD19^+$ DLBCL, FL, MCL	University of Pennsylvania
NCT03298828	CD19-specific CAR-T; PD-1 knockout		$CD19^+$ B-cell leukemia or lymphoma	Third Military Medical University
NCT03208556	CD19-specific CAR-T; PD-1 shRNA		$CD19^+$ B-cell lymphoma	Peking University

Autres source de cellules que les T: les cellules NK









Original Article

First-in-man clinical trial of CAR NK-92 cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia

Xiaowen Tang[2,3*], Lin Yang[3,4,5*], Zheng Li[1,2,3], Ansel P Nalin[6], Haiping Dai[1,2,3], Ting Xu[1,2,3], Jia Yin[1,2,3], Fengtao You[3,4,5], Mingqing Zhu[1,2,3], Wenhong Shen[1,2,3], Guanghua Chen[1,2,3], Xiaming Zhu[1,2,3], Depei Wu[1,2,3], Jianhua Yu[6,7,8]

2 RC/3 patients

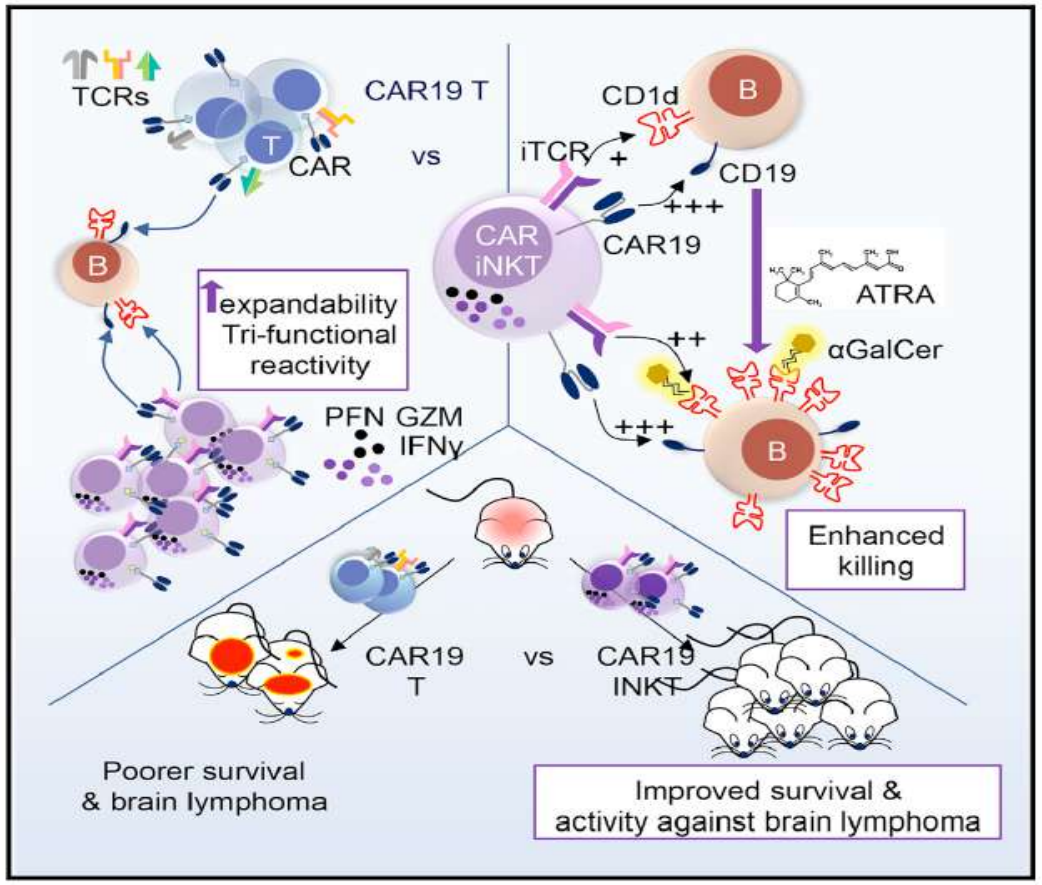
Rechute précoce chez les
2 répondeurs

Am J Cancer Res 2018;8(6):1083-1089
www.ajcr.us /ISSN:2156-6976/ajcr0078782

Autres source de cellules que les T: les cellules iNKT

Enhanced Anti-lymphoma Activity of CAR19-iNKT Cells Underpinned by Dual CD19 and CD1d Targeting

Graphical Abstract



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In Brief

Rotolo et al. show that anti-CD19 chimeric antigen receptor (CAR19)-engineered CD1d-restricted invariant NKT cells (iNKT) are more effective than CAR19-T cells against CD1d-expressing lymphomas, including those in the brain. De-repression of CD1d expression further enhances the anti-tumor efficacy of CAR19-iNKT.

