High-Throughput 2D and 3D Cell Panel screening to facilitate RAS target drug Discovery and development

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Introduction

High-throughput screening cell panel is very important tool in drug research and development, the tested sensitive cell lines can provide references for in vivo models or indication for the selection of clinical trial subjects. Cell Panel screening can be used to study the mechanism of action and selectivity of drugs, and combined with bioinformatics studies can help researchers discover biomarkers of drug response. Moreover, Cell Panel screening can be used to study whether combination therapy strategies improve cancer treatment effect and solve clinical drug resistance and other problems.

The Human RAS Oncogenes play a vital role in cancer development, including KRAS, NRAS and HRAS, KRAS is the most frequently mutated RAS isoform with a mutation incidence of >10% across all cancers, and up to 70% and 20% in pancreatic and non-small cell lung cancer (NSCLC), respectively.

ICE KRAS cell panel platform

ICE has developed the ICECP Pan KRAS 40 cell panel, which includes 40 cancer cell lines with diverse KRAS mutations across 11 cancer types. This panel supports both 2D and 3D experimental setups, catering to various research needs. The 2D model is ideal for rapid drug screening, while the 3D model provides a more complex, tumor-mimicking environment for in-depth drug action studies. The panel also incorporates pERK panel detection to assess the inhibition of signaling pathways, and bioinformatics analysis is used to explore mechanisms of drug resistance and resistance-related genomic signatures.

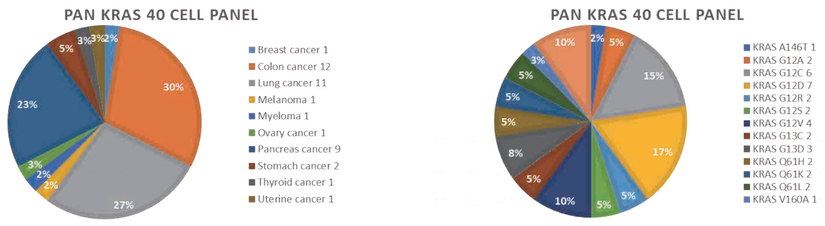


Figure1. A. cancer type distribution in KRAS cell panel. B. KRAS mutations distribution in KRAS cell panel.

Table 1. Differences Between 2D and 3D Cell Panel Screening

	2D cell panel	3D cell panel-Spheroids
Assay format	96-well or 384-well TC treated flat bottom plate	96 well or 384 well ultra-low attachment round bottom plate
Test reagents	2D cell-titer Glo	3D cell-titer Glo
Suitable cell lines	All adherent cell lines and suspension cell line	Most adherent cell lines and suspension cell lines
Support duration of cultivation	Long-term culture	Short-term culture
Throughput	High-throughput	Middle-throughput
Characteristic	Absence of cell-cell and cell-extracellular matrix interactions, unlimited access to nutrients, oxygen, and metabolites but easy to set up and analysis	More accurate representation of the in vivo scenarios, mirror tumor heterogeneity
Microplate reader	PHERAstar FSX	PHERAstar FSX
Choice guidance	According to the study goal and budget restraints, 2D assays offer simplicity and affordability, 3D assays provide more physiologically relevant environment, but at a greater cost and decreased throughput	

