

1. Abstract

Most cancer patients experiencing clinical benefit from targeted therapies in RTK-RAS driven cancers eventually develop acquired resistance (AR) and relapse. The mechanism of resistance in patients who initially respond to monoallelic KRAS G12C/G12D inhibitors is dominated by the accumulation of new mutations in KRAS itself, followed by mutations downstream in the MAP kinase pathway. Therapeutics that more broadly target this pathway, like the multi-KRAS isoform inhibitor daraxonrasib, offer promise in less common mutations (e.g., KRAS G12X/Q61X), however similar resistance mechanisms have been seen. Unfortunately, combining KRAS inhibitors with other therapies (e.g., EGFRi, other KRAS inhibitors, CPI) have been limited by overlapping toxicities, and new approaches to inhibit this pathway are needed.

To identify novel approaches for complex or resistant RTK-RAS driven cancers, we generated a murine CRC KRAS(G12D) checkpoint inhibitor (CPI) AR model and through transcriptional/proteomic analysis, identified the E3 ubiquitin ligase TRIM7 as a putative driver of resistance. TRIM7 is downstream in the RTK-RAS pathway and is upregulated in RAS mutant and MSI-high tumors, resulting in (1) aberrant cell proliferation through ubiquitination and stabilization of RACO-1 and activated cJun/AP1 transcription, and (2) dysregulated IFN responsiveness through ubiquitination and degradation of MAVS and STING. TRIM7 is not a known mutated oncogene; rather, its hyperactivation is a byproduct of increased RTK-RAS signaling due to upstream amplifications or mutations.

We developed a highly potent, selective, and covalent TRIM7 small-molecule inhibitor (KT-300) and showed it directly disrupted the ubiquitination and stability of RACO-1, resulting in decreased phosphorylation of cJun and STAT3, and altered transcription of cJun/AP1 targets. KT-300 reduced ubiquitination of MAVS, resulting in MAVS accumulation, and restored STING signaling in tumor cells where STING was impaired due to TRIM7 overexpression. KT-300 induced significant TGI in a range of KRAS/NRAS mutant tumor models (G12X, G13X, and Q61X) and in cell lines with amplification or mutation of EGFR or MAP kinase. TRIM7 specificity was confirmed using inactive enantiomers, which did not induce TGI. *In vivo*, KT-300 monotherapy delayed the growth of human RAS-mutant NSCLC, CRC, gastric, and PDAC tumor xenograft and PDX models. KT-300 anti-tumor activity was enhanced in combination with concurrent EGFR or KRAS inhibition, and KT-300 monotherapy delayed tumor growth in mice that acquired resistance and progressed on KRAS inhibitors.

TRIM7 inhibition via KT-300 may offer broad, mutation-agnostic activity in RTK-RAS driven cancers. This represents a novel “pan-RTK-RAS pathway inhibition” strategy that could extend efficacy to complex mutational backgrounds not addressed by current KRAS inhibitors, including in patients who have developed resistance to existing RTK-RAS targeted therapies.

