CoREST inhibition by TNG260 increases expression of immunomodulatory genes in STK11-mutant cancer and sensitizes to immune checkpoint blockade

Abstract #870

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INTRODUCTION

Loss of function mutations in STK11 drive immune evasion and cause resistance to immune checkpoint blockade.

TNG260 is a small molecule which selectively inhibits the CoREST complex and spares the other Class I HDAC complexes, NCoR, NuRD, and Sin3 (with 500-fold selectivity).

TNG260 treatment reverses the immune evasion phenotype caused by STK11 loss and induces tumor regressions in STK11-deficient models in combination with anti-PD1. Through inhibition of CoREST, TNG260 increases the expression of immunomodulatory genes in STK11-deficient cancer cells, such as up-regulation of genes involved in antigen presentation and T cell-recruiting cytokines. These results are consistent with T cell + tumor cell co-culture experiments which demonstrate TNG260 supports increased T cell migration and activity.

TNG260 enables a higher Teffector:Tregulatory cell ratio which drives an increase in immune-mediated tumor cell killing. In vitro, TNG260 decreases the immunosuppressive functions of Tregulatory cells, leading to improved Teffector cell expansion and activity. In mouse tumors, TNG260 + anti-PD1 results in decreased intratumoral Tregulatory cells.

LSD1 is a member of the CoREST complex. Previous reports have shown that combining an LSD1 inhibitor with anti-PD1 results in improved antitumor responses. We evaluated the combination of an LSD1 inhibitor with anti-PD1 to determine if LSD1 blockade can replicate the effects of TNG260 in an STK11-deficient mouse model that is typically resistant to anti-PD1 monotherapy. TNG260 with anti-PD1 induced tumor regressions in 75% of animals with STK11-deficient tumors. In contrast, an LSD1 inhibitor in combination with anti-PD1 provided limited tumor growth inhibition compared to anti-PD1 alone in an STK11-deficient syngeneic model.

We also profiled an AXL inhibitor, bemcentinib, which is currently in development for STK11-mutant cancer as a combination approach with pembrolizumab. Bemcentinib provided a minor enhancement to the tumor growth inhibition seen with anti-PD1 as a single agent in the STK11-deficient model. Unlike other small molecules being combined with anti-PD1 for STK11-deficient NSCLC, the combination of TNG260 with anti-PD1 drives complete tumor regressions in STK11-deficient models that are typically resistant to anti-PD1 monotherapy. TNG260 is under investigation in a Phase 1/2 study for patients with STK11-mutated, advanced solid tumors.

An in vivo tumor suppressor screen demonstrates that STK11 loss of function causes resistance to immune checkpoint blockade

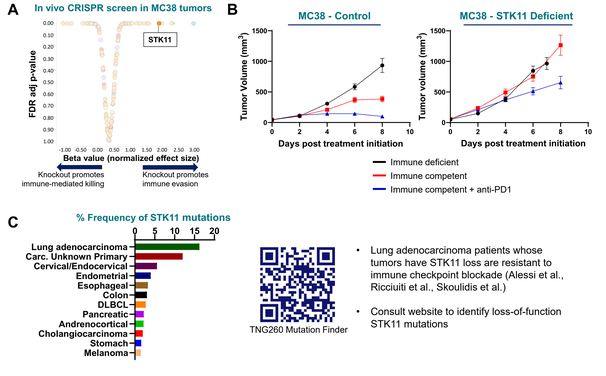

Figure 1: *STK11 loss drives immune evasion and anti-PD1 resistance.* (**A**) An *in vivo* CRISPR screen in MC38 tumors treated with anti-PD1 showed that STK11 loss promotes resistance to anti-PD1 (**B**) STK11 isogenic MC38 cells were generated by CRISPR using a non-targeting control or STK11-targeting sgRNA. STK11 knockout caused tumors to grow more quickly in an immune competent mouse model. The STK11-deficient tumors were more resistant to anti-PD1 treatment. (**C**) STK11 mutations occur most commonly in lung adenocarcinoma but are enriched in other solid tumor types (TCGA, Alexander et al., 2020*)*.

