

# Next-generation non-viral gene transfer to redirect T-cell specificity

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Cancer-Immunotherapy, Cancer Vaccines I

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# Disclosure

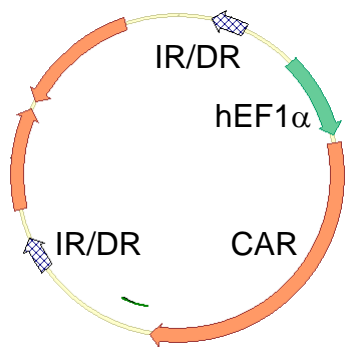
The technology being discussed has been licensed by MD Anderson to Ziopharm and Intrexon and MD Anderson, which is my employer, had an equity interest in both companies as a result of the transaction. As an inventor of the licensed technology, I shared in the proceeds of the consideration received by MD Anderson under the license in accordance with UT System Rules and MD Anderson policies and to that extent, I had financial interest in both companies.

# Overview

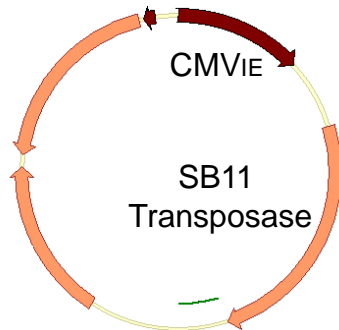
To improve therapeutic potential and shorten the time for *ex vivo* manufacture of T cells genetically modified using the *Sleeping Beauty* system to stably express CD19-specific CARs

# *Sleeping Beauty* (SB) system transposon/transposase

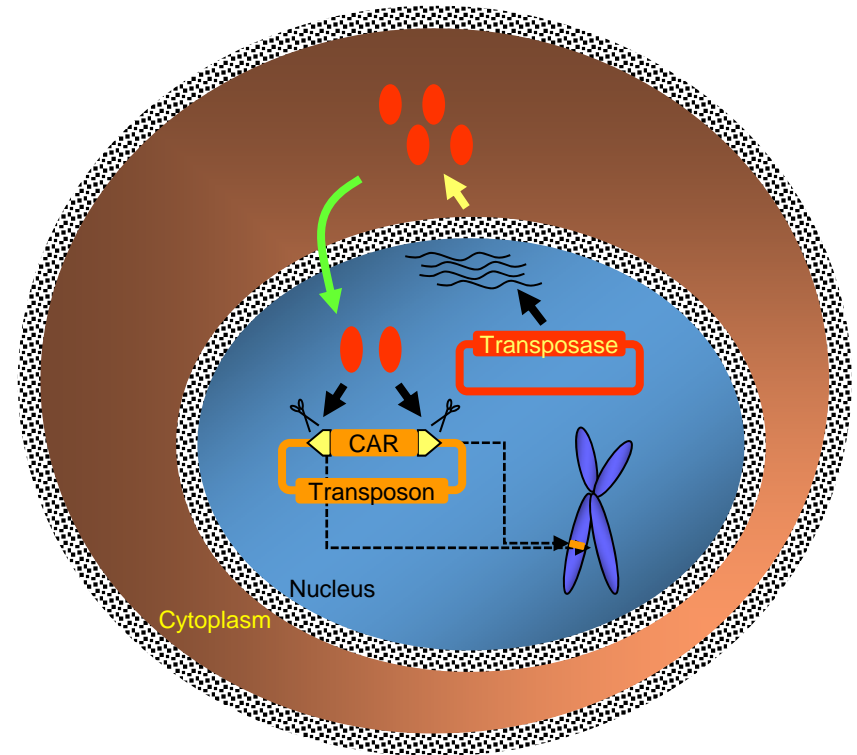
Transposon DNA plasmid



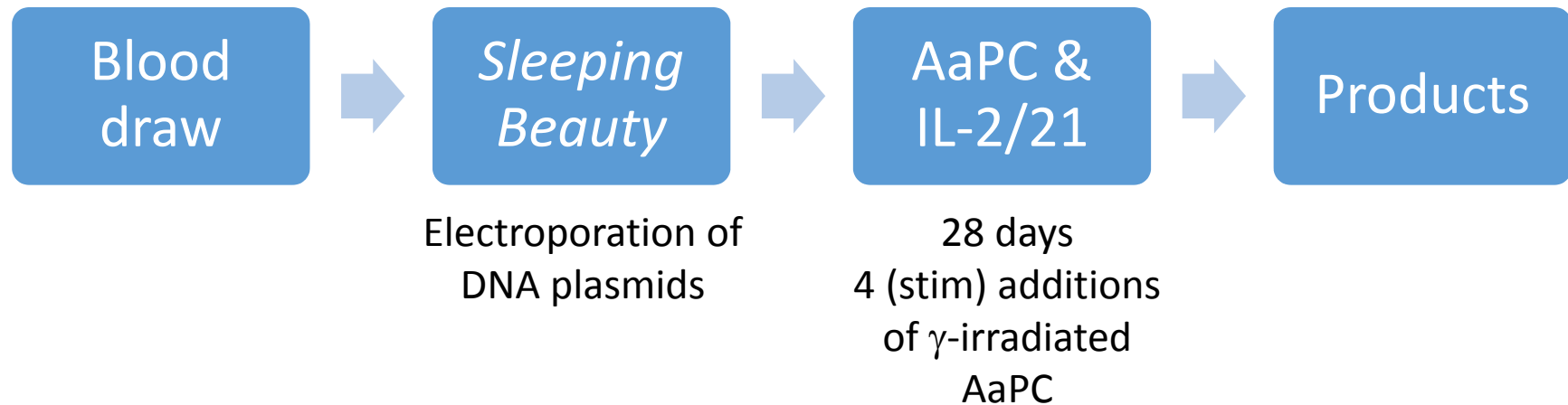
Transposase DNA plasmid  
(or *in vitro* transcribed mRNA)



Co-delivery into cells by nucleofection (Lonza)



