



# RAS(ON) G12D-Selective and Multi-Selective Inhibitor Doublet Drives Complete Responses in Combination with Anti-PD-1 in a Preclinical Model of MSS KRAS G12D Mutant CRC

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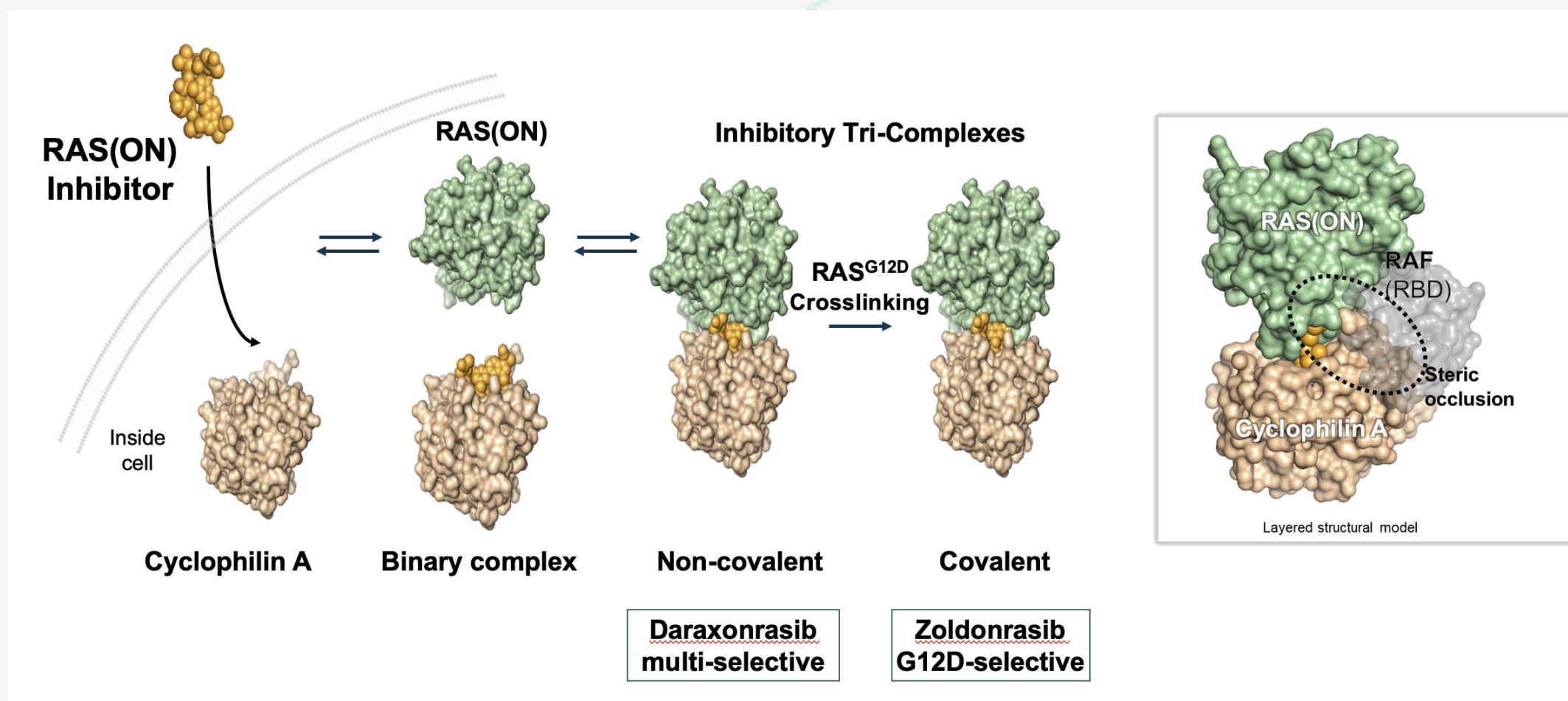
## Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the United States. About 50% of colorectal cancers harbor KRAS mutations, with KRAS G12D representing the largest population. Targeted therapies, as well as immune checkpoint inhibitor therapies, have limited efficacy as monotherapies for patients with microsatellite stable (MSS) CRC. However, recent clinical evidence in BRAF mutant MSS CRC suggests that BRAF inhibitors in combination with MEK or EGFR inhibitors and anti-PD-1 can increase the durability of the response. CRC is a heterogeneous disease, and the availability of preclinical models that recapitulate the complexity of human tumors is limited. Here we developed a syngeneic preclinical model representative of MSS RAS mutant CRC to investigate if RAS pathway inhibition with RAS(ON) inhibitors can sensitize CRC to immune checkpoint inhibition. CMT93 is a murine cell line derived from a polypoid carcinoma of the rectum that harbors a Ctnnb1 (gene that encodes B-catenin) gain of function mutation, Tp53 loss of function mutation, with no mutations in mismatch repair genes. We engineered the eCMT93 model using CRISPR-Cas9 editing to express homozygous Kras G12D to develop a RAS-driven MSS CRC preclinical model.