TNG260 is an inhibitor of the CoREST deacetylase complex

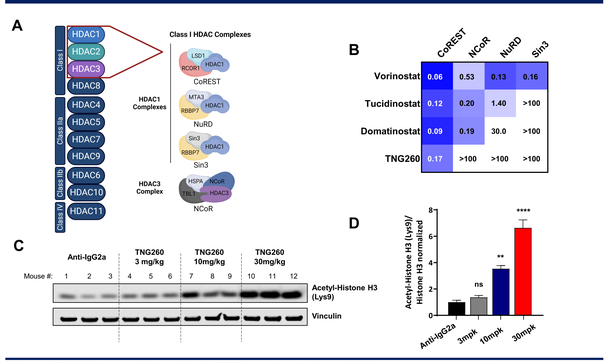

Figure 2: TNG260 is an inhibitor of the CoREST complex. (**A**) Class I HDAC enzymes are a subset of the zinc-dependent HDACs, which act in one of four complexes. (**B**) IC50s of the indicated inhibitors against each Class I HDAC complex in a biochemical deacetylase assay. TNG260 is selective for the CoREST complex. (**C**) Western blot showing increased acetylation of histone H3 at lysine 9 in mouse MC38 tumors treated with the indicated dose of TNG260 for 7 days. (**D**) Quantification of the western blot in (**C**) showing a dose-dependent increase in Acetyl-Histone H3 Lys 9 normalized to Histone H3.

CoREST inhibition by TNG260 reverses anti-PD1 resistance caused by loss of STK11

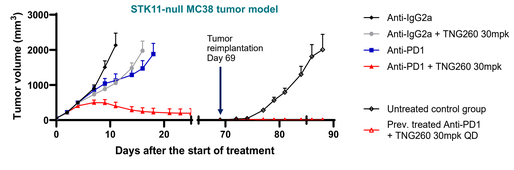

Figure 3: TNG260 + anti-PD1 induces regressions in an STK11-deficient tumor model. The STK11-null MC38 syngeneic tumor model was treated with Anti-IgG2a, TNG260 (30mg/kg, QD), Anti-PD1 (10mg/kg, BIW), or a combination of both. 5 animals exhibited complete tumor regressions at the end of treatment. Mice were observed off-treatment for 3 weeks to evaluate tumor regrowth. 0/5 mice had tumor regrowth following treatment cessation. 5/5 previously cured animals rejected tumor reimplantation. A cohort of naïve, previously untreated mice were injected with the same tumor cells and tumors grew as expected.

TNG260 increases expression of immunomodulatory genes in STK11-deficient cells and sensitizes to T cell killing

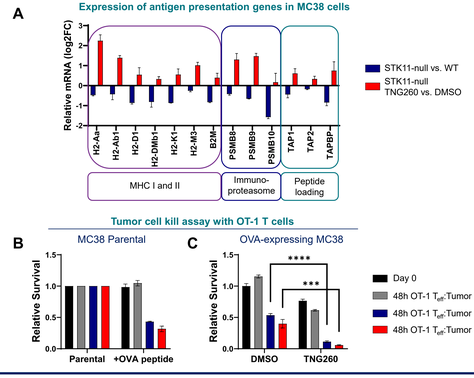

Figure 4: TNG260 reverses impaired antigen presentation in STK11-deficient cells, sensitizing them to T cell killing. (**A**) Relative mRNA levels of antigen presentation genes in STK11-null MC38 cells compared to wild-type MC38 (blue), and in STK11-null MC38 cells treated with 400nM TNG260 compared to DMSO (red). Similar effects have been observed in the human cell line H1975. (**B, C**) CD8+ T cells from OT-1 mice were collected and incubated with tumor cells at the indicated ratios. Tumor cell survival was evaluated after 48h. (**B**) MC38 parental cell survival either alone or supplemented with OVA peptide as a positive control to show on-target activity of OT-1 T cells. (**C**) MC38 cells expressing the OVA peptide were pre-treated with either DMSO or 500nM TNG260 for 96 hours prior to T cell co-culture. TNG260 pre-treatment caused enhanced killing of tumor cells by OT-1 T cells.