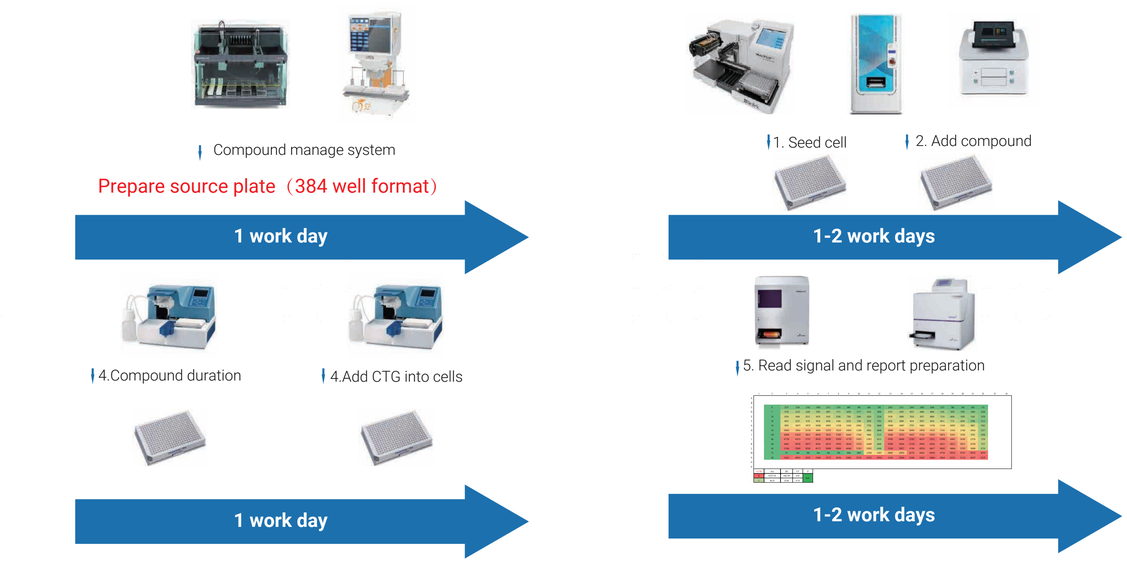


Figure2. High throughput work flow of KRAS cell panel platform.

2D and 3D CTG KRAS cell panel

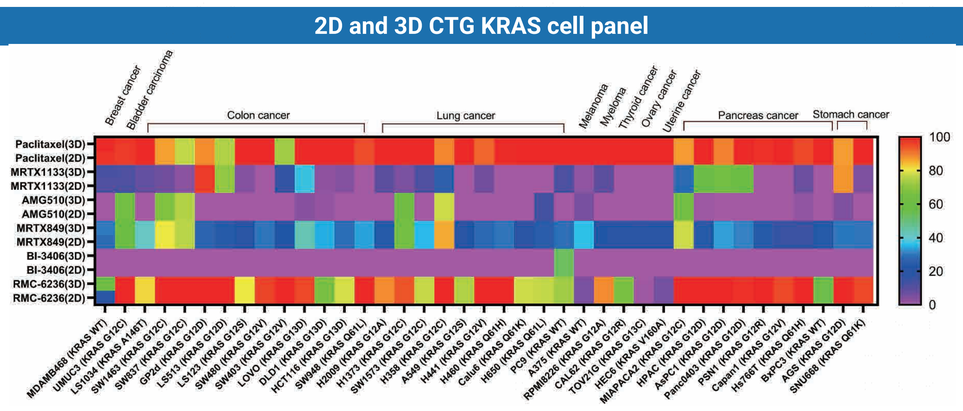


Figure3. The evaluation of KRAS inhibitors' IC50 in a broad KRAS cell panel reveals consistent potency across most cell lines when assessed using both 2D and 3D detection methods.

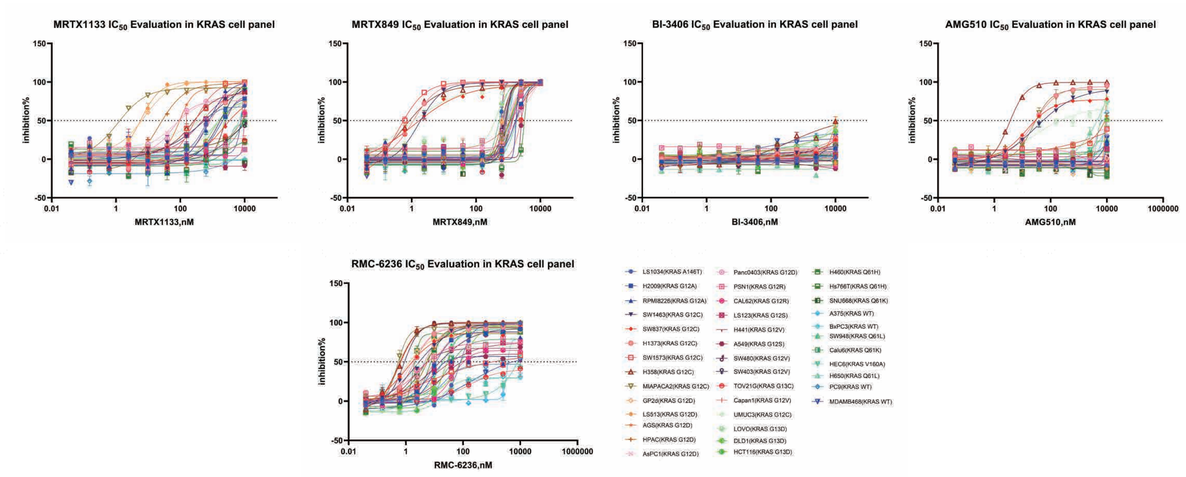
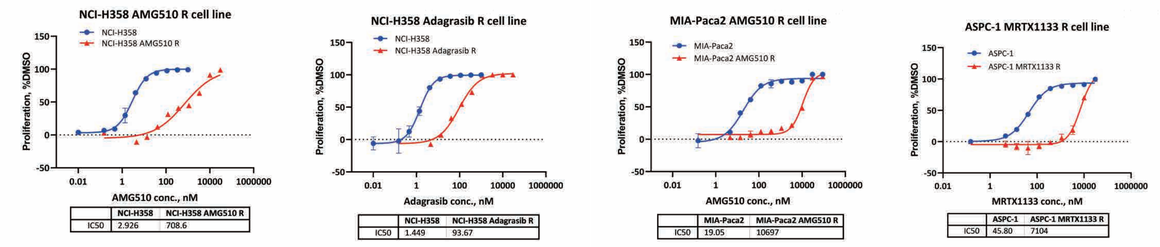


