Challenges in the Clinical Implementation of piggyBac Transposon mediated CAR-T cell Therapy

Shigeki Yagyu

A-SEEDS Co., Ltd.

Shinshu University Innovative Research & Liaison Organization

Today’s topic

1. T cell composition in the starting materials

- The composition of T cells in the starting materials is critical for determining the manufacturing efficiency and quality of ***piggyBac*** transposon CAR-T cell products.
- **Key Question 1:** What strategies can be employed to optimize T cell composition in patient-derived samples, ensuring manufacturing success and the high quality of final CAR-T cell products?

2. CAR-T cell driven second malignancies

- Multiple factors, including vector insertion and pre-existing malignant clones, may be associated with CAR-T driven second malignancies.
- **Key Question 2:** What are the key considerations for establishing robust vector copy number (VCN) criteria for piggyBac transposons to ensure long-term safety?

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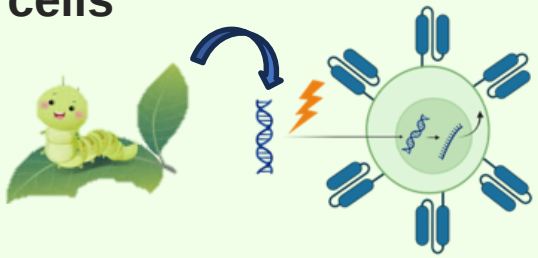
Breakthrough *piggyBac* CAR-T cells with superior efficacy and scalability

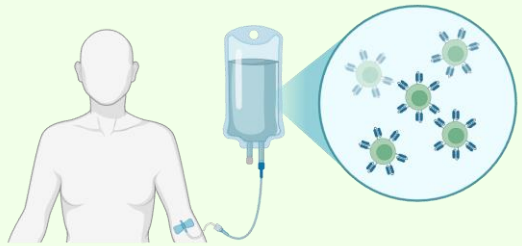
“ The piggyBac technology transforms CAR-T therapy with enhanced efficiency, durability, and reduced costs. “

Combating solid tumors with *piggyBac* transposon CAR-T cells



- ✓ Optimized **T_{scm} Cell Enrichment**
- ✓ Maximized **Therapeutic Effectiveness**
- ✓ Improved **Cellular Resilience**
- ✓ Streamlined **Manufacturing Workflow**
- ✓ **Cost-Effective** Production

Two P1 trials are ongoing



- ✓ **CARTiEr G111 (Japan)**

  GMR-redirected CAR-T cell therapy for AML

- ✓ **CARTiEr E211 (Japan)**

  EPHB4/EPHA2 dual specific CAR-T cell therapy for solid tumors

Acceralate global clinical development

- ✓ **CARTiEr E312 (Australia)**

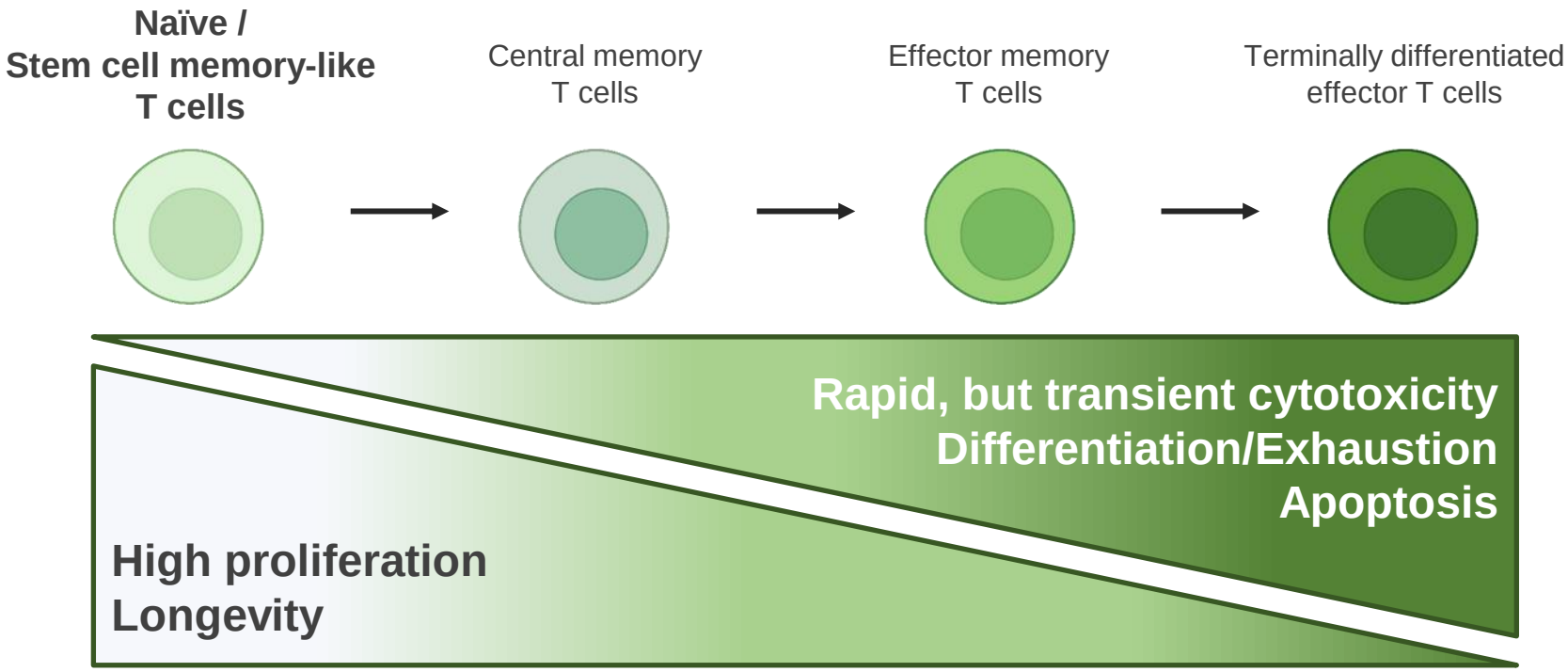
  Phase 1/2a trial of EPHB4/EPHA2 dual specific CAR-T cell therapy for colorectal cancer, hepatocellular carcinoma, and soft tissue sarcoma, on track for 2025

Viral vs. non-viral engineered CAR-T cells

	Retro/Lentivirus	Transposon	mRNA
Genome integration	+	+	-
Gene transfer to Non-dividing cells	Possible (Lentivirus)	+	+
Transgene expression	Stable	Stable	Transient
Immunogenicity	Immunogenic	Less immunogenic	Less immunogenic
Transfection efficiency	High	Low to mid	High
Oncogenic Activation	Posssible	Posssible	None
Pre-activation of T cells	Necessary	Not necessary	Not necessary
GMP manufacturing	High, hazardous to produce	Affordable?	Affordable?

The differentiation status of CAR-T cells determines the function

"Stem cell memory like CAR-T cells strongly correlated with the longe-term anti-tumor effect. "



Physiological
T cell stimulation and proliferation

Manufacturing process

Excessive
T cell activation

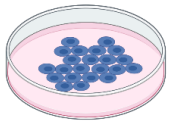
Engineering of PB-based T_{scm}-rich CAR-T cells – Our Strategy

