KRAS, BRAF, BRCA1 and EGFR Mutation-Specific Panels using Hydrogel-Based 3D *In Vitro* Tumor Models

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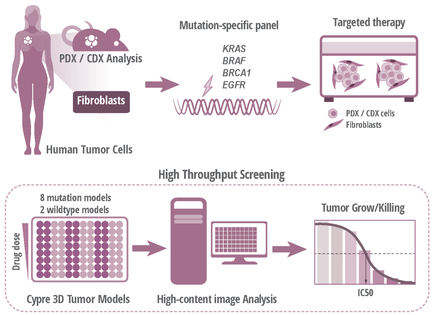
1 ABSTRACT

Mutation-targeting therapies have become a transformative technology and, in many ways, a new paradigm for cancer treatment for patients. The early development of Erlotinib for non-small cell lung cancer paved the way for EGFR treatment, irrespective of tumor indication subtype. And the more recent launch of Sotorasib for $KRAS^{G12C}$ mutation has marked a turning point for the once undruggable mutation. The advent of gene specific-treatments requires a reconfiguration of preclinical models which more effectively represent the said mutation in populations of diverse backgrounds and tumor indications. 3D models which have the ability to recreate the key hallmarks of cancer - such as growth, invasion, immune infiltration and suppression, stromal transformation, drug diffusion, as well as gene mutational signatures - are of exceptional interest to drive rapid and scalable compound efficacy screening.

Here, we developed four 3D *in vitro* tumor panels carrying mutations in one of the following genes - KRAS, EGFR, BRCA1, and BRAF - as well as wildtype models for comparison. The tumors were grown in a hydrogel matrix in 96-well plates with stromal fibroblasts and screened with the standard of care (e.g. Sotorasib for $KRAS^{G12C}$, Erlotinib for $EGFR^{mut}$, the PARP inhibitor Olaparib for $BRCA^{mut}$, and Vemurafenib for $BRAF^{V600E}$) and other known therapeutic compounds that target their respective mutations. The utility of a 3D hydrogel matrix and fibroblasts in the panels' models were key in recreating the stromal compartment of the tumor microenvironment. Endpoints were determined within four days of treatment, including tumor size reduction and tumor killing, via high-content image analysis. The Panels' robustness as demonstrated by the reproducible results in independent experiments suggests they can be utilized at *in vitro* pharmacology stages and earlier in Hit-to-lead screens.

2 PLATFORM



A streamlined workflow for growing 3D patient-derived xenograft tumors (PDX) or cell-line derived xenograft (CDX) models and assaying targeted drugs using high content analysis and advanced analytics. The standardized setup includes 96-well format and 6-dose in duplicates, and assay endpoints include tumor size and cell death using high content imaging.

**Visit: https://www.criver.com/cancer-model-database to learn the PDX lines in the panel.

1 RESULTS

Fig 1. Dose-response analysis of the 3D tumor growth/killing of Sotorasib and Vemurafenib in Cypre 3D platform

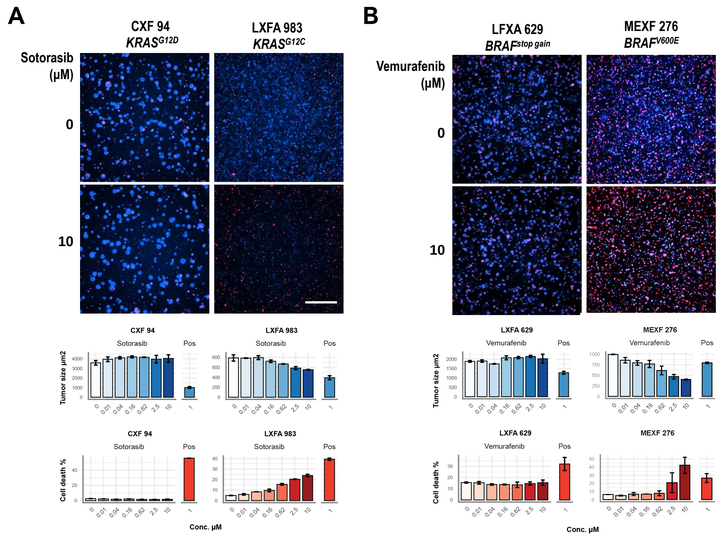


Fig 1. Dose-response profiles of Sotorasib (A) and Vemurafenib (B) with tumor size and the percentage of cell death. The mutation-specific panels were treated with 6 doses of the compound, and Staurosporine 1μM as positive control for 4 days. Subsequently, the 3D models were stained with Hoechst and DRAQ7, and subject to high-content imaging analysis. The results of a non-responder line and responder line from KRAS and BRAF mutation panel are shown. Scale bar = 200μm