## Revolution Medicines Potential Clinical Approaches to Target RAS<sup>G12D</sup> Mutant Cancers

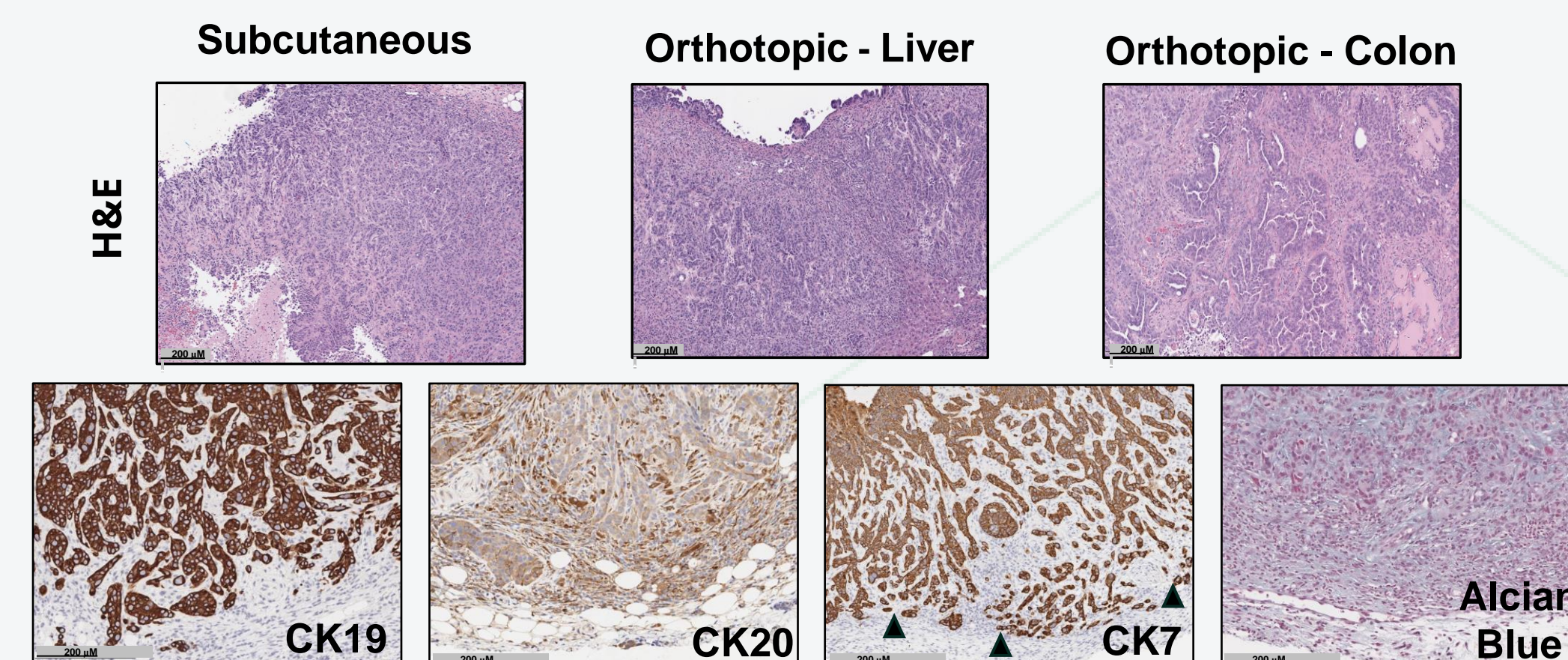
**Daraxonrasib (RMC-6236)**  
RAS(ON) Multi-Selective

