SITC 2019

NOVEMBER 6–10
NATIONAL HARBOR, MARYLAND
Gaylord National Hotel & Convention Center

Novel Multi-Targeted Therapeutic Platforms • Nov. 6, 2019

Wednesday, Nov. 6, 2019
2 – 6:30 p.m.

Francesco Marincola
Chief Science Officer, Refuge Biotechnologies
Franco.Marincola@refugebiotech.com

Session 2: Novel Platforms and Innovation

Time:	4:15 – 5:15 p.m.
4:15 p.m.	**Contextual reprogramming of T cells for multi-targeted therapeutics: checkpoint blockade, immune resilience, and stemness to overcome immune resistance and reduce toxicity, all in one cell product** Francesco M. Marincola, MD – *Refuge Biotechnologies*

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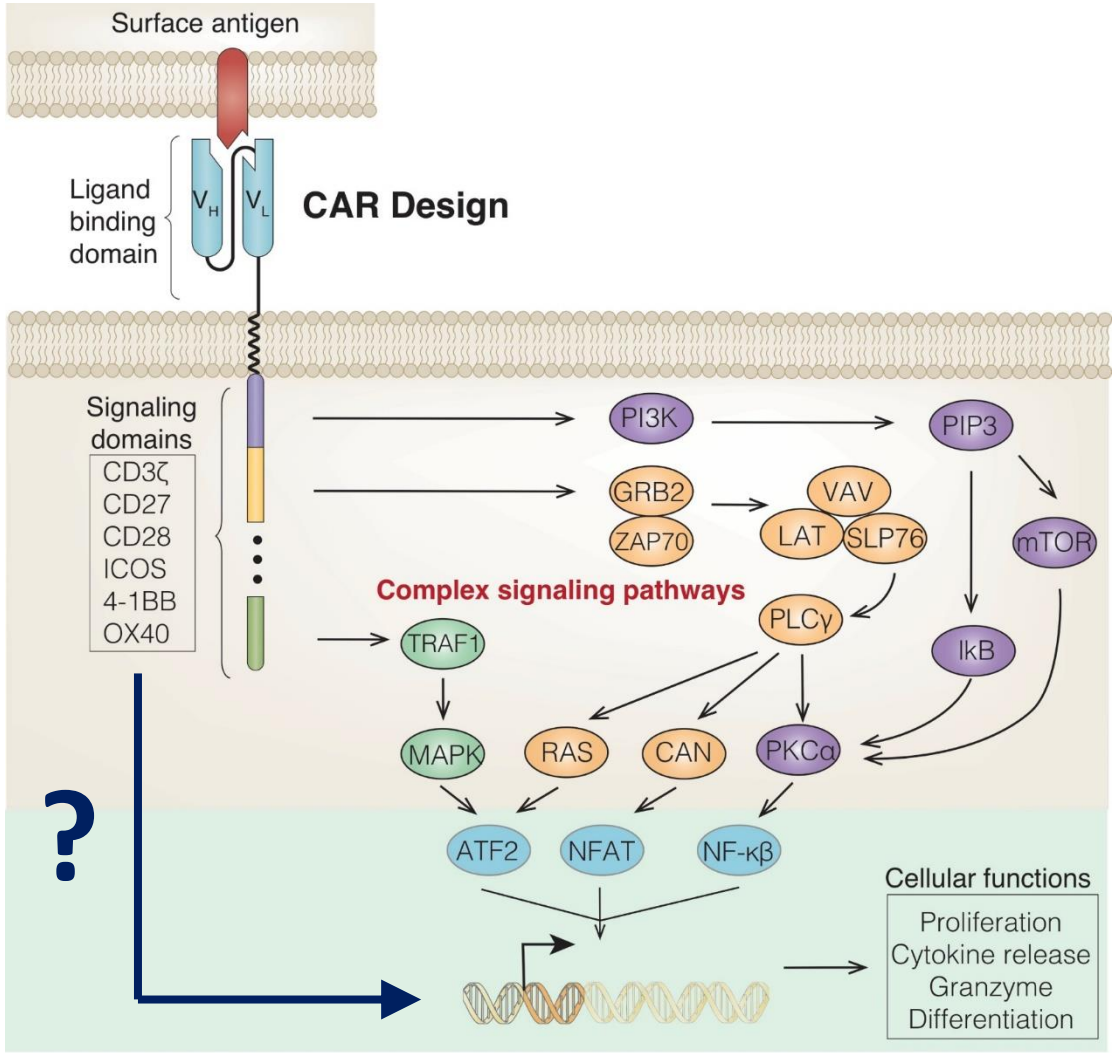
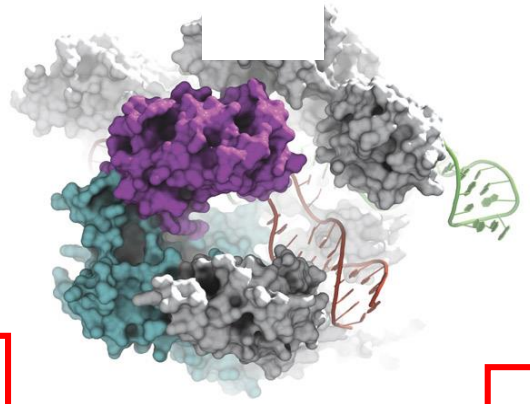
A bioengineer's perspective on CAR T cell design



