In vivo CRISPR screens identify HDAC1 as an immune sensitizer reversing immune resistance driven by STK11 loss

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ABSTRACT

Background:
Immunotherapy with checkpoint blockade is effective in only a subset of cancer patients and additional treatment strategies are needed. Here we used a pooled *in vivo* CRISPR-based genetic screening approach to discover cancer cell intrinsic regulators of immune resistance and sensitization. A two-step approach was taken to uncover tumor suppressor gene loss driving immune evasion as step one, and immune sensitizers specifically reversing the immune evasion driven by certain tumor suppressor loss as step two. Through this approach, we are determined to identify genetic context specific immune sensitizers, also known as synthetic lethal immune targets.

Methods:
We first performed a pooled CRISPR-Cas9-based *in vivo* genetic screen targeted to a pre-defined set of tumor suppressor genes to mimic loss-of-function mutations in syngeneic tumor models. CRISPR edited tumor cells were implanted into immune-deficient or immune-competent C57BL/6 mice, a subset of which were treated with anti-PD1 to simulate increased immune pressure *in vivo*. Tumor samples at the endpoint were subjected to next generation sequencing and statistical analysis to identify tumor suppressor genes driving immune evasion. Furthermore, a second pooled CRISPR-Cas9-based *in vivo* genetic screen targeted to a set of potentially druggable genes was performed in MC38/STK11-/- model vs MC38 WT model in the presence of increasing immune pressure *in vivo*. Next generation sequencing and statistical analysis were conducted to uncover STK11 loss specific immune sensitizers.

Results:
The screen confirmed previously identified immunotherapy targets such as CD47 and Adar as well as known drivers of immune resistance in the interferon signaling and antigen presentation pathways. Importantly, STK11 loss was identified as a driver of immune evasion in MC38 and 3LL syngeneic models. STK11 knockout in MC38 or 3LL tumor cells significantly accelerated tumor growth in immune-competent mice and in these mice treated with anti-PD1. Tumor infiltrating lymphocyte (TIL) profiling suggests that STK11 knockout induces an immune suppressive tumor microenvironment. Our second step target identification screen discovered HDAC1 as an immune sensitizer specific for STK11 loss context.

Conclusions:
CRISPR-based *in vivo* genetic screen is an effective approach to identify tumor cell-intrinsic drivers of immune resistance and sensitization. HDAC1 was identified as an immune sensitizer specifically reversing immune resistance driven by STK11 loss. *STK11* mutations are found in ~15% of non-squamous non-small cell lung cancer and have been reported as a major predictor of primary resistance to PD-1 blockade. Inhibitors selectively targeting HDAC1 & 2 in combination with anti-PD1 represent a promising therapeutic opportunity for non-small cell lung cancer patients with STK11 mutations.

TWO-STEP DISCOVERY STRATEGY

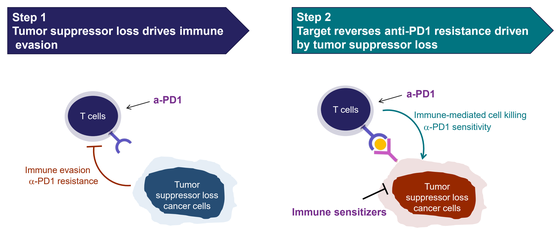
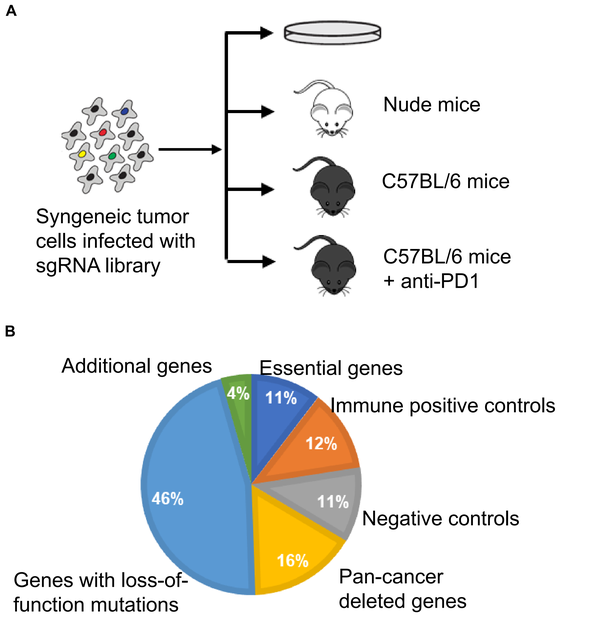