CAR allogéniques

2015 : **Première guérison d'une leucémie**

Cellectis : LT CAR multi-résistants et utilisables dans un contexte allogénique / utilisation des système d'édition du génome (TALEN/CRISPR)

- **Extinction des chaines αβ du TCR** → donneur sain allogénique
- **Extinction de l'enzyme dCK** → Résistance des cellules à la lymphodéplétion via Clofarabine, Fludarabine, Cytarabine
- **Expression d'un CAR CD19**
- **Gène suicide** → élimination des cellules en cas de problèmes

Fillette de 11 mois atteinte d'une LAL réfractaire et en rechute

« *Sa leucémie était tellement agressive qu'une telle réponse est presque un miracle* » Dr Paul Veys, directeur de l'unité de transplantation de moelle osseuse du Great Ormond Street Hospital (GOSH) à Londres

CAR universels: uCAR

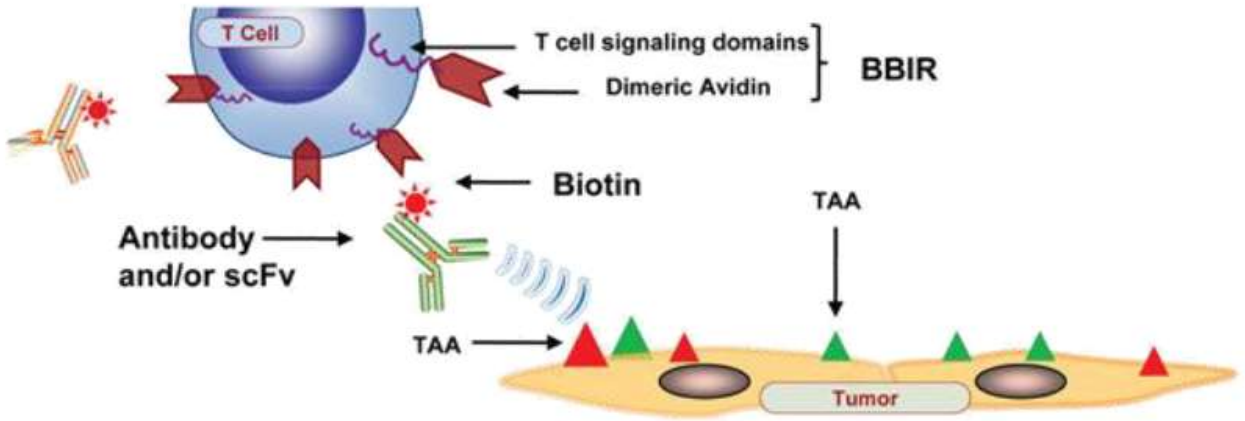
scFv **anti-FITC ou anti biotin** fusionné à une **domaine de transduction du signal**

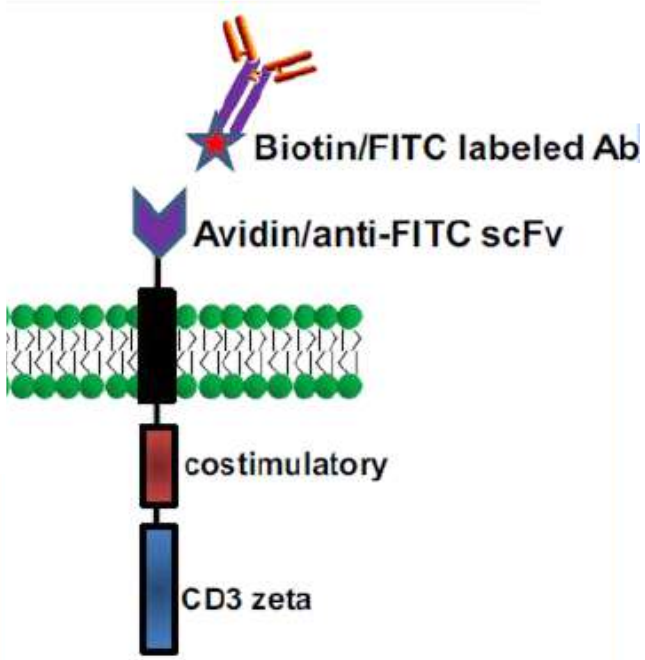
A combiner avec des **anticorps monoclonaux conjugués**

Reconnaissance du ligand (FITC ou biotin) entraine :

- **Lyse des cibles**
- **Prolifération des LT**
- **Production de cytokines**

Système **flexible**





Tamada et al., 2012 ; Urbanska et al., 2012 ; Shi et al, 2014

Conclusions

- Les CAR-T cells = allogreffe moderne avec moins de toxicité

- Les CAR-T utilisés en clinique actuellement sont des CAR de 2nd génération et ciblant un seul antigène tumoral: risque d'échappement immunitaire, de sélection de clones n'exprimant pas/plus l'antigène

- De nombreuses améliorations sont attendues de manière rapide:
  - ✓ CAR-T de 3eme génération
  - ✓ CAR TRUCK: pour favoriser l'action d'autres cellules de l'immunité anti-tumorale

- Différentes approches en cours de développement pour contre-carrer les mécanismes d'échappement:
  - Dual CAR (PHRC Nicolas Boissel avec dual CAR anti-CD19 et CD22 dans les LAL pédiatriques et AJA)
  - CAR et approches anti-PD1

- Intérêt d'utiliser des cellules à capacités cytotoxiques plus importantes que les lymphocytes T ?
  - ✓ CAR-NK
  - ✓ CAR-iNKT

- Intérêt de développer les CAR « universels »: banque de CAR prêts à l'emploi
  - ✓ CAR allogéniques
  - ✓ CAR anti-FITC ou biotin
  - ✓ CAR-CTL anti-viral