dCas: RNA-guided programmable gene regulator

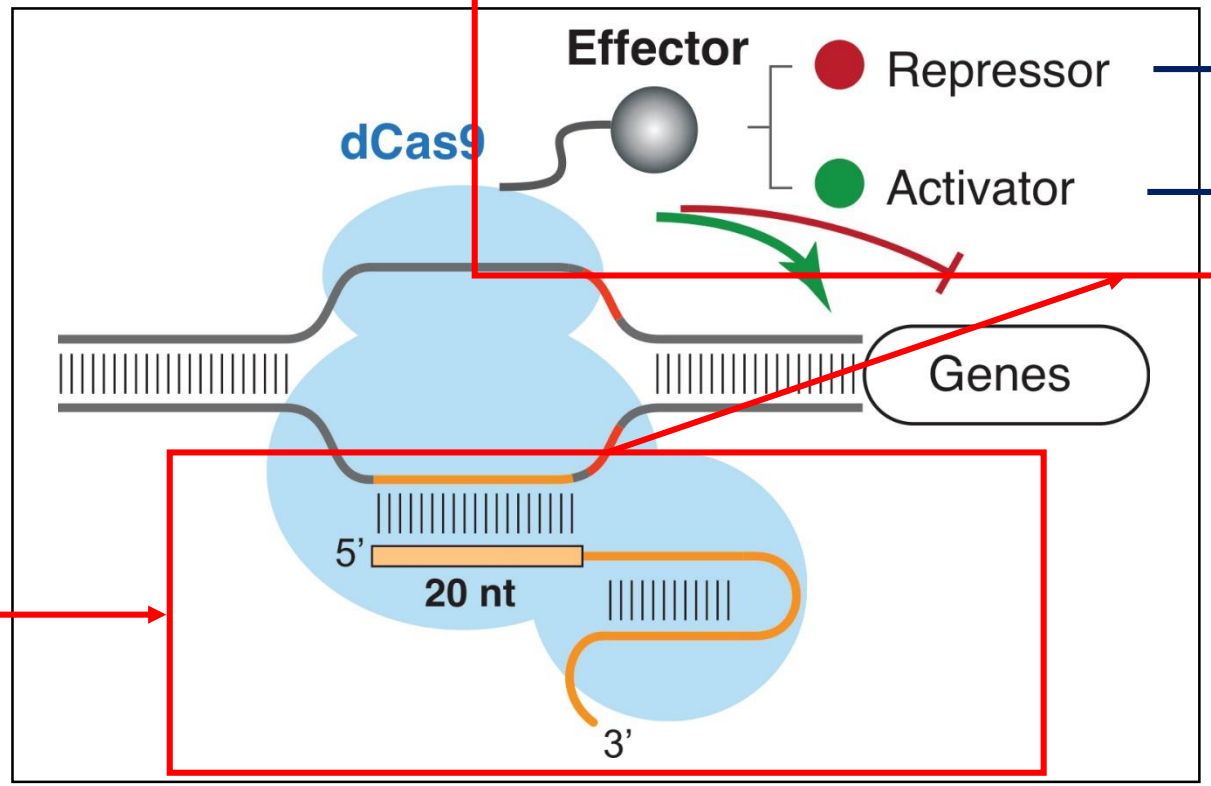
dCas9 →



Qi et al. *Cell* 2013
Gilbert et al. *Cell* 2013
Gao *Nature Method* 2016
Wang et al. *Cell* 2018
Wang et al. Science 2019

Stanley Qi
Refuge Co-Founder
Stanford University

d = nuclease-deactivated



CRISPRi, Interference

CRISPRa, Activation

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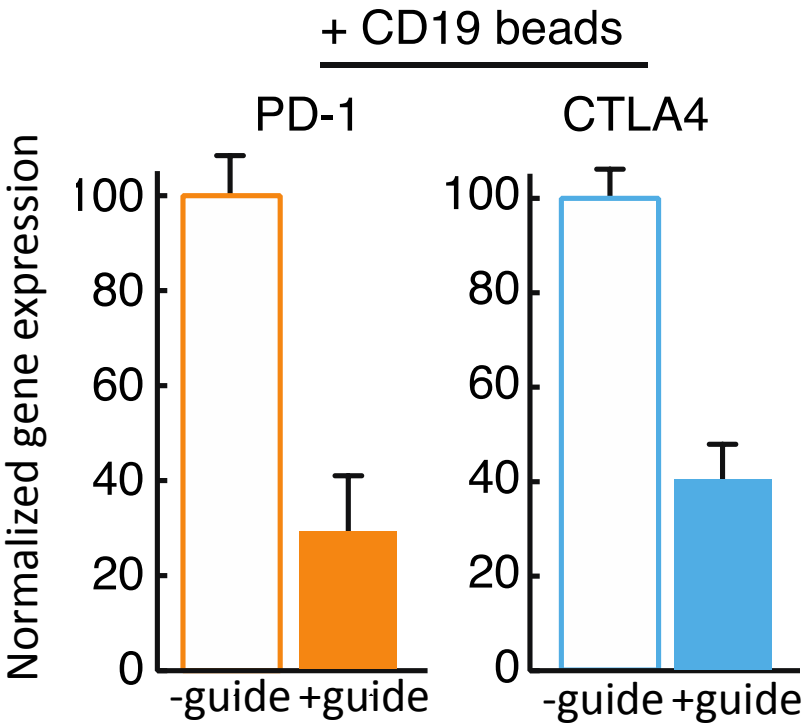
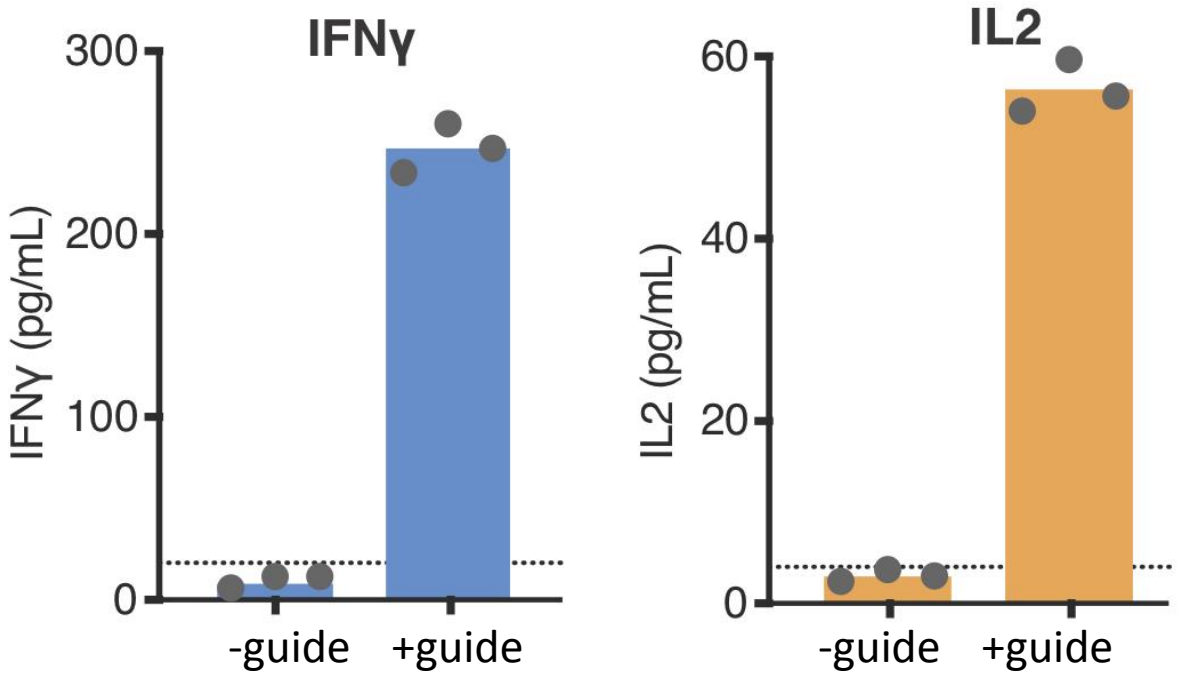
dCas can control expression of any gene

