



Noninvasive positron emission tomography (PET) imaging of *Sleeping Beauty* (SB) modified CD19-specific T cells expressing HSV1-Thymidine kinase

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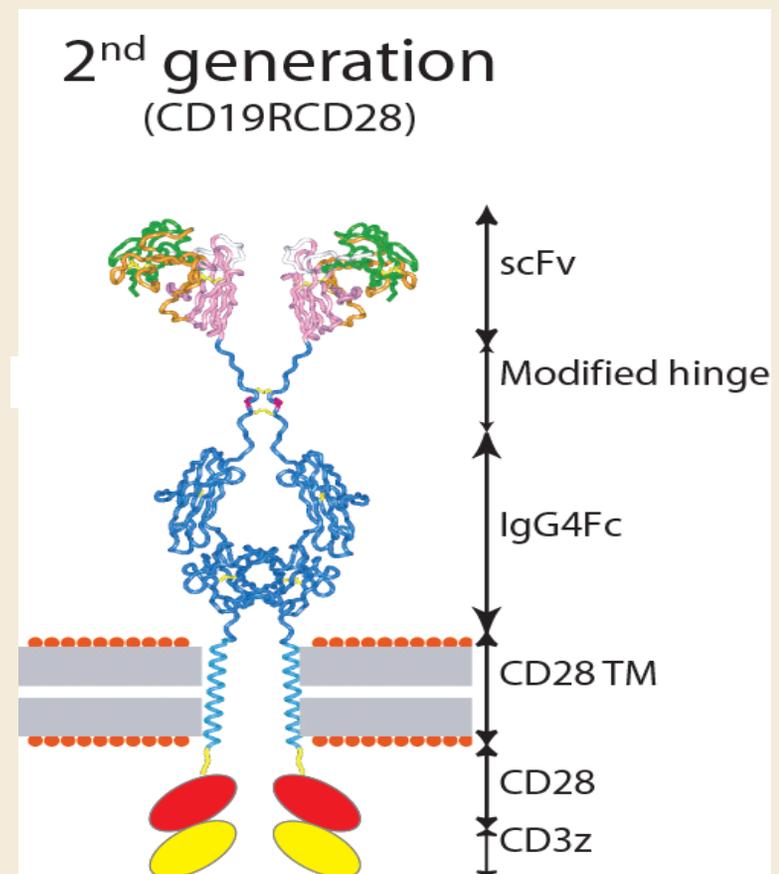
MD Anderson
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Making Cancer History®

Adoptive Cell Therapy

- Development of engineered T cells using
 - T-cell receptors
 - Chimeric antigen receptors (CARs)
- Methods of genetic modification
 - Viral
 - No-viral

CD19-specific CAR



Rationale

❖ However, to improve the design, application and evaluation of adoptive T-cell therapy requires monitoring methods that can

- Detect
- Locate and
- Serially quantify the cell-mediated immune responses

Rationale

Currently monitoring methods are chiefly invasive techniques

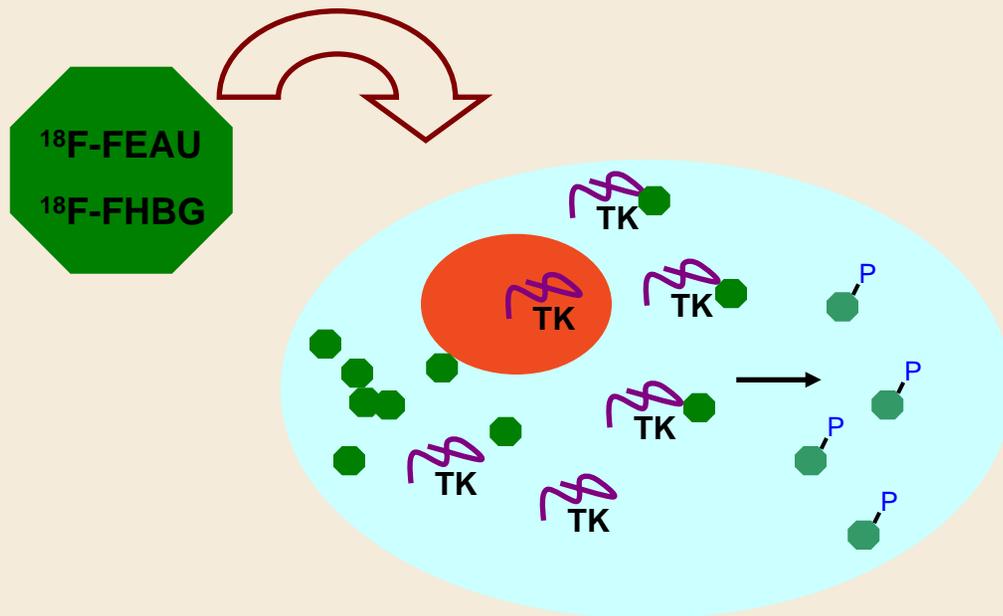
- Histology
- Flow cytometry
- Q-PCR and/cytokine analysis

