Poster No. 1942

Developing KRAS G12C Inhibitor-Resistant Tumor Models for Efficacy Evaluation of Next-Generation Anticancer Therapies

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Introduction

Targeted therapy has made remarkable progress against a broad spectrum of cancers and revolutionized the treatment of cancer. KRAS is one of the most frequent mutated oncogenes and has been recognized as undruggable for many years. AMG510, the first therapy to directly target KRAS, was approved by the FDA to treat non-small cell lung cancer (NSCLC) bearing KRAS G12C mutation. However, the emergence of resistance in patients remains a challenge and limits its clinical benefits, which calls for next-generation targeted therapy or combination strategies to overcome the resistance to KRAS G12C inhibitors. A variety of secondary mutations in KRAS attributing to the resistance have been identified, which requires robust *in vitro* and *in vivo* preclinical models to validate potential therapeutics targeting these mutations. Therefore, we generated a panel of KRAS G12C inhibitor-resistant tumor models to facilitate the development of possible strategies to overcome such resistance.

Methods

- First, a secondary KRAS mutation, including H95D, H95Q, H95R, Q61H and R68S, was introduced using CRISPR/Cas9 in MIA PaCa-2 cell line, which harbors a homozygous KRAS G12C mutation, in addition to Y96D, Y96C and Y96S previously published by us. Point mutation knock-in was validated by sanger sequencing, and cell identity was confirmed by SNP assay.
- Then, the parental and mutated cells were treated with either AMG510 and MRTX849. In addition, Y96D mutated cells were treated with BI-3406（SOS1 inhibitor) and Trametinib (MEK inhibitor) alone or in combination. Cell viability was measured by CellTiter-Glo. RAS–MAPK pathway activity was assessed by Western Blot.
- *In vivo* efficacy of AMG510, MRTX849, BI-3406 and Trametinib were evaluated in the Y96D mutated MIA PaCa-2 subcutaneous xenograft.

Results

- Homozygous secondary point mutation knock-in in MIA PaCa-2 cells was confirmed by sanger sequencing (Figure 1A). Similar growth rate was observed in selected clones compared to the parental line (Figure 1B).
- Similar to Y96D mutation previously published, R68S mutation was highly resistant to both KRAS G12C inhibitors, with IC50 increased more than 100 fold, whereas H95D, H95Q and H95R mutation was more resistant to MRTX849, while Q61H had a minimal effect on either one of the inhibitors (Figure 2A). Consistent with the effects on cell viability, persistent phosphorylated ERK (p-ERK) and p-RSK levels were also observed in KRAS G12C/Y96D and G12C/R68S expressing cells even at high concentrations of MRTX849, indicating sustained RAS-MAPK activity (Figure 2B).
- In addition, cells expressing KRAS G12C/Y96D were also resistant to AMG510 and MRTX849 in *in vivo* study, whereas combination of BI-3406 (single treatment: TGI of 27%) and Trametinib (single treatment: TGI of 76%) suppressed tumor growth significantly with TGI of 93% on day 17 after randomization compared to control group ($p<0.001$). Also, the combination showed significant improvement compared to BI-3406 single treatment ($p<0.001$) whereas no significant improvement was observed compared to Trametinib single treatment ($p>0.05$). (Figure 3).

References

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Figure 1. Generation point mutation in MIA PaCa-2 via CRISPR/Cas9

1A. Genomic DNA was extracted from MIA PaCa-2 and engineered clones. The target region was PCR-amplified followed by Sanger sequencing. **1B**. Cells were seeded in 96-well plate at 2000 cells per well and cell growth kinetics were monitored on day 1 to day 3 by CellTiter-Glo. N=3, error bars=standard deviation

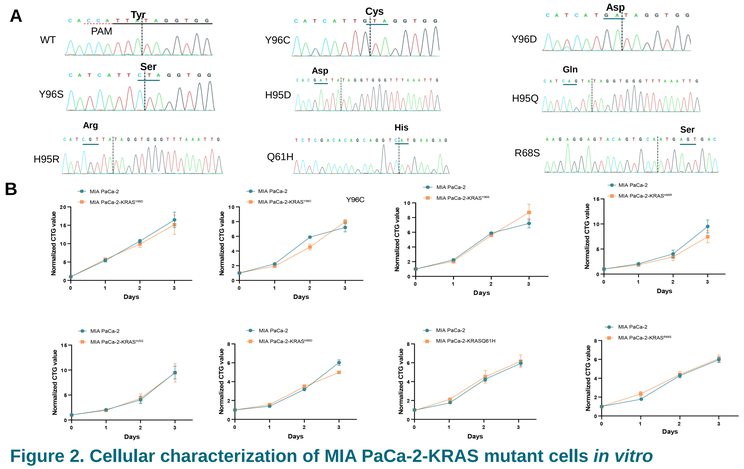
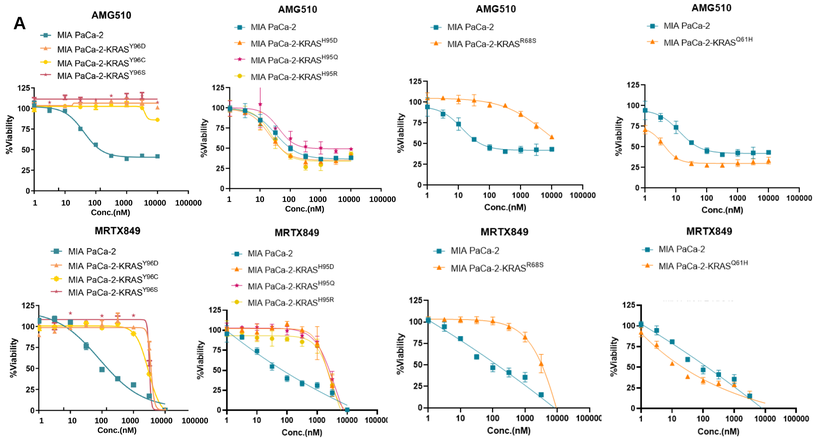