1. Leukapheresis

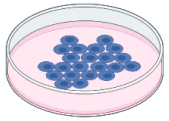
PBMC isolation

2. PBMC isokation

3. Preparation of PBMC for CAR-T and feeder cells



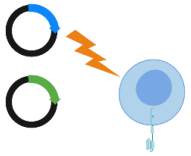
For CAR-T cells



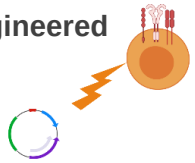
For genetically-engineered feeder cells

4. Electroporation

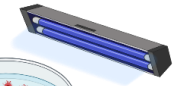
CAR-T cells



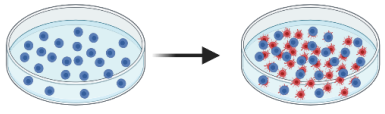
Genetically engineered feeder cells



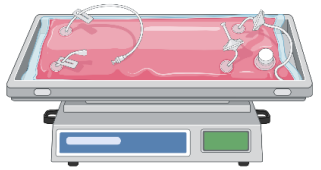
5. Inactivation by UV irradiation

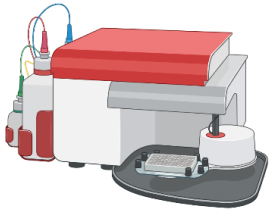


6. Co-culture with feeder cells



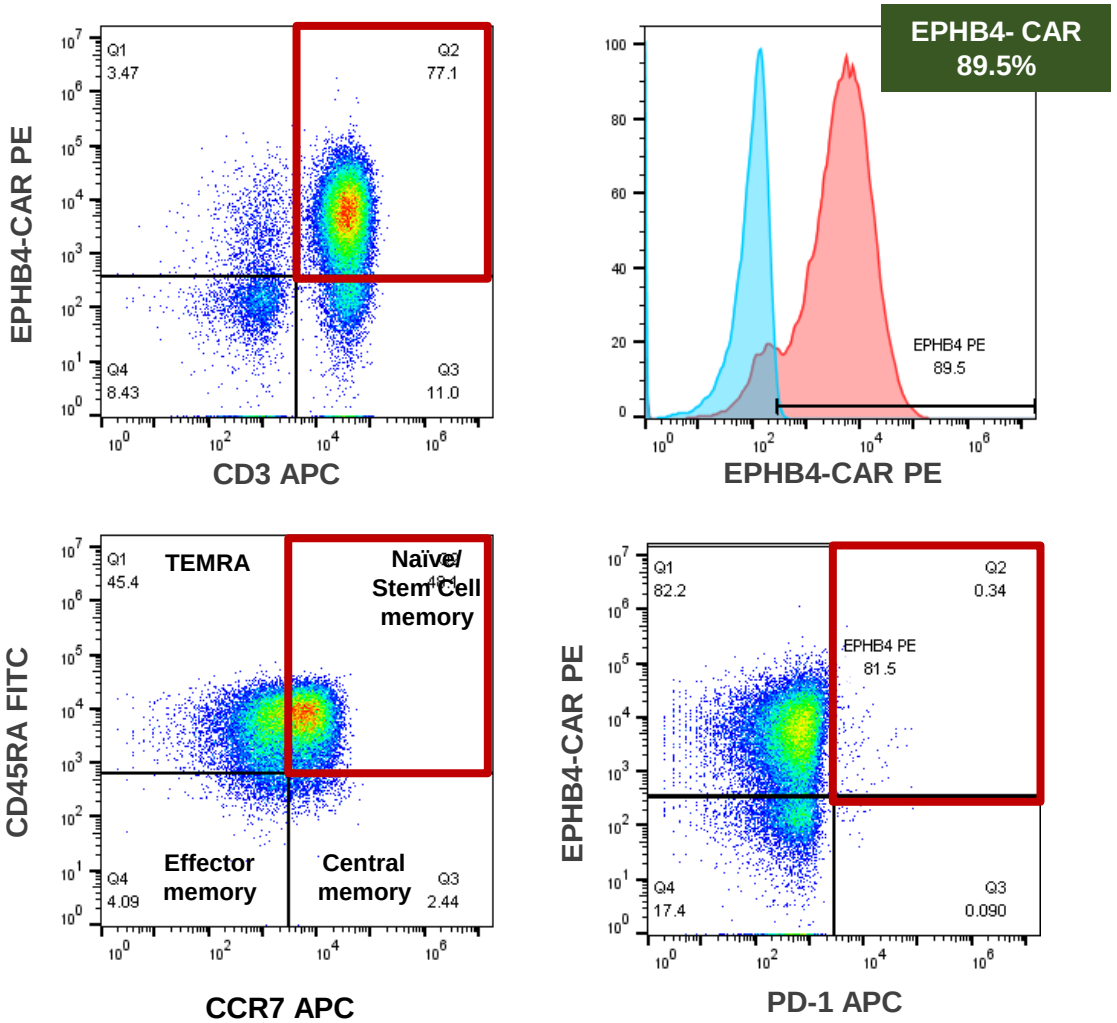
7. expansion

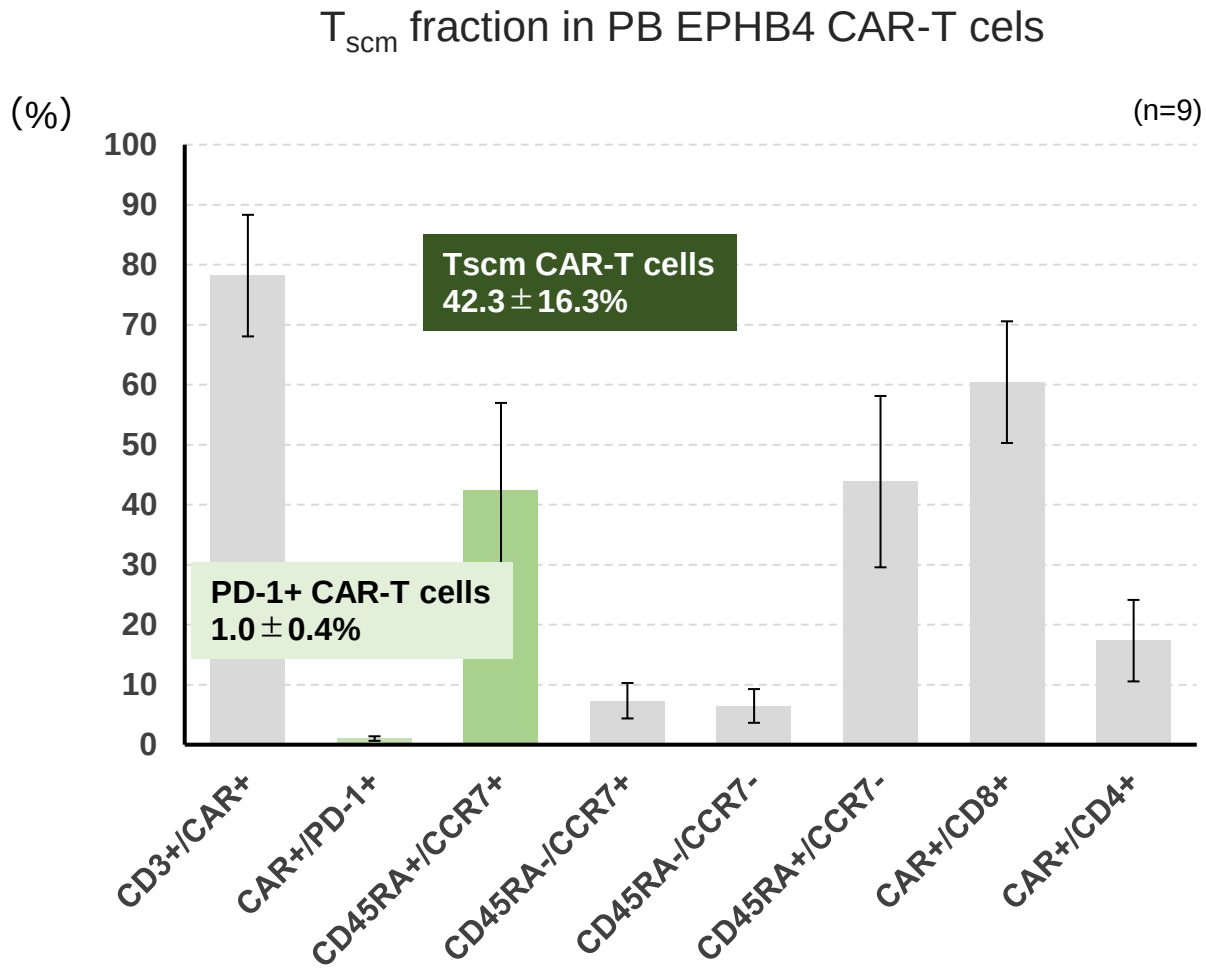




8. Quality assessment, cryopreservation

PB EPHB4 CAR-T cells exhibited enriched PD-1^{-} T_{scm} fraction





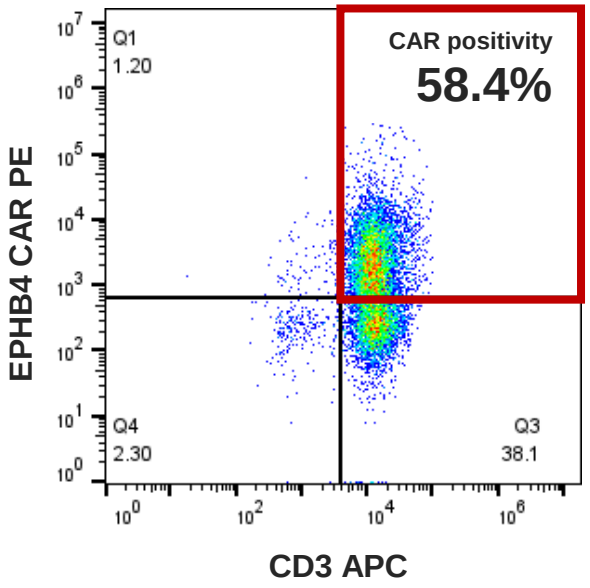
(Kubo H et al. Mol Ther Oncolytics 2021)

Unmatched Performance: piggyBac CAR-T Cells vs. Viral-Based CAR-T Cells

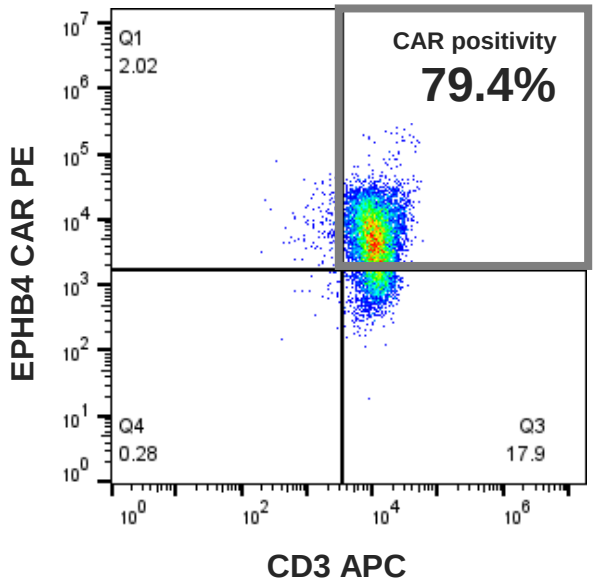
“ Our piggyBac CAR-T cells show superior resilience, efficiency, and longevity compared to viral CAR-T therapies. “

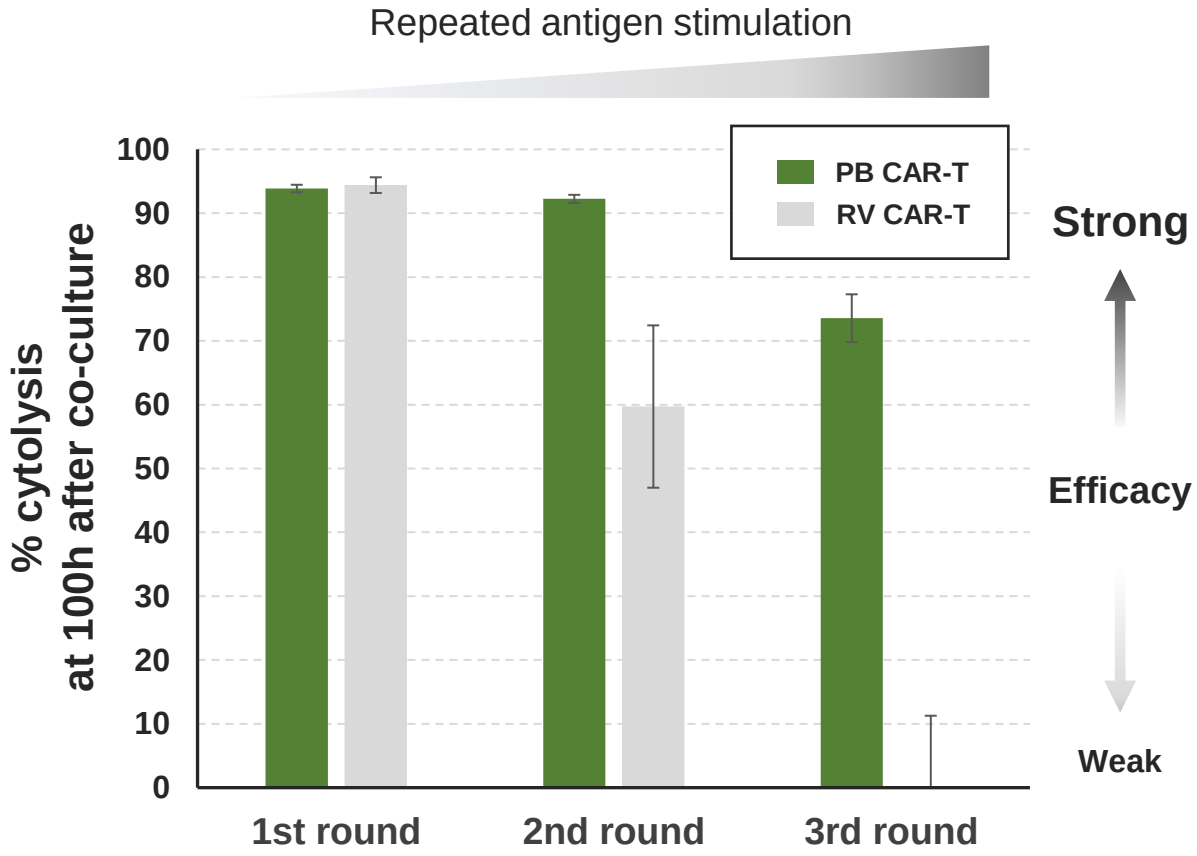
PB CAR-T cells showed superior anti-tumor efficacy than retroviral CAR-T cells in multiple tumor re-challenges

PB-transposon EPHB4 CAR-T cells (AP8901)