In contrast, Positron emission tomography (PET) is a

- Noninvasive, accurate, and
- Sensitive whole-body imaging technology allowing
- Repetitive measurement *in vivo*

Herpes Simplex Virus 1- thymidine kinase (HSV1-tk)

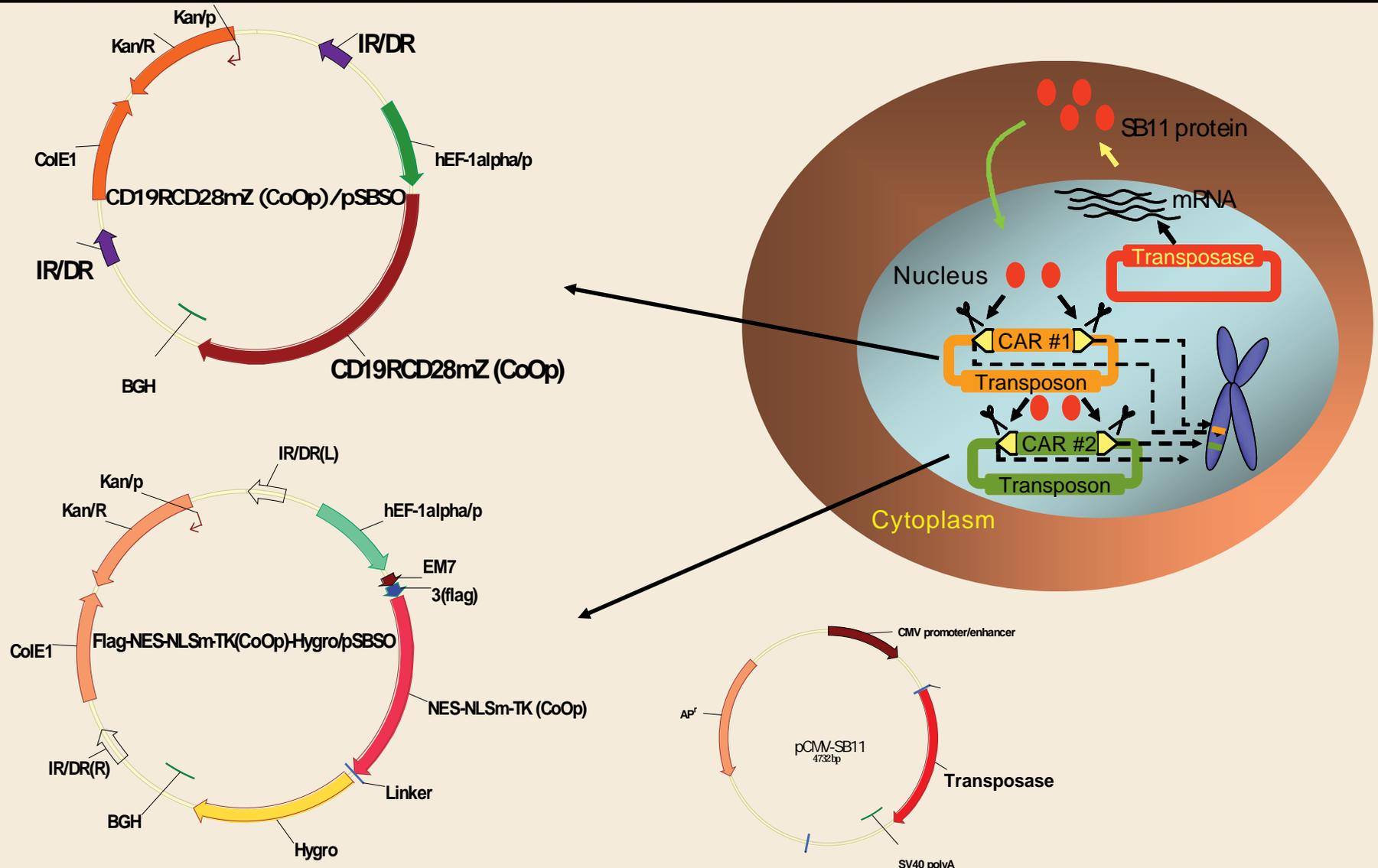
- Expression of reporter genes and use of corresponding reporter probes (radiotracers) labeled with positron-emitting radionuclides.



