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

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Cancer-Immunotherapy, Cancer Vaccines I

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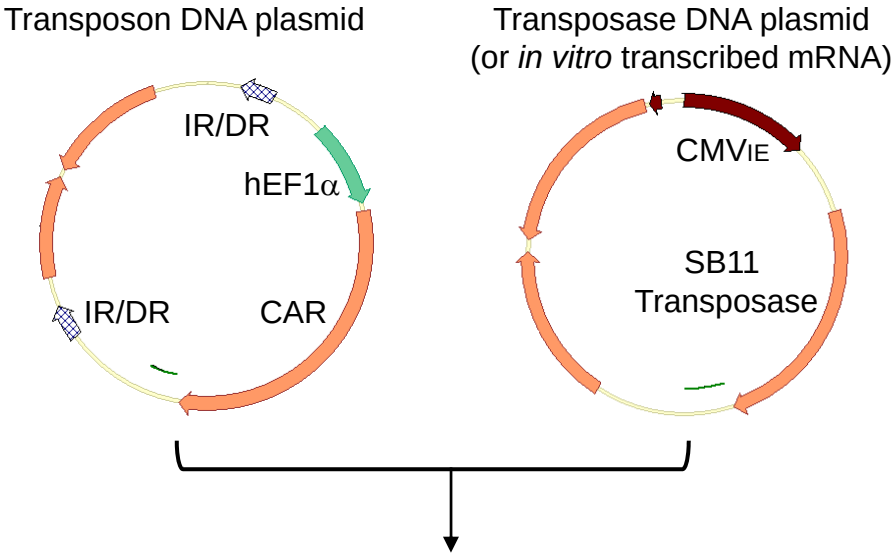
Disclosure

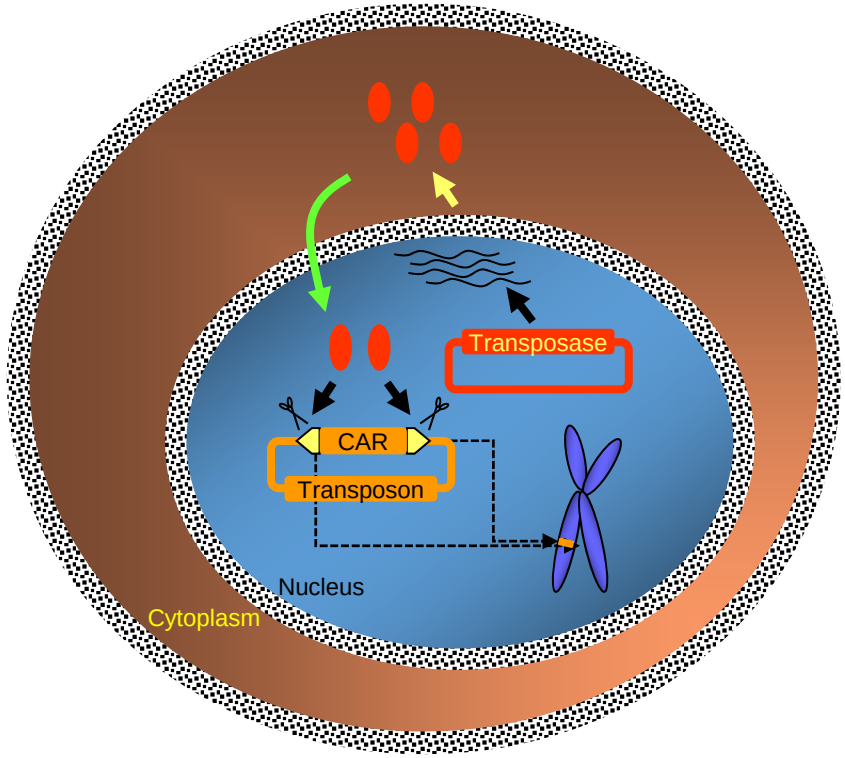
The technology being discussed has been licensed by MD Anderson to Ziopharm and Intrexon and MD Anderson, which is my employer, had an equity interest in both companies as a result of the transaction. As an inventor of the licensed technology, I shared in the proceeds of the consideration received by 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

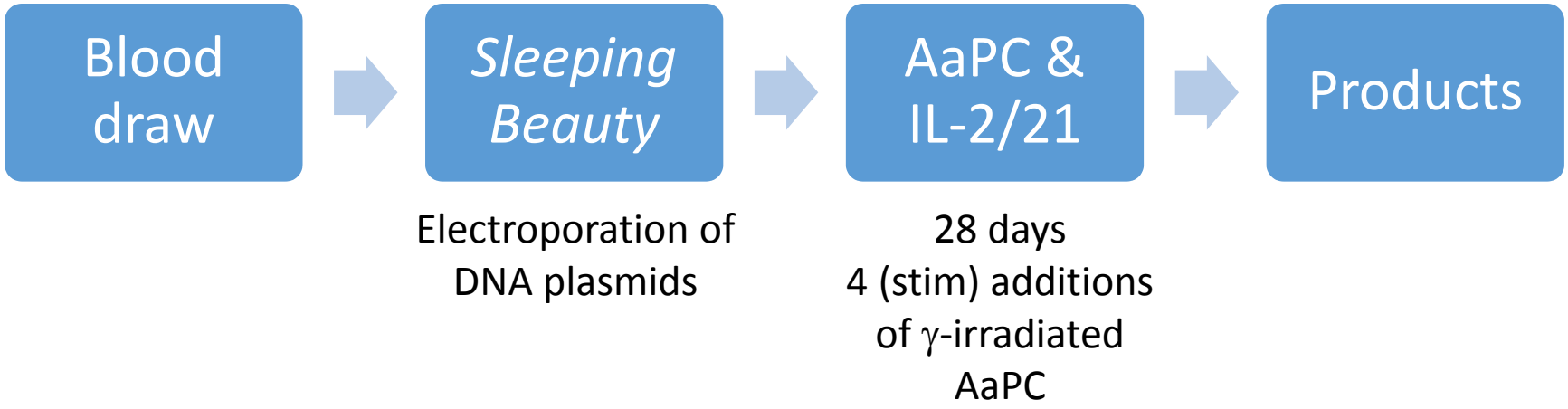
Sleeping Beauty (SB) system transposon/transposase





Co-delivery into cells by nucleofection (Lonza)

