An Immune-CRISPRomics platform enabling genome-scale and pair-wise combination in vivo T-cell function screens enables comprehensive identification of novel therapeutic targets

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Abstract

Background: Although immunotherapy with PD-1/PD-L1 antagonists has significantly advanced patient care, the majority of cancer patients currently do not benefit from checkpoint inhibitor therapies. To identify novel targets for the treatment of PD-1 insensitive cancers, we developed a novel Immune-CRISPRomics® platform that enabled genome-wide CRISPR/Cas9 screens in primary T cells in an in vivo setting.

Methods: In vivo genome-wide CRISPR/Cas9 screens were conducted by introducing sgRNA libraries into Cas9-expressing TCR-Tg T cells, followed by adoptive transfer into tumor-bearing hosts. Targets enabling enhanced TCR-Tg T cell function in the context of the tumor microenvironment were identified and validated by follow-on efficacy studies.

Results: Our CRISPRomics® platform identified clinically active molecules, such as PD-1, and also predicted recent clinical failures. In addition, we identified multiple targets that enhanced anti-tumor T-cell function similar to or better than PD-1 as a monotherapy. The anti-tumor activity of targets was assessed across a collection of PD-1 sensitive and PD-1 refractory syngeneic tumor models. One of the targets identified, IO-7, was found to possess robust activity across multiple PD-1 refractory models. We found that inhibition of IO-7 leads to long-term T-cell memory and prevents tumor growth upon re-challenge. Moreover, mechanistic follow-up studies demonstrate that IO-7 inhibition leads to an expansion of central memory T-cell subsets, which have been implicated in driving the durable clinical response of checkpoint inhibitors. We then extended these findings further with an in-vivo double CRISPR screen to examine more than 2,000 pairwise combinations of active sgRNAs in-vivo. These combination studies identified CD8 T-cell gene pairs that, when inactivated, gave anti-tumor activity that exceeded any single gene inactivation from the primary screen and greatly exceeded the efficacy of PD-1 monotherapy.

Conclusion: We describe an Immune-CRISPRomics® platform which 1) identified multiple functional targets with therapeutic promise and 2) functionally screened combinations of these targets to reveal the most potent T-cell target combinations for the treatment of PD-1 resistant solid tumors.

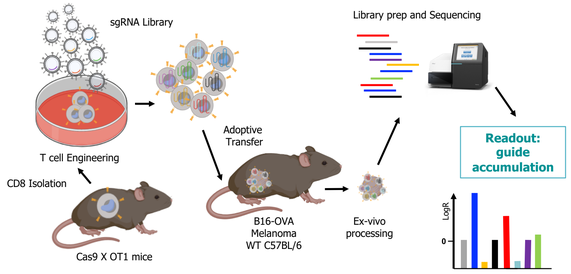
Methods

In vivo T-cell screen : CD8 T cell were isolated from OT1 x Cas9 Transgenic mice, transduced with a sgRNA library and adoptively transferred into B16-OVA tumor bearing mice. After in vivo expansion, tumor were collected, gDNA was isolated and sgRNA accumulation was evaluated by NGS.

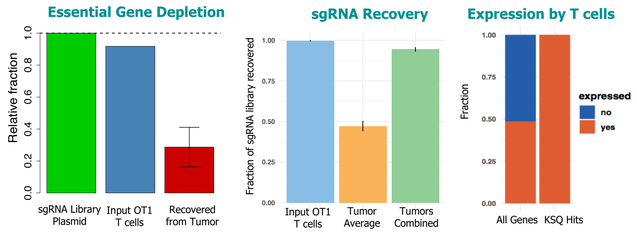
In vivo Target validation : B16-OVA melanoma or gp100 CRC cancer cells were injected s.c. into C57BL/6J mice. Mice were randomized for tumor volume (approx. mean 100mm^3 or 450mm^3) and either control, PD-1, IO-7, CT-1, IO-12 or dual edited OT1 or PMEL T cells were injected i.v. Tumor volume was measured twice a week.
For MOA study, Five days post adoptive-transfer of OT1 T cells, tumors were harvested, TILs were isolated and analyzed by Single-Cell RNA-seq (10X Genomics).
B16-F10 cells were injected i.v. into C57BL/6J mice. Three days later, control, PD-1 or IO-7 edited PMEL T cells were injected i.v. Twelve days following adoptive transfer of T cells, lungs were collected and images were taken.