**Zoldonrasib (RMC-9805)**  
RAS(ON) G12D-Selective



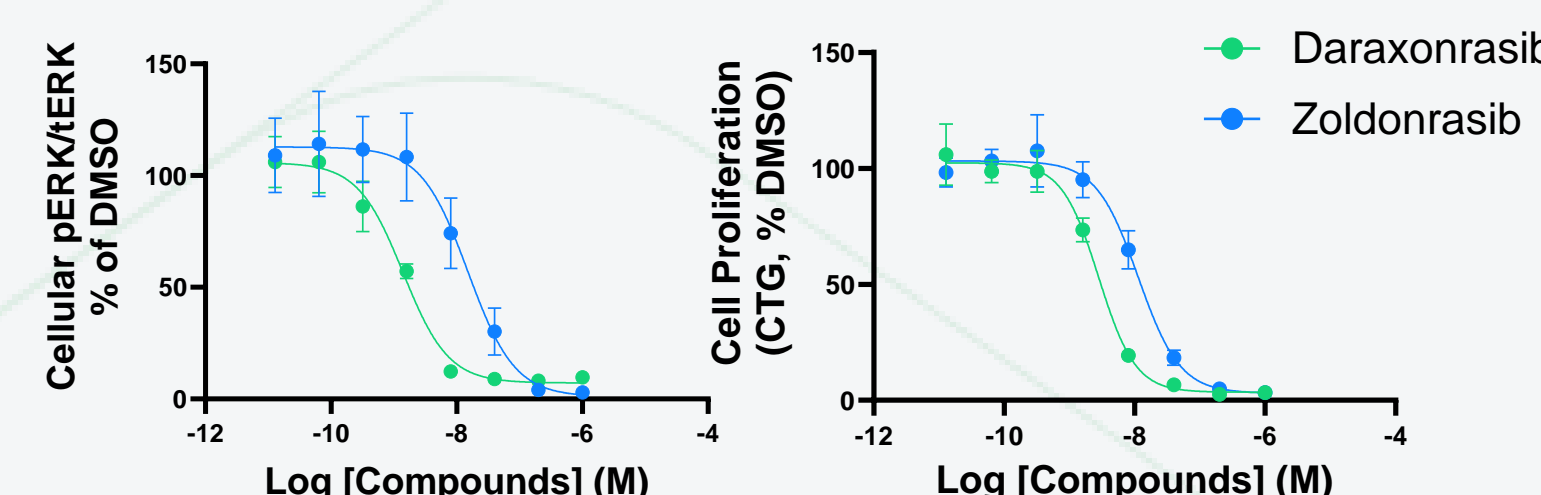
## Key Results

### eCMT93 Tumors Show Glandular Morphology and Budding Typical of Well Differentiated Invasive CRC Across Three Different Inoculation Sites



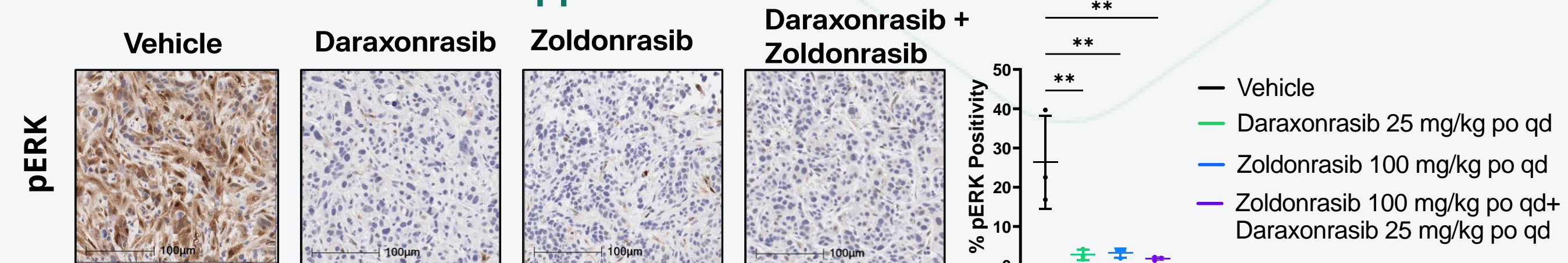
(Top) H&E staining of eCMT93 tumors at 3 different inoculation sites (subcutaneous, liver, colon). (Bottom) Subcutaneous eCMT93 tumors stained with CK19, CK20, CK7, and Alcian Blue. Black arrows indicating tumor budding at the invasive front.

