

Epigenetic and signaling-based engineering enhances CAR-T cell function in primary central nervous system lymphoma

Dr Marion ALCANTARA, MD, PhD
Medical Director of CellAction (Cell therapy Acceleration and Innovation)
Hematology Unit
Institut Curie, Saint-Cloud, France

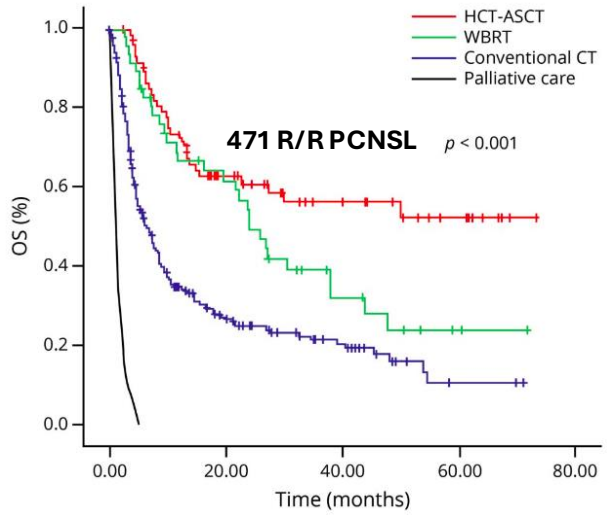
June 11, 2025

PCNSL: a brain lymphoma with poor prognosis

- Primary central nervous system lymphoma
- B-cell derived cancer
  - Large B-cell lymphoma
- Exclusive CNS involvement (brain, meninges, eye, spinal cord)
- Pathophysiology not fully understood
  - Recognition of CNS proteins?
- 300 new cases/year in France
- Median age: 67 years
- Poor prognosis



Symptoms at diagnosis	% of patients
Cognitive/behavioral disorders	57
Gait disorders	56
Motor/sensory deficit	41
Headache/intracranial hypertension	31
Blurred vision, floaters, decreased visual acuity	10
Epilepsy	9



- French national network
- 2011 Cancer Plan "rare cancer"
- Coord. Dr Soussain (Curie), Prof Hoang-Xuan (La Pitié)
- National database
- Recommendations
- Clinical trials and translational research
- Biobanking

LOC

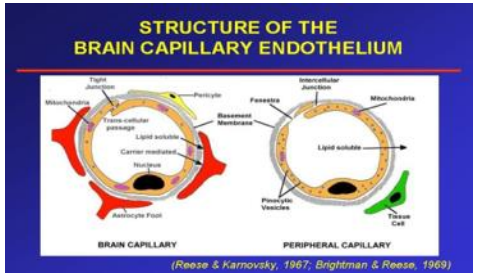
Montesinos et al, J Immunol 2015
Houillier et al, Neurology 2020

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Therapeutic challenges



Toxicity



Efficacy

Tumor cells

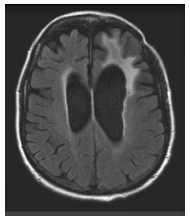
- Poor prognosis subgroups
- Heterogeneous

Microenvironment

- Immunosuppressive
- Heterogeneous

Blood-brain barrier

- Treatment accessibility

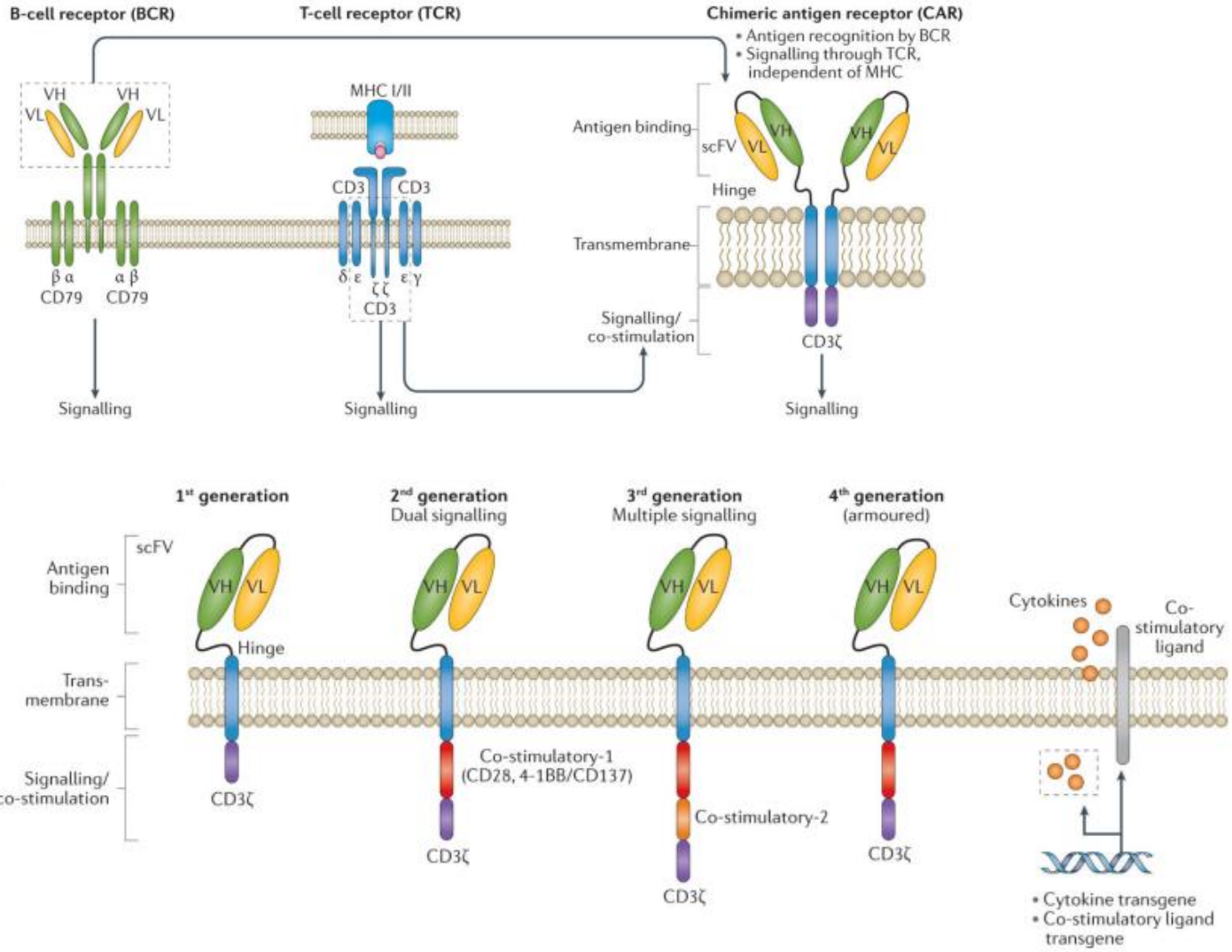


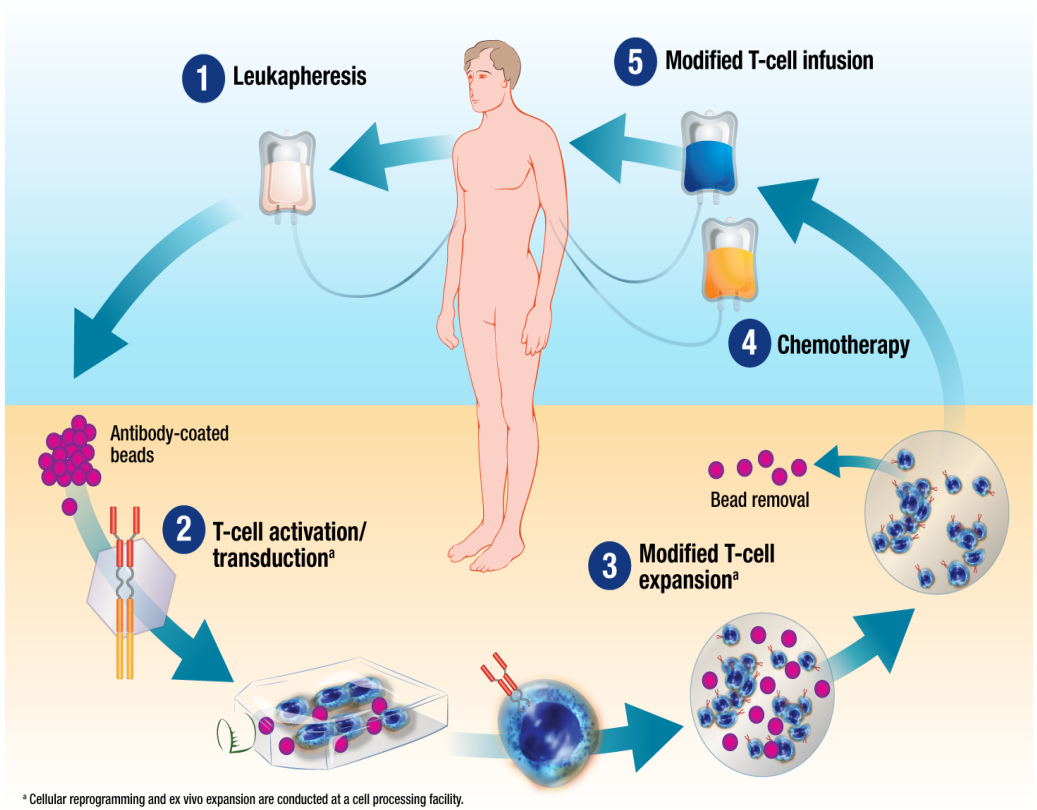
Irreversible cognitive disorders

- Radiotherapy: promoting factors?
- Chemotherapy?
- Immunotherapy?

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Autologous chimeric antigen receptor (CAR)-T cells

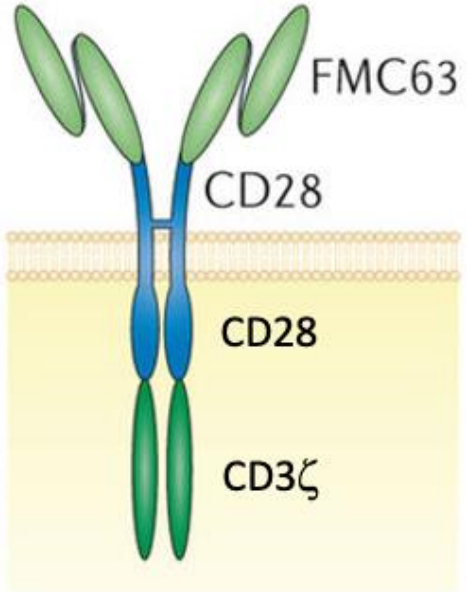
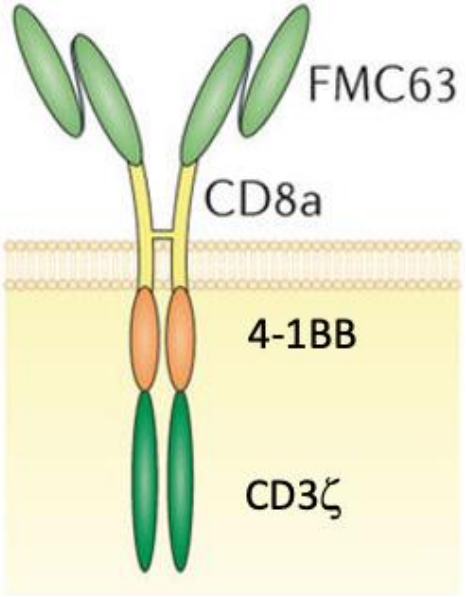
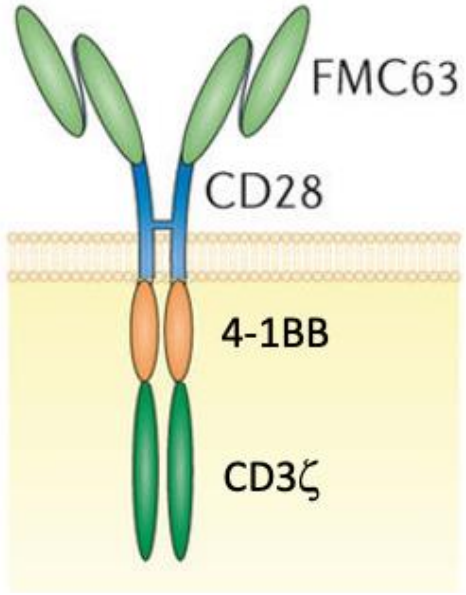




Batlevi et al, Nat Rev Clin Oncol. 2016

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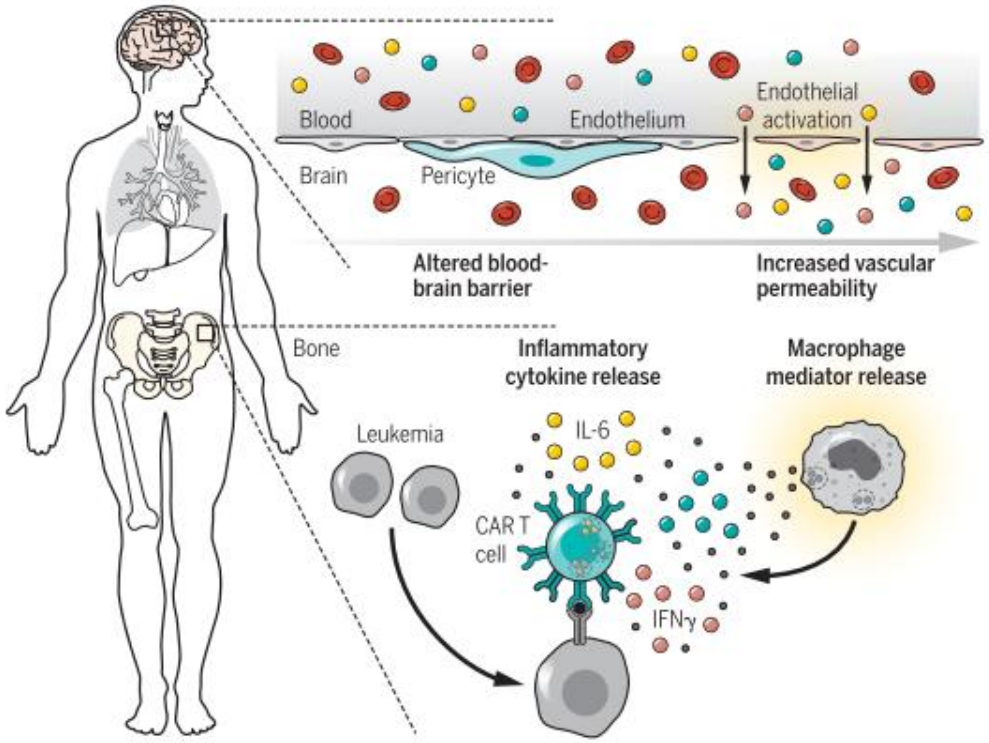
CD19 CAR-T cell constructs

	Axicabtagene ciloleucel (Axi-cel)	Tisagenlecleucel (Tisa-cel)	Lisocabtagene maraleucel (Liso-cel)
CD19 Ab			
Hinge			
Transmembrane			
Signal 2			
Signal 1			
Gene transfer	Retrovirus	Lentivirus	Lentivirus

Adapted from Van Der Steegen et al, Nat Rev Drug Discov 2015

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CAR-T toxicity: CRS & ICANS

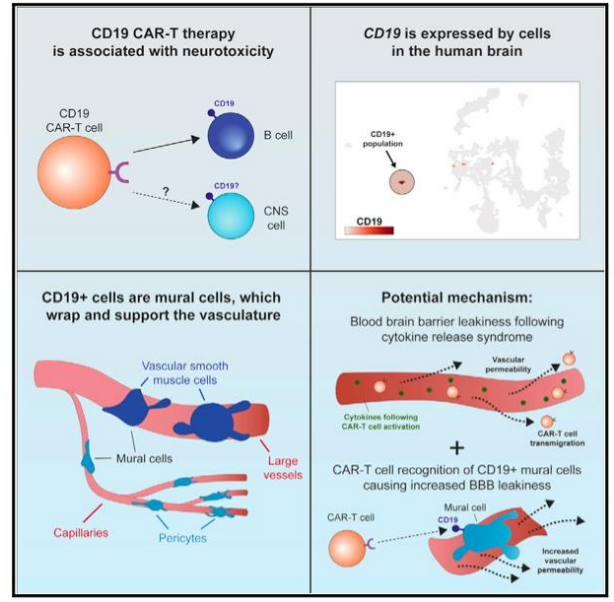


Neurotoxicity: ICANS

Immune effector cell-associated neurotoxicity syndrome

Impaired handwriting
Confusion
Cognitive impairment
Aphasia
Motor deficit
Seizures
Cerebral edema

On-target, off-tumor?



