

# Gene Modification of Hematopoietic Stem Cells for Enhancement of Graft-versus-Lymphoma Effect



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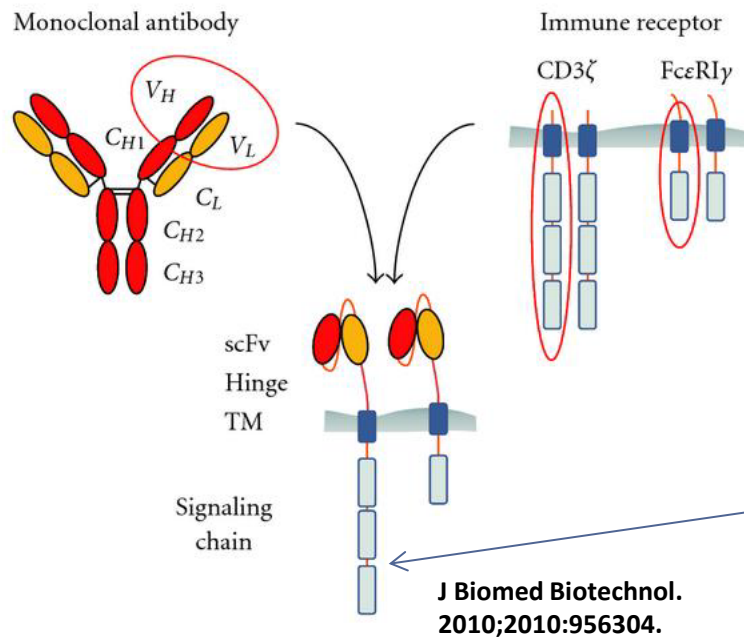
# Outline

1. Targeting CD19 with Chimeric Antigen Receptors (CAR)
2. CAR Modification of Hematopoietic Stem Cells
3. CAR-Engineered Specificity of HSC Progeny
4. Engraftment and Tumor Challenge in Animal Model
5. Next Steps



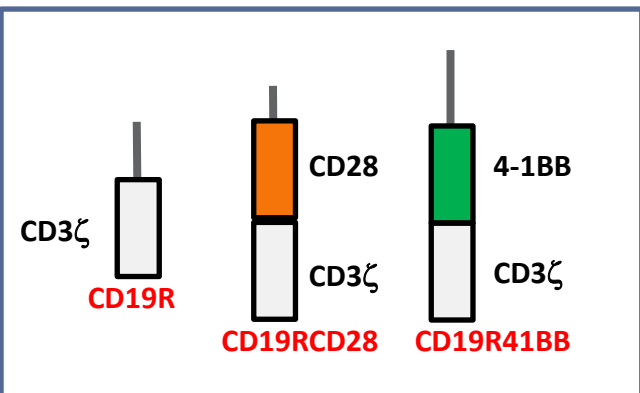
# Targeting CD19 with Chimeric Antigen Receptors

- **B-lineage NHL** account for ~80% of pediatric NHL and ~10% of childhood cancers (650 new cases/year)
- Recurrent disease: **less than 50%** of chance of cure