TNG260 increases expression of T cell attracting cytokines and stimulates T cell migration

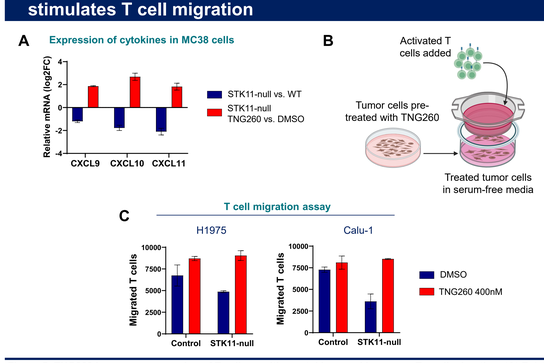

Figure 5: TNG260 increases expression of T cell-recruiting cytokines and increases T_{eff} cell migration. (**A**) Relative levels of cytokine mRNA in STK11-null MC38 cells compared to wild-type MC38, and in STK11-null MC38 cells treated with 400nM TNG260 compared to DMSO. Increased CXCL9-11 was previously observed in TNG260 + anti-PD1-treated tumors. (**B**) Assay scheme for T cell migration assay. (**C**) STK11 knockout in STK11 wild-type cancer cell lines causes decreased migration of activated T cells. Pre-treatment of tumor cells with 400nM TNG260 for 48 hours reverses the negative effects of STK11 loss on T_{eff} cell migration.

TNG260 decreases intratumoral T_{reg} recruitment in combination with anti-PD1

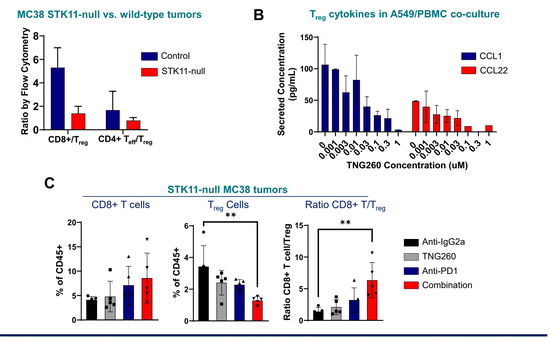

Figure 6: TNG260 combined with anti-PD1 reverses the low CD8+ T cell:T_{reg} cell ratio caused by STK11 loss. (**A**) STK11 loss in the MC38 mouse model decreases the intratumoral T_{eff}/T_{reg} ratio, favoring immunosuppression. (**B**) TNG260 causes dose-dependent decreases in secreted CCL1 and CCL22 in media from A549/PBMC co-culture. (**C**) Flow cytometry of CD8+ T cells and T_{reg} cells. TNG260 + anti-PD1 improves the T_{eff}/T_{reg} ratio in STK11-null tumors, favoring T_{eff} cell activity.

TNG260 inhibits effector T cell suppression by regulatory T cells

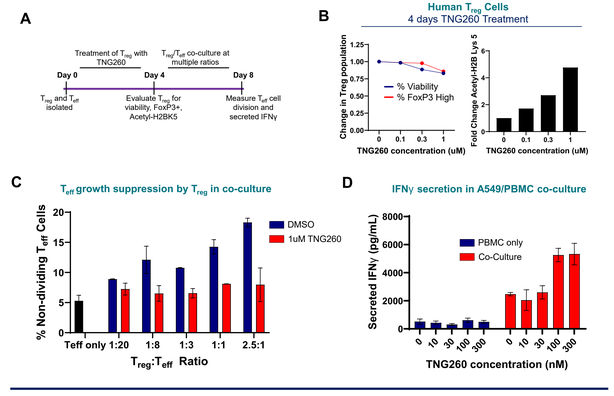

Figure 7: TNG260 reverses the immunosuppressive effects of T_{reg} cells, enabling increased T_{eff} activity. (**A**) Timeline of T_{reg}/T_{eff} co-culture assay. (**B**) TNG260 treatment of T_{reg} increases histone acetylation while minimally impacting T_{reg} cell viability or FOXP3+ status. (**C**) Using increasing ratios of T_{reg}:T_{eff} cells, 1uM of TNG260 suppresses T_{reg} activity. Accordingly, T_{eff} cell proliferation is increased. (**D**) TNG260 treatment leads to increased IFNγ secretion in co-cultures of A549 NSCLC tumor cells and PBMCs. Similar increases in IFNγ secretion are seen in T_{reg}/T_{eff} cell co-cultures treated with TNG260.

TNG260 outperforms AXL inhibitor in combination with anti-PD1 in STK11-null cancer model

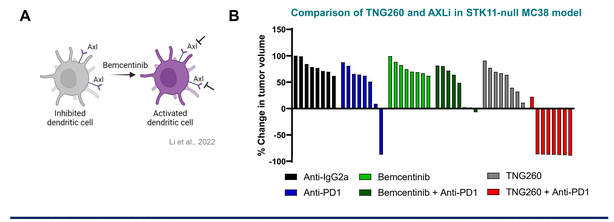

Figure 8: TNG260 + anti-PD1 induces stronger anti-tumor responses than bemcentinib (AXLi) + anti-PD1. (**B**) The percent maximal change in tumor volume of individual mice bearing STK11-null MC38 tumors. Mice were treated with Anti-IgG2a, TNG260 (30mg/kg, QD), Anti-PD1 (10mg/kg, BIW), Bemcentinib (50mg/kg, BID), or the indicated combination. Increases in tumor volume were normalized to the largest tumor, n=8 mice/group.