Cytokine release syndrome

Fever
Hypotension
Tachycardia
Capillary leak syndrome
Respiratory failure
Organ dysfunction

Treatment

- Symptomatic
- Tocilizumab (anti-IL6R)
- Anakinra (anti-IL1)
- Corticosteroids

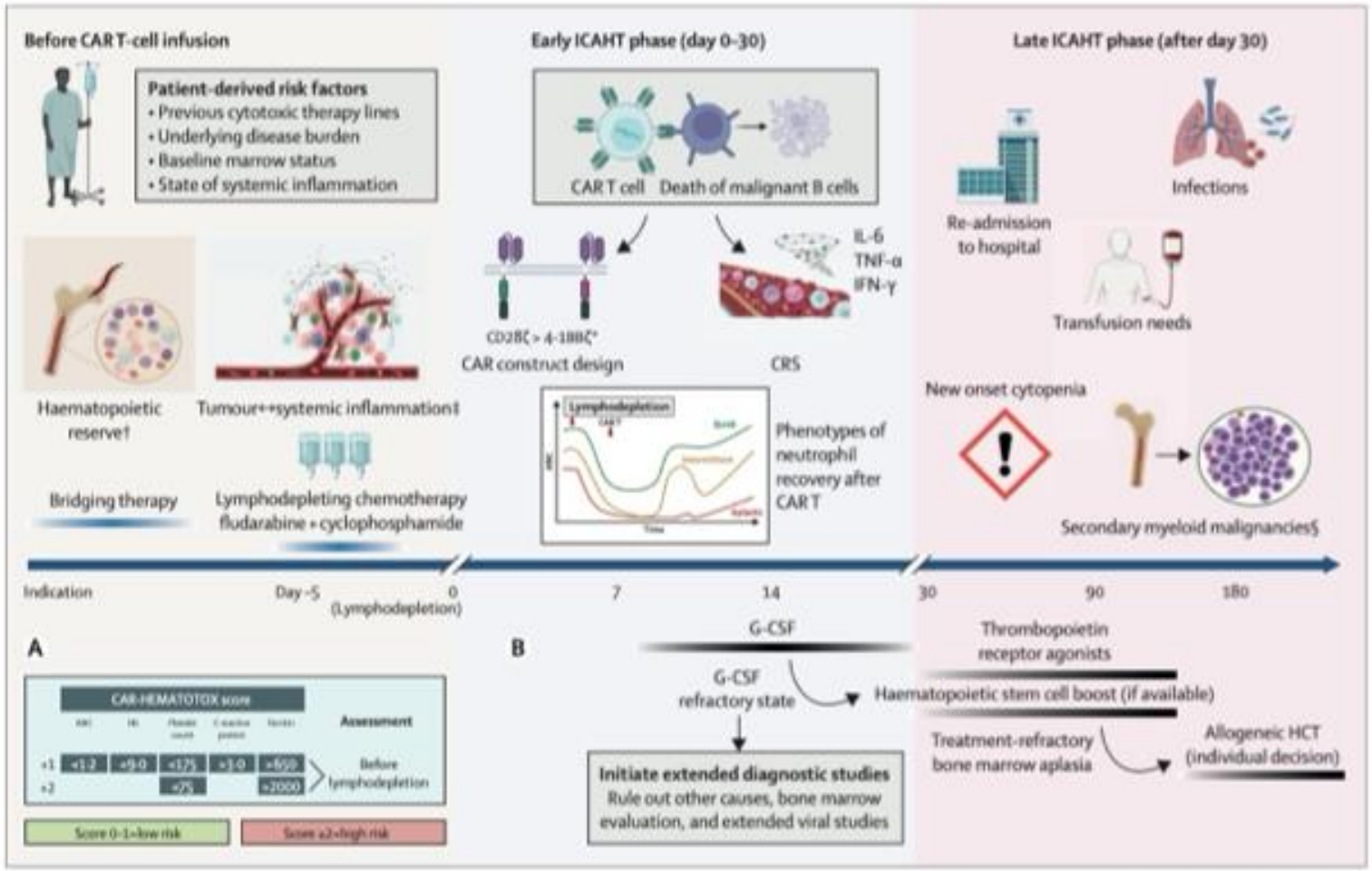
June et al, Science 2018
Parker et al, Cell 2020

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CAR-T toxicity: ICATH

Immune effector cell-associated hematotoxicity

	Grade 1	Grade 2	Grade 3	Grade 4
Common terminology criteria for adverse events				
Neutropenia (ANC/μL)	<LLN-1500	<1500–1000	<1000–500	<500
Anaemia (Hb g/dL)	<LLN-10·0	<10·0–8·0	<8·0; transfusion	Life-threatening intervention
Thrombocytopenia (platelet count G/L)	<LLN-75	<75–50	<50–25	<25
ICAHT grading				
Early; 0–30 days after CAR T-cell infusion (ANC/μL)	<500* for 1–6 days	<500 for 7–13 days	<500 for ≥14 days; <100† for ≥7 days‡	Never above 500; <100 for ≥14 days
Late; 30 or more days after CAR T-cell infusion (ANC/μL)	<1500	<1000	<500	<100



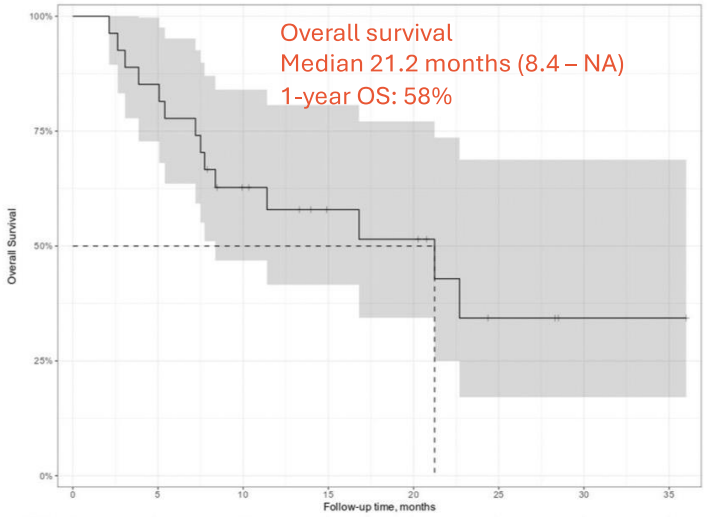
Rejeski et al, Lancet Haematol 2024

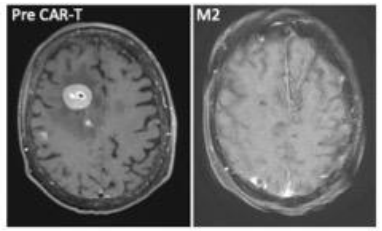
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CD19 CAR-T cells and PCNSL

Focus on the latest works of the LOC

- March 2020 – November 2022
- 25 patients infused with tisa-cel or axi-cel
- Median age 68 years
- Heavily pretreated patients





Response	CR	PR	PD
Best response	16 (64%)	4 (16%)	5 (20%)[a]
Best response in each CNS compartment			
Brain	12 (60%)	4 (20%)	4 (20%)
CSF	7 (87%)	0	1 (13%)
Eye	2 (67%)	0	1 (33%)

- High response rates
  - Similar to what is reported for systemic lymphomas
  - Responses observed in each CNS compartment
- Similar tolerance profile?
  - Ongoing study regarding the risk of prolonged neurotoxicity
- No commercial authorization
- **Need to improve long-term efficacy**

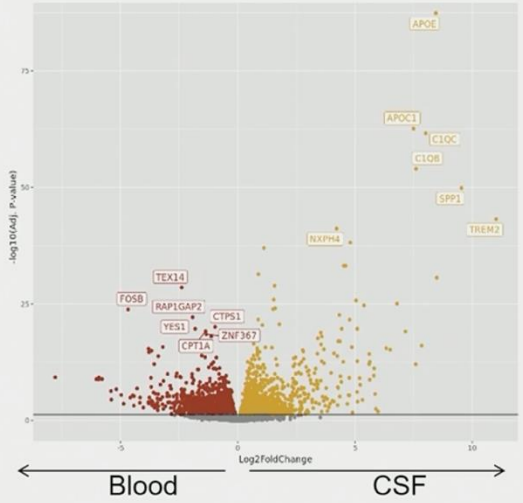
Alcantara et al, Blood 2021
Choquet et al, Am J Hematol. 2024

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Axi-cel: ongoing clinical trial

CD8 CSF CAR T cells show an enrichment for exhaustion associated genes

Differential Expression Blood vs CSF CAR-T cells at maximum expansion



Top 20 DE genes in CSF CAR-T cells include the T cell inhibitory genes:

- PD-1
- TIM-3
- BLIMP-1

DE analysis suggest a higher risk of CAR-T cell inhibition in the CSF

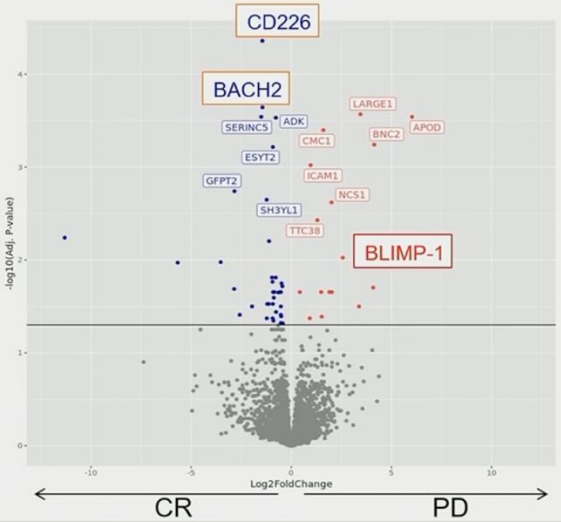
Nayak L. ASCO 2024
Dana Farber
N = 18 patients (13 PCNSL)

Upregulation of genes that counteract T cell inhibition in patients with CR

Differential Expression CSF CD8-CAR T cells
Complete Responders (CR) vs. Progressive Disease (PD)
at maximum expansion

Upregulated in durable CR:

- CD226
- BACH2



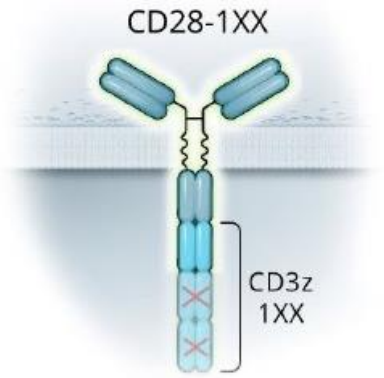
Upregulated in early PD:

- BLIMP-1

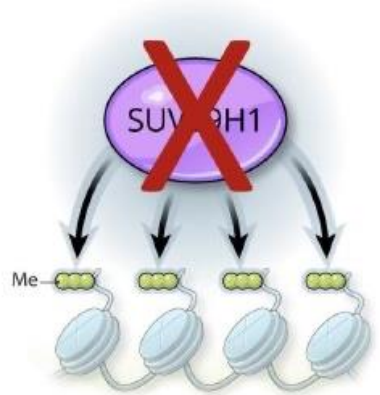
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Enhancing CAR-T cell function in PCNSL

Modification of the CAR design



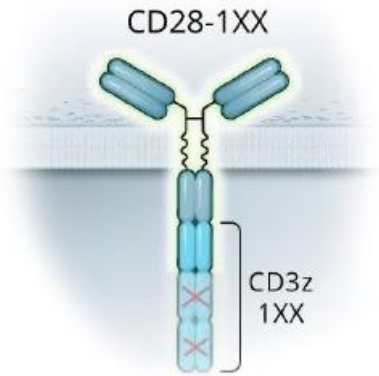
Epigenetic reprogramming



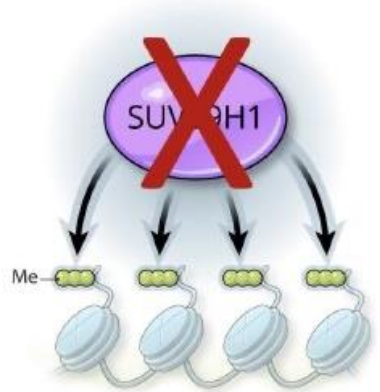
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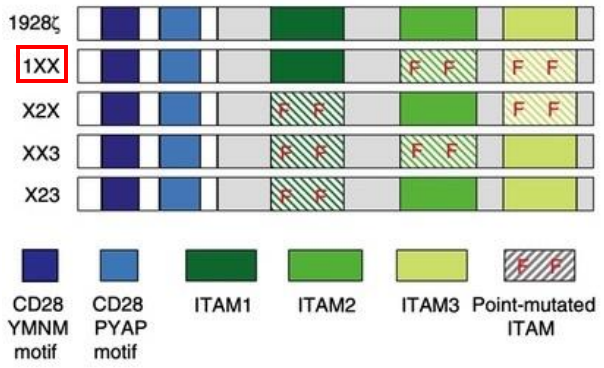
Epigenetic reprogramming