2. TRIM7 Identified As a Driver of Therapeutic Resistance

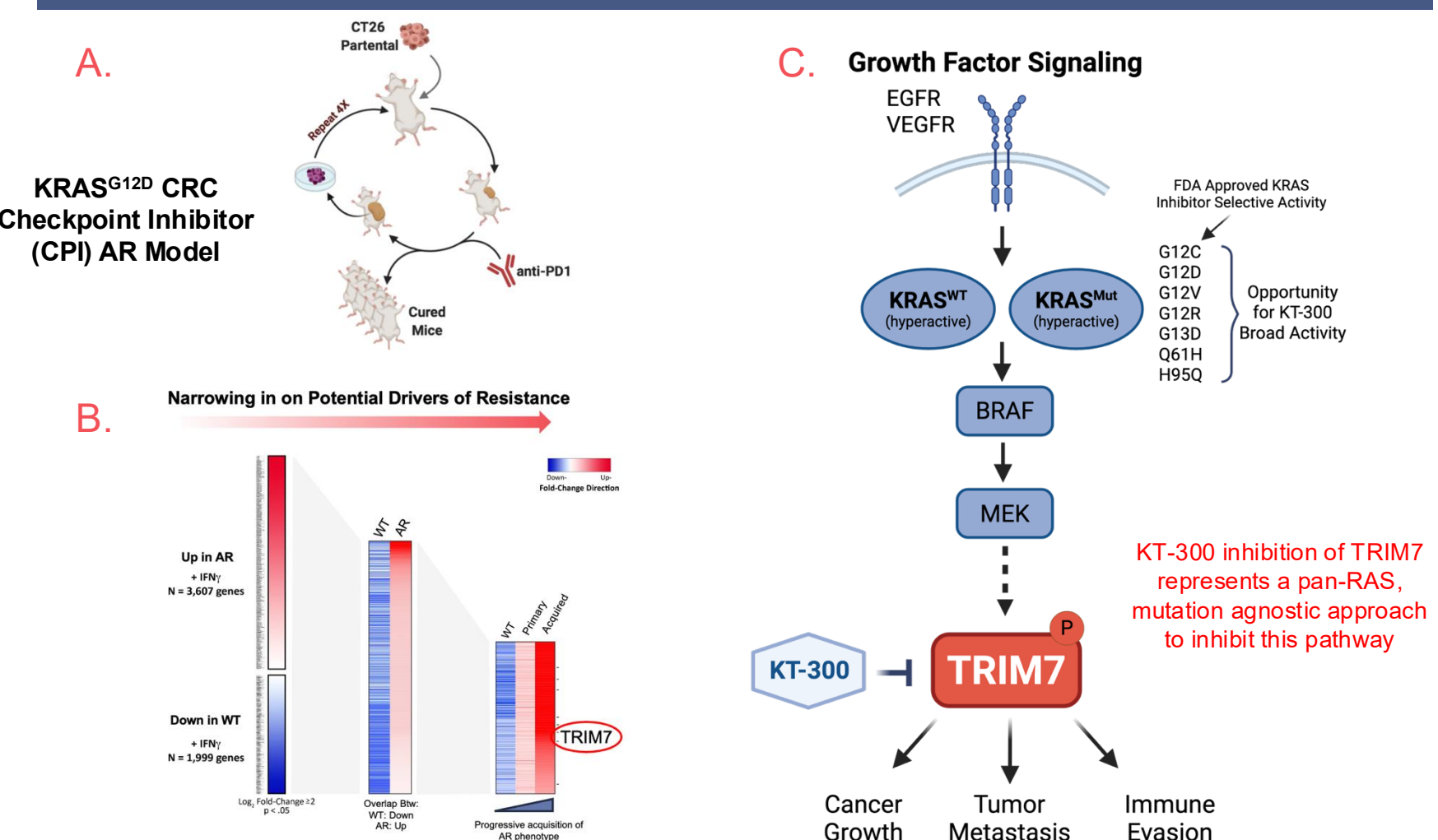


Figure 1. Identification of TRIM7. (A) Mice bearing CT26 CRC KRAS^{G12D} tumors were conditioned to develop acquired resistance to CPI therapy. (B) Through a series of *in vitro* and *in vivo* transcriptomic studies, TRIM7 was identified as a potential driver of the AR phenotype (Memon D, Fromm G, Hellmann MD. 2024. Cancer Cell). (C) TRIM7 is an E3 ubiquitin ligase that sits downstream of RTK/RAS signaling. TRIM7 is not a known mutated oncogene, rather it is hyperactivated when the pathway is dysregulated upstream due to mutation or amplification (e.g., EGFR, RAS, etc.).