Figure4. Dose-response curves depict the IC50 evaluation of KRAS inhibitors across a panel of 3D cultured Pan KRAS cell lines. The results indicate that RMC-6236, as a molecular glue, can inhibit a broader spectrum of KRAS-mutated tumor cells compared to other small molecule inhibitors.

KRAS resistant cell line



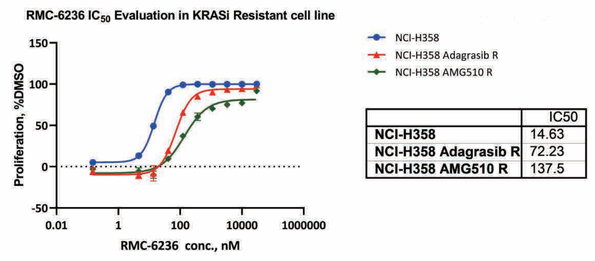
	NCI-H358	NCI-H358 AMG510 R
IC50	2.926	708.6

	NCI-H358	NCI-H358 Adagrasib R
IC50	1.449	93.67

	MIA-Paca2	MIA-Paca2 AMG510 R
IC50	19.05	10697

	ASPC-1	ASPC-1 MRTX1133 R
IC50	45.80	7104

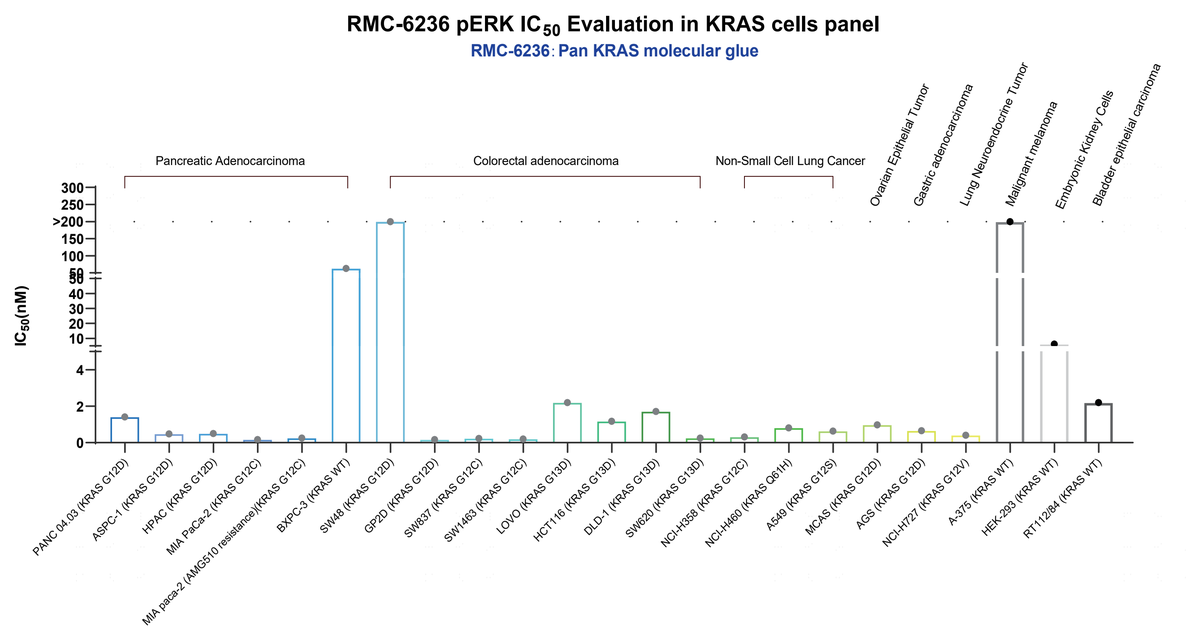
Figure5. KRAS G12C drug resistant cell line and KRAS G12D drug resistant cell line.