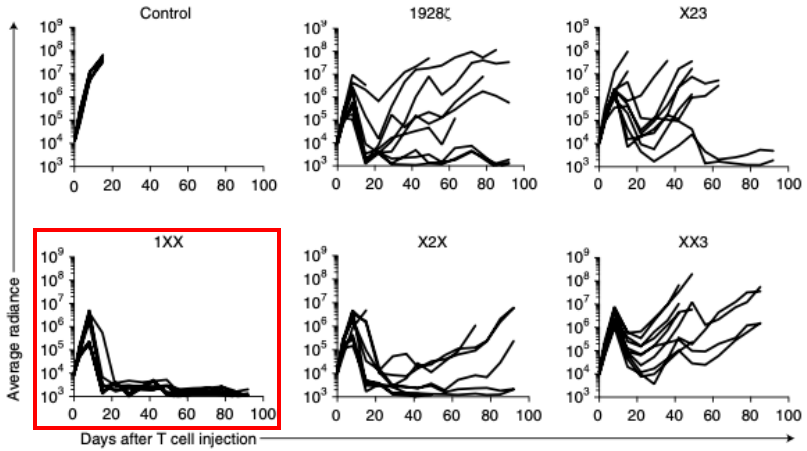


Calibrating CAR activation potency to increase persistence

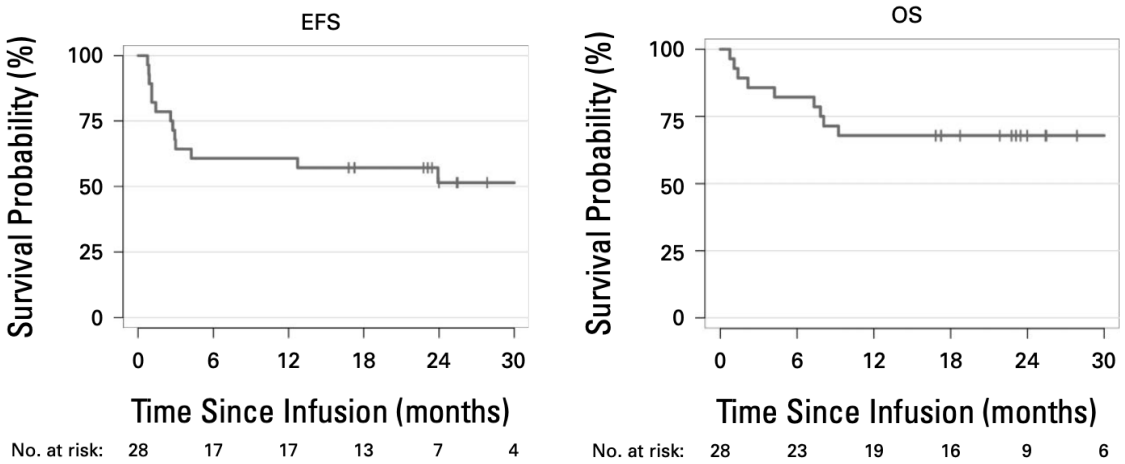
1XX CAR: sequential mutation of the intracellular ITAM domains of the CAR CD3ζ

The Sadelain laboratory hypothesized that the redundancy of CD28 and CD3ζ signaling in a CAR design incorporating all three CD3 ITAMs might foster counterproductive T cell differentiation and exhaustion and therefore created a new CAR construct with calibrated CAR activation potential by mutating 2 of the 3 ITAMs, termed 1XX





- **Balancing effector and memory programs**
- **Enhanced therapeutic efficacy**
  - Phase I dose-escalation study (MSKCC)
  - 28 patients (DLBCL, HGBCL, tFL)
  - RP2D **25x10^6 CAR-T**
  - ≥ Grade 3 CRS 4%, ICANS 7%
  - ORR 82%, CR 71%

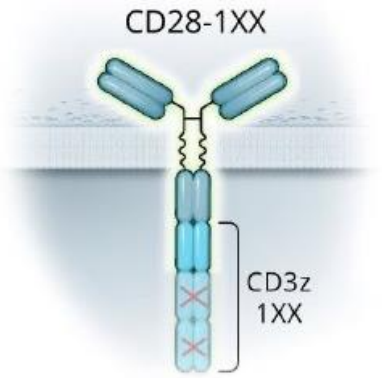


Feucht et al, Nature Med 2019
Park et al, JCO 2025

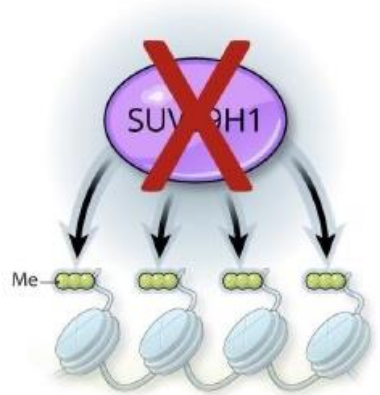
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Enhancing CAR-T cell function in PCNSL

Modification of the CAR design

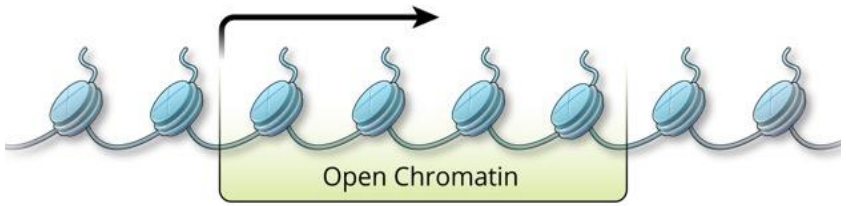


Epigenetic reprogramming

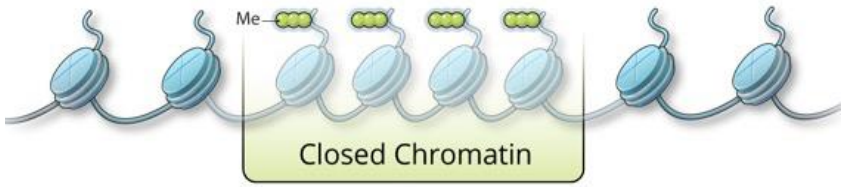


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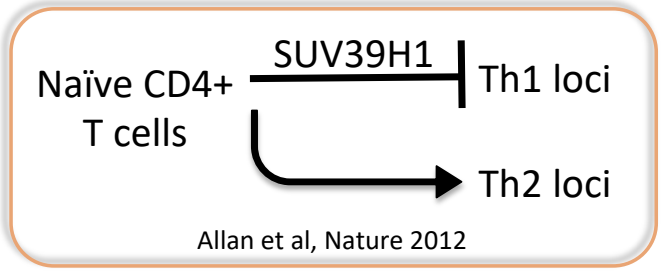
SUV39H1: a histone methyltransferase







Catalyzes the trimethylation of H3K9 leading to chromatin compaction, gene silencing, and lineage commitment

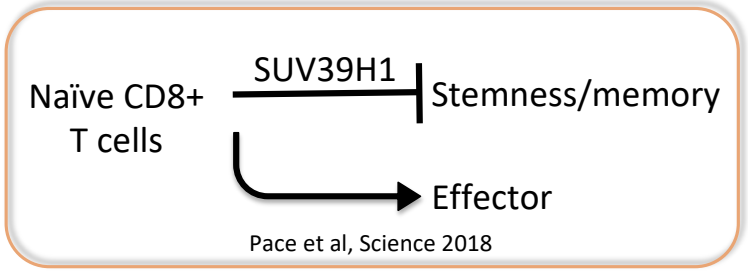


Allan et al, Nature 2012

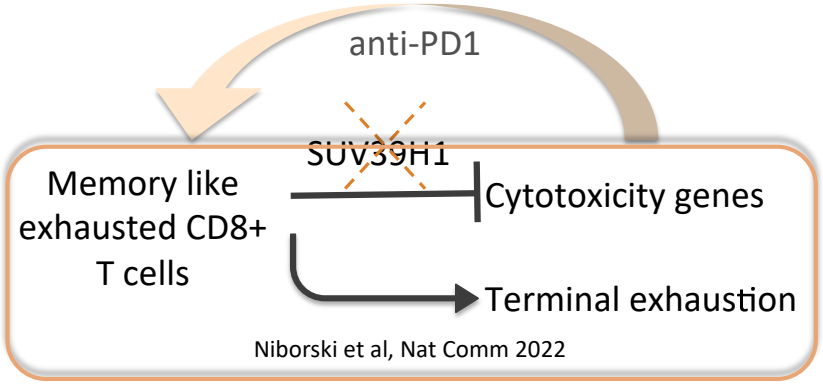
Maintaining the silencing of TH1 loci, ensuring TH2 lineage stability



Silencing memory and stem cell programs during the terminal differentiation of effector CD8+ T cells



Pace et al, Science 2018



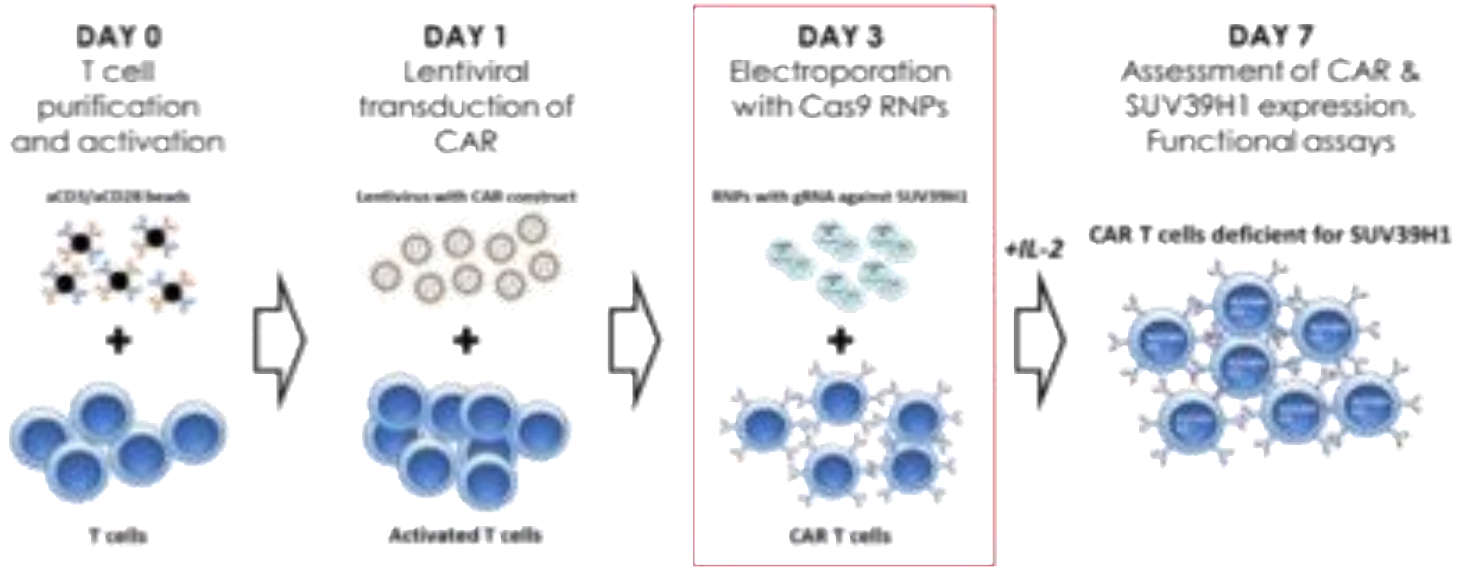
Niborski et al, Nat Comm 2022

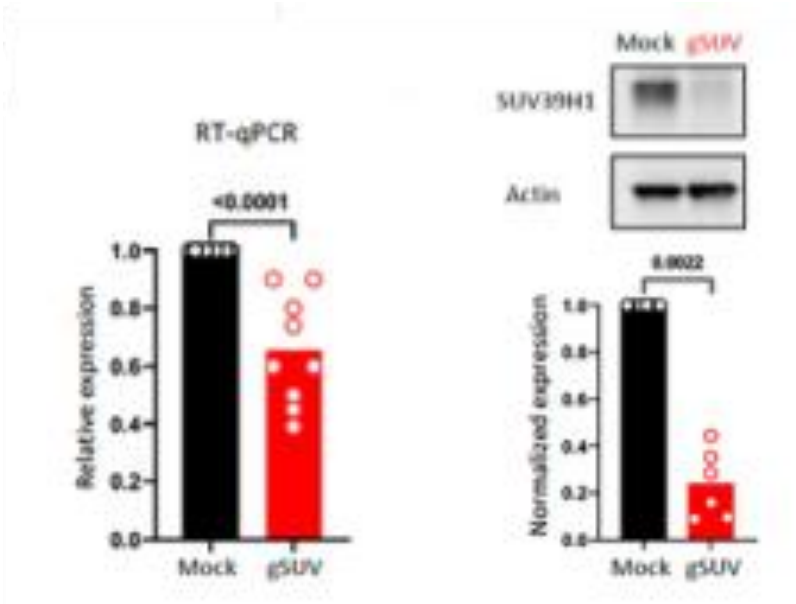
SUV39H1 deficiency enhances antitumor CD8+ T cell responses, alone or combined with anti-PD1 treatment

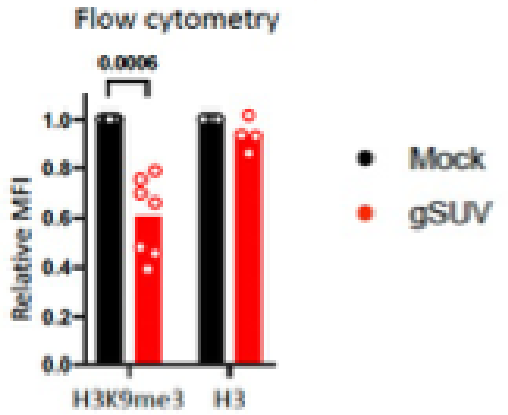
Role in human CAR-T cells?

