TNG260, a novel small-molecule CoREST inhibitor, sensitizes STK11-mutant tumors to anti-PD-1 therapy

Poster #681

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Abstract

Background: Non-small cell lung cancer (NSCLC) patients with *STK11* loss-of-function mutations (which account for ~20% of all cases) respond poorly to immune checkpoint-based therapies compared to *STK11* wild-type lung cancers. Additionally, *STK11* mutations are typically mutually exclusive with druggable alterations in EGFR or ALK, leaving these patients underserved by recent advancements in targeted therapies. To address this unmet medical need, we developed TNG260; a novel, highly selective CoREST complex inhibitor, that is being investigated in a Ph1/2 clinical study for evaluation of safety and efficacy in combination with pembrolizumab in advanced STK11-mutant cancers (NCT05887492).

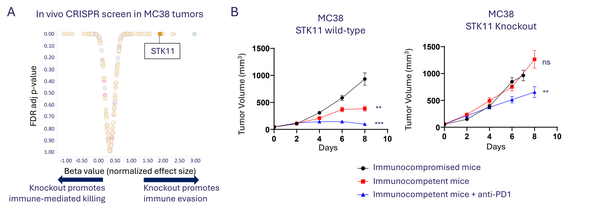
Methods: Informed by in vivo CRISPR screens, we identified CoREST as a target that, when inhibited, reverses the immune evasion caused by loss of *STK11*. We developed TNG260, a molecule that, in preclinical studies, was shown to potently inhibit the HDAC1 enzyme within the CoREST complex. It spares HDAC enzymes 3-11, and other Class I HDAC complexes, NCoR, NuRD, and Sin3, which contribute to pan-HDAC inhibitor toxicity.

Results: Inhibition of CoREST by TNG260 led to increased expression of immunomodulatory genes in *STK11*-deficient cancer cells in vitro and in vivo. Interestingly, TNG260 increased the expression of interferon pathway and inflammation genes to a greater extent in the *STK11*-deficient setting than in an *STK11* wild-type model that is similarly anti-PD-1 resistant. ChIP-sequencing in these models showed a TNG260-induced enrichment of histone acetylation at immune-relevant gene loci in the *STK11*-deficient setting compared to wild-type.

Syngeneic models engineered to have *STK11* knockout respond poorly to anti-PD-1, consistent with clinical observations that patients with *STK11*-mutant tumors respond poorly to immune checkpoint blockade. The addition of TNG260 to anti-PD-1 caused strong anti-tumor responses, including regressions in most animals. As the majority of *STK11*-mutated cancers are in NSCLC, we sought to verify our findings in murine lung models. The combination of TNG260 and anti-PD-1 led to complete control of tumor growth in *KRAS/STK11* co-mutated NSCLC allograft and autochthonous models.

Conclusion: This study describes TNG260, a CoREST inhibitor which modulates the expression of key immune genes in *STK11*-null models. Through this mechanism, TNG260 sensitizes immunotherapy-resistant mouse models to anti-PD-1 treatment, illustrating a potential treatment strategy for patients with *STK11*-mutated tumors. TNG260 in combination with pembrolizumab is currently being evaluated in patients with *STK11*-mutated, advanced solid tumors (NCT05887492).

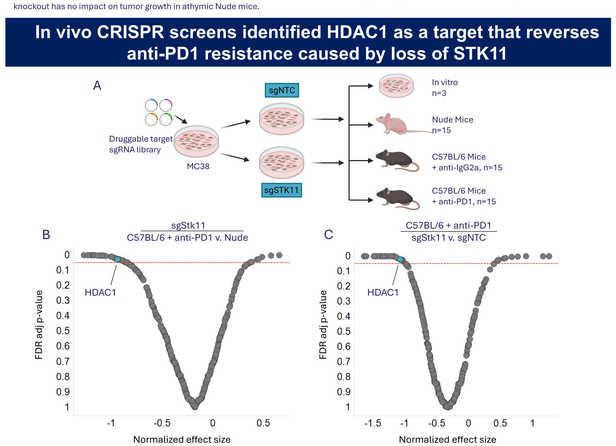
STK11 loss of function mutations cause resistance to immune checkpoint blockade



- **STK11 mutations are in 15-20% of NSCLC**
- **Patients with STK11-mutant lung adenocarcinoma are resistant to immune checkpoint blockade**
- **These patients are a high ummet medical need**