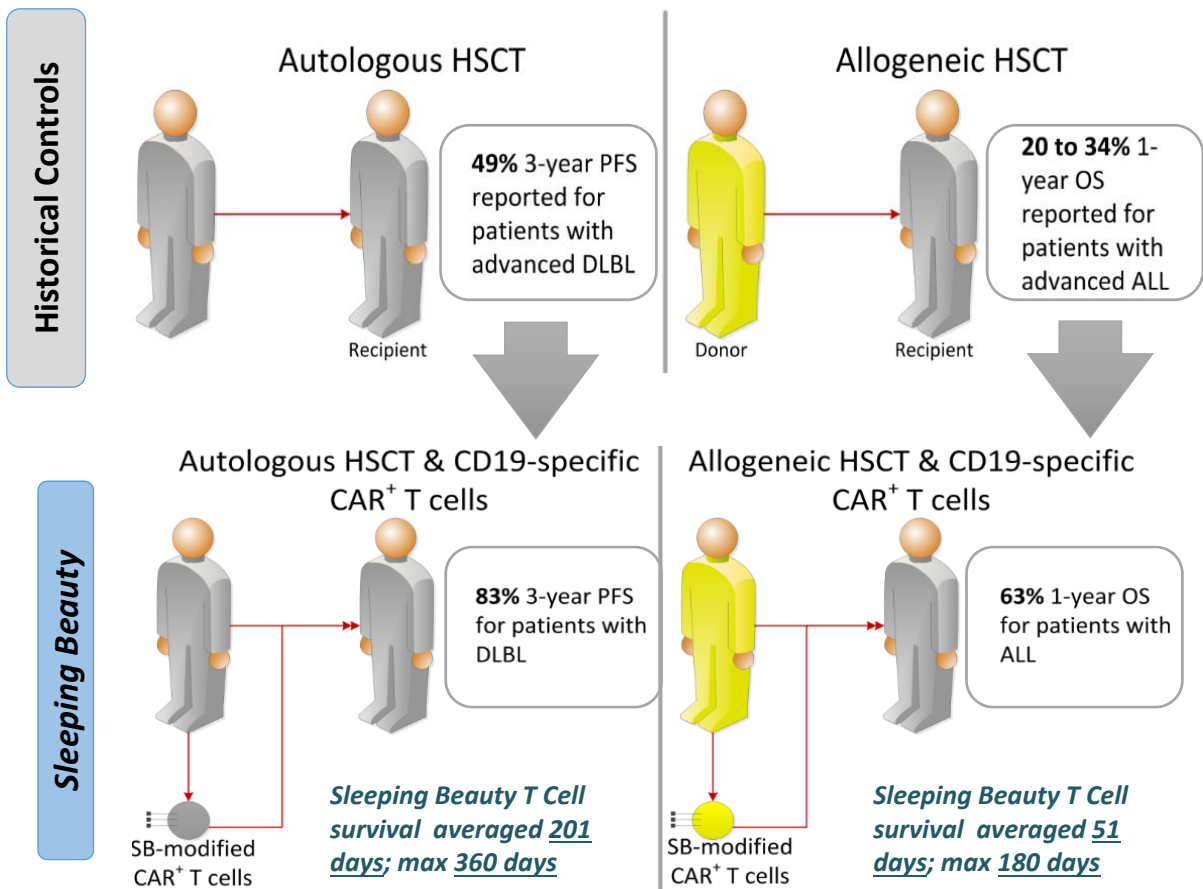
# Non-viral gene transfer in compliance with current good manufacturing practice (cGMP)



# Non-viral delivery: *Sleeping Beauty* CAR<sup>+</sup> T-cell platform (first-in-human studies)

Long term follow-up data from 1<sup>st</sup> generation *Sleeping Beauty* platform in two trials infusing CAR<sup>+</sup> T cells *after* hematopoietic stem-cell transplantation (HSCT)

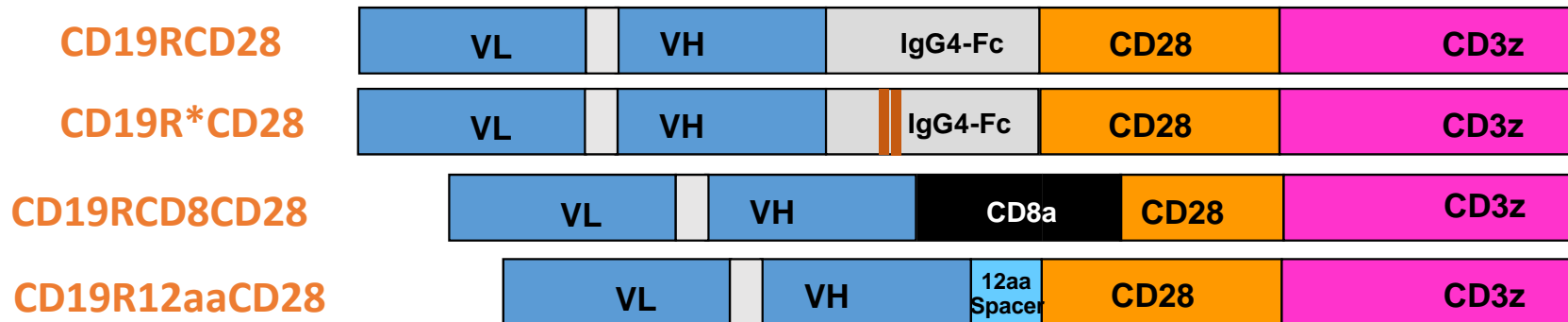
- Showed approximate doubling PFS or OS in both autologous and allogeneic cohorts
- Non-viral *Sleeping Beauty* T-cell survival compared favorably versus viral approaches



