#1888 Unveiling mechanisms of MRTX1133 resistance in PDAC models

Qin Li, Feifei Fan, Xun Huang, Xuebing Yan, Xiaoxu Jiang, Wen Zhang, Qian Zhang, Yan Lei, Jian Xiang, Zhixiang Zhang
In Vivo Pharmacology Unit, WuXi Biology, WuXi AppTec, China

WuXi AppTec

WuXi Biology

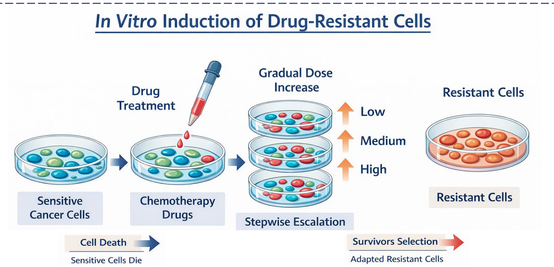
Introduction

Recent years have witnessed significant advances in the development of therapeutics targeting KRAS G12D in cancer patients. However, the emergence of drug resistance remains a formidable clinical challenge, and the underlying mechanisms remain largely unknown. To investigate these mechanisms, we established resistant cell lines through stepwise escalation of MRTX1133, the first covalent inhibitor of KRAS G12D to enter clinical evaluation, in KRAS G12D–mutant models.

These resistant cells maintain stable phenotypes to MRTX-1133 both *in vitro* and *in vivo*, and exhibited broad cross-resistance to other KRAS G12D inhibitors, such as RMC9805 and HRS-4642, with partial resistance observed toward the pan-RAS inhibitor RMC6236.Bioinformatic analysis of both resistant models revealed a spectrum of convergent adaptive mechanisms, including: (1) altered oncogenic signaling networks, (2) upregulation of cell cycle and drug efflux regulators, and (3) epithelial–mesenchymal transition (EMT). Interestingly, the MRTX1133-R-GP2D model acquired secondary KRAS mutations (specifically H95Q, Y96H, Y96N, and D92N), indicating on-target resistance. In a contrast, the MRTX1133-R-KPC model exhibited chromosome 5 amplifications encompassing key metabolic and detoxification genes (notably CYP51 and CYP3A), accompanied by tumor microenvironment (TME) reprogramming and immune evasion.

Together, these findings reveal comprehensive mechanisms of resistance to KRAS G12D inhibition. The established resistant models serve as physiologically relevant platforms for biomarker discovery and the development of rational combination therapies. Using these models, functional studies demonstrated that targeting core vulnerabilities—including the cell cycle, DNA damage repair, compensatory signaling pathways, metabolic modulators may overcome resistance phenotype.

Experimental Design



GP2D or KPC cells were continuously exposed to stepwise increasing concentrations of MRTX1133. The resistant phenotype was confirmed by cell viability assays and xenograft studies. The image was created with BioRender.

