Poster #: 127

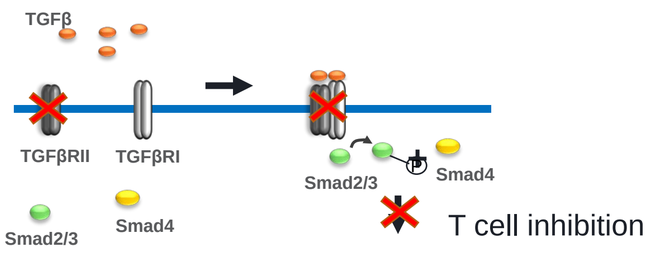
Improving Efficacy of CAR T Cells through CRISPR/Cas9 Mediated Knockout of TGFβRII

G. Grant Welstead[1], Queenie Vong[2], Chris Nye[2], Ron Hause[2], Chris Clouser[2], Jon Jones[2], Stephen Burleigh[2], Christopher M. Borges[1], Melissa Chin[1], Eugenio Marco[1], Jen Da Silva[1], Fred Harbinski[1], Georgia Giannoukos[1], Vidya Dhanapal[1], Yue Jiang[2], Ruth Salmon[2], Christopher J. Wilson[1], Vic Myer[1], Christopher J. Bond[2], Blythe D. Sather[2]

[1]Editas Medicine, Inc, Cambridge, MA, [2]Juno Therapeutics, a Celgene company, Seattle, WA

Introduction

- CD19-directed chimeric antigen receptor (CAR) T cells have shown efficacy in the treatment of certain B cell malignancies resulting in the recent FDA approval of two CD19-CAR T cell therapies.
- The immunosuppressive nature of certain tumor micro-environments may blunt the anti-tumor activity of certain adoptively transferred T cells.
- The anti-inflammatory cytokine, transforming growth factor β (TGFβ) is elevated in the tumor microenvironment for a variety of tumors and is a potent suppressor of T cell proliferation and inhibitor of effector function.
- The suppressive effect of TGFβ involves signaling through TGFβ Receptor 2 (TGFβRII):

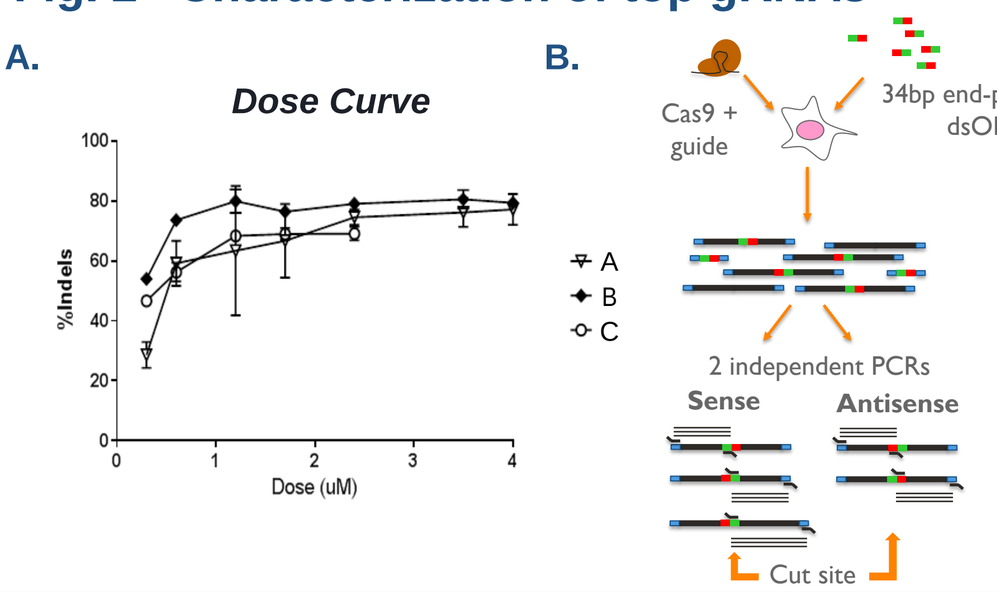


- Removal of the TGFβRII from the surface of CAR T cells via CRISPR gene editing could allow them to escape the suppressive effects of TGFβ and have increased function in the tumor microenvironment.