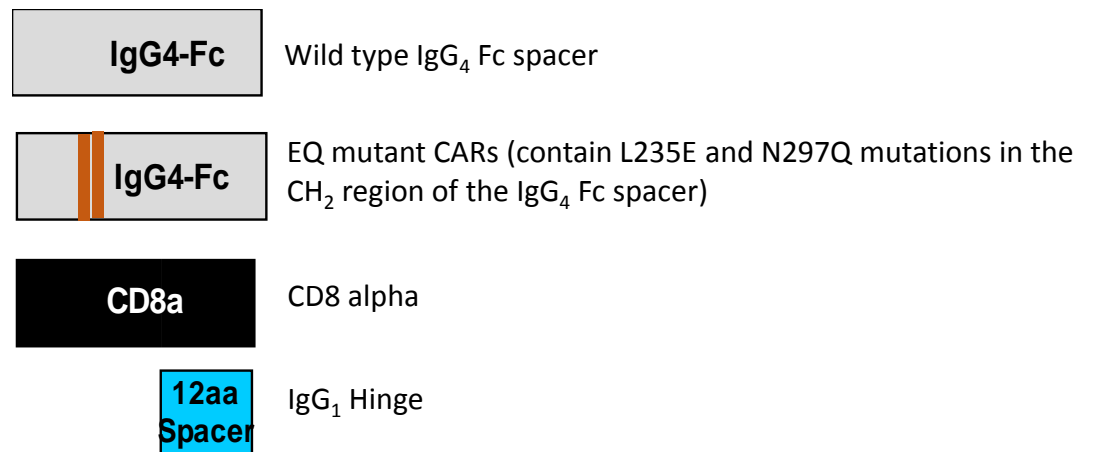
CAR = CD19RCD28



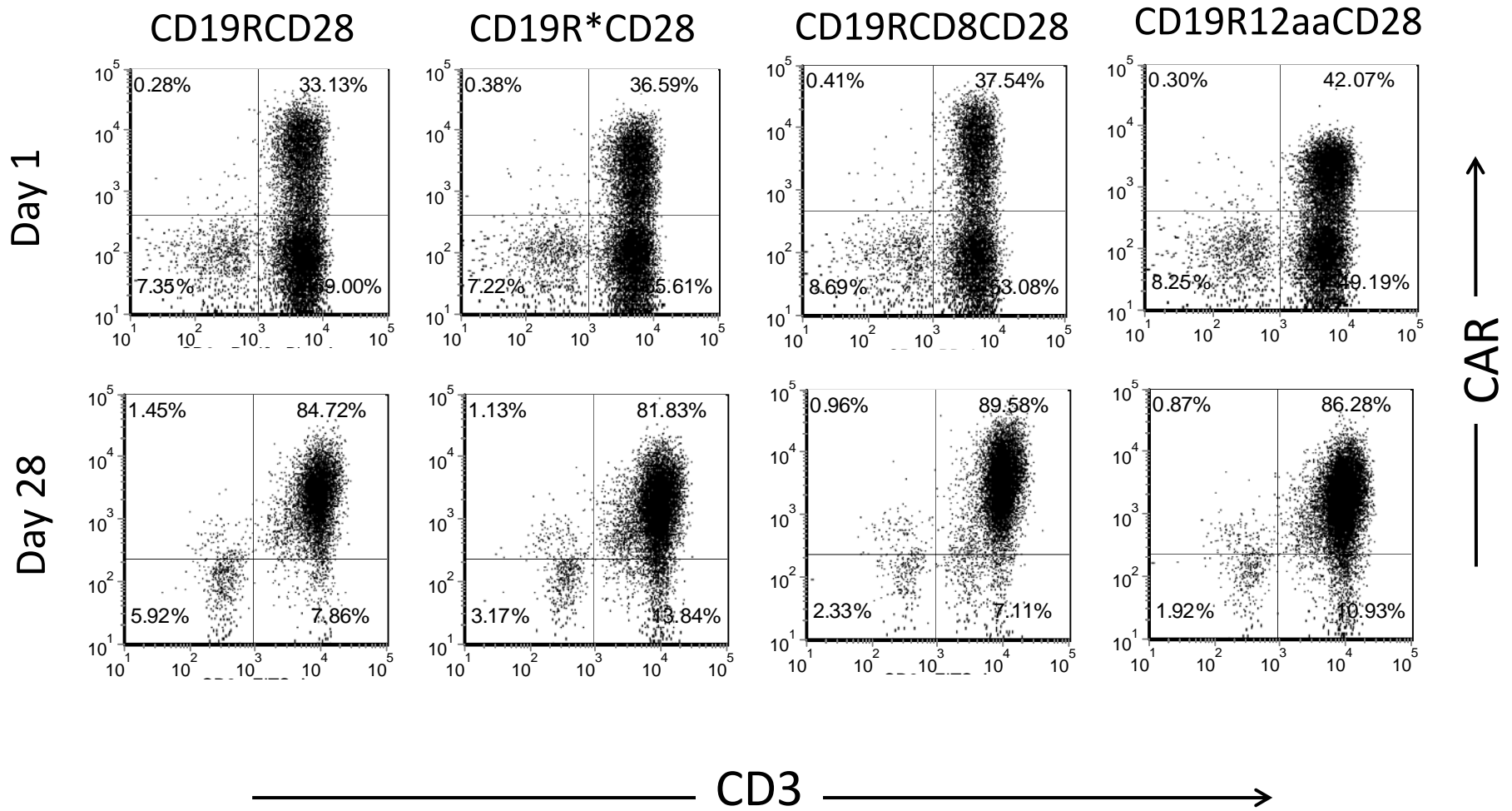
# Designs of CARs



- CARs signal through chimeric CD28 and CD3- $\zeta$
- CARs differ in the type and length of extracellular spacer

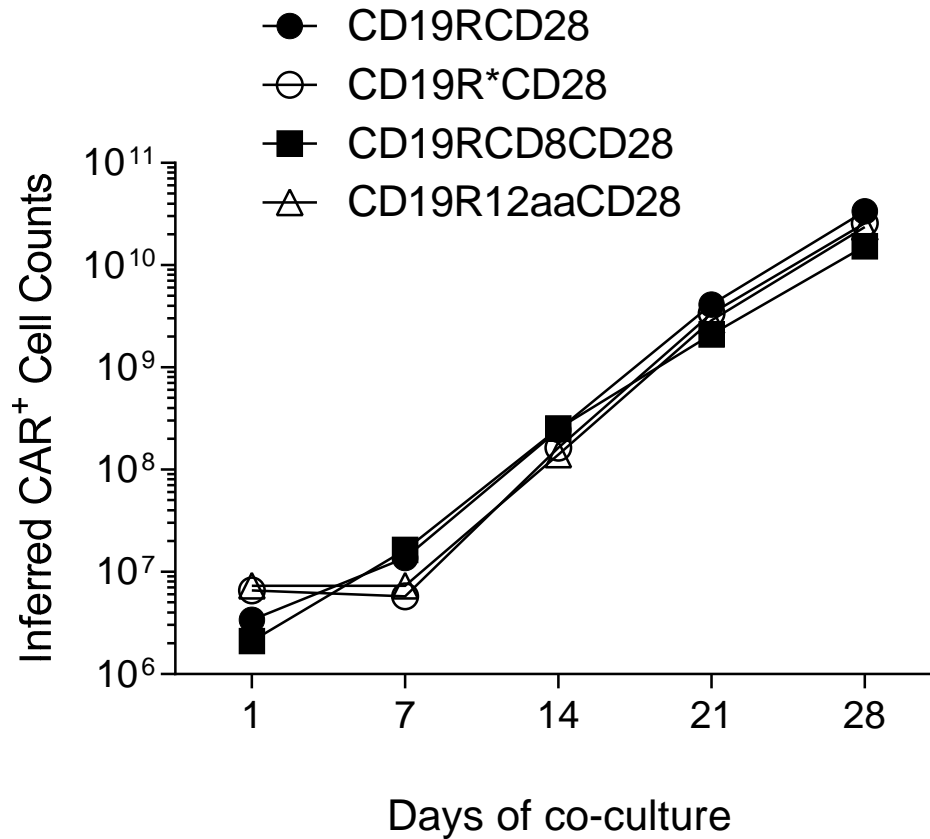


# Phenotype of 28-day AaPC (4-stim) propagated CAR<sup>+</sup> T cells

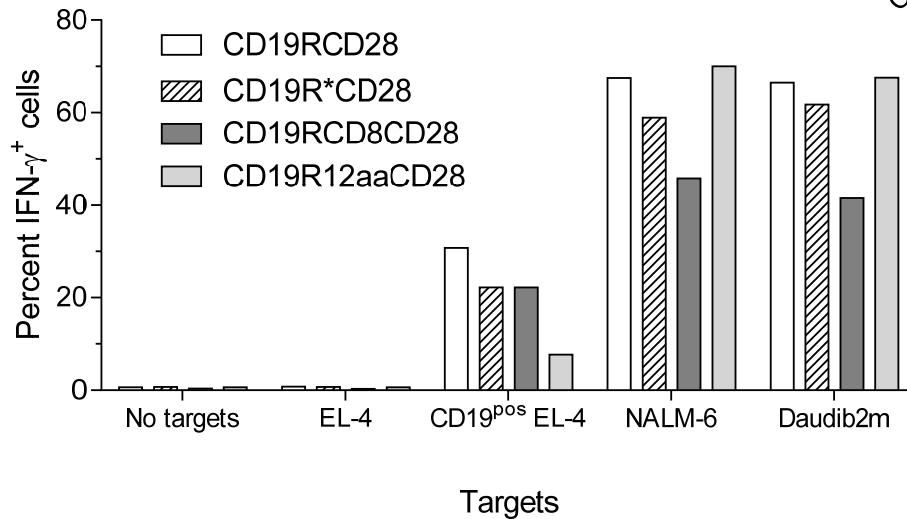
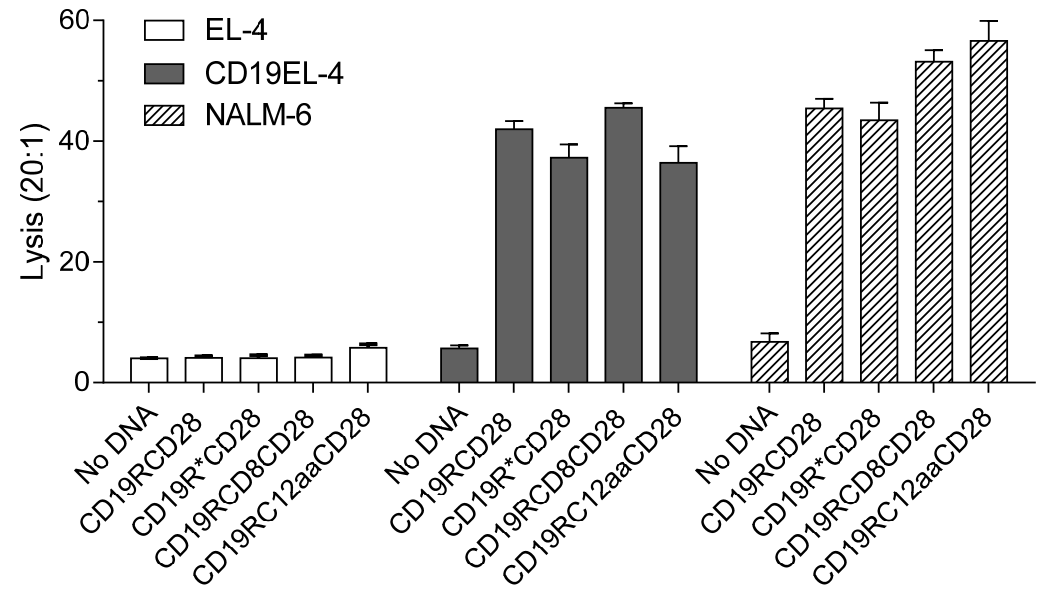