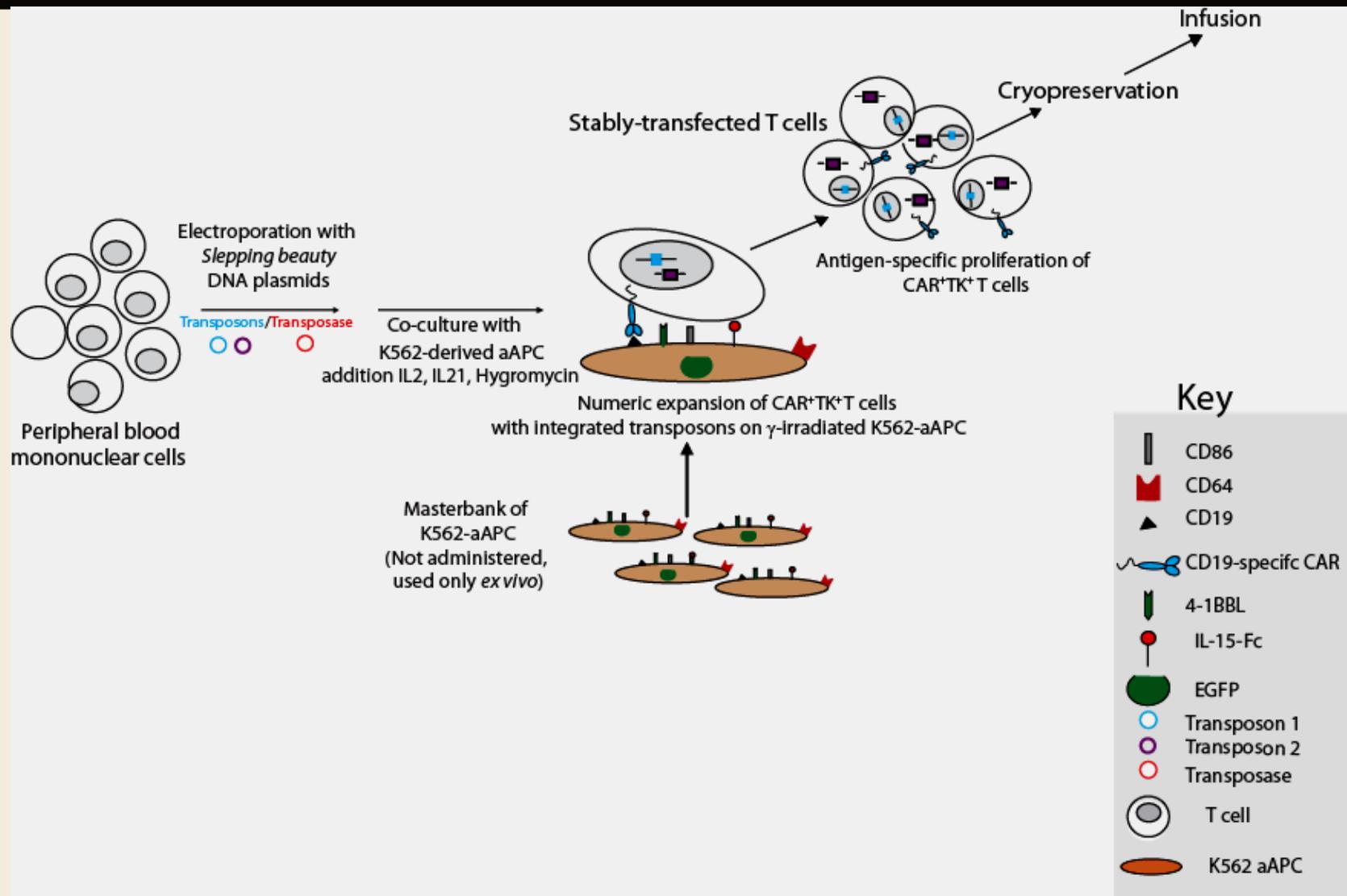
The specificity and/or sensitivity of HSV1-tk were altered by

- Mutations in the nucleoside binding region (HSV1-sr39tk)
- Inactivation of the nuclear localization signal (NLS) of HSV1-tk Arg25-26 were replaced by Gly25-26
- Addition of the nuclear export sequence (NES)

Generation of CD19-specific T cells capable of being imaged non-invasively by PET

