Inhibiting CD73 and KRAS enhances the effect of anti-PD-1 therapy in a KRAS^{G12C}/TP53$^{R172H/+}$ pancreatic cancer model

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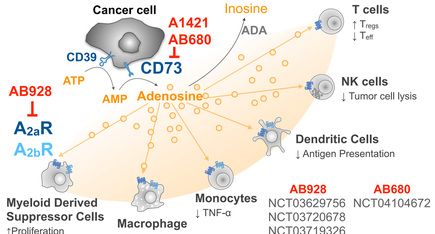
INTRODUCTION

CD73 catalyzes the extracellular generation of adenosine (ADO) from adenosine monophosphate (AMP). High levels of ADO, found in the tumor microenvironment (TME), have been shown to suppress immune responses and curtail T cell activation in the presence of anti-PD-1/PD-L1 blocking antibodies. These immunosuppressive effects can be counteracted by CD73 inhibitors (A1421 and AB680) or by a dual ADO receptor (A_{2a}R/A_{2b}R) antagonist (AB928).

Fig. 1. Adenosine (ADO) production and immunosuppressive effects



METHODS

- Linear models were used to evaluate the ability of 299 pan-cancer consensus oncogenic drivers to predict CD73 expression independent of tumor type in the TCGA dataset.
- Changes in gene expression induced by KRAS inhibition were determined by RNAseq.
- Metabolic and proteomic alterations induced by KRAS inhibition were determined by LC-MS.
- C57BL/6J mice bearing established KP4662-G12C tumors (>150 mm^3) were treated with A1421 (CD73i; 30 mg/kg/day, s.c.) and anti-PD1 (Clone RMP 1-14; 10 mg/kg; twice per week, i.p) ± MRTX-1257 (KRASi, 100 mg/kg/day, p.o.). Treatment efficacy was monitored using micro computed tomography (mCT).
- A1412 and MRTX-1257 were provided by Arcus Biosciences.

RESULTS

KRAS mutations, of which 60% were derived from pancreatic ductal adenocarcinoma (PDAC) samples, significantly up-regulated CD73 expression and resulted in worsening prognosis. Direct KRAS inhibition reduced but did not abolish CD73 and A_{2a}R/A_{2b}R expression in multiple PDAC models. Metabolic analyses indicated that KRAS inhibition increased AMP and ADO levels. KRAS inhibition in PDAC models induced gene expression changes consistent with increased tumor immunogencity. In a murine model of pancreatic cancer bearing the KRAS^{G12C} mutation, co-administration of a CD73 inhibitor with anti-PD-1 in established tumors resulted in significant tumor growth retardation, comparable to KRAS G12C inhibition alone. Durable tumor regressions was observed when mice were treated with the triple combination therapy. These data support the rationale for the clinical development of modulators of immunosuppressive adenosine in pancreatic cancer.

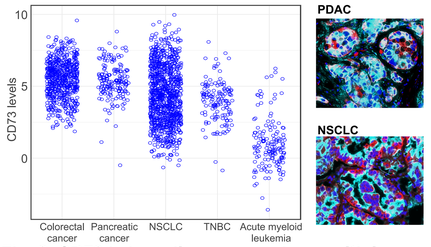


Fig. 2. CD73 is broadly expressed on multiple tumor types

CD73 is broadly expressed on multiple tumor types. CD73 IHC on formalin fixed paraffin embedded samples using primary CD73 antibody (Cell Signaling, D7F9A) was detected by DAB chromogen and quantified using QuPath Software. NSCLC: non-small cell lung cancer. TNBC: triple negative breast cancer

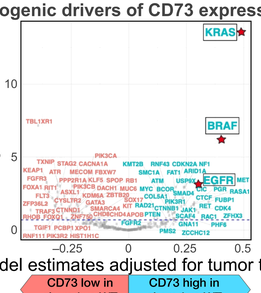


Fig. 3. Oncogenic drivers of CD73 expression

The linear model evaluated 299 pan-cancer consensus oncogenic drivers to predict CD73 expression independent of tumor type in the TCGA dataset.