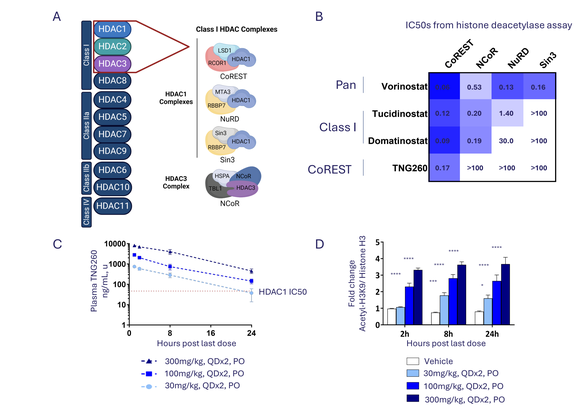
(**A**) Volcano plot illustrates knockout of STK11 in tumor cells caused resistance to anti-PD1 in immunocompetent animals.
(**B**) STK11 knockout leads to increased tumor growth and resistance to anti-PD1 in MC38 isogenic syngeneic tumors in immunocompetent mice. STK11 knockout has no impact on tumor growth in athymic Nude mice.

In vivo CRISPR screens identified HDAC1 as a target that reverses anti-PD1 resistance caused by loss of STK11



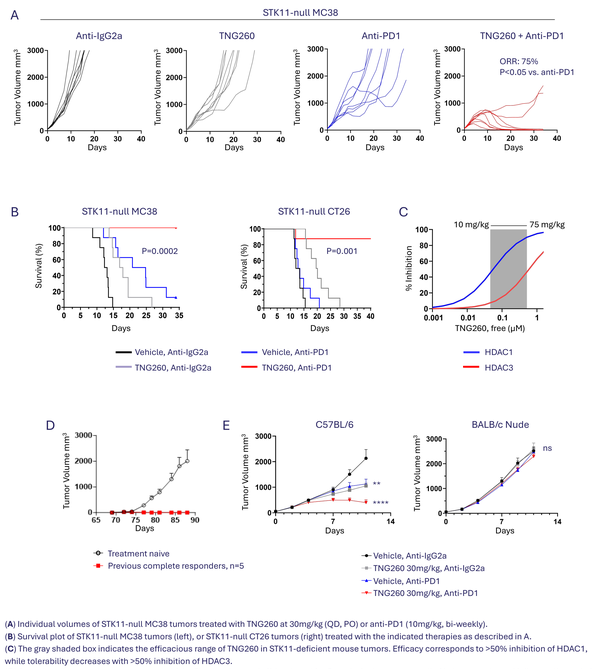
(**A**) In vivo CRISPR screen approach used to identify targets that would sensitize STK11-mutant tumors to anti-PD1. Figure created with BioRender.com.
(**B**) Volcano plot showing that cells with HDAC1 knockout are depleted in STK11-deficient mouse tumors treated with anti-PD1 compared to immunocompromised mice. This indicates adaptive immunity is required for the effect seen with HDAC1 knockout.
(**C**) Volcano plot showing that cells with HDAC1 knockout are depleted in STK11-deficient mouse tumors treated with anti-PD1 compared to STK11 wild-type tumors treated with anti-PD1. This comparison indicates that the effect is selective for STK11-deficient cells compared to wild-type.

TNG260 is a small molecule inhibitor of CoREST



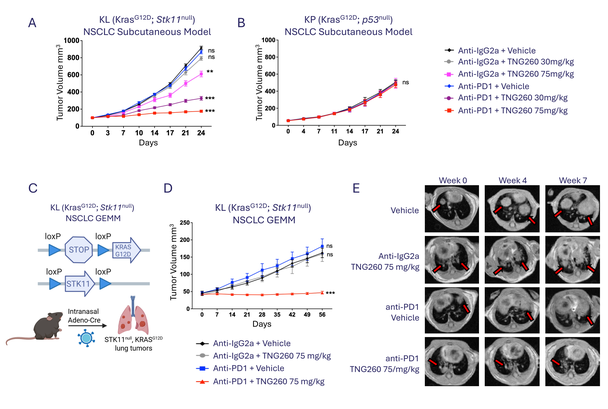
(**A**) HDAC1 is a Class I deacetylase enzyme that participates in 3 complexes, including CoREST. CoREST is a complex with both demethylase and deacetylase activity. Figure created with BioRender.com
(**B**) TNG260 and less-selective HDAC inhibitors were tested in a deacetylase assay with 4 Class I HDAC complexes. TNG260 is selective for CoREST.
(**C**) Two days of once daily (QD) oral administration (PO) of TNG260 at 30 mg/kg and above resulted in stable pharmacokinetics that sufficiently covered the in vitro IC50 of HDAC1. u = unbound
(**D**) Two days of once daily (QD) oral administration (PO) of TNG260 induced dose-dependent increases in acetyl-histone H3 Lys 9 in MC38 mouse tumor tissue.