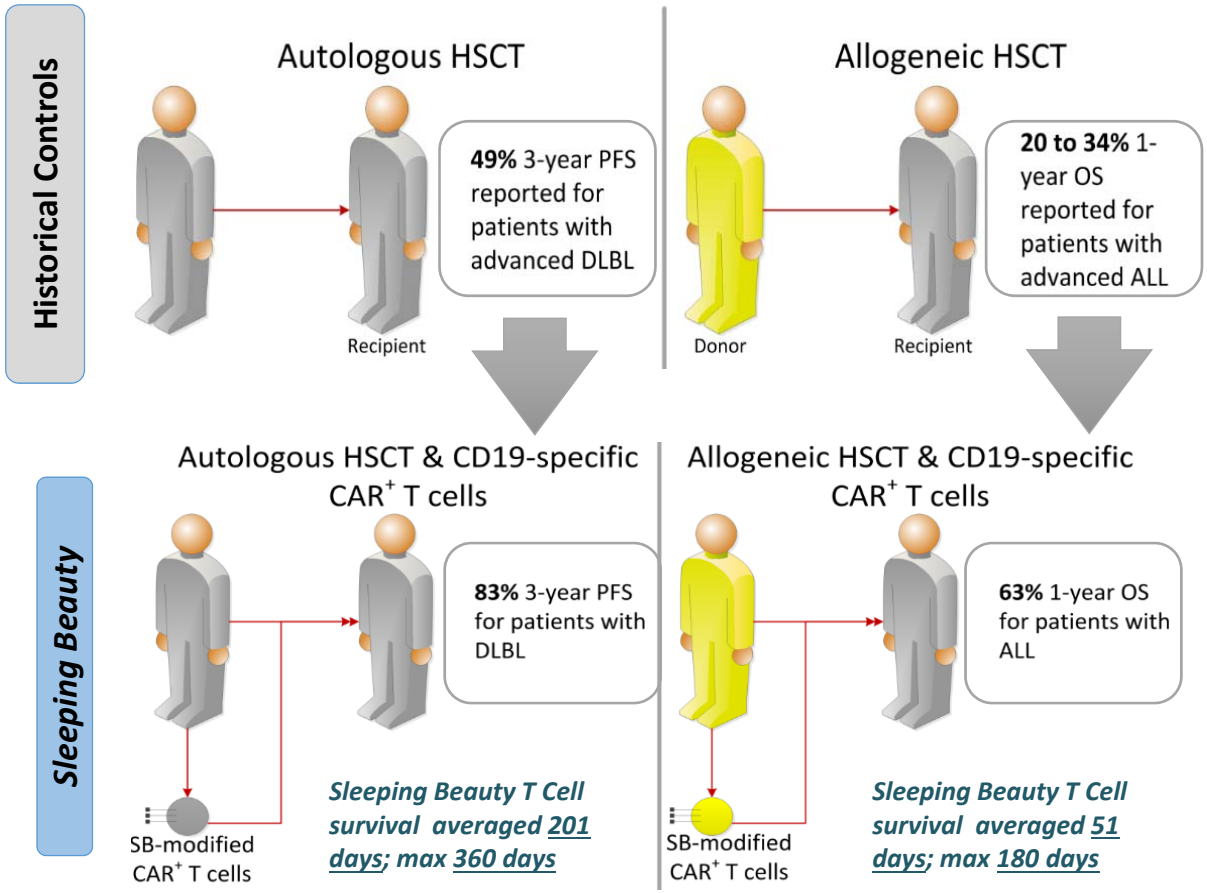
Non-viral gene transfer in compliance with current good manufacturing practice (cGMP)



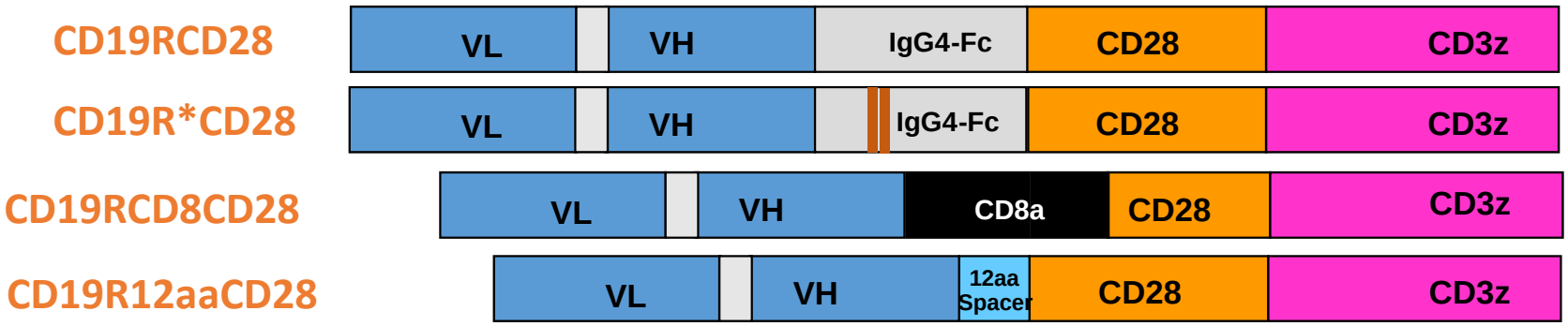
Non-viral delivery: *Sleeping Beauty* CAR$^+$ T-cell platform (first-in-human studies)

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

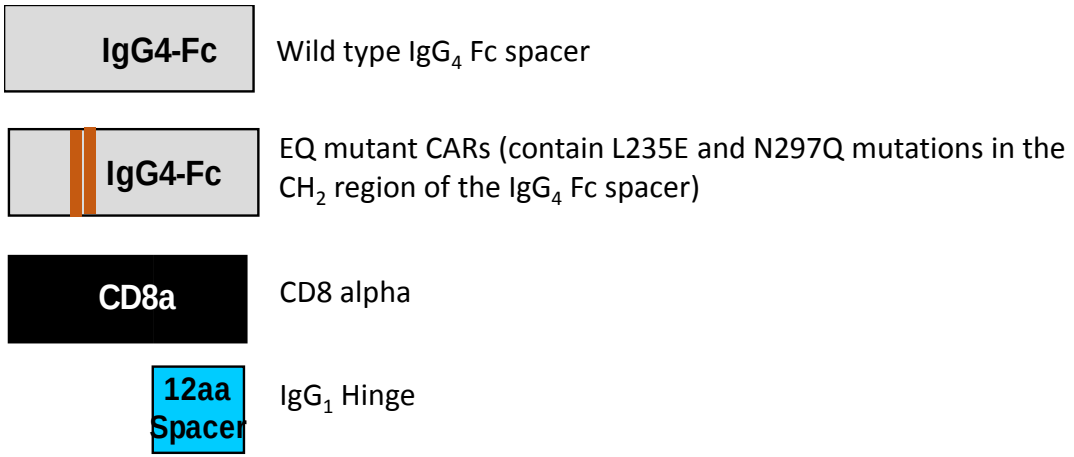
- Showed approximate doubling PFS or OS in both autologous and allogeneic cohorts
- Non-viral *Sleeping Beauty* T-cell survival compared favorably versus viral approaches