CRISPRi (interference)



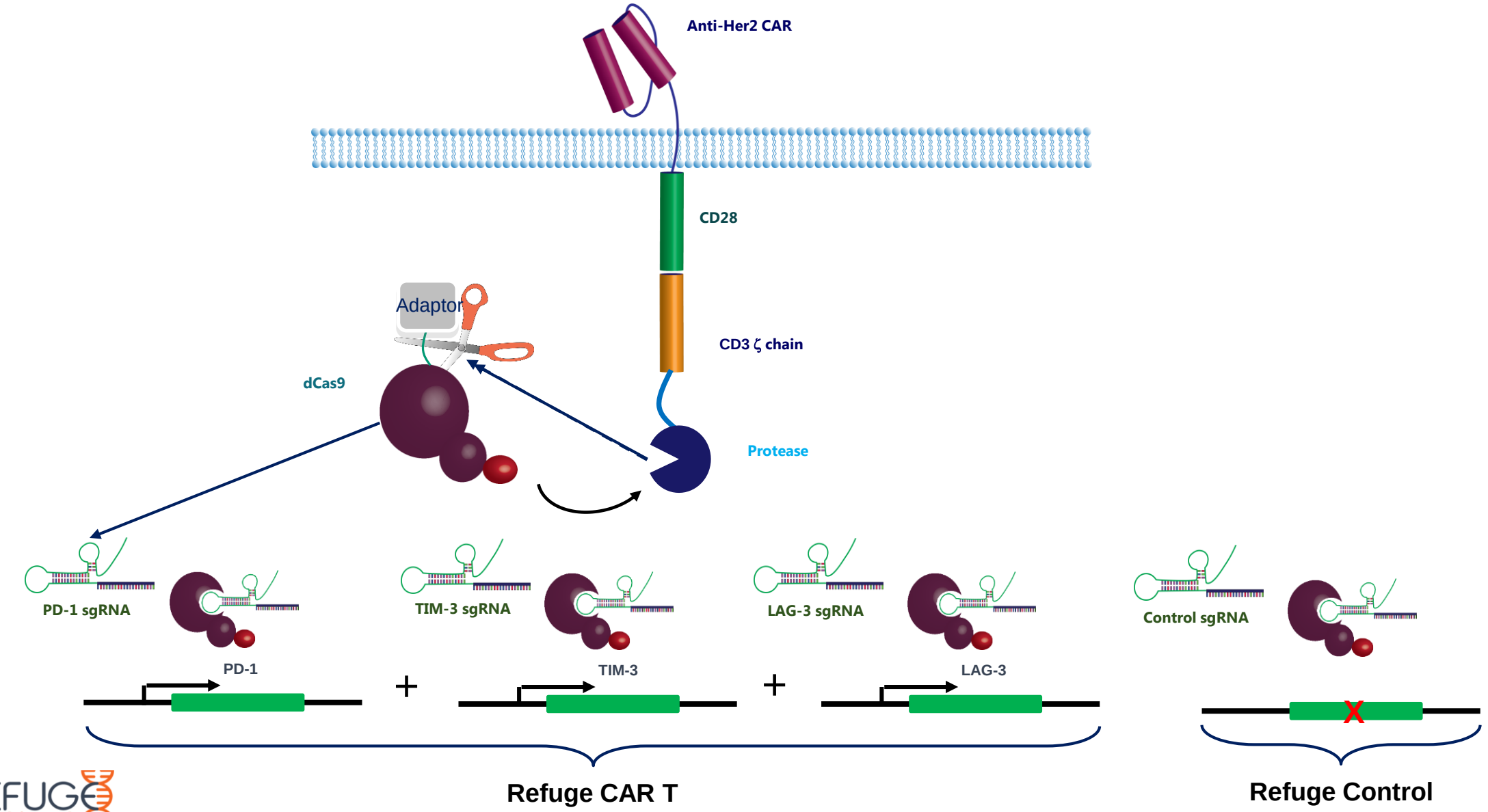
CRISPRa (activation)



- Primary human T cells
- Simultaneous repression or activation

CRISPR Interference in IO: the CAR T cell model

Reprogramming T cells to overcome “compensatory” immune resistance



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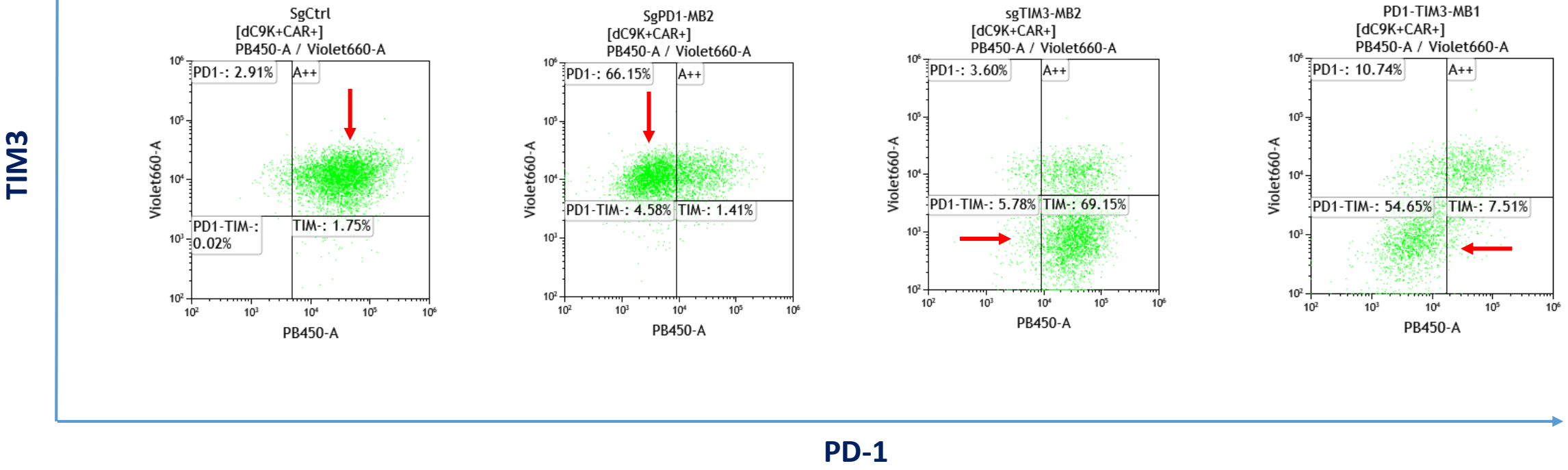
CRISPRi (interference) for dual knock down of PD-1 and TIM3