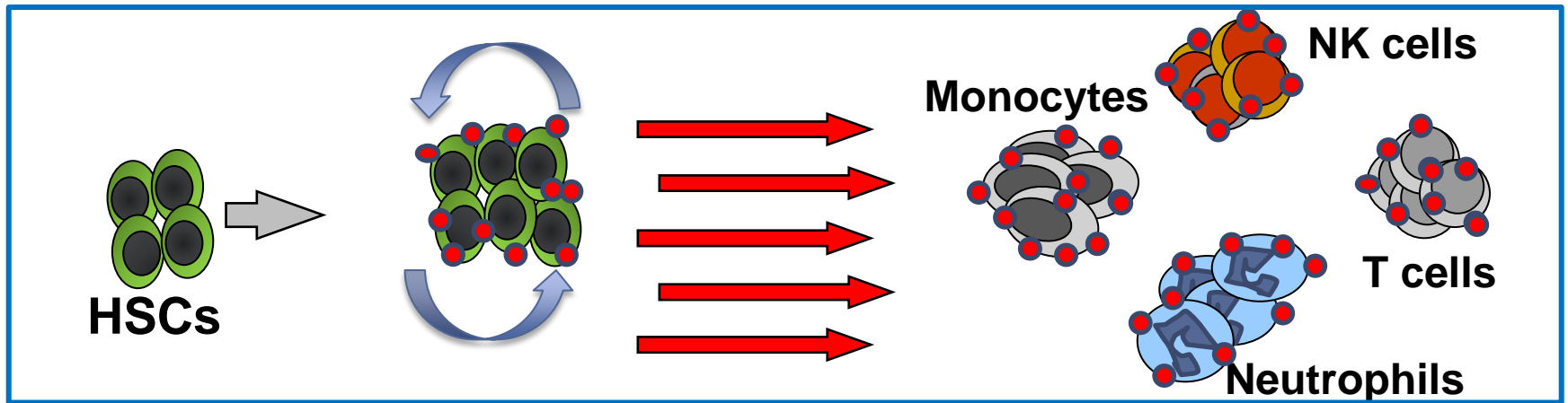
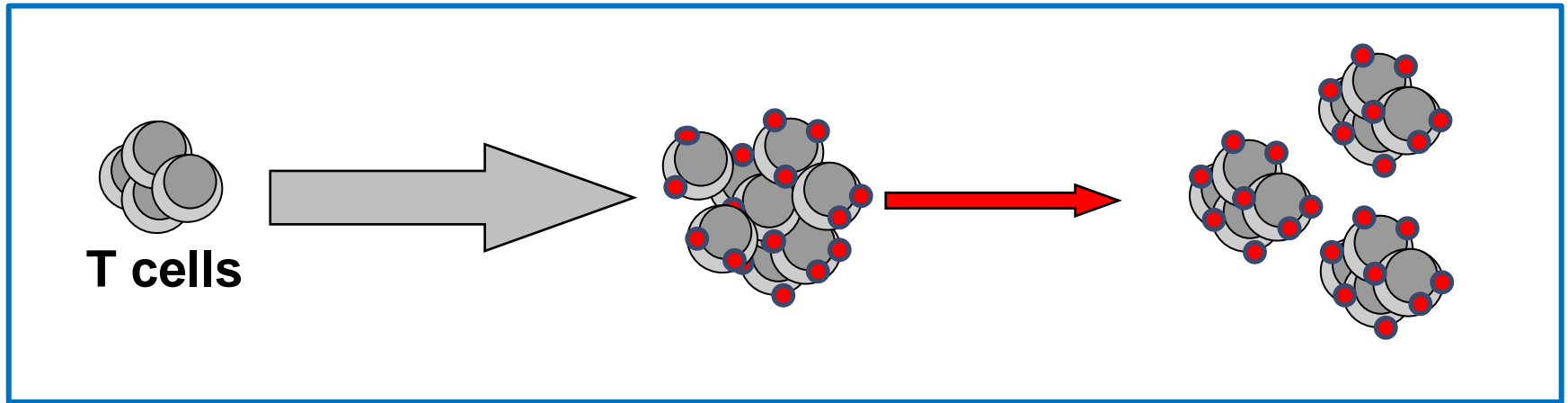
## CD19:

- *Not present on HSC*
- *Ablation is compatible w/ life*

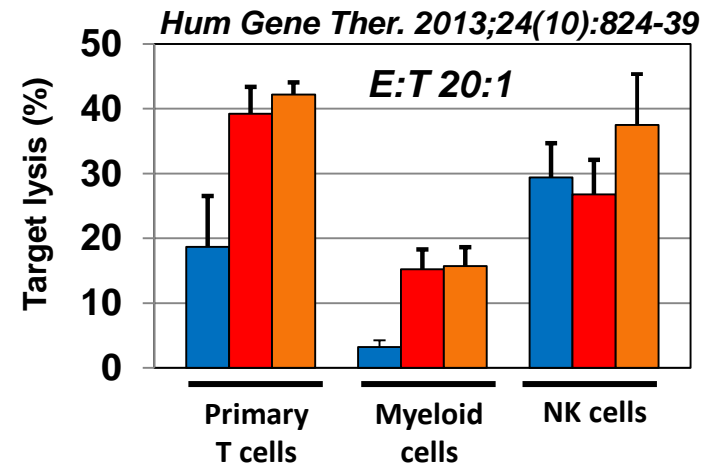
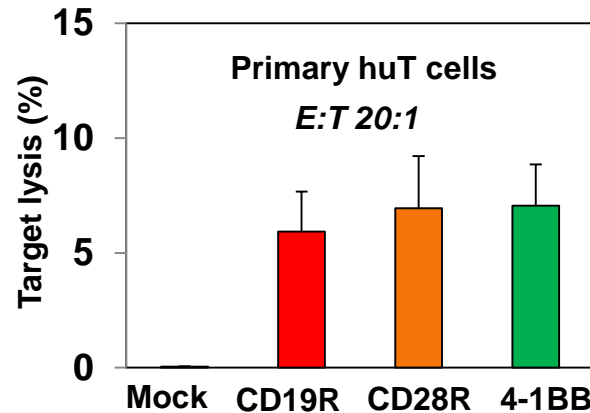
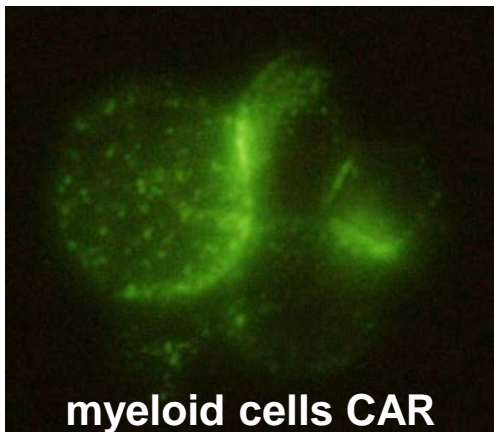
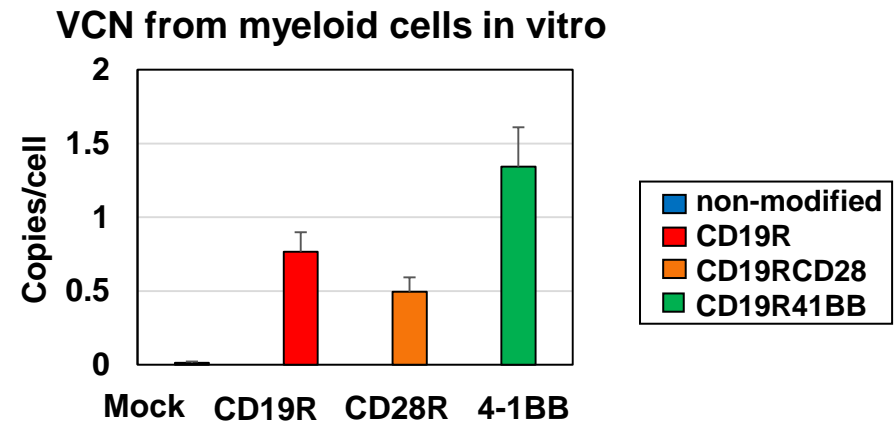
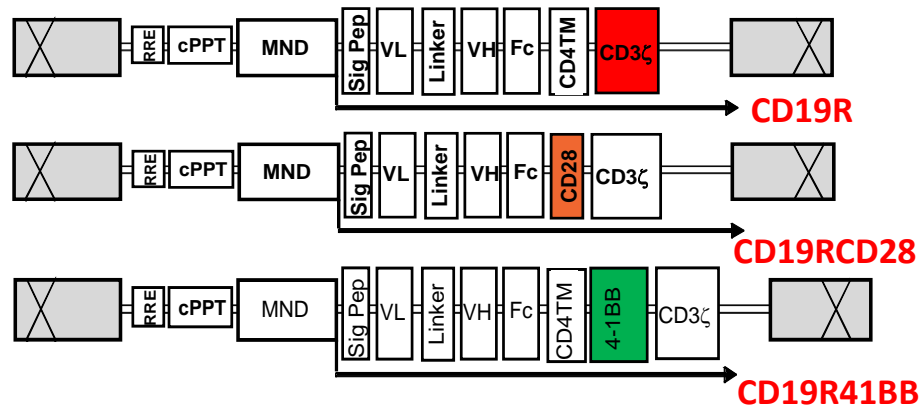