Retroviral EPHB4 CAR-T cells

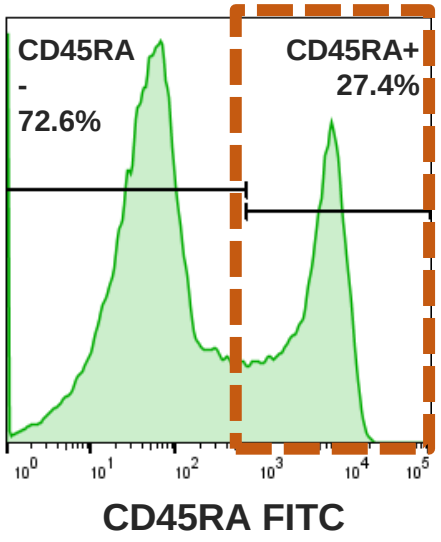




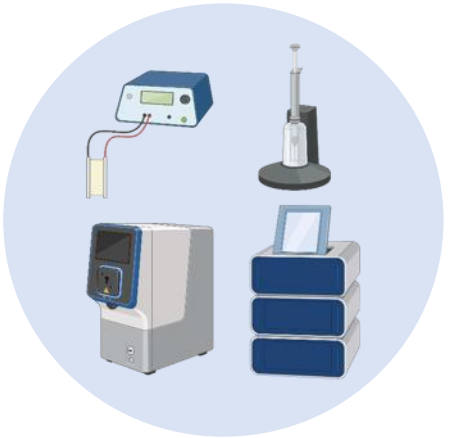
(Co-culture at E:T ratio of 2:1 in each round)

Our manufacturing system enriched stem cell memory-like CAR-T cells

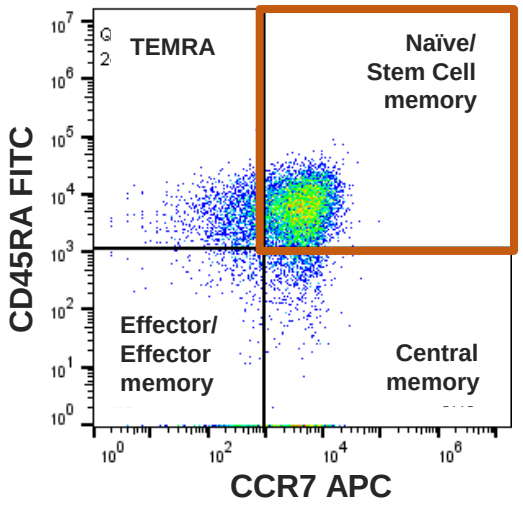
Fresh PBMC
Before electroporation



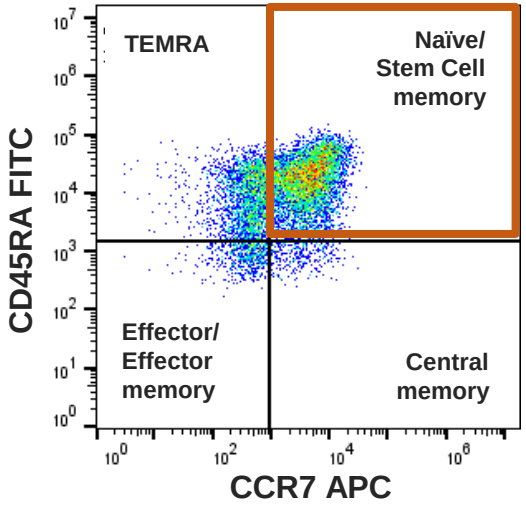
CAR-T manufacturing
- Electroporation
- Expansion



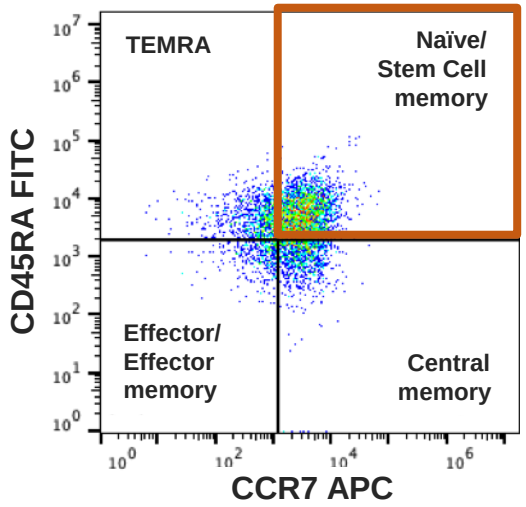
CD19 CAR-T cells



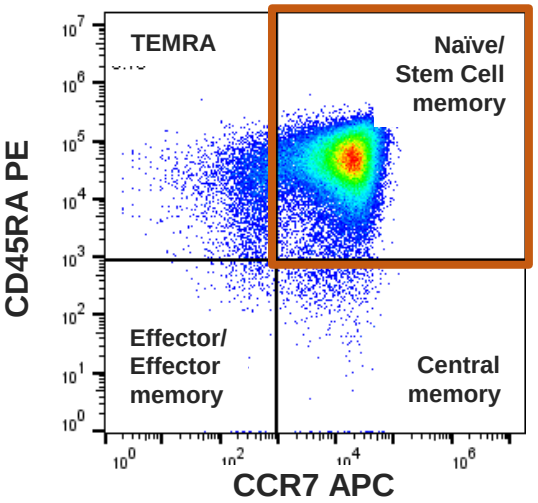
GD2 CAR-T cells



EPHB4 CAR-T cells



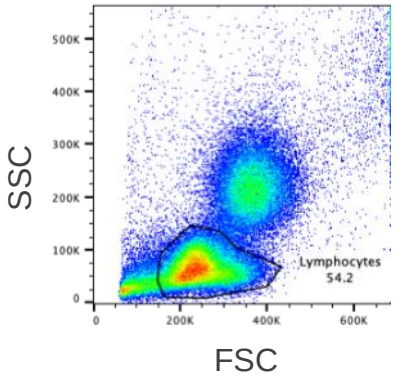
FLT3 CAR-T cells



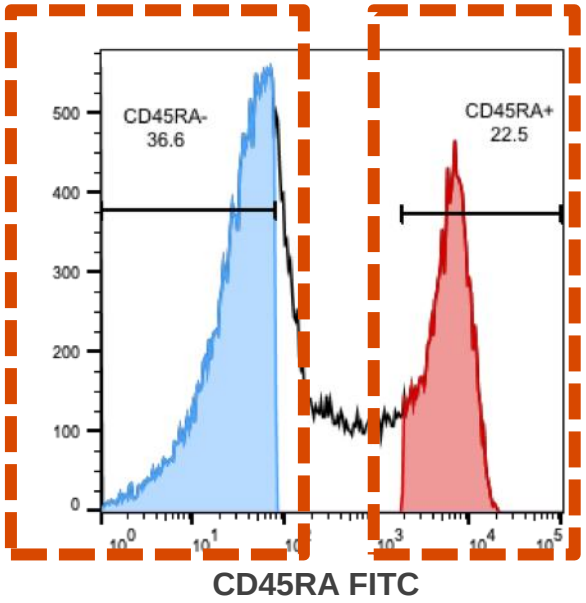
(Tomida A et al. Cancer Sci. 2021, Kubo H et al. Mol Ther Oncolytics. 2021, Suematsu M et al. Front Immunol. 2022)