Methods Overview

- RNPs comprising a purified *S.pyogenes* Cas9 protein and a 100nt gRNAs (~60 gRNAs were screened) were used to target different regions of the TGFβR2 gene in primary T cells (Fig. 1a)
- A T7E1 assay or next generation sequencing (NGS) was used to assess indel rates when primary T cells were treated with RNP alone (Fig. 1b, 2) or in combination with lentiviral transduction of a CAR (Figure 3,4)
- Optimal amount of RNP for editing was identified by a dose titration of our top three gRNA complexed with Cas9 and electroporated onto primary T cells (Fig. 2a)
- GUIDE-seq was used to detect off target cutting of a subset of active RNPs (Fig. 2c)
- Characterization and functionality of T cells was assessed in vitro. Cells were grown in the presence or absence of TGFβ and the following was assessed:
  - Cell health and CD4/CD8 T cell ratio (Fig. 3c/d)
  - SMAD2/3 signaling by flow cytometry (Fig. 3b)
  - T cell proliferation (Fig. 4a)
  - Cytokine production and cell killing in response to target+ cell lines (Fig. 4b, c, d, e)
  - Serial killing of target+ cells (Fig. 4f, 5)

Fig. 2 Characterization of top gRNAs



C. *GUIDE-seq Analysis*

Guide-Seq Data	Number of off-target sites identified			
	Donor 1	Donor 2	Donor 3	Unique Sites
Guide A	0	0	0	0
Guide B	1	1	0	2
Guide C	1	1	1	2