# Hypothesis:

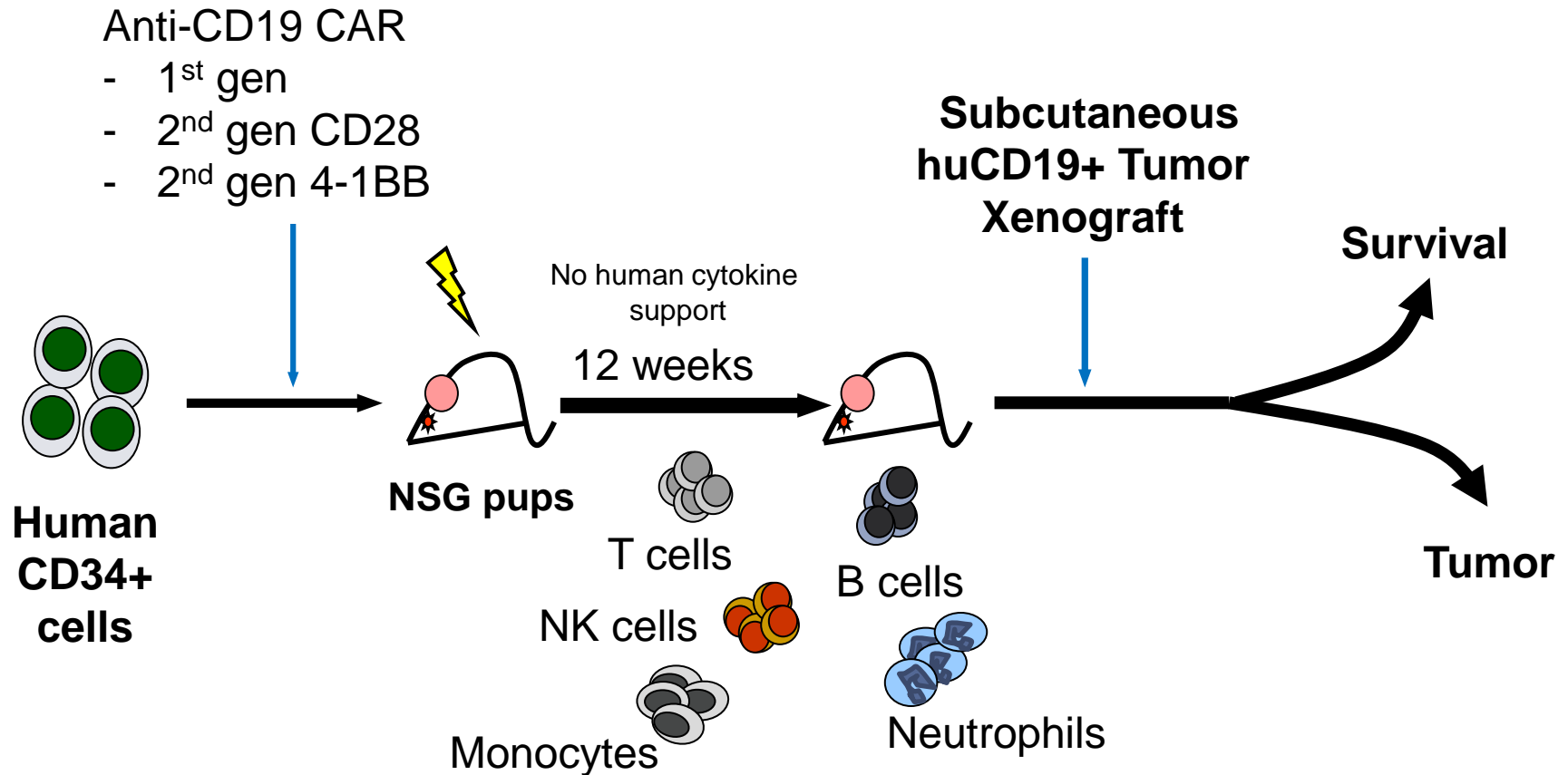
Development of persistent multilineage anti-leukemic effect



# CAR-Engineered Specificity of HSC Progeny



# *In vivo* model of CAR Modification of Human HSC



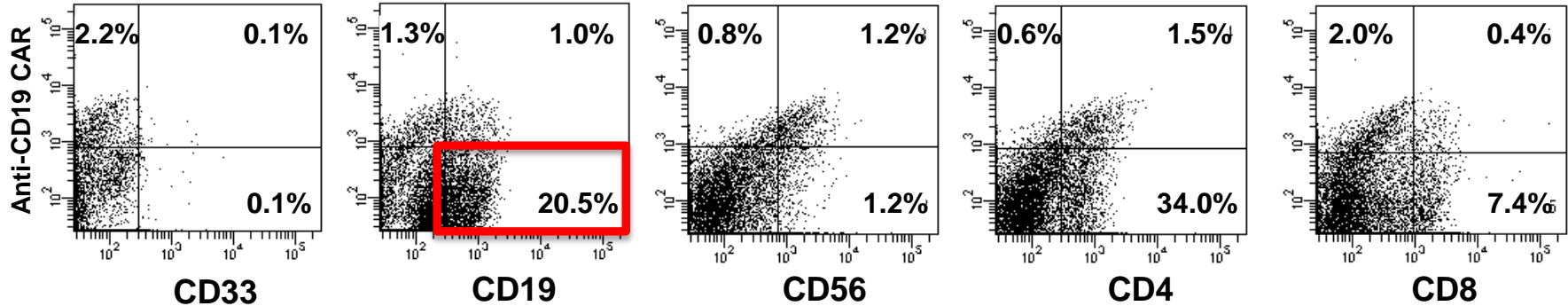
*De Oliveira et al. Hum Gene Ther. 2013;24(10):824-39*