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Generation of SUV39H1 deficient human CAR-T cells







López-Cobo, Fuentealba et al, Cancer Discov 2024

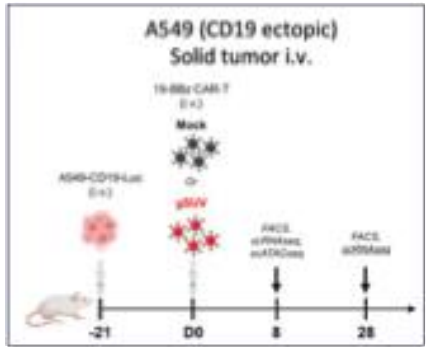
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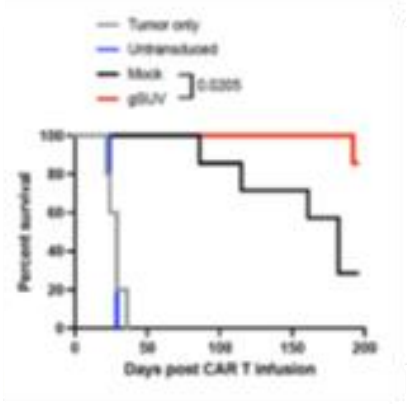
Inhibition of SUV39H1 improves the functional persistence of CAR-T cells

RESEARCH ARTICLES | JANUARY 12 2024

SUV39H1 Ablation Enhances Long-term CAR T Function in Solid Tumors

Sheila López-Cobo ; Jaime R. Fuentealba ; Paul Gueguen ; Pierre-Emmanuel Bonté ; Kyriaki Tsalkitzi ; Irena Chacón ; Salomé Glauzy ; Armelle Bohineust ; Ariane Biquand ; Lisseth Silva ; Zelia Gouveia ; Christel Goudot ; Franck Perez ; Michael Saitakis ; Sebastian Amigorena

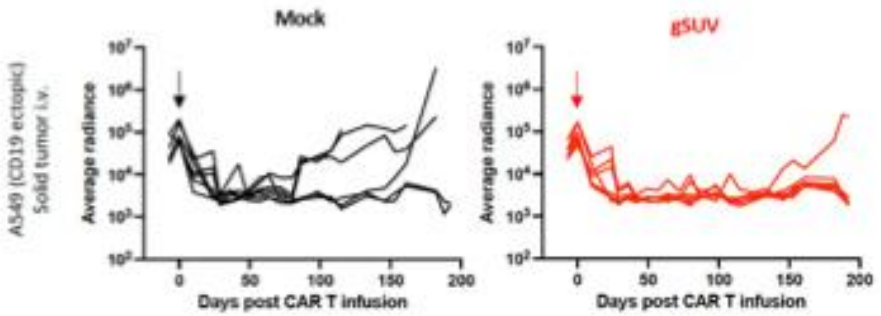


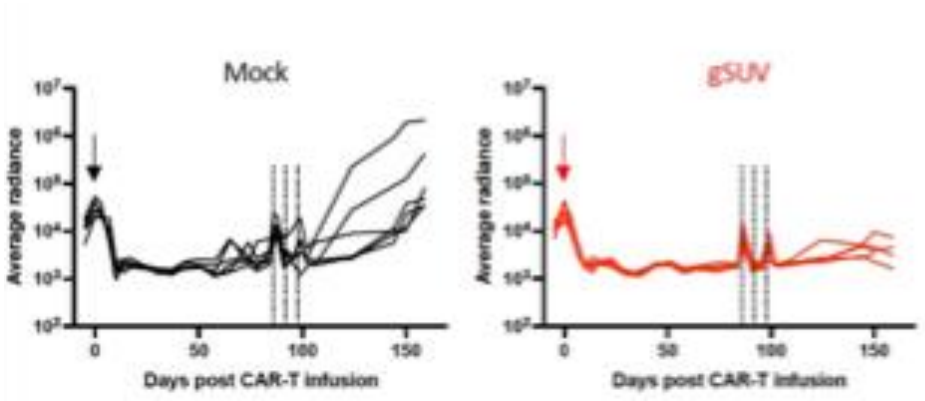


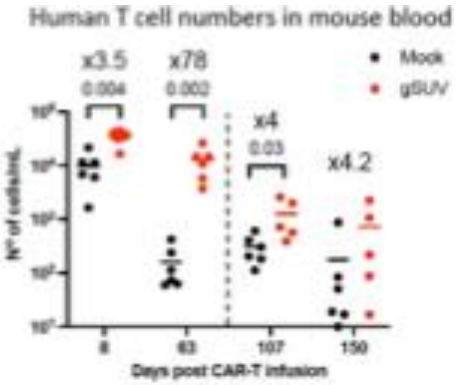
RESEARCH ARTICLES | JANUARY 12 2024

Disruption of *SUV39H1*-Mediated H3K9 Methylation Sustains CAR T-cell Function

Nayan Jain ; Zeguo Zhao ; Richard P. Koche ; Chenling Antelope ; Yosi Gozlan ; Antonino Montalbano ; David Brocks ; Michael Lopez ; Anton Dobrin ; Yuzhe Shi ; Gertrude Gunset ; Theodoros Giavridis ; Michel Sadelain





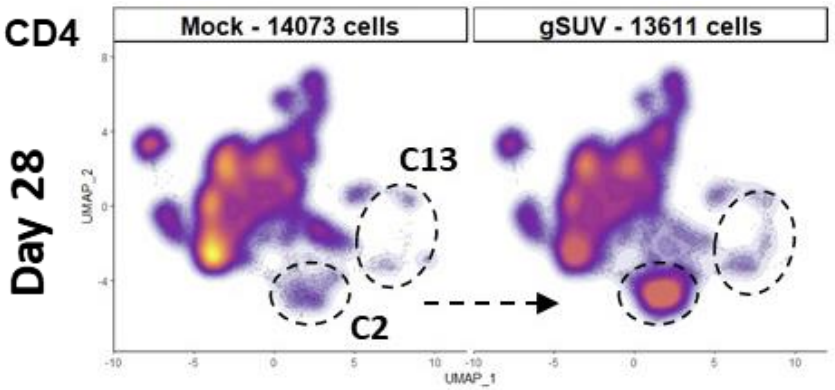


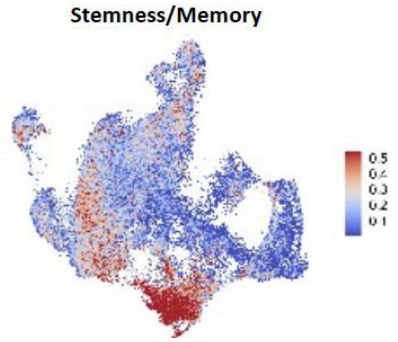
SUV39H1 KO CAR-T cells promote complete, long-lasting tumor rejection even at low CAR-T doses

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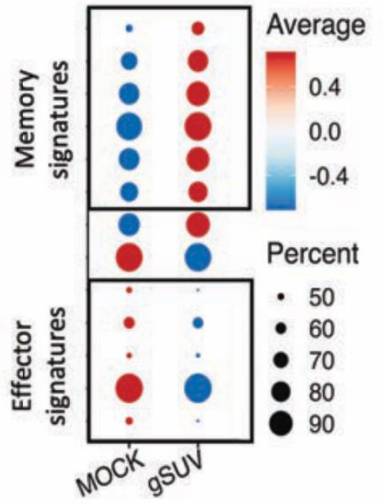
Inhibition of SUV39H1 promotes long-term memory persistence

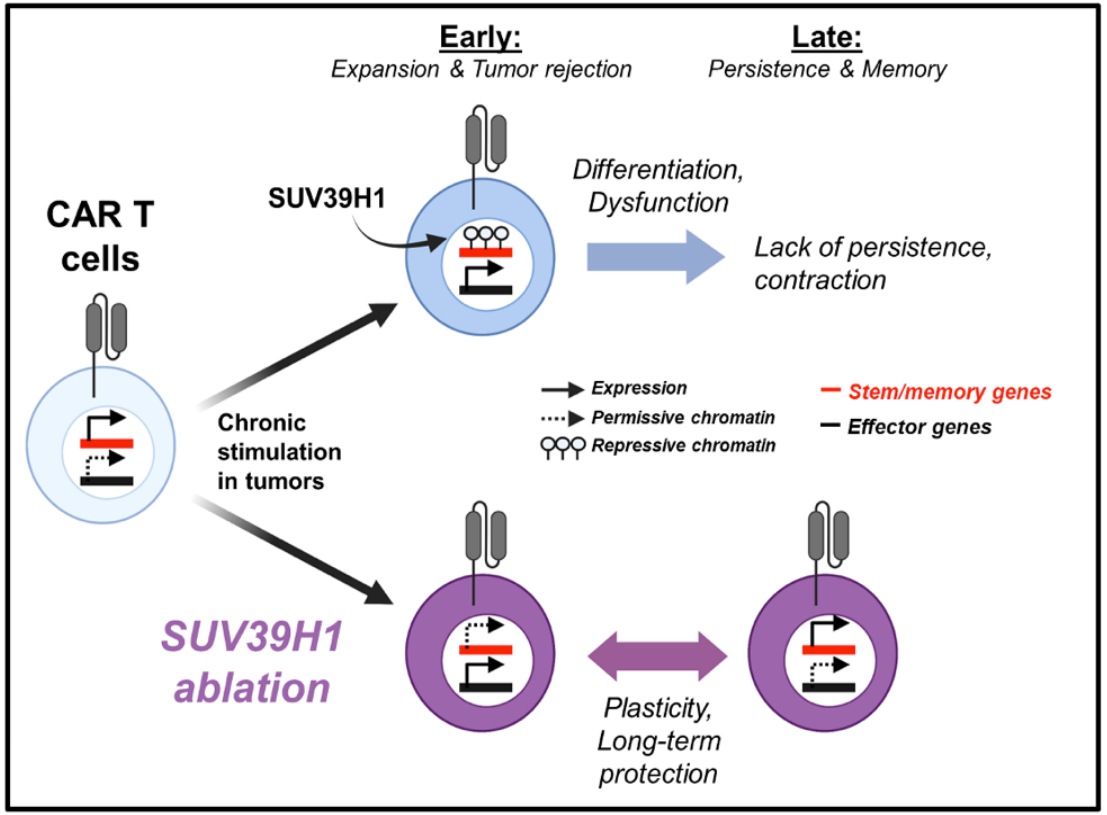
scRNAseq





scATAQseq

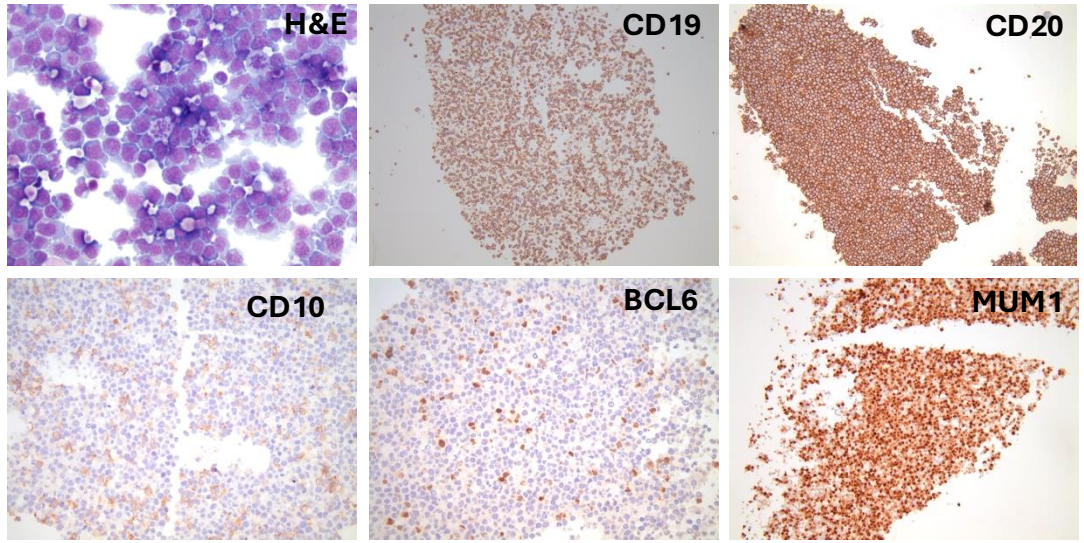


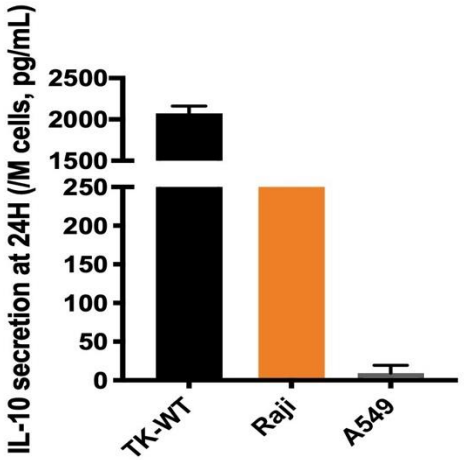


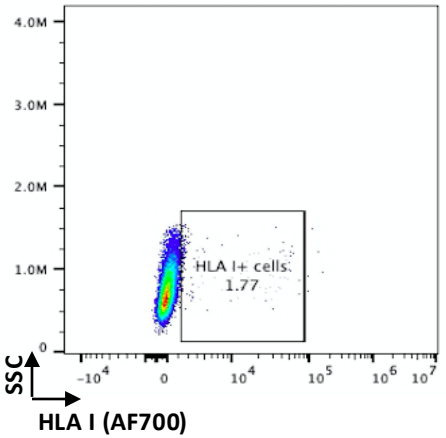
López-Cobo, Fuentealba et al, Cancer Discov 2024

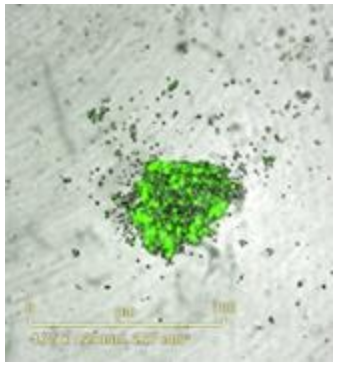
In vitro and *in vivo* models of oculo-cerebral lymphomas