- **Day 0: Pan-T cells activated on coated plates**
- **Day 5: measure activation markers**

CTR sgRNA → **PD-1 sgRNA** → **TIM3 sgRNA** → **PD-1+TIM3 sgRNA**



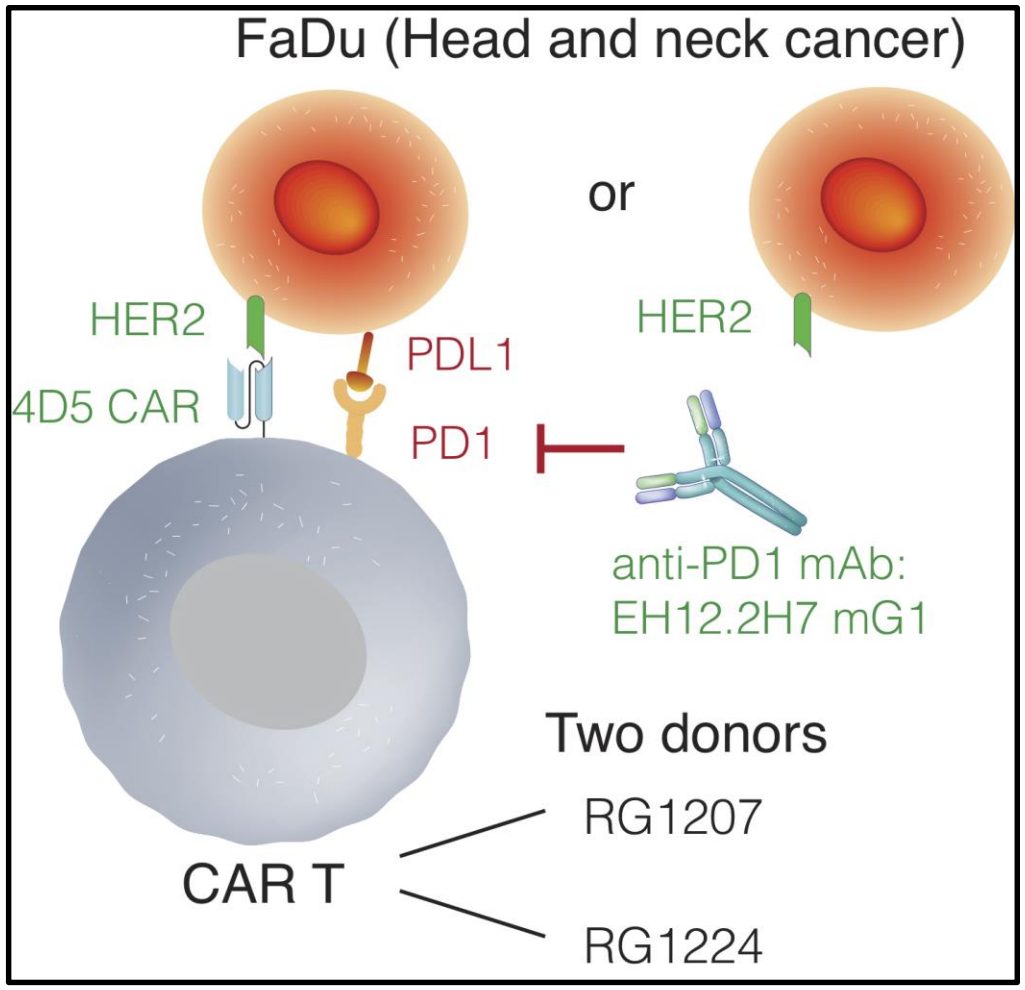
First-in-human trial to assess of feasibility and efficacy of delivering context-reactive CAR-T cells: the HER2/PD1 model.

RB-340-1 autologous Her2(4D5) cFv.CD28ζ/PD1sgRNA CAR T-cells

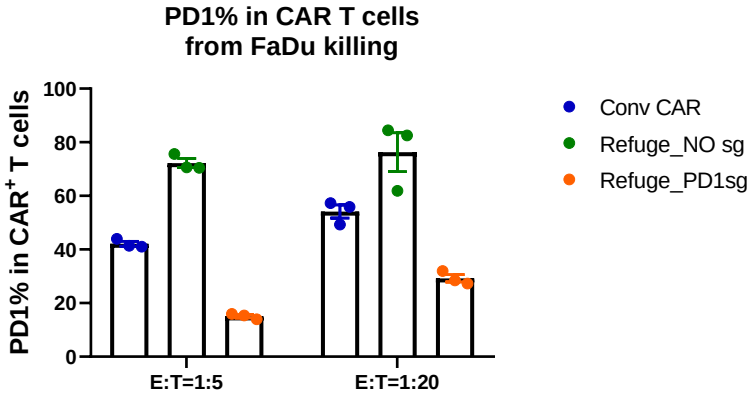
Pre-clinical Development

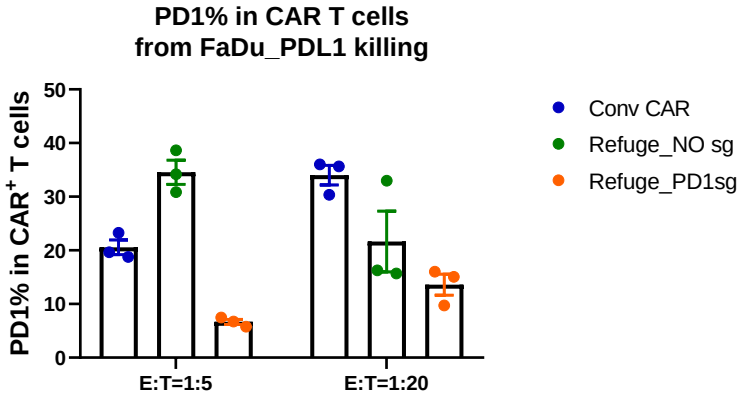
To assess:

Feasibility
Scalability
Tolerability
Immunogenicity
Trafficking
Efficacy



Mechanism of Efficacy – Effect of PD-1 regulation on proliferation and cytotoxicity in a three day exposure assay

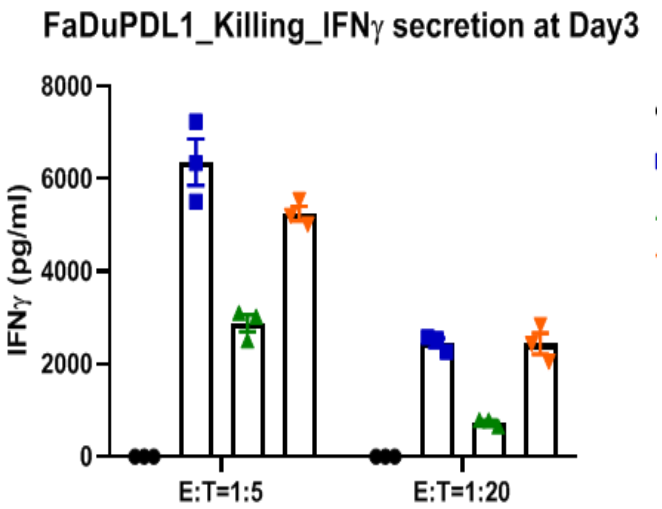


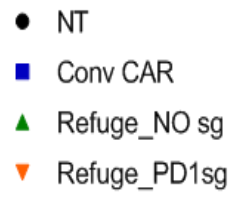


Enhanced cytokine production by Refuge CAR T cells

E:T ratio = 1:5 or 1:20
At day 3 of killing

IFN-γ





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Proof of Concept. *In vivo* activity of RB-340-1

- **Experimental Cohorts**
  (6 mice per group)

60 mice total

1. PBS > **Tumor Only**
2. Conventional HER2.CAR T cells (**0.25** million CAR+ T cells)
3. Conventional HER2.CAR T cells (**0.1** million CAR+ T cells)

Conventional HER2 CAR

4. Refuge HER2.CAR/No sgRNA T cells (**0.25** million CAR+ T cells)
5. Refuge HER2.CAR/No sgRNA T cells plus **Atezo*** (**0.25** million CAR+ T cells)
6. Refuge HER2.CAR/No sgRNA T cells (**0.1** million CAR+ T cells)
7. Refuge HER2.CAR/No sgRNA T cells plus **Atezo*** (**0.1** million CAR+ T cells)

Control HER2 CAR

8. Refuge HER2.CAR/PD1sgRNA T cells (**0.25** million CAR+ T cells)
9. Refuge HER2.CAR/PD1sgRNA T cells (**0.1** million CAR+ T cells)

Refuge HER2/PD-1 CAR

*Atezolizumab (PD-L1 blocking antibody, 5 mg/kg) i.v. twice a week following T cells injection

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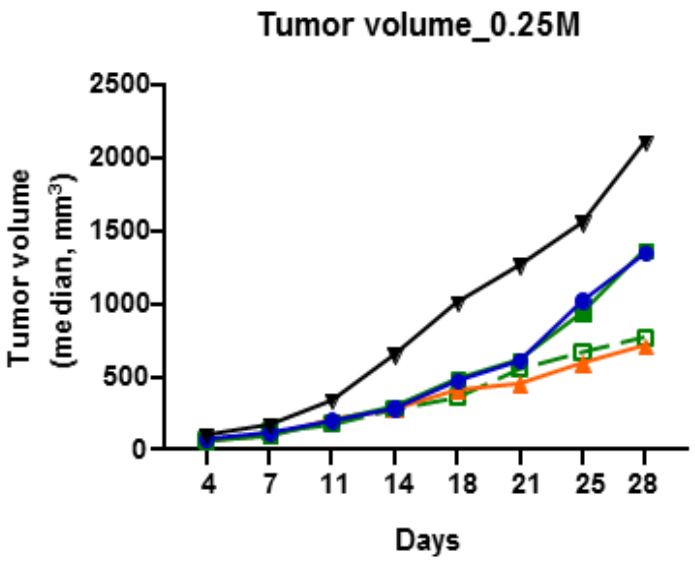
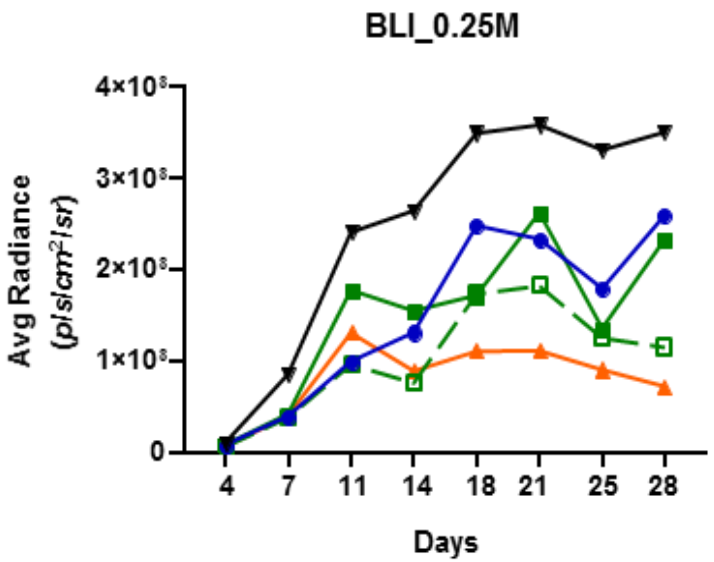
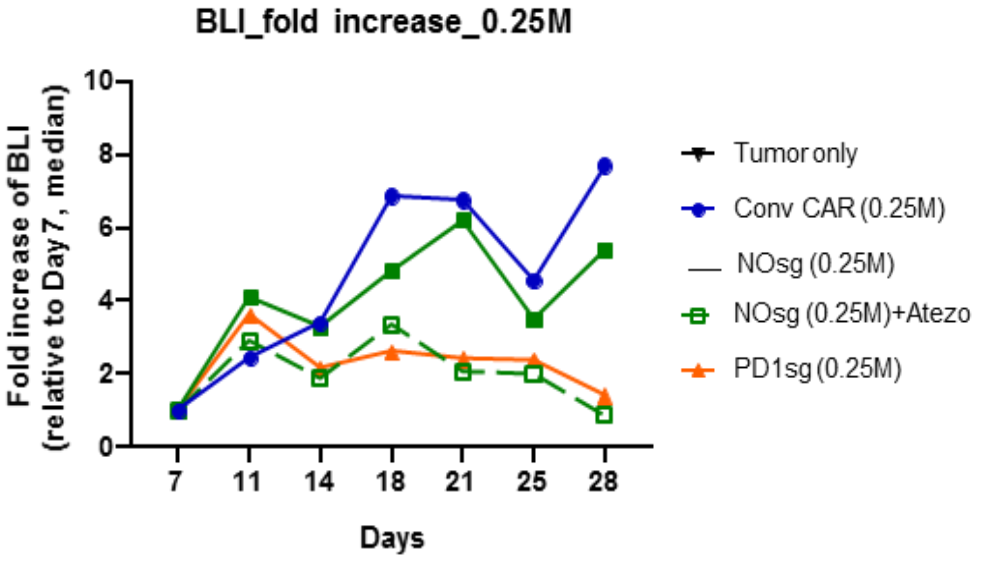
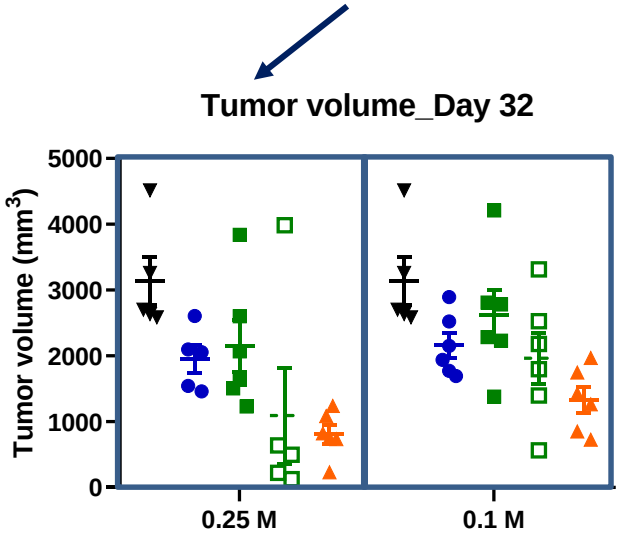
PD-L1 blockade enhances anti-tumor efficacy of NK cells
Oyer JL, et.al, ONCOIMMUNOLOGY 2018