3. TRIM7 Expression is Enriched in RAS Mutant Cancers

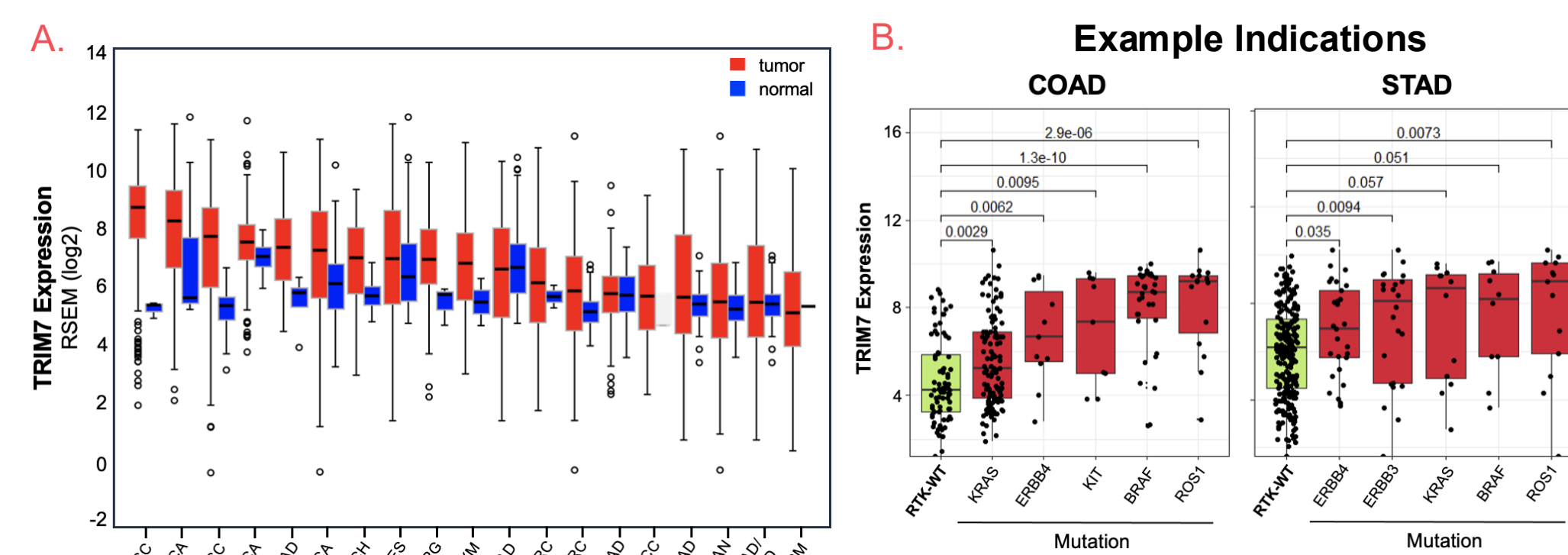


Figure 2. TRIM7 expression in TCGA. (A) TRIM7 is highly expressed in many cancers (including lung, pancreatic, and colorectal cancers) and is enriched in the tumor as compared to normal adjacent tissue. (B) In many cancers, TRIM7 expression is significantly higher in patients with mutations in the RTK-RAS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