CD45RA+ PBMC-derived CAR showed higher transduction capacity

CD45RA+/- PBMC sorting



CD45RA-　　CD45RA+

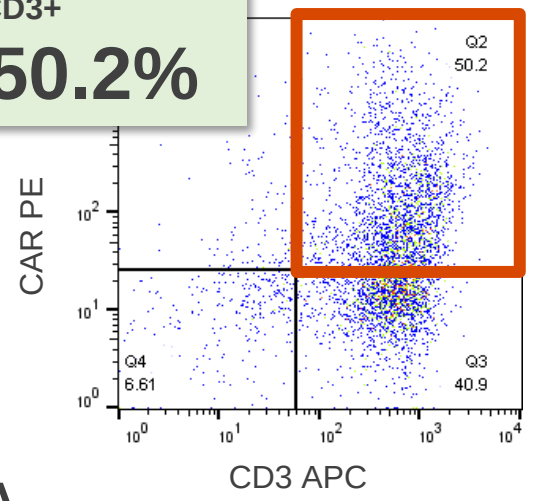


PB CD19 CAR-T cell manufacturing

CD45RA+

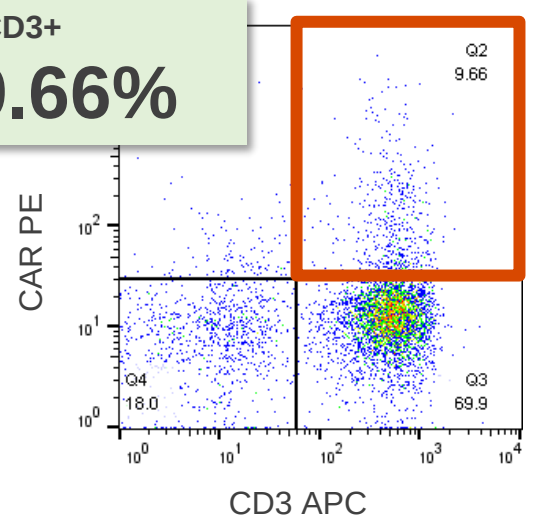
24 hours after EP

CAR+/CD3+
50.2%

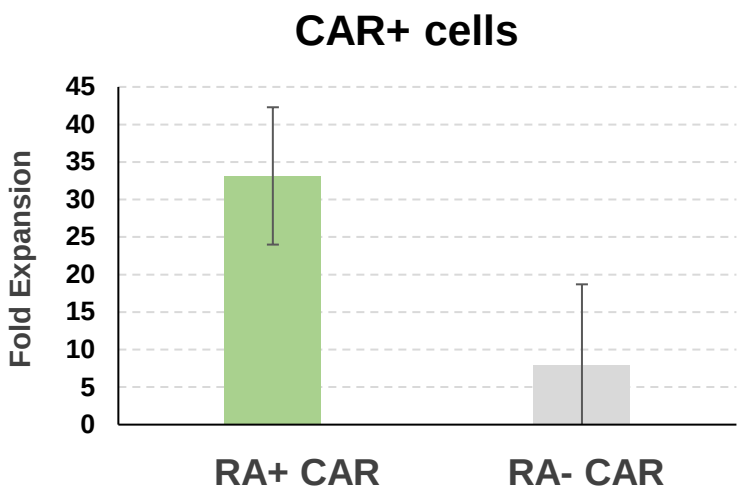


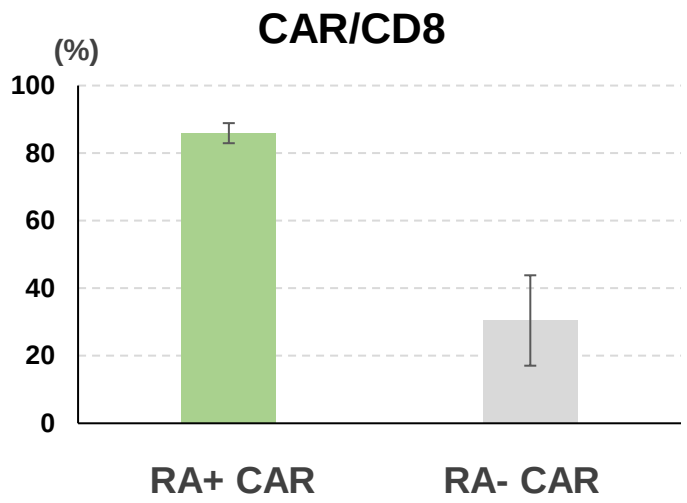
CD45RA-

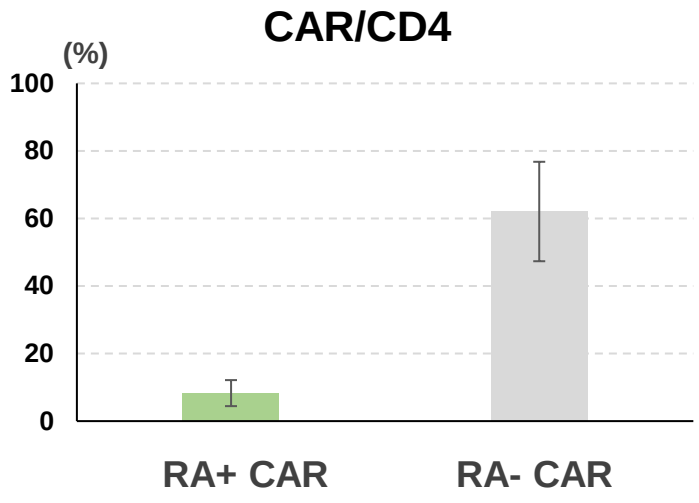
CAR+/CD3+
9.66%

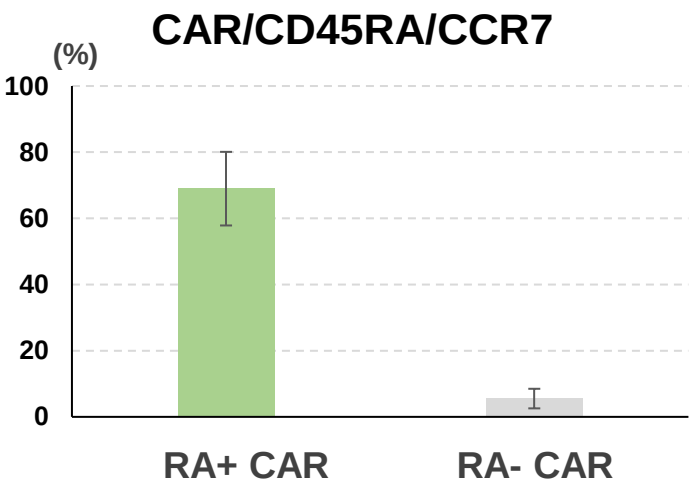


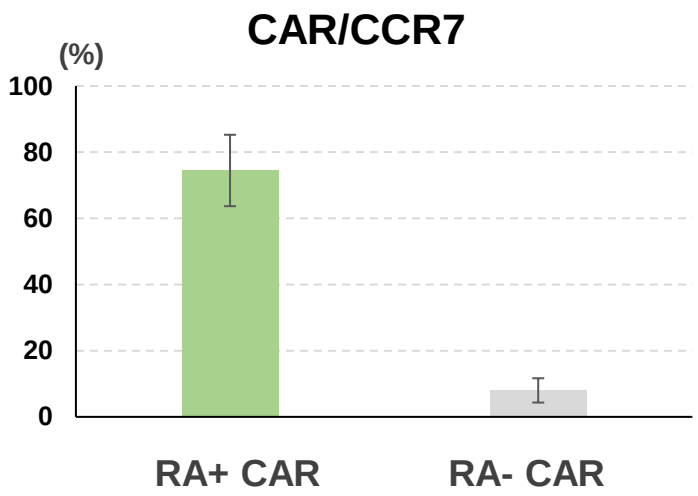
Tscm phenotype was enriched in RA+ CAR-T cells

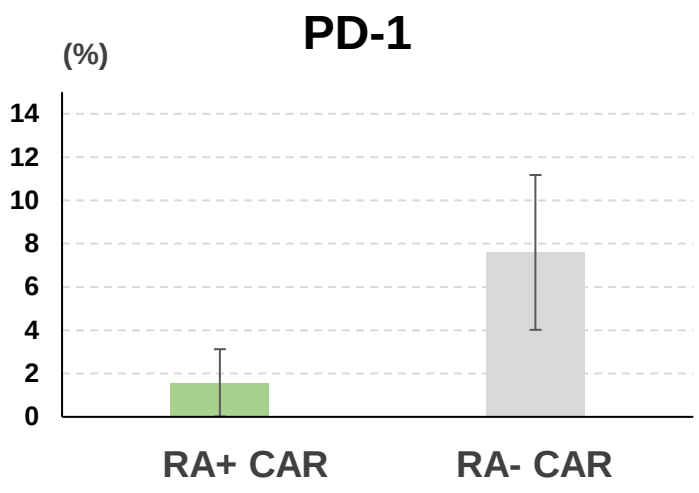






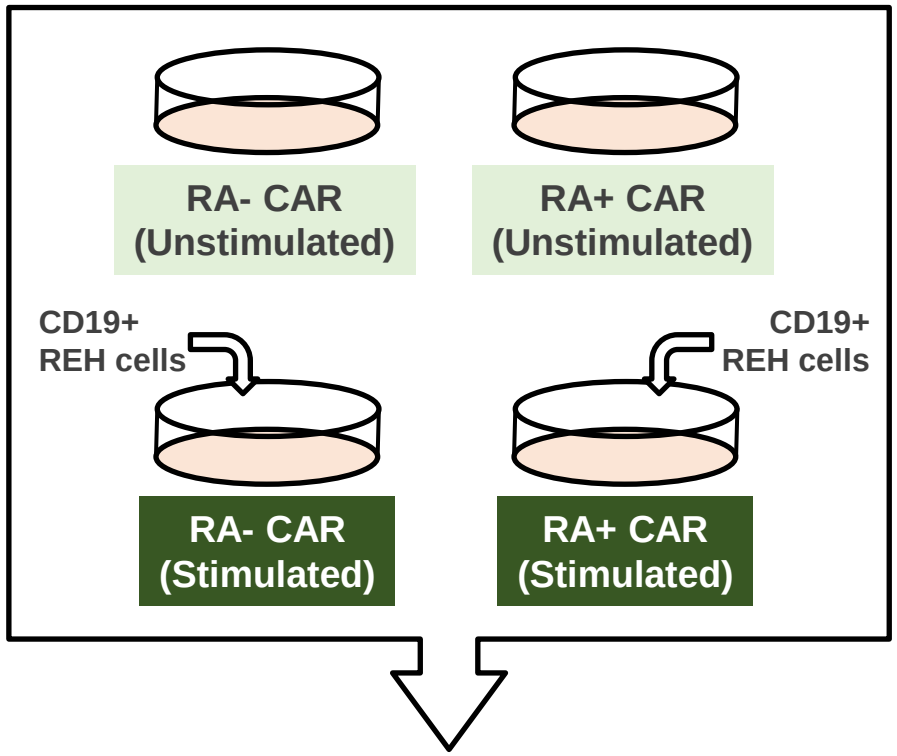


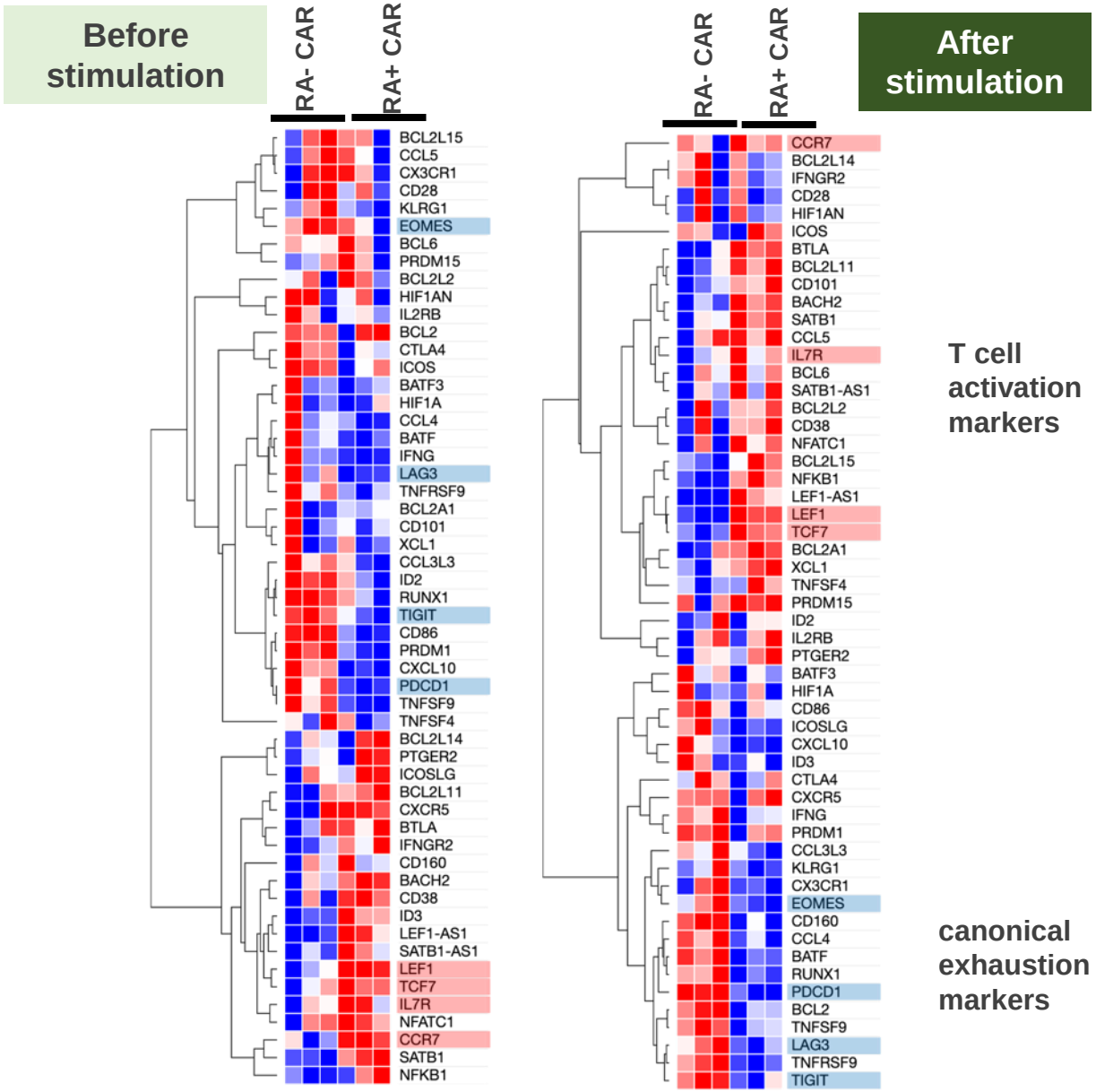




(Suematsu M et al. Front Immunol. 2022)

