#1167

TRIM7 Inhibition Blocks RTK/RAS Pathway Driven Tumor Cell Proliferation Independent of Mutation and Restores Tumor-Intrinsic IFN Responsiveness

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1. Abstract

Background: Most cancer patients experiencing clinical benefit from $KRAS^{G12C}$ blockade or immune checkpoint inhibitor (CPI) therapy eventually develop acquired resistance (AR) and relapse. Mechanisms of AR have been shown to include the development of new KRAS/MAPK mutations, and the transcriptional hyperactivation of genes associated with chronic IFN signaling. Analysis of preclinical AR models and human cancer transcriptomics identified the E3 ubiquitin ligase **TRIM7**, as a driver of the AR phenotype. **TRIM7** is upregulated in KRAS mutant and MSI-high tumors, and (1) mediates cell proliferation downstream of aberrant RTK/RAS/RAF signaling through ubiquitination and stabilization of RACO-1 and (2) modulates IFN responsiveness through ubiquitination and degradation of MAVS. Here we describe the development of the lead **TRIM7** small molecule inhibitor (SMI) **KT-300** and validate it as a novel therapeutic in RTK/RAS driven cancers.

Methods: MST, fluorescent polarization, and mass spec determined binding specificity/affinity of >700 **TRIM7** SMI. Solubility, stability, and half-life were assessed in plasma. Membrane permeability/efflux were determined using MDCK-MDR1. Potency was evaluated using target ubiquitination/stabilization/degradation, human tumor growth inhibition (TGI), transcriptional profiling, and *in vivo* efficacy in human xenograft models.

Results: **TRIM7** inhibition disrupted the ubiquitination and stability of RACO-1, resulting in decreased phosphorylation of c-Jun and STAT3, and altered transcription of c-Jun/AP-1 targets. **KT-300**-mediated degradation of RACO-1 resulted in significant TGI in both EGFR/KRAS wild-type and mutant tumor cell lines, and outperformed other KRAS inhibitors like sotorasib and zoldonrasib, without selectivity to specific KRAS mutations. **TRIM7** specificity was confirmed using inactive enantiomers of lead SMI, which did not induce TGI. In addition, **TRIM7** inhibition reduced ubiquitination of MAVS, resulting in MAVS accumulation, and transcriptional changes in interferon responsiveness genes. *In vivo*, **KT-300** monotherapy delayed the growth of aggressive CT26/AR tumors ($KRAS^{G12D}$), and KRAS mutant human NSCLC and CRC tumor xenografts. **TRIM7** over-expression in NCI-H1299 lung cancer cells resulted in the downregulation of previously defined 'good' interferon stimulatory genes (ISGs), including IDO1, IFIT2, and ISG20, and upregulation of 'bad' ISGs, including STAT1, CXCL1, and OAS3. **TRIM7** inhibition reversed these transcriptional patterns and downregulated genes associated with KRAS pathway activation, including RACO-1(RNF187), MAP2K2, and RASA1.

Conclusions: TRIM7 controls cell proliferation and IFN responsiveness downstream of RTK/RAS/RAF and was identified as a candidate mediator of CPI acquired resistance. Therapeutic inhibition of **TRIM7** with **KT-300**, reduced tumor growth *in vitro* and *in vivo* and outperformed multiple KRAS inhibitors as monotherapy. **KT-300** warrants further development to address the unmet need in patients with cancers that evolve various therapeutic resistance mechanisms, including aberrant RTK/RAS/RAF signaling due to gene amplifications and mutations.

Ethics Approval: Animal studies were approved by an Institutional Animal Care and Use Committee (IACUC) and a licensed veterinarian.