4. KT-300 Identified as Lead Covalent TRIM7 Inhibitor

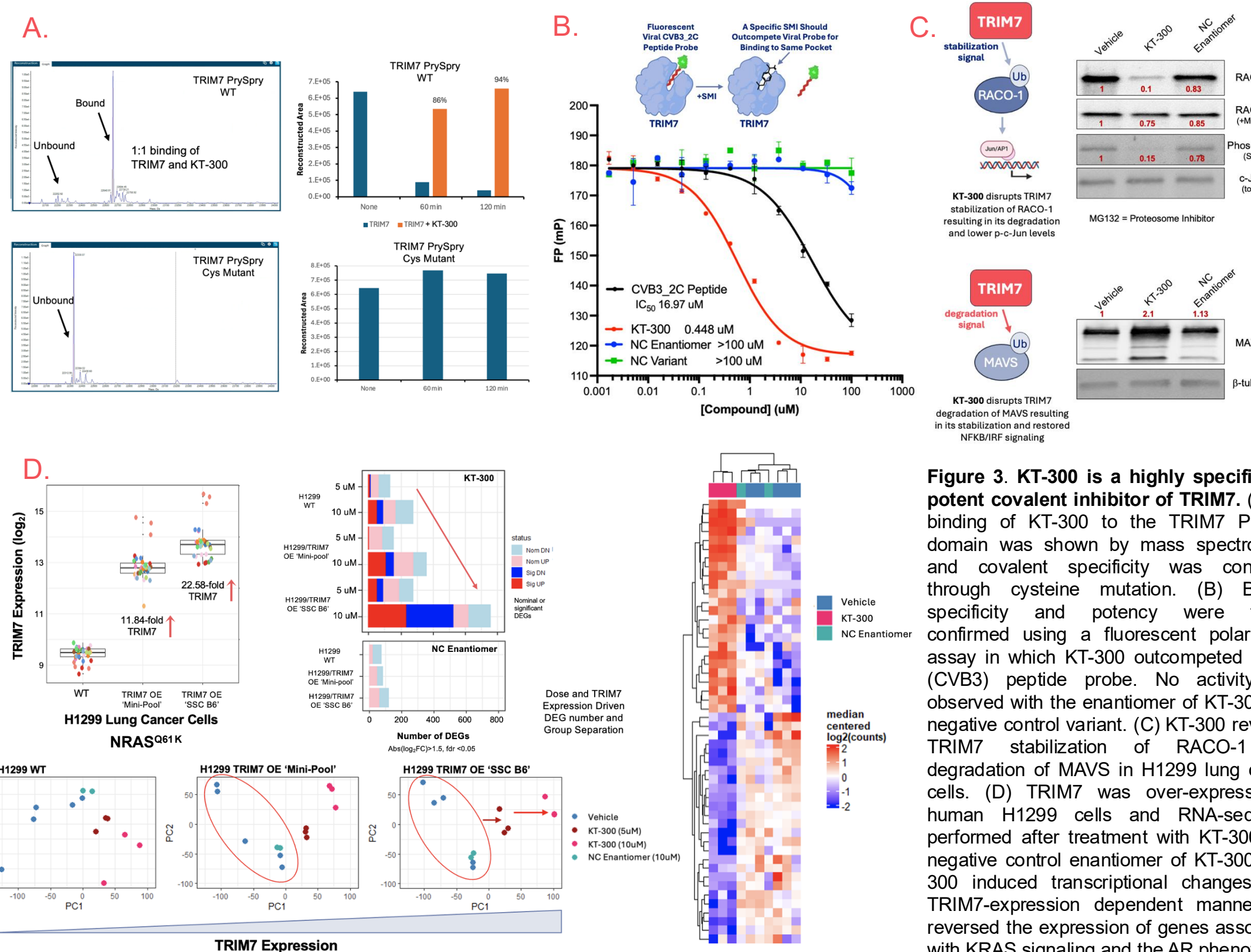


Figure 3. KT-300 is a highly specific and potent covalent inhibitor of TRIM7. (A) 1:1 binding of KT-300 to the TRIM7 Pryspry domain was shown by mass spectrometry and covalent specificity was confirmed through cysteine mutation. (B) Binding specificity and potency were further confirmed using a fluorescent 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 Exerts Potent and Broad Anti-Tumor Activity