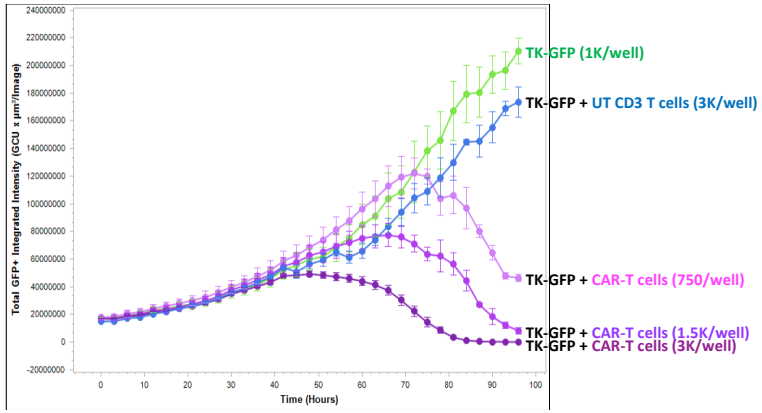
TK cell line

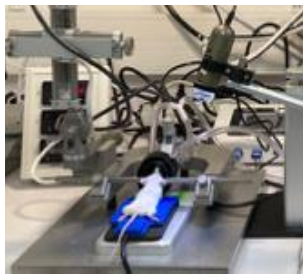


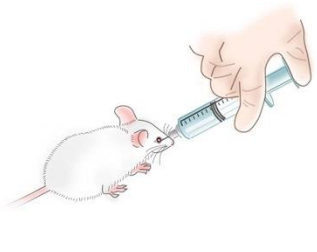


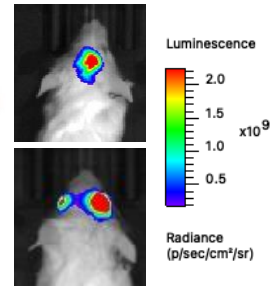








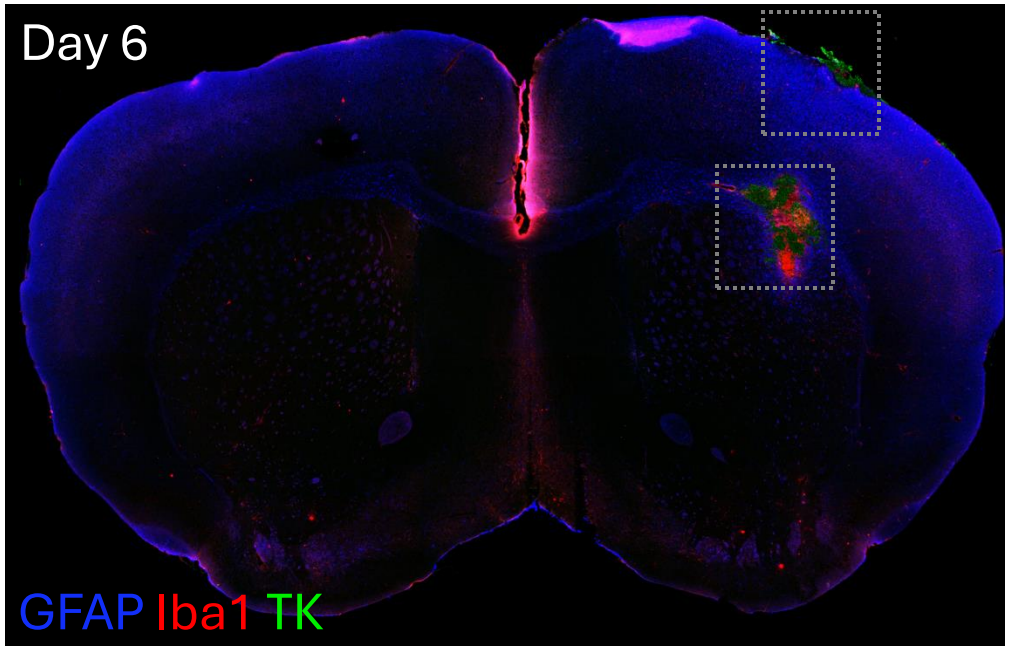




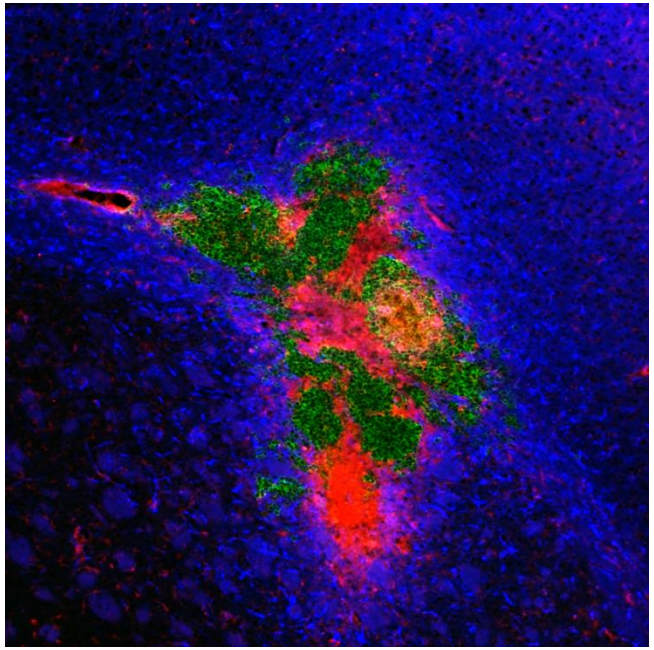
Collab. Denis Malaise,
Institut Curie

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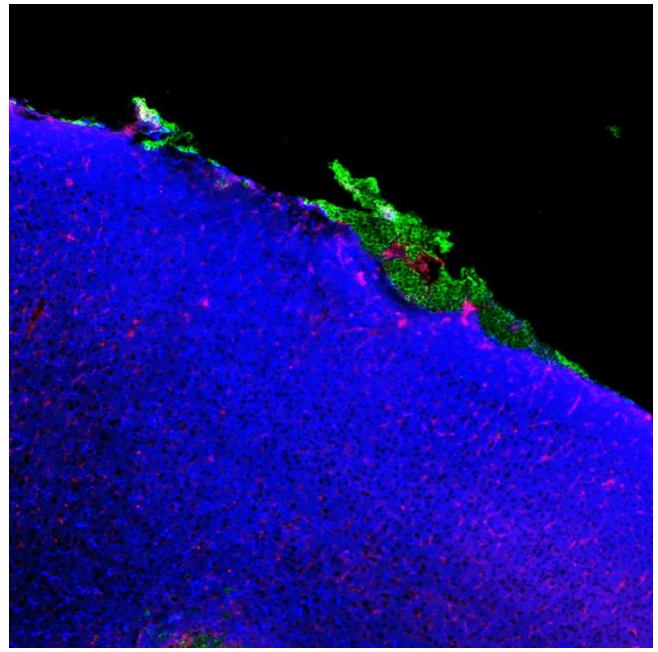
TK cells induce a strong astrogliosis and microglial activation



Brain parenchyma

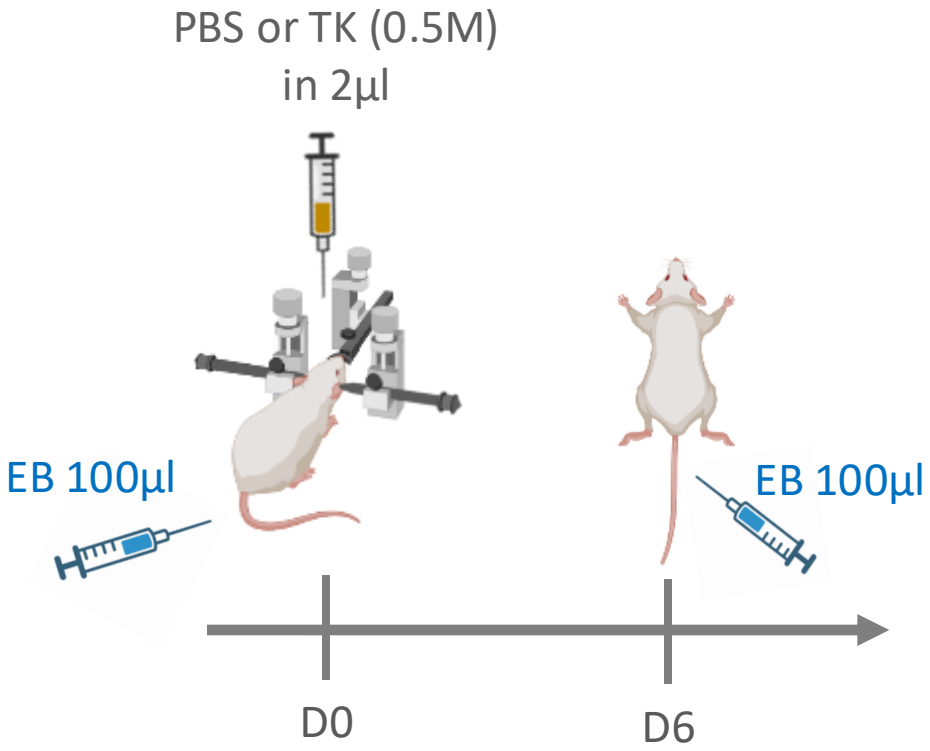


Leptomeninges

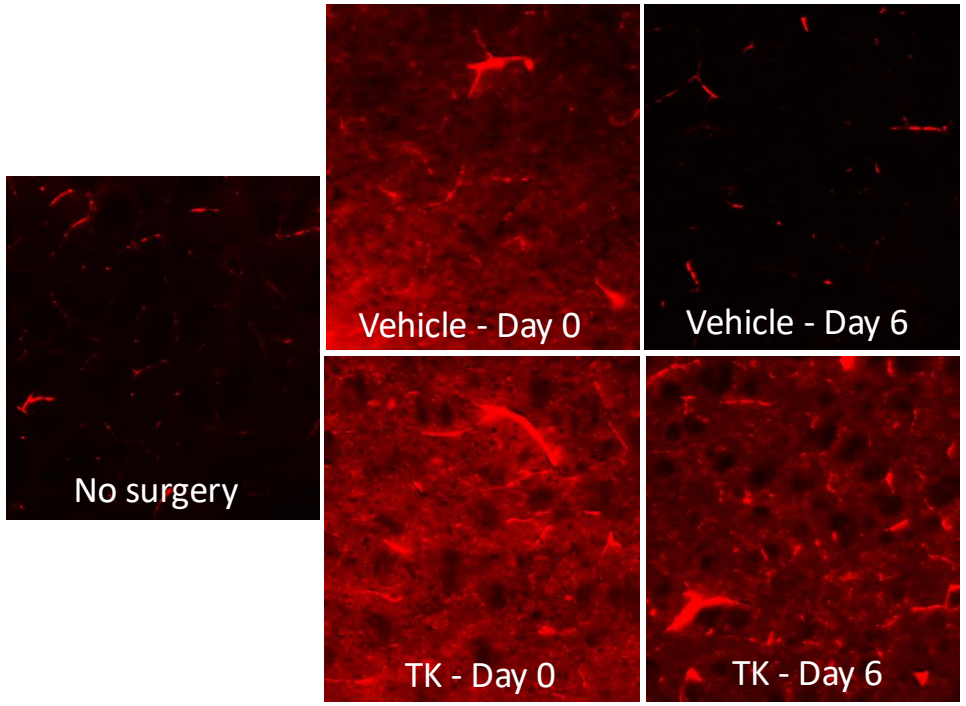


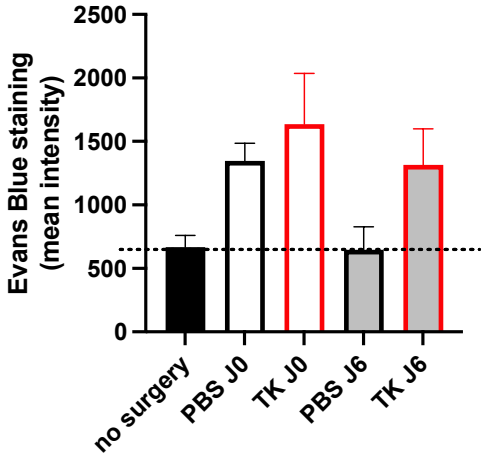
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Evaluation of the BBB integrity in TK bearing mice



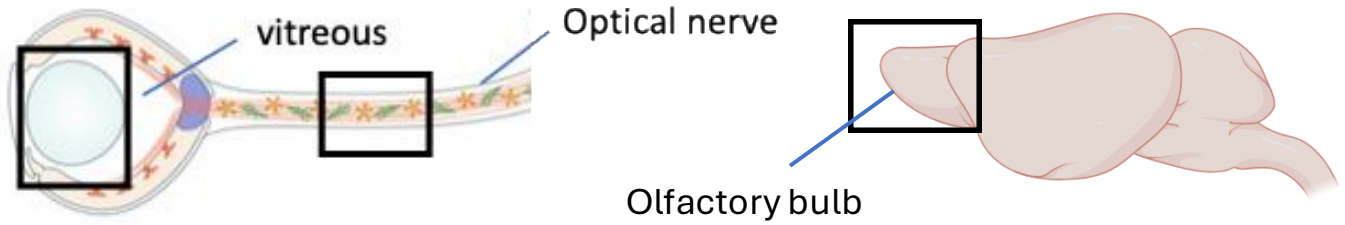
Evans blue extravasation



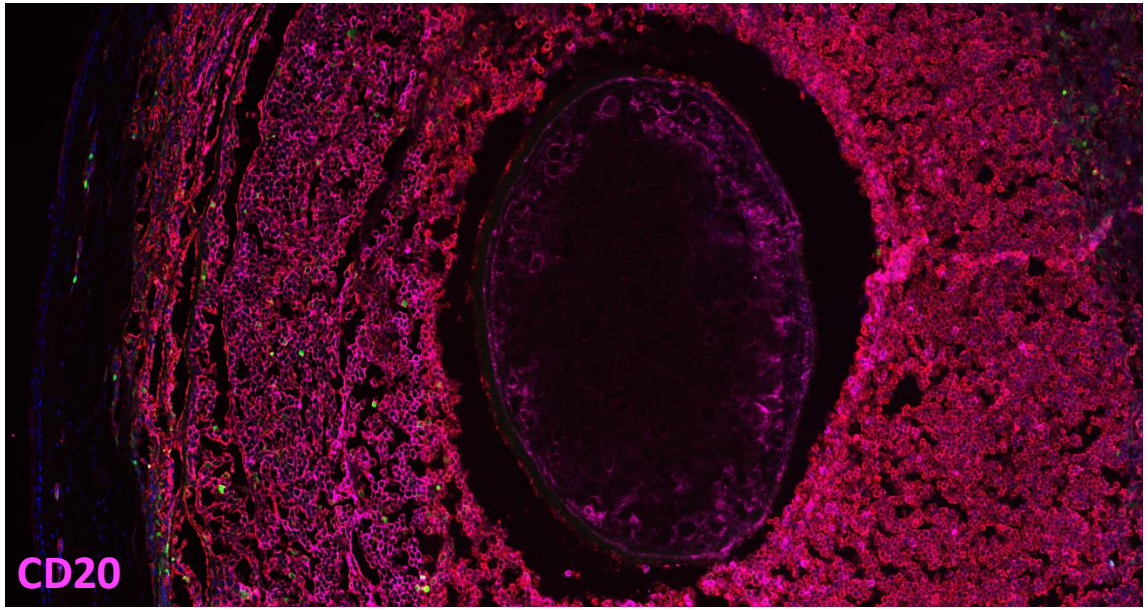


The BBB integrity is compromised at day 6 due to TK cell line and not to mechanical disruption due to the surgery

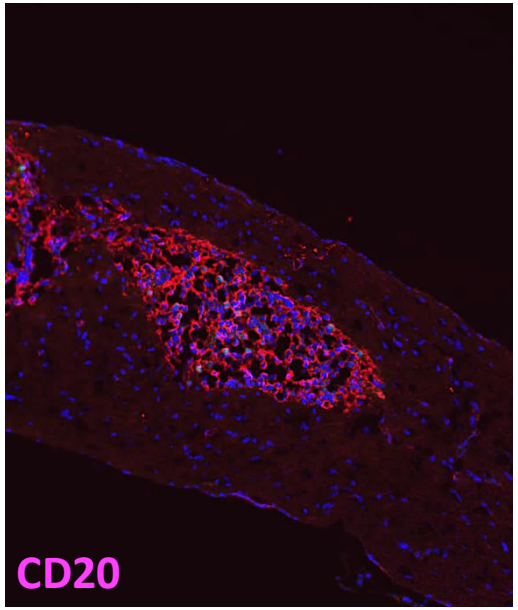
TK cells can migrate from the eye to the brain