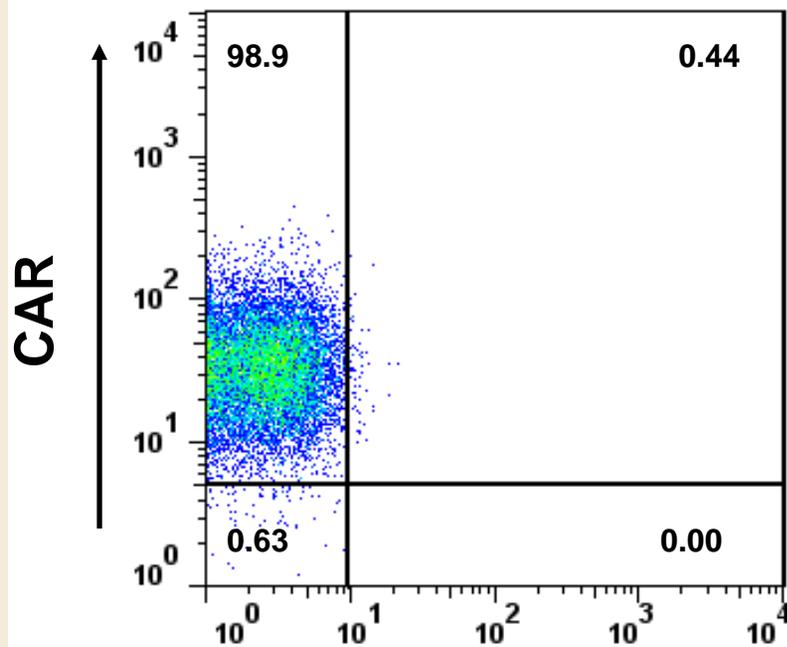


Scheme of expansion of T cells on artificial antigen presenting cells

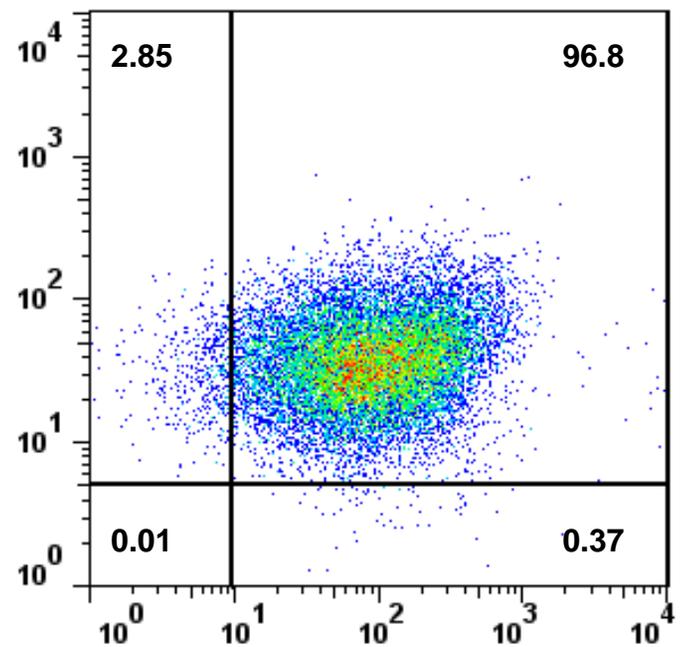


Co-expression of CD19-specific CAR and TK