Figure 2. Cellular characterization of MIA PaCa-2-KRAS mutant cells *in vitro*



Cell Line Name	*Kras* Mutation	AMG510		MRTX849	
		Response	IC_{50} (μM)	Response	IC_{50} (μM)
MIA PaCa-2	G12C	Sensitive	0.1628	Sensitive	0.067
MIA PaCa-2-KRAS^{Y96D}	G12C+Y96D	Resistant	>10	Partial response	4.1657
MIA PaCa-2-KRAS^{Y96C}	G12C+Y96C	Resistant	>10	Partial response	5.0693
MIA PaCa-2-KRAS^{Y96S}	G12C+Y96S	Resistant	>10	Partial response	7.5189
MIA PaCa-2-KRAS^{H95D}	G12C+H95D	Sensitive	0.0792	Partial response	2.4613
MIA PaCa-2-KRAS^{H95Q}	G12C+H95Q	Sensitive	0.1795	Partial response	2.8872
MIA PaCa-2-KRAS^{H95R}	G12C+H95R	Sensitive	0.08	Partial response	2.6205
MIA PaCa-2-KRAS^{R68S}	G12C+R68S	Resistant	>10	Partial response	3.6237
MIA PaCa-2-KRAS^{Q61H}	G12C+Q61H	Sensitive	0.0052	Sensitive	0.0324

Note: IC_{50} <1 μM sensitive, 1uM<IC_{50}<10 μM partial response, IC_{50}>10 μM resistant

2A. Cell viability assays performed in MIA PaCa-2 and MIA PaCa-2 mutant cell lines. Cells were treated with indicated drugs and the viabilities were measured with CellTiter-Glo. IC_{50} was summarized in the table. N=3, error bars=standard deviation. **2B.** Western blot analysis was performed after treating MIA PaCa-2 cells or MIA PaCa-2 mutant cell with vehicle or indicated concentration of AMG510 or MRTX849 for 4 hours.

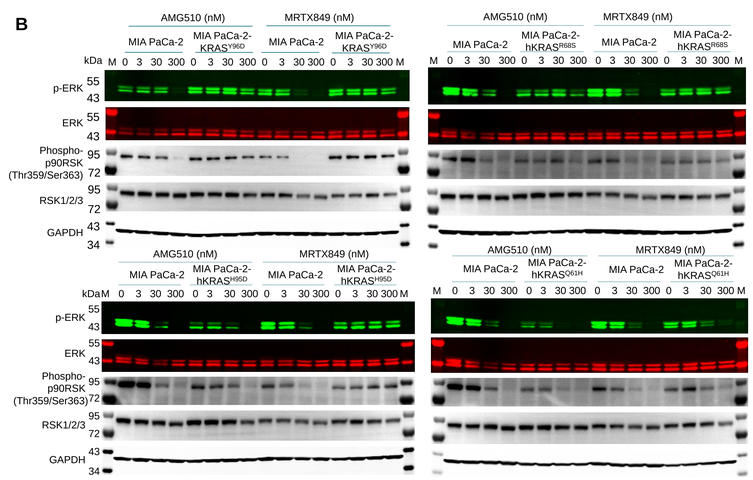
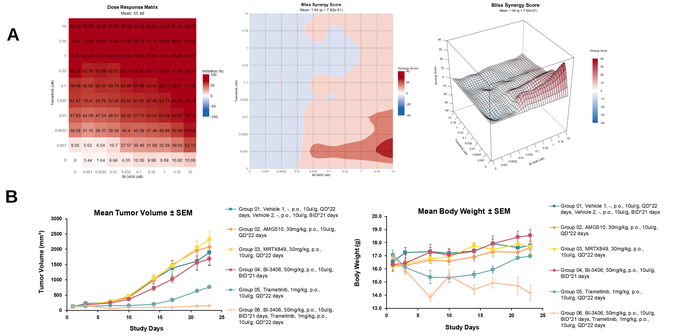


Figure 3. Combination treatment for MIA Paca-2-KRAS mutant cell *in vitro* and *in vivo*

3A. Synergistic effect of BI3406 and trametinib on MIA Paca-2 hKRAS^{Y96D} *in vitro* was analyzed by CrownSyn^{TM}. Dose response matrix shows the inhibition rate in the drug treatment (Fig 3A left). Synergy scores calculated Bliss independence model. A score higher than 5 indicates synergy, and a score less than -5 indicates antagonism (Fig 3A middle and right). **3B.** MIA PaCa-2-KRAS^{Y96D} was inoculated into BALB/c nude mice and tumor volume or body weight was measured every 3 or 4 days. N=6, error bars=standard error mean.



Conclusion

CRISPR/Cas9 engineered second site KRAS mutations in cells harboring KRAS G12C mutation displayed a differentially resistant profile to KRAS G12C inhibitors. Thus, H95D/Q/R, R68S and Y96D can be used as preclinical inhibitor-resistant models to evaluate clinical strategies to overcome resistance to KRAS-targeted therapies.

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