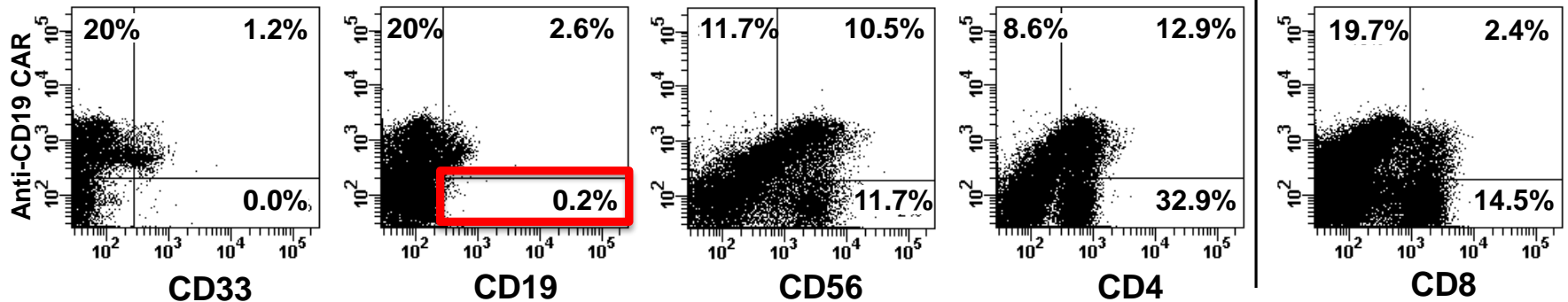
# Engraftment and CAR Expression in Humanized NSG

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## Mock Spleen



## CD19<sup>+</sup>CD28<sup>+</sup> Spleen



*Immunol Rev* 2013;257(1):237-49



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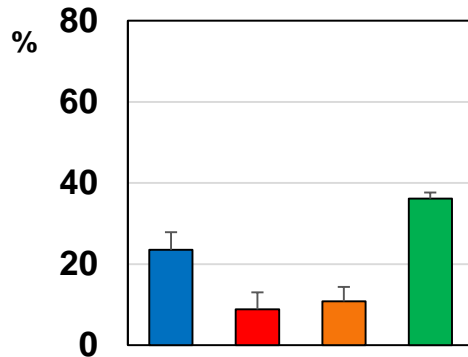
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# Engraftment and CAR Expression in Humanized NSG

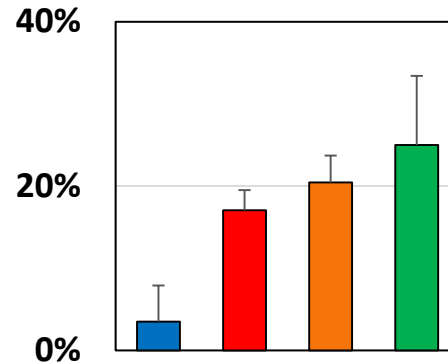
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**BM**