Proof of Concept (POC) – *In vivo* activity of RB-340-1

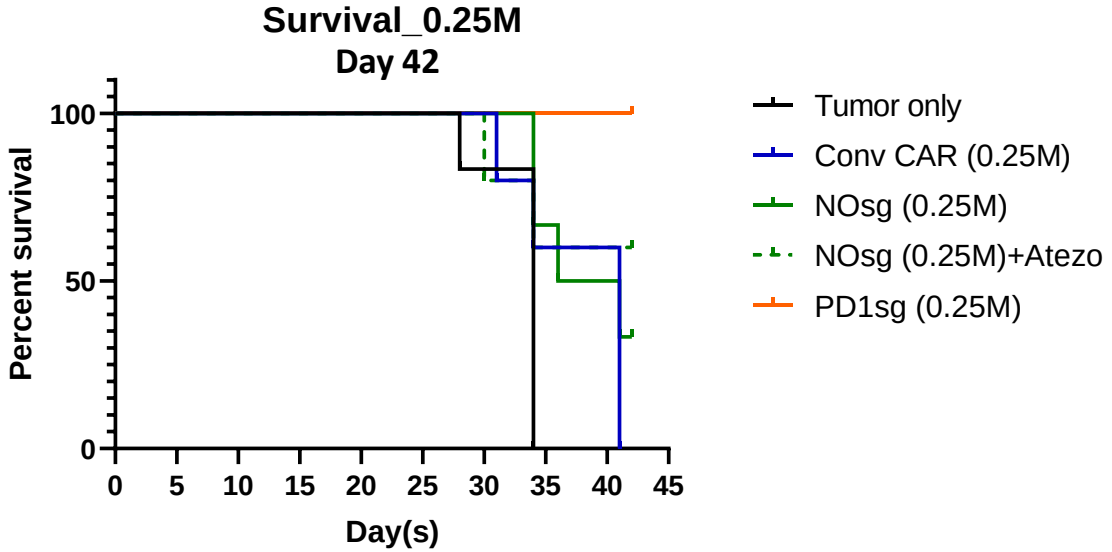






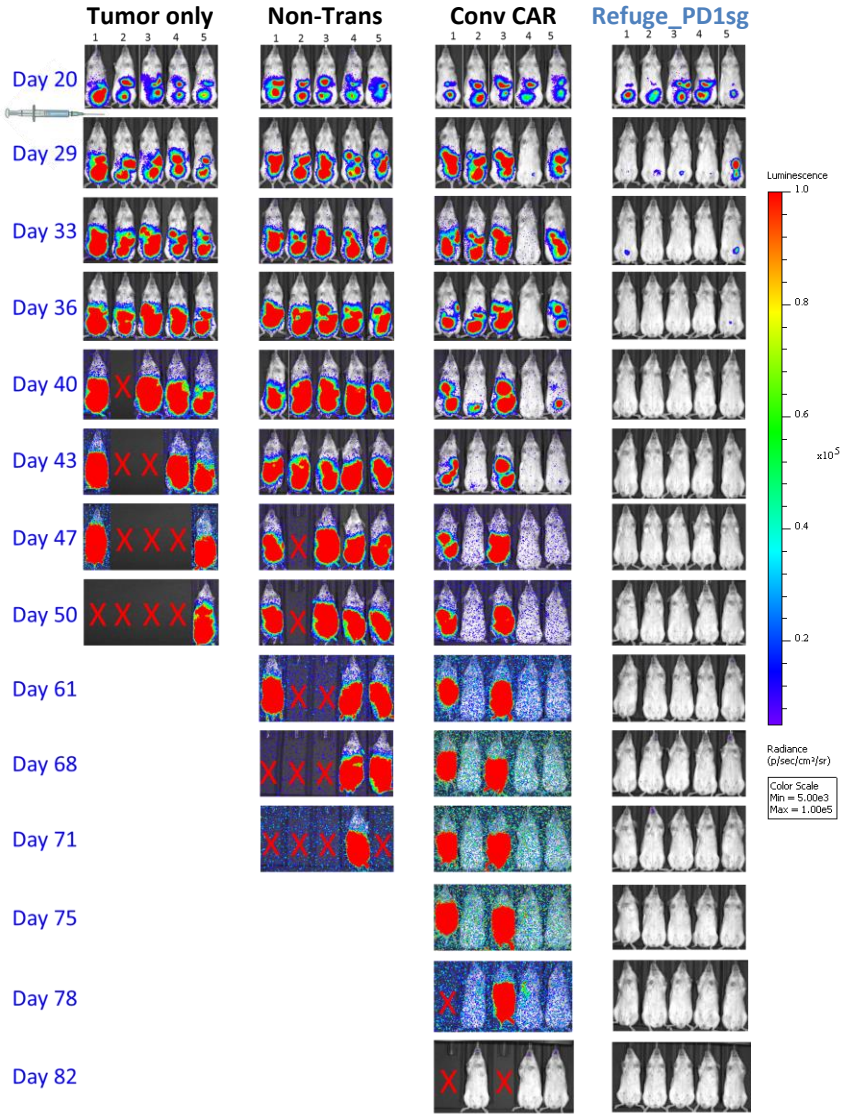


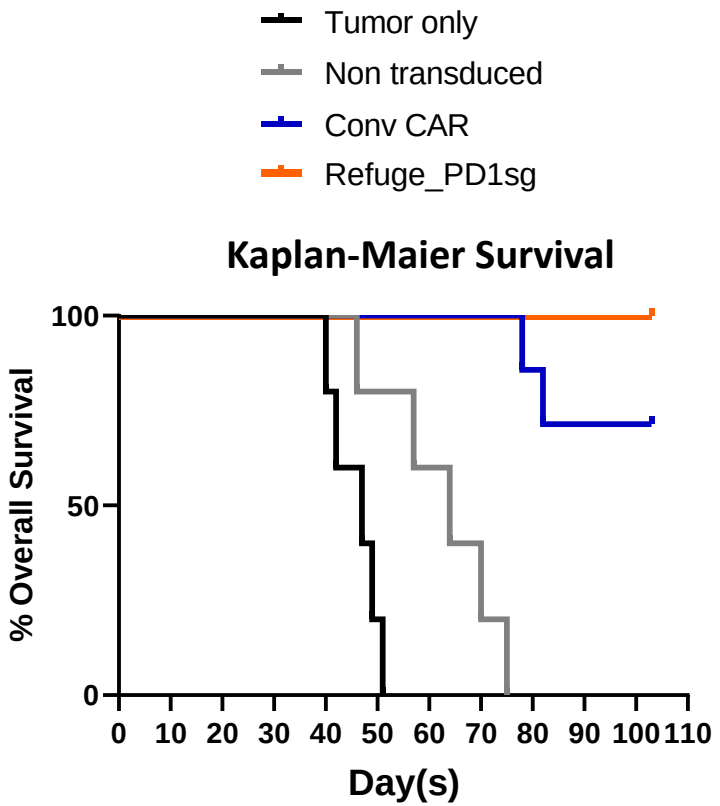
NT vs Refuge	0.004
CONV vs Refuge	0.012
CONTR vs Refuge	0.018
CONTR + Atezo vs Refuge	N.S.



Her2.CAR T in a Preclinical Aggressive * SCOV 3 Ovarian Animal Model

Day 21:
T cell
10M i.p.