LSD1 inhibition is not redundant for TNG260 when combined with anti-PD1 in STK11-deleted cancer

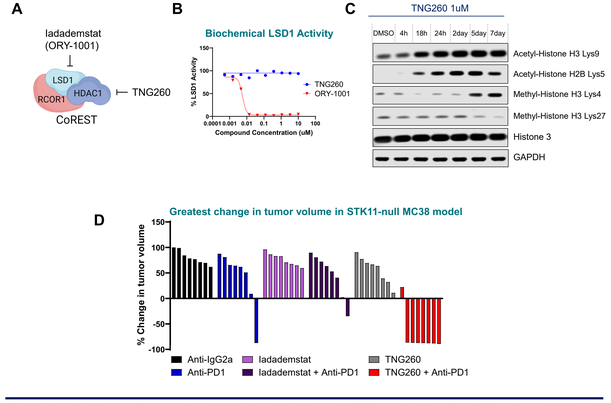

Figure 9: Iadademstat (LSD1 inhibitor) does not replicate the effects of TNG260 with anti-PD1 in STK11-null tumors. (**A**) Diagram of the CoREST complex featuring both LSD1 and HDAC enzymes. (**B**) TNG260 has no biochemical activity against LSD1. (**C**) TNG260 does not directly influence histone methyl marks, while it does increase histone acetylation. (**D**) The percent maximal change in tumor volume of individual mice bearing STK11-null MC38 tumors. Mice were treated with Anti-IgG2a, TNG260 (30mg/kg, QD), Anti-PD1 (10mg/kg, BIW), Iadademstat (0.03mg/kg, 5 day on/2 day off), or the indicated combination. Increases in tumor volume were normalized to the largest tumor, n=8 mice/group. Control animals and TNG260-treated groups are from the same experiment as Figure 8, so these data are replicated.

TNG260 Phase 1/2 Clinical Trial

A Phase 1/2, Open-Label Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Efficacy of TNG260 as Single Agent and in Combination With an Anti-PD-1 Antibody In Patients With STK11 Mutated Advanced Solid Tumors

NCT05887492
Clinicaltrials.gov

- Currently enrolling patients

SUMMARY

- **Tumors with STK11 loss of function are resistant to immune checkpoint blockade**
- **TNG260 is an oral small molecule inhibitor of the CoREST complex**
- **CoREST inhibition by TNG260 reverses resistance to anti-PD1 that is caused by STK11 loss**
- **TNG260 increases the expression of antigen presentation genes and T cell-stimulating cytokines which are decreased with loss of STK11.**
- **Treatment of tumor cells with TNG260 increases their susceptibility to killing in a T cell co-culture assay and improves effector T cell migration.**
- **TNG260 + Anti-PD1 improves the ratio of $T_{effector}$:$T_{regulatory}$ cells and decreases the immunosuppressive effect of $T_{regulatory}$ cells.**
- **While LSD1 is a member of the CoREST complex, LSD1 inhibition and TNG260 treatment are not redundant**
- **TNG260 outperforms LSD1 and AXL inhibitors in combination with anti-PD1 in an STK11-deficient mouse model**

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REFERENCES

Alessi JV, Elkrief A, Ricciuti B, Wang X, Cortellini A, Vaz VR, et al. *J Thorac Oncol*. 2023;18:731–43.

Alexander, B., Sokol, E. S., Danziger, N. A., Pavlick, D. C., Elvin, J., Killian, J. K., et al. *Annals of Oncology* 2020;31, S283–S284.

Li H, Liu Z, Liu L, Zhang H, Han C, Girard L, et al. *Reports Medicine*. 2022;3:100554.

Ricciuti B, Arbour KC, Lin JJ, Vajdi A, Vokes N, Hong L, et al. *J Thorac Oncol*. 2022;17:399–410.

Skoulidis F, Goldberg ME, Greenawalt DM, Hellmann MD, Awad MM, Gainor JF, et al. *Cancer Discov*. 2018;8:CD-18-0099.