CAR+TK-

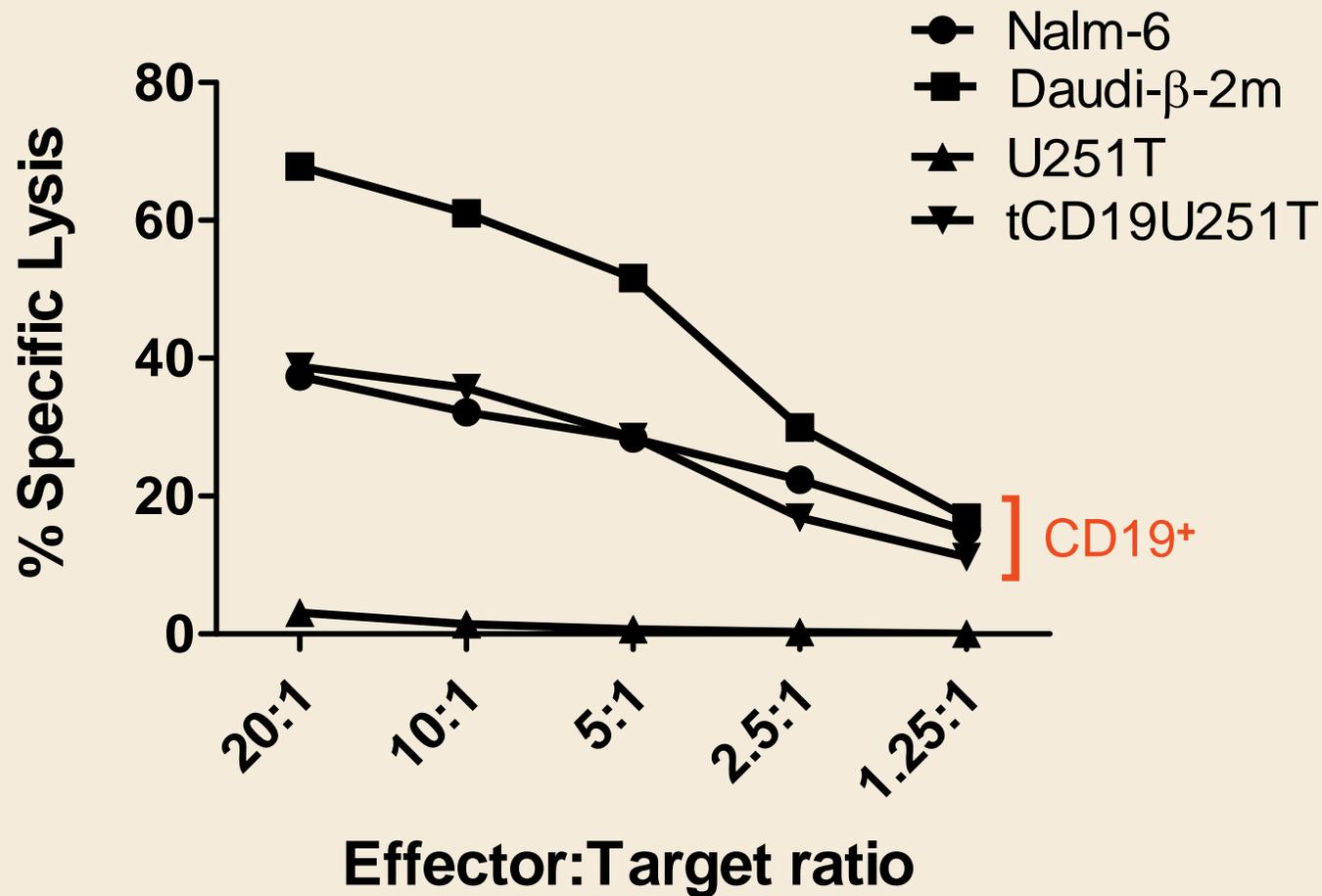


CAR+TK+



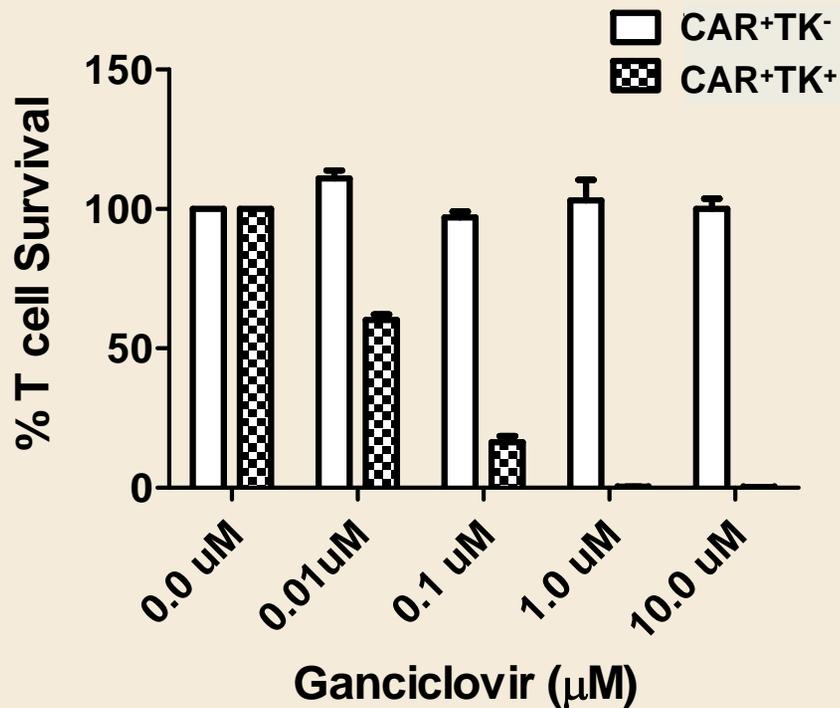
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Redirected specificity of CD19CAR+TK+ T cells