Results

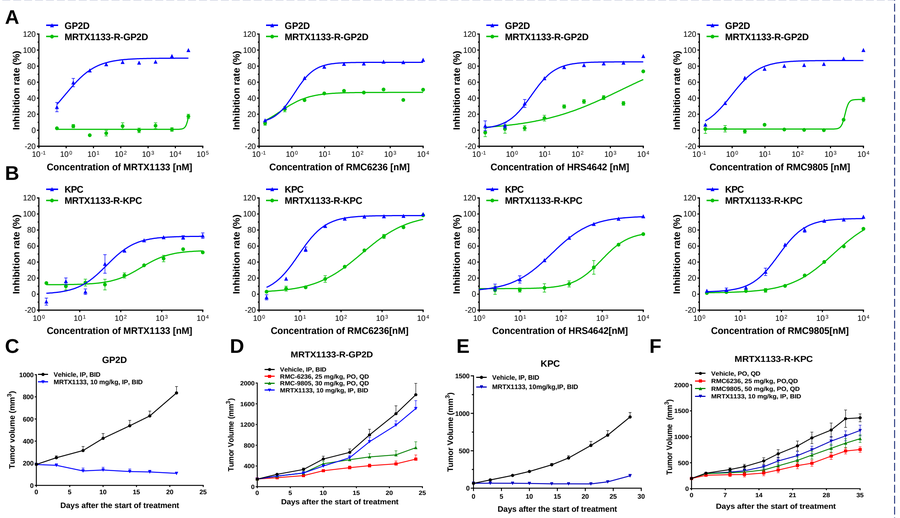
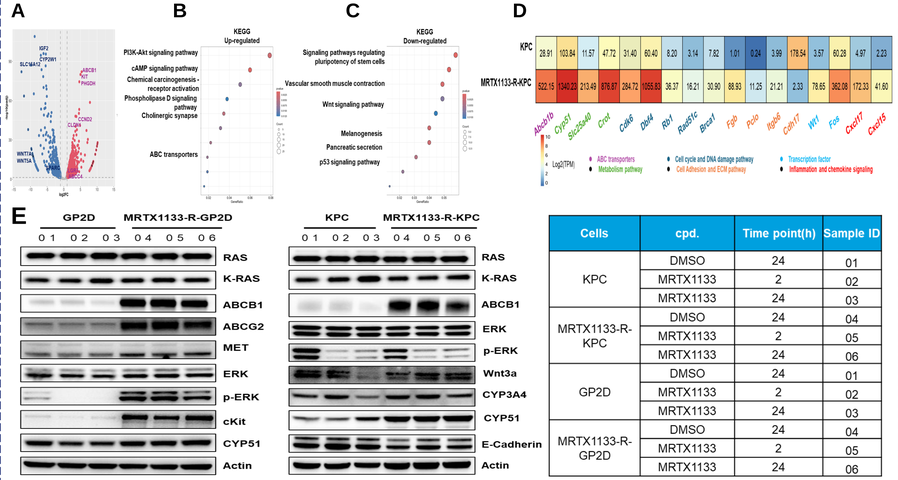


Figure 1. *In vitro* and *in vivo* validation of MRTX1133-induced resistant GP2D and KPC cells. (A) *In vitro* validation of GP2D and MRTX1133-R-GP2D cells treated with MRTX1133, RMC6236, HRS4642, and RMC9805. (B) *In vitro* validation of KPC and MRTX1133-R-KPC cells treated with MRTX1133, RMC6236, HRS4642, and RMC9805. (C-F) *In vivo* validation of MRTX1133 resistant models (C) GP2D, (D) MRTX1133-R-GP2D, (E) KPC, and (F) MRTX1133-R-KPC.



Cells	cpd.	Time point(h)	Sample ID
KPC	DMSO	24	01
	MRTX1133	2	02
	MRTX1133	24	03
MRTX1133-R-KPC	DMSO	24	04
	MRTX1133	2	05
	MRTX1133	24	06
GP2D	DMSO	24	01
	MRTX1133	2	02
	MRTX1133	24	03
MRTX1133-R-GP2D	DMSO	24	04
	MRTX1133	2	05
	MRTX1133	24	06

Figure 2. The mechanisms underlying MRTX1133 resistance in MRTX1133-resistant GP2D and KPC cells. (A) Volcano plot of differentially expressed genes in RNA-seq of GP2D and MRTX1133-R-GP2D cells. KEGG enrichment of up-regulated (B) and down-regulated (C) signaling pathways in MRTX1133-R- GP2D cells. (D) Heatmap of differentially expressed genes in RNA-seq of KPC and MRTX1133-R-KPC cells. (E) WB validation of potential targets.