Figure 1: Identification of Novel Targets by CRISPR/Cas9 *in vivo* T- cell screen

Experimental schematic of OT1 / B16-OVA in vivo T cell CRISPR screen

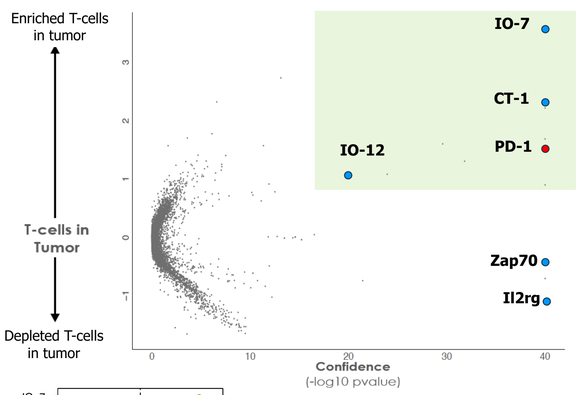


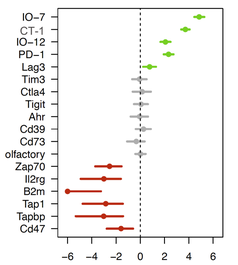
Screen is robustly powered and validated by multiple quality-control checks



- Robust depletion of essential genes in tumor infiltrating T cells
- Robust sgRNA recovery from individual tumors
- All KSQ hits are expressed at the mRNA level by T cells

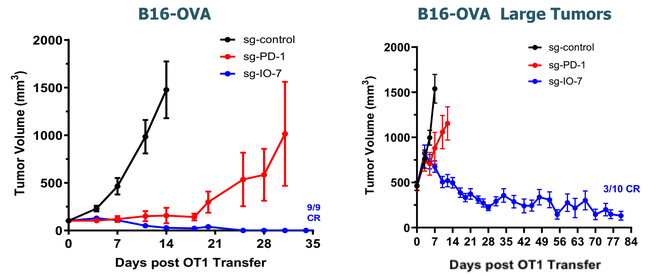
Genome-Scale in vivo Screen Identifies Novel T cell Targets

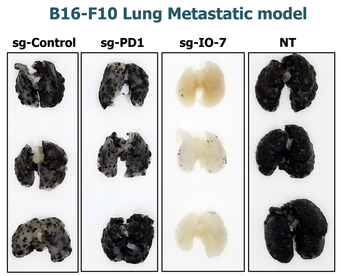


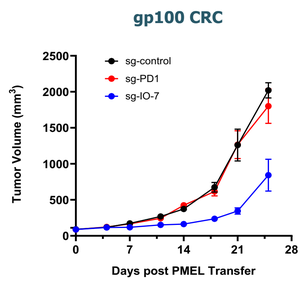


- Multiple targets identified that drive T cell enrichment similar or greater than PD-1 (green box above)
- PD-1 serves as a positive control. IO-7, CT-1 and IO-12 are KSQ portfolio targets.
- Critical components of T-cell activation (Zap70) and survival pathways (Il2rg) are identified as depleted targets in the screen (left graph)

Figure 2: KSQ Target IO-7 demonstrate robust efficacy in PD-1 sensitive and insensitive syngeneic tumor models



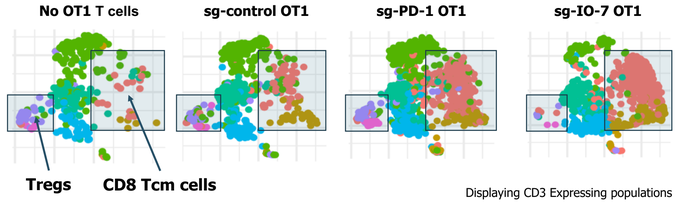




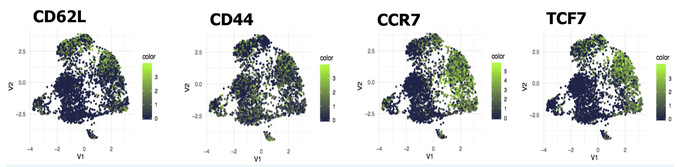
- IO-7 is shown as an example for targets identified in our genome-scale screen that possess robust activity across multiple PD-1 refractory models
- Adoptive transfer of IO-7 edited OT1 T cells into mice bearing established 100mm^3 and 450mm^3 B16-OVA tumors demonstrate efficacy greater than PD-1-edited OT1 T cells
- Transfer of IO-7 edited PMEL T cells show efficacy in two additional PD1-refractory models (B16-F10 lung met model and a gp100 CRC model)