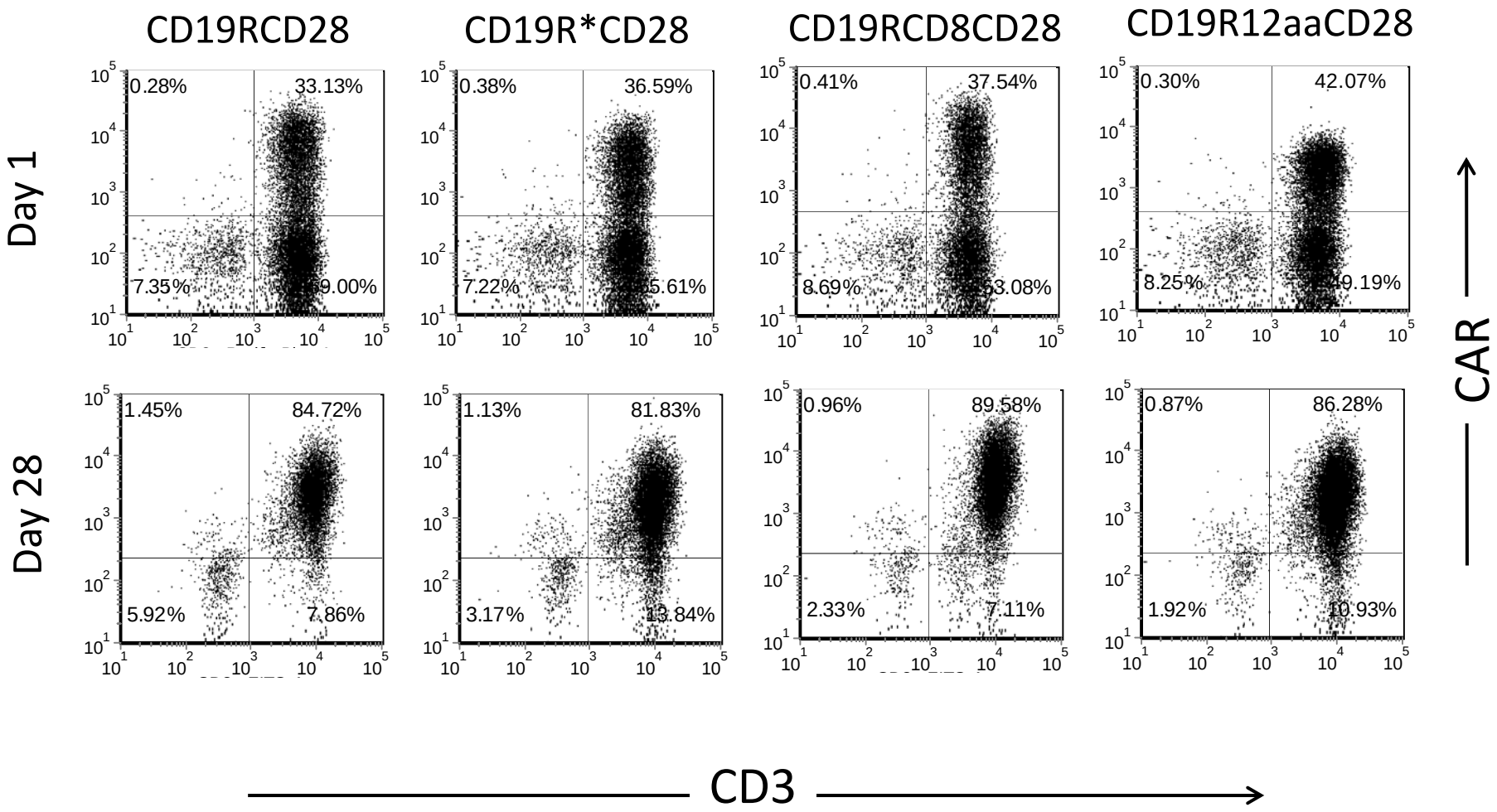
Designs of CARs



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



Phenotype of 28-day AaPC (4-stim) propagated CAR^{+} T cells



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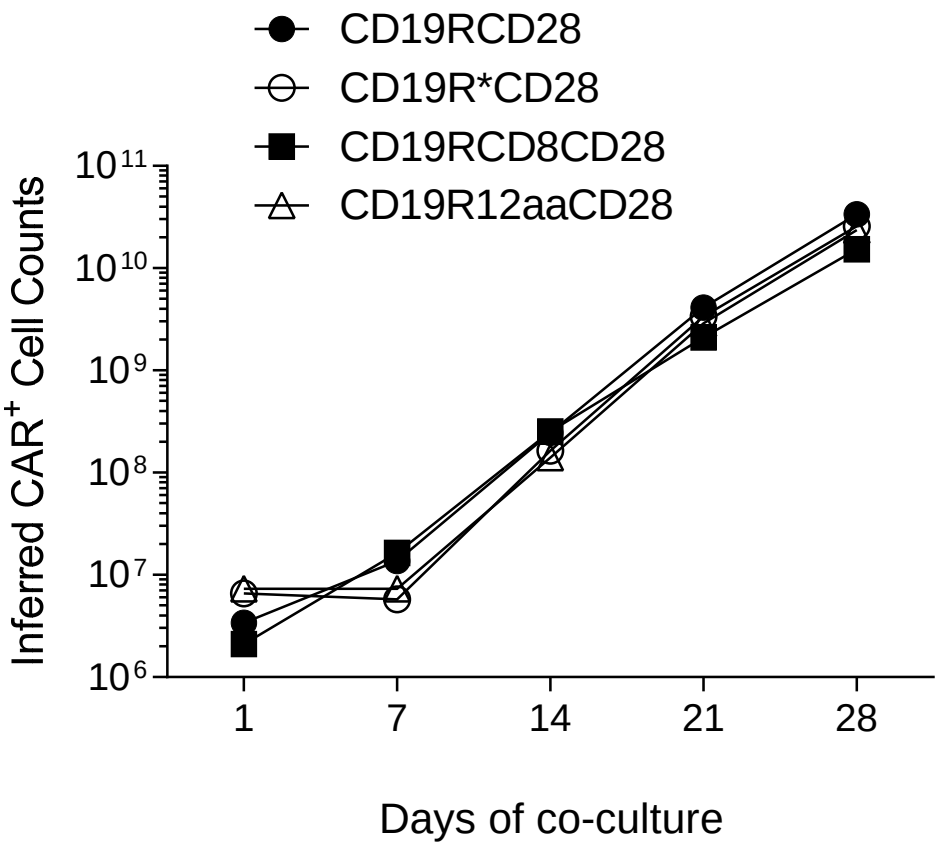
