#3153 An integrated *in vitro* profiling platform for the simultaneous assessment of anti cancer efficacy and multi organ toxicity

Xia Tu, Hao Wen, Dawei Yan, Jingna Ren, Qin Guo, Peichuan Zhang, Haiheng Dong
WuXi Biology, WuXi AppTec, Shanghai, China

WuXi AppTec

WuXi Biology

Introduction

One major reason for the high attrition rate of oncology candidates is attributed to insufficient understanding of the therapeutic index (TI) [1,2] early in development. Many candidate molecules with promising efficacy later fail because of unanticipated safety issues, highlighting the need for a comprehensive *in vitro* strategy that simultaneously evaluates anti-cancer activity and organ-specific toxicity risks. In this study, WuXi Biology developed an integrated *in vitro* profiling platform with a comprehensive early safety and toxicity assessment module. Here, we applied this platform to profile several KRAS inhibitors, including the approved agent Sotorasib (AMG-510) and several investigational candidates, demonstrating its utility in generating a comprehensive efficacy-safety profile for informed, de-risked candidate selection. The platform aims to identify efficacy and safety risks concurrently at an early stage to enable more efficient, lower risk candidate selection and clinical translation.

Methods

Seven KRAS inhibitors were profiled, including the approved agent Sotorasib (AMG-510) and six investigational candidates (Compounds A–F). Cell viability was evaluated in 2D culture across 12 lung and pancreatic cancer cell lines (designated CL#1–CL#12; see table for KRAS mutation status) following 3-day compound treatment using the CellTiter-Glo® Luminescent Cell Viability Assay. To model the tumor microenvironment, 3D spheroid cultures were established using an integrated biomimetic array chip[3] in four cell lines, with spheroids formed over 4 days prior to 3-day compound exposure and viability assessment. For safety profiling, organ-specific toxicity was assessed in multiple normal human cell models: hepatotoxicity[4] was evaluated in 3D-cultured primary human hepatocytes using an integrated biomimetic array chip over 7 days, nephrotoxicity in HK-2 cells over 3 days, and pulmonary toxicity in HFL-1, MRC-5, and BEAS-2B cells over 3 days, with the average CC_{50} among the three lung cell lines used as the pulmonary toxicity reference. Mitochondrial toxicity[5] was assessed in HepG2 cells using glucose versus galactose culture conditions, where an IC_{50} ratio (glucose/galactose) >3 indicates mitochondrial liability[6], and phototoxicity by the 3T3 Neutral Red Uptake assay[7] with Photo-Irritation Factor (PIF) calculation. All viability readouts were obtained using the CellTiter-Glo® Luminescent Cell Viability Assay. *In vitro* Therapeutic Index (TI) is defined as the ratio of normal cell toxicity (CC_{50}) to the EC_{50} in each cancer cell line. A TI ≥ 30 was considered indicative of a wide safety window suitable for further development, based on the high selectivity observed in our data.

Results

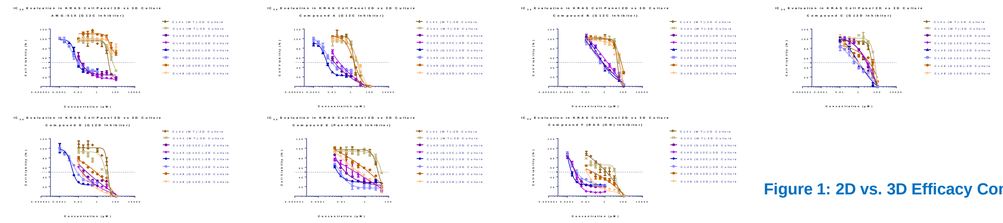

Figure 1: 2D vs. 3D Efficacy Comparison

- G12C inhibitors (AMG-510, Compounds A–B) and the RAS(ON) inhibitor (Compound F) remained highly potent in G12C-mutant cells under both 2D and 3D conditions, with minimal potency loss in 3D. G12D inhibitors (Compounds C–D) showed improved activity in 3D versus 2D specifically in G12D-mutant cells. The pan-KRAS inhibitor (Compound E) displayed broad activity across mutation types, though with slight reduction in 3D for G12C cells. Wild-type cells were generally less sensitive, with further decreased sensitivity in 3D. These results highlight that 3D culture can reveal compound-specific potency differences not apparent in 2D assays.

