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The KRAS^{G12C} Inhibitor MRTX849 Reconditions the Tumor Immune Microenvironment and Leads to Durable Complete Responses in Combination with Anti-PD-1 Therapy in a Syngeneic Mouse Model

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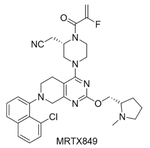
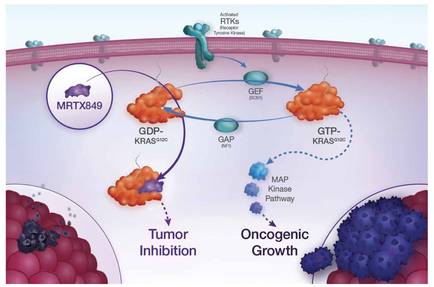
ABSTRACT

After decades of research, covalent inhibitors targeting KRAS^{G12C} are entering clinical trials. KRAS^{G12C} mutations are found in 14% of non-small cell lung cancer (NSCLC) adenocarcinoma as well as several other cancer types at lower frequencies. KRAS^{G12C} mutations are smoking-associated transversion mutations that are associated with a relatively high total mutation burden (TMB) and PD-L1 positivity. Although pembrolizumab is clinically active in KRAS-mutant NSCLC, response rates remain modest and strategies to augment the clinical activity of checkpoint inhibitor (CPI) therapy is an area of major clinical investigation. MRTX849 was identified as a potent, selective, and covalent KRAS^{G12C} inhibitor presently in clinical development. To evaluate the potential of MRTX849 to augment CPI therapy, the impact of MRTX849 on immune signaling molecules and response to anti-PD-1 therapy was evaluated. In a panel of human xenograft models, MRTX849 increased MHC Class I protein expression and decreased RNA and circulating protein expression of signaling molecules including VEGFA, CXCL1 and CXCL8, demonstrating MRTX849 modulates factors that are implicated in antigen presentation or an immunosuppressive tumor microenvironment through a tumor cell-mediated mechanism. In a CT26 syngeneic mouse model engineered to express KRAS^{G12C}, MRTX849 decreased intratumoral immunosuppressive myeloid-derived suppressor cell (MDSC) populations and increased immune-enhancing M1-polarized macrophages, dendritic cells, CD4+ and CD8+ T cell populations when administered as a single agent. These effects were also observed in tumors from MRTX849 plus anti-PD-1 treated mice. In efficacy studies, MRTX849 plus anti-PD-1 antibody treatment resulted in durable, complete responses in six out of ten animals whereas all but one of the tumors eventually progressed in the anti-PD-1 or MRTX849 single agent treatment groups. To further interrogate the mechanism of response to the combination, the six mice with complete responses were re-implanted with CT26KRAS^{G12C} cell inoculum and tumors failed to form, demonstrating combination-treated mice developed durable anti-tumor immunity. In summary, these data demonstrate MRTX849 in combination with anti-PD-1 therapy leads to durable complete regressions through an immune-mediated anti-tumor response.

MRTX849 is a Novel, Covalent KRAS^{G12C} Inhibitor



MRTX849 is a KRAS^{G12C} inhibitor that covalently binds mutant Cys 12 in inactive GDP-loaded KRAS^{G12C}, blocks KRAS signaling and inhibits tumor growth.