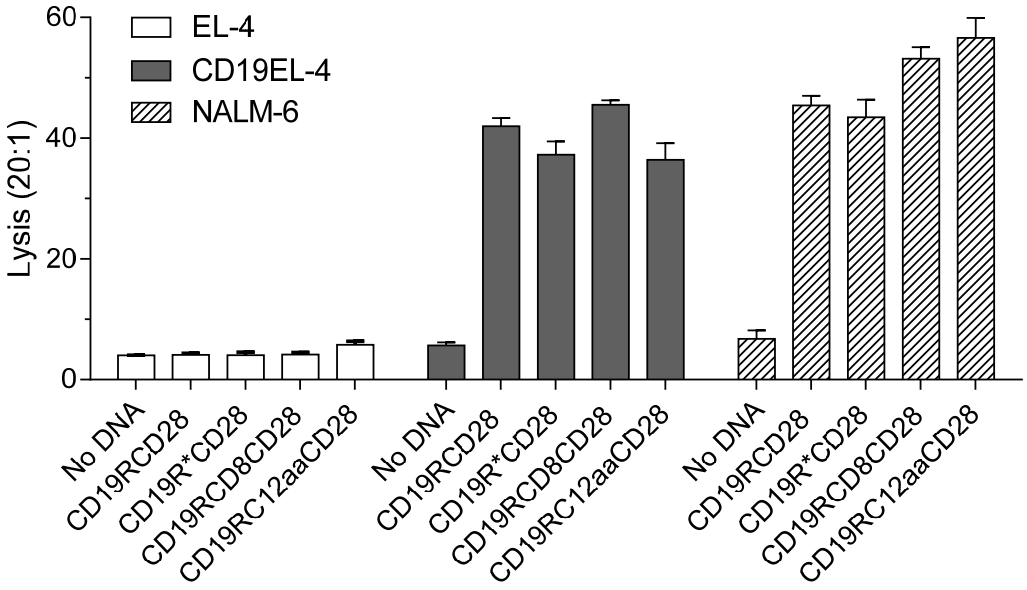
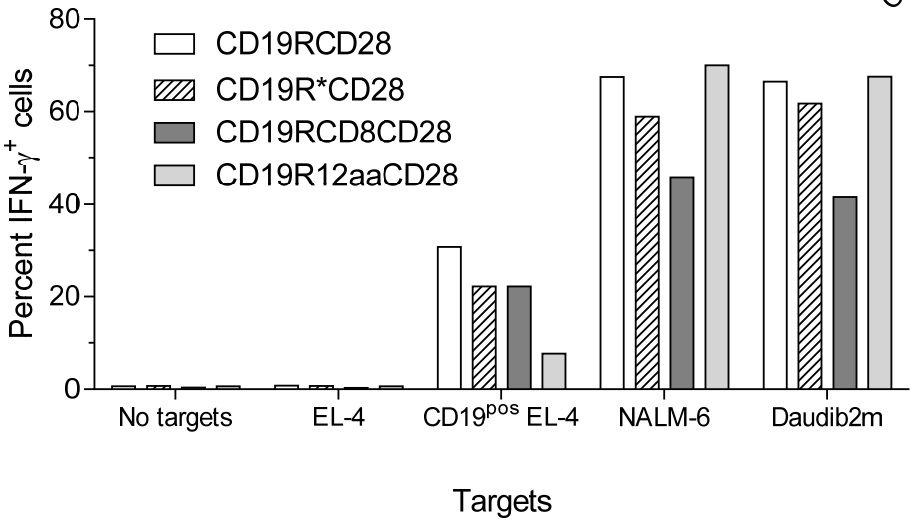
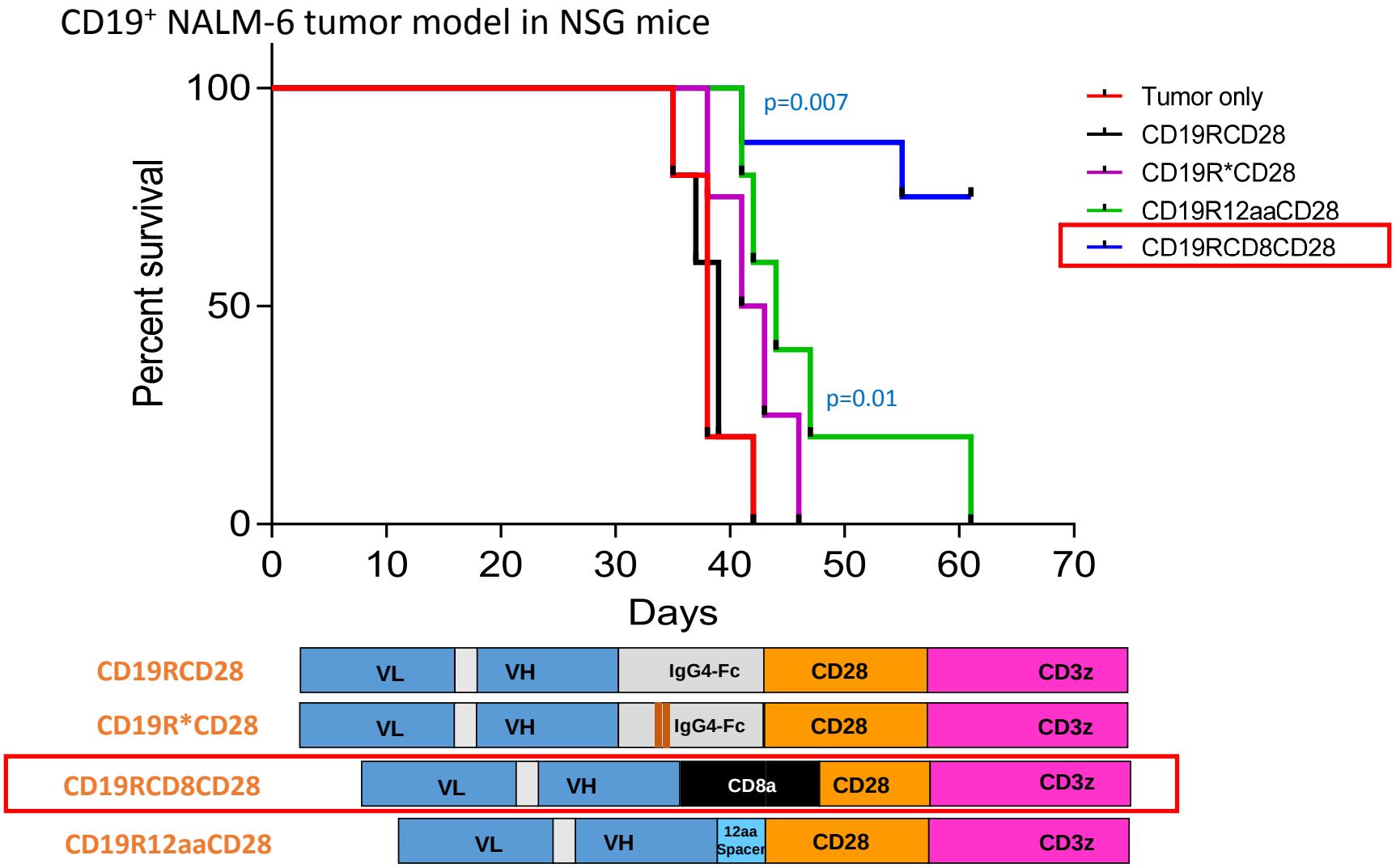


Specificity for CD19 by CAR^{+} T cells after 28 days (4-stim) of co-culture on AaPC