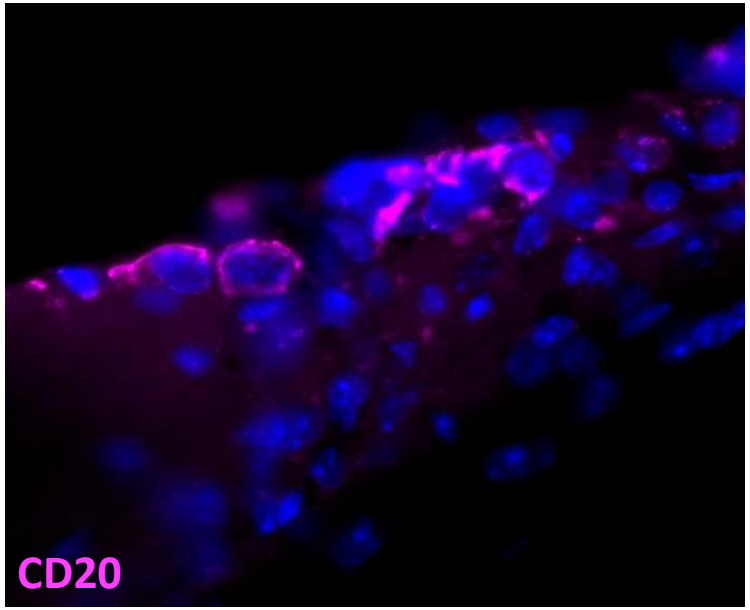
Eye



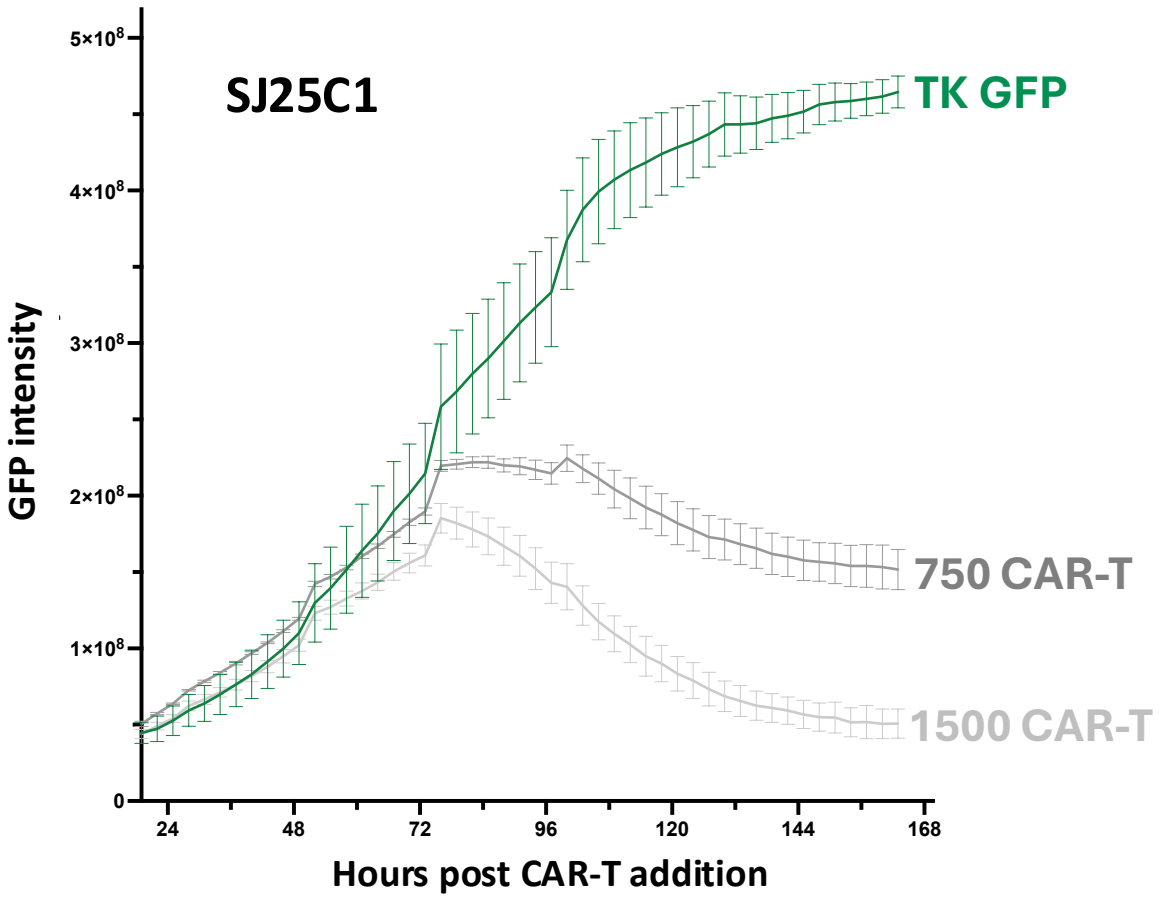
Optical nerve

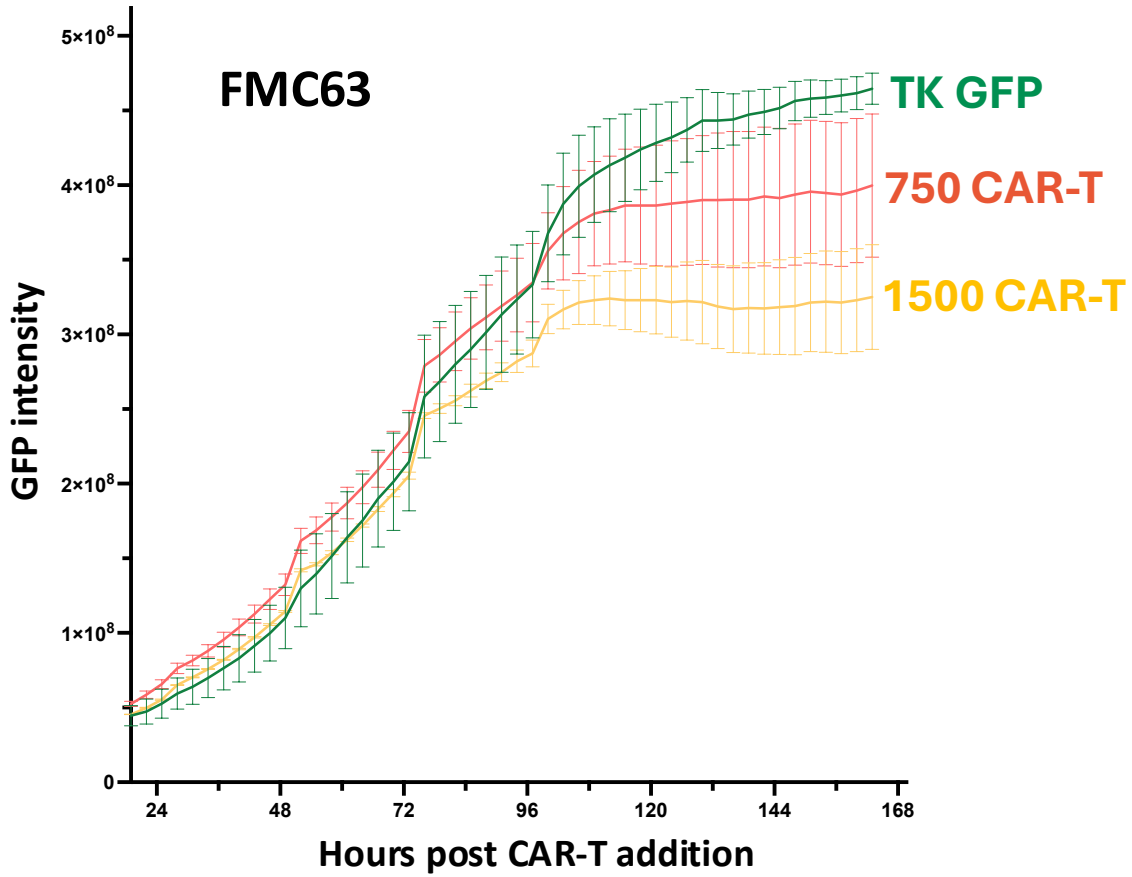


Brain (Olfactory bulb)



SJ25C1-derived outperform FMC63-derived 1XX CAR-T cells



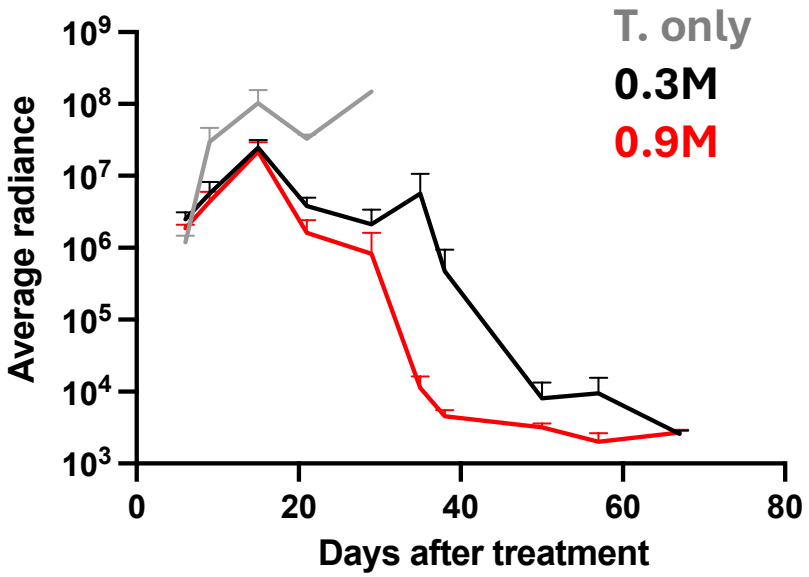


- Comparable CAR expression and MFI
- Similar results were observed in a 2D killing against Nalm6 cell line

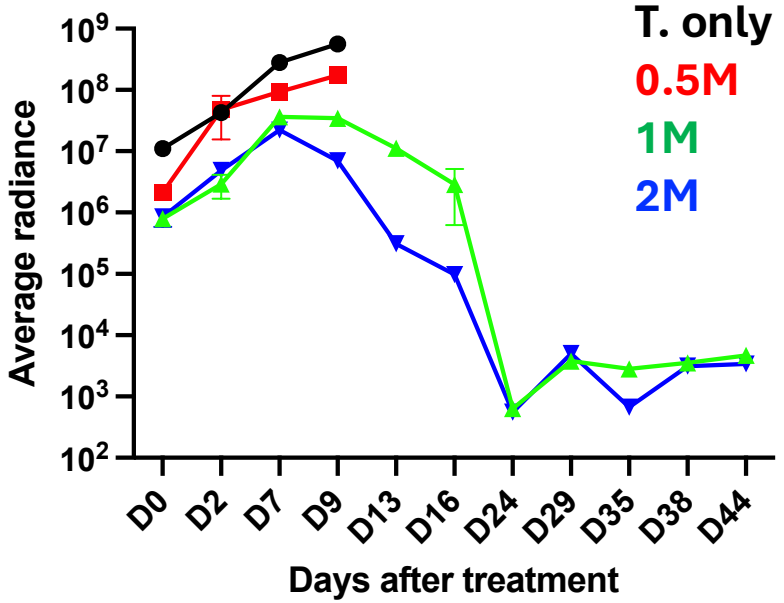
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SJ25C1-derived outperform FMC63-derived 1XX CAR-T cells

SJ25C1-based 1XX CAR



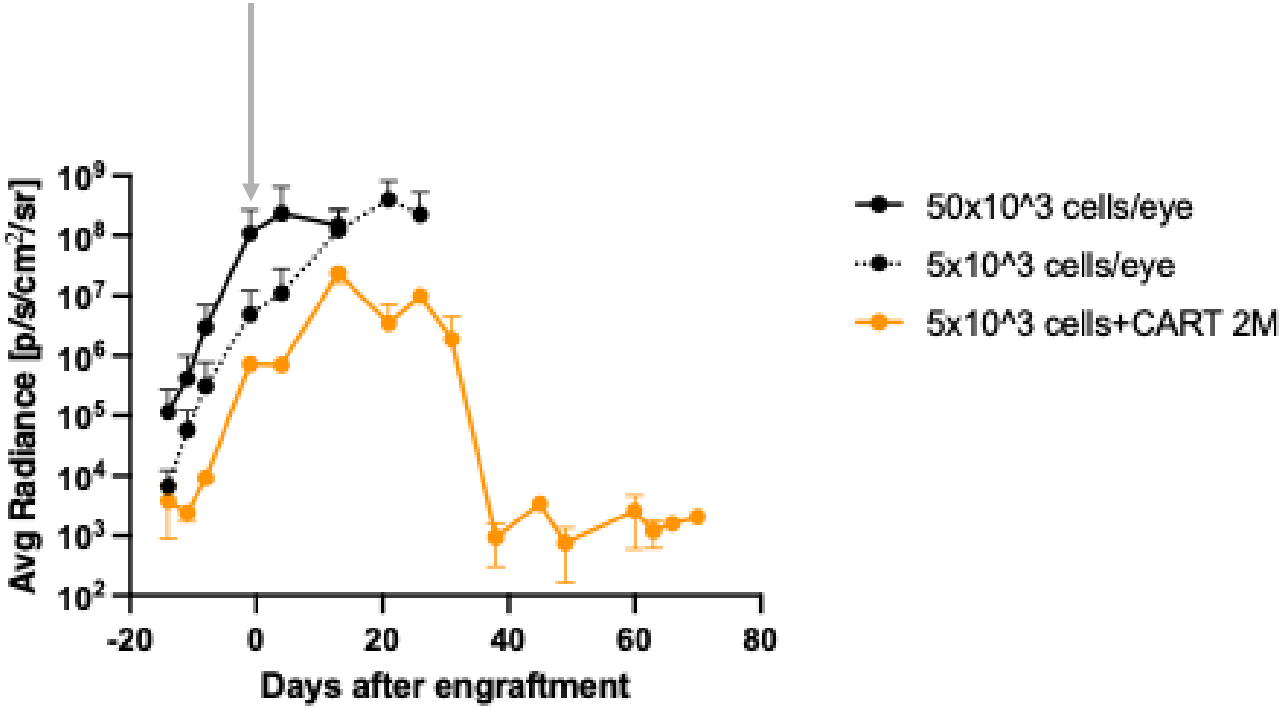
FMC63-based 1XX CAR

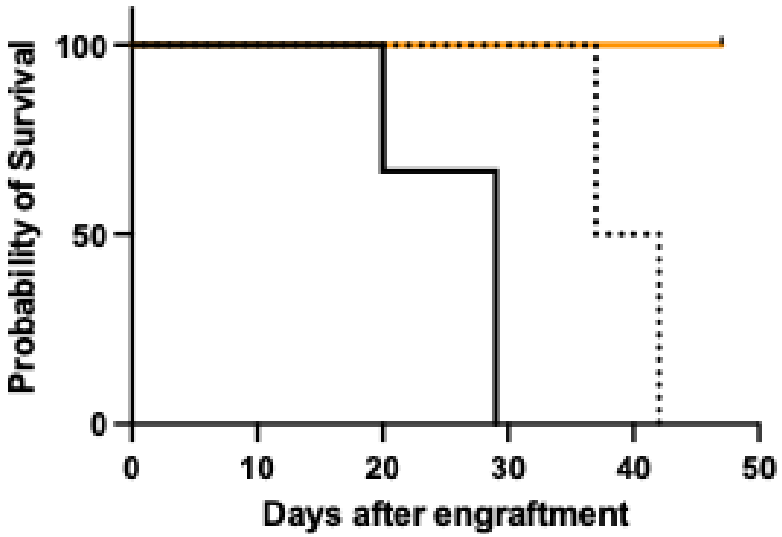


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CD19 CAR-T efficacy in a PVRL model: preliminary results

CD19 CAR-T cells (IV infusion, 2M)
16 days after TK cells intravitreal engraftment

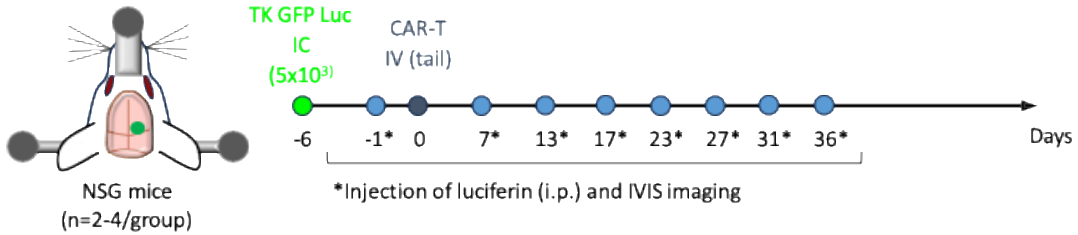




- Antitumor efficacy
- No side effect, no exophthalmos
- Needs to be repeated, lower CAR-T doses

