Dual inactivation of SOCS1 and Regnase-1 in T Cell Therapies Demonstrated Enhanced Anti-tumor Activity by Expanding Discrete Cell States in Pre-clinical Syngeneic Mouse Models

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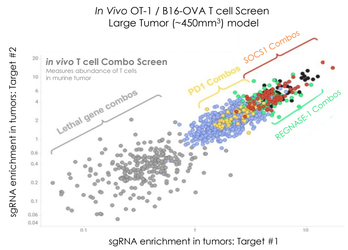
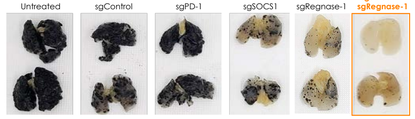
BACKGROUND

The use of CRISPR/Cas9-gene-editing to enhance the anti-tumor activity of T cell therapies is a promising approach in the treatment of patients with solid tumors. We previously reported the utility of in vivo pair-wise CRISPR/Cas9 screens to identify the best target combinations in T cells able to enhance anti-tumor function, with SOCS1 and Regnase-1 identified as a top combination. In these studies, we further explore the mechanisms by which the dual-inactivation of SOCS1 and Regnase-1 in T cells enhances anti-tumor activity.

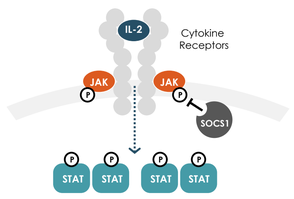
CRISPR² screens identify SOCS1 x Regnase-1 dual edit as a top combination in enhancing T cell infiltration into syngeneic tumors



Dual inactivation of SOCS1 and **Regnase-1** in edited PMEL CD8 TCR-Tg T cells transferred into a high-bar, PD1-refractory B16F10 lung-met model drives enhanced anti-tumor activity



SOCS1



Regnase-1

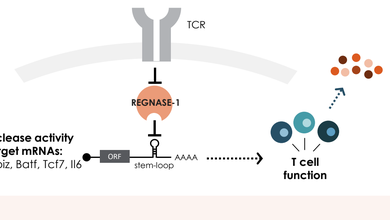


Figure 1: Inactivation of SOCS1 and Regnase-1 in transferred CD8 T cells drives significant tumor growth inhibition in high bar B16-OVA subcutaneous tumor model

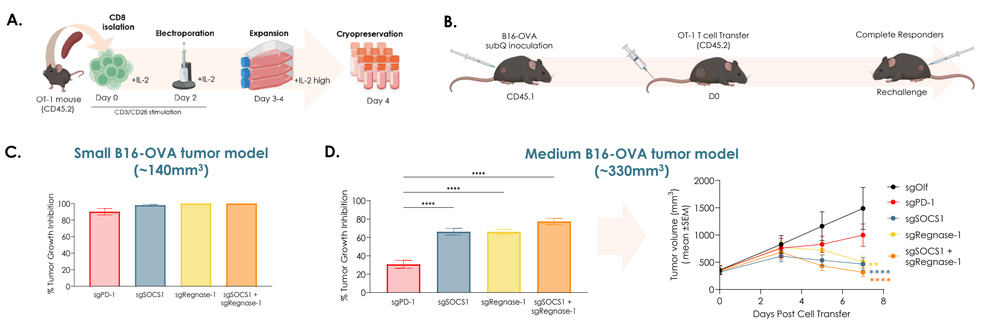


Figure 1. B16-OVA tumor-bearing mice were treated with 3x10⁶ OT-1 T cells edited using CRISPR/Cas9 targeting either *Olf1a* (sgOlf), *Pdcd1* (sgPD-1), *SOCS1* (sgSOCS1), *Zc3h12a* (sgRegnase-1) or *SOCS1+ Zc3h12a* (sgSOCS1+sgRegnase-1) on day 9 (small tumor model) or day 18 (medium tumor model) after B16-OVA inoculation. **(A, B)** Schematics illustrating the generation of edited OT-1 T cells and the experimental design assessing their activity in a medium B16-OVA subcutaneous tumor model. Created with BioRender.com **(C)** Tumor growth inhibition (TGI) in small B16-OVA tumor model compared to sgOlf is calculated on Day 16. **(D)** *(left)* TGI in a medium B16-OVA tumor model calculated on Day 7, *(right)* with the average tumor growth curves of each group depicted. Results of statistical analysis depicted between sgOlf versus sgPD-1, sgOlf versus sgSOCS1, sgOlf versus sgRegnase-1, sgOlf versus sgPD-1 or sgOlf versus sgSOCS1+sgRegnase-1 on Day 7 using One-way ANOVA. For **C** and **D**, TGI is calculated using formula: % TGI = (1-(Tt/T0/Ct/C0)) x 100% where C represents sgOlf, T represent the individual edits, t is the final day of all sgOlf mice on study and 0 represents the tumor volume on the day of cell transfer. One-way ANOVA with Tukey's multiple comparison test was used for evaluation of statistical differences between treatment groups. $^{**}P<0.005$, $^{****}P<0.0001$