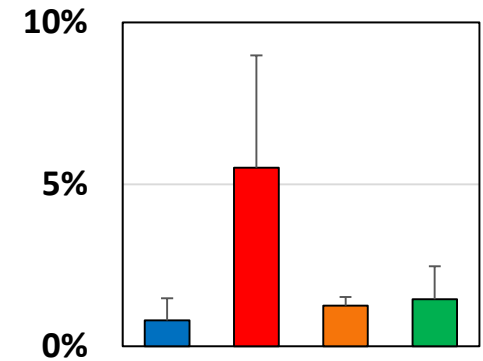
**huCD45%**



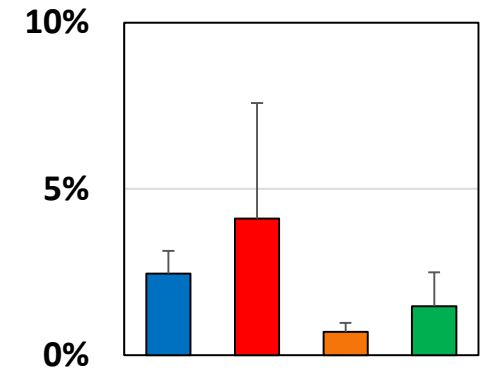
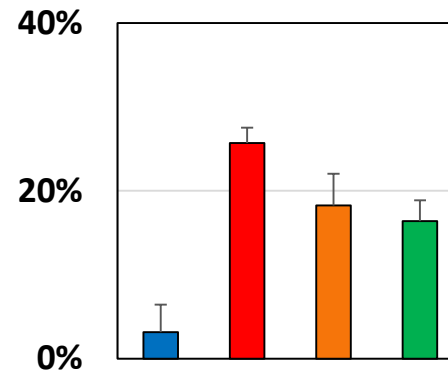
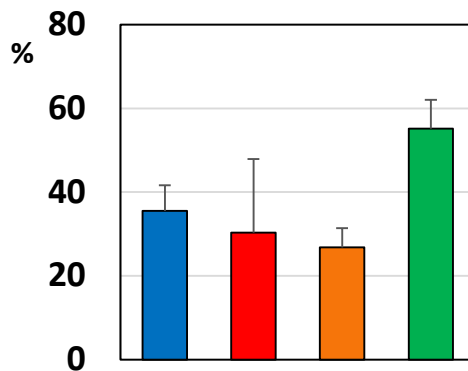
**CAR% in huCD45**



**CD19% in huCD45**



**Spleen**



- MOCK
- CD19R
- CD19RCD28
- CD19R-41BB



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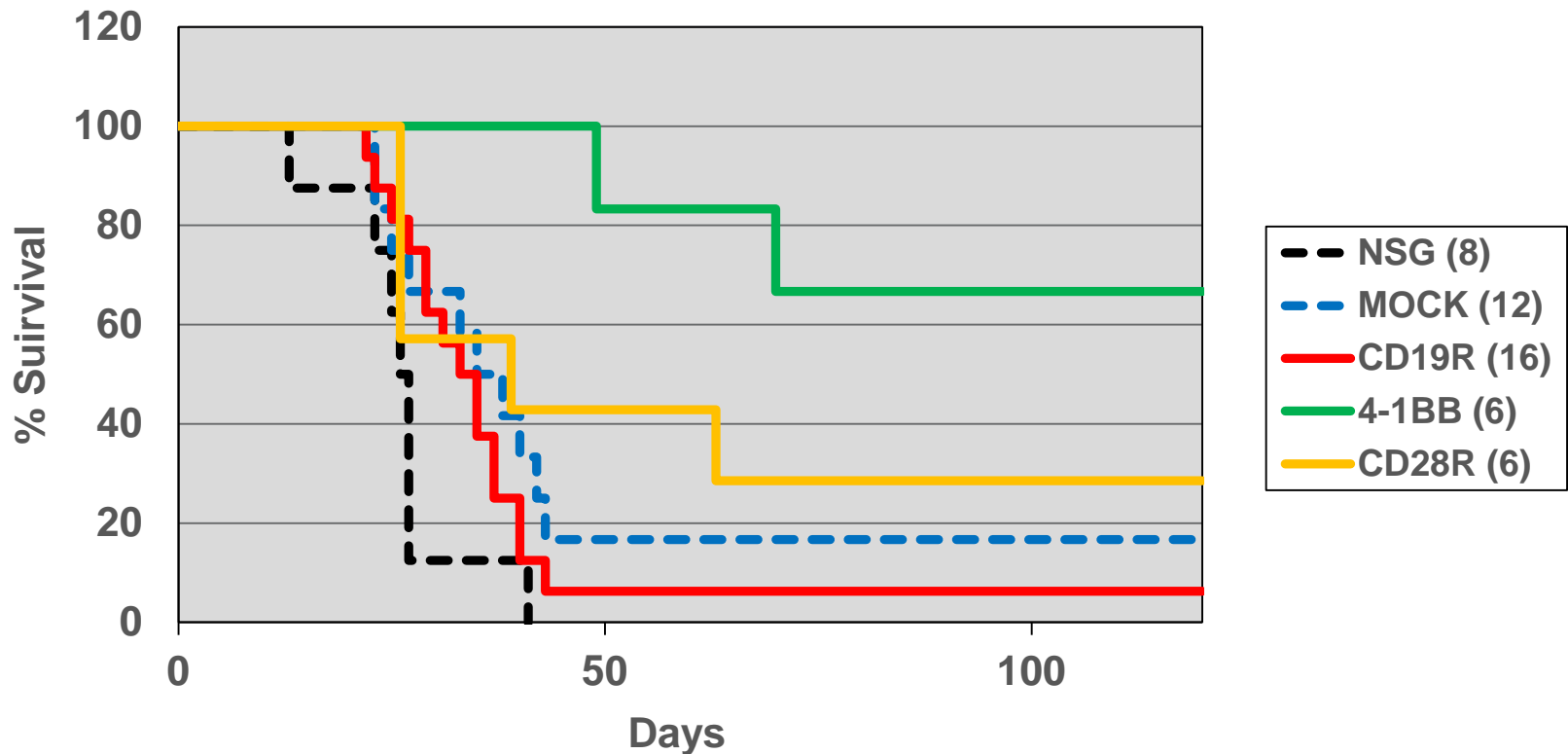
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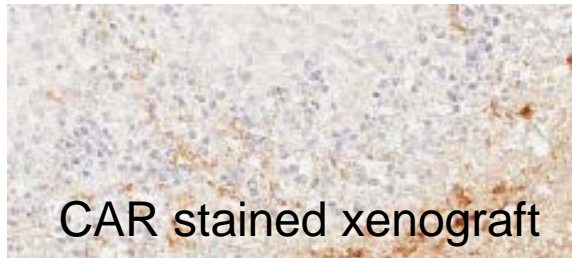
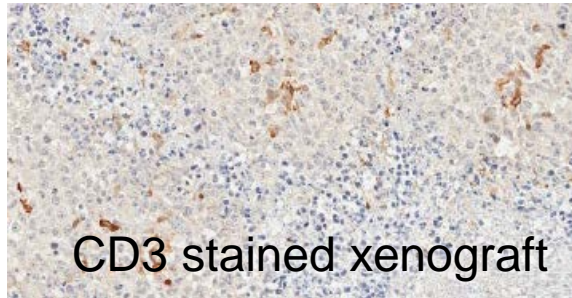
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# CAR-modified HSC Elicit Survival Advantage Against CD19+ Tumor