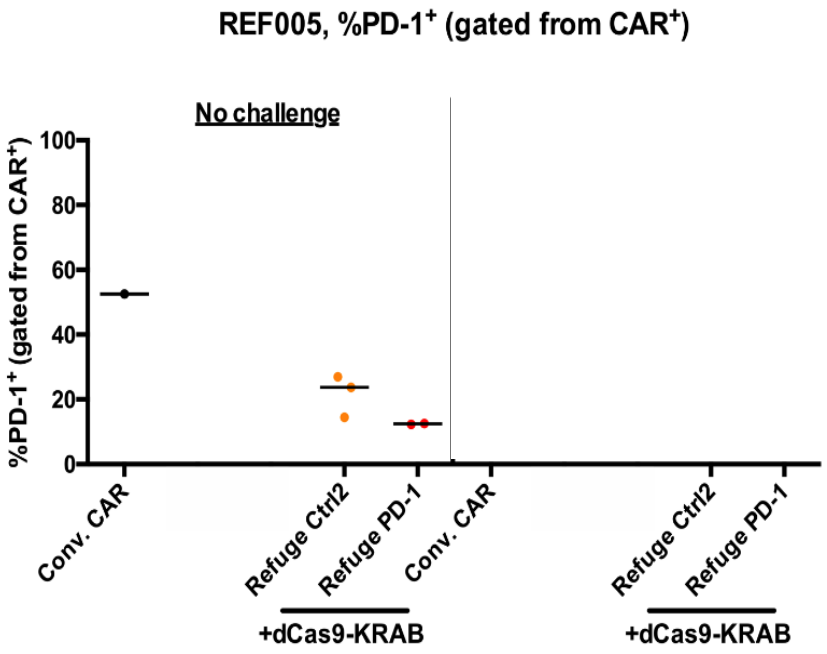




Prolonged *In vivo* persistence of RB-340-1

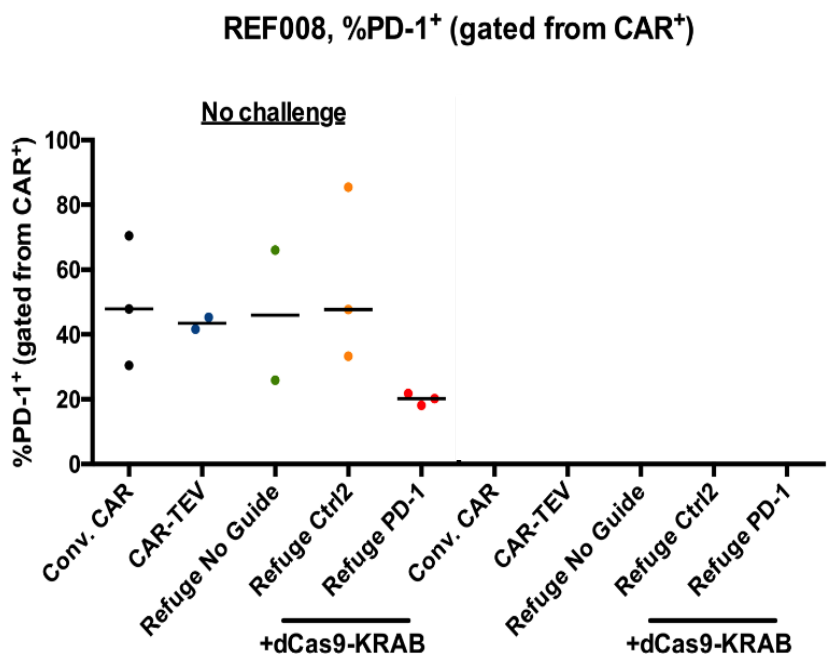
SKOV3 ovarian animal model

Donor 1



Mice re-challenged on day 159
Assay performed at day 161

Donor 2



Mice re-challenged on day 87
Assay performed at day 89

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First-in-human trial to assess of feasibility and efficacy of delivering context-reactive CAR-T cells: the HER2/PD1 model.

RB-340-1 autologous Her2(4D5) cFv.CD28ζ/PD1sgRNA CAR T-cells

Clinical Development:
Phase I dose escalation study in HER2+ / PD-L1+ breast cancer patients

Clinical partnership with:

- **Gene and Cell Therapy Lab**, Institute of Translational Health Sciences, University of Washington Medical Center - Dr. Donovan Farris
- **University of Washington Cancer Center**, Seattle –Dr. William R. Gwin and Dr. Mary L. (Nora) Disis

- HER2 (4D5) scFV/PD1 sgRNA
- Route intravenous
- Dose escalation following 3x3 trial design
  - 100,000 per kilo
  - 500,000 per kilo
  - 2M per kilo
  - Max of 200M per patient
- Time of expansion 10 days

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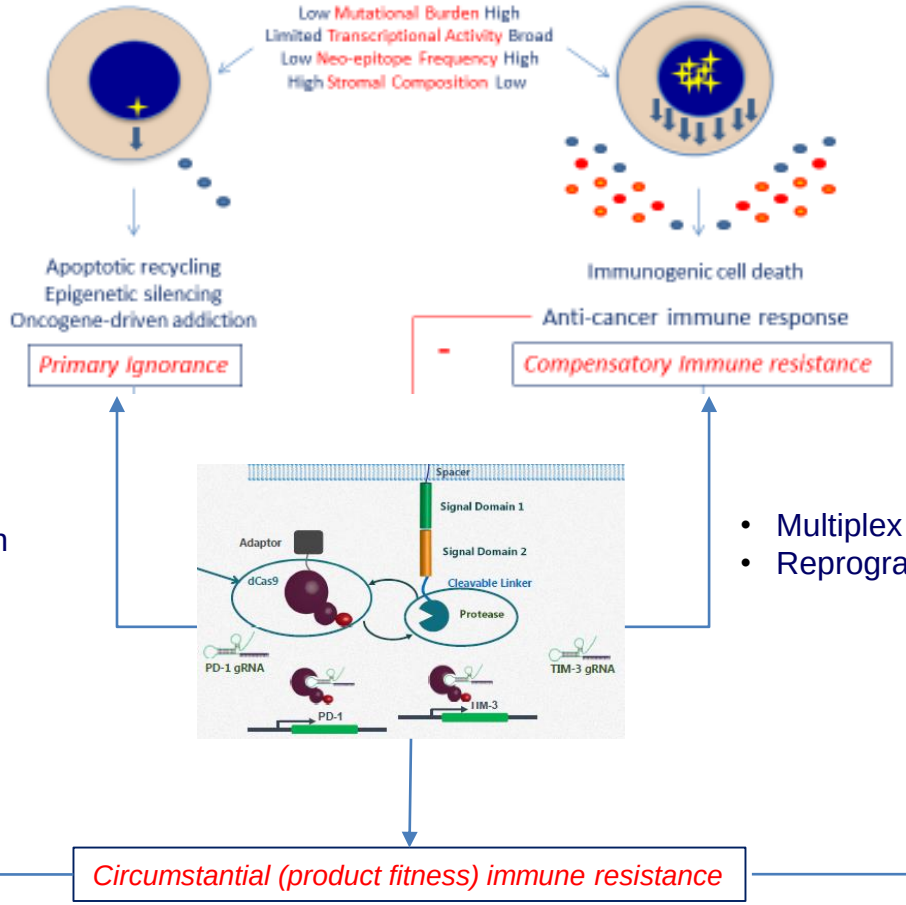
Contextual regulation of CAR T cells

Turan et al. Journal for ImmunoTherapy of Cancer (2018) 6:50
https://doi.org/10.1186/s40425-018-0355-5

Journal for ImmunoTherapy of Cancer

Immune oncology, immune responsiveness and the theory of everything