TNG260 re-sensitizes STK11-deficient syngeneic tumors to anti-PD1 in an immune-mediated manner



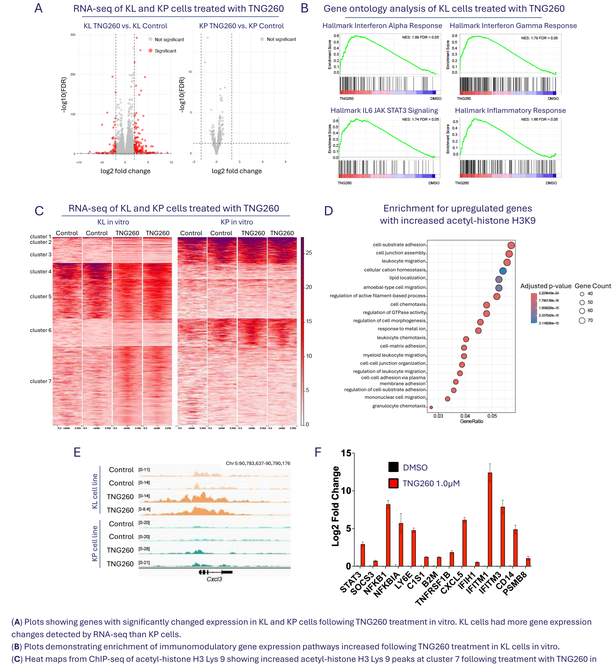
(**A**) Individual volumes of STK11-null MC38 tumors treated with TNG260 at 30mg/kg (QD, PO) or anti-PD1 (10mg/kg, bi-weekly).
(**B**) Survival plot of STK11-null MC38 tumors (left), or STK11-null CT26 tumors (right) treated with the indicated therapies as described in A.
(**C**) The gray shaded box indicates the efficacious range of TNG260 in STK11-deficient mouse tumors. Efficacy corresponds to >50% inhibition of HDAC1, while tolerability decreases with >50% inhibition of HDAC3.
(**D**) Animals with compete responses in part A (n=5) were observed off-treatment for 3 weeks for tumor re-growth. No tumors returned following the end of treatment. These mice were re-injected with STK11-deficient MC38 tumors at the same time as a cohort of naive mice. Animals with previous responses to TNG260 + anti-PD1 rejected tumor formation, indicating the presence of an immune memory.
(**E**) Antitumor activity of TNG260 + anti-PD1 is observed in immunocompetent mice (left), but not in athymic nude mice (right), indicating that an intact T cell compartment is required for efficacy with TNG260.

TNG260 sensitizes highly immunotherapy resistant STK11-deficient GEM mouse models to anti-PD-1



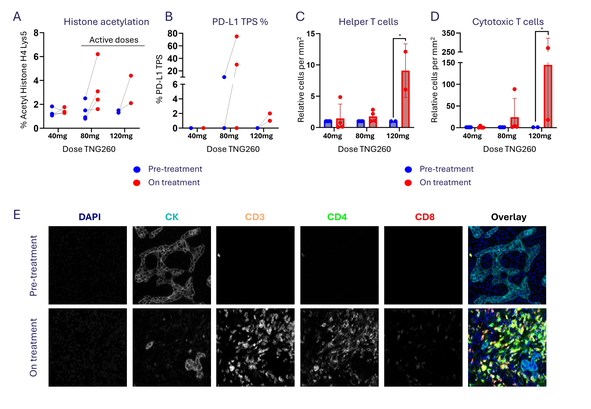
For each mouse study, TNG260 is administered QD, PO. Anti-PD1 is administered intravenously, twice per week at 10mg/mL.
(**A**) Average volume of KL subcutaneous allograft tumors generated with cells derived from LSL-KRAS^{G12D}, STK1 1^{null} mice.
(**B**) Average volume of KP subcutaneous allograft tumors generated with cells derived from LSL-KRAS^{G12D}, p53^{null} mice.
(**C**) Diagram describing KL autochthonous GEM model where intranasal delivery of adeno-cre induces genetic recombination leading to expression of oncogenic KRAS and knockout of STK11 in lung tissue, leading to lung tumor formation. Figure created with BioRender.com
(**D**) Efficacy of the indicated treatment in autochthonous KRAS^{G12D}, STK11^{null} tumors generated as described in C.
(**E**) Exemplar MRIs of mice from each cohort in D showing lung tumor burden over the course of treatment.