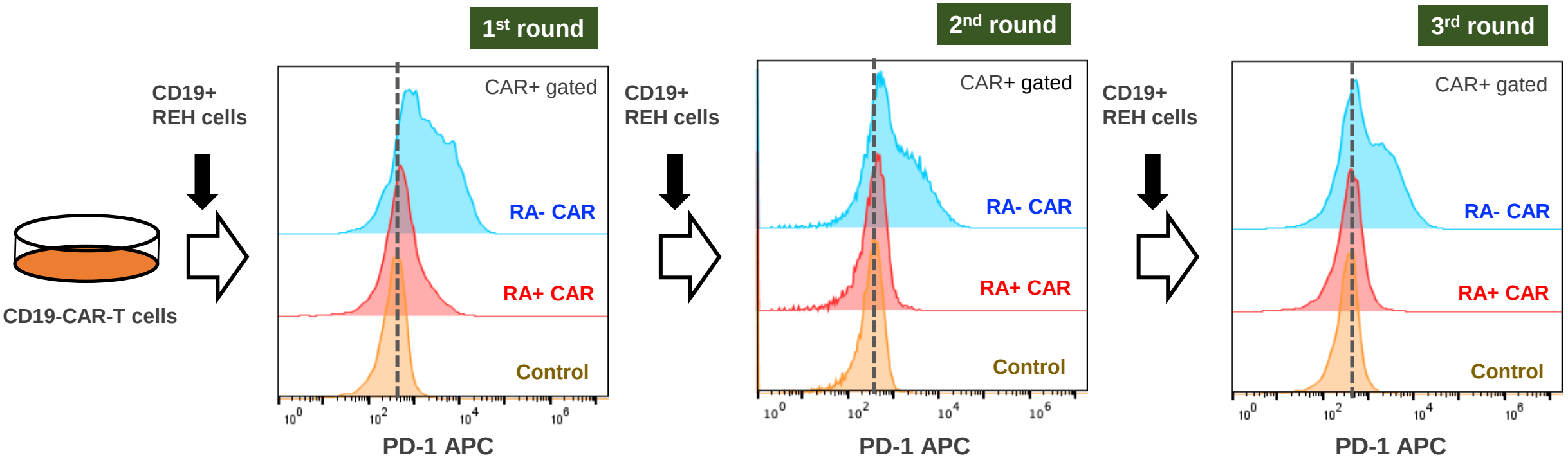
RA+ CAR-T cells were less exhausted after antigen exposure





RA+ CAR-T cells were less exhausted by serial antigen stimulation

PD-1 expression on CAR-T cells during multiple tumor re-challenges



(Suematsu M et al. Front Immunol. 2022)

RA+ CAR-T cells exhibited superior tumor control in xenogradt model

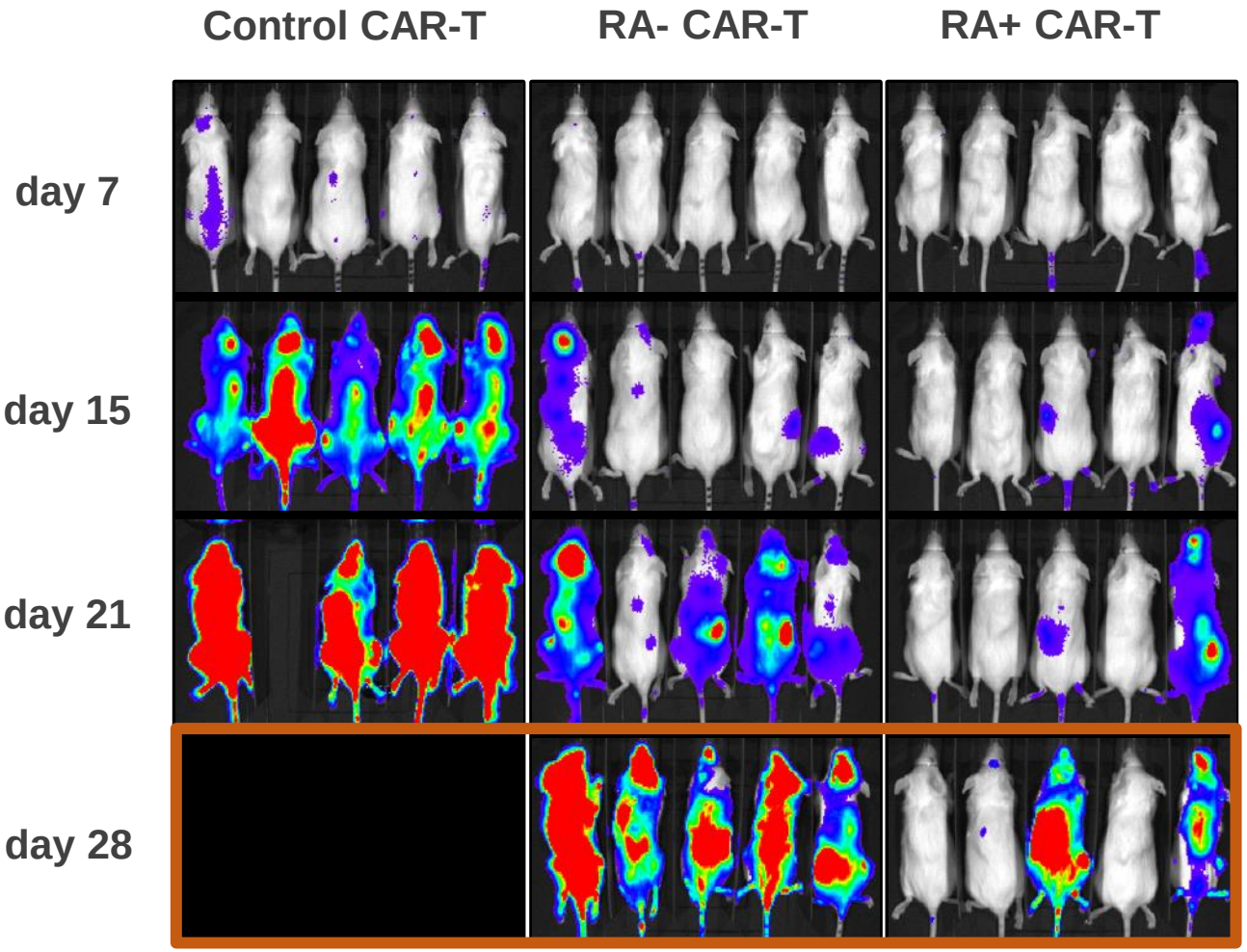
“*in vivo* stress test”

Reh-FFLuc ($5x10^5$)
i.v.
▼
day -6

- Control CAR-T ($1x10^5$)
- RA- CAR-T ($1x10^5$)
- RA+ CAR-T ($1x10^5$)

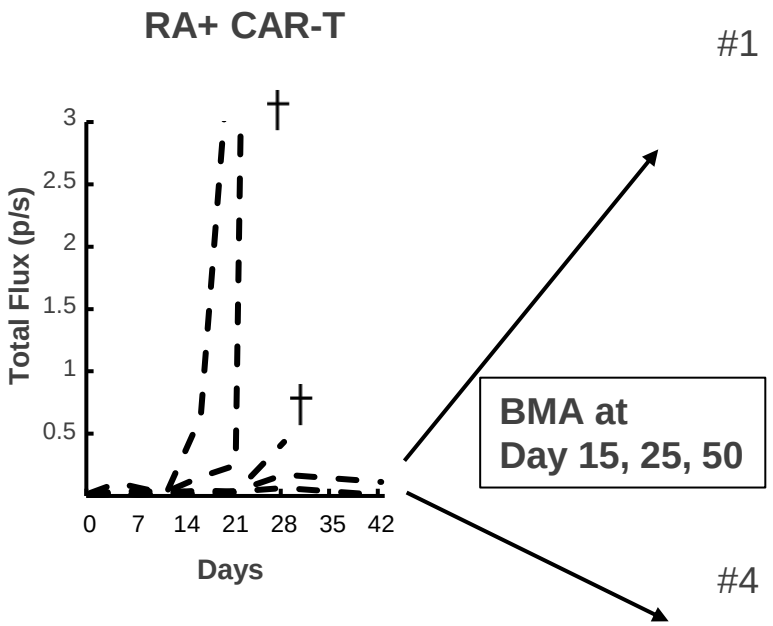
i.v.
▼
day 0

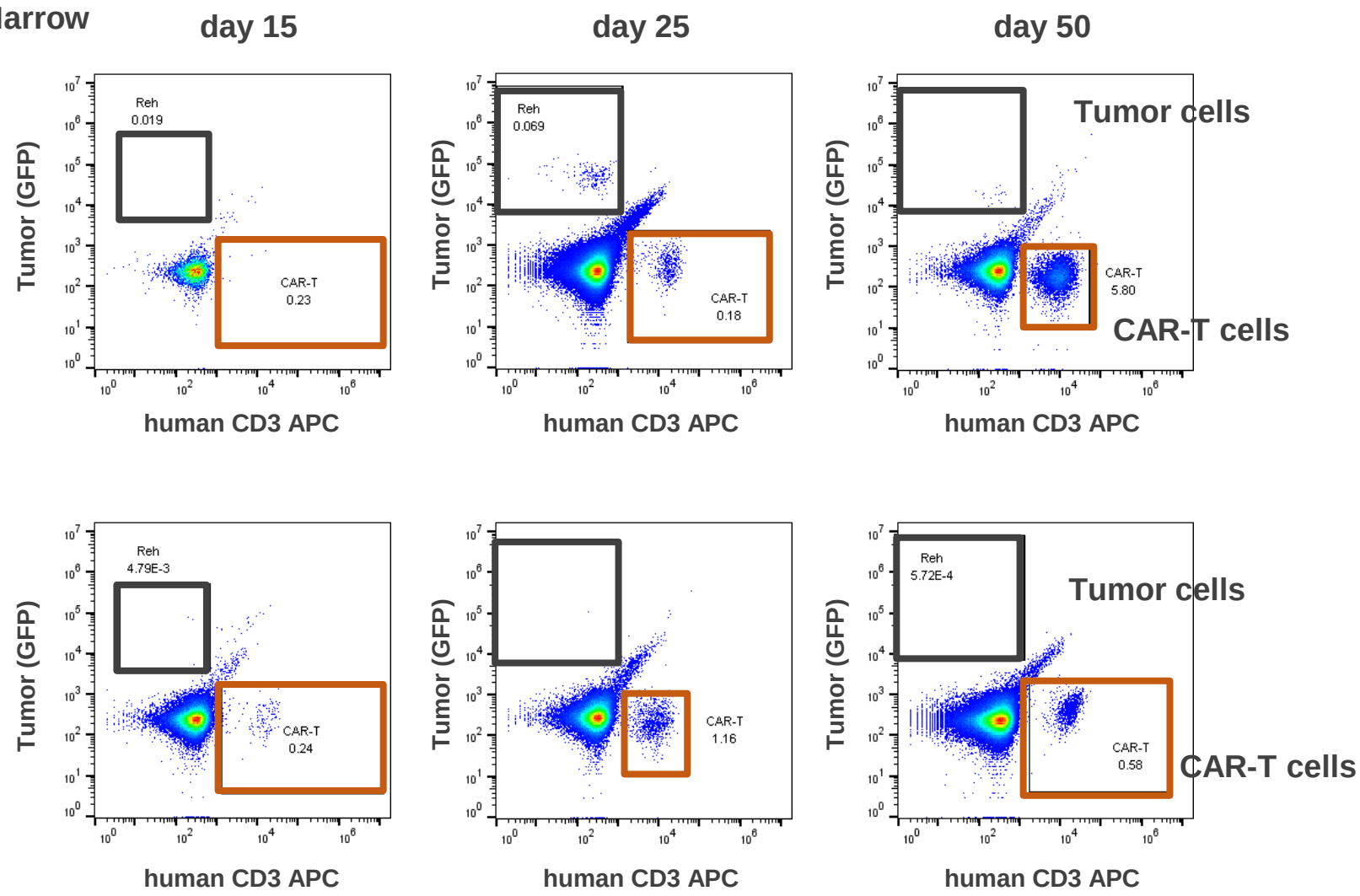
Monitoring
- IVIS imaging
- CAR expansion in bone marrow



(Suematsu M et al. Front Immunol. 2022)