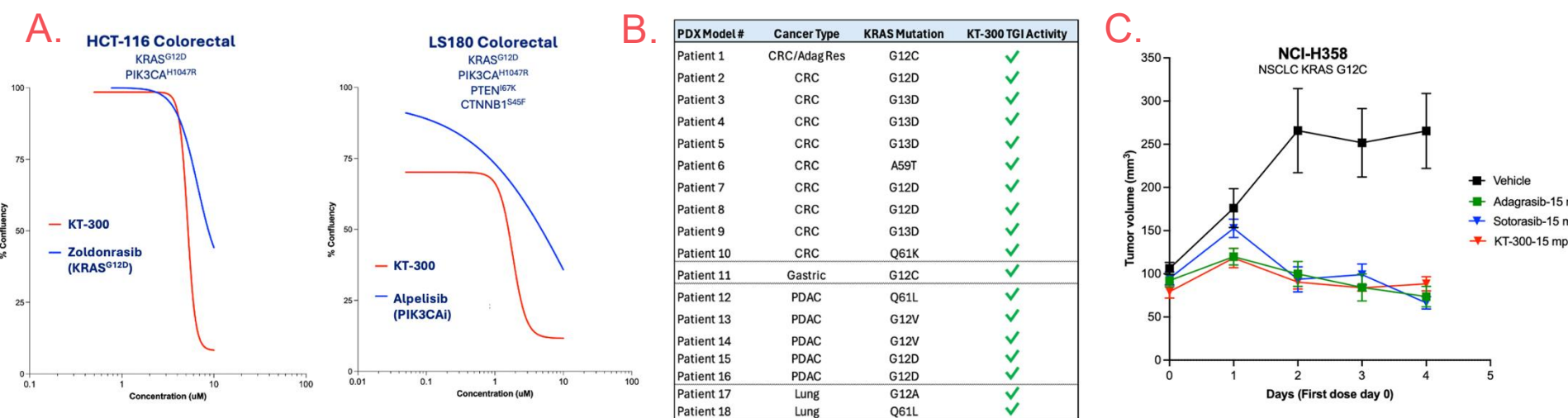


Figure 4. KT-300 monotherapy activity in (A) complex mutation backgrounds (*in vitro* 2D CDX TGI), (B) 3D spheroid PDX models regardless of KRAS mutation, and (C) *in vivo* in mouse KRAS mutant CDX models.