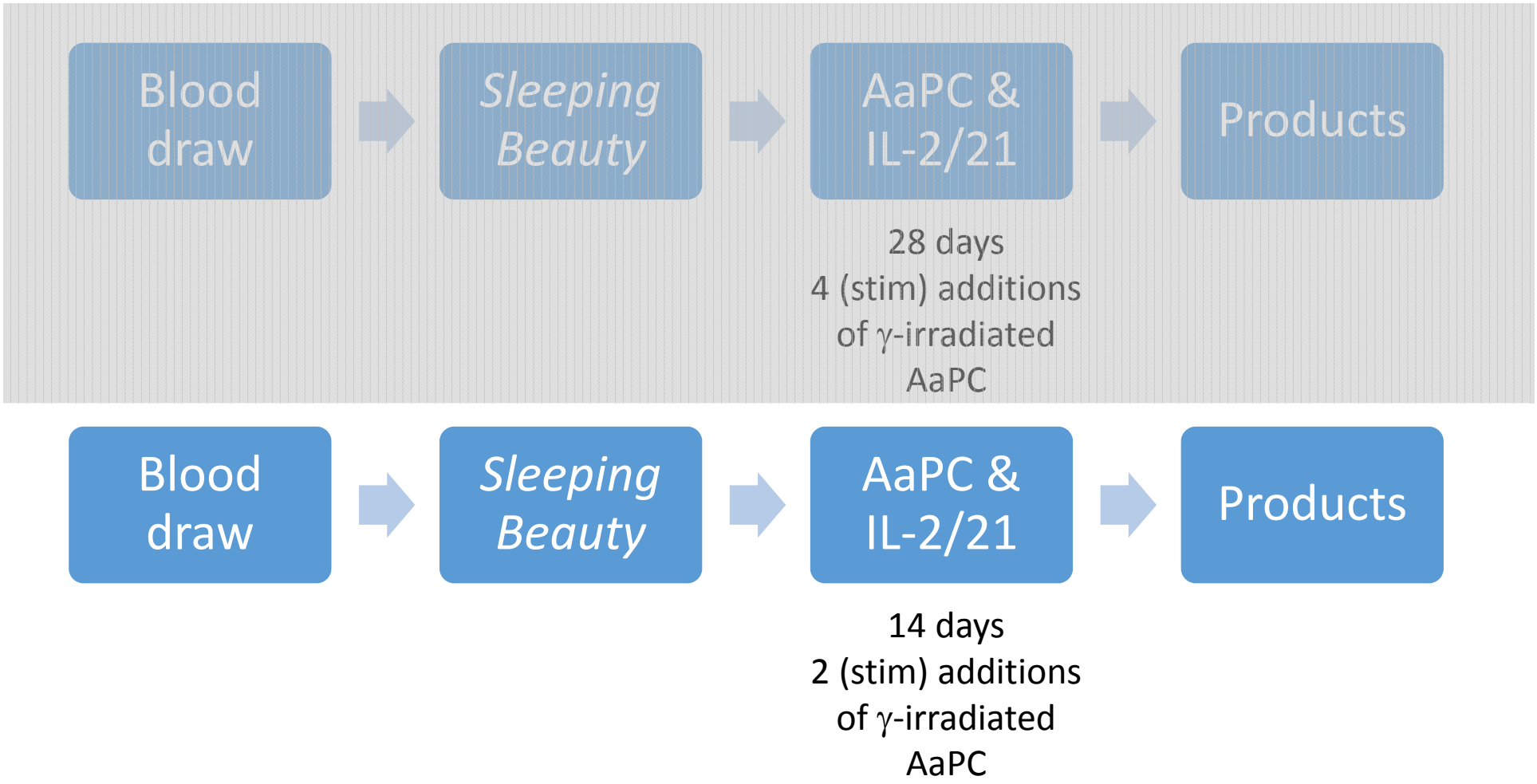




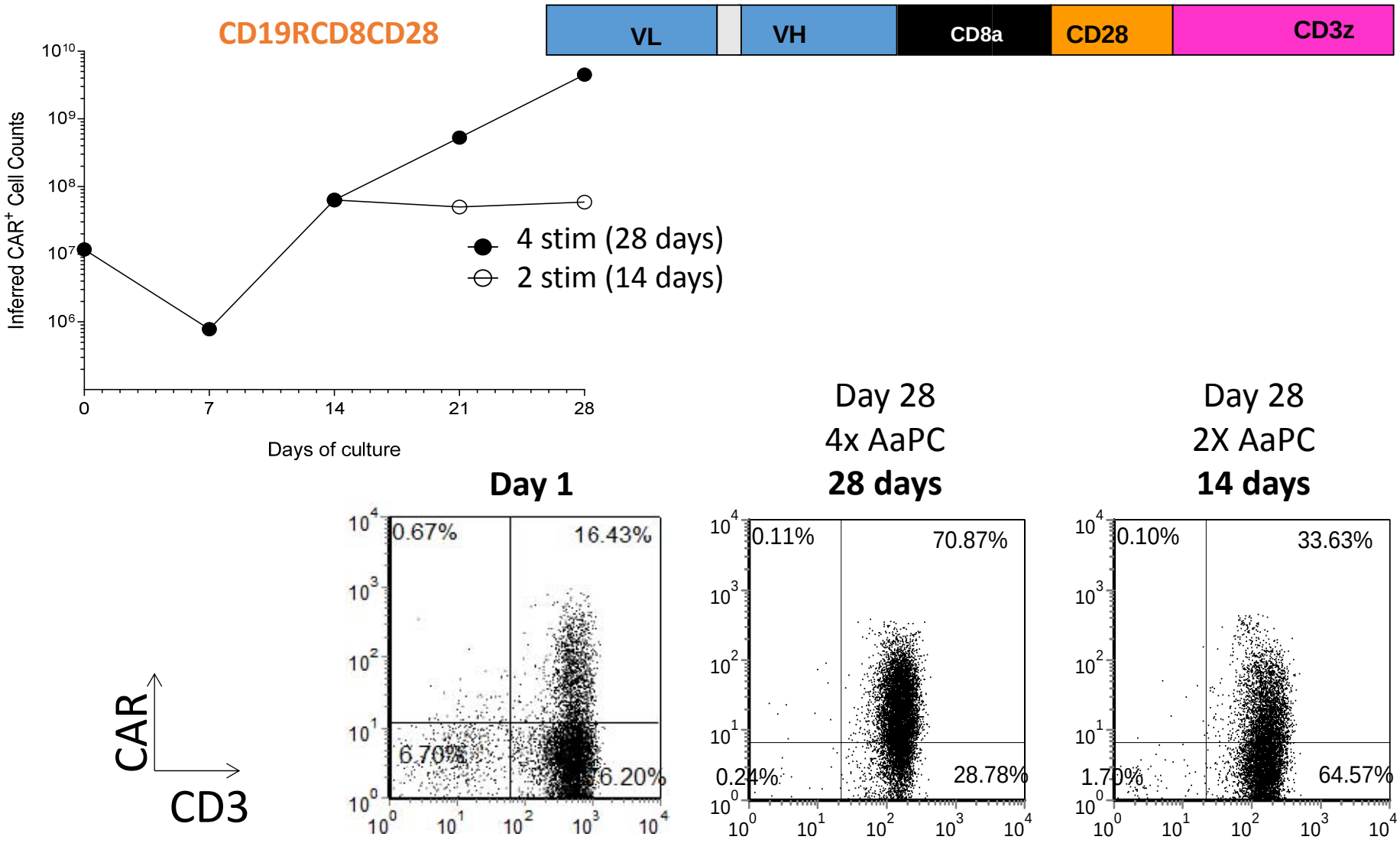
CD8 stalk improves *in vivo* anti-tumor effect of CAR$^+$ T cells propagated for 28 days (4 stim) on AaPC