RA+ CAR-T cells expanded and persisted *in vivo*

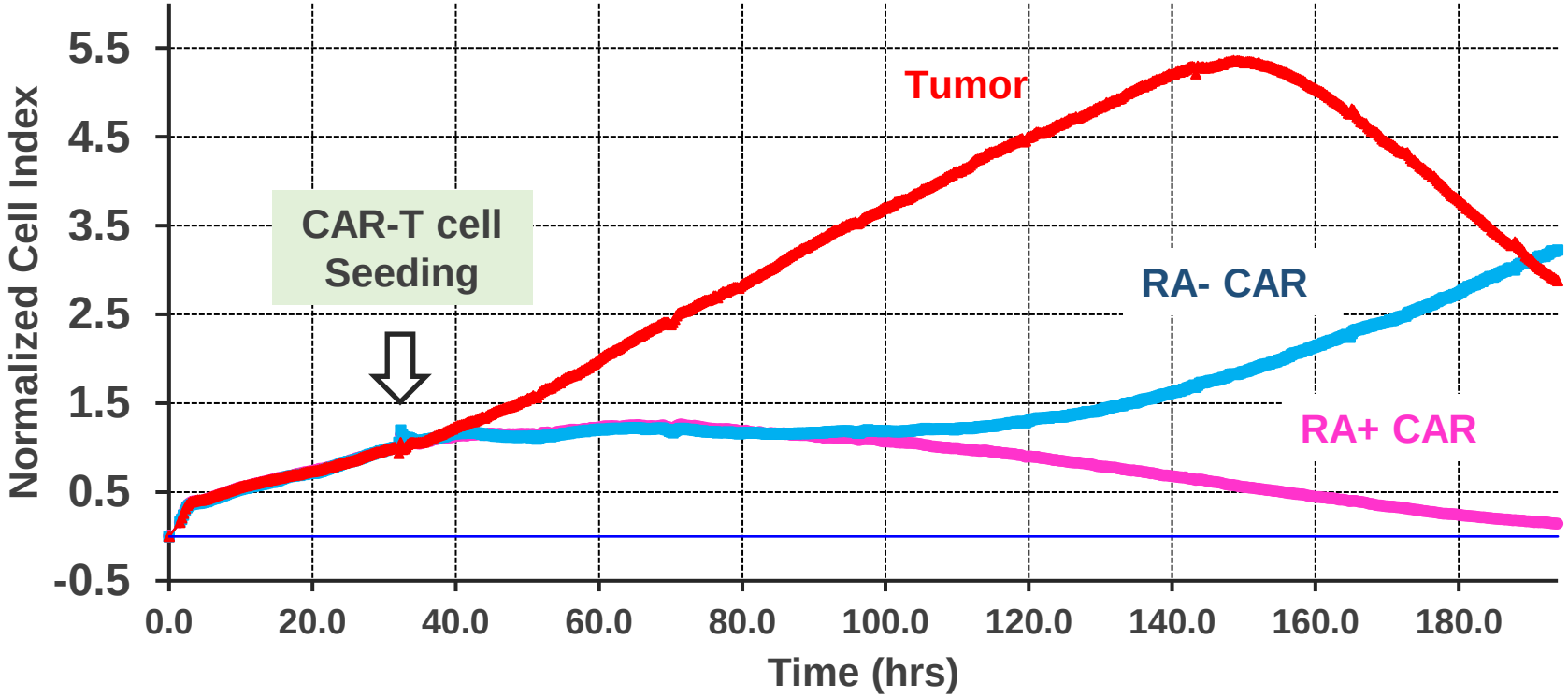


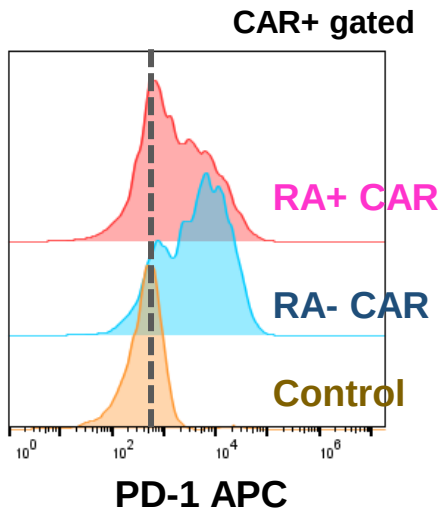


(Suematsu M et al. Front Immunol. 2022)

RA+ CAR-T cells outperformed RA- CAR-T cells in sarcoma model

Real time co-culture of EPHB4+ sarcoma cells with RA+ or RA- EPHB4 CAR-T cells





T cell composition in the starting materials determines the quality of PB CAR-T cells

Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products

Guidance for Industry

B. Collection, Handling, and Testing of Cellular Starting Material

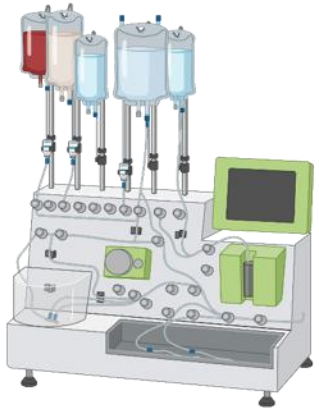
Due to patient or donor variability, the cellular starting material can represent a major source of lot-to-lot variability in CAR T cell quality and function. The probability of manufacturing success may be increased by establishing acceptance criteria for the leukapheresis starting material used in CAR T cell manufacturing, as experience is gained throughout product development. For example, you may specify a minimum cell number, viability, and percent CD3+ cells. To aid in manufacturing failure investigations, we recommend that you test the leukapheresis starting material for microbial contamination (e.g., sterility or bioburden) prior to initiating CAR T cell manufacturing or that you retain a sample for post hoc testing in the event of a DP sterility test failure. Additional characterization of the leukapheresis starting material (e.g., for percent and absolute number of CD4+ and CD8+ T cells, NK cells, monocytes, B cells) may inform the CAR T cell manufacturing process as these characteristics may influence T cell selection and expansion and final CAR T cell quality (Refs. 21, 22, 23).

- ✓ The composition of T cells in the starting materials is critical for determining the manufacturing efficiency and quality of ***piggyBac*** transposon CAR-T cell products.
- ✓ **Key Question 1:**
  What methods can be used to effectively regulate T cell composition in patient-derived samples?

CD45RA^{+} PBMCs are favorable for manufacturing Tscm-like CAR-T cells

1. Leukapheresis

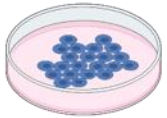
2. CD45RA PBMC isolation



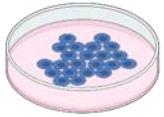
CD45RA-

CD45RA+ PBMC

3. CAR-T manufacturing



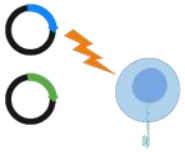
For CAR-T manufacturing



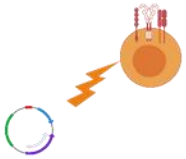
For feeder cell manufacturing

4. Electroporation

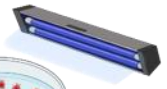
CAR-T cells



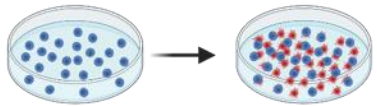
Feeder cells



5. Inactivation



6. Stimulation



7. Ex vivo expansion

8. Cryo-preservation

Today’s topic

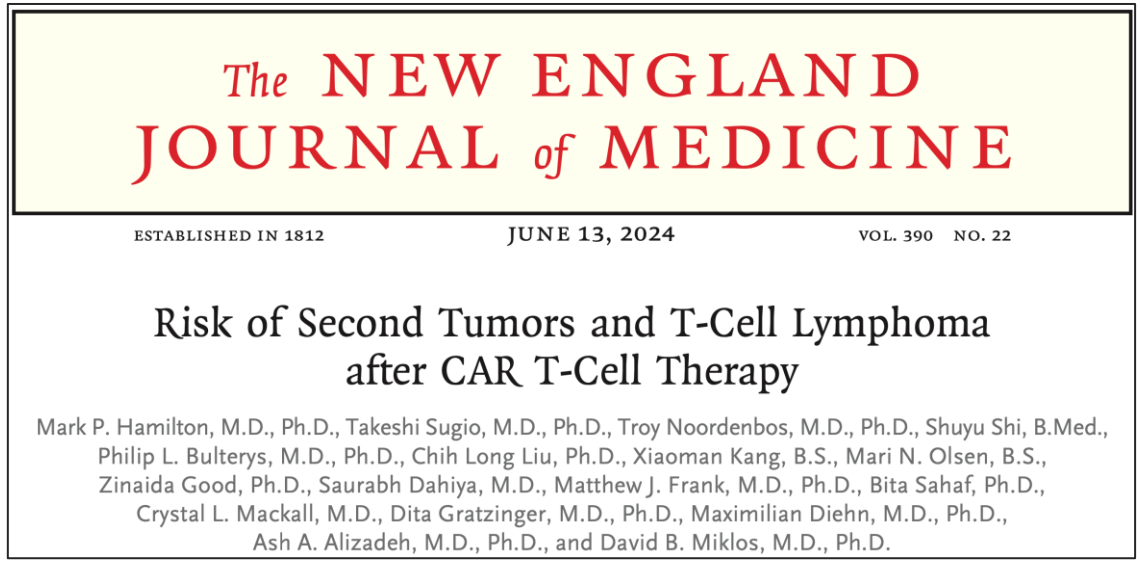
1. T cell composition in the starting materials

- The composition of T cells in the starting materials is critical for determining the manufacturing efficiency and quality of ***piggyBac*** transposon CAR-T cell products.
- **Key Question 1:** What strategies can be employed to optimize T cell composition in patient-derived samples, ensuring manufacturing success and the high quality of final CAR-T cell products?

2. CAR-T cell driven second malignancies

- Multiple factors, including vector insertion and pre-existing malignant clones, may be associated with CAR-T driven second malignancies.
- **Key Question 2:** What are the key considerations for establishing robust vector copy number (VCN) criteria for piggyBac transposons to ensure long-term safety?

Long-term follow-up on second tumors after CAR-T therapy



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Risk of Second Tumors and T-Cell Lymphoma after CAR T-Cell Therapy

Mark P. Hamilton, M.D., Ph.D., Takeshi Sugio, M.D., Ph.D., Troy Noordenbos, M.D., Ph.D., Shuyu Shi, B.Med., Philip L. Bulterys, M.D., Ph.D., Chih Long Liu, Ph.D., Xiaoman Kang, B.S., Mari N. Olsen, B.S., Zinaida Good, Ph.D., Saurabh Dahiya, M.D., Matthew J. Frank, M.D., Ph.D., Bita Sahaf, Ph.D., Crystal L. Mackall, M.D., Dita Gratzinger, M.D., Ph.D., Maximilian Diehn, M.D., Ph.D., Ash A. Alizadeh, M.D., Ph.D., and David B. Miklos, M.D., Ph.D.

Study Overview

- ✓ **Participants:** 724 patients treated with CAR-T cell therapy
- ✓ **Focus:** Identification of second cancers post-CAR-T therapy
- ✓ **Findings:** **25 cases of second cancers** detected (Solid tumor: 11 cases, Hematological malignancies: 14 cases)
- ✓ 1 suspected **CAR-T-derived T-NHL** cases

T-cell Non-Hodgkin Lymphoma (T-NHL) case

- **Patient:** 59-year-old woman
- **Timeline:** T-NHL developed 54 days post-CAR-T infusion
- **Suspected Cause:** CAR-T-derived T-NHL
  - T-NHL clone was detected at low levels in the patient's blood before CAR-T therapy
  - **Pre-existing clonal hematopoiesis** and its malignant transformation during CAR-T cell therapy.