Figure 1: Schematic diagram of a two-step discovery strategy. Immuno-oncology discovery focuses on cancer cell intrinsic immune biology. Step 1 focuses on discovery of tumor suppressor loss driving immune evasion and resistance to immune pressure. Step 2 focuses on discovery of immune sensitizers that can reverse the tumor suppressor gene-mediated immune evasion.

IN VIVO CONTEXT IDENTIFICATION SCREEN DESIGN



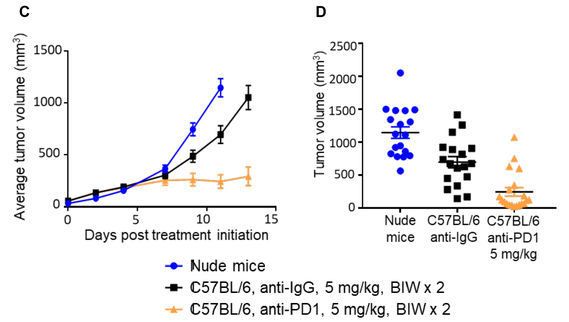


Figure 2: In vivo context identification screen design. (A) Diagram of in vivo screen platform and design. Syngeneic tumor cells were transduced with CRISPR tumor suppressor library and stable cells inoculated into nude mice, C57BL/6 mice and C57BL/6 mice treated with anti-PD1. (B) CRISPR tumor suppressor library. (C) Tumors grow slower with increasing immune pressure in vivo. Gradually decreasing tumor growth from nude mice to C57BL/6 treated with anti-IgG to C57BL/6 mice treated with anti-PD1 reflects increased anti-tumor immunity in these three in vivo conditions.

STK11 IS AMONG THE TOP IMMUNE EVASION GENES

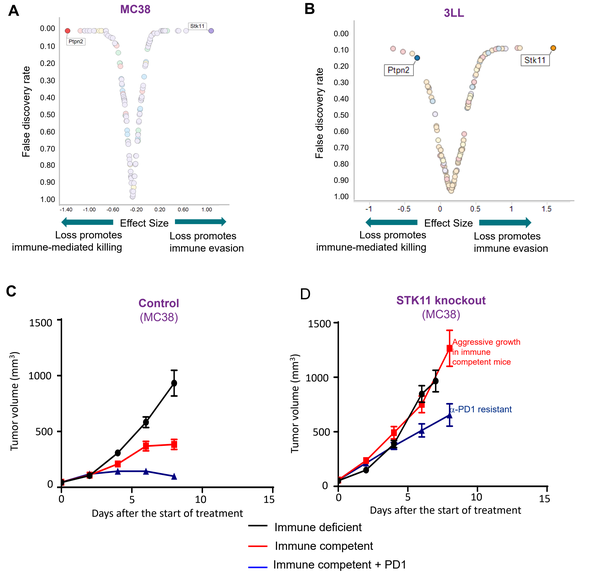


Figure 3: STK11 is among the top resistant genes from the context screen. (A & B) sgRNA depletion or enrichment from tumors of C57BL/6 mice treated with anti-PD1 vs. C57BL/6 mice treated with anti-IgG is represented in a volcano plot for MC38 (A) and 3LL (B) models. sgRNAs for STK11 are the top enriched in C57BL/6 treated with anti-PD1 vs anti-IgG, whereas sgRNAs for Ptpn2 are among the most depleted as expected. (C & D) MC38 cells were transduced with sgRNAs targeting STK11 or non-targeting control (NTC). Derivatives were inoculated into C57BL/6 mice treated with anti-PD1 or anti-IgG or nude mice. Depletion of STK11 drives resistance to increasing immune pressure in vivo.