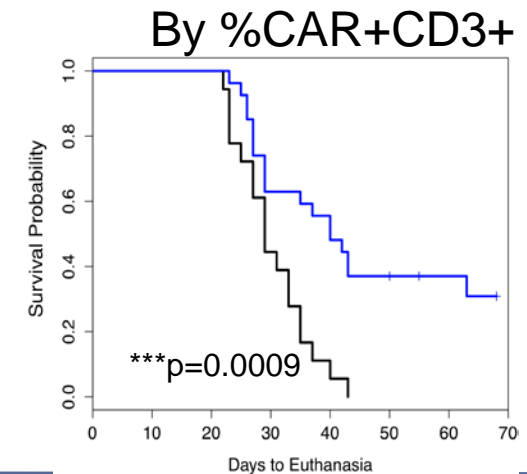
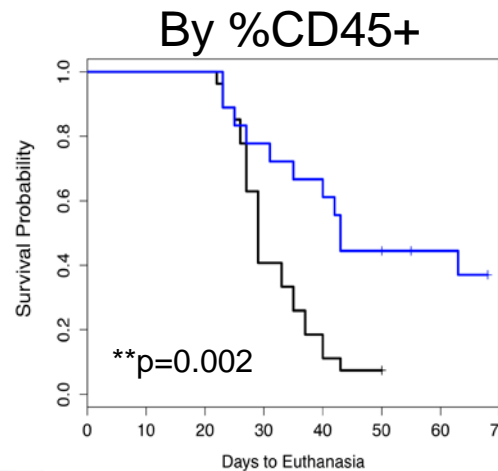
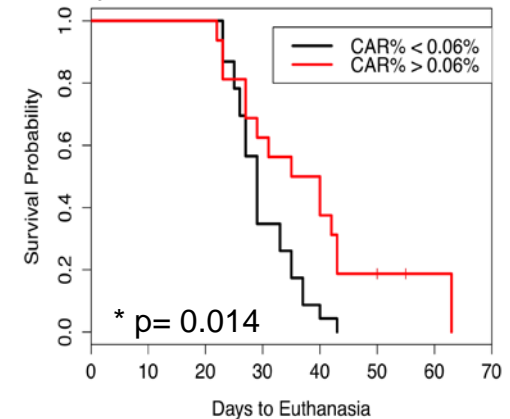
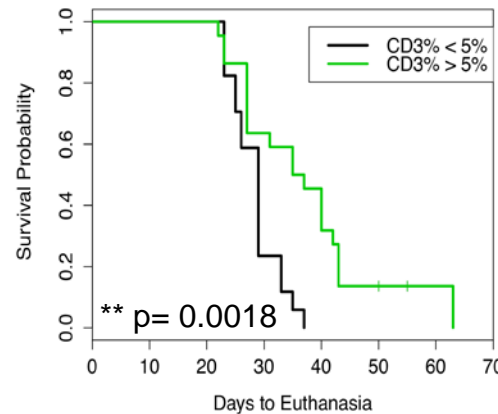
# CAR Engraftment and Tumor Infiltration Determine Survival Advantage



10X Magnification

Survival advantage of higher engraftment of huCD45 and CAR+ CD3+ cells.