# Phenotype of 28-day AaPC (4-stim) propagated CAR<sup>+</sup> T cells

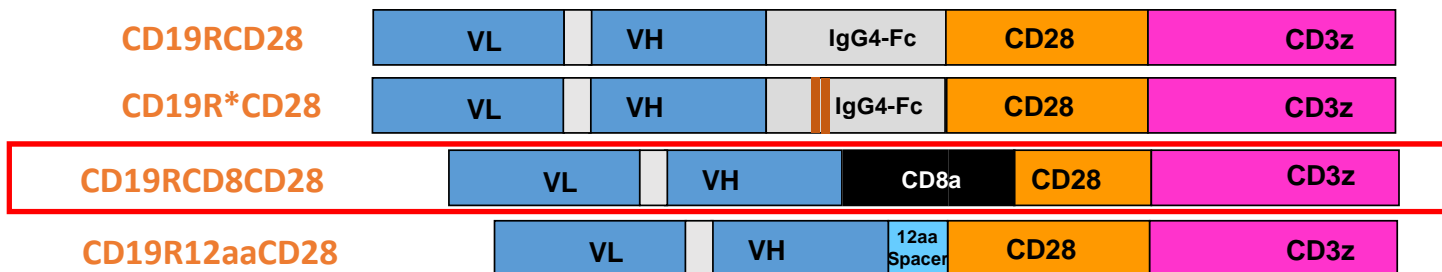
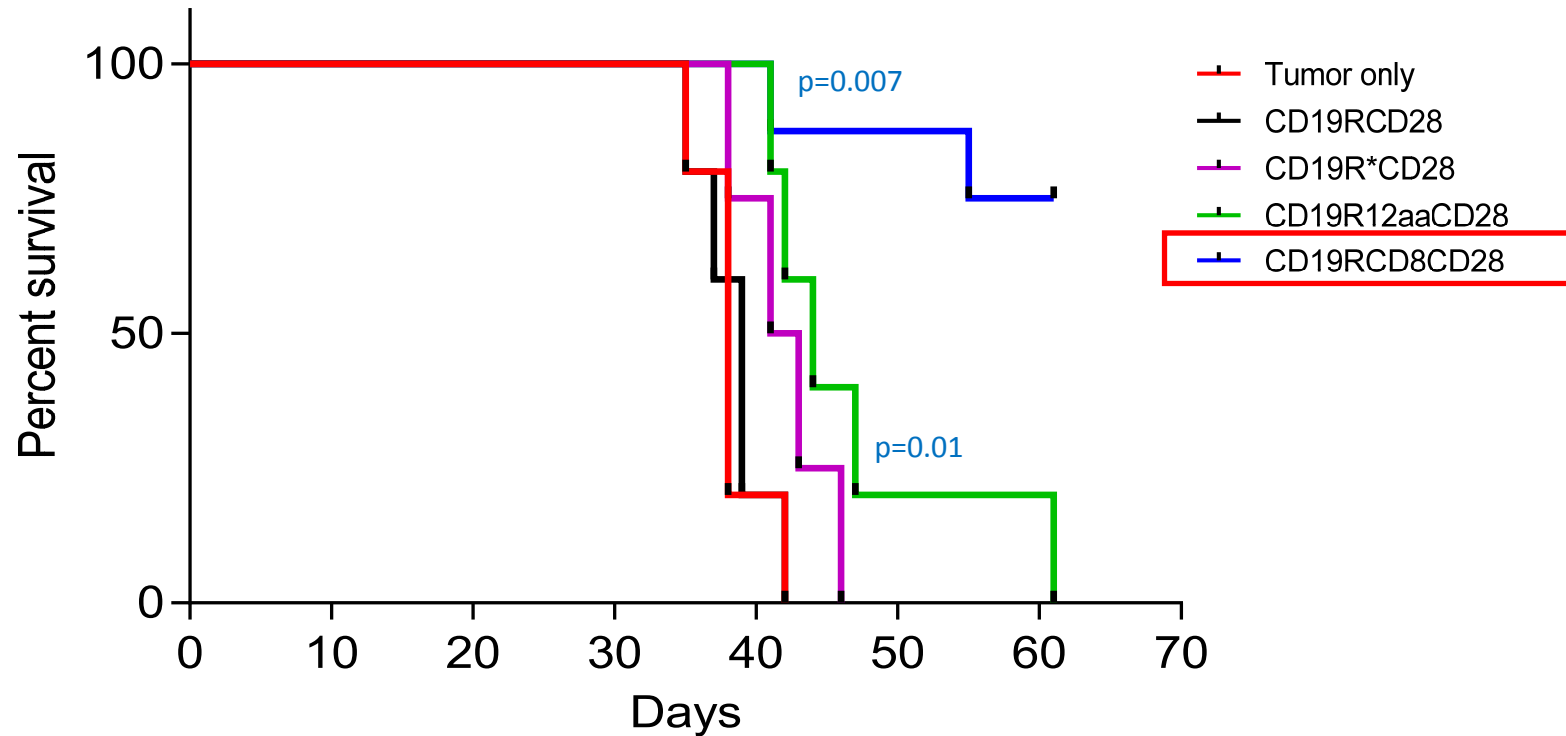