TNG260 induces epigenetic changes at immunomodulatory gene loci in STK11-mutant lung cancer cells



(**A**) Plots showing genes with significantly changed expression in KL and KP cells following TNG260 treatment in vitro. KL cells had more gene expression changes detected by RNA-seq than KP cells.
(**B**) Plots demonstrating enrichment of immunomodulatory gene expression pathways increased following TNG260 treatment in KL cells in vitro.
(**C**) Heat maps from ChIP-seq of acetyl-histone H3 Lys 9 showing increased acetyl-histone H3 Lys 9 peaks at cluster 7 following treatment with TNG260 in KL cells. This cluster is unaffected by TNG260 in KP cells.
(**D**) Pathways enriched from the list of genes with both increased acetyl-histone H3 Lys 9 and increased mRNA level following TNG260 treatment of KL tumors. Pathways include immune cell chemotaxis and cell adhesion.
(**E**) Genome browser view showing that TNG260 causes increased acetyl-histone h3 lys 9 peaks at the CXCL3 gene compared to control in KL cells. This enrichment is minimal in KP cells, showing that TNG260 can selectively modulate loci of immune genes.
(**F**) Relative mRNA levels of selected transcripts from immunomodulatory pathways from B that were increased in KL cells after treatment with TNG260.

TNG260 remodels the tumor immune microenvironment in patients with STK11-mutant tumors



Paired biopsies collected during Ph1/2 clinical trial of TNG260 + pembrolizumab were assessed for the indicated analytes. On-treatment biopsies were collected after 2 cycles of combination treatment.
(**A**) TNG260 administered at 80 or 120 mg increased histone acetylation in tumor tissue. 40 mg did not increase histone acetylation and is considered an inactive dose.
(**B**) The PD-L1 tumor proportion score (TPS) increased in tumors from patients treated with 80 or 120 mg (active doses) of TNG260 and pembrolizumab.
(**C**) TNG260 increases the number of intratumoral helper T cells as evaluated by multiplex immunofluorescence for CD3/CD4 positivity. CD4/FOXP3 positive cells (Treg) were generally rare in these samples and do not account for the changes in CD3/CD4 positive population.
(**D**) TNG260 increases the number of cytotoxic T cells as evaluated by multiplex immunofluorescence for CD3/CD8 positivity.
(**E**) Multiplex immunofluorescence panel showing the indicated markers. CK = cytokeratin (tumor cells).

Summary

- **Tumors with STK11 loss-of-function mutations are resistant to immune checkpoint blockade**
- **In vivo CRISPR screening identified CoREST as a target that can sensitize STK11-mutant tumors to anti-PD1**
- **TNG260, a small molecule inhibitor of CoREST, sensitizes STK11-mutant tumors to anti-PD1**
- **TNG260 changes histone acetylation patterns in STK11-mutant tumor cells, causing increased expression of immunomodulatory genes**
- **TNG260 increases intratumoral infiltration of helper and cytotoxic T cells, leading to remodeling of the tumor immune microenvironment**
- **A phase 1/2 study (NCT05887492) evaluating TNG260 and pembrolizumab in STK11-mutant NSCLC patients is currently enrolling patients**
- **See poster 561 for the first clinical disclosure for the phase 1/2 study of TNG260 and pembrolizumab for STK11-mutant cancer**

Acknowledgements

The authors would like to acknowledge the valuable collaboration with Wenrong Zhou and Wenting Shi at Wuxi Apptec. Tango Therapeutics would like to thank patients and their families for participating in the TNG260 clinical trial, and trial investigators for their contributions.

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