Fig. 3 Molecular characterization of edited CAR T cells

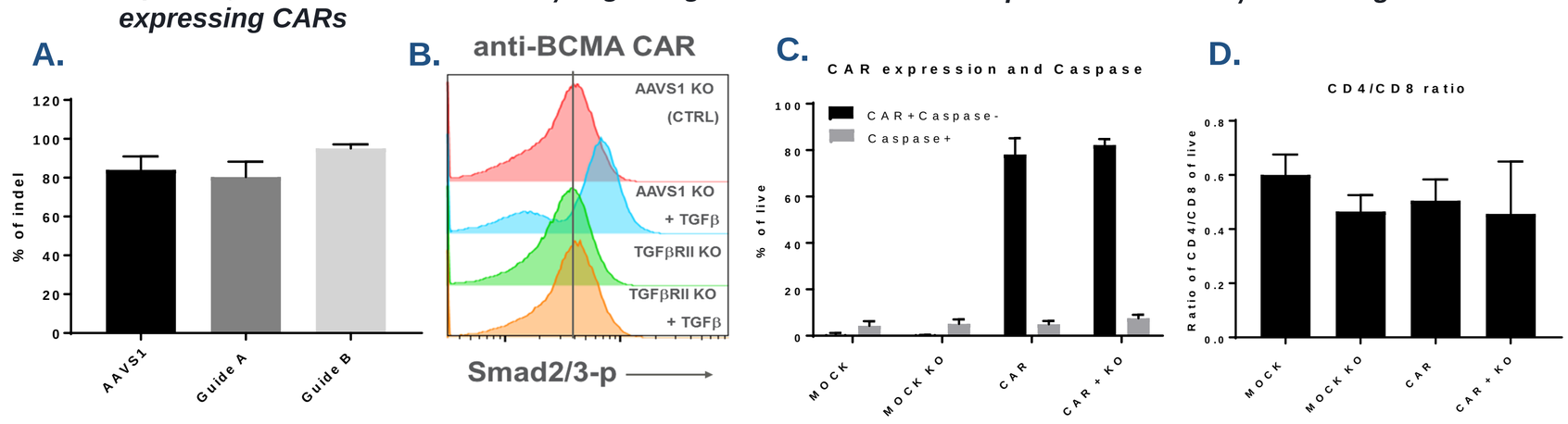


Fig. 4 TGFβRII edited CAR T cells are resistant to TGFβ suppression

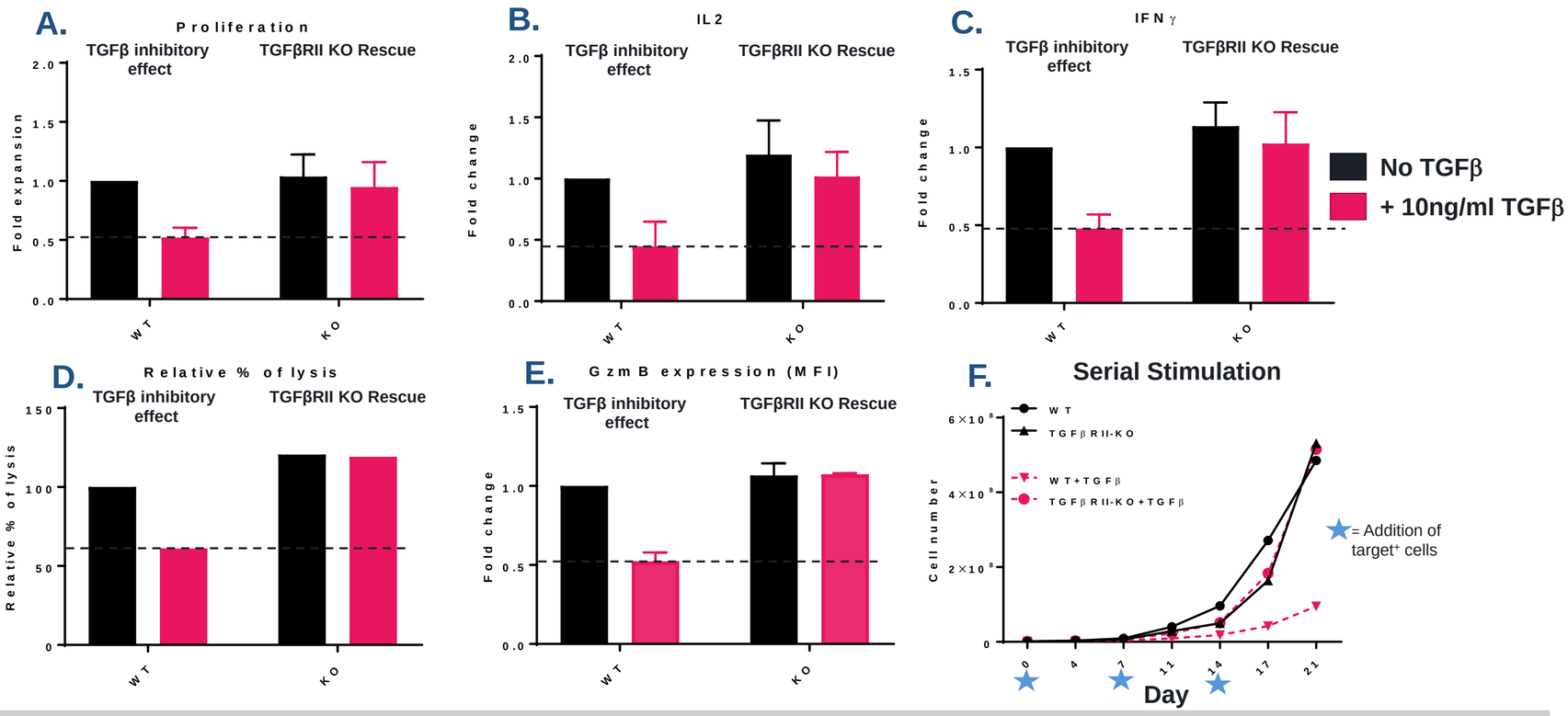
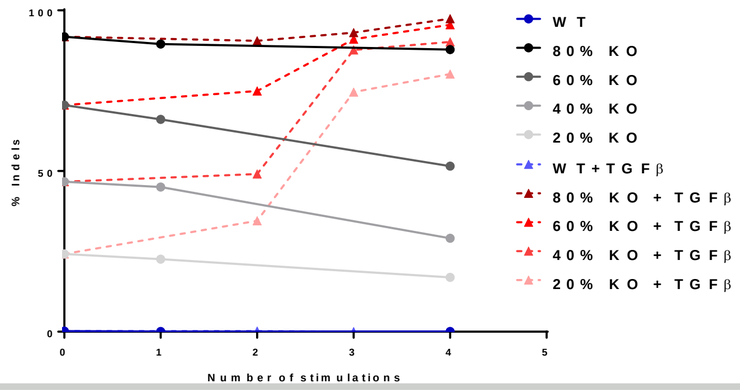
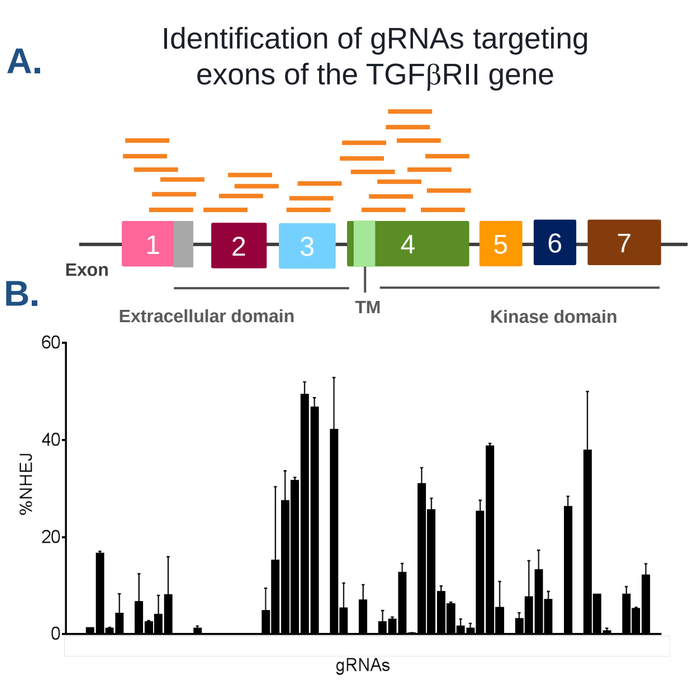