# Specificity for CD19 by CAR<sup>+</sup> T cells after 28 days (4-stim) of co-culture on AaPC

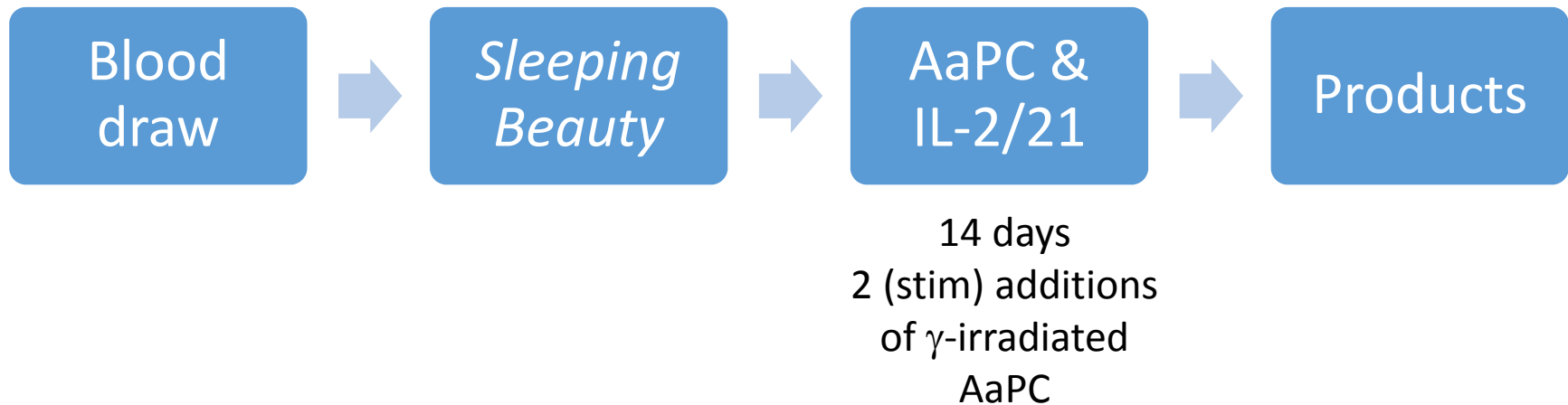
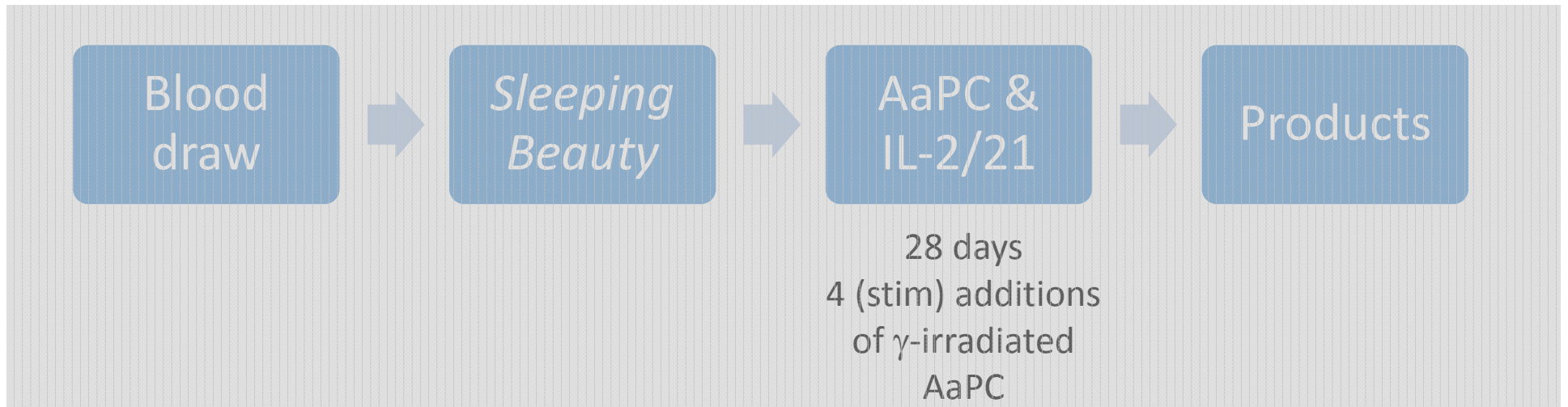


# CD8 stalk improves *in vivo* anti-tumor effect of CAR<sup>+</sup> T cells propagated for 28 days (4 stim) on AaPC

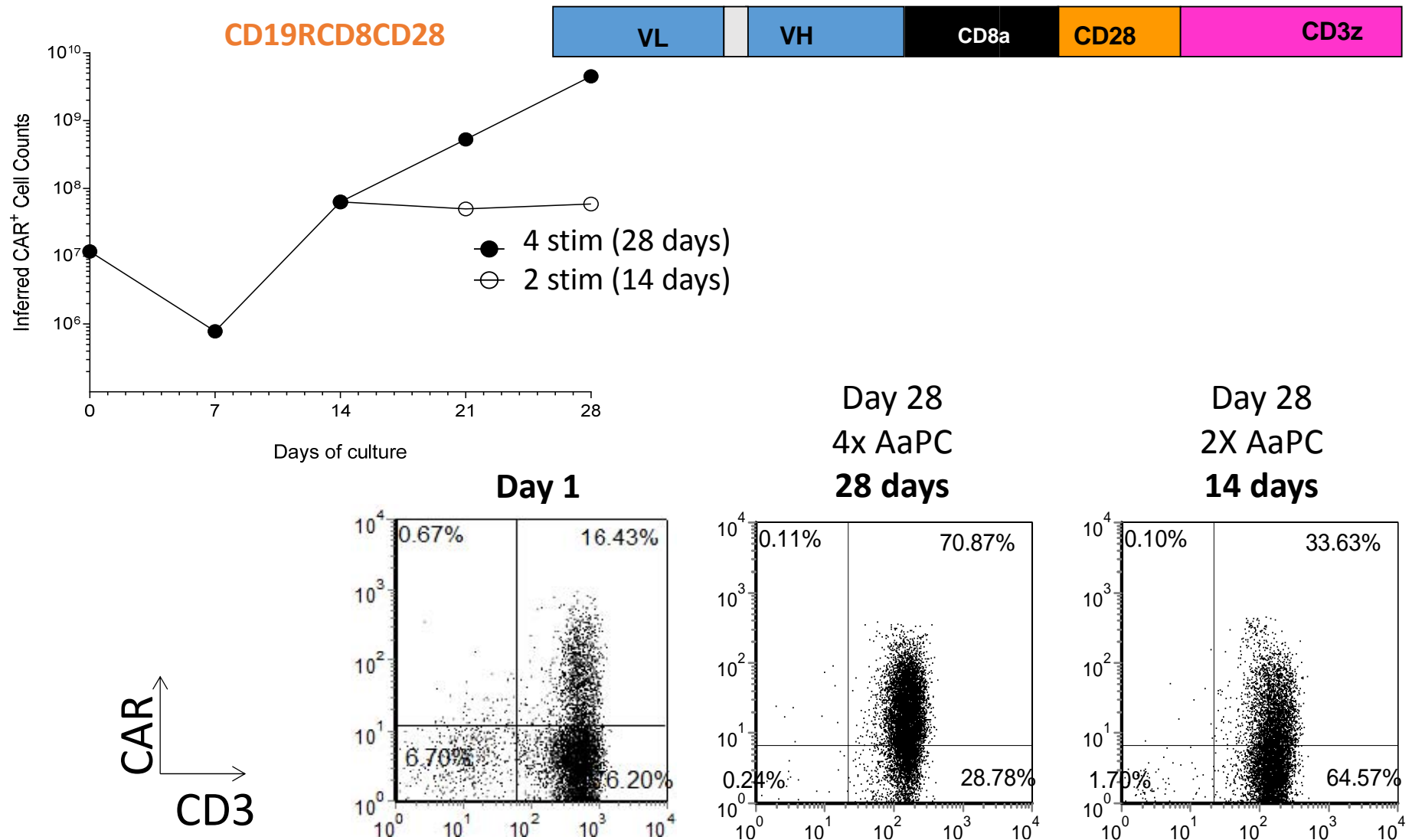
CD19<sup>+</sup> NALM-6 tumor model in NSG mice