### The eCMT93 Model Is Dependent On KRAS G12D In Vitro



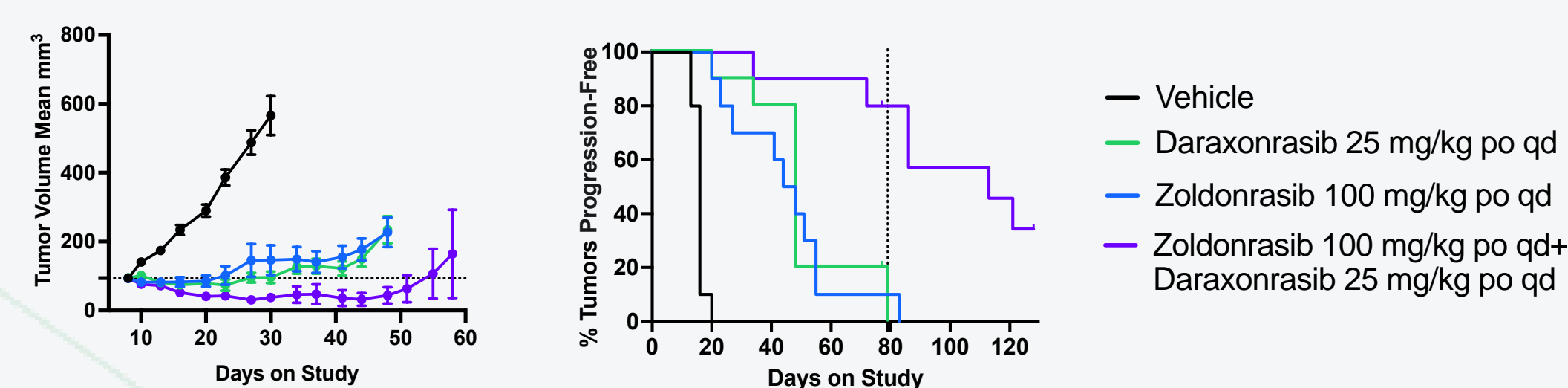
eCMT93 EC <sub>50</sub> (nM)	Daraxonrasib	Zoldonrasib
5 Day 3D-CTG	2.8	11.3
4-hour pERK	1.4	15.1