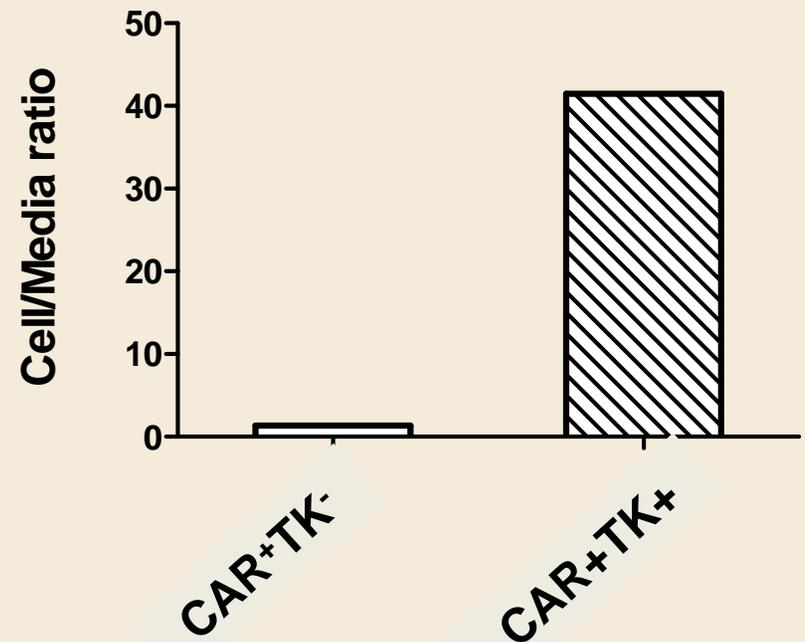


Specificity towards nucleoside analogs

Sensitivity towards Ganciclovir



In vitro accumulation of ³H-FEAU



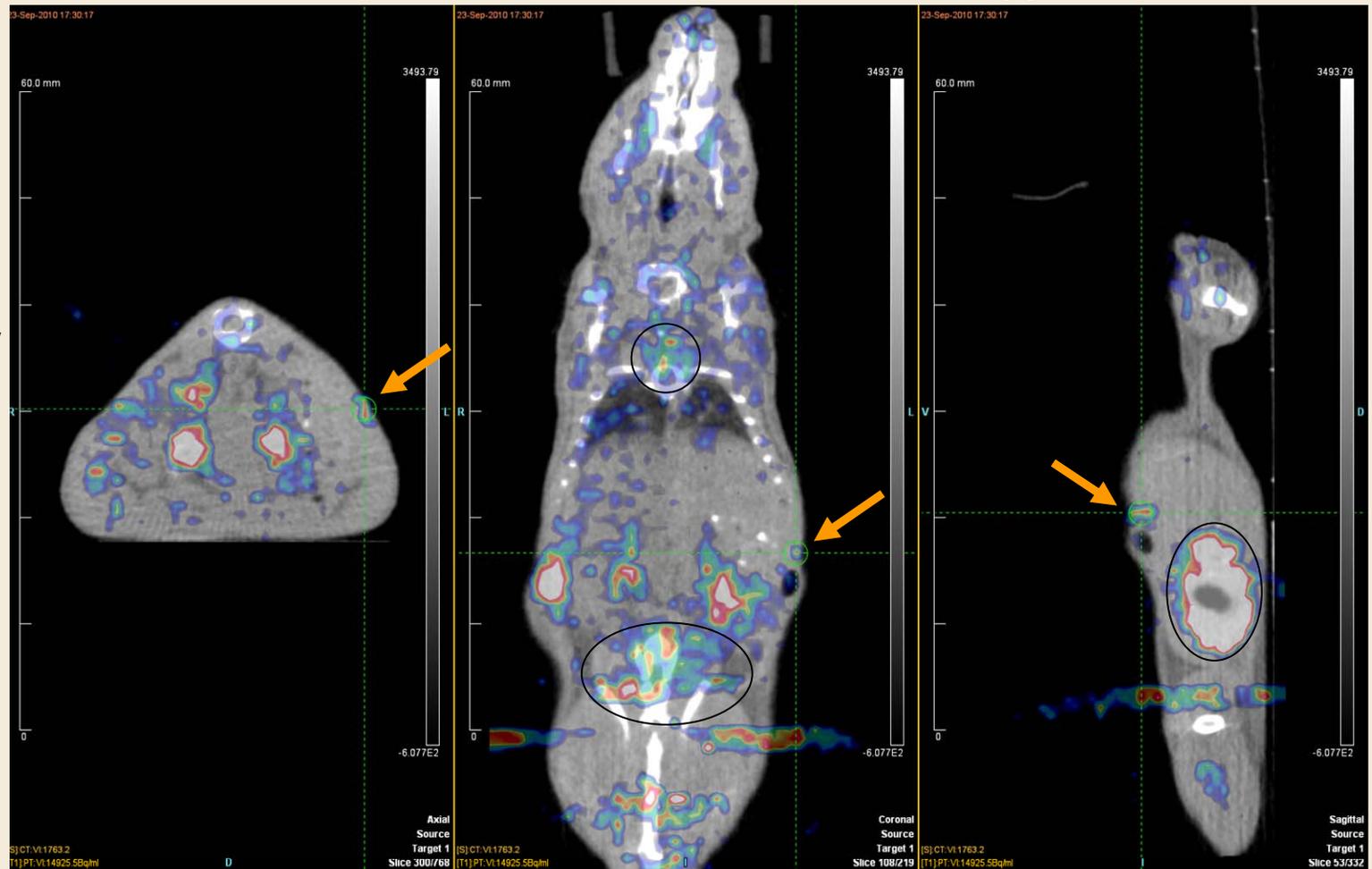
In Vivo Imaging of CD19CAR⁺TK⁺ T cells with PET

