

THE KRAS^{G12D}(ON) COVALENT TRICOMPLEX INHIBITOR RM-044 INCREASES TUMOR ANTIGEN PRESENTATION AND TCR REPERTOIRE DIVERSITY TO PROMOTE ANTI-TUMOR IMMUNITY IN A PRECLINICAL MODEL OF KRAS^{G12D} MUTANT CANCER

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Background KRAS^{G12D} mutations most commonly occur in pancreatic and colorectal cancers, which typically do not respond to immunotherapy. Lack of response to anti-PD-1 is often associated with a low neoantigen burden and/or low antigen presentation by tumors, resulting in paucity of tumor-specific T cells.¹

We previously showed that mutant-selective, covalent, active-state KRAS^{G12D} (KRAS^{G12D}(ON)) inhibitors transform the tumor microenvironment in favor of anti-tumor immunity, leading to complete tumor regressions and immunological memory in a KRAS^{G12D} preclinical cancer model where immunotherapy exhibits limited activity.²

Here, we investigate the impact of RM-044, a KRAS^{G12D}(ON) preclinical tool compound, representative of the clinical candidate RMC-9805, on tumor antigen presentation and the tumor-specific TCR repertoire *in vivo*.

Methods Flow cytometry, NanoString, and TCR sequencing were used to evaluate the impact of KRAS^{G12D}(ON) inhibition on antigen presentation and the anti-tumor T cell response by conducting a longitudinal assessment of intratumoral and peripheral TCR repertoires *in vivo* in the KRAS^{G12D} CT26 tumor model. CT26 is a representative model of immunogenic KRAS^{G12D} mutant cancer with limited response to anti-PD-1.

Results Treatment of KRAS^{G12D}-mutant murine and human cancer cells with a KRAS^{G12D}(ON) inhibitor increased cell surface expression of MHC class I proteins *in vitro*. Additionally, transcriptomic analysis showed significant upregulation of antigen processing and presentation machinery and lymphocyte related genes in CT26 tumors treated with RM-044 for 8 days *in vivo*, suggesting increased tumor antigen presentation and recognition.

Analysis of the intratumoral TCR repertoire following 8-days of treatment with RM-044 demonstrated that RAS(ON) inhibition in tumor cells leads to a profound increase of both T cell frequency and TCR repertoire richness in the tumor.

Longitudinal analysis of blood samples during treatment with a RAS(ON) inhibitor revealed a modulation of the peripheral TCR repertoire, with significant treatment-induced expansion of newly detectable, tumor-associated T cell clones over time. Richness of the intratumoral TCR repertoire was inversely correlated with the tumor volume, with greater TCR diversity in mice showing tumor regression.

Conclusions Direct inhibition of RAS signaling by RM-044 in tumor cells leads to increased anti-tumor immune response, including expansion of treatment-induced T cell clones that can be detected in both the tumor compartment and the blood, highlighting the interplay between RAS signaling in tumors and the immune system.

These preclinical data suggest that the potential therapeutic impact of mutant KRAS inhibitors, particularly KRAS(ON) inhibitors, may be complemented by, and possibly enhanced by, combination with immunologic therapies including neoantigen targeting technologies.

REFERENCES

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