### Daraxonrasib, Zoldonrasib and RAS(ON) Doublet Achieve RAS Pathway Suppression *In Vivo*



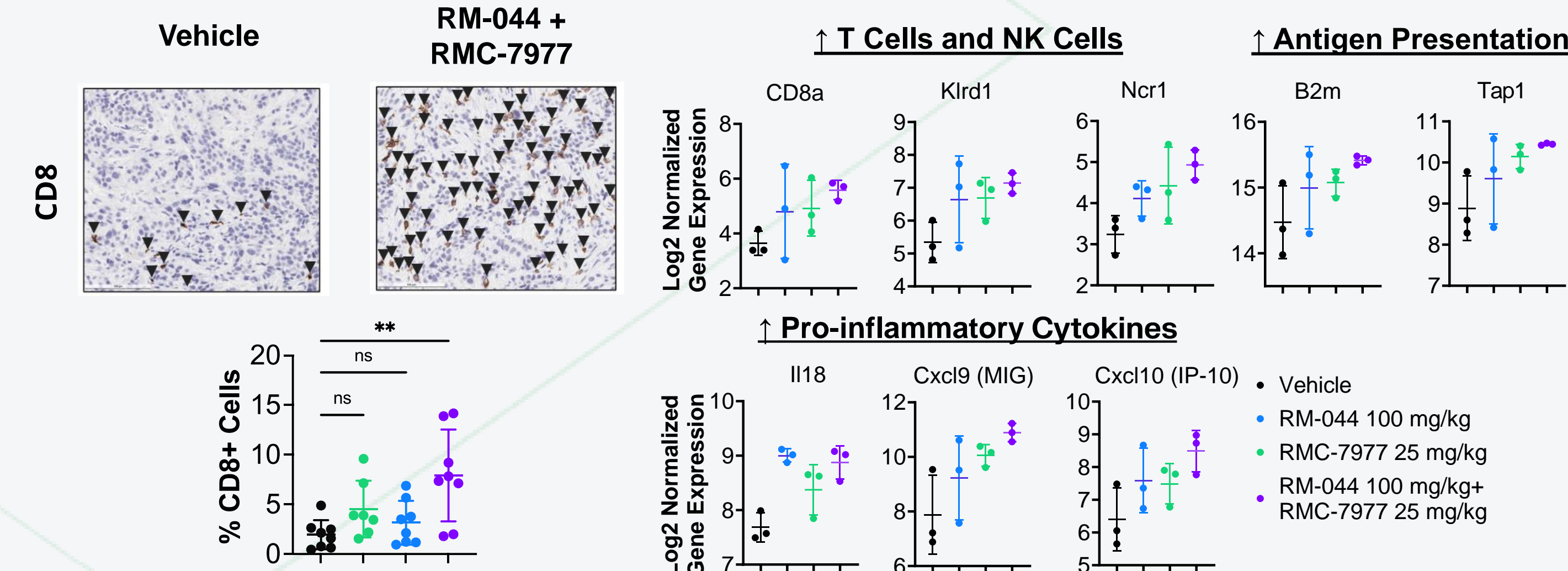
Assessment of pERK in eCMT93 tumors at 24 hours post 4 days of treatment with vehicle, daraxonrasib at 25 mg/kg PO QD, zoldonrasib at 100 mg/kg PO QD, or daraxonrasib 25mg/kg PO QD and zoldonrasib at 100 mg/kg PO QD. Quantification of pERK staining is defined by AI identified tumor regions. Unpaired T test (\*\*p<0.01).

### Daraxonrasib and Zoldonrasib Induce Transient Regressions, While The Combination Further Improves the Depth and Durability of Response