Axial view

Coronal view

Saggital view

7.5x 10⁶ T cells subcutaneously and 100 μ Ci of ¹⁸F-FEAU intravenously



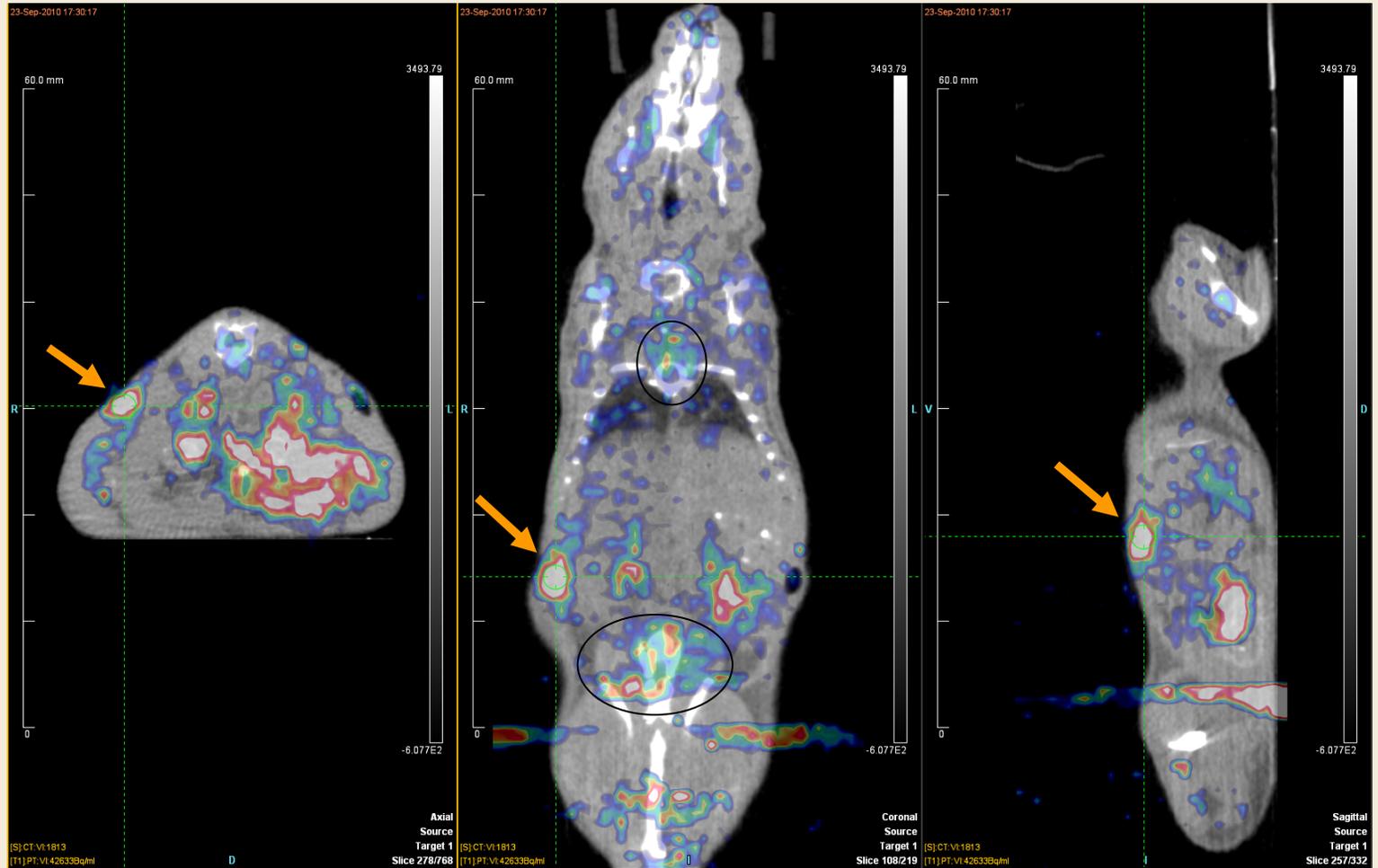
In Vivo Imaging of CD19CAR⁺TK⁺ T cells with PET