2. TRIM7 Identified As a Driver of Therapeutic Resistance

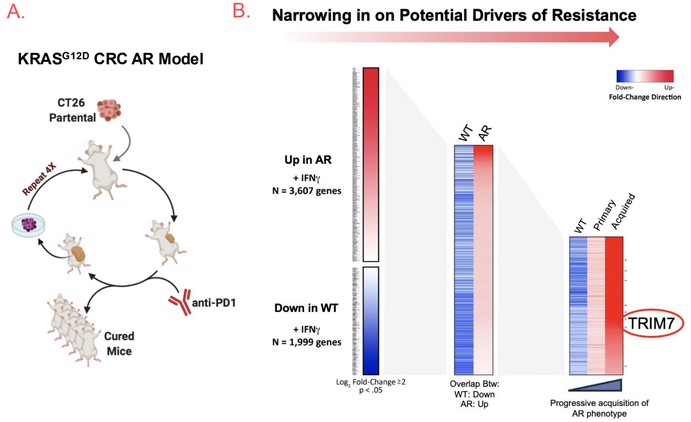


Figure 1. Identification of TRIM7. (A) Mice bearing CT26 CRC $KRAS^{G12D}$ tumors were conditioned to develop acquired resistance to CPI therapy. (B) Through a series of *in vitro* and *in vivo* transcriptomic studies using CT26-AR cells and tumors, TRIM7 was identified as a potential driver of the AR phenotype (*Memon D, Fromm G, Hellmann MD. 2024. Cancer Cell*).

3. TRIM7 Expression is Enriched in KRAS Mutant Cancers

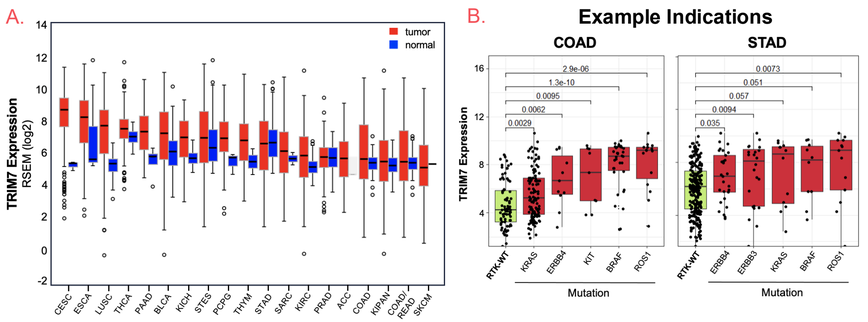


Figure 2. TRIM7 expression in TCGA. (A) TRIM7 is highly expressed in many cancers (including lung, pancreatic, and colorectal cancers) and is enriched in the tumor as compared to normal adjacent tissue. (B) In many cancers, TRIM7 expression is significantly higher in patients with mutations in the RTK-KRAS pathway.