BACKGROUND

- KRAS G12C mutations are transversion mutations, which are linked to the pathogenesis of smoking-related lung adenocarcinoma and are enriched for relatively high tumor mutation burden and PD-L1 expression, factors which increase likelihood of clinical benefit from checkpoint inhibitor therapy (CIT) (Campbell-2016, Goodman-2017).
- Checkpoint inhibitors that block Programmed Cell Death 1 (PD-1)/Programmed Cell Death 1 Ligand 1 (PD-L1) signaling have demonstrated clinical activity in NSCLC, including in KRAS-mutant NSCLC (Borghaei, 2015; Garon, 2015).
- However, a large percentage of patients do not respond or develop resistance to checkpoint inhibitor therapy.
- Several mechanisms responsible for checkpoint inhibitor resistance mediated by oncogenic KRAS have been hypothesized including silencing of antigen presentation and development of an immune suppressed tumor microenvironment (Liao, 2019; Sunaga N, 2012; Coehlo, 2017; Kortlever, 2017; Ancrile, 2008; El-Jawhari, 2013).
- KRAS inhibition may therefore reverse the immunosuppressive tumor microenvironment and sensitize tumors to checkpoint inhibitor therapy.

MRTX849 Modifies Tumor Cell-Intrinsic Factors that Regulate Antigen Presentation and an Immunosuppressive Tumor Microenvironment

Fig. 1A: RNA Expression of Secreted Immune-Modulating Genes are Down Regulated by MRTX849 in Human Xenografts

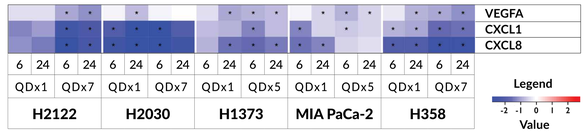


Fig. 1B: Plasma Concentration of Secreted Immune-Modulating Proteins are Decreased in Human Xenograft-Bearing Mice Treated with MRTX849

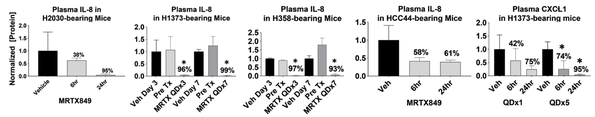
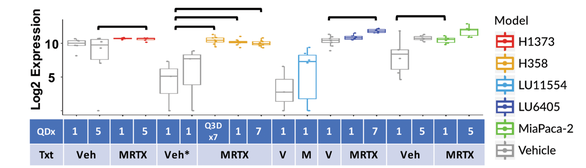


Fig. 1C: MHC Class 1 Protein Expression is Up Regulated by MRTX849 in Human Xenografts



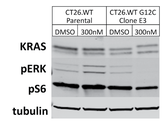
1A. RNA was collected from human xenograft models grown in immunocompromised mice and dosed orally with vehicle or 100 mg/kg MRTX849 once (QDx1) or daily for 5 or 7 days (QDx5 or QDx7; n=3 per treatment condition). Tumors were collected after 6 or 24 hours. RNAseq data was generated and log2 fold changes in expression in MRTX849 versus vehicle-treated xenografts was determined.

1B. Plasma was collected from control and MRTX849-treated (100 mg/kg) mice dosed orally for the study duration as indicated (n=3 per treatment group). Protein concentration was determined using a QuantiGlo ELISA kit (R + D Systems, Human CXCL8 (IL-8) #Q8000B; Human CXCL1 # DGR00B) and data were normalized to concentration in control mice and normalized to tumor volume (except for H1373 CXCL1). Plasma was collected at 24 hrs post last dose for H358 and H1373 and as indicated for other models. "*" indicates Student's test p-value < 0.05 MRTX849 compared to Vehicle or pretreatment. Veh = vehicle; Pre Tx = pretreatment.

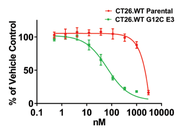
1C. Protein lysates were generated from human xenograft models grown in immunocompromised mice dosed orally with vehicle or 100 mg/kg MRTX849 (n=3 per treatment group). MHC Class I protein expression was determined using a reverse phase protein array (RPPA) and log2 expression was determined. Vehicle samples are shown in grey to the left of the treated sample. Brackets indicates statistical significance in expression between the groups by Student's t-test, p-value < 0.05. "*" - two different tumor volumes (300 and 500 mm^3) were analyzed in the H358 study.