Axial view

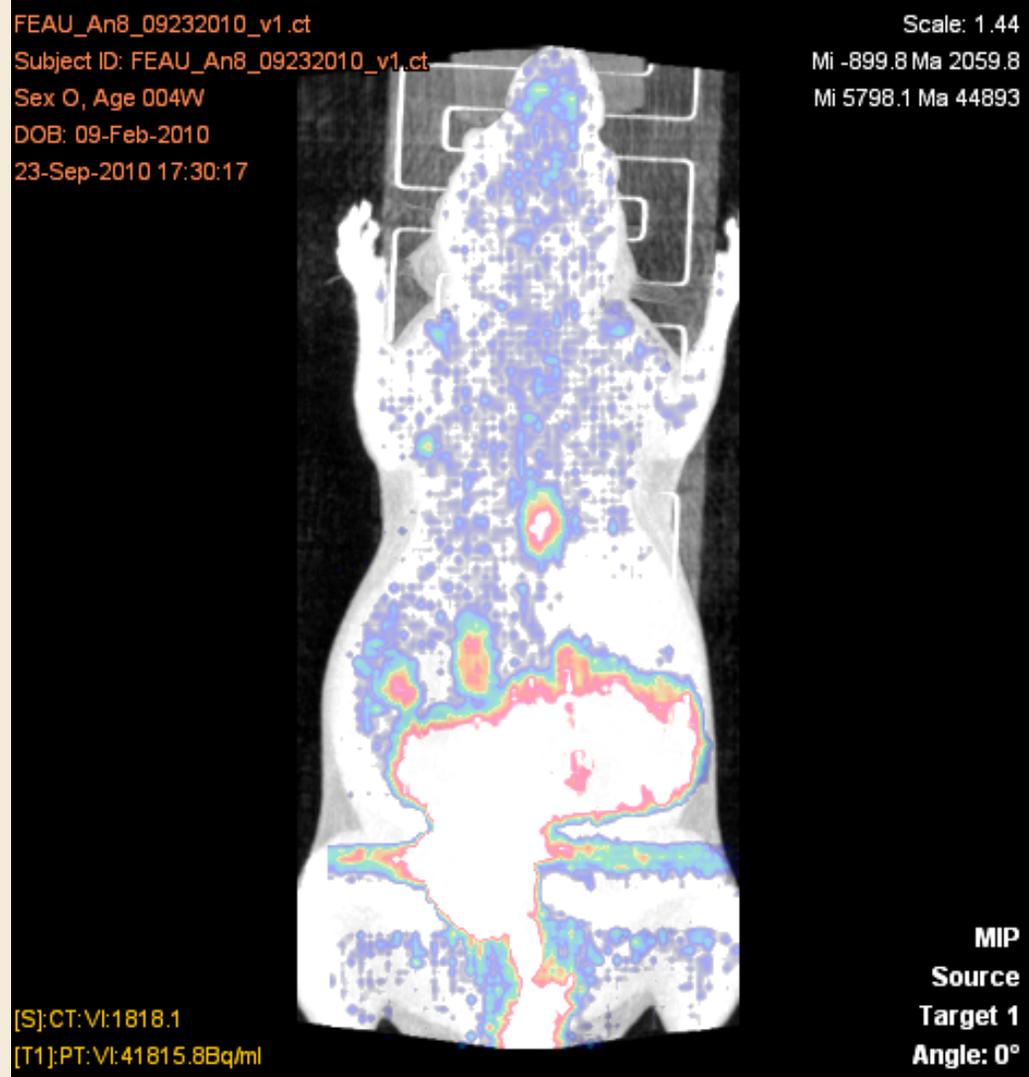
Coronal view

Saggital view

15x 10⁶ T cells
subcutaneously



3 dimensional reconstruction of CD19CAR+TK+T cells imaged by PET



Summary

- Co-expression of a CD19-specific CAR and HSV1-tk by *SB* transposition.
- *SB* modified CD19CAR⁺TK⁺ T cells
 - visualized spatio-temporally by μ PET using ¹⁸F-FEAU
 - ablated in the presence of ganciclovir and also
 - Have the ability to kill CD19⁺ tumor targets.

Implication

Clinical Significance: in non-invasively monitoring the persistence and trafficking SB modified adoptively transferred CD19-specific T cells.

Ongoing work: *in vivo* tumor model is being worked out

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