4. KT-300 Identified as Lead TRIM7 Inhibitor

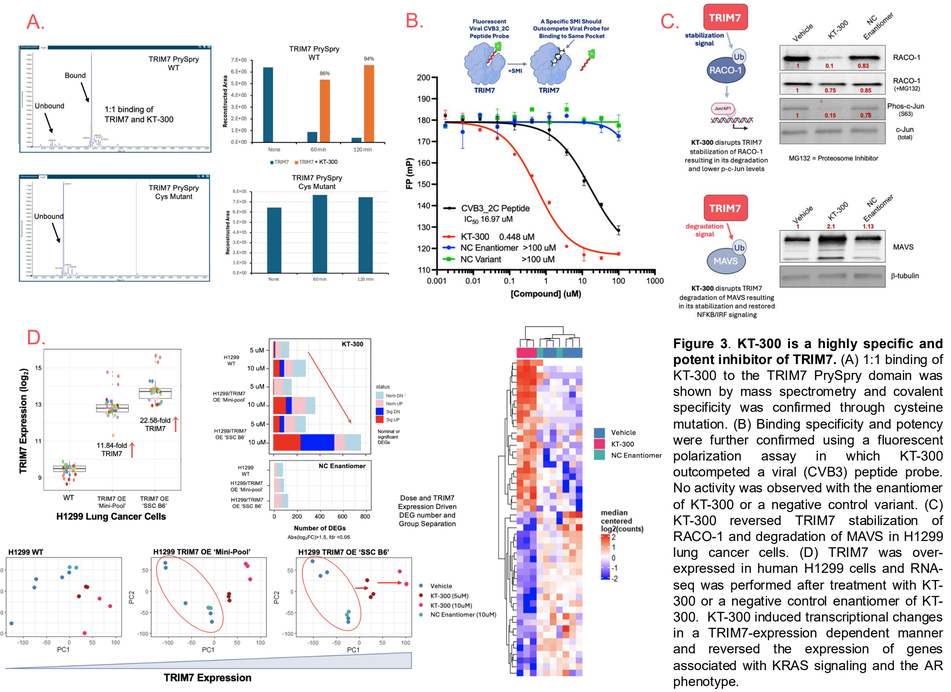


Figure 3. KT-300 is a highly specific and potent inhibitor of TRIM7. (A) 1:1 binding of KT-300 to the TRIM7 PrySpry domain was shown by mass spectrometry and covalent specificity was confirmed through cysteine mutation. (B) Binding specificity and potency were further confirmed using a fluorescent polarization assay in which KT-300 outcompeted a viral (CVB3) peptide probe. No activity was observed with the enantiomer of KT-300 or a negative control variant. (C) KT-300 reversed TRIM7 stabilization of RACO-1 and degradation of MAVS in H1299 lung cancer cells. (D) TRIM7 was over-expressed in human H1299 cells and RNA-seq was performed after treatment with KT-300 or a negative control enantiomer of KT-300. KT-300 induced transcriptional changes in a TRIM7-expression dependent manner and reversed the expression of genes associated with KRAS signaling and the AR phenotype.

5. KT-300 Induced Monotherapy Anti-Tumor Activity

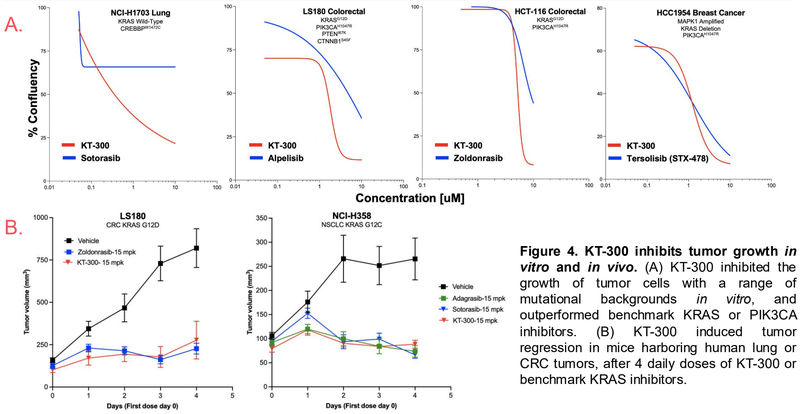
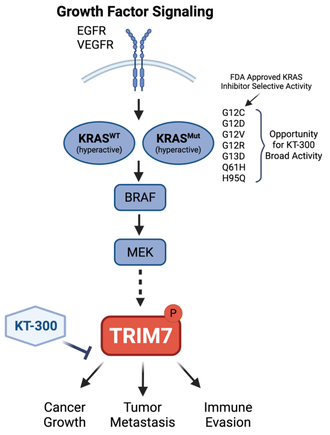


Figure 4. KT-300 inhibits tumor growth *in vitro* and *in vivo*. (A) KT-300 inhibited the growth of tumor cells with a range of mutational backgrounds *in vitro*, and outperformed benchmark KRAS or PIK3CA inhibitors. (B) KT-300 induced tumor regression in mice harboring human lung or CRC tumors, after 4 daily doses of KT-300 or benchmark KRAS inhibitors.

6. Summary



- **TRIM7,** an E3 ubiquitin ligase downstream of RTK-RAS signaling, was identified as a driver of CPI acquired resistance, and is upregulated in KRAS mutant/amplified cancers whenever the RTK-RAS pathway is activated
- **KT-300** is a high affinity covalent inhibitor of TRIM7, and was shown to exhibit strong on-target monotherapy tumor growth inhibition both *in vitro* and *in vivo*
- **KT-300** activity is mutation-agnostic, is broadly active across a range of RTK-RAS mutational backgrounds, and inhibits a novel target that may overcome resistance mechanisms as new mutations develop in patients on existing therapies (e.g., new KRAS/MAPK mutations seen in patients treated with $KRAS^{G12C}$ inhibitors)
- **KT-300** is expected to enter IND-enabling activities in 2026

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