CONTEXT SPECIFIC TARGET IDENTIFICATION SCREEN DESIGN

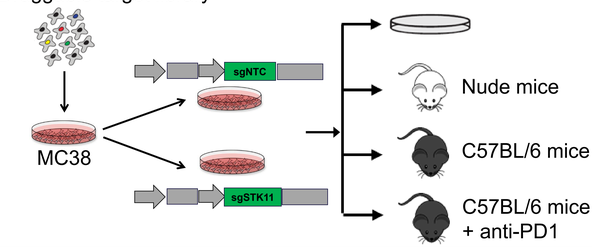
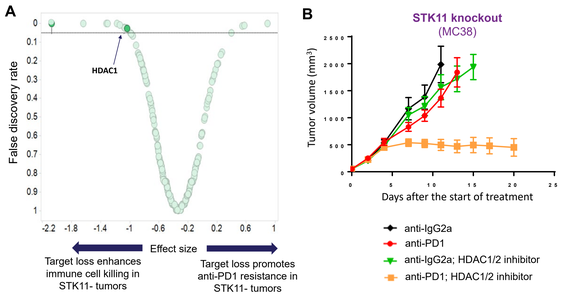


Figure 4: Context specific target identification screen design. MC38 cells were transduced with CRISPR-based druggable target library as step 1 and then transduced with guide RNA for STK11 or NTC control as step 2. Stable cells were inoculated into nude mice, C57BL/6 mice and C57BL/6 mice treated with anti-PD1, representing increasing degrees of anti-tumor immunity

HDAC1 WAS IDENTIFIED AS AN IMMUNE SENSITIZER REVERSING STK11-DRIVEN IMMUNE EVASION



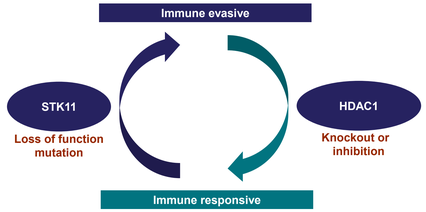


Figure 5: HDAC1 was identified as an immune sensitizer reversing STK11-driven immune evasion. (A) sgRNA depletion from sgSTK11 tumors treated with anti-PD1 vs sgNTC tumors treated with anti-PD1 is presented in a volcano plot. sgRNAs for HDAC1 are among the most depleted. In addition, sgRNAs for HDAC1 are among the most depleted from the comparison of STK11-null tumors in C57BL/6 mice treated with anti-PD1 vs nude mice. Together, these results indicated HDAC1 is an immune sensitizer specific for STK11-null tumors. (B) In vivo efficacy study in MC38/STK11-/- tumor model. Synergistic combo effect of anti-PD1 with HDAC1/2 inhibitor on STK11-/- tumors was observed. (C) The discovery and validation of HDAC1 reversing STK11 deficiency driven immune evasion completes one cycle of Tango's immuno-oncology discovery strategy.

CONCLUSION/SUMMARY

- A pooled CRISPR-Cas9-based in vivo screening platform was established for conditions reflecting increased anti-tumor immunity
- STK11 was identified as the top immune evasion context and association of STK11 mutations with poor response to checkpoint blockade has been reported in non small cell lung cancer patients
- Depletion of STK11 drives resistance to immune pressure in immune competent mice
- A target identification screen for STK11-/- context was performed successfully and HDAC1 was identified as an immune sensitizer reversing STK11-driven immune evasion
- HDAC1/2 inhibitor re-sensitizes STK11 deficient tumors to anti-PD1 treatment
- A CoreDAC inhibitor is under development for non small cell lung cancer patients with STK11 mutations (see Poster 444)