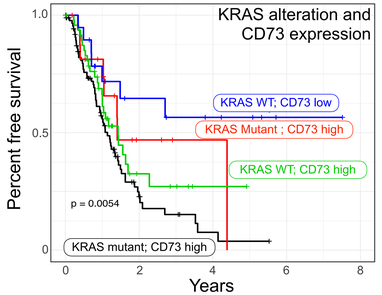
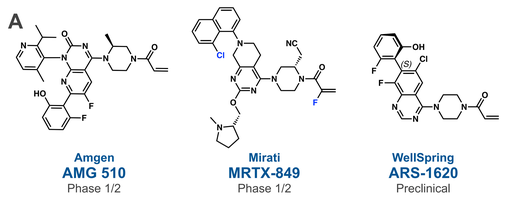
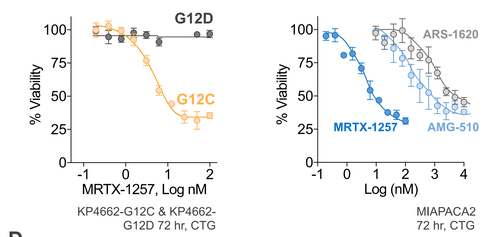


Fig. 4. High CD73 is associated with poor overall survival in pancreatic cancer

Kaplan-Meier curve shows the impact of CD73 expression on the altered status of KRAS in PDAC patients.





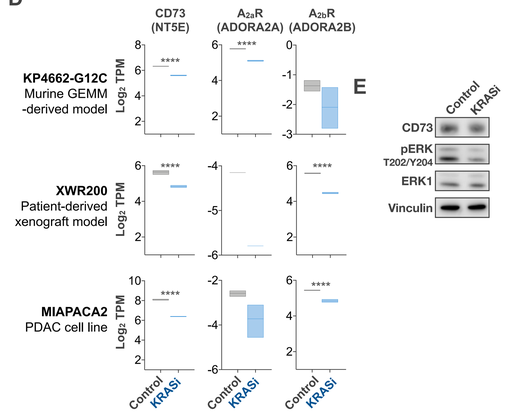


Fig. 5. Mutant KRAS inhibitors reduce but do not abolish CD73 and ADO receptor expression in PDAC models

(A) KRAS G12C can be targeted by new allele-specific inhibitors that are currently advancing in clinical trials. **(B)** MRTX-1257 significantly impacts the growth of KP4662-G12C (KRAS G12C/TP53$^{-/-}$) cells while the parental G12D cells KP4662 cells are not affected. **(C)** dose-response curves of three KRAS inhibitors against MIAPACA2 PDAC cells. Cell viability was measured by CellTiterGlo$^{(R)}$ assay after 72 hr treatment. **(D)** Effects of KRAS inhibition on CD73, A_{2a}R and A_{2b}R genes in three KRAS G12C pancreatic cancer models. 5 μM ARS-1620 for 72 hr (mean ± SD, n = 3, FDR ≤ 1%). * P < 0.05; ** P < 0.001; **** P < 0.0001. **(E)** Western blot analysis of CD73, pERK (T202/Y204), total ERK and Vinculin (loading control) levels following 24 hr treatment of MIAPACA2 cells with ARS-1620 (KRASi, 5 μM).

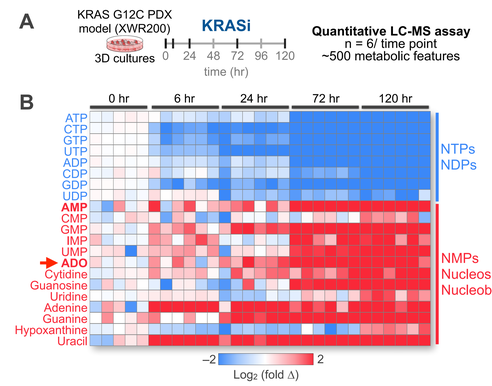


Fig. 6. KRAS inhibition impacts nucleotide biosynthesis and increases ADO levels in a KRAS G12C PDX model

(A) Experimental design to analyze metabolic alterations induced by KRAS inhibition in XWR200, a KRAS G12C PDX model derived from a PDAC patient who underwent surgery at UCLA. Dark-colored ticks indicate selected time points for quantitative metabolomic LC-MS analysis. **(B)** Fold change (log$_2$) in nucleotides. All nucleotides exhibit significance, FDR < 1%, one-way ANOVA.