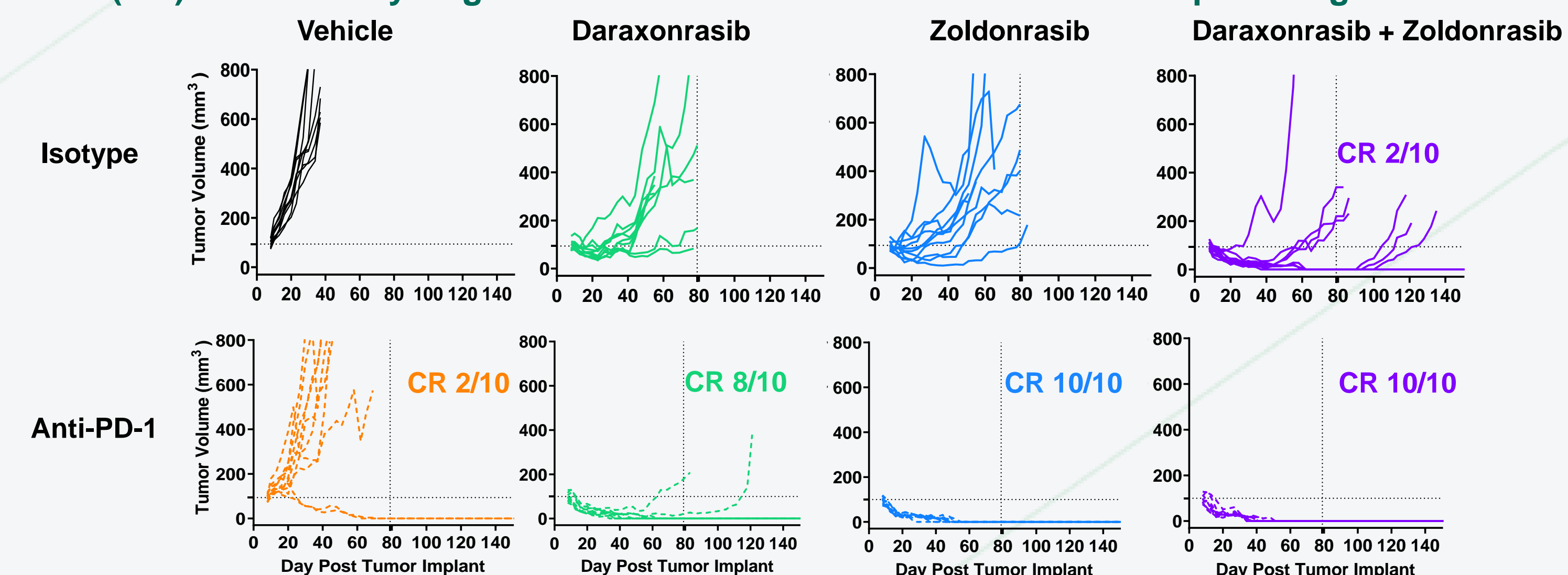
eCMT93 implanted subcutaneously in C57BL/6 mice, treated for 72 days with vehicle, daraxonrasib at 25 mg/kg PO QD, zoldonrasib at 100 mg/kg PO QD, or daraxonrasib 25mg/kg PO QD and zoldonrasib at 100 mg/kg PO QD. Progression defined as tumor doubling from baseline. Horizontal dotted line indicates tumor size at which randomization and dosing started (100mm³). Vertical dotted line indicates treatment stop.

### RAS(ON) Inhibitors Modulate the Tumor Microenvironment in Favor of Anti-Tumor Immunity



(Left) Histological quantification of CD8+ T cells in eCMT93 tumors at 24 hours post 4 days of treatment with RM-044 KRAS G12D mutant-selective inhibitor, RMC-7977 RAS multi-selective inhibitor, or the combination. Black arrows indicate CD8+ T cells in the tumor core. Unpaired T test (\*\*p<0.01). (Right) Transcriptomic analysis of whole tumor tissue at 24 hours post 4 days of treatment with RM-044, RMC-7977, or the combination. Gene set analysis evaluated by Rosalind.

### RAS(ON) Inhibitors Synergize with anti-PD-1 To Achieve Durable Complete Regressions



eCMT93 implanted subcutaneously in C57BL/6 mice, treated for 72 days with vehicle, daraxonrasib at 25 mg/kg PO QD +/- anti-PD-1 10 mg/kg IP BIW, zoldonrasib at 100 mg/kg PO QD +/- anti-PD-1 10 mg/kg IP BIW, or daraxonrasib 25 mg/kg PO QD and zoldonrasib at 100 mg/kg PO QD +/- anti-PD-1 10 mg/kg IP BIW. Progression defined as tumor doubling from baseline. Horizontal dotted line indicates tumor size at which randomization and dosing started (100mm³). Vertical dotted line indicates treatment stop. CR = Complete regression.

## Conclusion

- We have developed a RAS-dependent syngeneic model, representative of invasive MSS CRC
- Daraxonrasib, a RAS(ON) multi-selective inhibitor, and zoldonrasib, a KRAS G12D mutant-selective inhibitor, induced transient tumor regressions, and the RAS(ON) doublet increased the depth and durability of response *in vivo*
- RAS(ON) Inhibitors increased tumor T cell infiltration significantly, potentiated T cell function by enhancing cytokine signaling, and upregulated the antigen presentation machinery
- RAS(ON) inhibitors synergize with anti-PD-1 to achieve durable complete regressions
- The eCMT93 model highlights the potential benefit of the RAS(ON) doublet therapy and showcases how deep RAS pathway inhibition may transform the tumor microenvironment and sensitize colorectal tumors to anti-PD-1