Tolga Turan[1], Deepti Kannan[1], Maulik Patel[2], J. Matthew Barnes[1], Sonia G. Tanlimco[1], Rongze Lu[1], Kyle Halliwill[1], Sarah Kongpachith[1], Douglas E. Kline[3], Wouter Hendrickx[4], Alessandra Cesano[5], Lisa H. Butterfield[6], Howard L. Kaufman[7], Thomas J. Hudson[1], Davide Bedognetti[4], Francesco Marincola[1] and Josue Samayoa[1*]



- Overcome immune exclusion

- Multiplex CPI modulation
- Reprogramming of T cell differentiation

- T cell stemness to increase persistence (targeting master regulators of T cell differentiation)
- Reduction of toxicity
- Increases consistency, comparability and potency
- Conditional modulation of immunogenicity

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Refuge Biotechnologies Inc. US Team:

Zhifen Yang, Ph.D.

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Key Management

Bing C Wang
CEO

Franco Marincola
CSO

Jing Zhao
CBO

Co-Founders

Stanley Qi
Stanford University

Bing C Wang
CEO

David Parkinson
Chairman of Board of Directors

- **Particular thanks to:**
- **Gene and Cell Therapy Lab**, Institute of Translational Health Sciences, University of Washington Medical Center, Seattle, WA - Dr. Donovan Farris
- **University of Washington Cancer Center**, Seattle WA - Dr. William R. Gwin and Dr. Mary L. (Nora) Disis
- **National Center for Cellular Engineering**, National Institutes of Health, Bethesda, MD – Dr. David F. Stroncek, Dr. Ping Jing and Dr. Steven Highfill

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