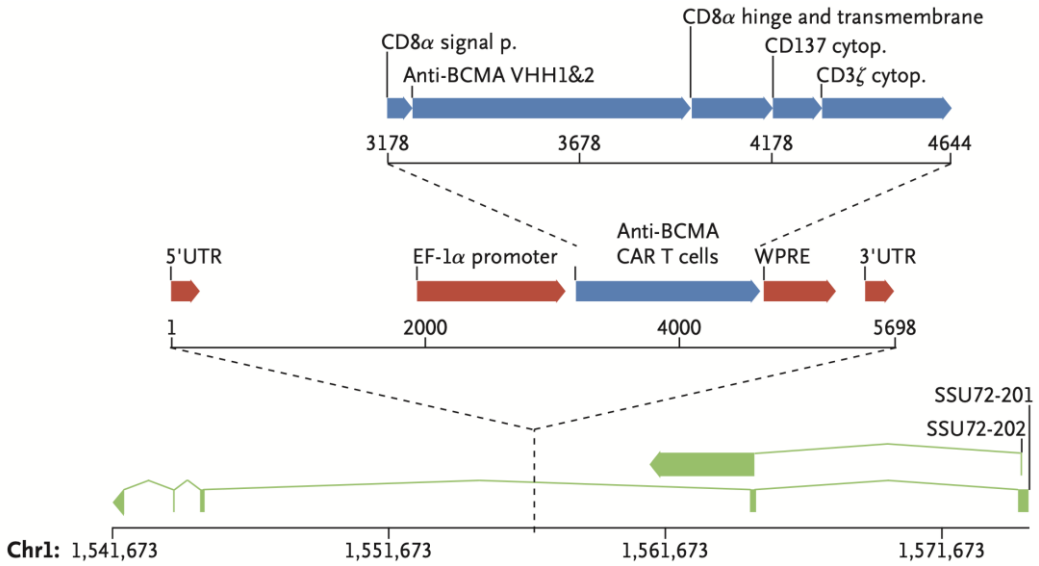
(Hamilton MP et al. N Eng J Med. 2024)

Secondary CAR-T driven T-NHL

BRIEF REPORT

Indolent CD4+ CAR T-Cell Lymphoma after Cilta-cel CAR T-Cell Therapy

Metin Ozdemirli, M.D., Ph.D., Thomas M. Loughney, M.D., Emre Deniz, Ph.D., Joeffrey J. Chahine, Ph.D., Maher Albitar, M.D., Stefania Pittaluga, M.D., Sam Sadigh, M.D., Philippe Armand, M.D., Ph.D., Aykut Uren, M.D., and Kenneth C. Anderson, M.D.



Overview

- ✓ **Indolent lymphoma diagnosed 5 months post-CAR-T infusion** with persistent monoclonal CD4+ T-cell infiltrates.

Molecular Analysis

- ✓ **Lentiviral vector integration into the SSU72 gene** identified as a potential driver of lymphomagenesis.
- ✓ **High levels of CAR T cell-specific RNA fusion transcripts** detected in tumor cells.
- ✓ **Numerous genetic alterations** that may have contributed to malignant transformation

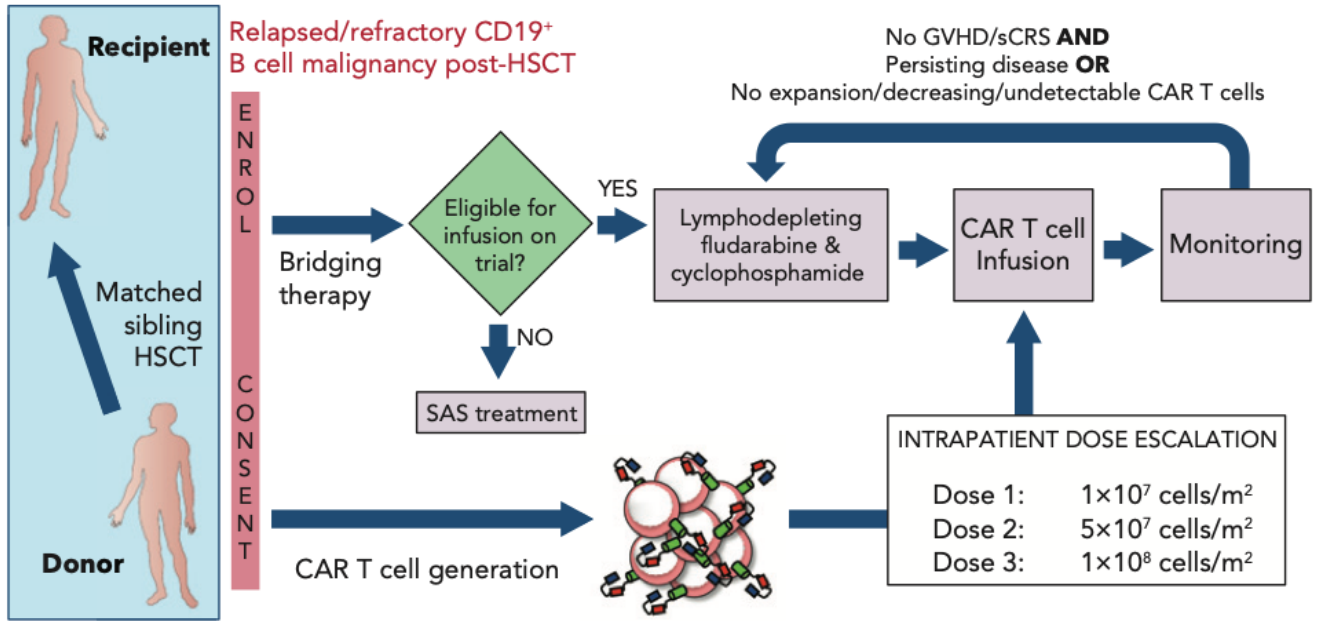
Conclusion

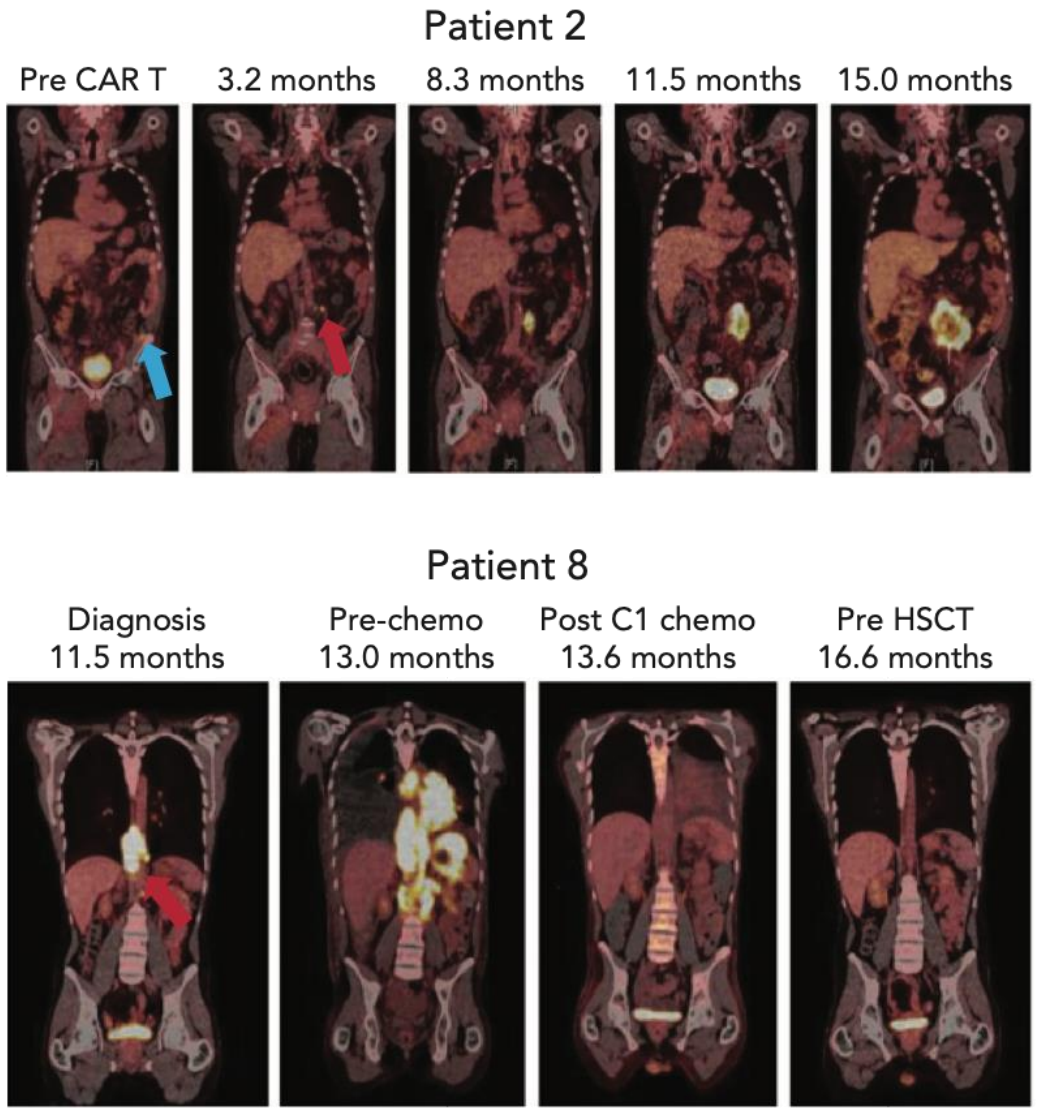
- ✓ The potential for secondary malignancies associated with **lentiviral-based CAR-T products**.

(Ozdemirli M et al. N Eng J Med. 2024)

CAR-T cell-derived lymphoma in PB CD19-CAR clinical trial

CARTELL study





(Micklethwaite KP, el al. Blood. 2021, Biship DC, et al. Blood. 2021)

Product-derived lymphoma in PB-CD19-CAR (CARTELL) trial

Parameters	Strategies
Cell Type	- **Allogeneic PBMC** no chemo-induced or disease-specific factors
Transgene	- CD19-CAR with 4-1BB co-stumulation - **EF1α** promoter
Transposase	- **Hyperactive form PB transposase (Super PB) mRNA** achieved higher genome integration
Electroporation	- **Single, and high voltage pulse**
Post-activation	- Donor-derived irradiated PBMC feeder
Expansion	- AIM-V with human serum/IL-15 supplementation - 15-23 days of expansion