CRISPR/Cas9-Engineered CT26$^{KRAS\ G12C}$ Cell Line is Sensitive to MRTX849 Treatment

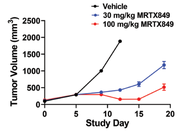
2A. **MRTX849 24 hour Treatment**



2B. **MRTX849 3 Day Proliferation**



2C. **CT26.WT G12C Clone E3 TGI *nu/nu* Strain**



2D. **CT26.WT G12C Clone E3 TGI *balb/c* Strain**

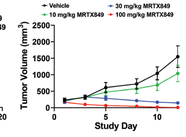


Fig. 2A-2D

2A. Parental CT26.WT and G12C engineered CT26.WT Clone E3 were incubated with 300 nM of MRTX849 for 24 hours and then probed via western blot with KRAS, pERK, p6 and the loading control alpha tubulin.

2B. 2000 Parental and Clone E3 cells per well were seeded and treated with MRTX849. On day 3, cells were treated with cell titer glo and IC50 determined by graphpad.

2C. 1e6 CT26.WT Clone E3 cells were inoculated into female, nu/nu athymic mice. ~100mm3 tumors were treated PO, daily with vehicle, 30 mg/kg and 100 mg/kg of MRTX849.

2D. 1e6 CT26.WT Clone E3 cells inoculated into female, balbc mice. ~150mm3 tumors were treated PO, daily with vehicle, 10 mg/kg, 30 mg/kg and 100 mg/kg of MRTX849

MRTX849 Treatment Reconditions the Tumor Immune Microenvironment by Modulating Key Immune Cell Types

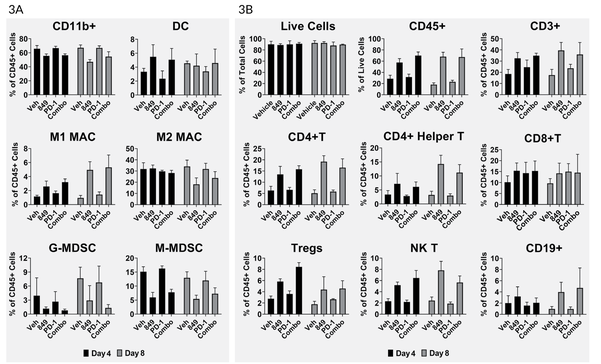


Fig. 3A-4B

MRTX849 at 100 mg/kg, rat IgG2a Isotype Control antibody (Bio X cell lot # 686318F1B) at 10 mg/kg, and mouse PD-1 antibody (Bio X cell clone 29F.1A12) at 10 mg/kg were administered orally, daily (MRTX849) and intraperitoneally, Q3D (PD-1 and Isotype Control) to mice bearing established, subcutaneous CT26 KRAS G12C tumors. Tumors were shipped to MI Bioresearch overnight on wet ice, the tissues were stored in Lifor solution.

3A. Represents the myeloid panel.

3B. Represents the leukocyte panel. The cells were processed and stained according to MI BioResearch's protocol and acquired on the Attune NxT Acoustic Focusing Cytometer.

MRTX849 and Anti-PD-1 Treatment Leads to Durable Complete Responses in the Majority of Treated Mice