Figure 3: scRNA-SEQ profiling of tumor shows IO-7 edited T cells drive expansion of CD8 Tscm cells

IO-7 edited OT1 drive expansion of CD8 Tcm cells and depletion of Tregs

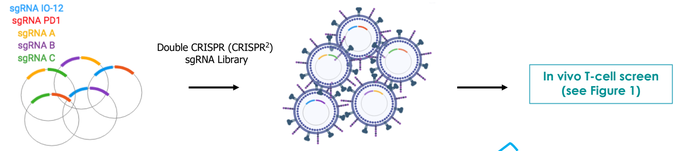


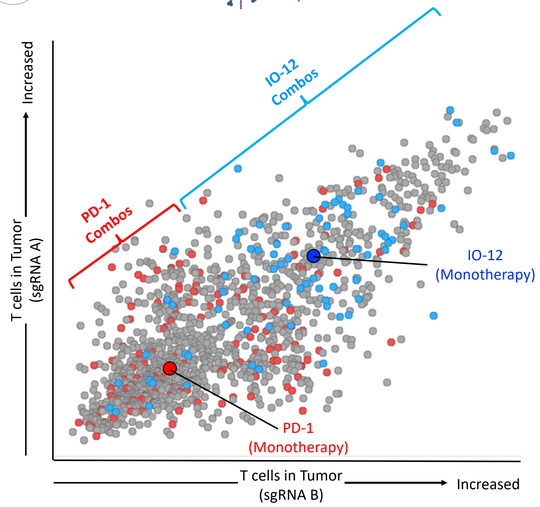
CD8 Tcm cells express TCF7 and resemble Tscm cells



- Adoptive transfer of IO-7 edited OT1 into tumor bearing mice expand a CCR7$^+$TCF7$^+$ Tscm population and deplete Tregs from the tumor microenvironment

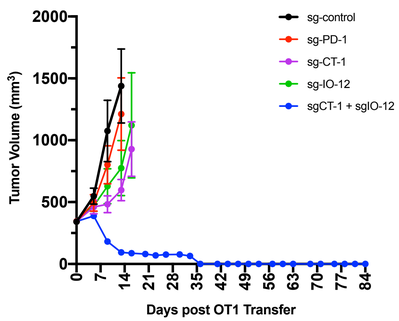
Figure 4: In vivo CRISPR^{2TM} screen identifies novel target combinations driving CRs in a PD-1 refractory solid tumor model

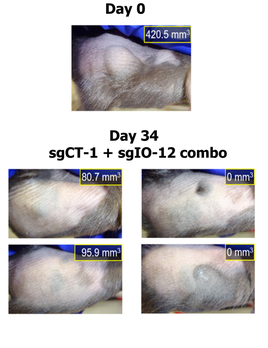




- Developed CRISPR^2 technology that enables in vivo T-cell screen of 400 combination targets in parallel; IO-12 scores as a top combination partner
- Exemplary combo efficacy study: Adoptive transfer of CT-1 and IO-12 dual edited OT1 leads to robust and durable efficacy in PD-1 refractory tumor model (large tumors ~350mm^3 at time of treatment)

B16-OVA Large Tumors





Conclusions

- An in vivo genome-wide CRISPR T-cell screen identifies multiple targets scoring similarly or greater than PD-1 in multiple syngeneic models
- Inhibition of IO-7 in CD8 T cells translated to robust activity across multiple syngeneic models where PD-1 inhibition showed no activity
- CRISPR^2 unveils top T cell target combinations, with CT-1 + IO-12 combo demonstrating robust activity
- **KSQ is developing novel therapeutics against in vivo validated targets for the treatment of checkpoint-inhibitor-refractory patients**