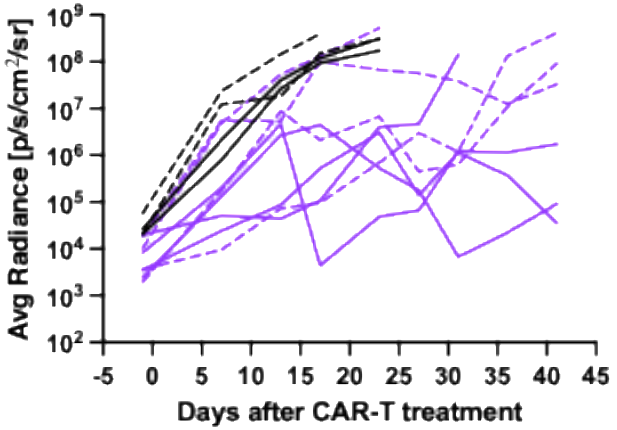
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CD19 28z1XX SUV39H1 KO CAR-T cells outperform commercial CAR-T cells



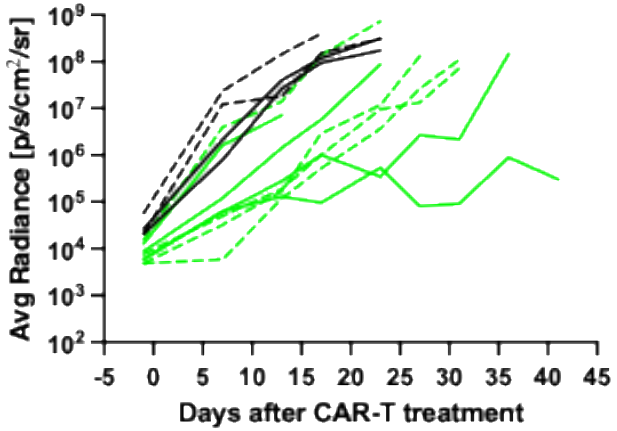
--- Tumor only

— Untransduced T-cells



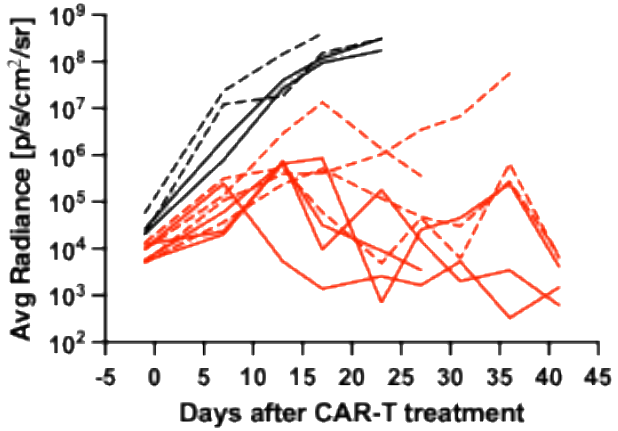
..... 19BBz-CAR non nucleof 0.3M

— 19BBz-CAR non nucleof 0.9M



..... 19CD28-CAR non nucleof 0.3M

— 19CD28-CAR non nucleof 0.9M

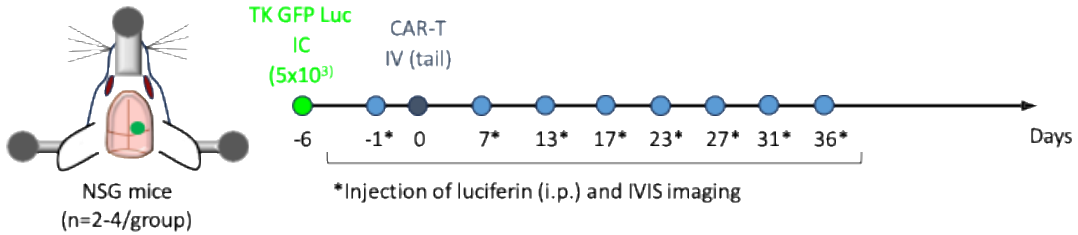


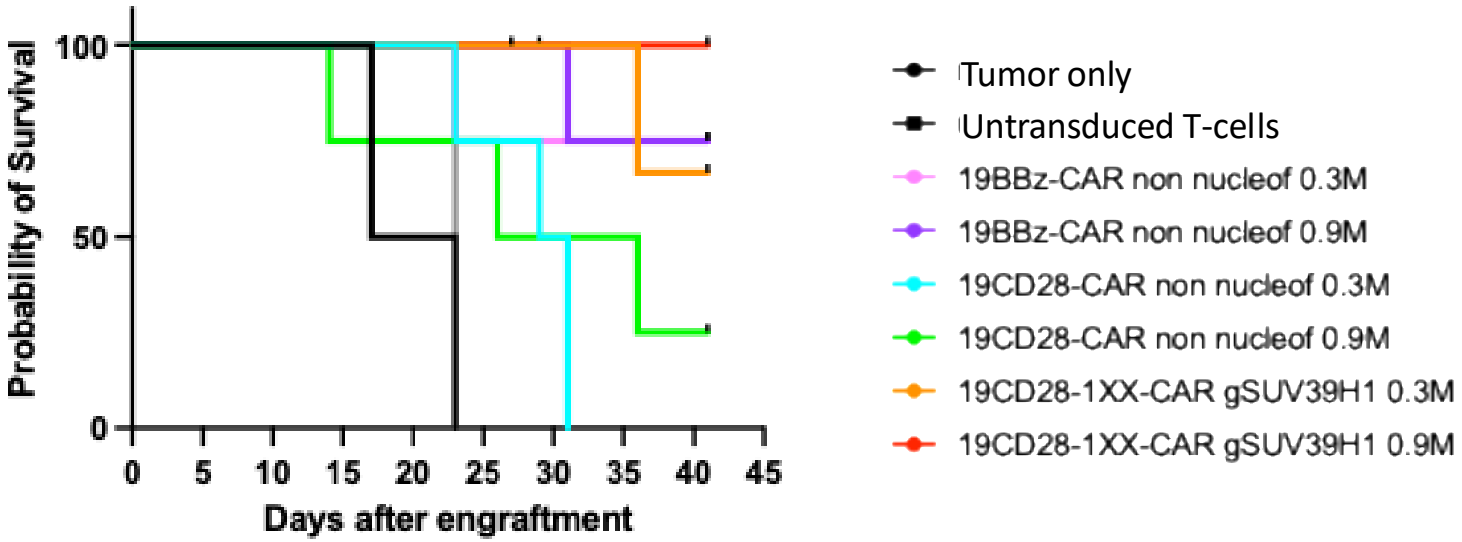
..... 19CD28-1XX-CAR gSUV39H1 0.3M

— 19CD28-1XX-CAR gSUV39H1 0.9M

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CD19 28z1XX SUV39H1 KO CAR-T cells outperform commercial CAR-T cells





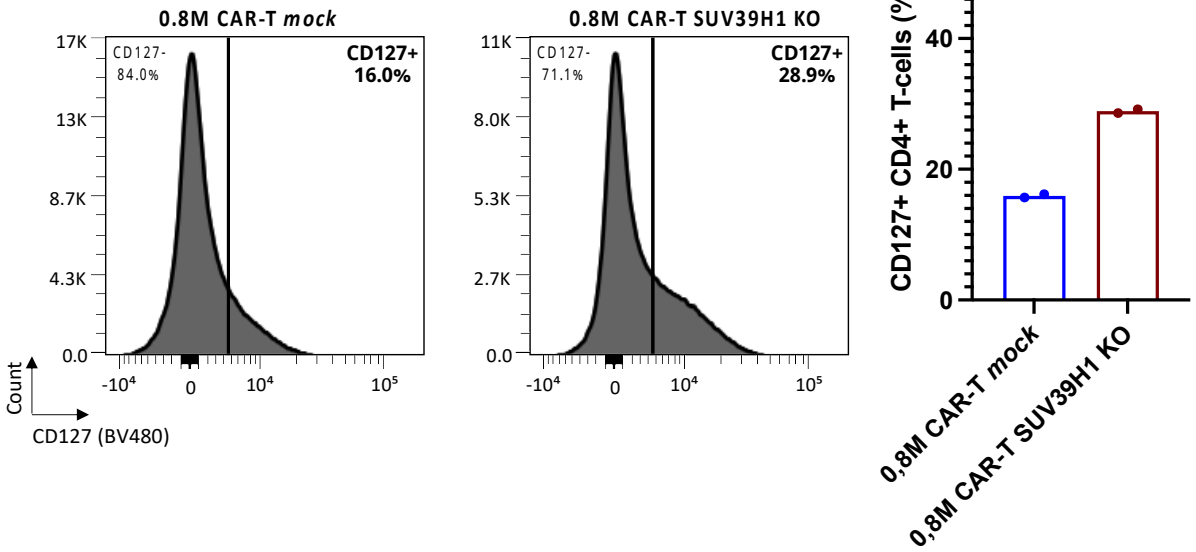
Phenotypic differences between 28z1XX SUV39H1 KO and control CAR-T cells in PCNSL

***Ex vivo* CAR-T analysis after brain dissociation**

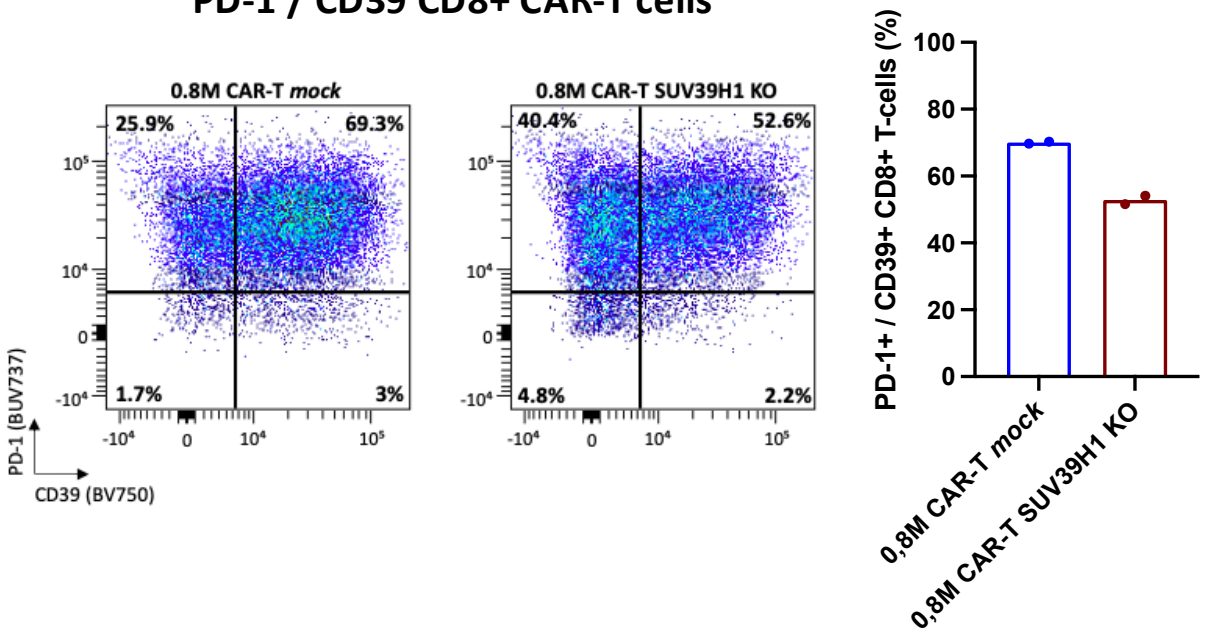
30 colors spectral flow cytometry

Day 25

CD127 CD4+ CAR-T cells



PD-1 / CD39 CD8+ CAR-T cells



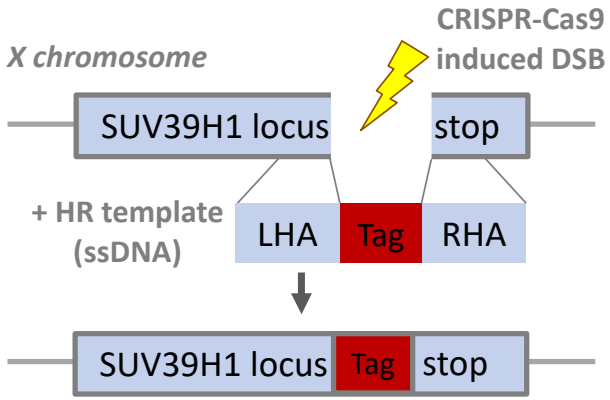
Silencing SUV39H1 in CD19 28z1XX CAR-T cells

- Increases the proportion of cells expressing memory markers
- Reduces dysfunctional/exhausted phenotypes
- In an *in vivo* model of PCNSL

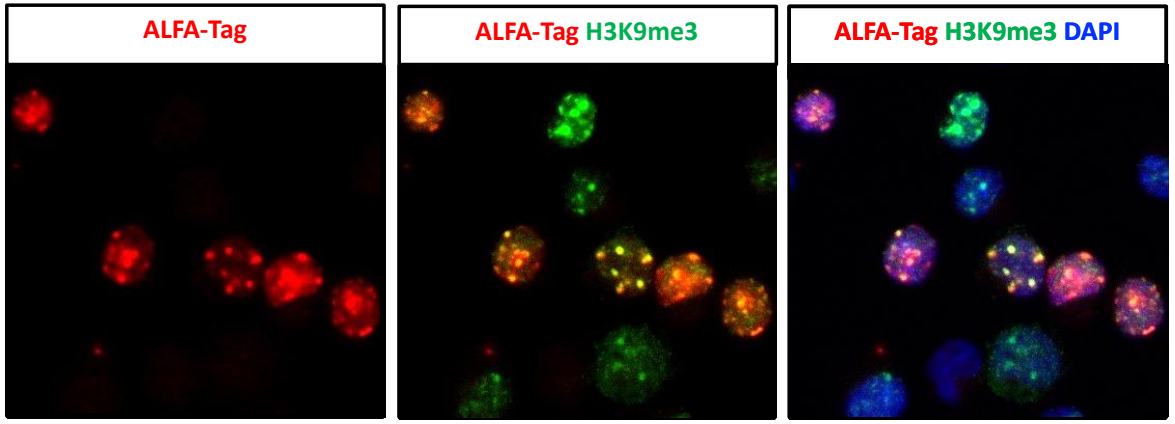
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MoA: Which genes loci are directly silenced by SUV39H1?