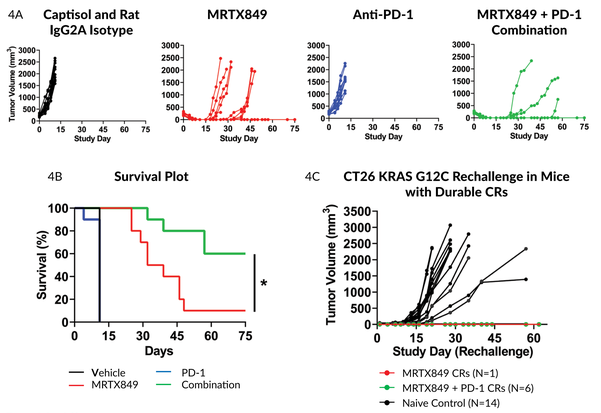


Fig. 4A-4C

4A. MRTX849 at 100 mg/kg (lot # EW5243-1094-P1), rat IgG2a Isotype Control antibody (Bio X cell lot # 686318F1B) at 10 mg/kg, and mouse PD-1 antibody (Bio X cell clone 29F.1A12) at 10 mg/kg were administered orally, daily (MRTX849) and intraperitoneally, Q3D x 3 doses (PD-1 and Isotype Control) to mice bearing established, subcutaneous CT26 KRAS G12C tumor grafts on the right flank with an average starting tumor volume of ~220mm3. Data are shown as individual tumor volumes for the efficacy and re-challenge studies.

4B. Survival in the combination treated cohort was statistically significant compared to the MRTX849 treated cohort by the Mantel-Cox test. * denotes adjusted P value < 0.05.

4C. Data in the rechallenge plot depict the individual tumor volumes from the re-implant of 1e6 CT26 KRAS G12C cells into the left flank in the one MRTX849-treated mouse and the six combination-treated mice with durable complete responses (CRs) of the tumors from the first implant. A cohort of naïve mice were also implanted as a control and tumors developed normally in thirteen of fourteen mice.

T Cell Frequency and Diversity are Increased in Response to MRTX849 and PD-1 Combination Treated CT26.WT Clone E3 Tumor Bearing Mice

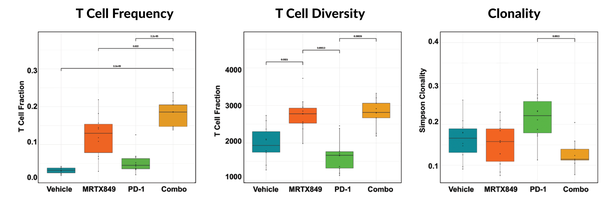


Fig. 5

MRTX849 at 100 mg/kg (lot # EW5243-1094-P1), rat IgG2a Isotype Control antibody (Bio X cell lot # 686318F1B) at 10 mg/kg, and mouse PD-1 antibody (Bio X cell clone 29F.1A12) at 10 mg/kg were administered orally, daily (MRTX849) and intraperitoneally, Q3D x 3 doses (PD-1 and Isotype Control) to mice bearing established, subcutaneous CT26 KRAS G12C tumor grafts on the right flank for a total of 8 days. Tumors were then sent to Adaptive Biotechnologies for processing. Horizontal bars represent significance with a P value < 0.05.

CONCLUSIONS

- MRTX849 is a novel, mutant-selective, covalent KRAS^{G12C} inhibitor in clinical development with strong rationale for combining with checkpoint inhibitor therapy.
- MRTX849 treatment alters tumor RNA and protein expression of factors implicated in presentation of tumor antigens and/or mediating an immunosuppressive tumor microenvironment in multiple KRAS^{G12C}-mutant human xenografts.
- A CT26 model engineered to express KRAS^{G12C} was dependent on KRAS for tumor cell growth and survival and was sensitive to MRTX849 treatment in vitro and in vivo.
- MRTX849 alone and in combination with an anti-PD-1 antibody decreased intra-tumoral immune-suppressive M2-polarized macrophages, M- and G-MDSCs and increased immune promoting M1-polarized macrophages, dendritic cells, and CD4 and NK T cells in CT26 KRAS^{G12C} tumors.
- MRTX849 plus anti-PD-1 leads to durable complete responses in the majority of mice and a survival advantage relative to either single agent therapy.
- MRTX849 / PD-1 combination-treated mice with durable, complete responses did not form tumors when re-challenged with CT26 KRAS^{G12C} cells, whereas naïve mice developed tumors normally, demonstrating the combination resulted in an anti-tumor adaptive immune response.

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WE WOULD LIKE TO THANK:

- MI Bioresearch for the tumor processing and FACS analysis
- Adaptive Biotechnologies for the TCRB diversity and clonality data set

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