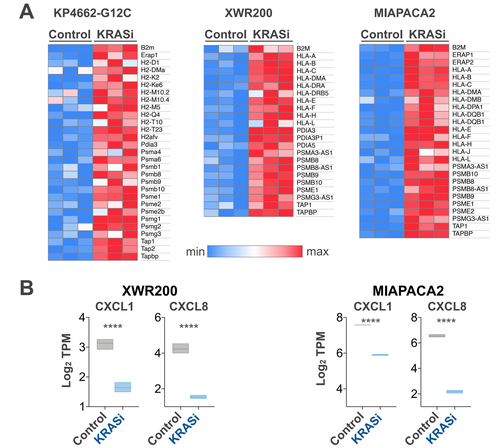
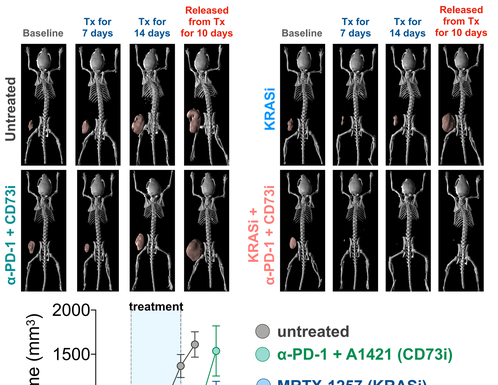


Fig. 7. KRAS inhibition in PDAC models induces gene expression changes consistent with increased tumor immunogenicity

(A) Effects of KRAS inhibition on the expression of genes involved in antigen presentation. **(B)** KRAS inhibition reduces the expression of immunosuppressive chemokines in XWR200 and MIAPACA2 PDAC models. (mean ± SD, n = 3). PDAC models were treated with 5 μM ARS-1620 for 72 hr in anchorage independent cultures and then subjected to RNAseq.



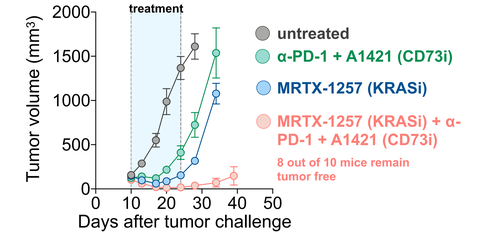


Fig. 8. Co-targeting KRAS and CD73 enhances immunotherapy in a KRAS^{G12C}/TP53$^{R172H/+}$ PDAC model

C57BL/6J mice (N = 10/group) bearing established KP4662-G12C tumors (>150 mm^3) were treated with A1421 (CD73i; 30 mg/kg/day, s.c.) and anti-PD1 (Clone RMP 1-14; 10 mg/kg; twice a week, i.p) ± MRTX-1257 (KRASi, 100 mg/kg/day, p.o.). Mice were treated for two weeks starting from day 10 post-tumor cell challenge. Treatment efficacy was monitored in a blinded manner using micro computed tomography (mCT, Genisys PET/CT G8; Sofie Biosciences).

CONCLUSIONS

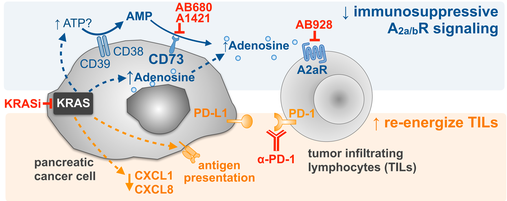


Fig. 9. Model for targeting immunosuppressive ADO signaling in KRAS G12C driven malignancies

Here we show that direct inhibition of mutant KRAS in pancreatic cancer models yields complex immunomodulatory effects. While antigen presentation pathways are transcriptionally upregulated and the expression of immunosuppressive chemokine is reduced, KRAS inhibition also reprograms nucleotide metabolism leading to elevated levels of ADO. These findings suggest that co-targeting mutant KRAS and adenosine signaling may enhance immunotherapy against pancreatic cancer and potentially other KRAS driven malignancies.