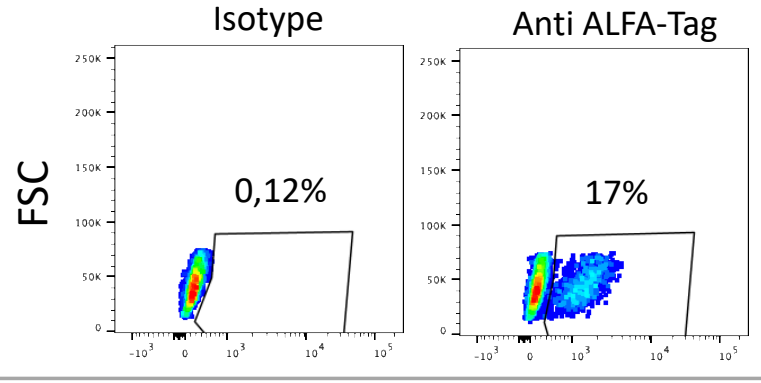
Tag insertion via homologous recombination (HR)



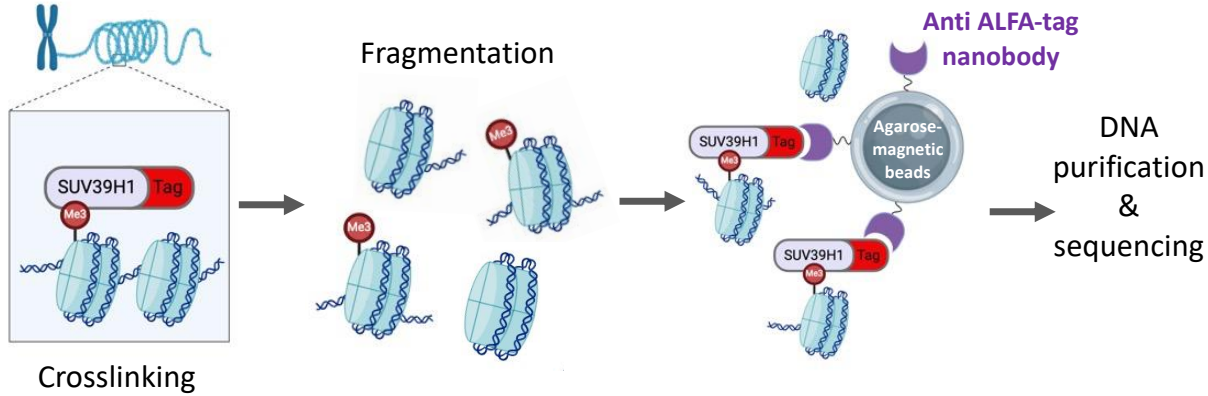
Colocalization of ALFA-tag and H3K9me3 immunostainings



Flow cytometry detection of tagged SUV39H1

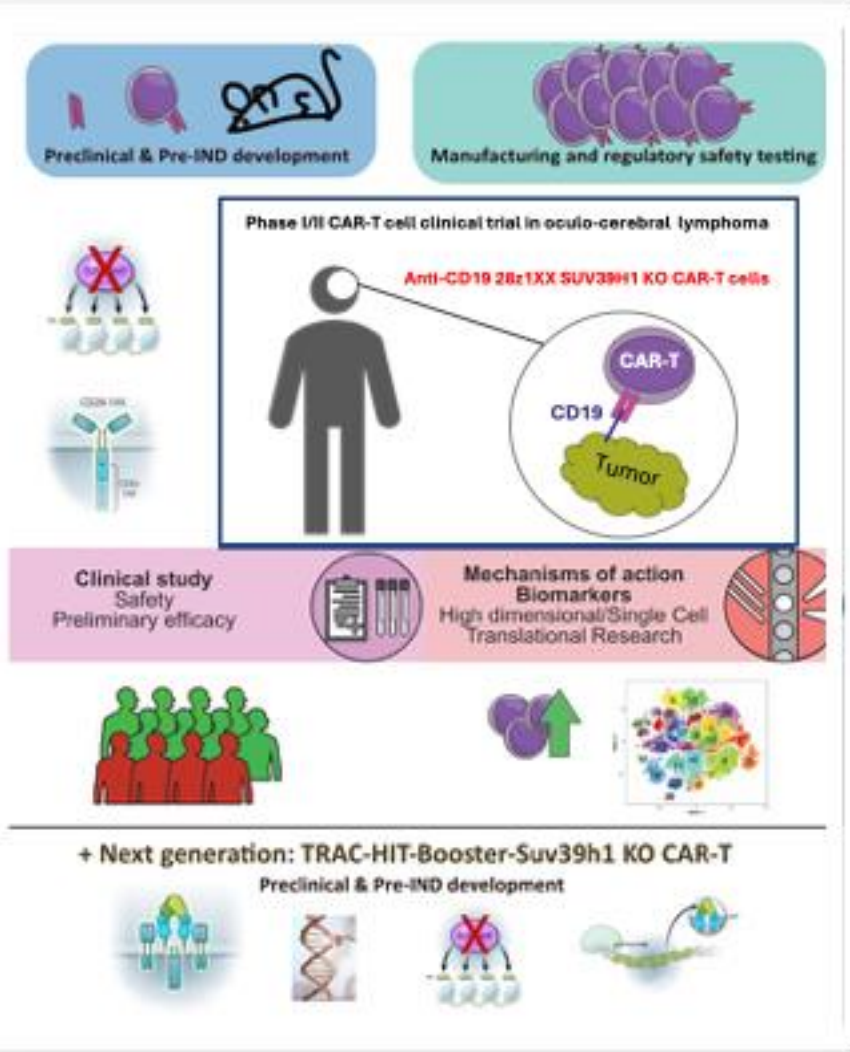


ChIP-seq against tagged SUV39H1 ("endogenous expression")



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Epigenetic reprogramming of CAR-T cells, the RHU EpCART program



Partner 1: Insitut Curie Research and Hospital Center

CellAction

FRANCE 2030

RHU Laureate

Fundamental and translational research
Patents

institut Curie

GMP CAR T cell product

EpCART

Partner 2: Mnemo Therapeutics

Memorial Sloan Kettering Cancer Center

UCSF

institut Curie

MNEMO THERAPEUTICS

MEARY CENTER

Partner 3: MEARY Cell and Gene Center AP-HP

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

CAR T cell design
Manufacturing process

Process development of CD19 28z1XX SUV39H1 KO CAR-T cells

Development strategy

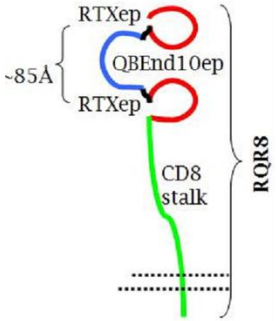
Manufacturing & analytical steps

CliniMacs® Prodigy (*Miltenyi*) & MaxCyte®





- Summary of the manufacturing process for **CD19 (SJ25C1) 28z1XX SUV39H1 KO CAR-T cells**
  - Introduce **RQR8**
    - Better define cell therapy product (CD34+ selection)
    - Safety switch (rituximab)



Autologous T-cell isolation (CD4+/CD8+) from leukapheresis and activation → **LVV transduction to introduce 28z1XX CAR** → **Purification of the RQR8+ cells (CD34+ selection)** → **SUV39H1 KO via CRISPR Cas9 (MaxCyte®)** → **Culture and expansion (IL-7 & IL-15)** → **Harvest & cryopreservation**

?

Clinical Product release:
CAR+, SUVKO%, VCN, viability, sterility, endotoxins, aspect, potency

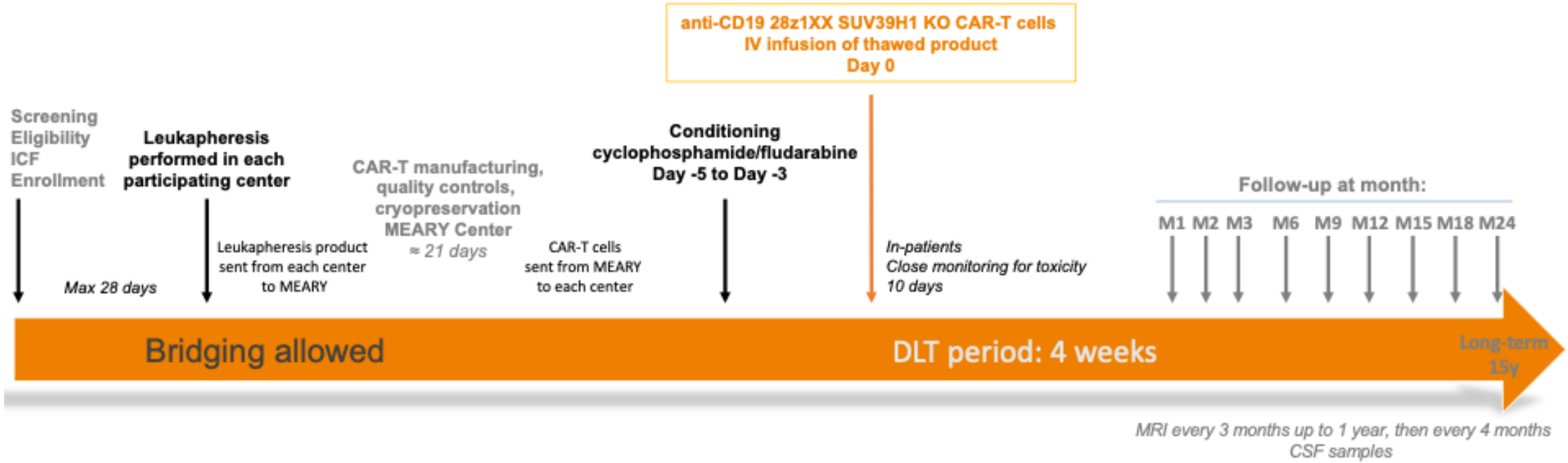
Our objective: getting a GMP-ready CD19 28z1XX SUV39H1 KO CAR-T product for Q1 2026

Philip et al, Blood 2014

Study design of the RHU EpCART program

- First-in-human phase I/II, multicentric, international study
- N = 30 patients
- R/R CD19+ oculo-cerebral lymphomas
- ≥ 1 prior therapy
- Ineligible for ASCT

PSL UNIVERSITÉ PARIS — PSL-USP chair



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Methodology of the RHU EpCART trial

- **Multicentric international study**

- **First-in-human phase I/II being conducted in two parts**

  - **Part 1: Dose-validation**
    - Relapsed/refractory PCNSL and primary vitreoretinal lymphoma (PVRL)
    - After 1 line or more of therapy including a methotrexate-containing regimen
    - Ineligible for autologous stem cell transplantation (ASCT):
      - Age ≥ 65 years or
      - Age ≥ 18 years and hematopoietic cell transplantation-comorbidity index (HCT-CI) score ≥ 3 or
      - Age ≥ 18 years and prior ASCT
    - Eligible for CAR-T cells
    - **Dose K (=RP2D as determined from NCT04464200) $25x10^6$ CAR+ T cells**
    - **Dose K-1 if needed**

  - **Part 2: Expansion to validate the clinical activity**
    - Same patient population

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Objectives of the RHU EpCART trial

Primary objective

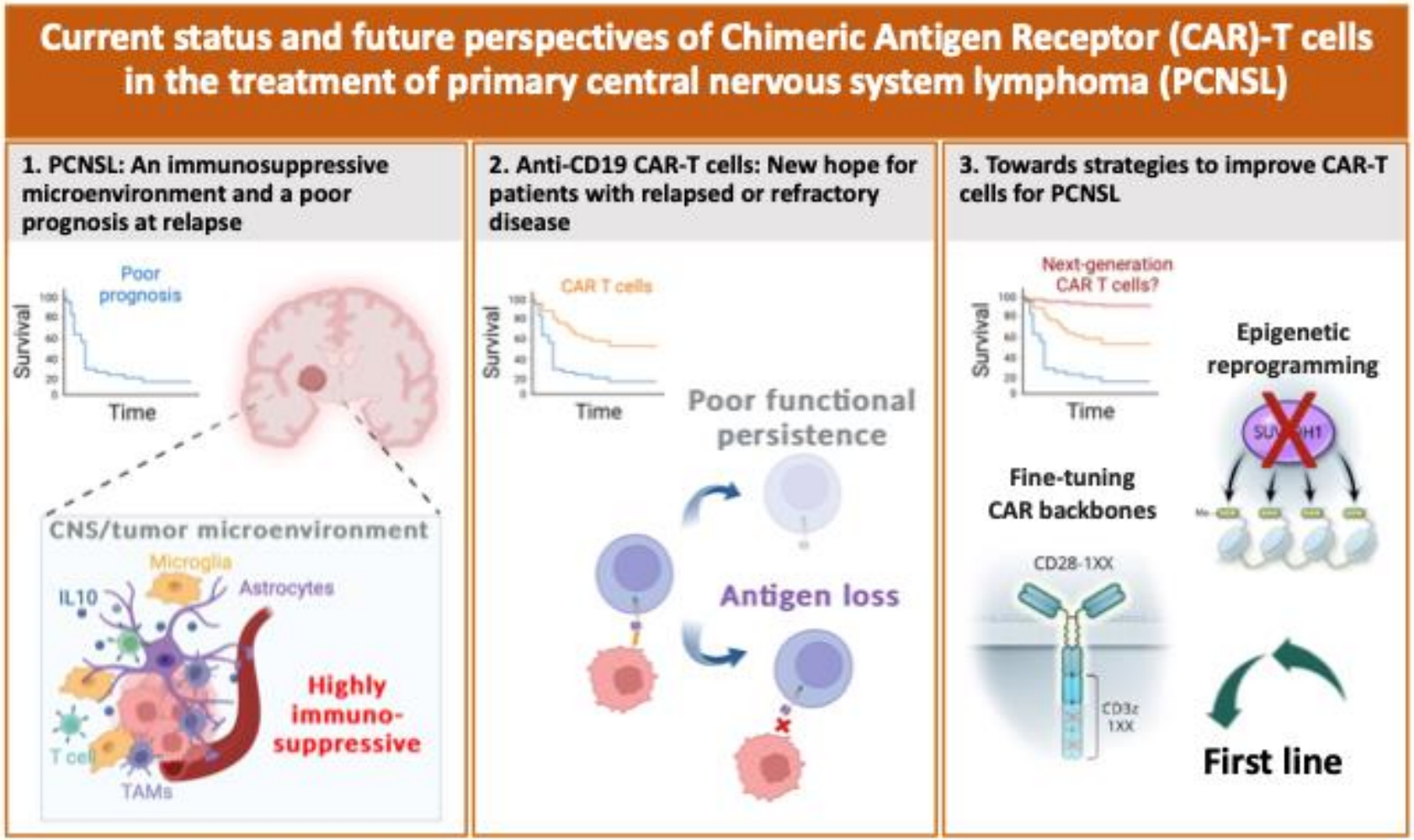
- **To validate 25x10^6 CAR+ T cells as a safe and active dose**
  - To document the safety
  - To determine whether the objective response rate (ORR) is higher than 60%, as determined by IPCG criteria at M1

Secondary objectives

- To document the **activity**
  - Time spent in response, progression-free survival, event-free survival and overall survival
- To assess **long-term toxicity**
  - Cytopenia, infections, T-cell lymphoma, secondary primary malignancy that occur after 4 weeks post CAR-T infusion
- To determine the **expansion**, **immunophenotype** and **persistence** of CD19 28z1XX SUV39H1 KO CAR-T cells
  - Flow cytometry and digital PCR
  - Correlation with clinical outcomes

Extensive ancillary studies

Conclusion



Looking forward to collaborate with you in this promising program!

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Acknowledgment

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Alice Léger
Amaury Leruste
Lucille Lew-Derivry
Silvia Menegatti
Cécile Pierre-Eugene
Valentine Pottez-Jouatte
Virginie Premel
Anne-Laure Privat
Kyle Raymond
Lisseth Silva



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