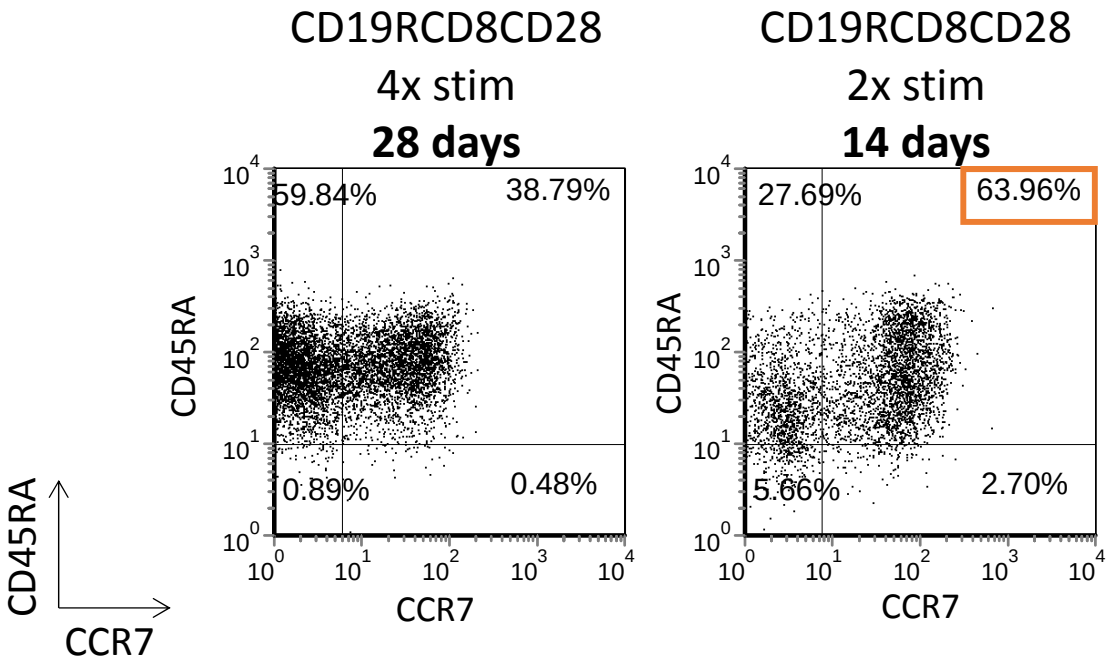
Shorten *ex vivo* time in culture from 28 to 14 days

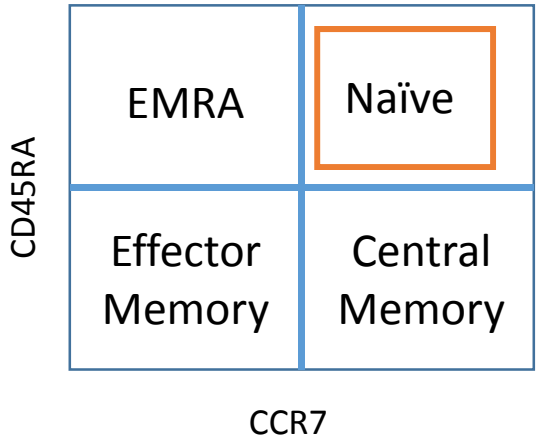


Phenotype of CAR+ T cells propagated for 28- (4 stim) versus 14- (2 stim) days on AaPC