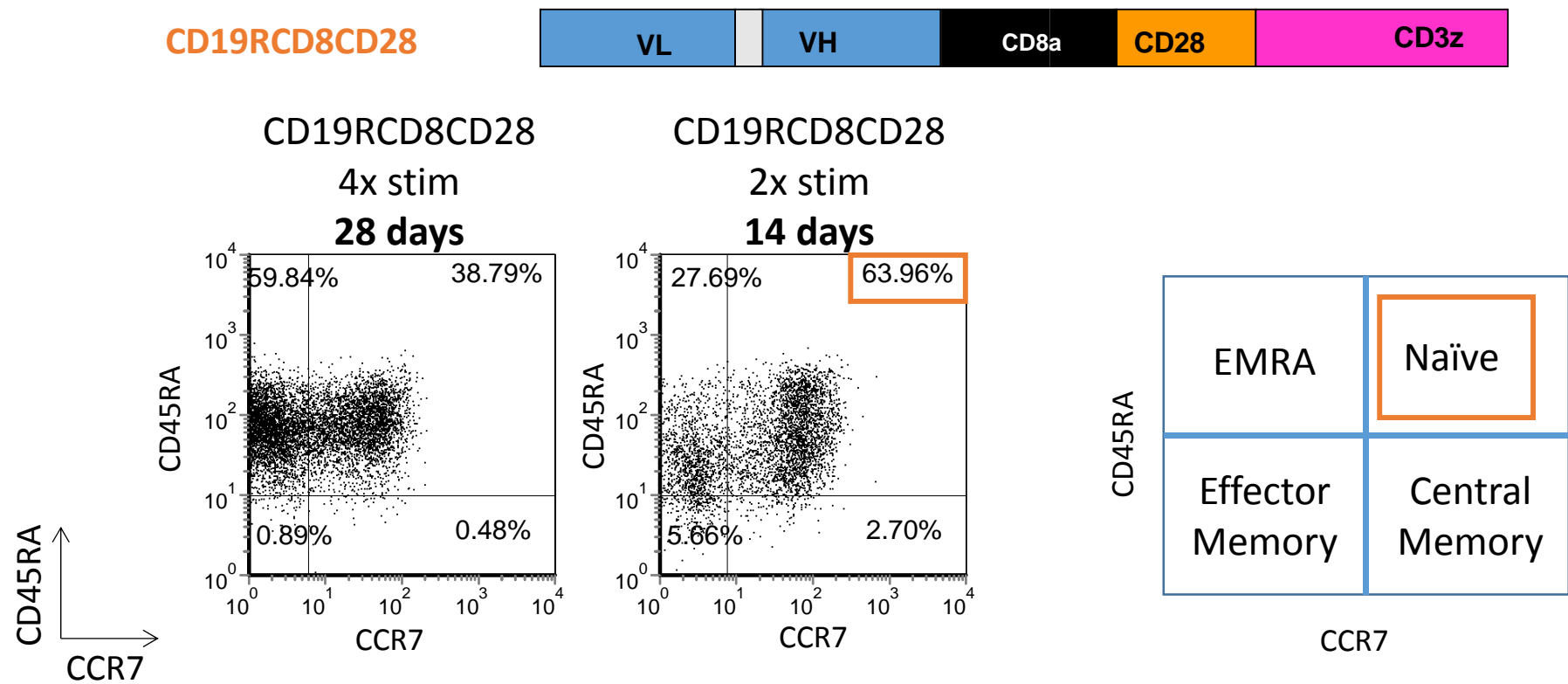
# Shorten *ex vivo* time in culture from 28 to 14 days



# Phenotype of CAR<sup>+</sup> T cells propagated for 28- (4 stim) versus 14- (2 stim) days on AaPC



# Propagation of CAR<sup>+</sup> T cells after 14 days (2-stim) on AaPC leads to improved outgrowth of naïve/memory populations

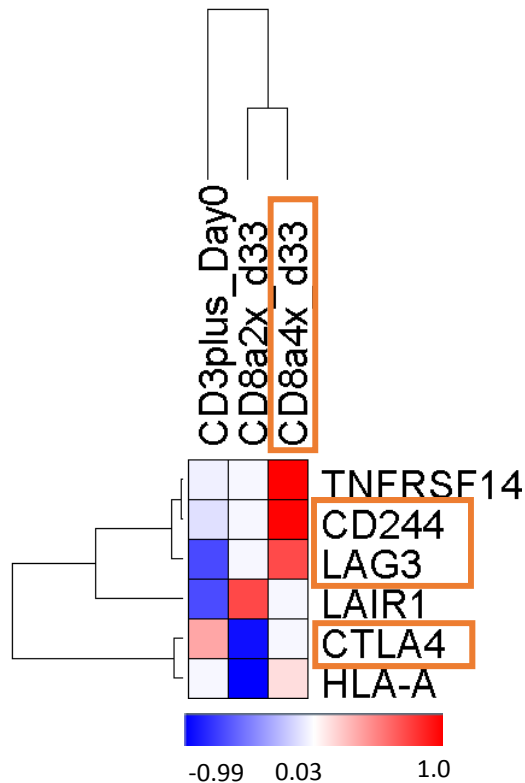


Cells gated on CAR<sup>+</sup> cells

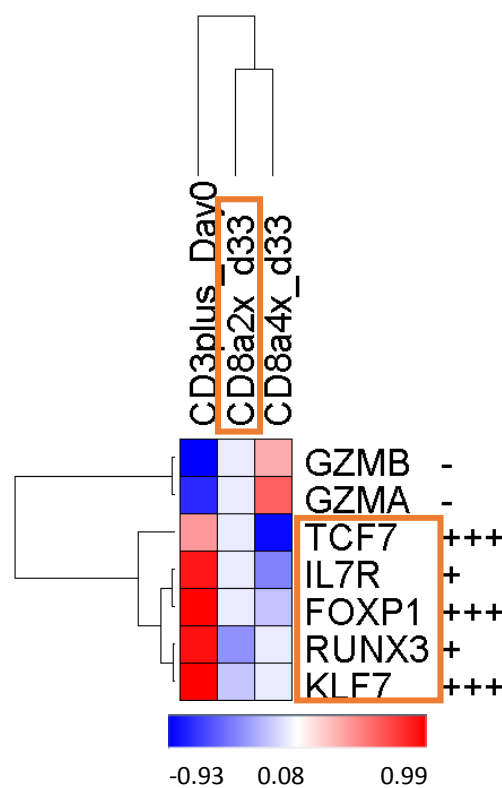
Sallusto F, et al. Nature. 401(6754):708-12.

# Propagation of CAR<sup>+</sup> T cells after 14 days (2-stim) on AaPC maintains a naïve-memory and less exhausted transcriptional profiles