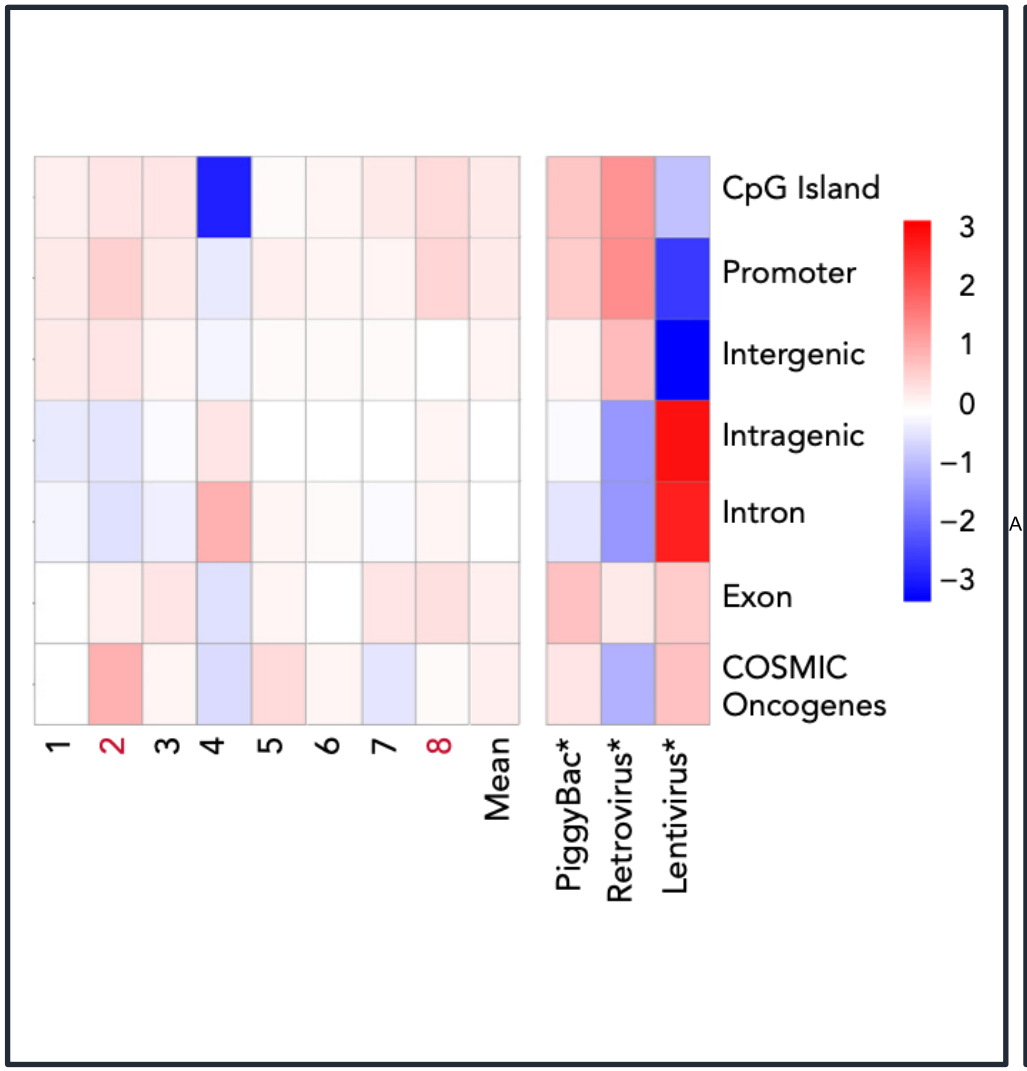
Product-derived lymphoma

Observed in lymphoma cells

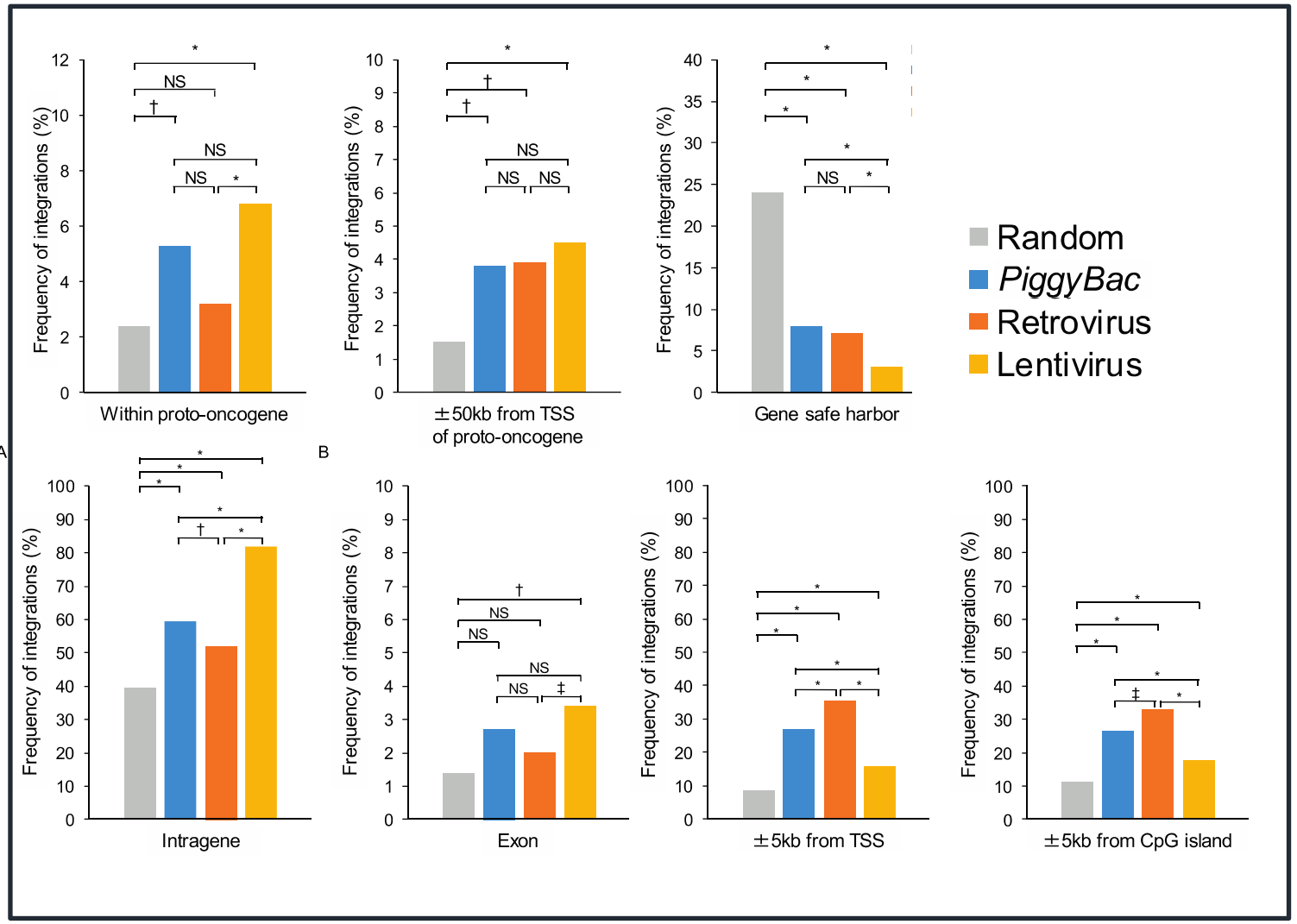
- **High number of CAR transgene integration**
- **Structual variant and copy number alteration**
- **Transcriptional readthrough with 4 genes (FAM11D, COL8A1, HIVEP1, and FYN)**
- **CAR transgene integration in *BACH2* locus**

(Micklethwaite KP, el al. Blood. 2021, Biship DC, et al. Blood. 2021)

Similar Integration Mapping in PB- and Retro- CAR-T cells



(Micklethwaite KP, el al. Blood. 2021)



(Hamada M, et al. eBioMedicine. 2018)

CAR-T derived T cell malignancies: potential mechanisms

1. **Vector integration**
   - The vector used to engineer CAR T-cells could potentially integrate into oncogenic regions of the T-cell genome
2. **Pre-existing malignant clones**
   - Pre-malignant cells or genetic predispositions in patients before CAR-T cell therapy, suggesting that the treatment may accelerate or unmask existing oncogenic potential
3. **EBV association**
   - The involvement of Epstein-Barr virus in some cases points to a potential role of viral-driven lymphoproliferation
4. **Genetic factors**
   - Mutations in genes such as DNMT3A and TET2, associated with clonal hematopoiesis, have been observed in some cases.

CAR-T derived T cell malignancies: vecter insertion and malignant transformation

b. Vector Copy Number (VCN)

Vector integration can potentially alter expression of cellular genes and contribute to tumorigenicity (Refs. 31 and 32). Therefore, vector integration in the DP is an important safety attribute to measure for CAR T cell release. For integrating vector systems, the average number of integrations per CAR-positive cell, generally referred to as VCN, should be determined and reported on the Certificate of Analysis (COA) for each lot. Determining VCN as a function of total cells includes CAR-negative cells in the denominator and lowers the reported vector integration rate.

Using the percentage of CAR-positive cells, the average VCN per CAR-positive cell can be calculated. VCN as a function of CAR-positive cells will provide a more accurate representation of the VCN in modified cells and thus a more accurate representation of product risk for insertional mutagenesis. We recommend that the manufacturing process be optimized to control VCN while meeting the target CAR-positive cell frequency.

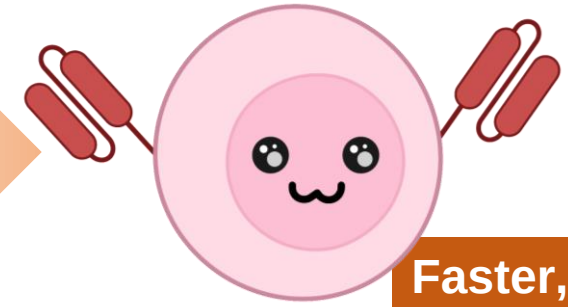
We recommend that the VCN release criterion be justified based on a risk assessment. The risk assessment may include supporting data from studies such as insertion site analysis, clonal dominance, dose, indication, study population, etc. Supporting experimental data may be obtained from developmental and engineering manufacturing runs.

- ✓ Multiple factors, including vector insertion and pre-existing malignant clones, may be associated with CAR-T driven second malignancies.
- ✓ **Key Question 2:**
  What are the key considerations for establishing robust vector copy number (VCN) criteria for piggyBac transposons to ensure long-term safety?

Long-term safety assessment of CAR-T cell products

Unexpected malignant potential

- ✓ Optimization of manufacture
- ✓ Safety assessment for the final product
- ✓ Design of trial to monitor the safety issue



Faster, cheaper and "SAFER" CAR

Parameters	Strategies
Genotoxicity - **High Copy Numbers** - **Harsh electroporation condition** - **Promoter-induced readthrough**	✓ Reduce the transgene copies (use of non-hyperactive transposase ?) ✓ Optimization of electroporation condition ✓ Optimization of promoter/enhancer
Insertional dysregulation - **Disruption of proto-oncogene**	✓ The transgene integration map (Tag-PCR based NGS) ✓ Development of site-specific transgene introduction
Monitoring of malignant transformation - **TCR repertoire assessment** - **Screening** - **Animal model ??**	✓ Check the possibility of monoclonal proliferation ✓ Lymphoma screening in early-phase clinical trial ✓ Development of reliable assessment tool for long-term safety

(Wilson MH et al, Blood 2021, Schambach A et al, Mol Ther 2021)

Conclusion

- ✓ The piggyBac transposon system redefines CAR-T cell therapy with improved scalability, efficiency, and cost-effectiveness.
- ✓ Optimized T cell composition significantly contributes to stable and robust manufacturing efficiency, ensuring consistent product quality.
- ✓ Leveraging stem-cell memory-like T cells enhances long-term efficacy and durability, critical for addressing solid tumors.
- ✓ Long-term safety monitoring through robust VCN criteria and advanced strategies remains essential for clinical success.

A-SEEDS

To redefine the landscape of cancer treatment and ignite a ray of hope for a brighter future,

WE HAVE THE SOLUTION.

#207 CSMIT 3-1-1 Asahi
Matsumoton Nagano Japan
390-8621

Official site

Linkedin

Contact