## Survival by % Tumor Infiltration by CD3+ and CAR+ Cells



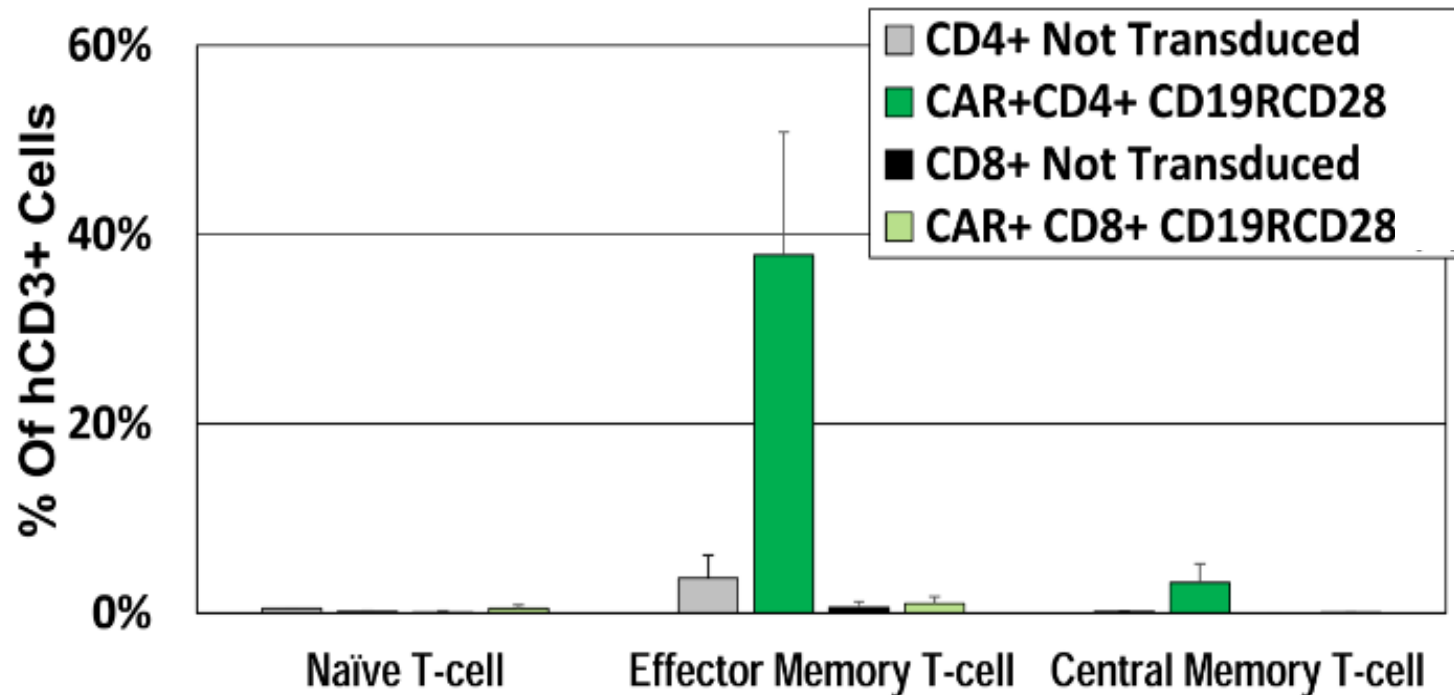
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# CAR-expressing T cell Memory Subsets



Naïve T cell: CD3+CD45RA+CD45RO-CD62L+CCR7+  
 Effector T cell: CD3+CD45RA+CD45RO-CD62L-  
 T cell Effector Memory: CD3+ CD45RO+ CD62L- CCR7-  
 T cell Central Memory: CD3+ CD45RO+ CD62L+ CCR7+



# Summary and Next Steps

## Summary:

- CAR-modification of HSC is feasible, does not impair engraftment and directs immune specificity
- 2<sup>nd</sup> generation CAR determines anti-lymphoma protection and generates T cell memory phenotypes
- Easy application in the clinical setting for patients requiring autologous stem cell infusion as standard of care.

## Next steps:

- Addition of Suicide Gene approaches
- Regulatory steps and “first-in-human” studies



# Acknowledgments



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