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

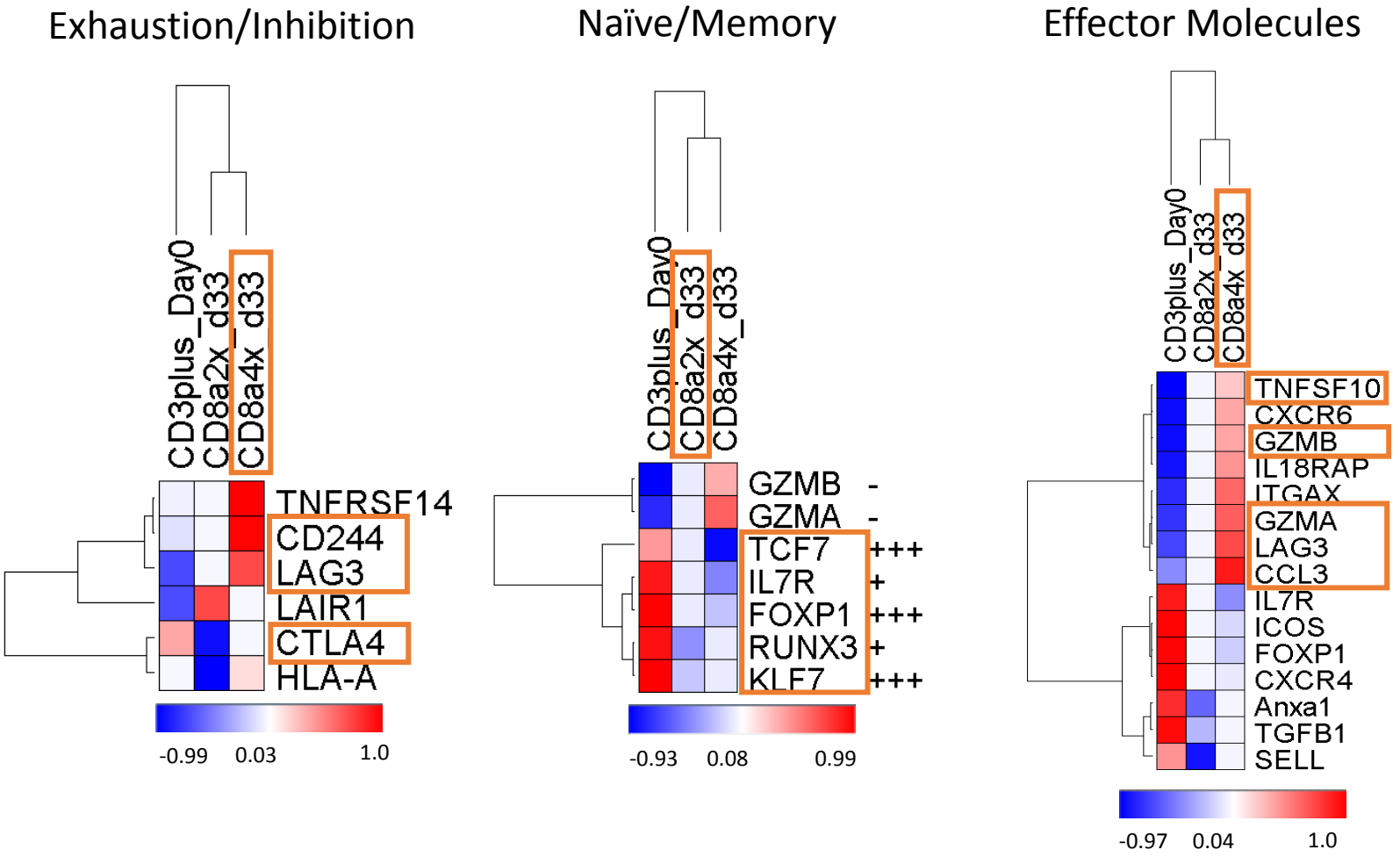




Cells gated on CAR$^+$ cells

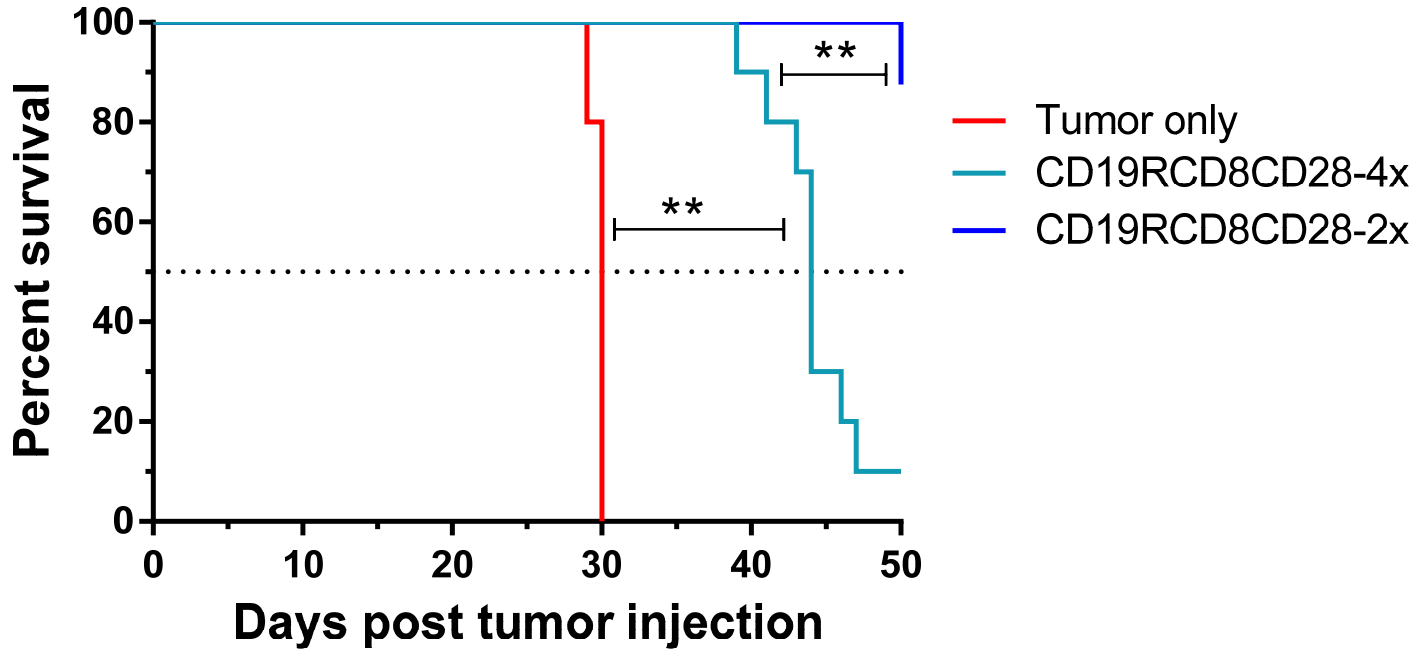
Sallusto F, et al. Nature. 401(6754):708-12.

Propagation of CAR^{+} T cells after 14 days (2-stim) on AaPC maintains a naïve-memory and less exhausted transcriptional profiles



Propagation of CAR^{+} T cells after 14 days (2-stim) on AaPC improves anti-tumor effect

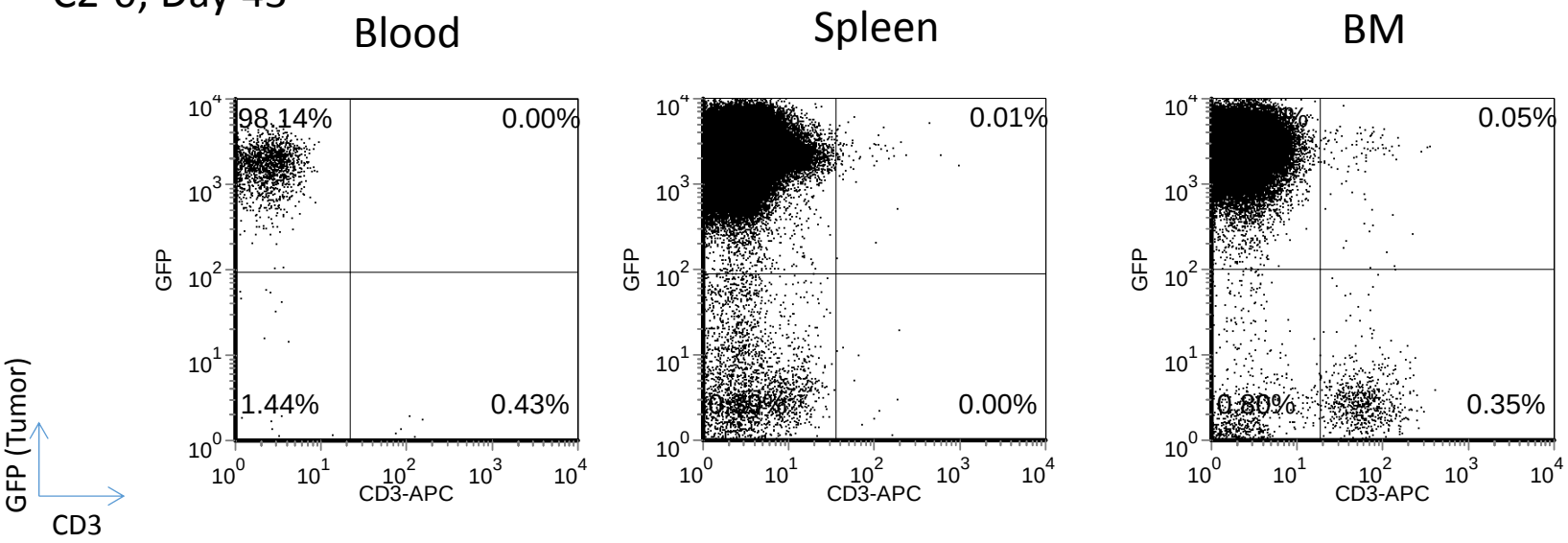
CD19^{+} NALM-6 tumor model in NSG mice



**p<0.01

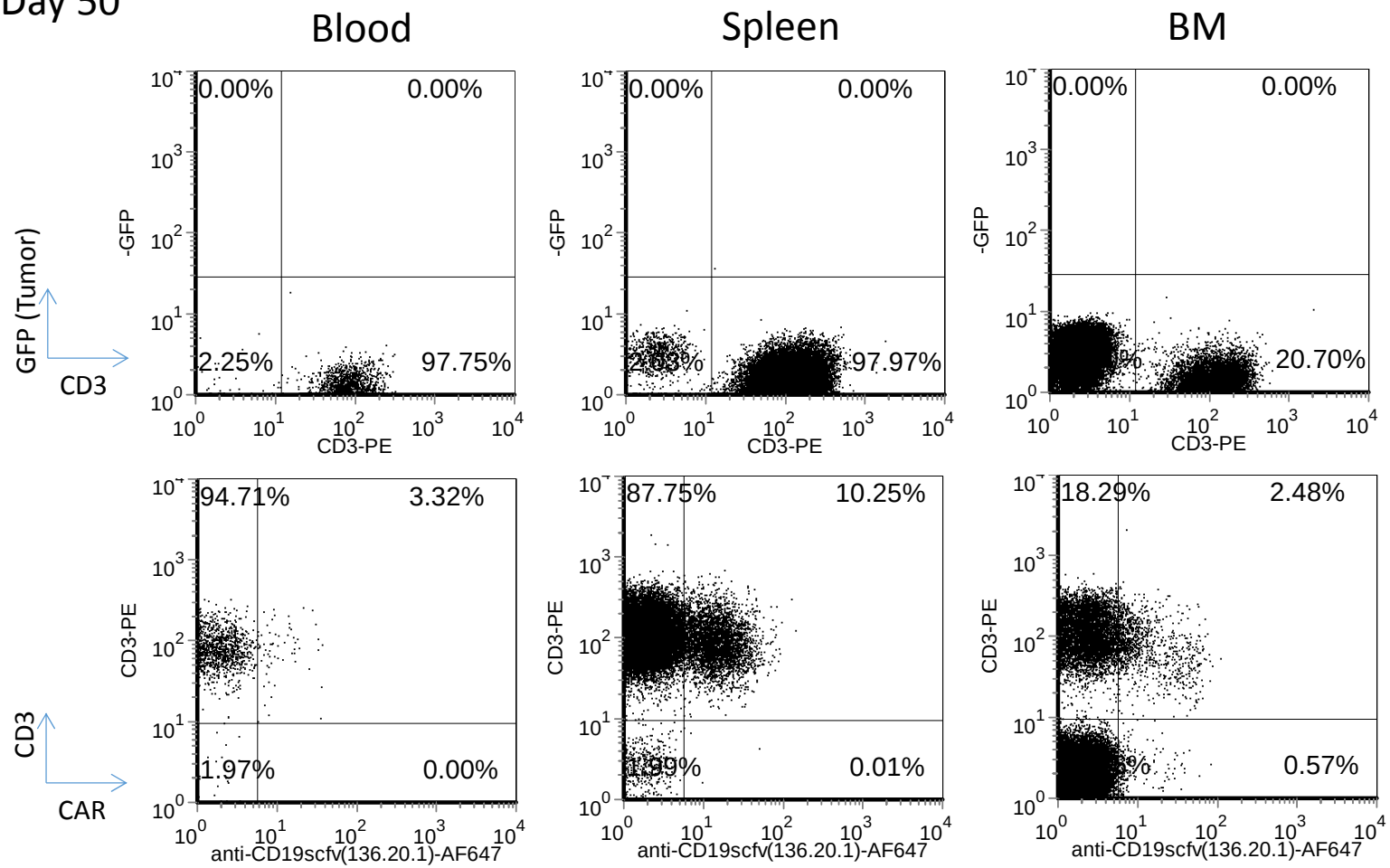
Propagation of CAR^{+} T cells after 14 days (2-stim) on AaPC improves persistence