Figure 2: SOCS1 and Regnase-1 dKO promotes the expansion of discrete subsets in a tissue specific manner during anti-tumor response

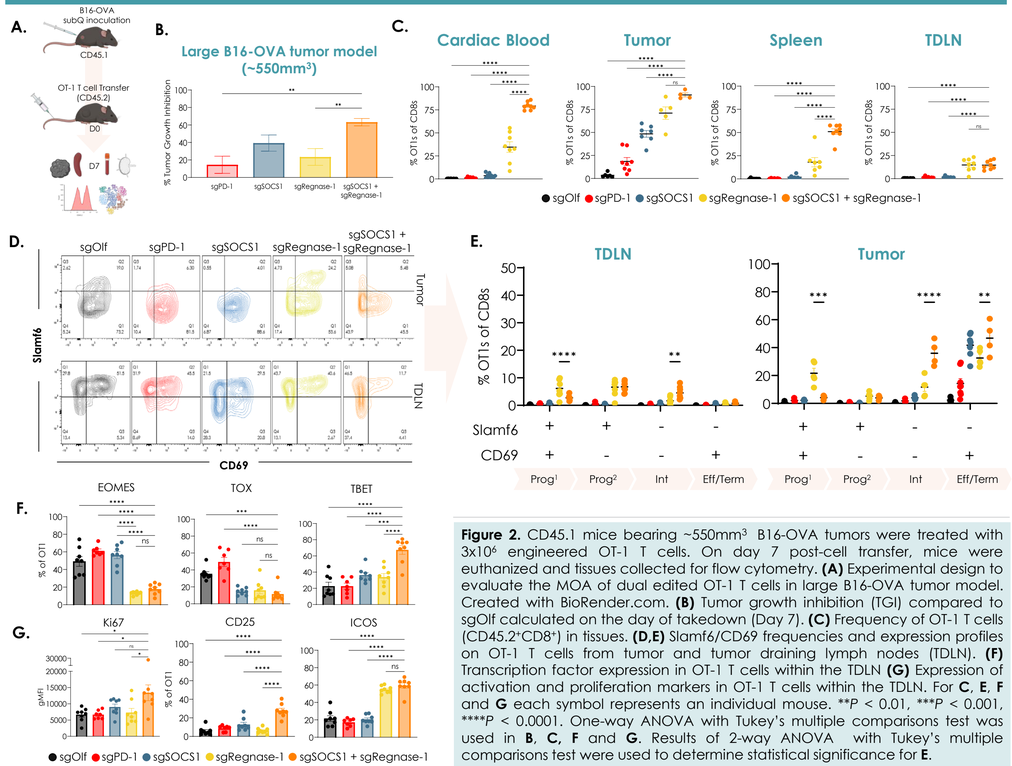


Figure 2. CD45.1 mice bearing ~550mm³ B16-OVA tumors were treated with 3x10⁶ engineered OT-1 T cells. On day 7 post-cell transfer, mice were euthanized and tissues collected for flow cytometry. **(A)** Experimental design to evaluate the MOA of dual edited OT-1 T cells in large B16-OVA tumor model. Created with BioRender.com. **(B)** Tumor growth inhibition (TGI) compared to sgOlf calculated on the day of takedown (Day 7). **(C)** Frequency of OT-1 T cells (CD45.2⁺CD8⁺) in tissues. **(D,E)** Slamf6/CD69 frequencies and expression profiles on OT-1 T cells from tumor and tumor draining lymph nodes (TDLN). **(F)** Transcription factor expression in OT-1 T cells within the TDLN **(G)** Expression of activation and proliferation markers in OT-1 T cells within the TDLN. For **C**, **E**, **F** and **G** each symbol represents an individual mouse. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. One-way ANOVA with Tukey's multiple comparisons test was used in **B**, **C**, **F** and **G**. Results of 2-way ANOVA with Tukey's multiple comparisons test were used to determine statistical significance for **E**.