Fig. 5 Selective advantage for TGFβRII KO cells in the presence of TGFβ



Different ratios of anti-BCMA CAR TGFβRII-KO cells with WT cells (1, 0.75, 0.5, 0.25) were co-cultured with RPMI-8226 in 1:1 Effector to Target (E:T) ratio ± 10ng/ml TGFβ. Cells were collected every 7 days for analysis of indel % by high throughput sequencing. Cells are re-stimulated with fresh RPMI-8226 and re-adjusted to 1:1 E to T ratio weekly.

Fig.1 CRISPR Targeting of TGFβRII



Conclusions

- Demonstration of >80% editing at the TGFβRII locus by delivery of Spy Cas9 RNPs to a 50:50 mix of CD3/CD28 stimulated CD4 and CD8 T cells.
- GUIDE-seq analysis of edited T cells identified a gRNA with no detectable off targets
- TGFβRII KO CAR T cells are resistant to TGFβ suppression
- TGFβRII KO CAR T cells have increased cell killing vs wildtype cells in the presence of TGFβ

Author Disclosures: GGW, CMB, MC, EN, JD, FH, GG, VD, CJW, VM are employees of Editas Medicine and have equity interest in the company; CN, RH, CC, JJ, SB, YJ, RS, CJB, BDS are employees of Celgene/Juno Therapeutics and have equity interest in the company; QV is presently an employee of Bluebird Bio and has equity interest in the company. QV contributed to the work on this poster as an employee of Juno Therapeutics