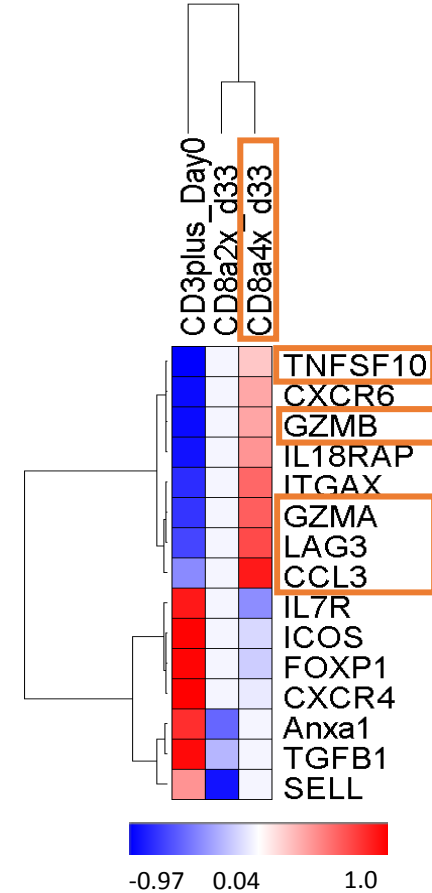
Exhaustion/Inhibition



Naïve/Memory

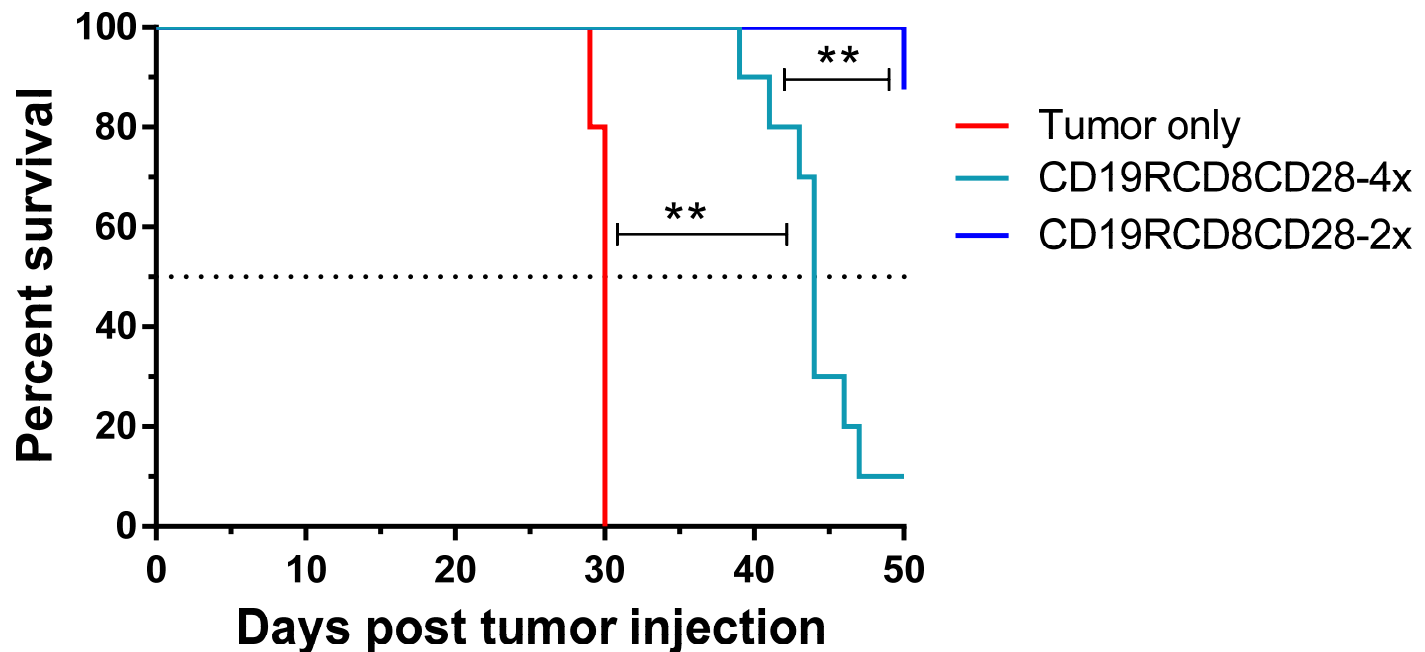


Effector Molecules



# Propagation of CAR<sup>+</sup> T cells after 14 days (2-stim) on AaPC improves anti-tumor effect

CD19<sup>+</sup> NALM-6 tumor model in NSG mice

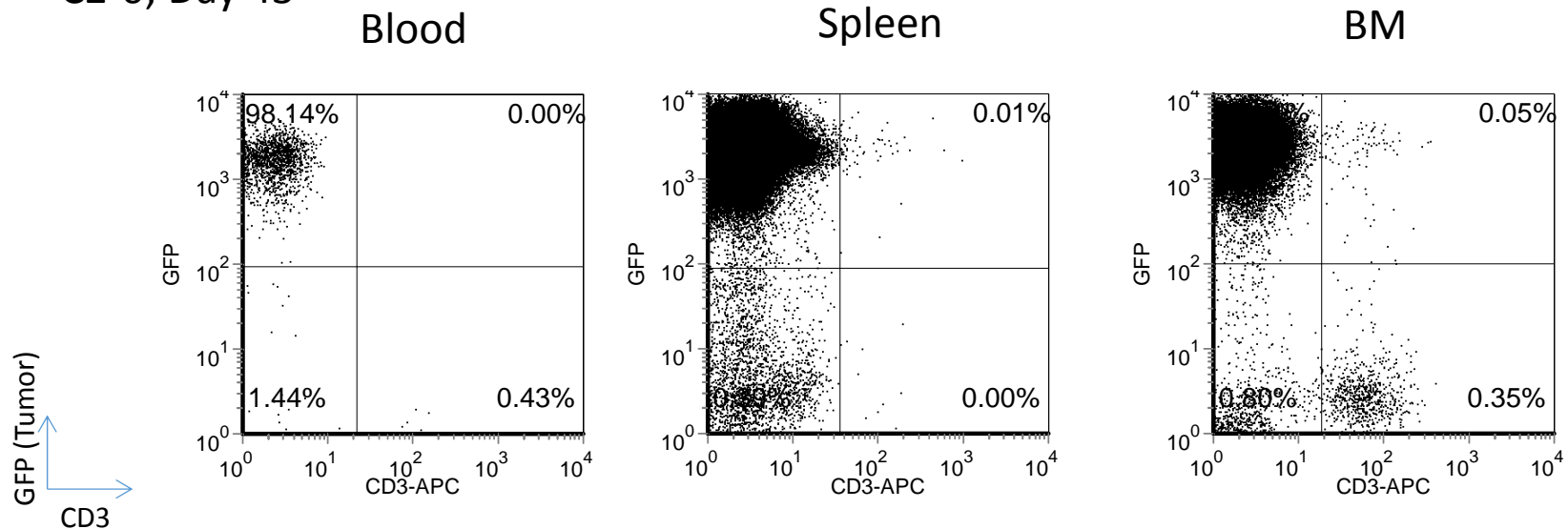


\*\*p<0.01



# Propagation of CAR<sup>+</sup> T cells after 14 days (2-stim) on AaPC improves persistence

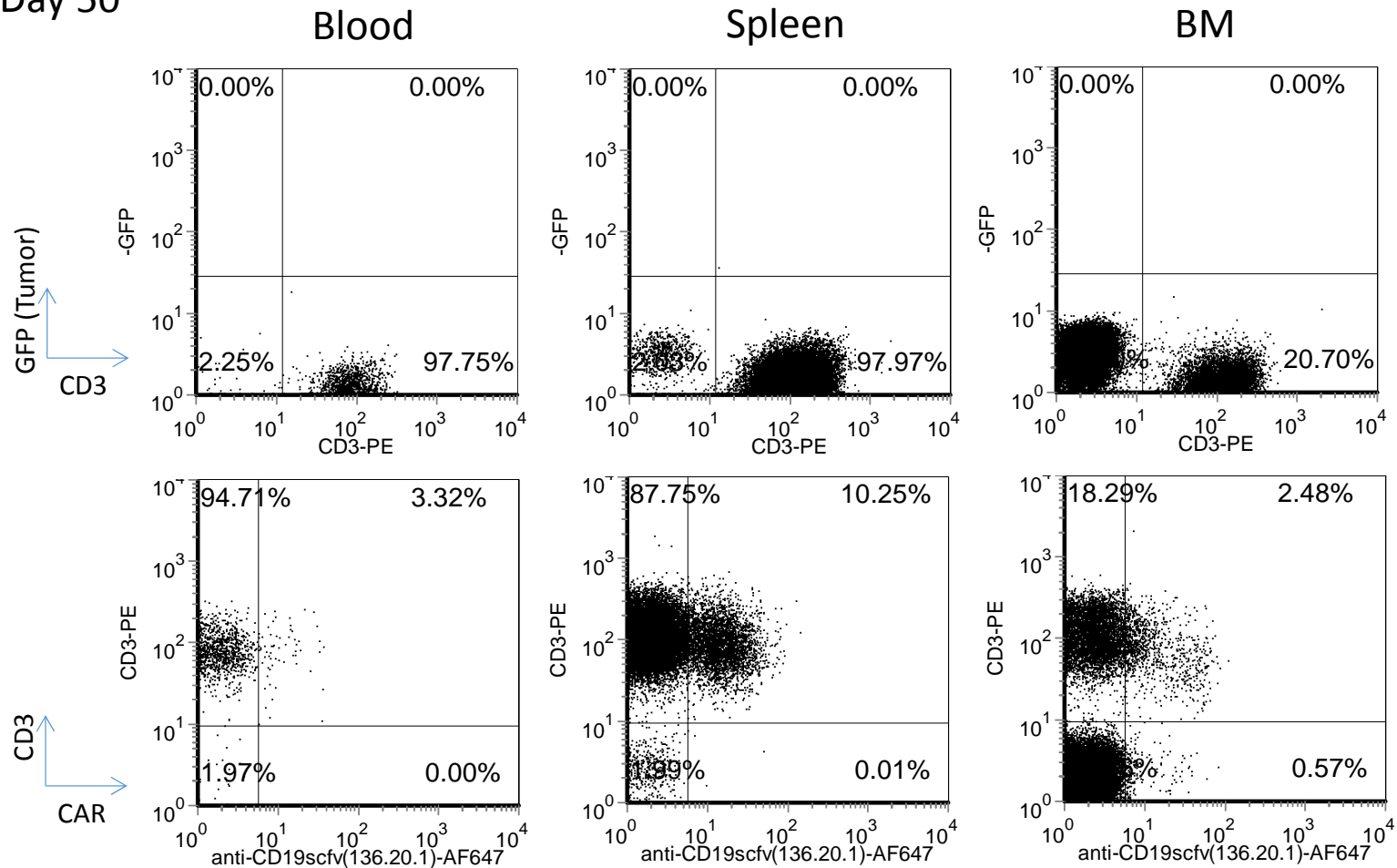
CD19RC<sup>+</sup>CD8<sup>+</sup>CD28-4x  
C2-0; Day 43