CD19RCD8CD28-4x
C2-0; Day 43



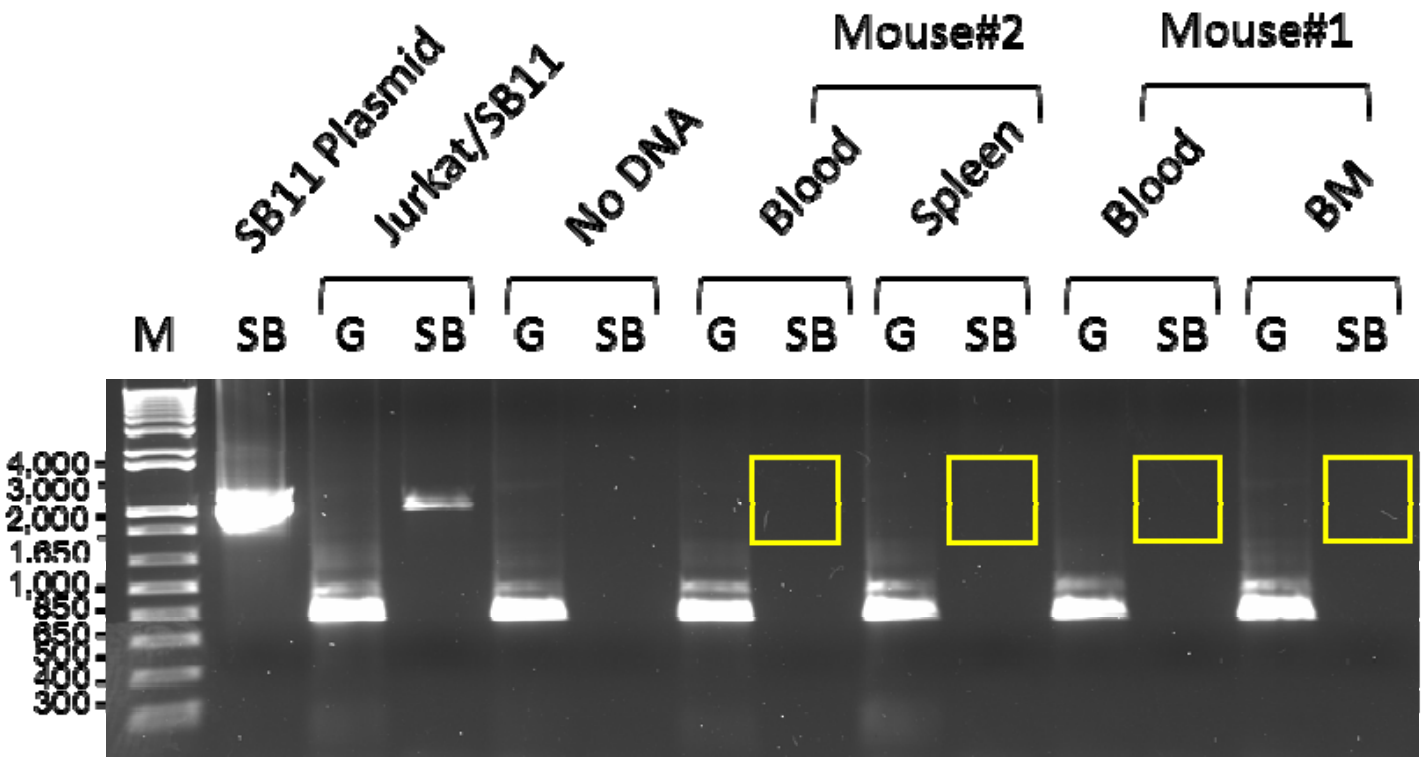
Propagation of CAR^{+} T cells after 14 days (2-stim) on AaPC improves persistence

CD19RCD8CD28-2x
D1-2; Day 50



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

Tissues from mice infused with CAR^{+} T cells after 14 days (2-stim) on AaPC



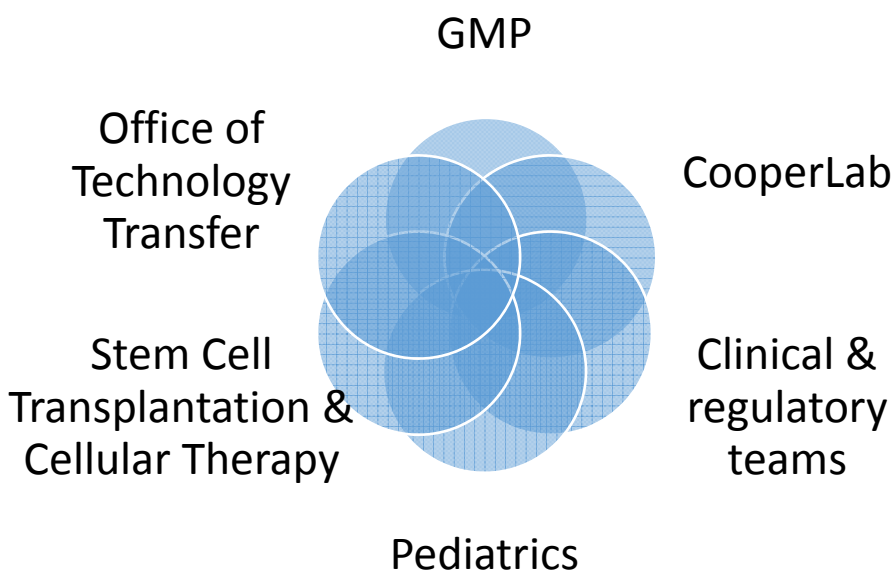
G = GAPDH Primers

SB = SB11 Primers

Summary

- CD8 stalk improves anti-tumor effect of CD19-specific CAR^+ T cells
- Propagating with 2-stim (14-day) on γ-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



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