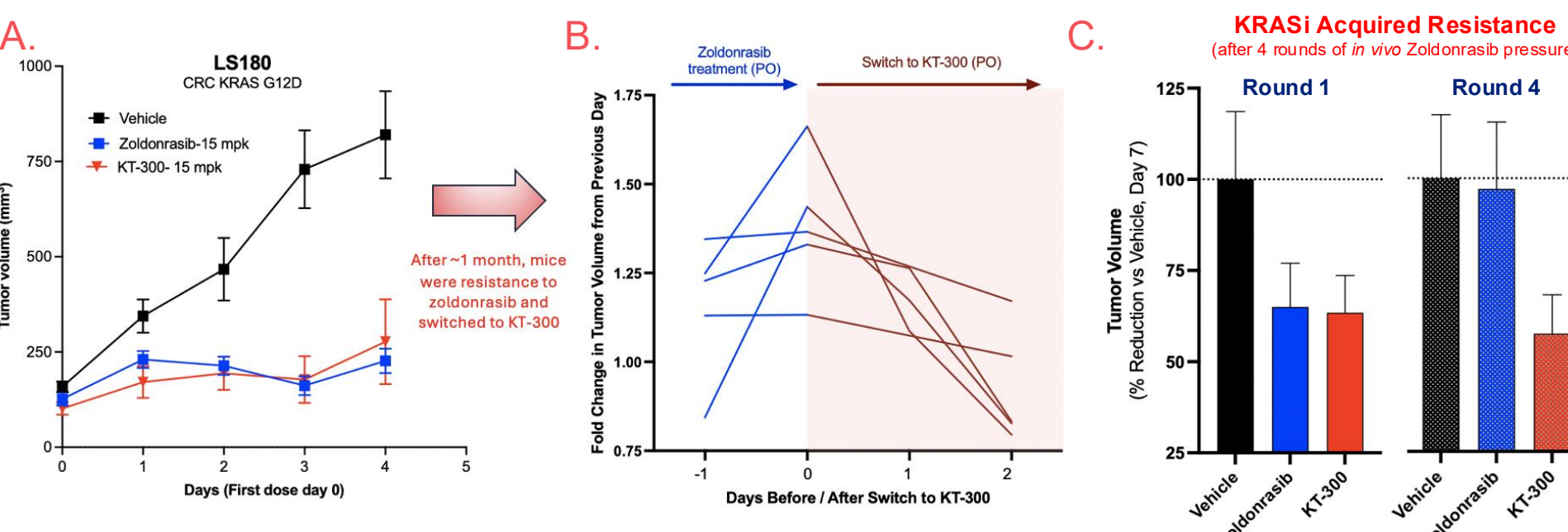


Figure 5. KT-300 *in vivo* anti-tumor activity in (A) KRAS^{G12D} mutant CRC, when (B) zolndonrasib responsive mice developed resistance to initial zolndonrasib treatment, progressed on treatment, and were switched to KT-300 (which restored tumor regression), and (C) when mice acquired resistance to Zolndonrasib longer term (after 4 rounds of *in vivo* passage followed by treatment).

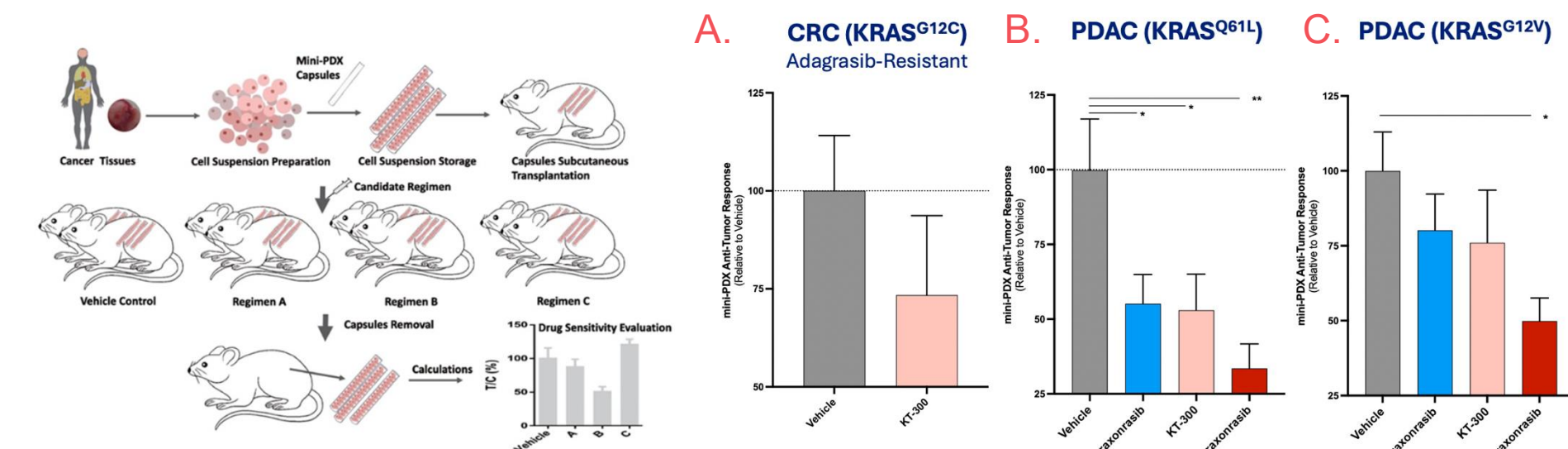


Figure 6. KT-300 *in vivo* PDX activity in (A) adagrasib-resistant KRAS^{G12C} CRC, and as monotherapy and in combination with daraxonrasib in (B) KRAS^{Q61L} and (C) KRAS^{G12V} pancreatic cancer (PDAC).

- **TRIM7**, an E3 ubiquitin ligase downstream of RTK-RAS signaling, was identified as a driver of CPI acquired resistance, and is upregulated in RAS 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**: (1) provides mutation-agnostic monotherapy activity, (2) overcomes resistance to KRAS inhibitors, (3) improves anti-tumor efficacy in combination with a range of other therapies, including EGFRi, KRASi, and checkpoint inhibitors, and (4) achieves high systemic exposures, a broad therapeutic index, and was well-tolerated in large animal toxicology studies.
- **KT-300** first-in-human IND filing expected 2H 2026

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