Results

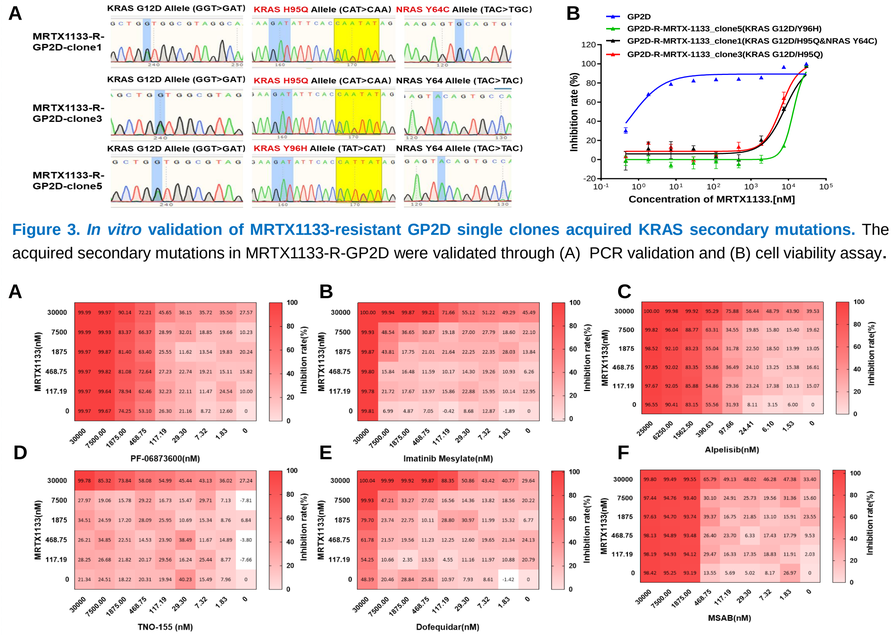


Figure 3. *In vitro* validation of MRTX1133-resistant GP2D single clones acquired KRAS secondary mutations. The acquired secondary mutations in MRTX1133-R-GP2D were validated through (A) PCR validation and (B) cell viability assay.

Figure 4. Combinatorial strategies to overcome MRTX1133 resistance. (A), (B), (C), (D), (E), and (F) *in vitro* matrix CTG analysis of MRTX1133-R-GP2D cells treated with CDK2/4/6 inhibitor (PF-06873600), RTK inhibitor (Imatinib Mesylate), PI3Kα inhibitor (Alpelisib), SHP2 inhibitor (TNO-155), ABCB1 inhibitor (Dofequidar) and β-catenin inhibitor (MASB) in combination with MRTX1133.

Conclusion

We established MRTX1133-resistant GP2D and KPC models with stable *in vitro* and *in vivo* resistance and cross-resistance to other KRAS G12D inhibitors (RMC9805, HRS-4642). Integrated bioinformatic and western blot analyses revealed convergent mechanisms, including ABC transporter upregulation, bypass signaling activation, WNT dysregulation, and EMT/stemness. Notably, GP2D resistant clones acquired KRAS secondary mutations confirmed by PCR and maintained stable resistance upon passaging. Combination strategies targeting cell cycle, compensatory signaling (PI3Kα, SHP2, RTK), drug efflux (ABCB1), and WNT/β-catenin showed synergistic effects with MRTX1133. These models support biomarker discovery and rational combination therapies to overcome KRAS G12D inhibitor resistance.

Reference:
1. Dilly J, Hoffman MT, Abbassi L, Li Z, Aguirre AJ. Mechanisms of Resistance to Oncogenic KRAS Inhibition in Pancreatic Cancer. Cancer Discov.
2. Zhou C, Li C. Anti-tumor efficacy of HRS-4642 and its potential combination with proteasome inhibition in KRAS G12D-mutant cancer. Cancer Cell.
3. Li C, Liu Y, Liu C. AGER-dependent macropinocytosis drives resistance to KRAS-G12D-targeted therapy in advanced pancreatic cancer. Sci Transl Med.

Contact Us USA: mahnaz_arjomand@wuxiapptec.com EU/UK/Israel: dave_madge@wuxiapptec.com Japan: fumio_itoh@wuxiapptec.com Korea: sycho@wuxiapptec.com China/Singapore/Australia: xu_longji@wuxiapptec.com