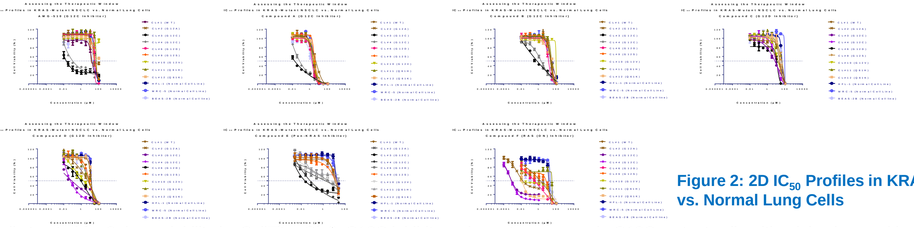

Figure 2: 2D IC_{50} Profiles in KRAS-Mutant NSCLC vs. Normal Lung Cells

- Cells treated for 3 days; viability by CellTiter-Glo®. G12C inhibitors show steep curves in G12C-mutant cells with minimal normal lung toxicity (wide window). G12D inhibitors are selective for G12D-mutant cells but with weaker potency shift; normal lung curves overlap non-cognate lines. Pan-KRAS inhibitor gives similar curves in mutant and normal cells (wild-type KRAS liability). RAS(ON) inhibitor is extremely potent in G12A/G12C/G12D/G12V/Q61K cells but much weaker in other contexts; normal lung cell curves align with those in non-sensitive lines.

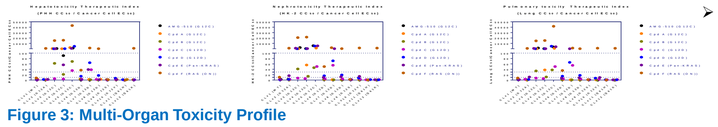

Figure 3: Multi-Organ Toxicity Profile

- TI = CC_{50}(normal cells)/EC_{50}(cancer cells) for liver, kidney, and lung. G12C and RAS(ON) inhibitors showed high TI exclusively in G12C-mutant cells; G12D inhibitors show moderate TI only in G12D-mutant cells; the pan-KRAS inhibitor has TI < 1 across most lines and organs. Mutation-selective inhibitors require precise patient stratification to achieve a favorable safety window.

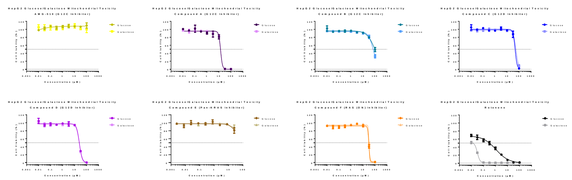

Figure 4: Mitochondrial Toxicity Assessment

- Cell viability curves in HepG2 cells cultured in glucose (normal metabolism) vs. galactose (oxidative phosphorylation-dependent) medium. All seven KRAS inhibitors exhibit IC_{50} ratios (glucose/galactose) <3, indicating no mitochondrial toxicity. Rotenone, a known mitochondrial complex I inhibitor, was included as a positive control.

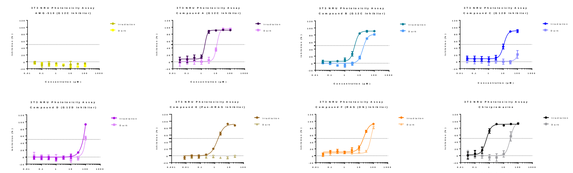

Figure 5: Phototoxicity evaluation by 3T3 NRU assay

- Cell viability curves under UVA-irradiated and non-irradiated (dark) conditions. AMG-510 showed no phototoxicity, consistent with published data[8], and compound D showed no phototoxicity (PIF<2). Compounds B, F were weak positive, with A, C and E positive (PIF>5). Chlorpromazine (positive control) confirmed assay validity. These results indicate that phototoxicity is a common liability among investigational KRAS inhibitors, emphasizing the need for early photosafety evaluation.