Figure 3: Inactivation of SOCS1 and Regnase-1 in intra-tumoral CD8 T cells expands stem-like T_{ex}^{prog} and effector T_{ex}^{eff} subsets

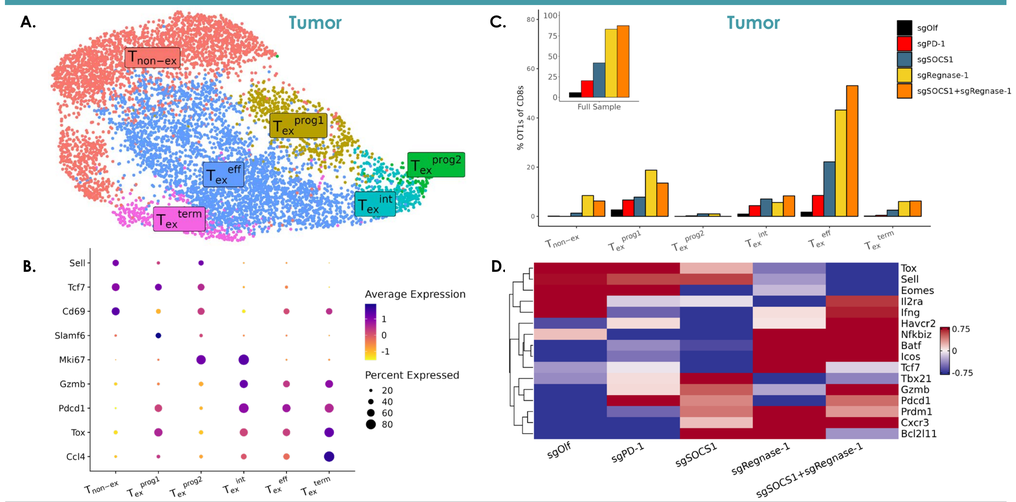


Figure 3. Paired single-cell RNA/TCR sequencing was performed on CD45+ cells isolated from B16-OVA tumors at day 7 post-cell transfer and CD8 T cells were isolated using computational gating. **(A,B)** Location of T_{ex} subsets on UMAP (A) annotated using expression of T_{ex} subset-defining transcripts (B). **(C)** Frequencies of transferred OT-1 cells identified by TCR sequencing as a percent of CD8 T cells within each T_{ex} subset by treatment group; inset shows frequencies in the full samples. **(D)** Expression of genes of interest in OT-1 cells by treatment group.

Figure 4: Inactivation of SOCS1 and Regnase-1 in transferred CD8 T cells promotes the persistence of CD62L⁻CD44⁺ effector memory cells

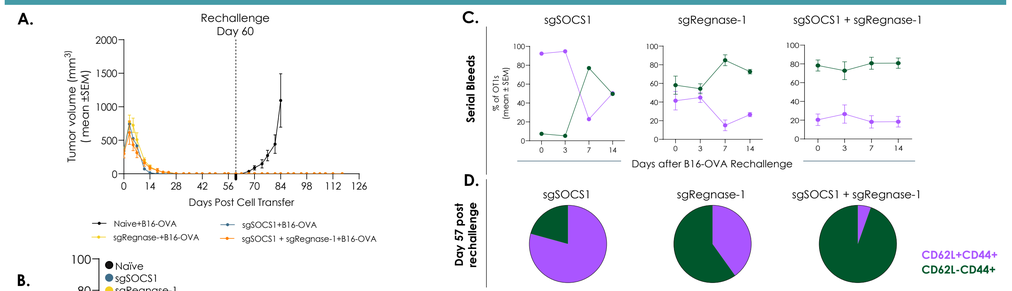


Figure 4. Mice treated with sgSOCS1, sgRegnase-1 and sgSOCS1 + sgRegnase-1 OT-1 T cells that have shown complete tumor rejection were rechallenged with B16-OVA tumor cells 60 days post initial cell transfer. **(A)** Tumor growth of the indicated treatment groups is depicted after primary and secondary challenge. **(B)** Frequency of OT-1 cells detected in peripheral blood as a percentage of CD8⁺ T cells prior to (Day 0) and following (D3, D7 and D14) B16-OVA rechallenge. **(C)** CD44⁺CD62L⁺ and CD44⁺CD62L⁻ phenotypes of edited OT-1 cells following B16-OVA rechallenge (Day 0, 3, 7 and 14) were quantified by flow analysis. **(D)** Day 57 post rechallenge mice were euthanized and CD44⁺CD62L⁺ and CD44⁺CD62L⁻ phenotypes of edited OT-1 T cells from cardiac blood characterized by flow.

CONCLUSIONS

OT-1 T cells with dual inactivation of SOCS1 and Regnase-1 inherit properties unique to each single edit and occupy a wider range of cell states in the TME and periphery compared to either single edit alone.

SOCS1 inactivation:
- ✓ Fosters an effector and intermediate T_{ex} subset with increased proliferative potential
- ✓ Drives transferred OT-1 cells towards a Granzyme B-expressing effector-like phenotype

Regnase-1 inactivation:
- ✓ Expands progenitor and stem-like T_{ex} subsets in the tumor and secondary lymphoid tissues
- ✓ Reduces expression of exhaustion regulators

↑ Enhanced infiltration in the TME ↑ Increased cytolytic function ↑ Persistent effector memory phenotype ↓ Exhaustion