# Propagation of CAR<sup>+</sup> T cells after 14 days (2-stim) on AaPC improves persistence

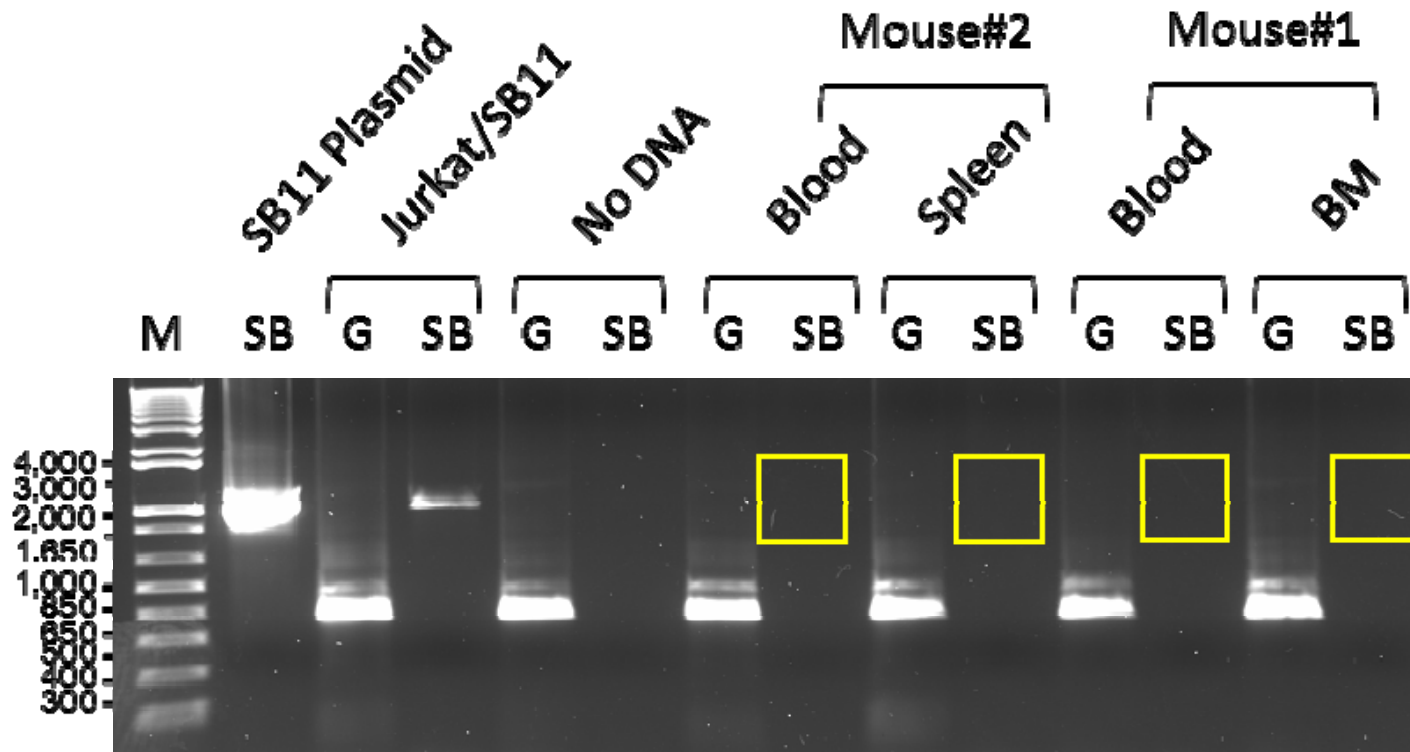
CD19RCD8CD28-2x

D1-2; Day 50



# SB11 was not detected in genetically modified T cells recovered from mice

Tissues from mice infused with CAR<sup>+</sup> T cells after 14 days (2-stim) on AaPC



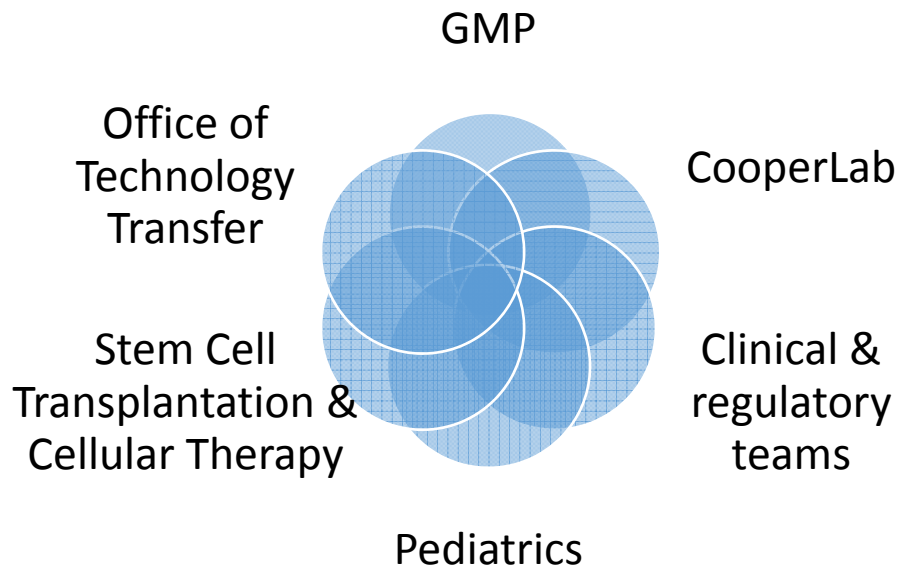
G = GAPDH Primers

SB = SB11 Primers

# Summary

- CD8 stalk improves anti-tumor effect of CD19-specific CAR<sup>+</sup> T cells
- Propagating with 2-stim (14-day) on  $\gamma$ -irradiated AaPC results in improved:
  - Outgrowth of T cells with naïve/memory phenotype and genotype
  - Persistence of administered T cells
  - Anti-tumor effect *in vivo*

# Acknowledgments



THE UNIVERSITY OF TEXAS  
**MD Anderson**  
~~Cancer Center~~

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