	IC50
NCI-H358	14.63
NCI-H358 Adagrasib R	72.23
NCI-H358 AMG510 R	137.5

Figure6. The IC50 evaluation of RMC-6236 in NCI-H358 Adagrasib-resistant (Adagrasib R) and NCI-H358 AMG510-resistant (AMG510 R) cell lines suggests that the compound does not exhibit significant resistance.

KRAS Signaling pathway-phospho-ERK panel



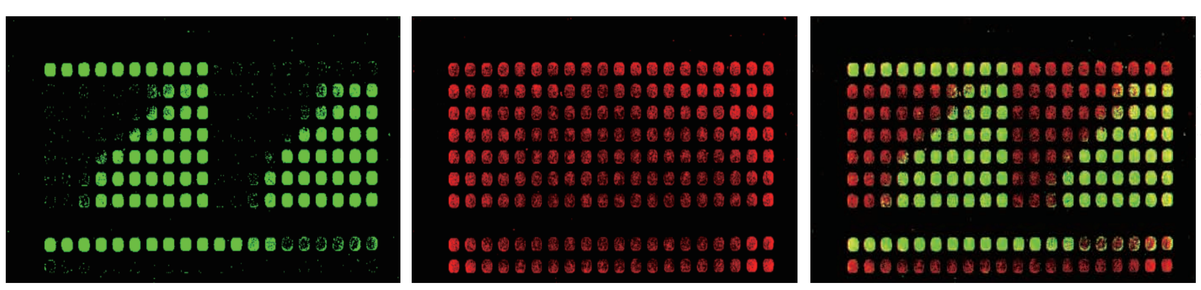


Figure7. The phospho-ERK panel assay detection using In-cell western method, indicating that RMC-6236 exhibits a strong inhibitory effect on the signaling pathways in the majority of cell lines carrying different KRAS mutations.

Bioinformatics analysis for KRAS cell panel

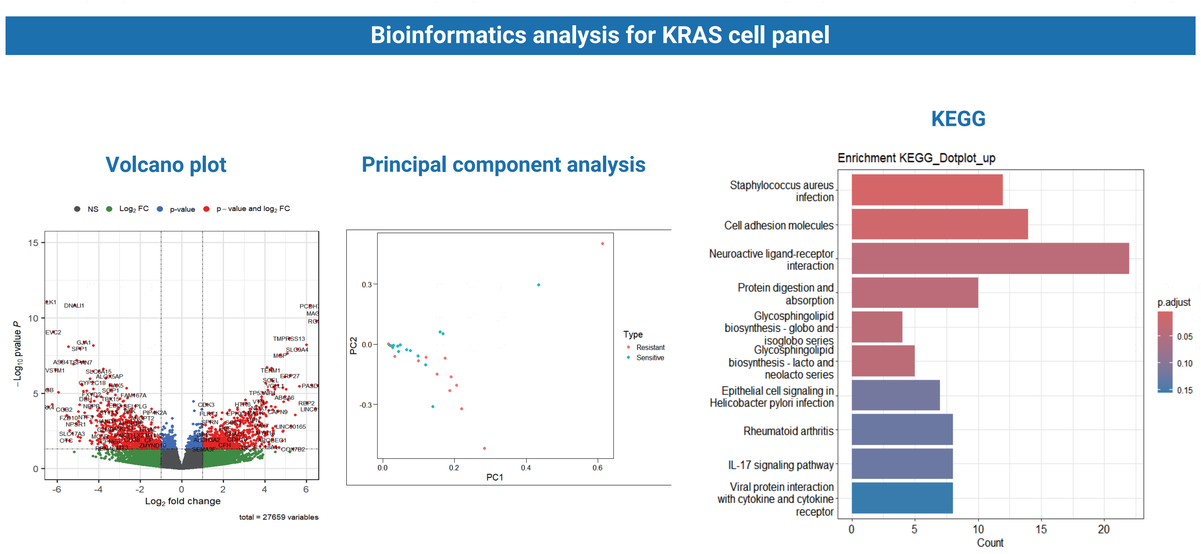


Figure8. bioinformatic analysis for RMC-6236 sensitive and resistant cell lines. Differential gene analysis and pathway enrichment results after grouping based on pharmacodynamic outcomes.

Conclusions

In summary, our high-throughput screening service utilizing 2D and 3D cell panels has identified compounds with selectivity for various RAS mutations, showing enhanced efficacy in 3D conditions. The integration of phospho-ERK (pERK) panel detection allows for a comprehensive assessment of drug action on signaling pathways. Bioinformatics analysis provides insights into drug efficacy, potency, mechanisms of action, and predictive biomarkers for clinical indications and resistance, making this panel a valuable asset for RAS drug development..

Acknowledgements

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