Table 1: Cell Line Code Table

Code	Tissue Origin	KRAS Mutation	Tumor Type
CL#1	Lung	Wild Type	NSCLC
CL#2	Lung	G12A	NSCLC
CL#3	Lung	G12C	NSCLC
CL#4	Lung	G12C	NSCLC
CL#5	Pancreas	G12C	PDAC
CL#6	Lung	G12D	NSCLC
CL#7	Pancreas	G12D	PDAC
CL#8	Pancreas	G12D	PDAC
CL#9	Lung	G12S	NSCLC
CL#10	Lung	G12V	NSCLC
CL#11	Lung	Q61H	NSCLC
CL#12	Lung	Q61K	NSCLC

Table 2: Compound Code Table

Compound Code	Inhibitor Type
AMG-510	KRAS G12C inhibitor
Compound A	KRAS G12C inhibitor
Compound B	KRAS G12C inhibitor
Compound C	KRAS G12D inhibitor
Compound D	KRAS G12D inhibitor
Compound E	Pan-KRAS inhibitor
Compound F	RAS (ON) Inhibitor

Summary

The integrated efficacy-safety platform successfully distinguished seven KRAS inhibitors based on their therapeutic windows, validating its utility for early-stage drug candidate profiling. Notably, mutation-selective inhibitors achieved high therapeutic indices only in cognate mutant cells, underscoring the critical need for precise patient stratification, whereas pan-KRAS inhibitors offered broader applicability but faced wild-type toxicity liabilities. All compounds tested negative for mitochondrial toxicity. In contrast, five of seven compounds exhibited weak or strong phototoxicity, with only Sotorasib (AMG-510) and compound D showing a negative result - highlighting the importance of early photosafety evaluation. While the present case study focused on KRAS inhibitors, our integrated platform encompasses a broader suite of assays that have been validated in other compound classes. These include mechanism-of-action (MOA) investigations (e.g., qPCR, Western Blot for pathway modulation), functional phenotyping for anti-metastatic potential (angiogenesis, migration, invasion), and comprehensive safety profiling covering cardiotoxicity, neurotoxicity, and genotoxicity. The modular nature of this platform allows for flexible adaptation to diverse drug discovery programs, enabling a holistic efficacy-safety assessment tailored to the specific needs of each project.

References

1. Muller PY, Milton MN. The determination and interpretation of the therapeutic index in drug development. Nat Rev Drug Discov. 2012 Oct;11(10):751-61.
2. Copple IM, Shelton LM, Walsh J, Kratschmar DV, Lister A, Odermatt A, Goldring CE, Dinkova-Kostova AT, Honda T, Park BK. Chemical tuning enhances both potency toward nrf2 and *in vitro* therapeutic index of triterpenoids. Toxicol Sci. 2014 Aug 1;140(2):462-9.
3. Xiao RR, Jin L, Xie N, Luo P, Gao W, Tu P, Ai X. Establishment and large-scale validation of a three-dimensional tumor model on an array chip for anticancer drug evaluation. Front Pharmacol. 2022 Oct 12;13:1032975.
4. Xiao RR, Lv T, Tu X, Li P, Wang T, Dong H, Tu P, Ai X. An integrated biomimetic array chip for establishment of collagen-based 3D primary human hepatocyte model for prediction of clinical drug-induced liver injury. Biotechnol Bioeng. 2021 Dec;118(12):4687-4698.
5. Marroquin LD, Hynes J, Dykens JA, Jamieson JD, Will Y. Circumventing the Crabtree effect: replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. Toxicol Sci. 2007 Jun;97(2):539-47.
6. Swiss R, Will Y. Assessment of mitochondrial toxicity in HepG2 cells cultured in high-glucose- or galactose-containing media. Curr Protoc Toxicol. 2011 Aug;Chapter 2:Unit2.20.
7. OECD Guideline for the Testing of Chemicals 432: *In Vitro* 3T3 NRU Phototoxicity Test (2004).
8. Ishida K, Werner JA, Davies R, Fan F, Thomas B, Wahlstrom J, Lipford JR, Monticello T. Nonclinical Safety Profile of Sotorasib, a $KRAS^{G12C}$-Specific Covalent Inhibitor for the Treatment of *KRAS p.G12C*-Mutated Cancer. Int J Toxicol. 2021 Oct;40(5):427-441.

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