Fig 3. High data reproducibility in Cypre 3D platform

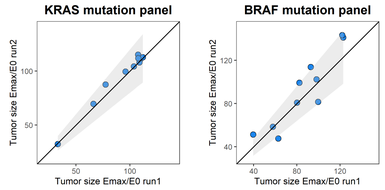
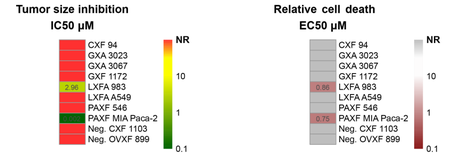


Fig 3. Duplicate testing of BRAF mutation and KRAS mutation panels shows consistent Emax/E0 values for tumor size in each 3D tumor model.

Fig 2. The list of each fixed Mutation-specific panel and their response to the targeted therapies

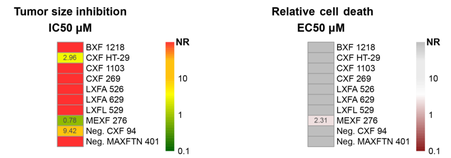
A KRAS mutation panel – Sotorasib treatment

Model	Tumor Type	Mutation
CXF 94	colon cancer	G12D
GXA 3023	gastric cancer	G13D
GXA 3067	gastric cancer	G12V
GXF 1172	gastric cancer	G12D
LXFA 983	NSCLC	G12C
LXFA A549	NSCLC	G12S
PAXF 546	pancreatic cancer	G12V
PAXF MIA-Pa-Ca-2_CL	Pancreatic	G12C
OVXF 899	ovarian cancer	
CXF 1103	colon cancer	



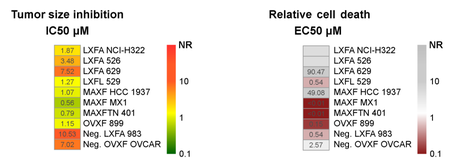
B BRAF mutation panel – Vemurafenib

Model	Tumor Type	Mutation
BXF 1218	Bladder	Y519F
CXF HT-29	Colon cancer	V600E| T119S
CXF 1103	colon cancer	T395I
CXF 269	colon cancer	
LXFA 526	NSCLC	
LXFA 629	NSCLC	
LXFL 529	NSCLC	A81G
MEXF 276	melanoma	V600E
CXF 94	colon cancer	
MAXFTN 401	breast cancer	



C BRCA1 mutation panel – Olaparib

Model	Tumor Type	Mutation
LXFA NCI H322	NSCLC	M1014I
LXFA 526	NSCLC	N1403T
LXFA 629	NSCLC	N1403T|I620V
LXFL 529	NSCLC	N1403T
MAXFTN_HCC-1937_CL	Breast cancer	
MAXFTN_MX1_CL	Breast cancer	
MAXFTN 401	breast cancer	
OVXF 899	ovarian cancer	I1044V
LXFA 983	NSCLC	
OVXF_OVCAR-5_CL	Ovarian	



D EGFR mutation panel – Erlotinib

Model	Tumor Type	Mutation
CXF 1103	colon cancer	
CXF 269	colon cancer	
GXA_MKN45_CL	NSCLC	A1048V
LEXFAL CCRF-CEM	NSCLC	A1118T
LXFA NCI-H1975	NSCLC	T790M|L858R
LXFA 526	Gastric	D347E
LXFA 629	ALL Leukemia	H383D
LXFL529	NSCLC	S116A|T678R
CXF 94	colon cancer	
GXA 3023	gastric cancer	

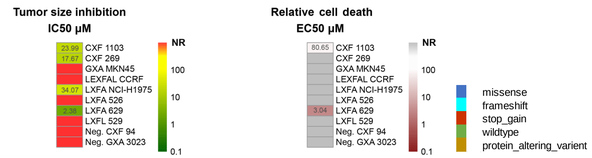


Fig 2. Each Mutation-specific panel is comprised of 8 mutant models and 2 wildtype models across multiple tumor types. KRAS (A), BRAF (B), BRCA1 (C) and EGFR (D) panels were treated with their targeted therapies Sotorasib, Vemurafenib, Olaparib and Erlotinib, respectively. The drug efficacy was visualized using heatmap showing IC50 value of relative tumor size inhibition and EC50 value of cell death induction (comparison to vehicle and positive control).

4 CONCLUSION

- ❑ **Cypre 3D Mutational Panels comprising PDX/CDX *in vitro* models in 3D hydrogels with fibroblasts screen for anti-tumor effects of targeted compounds.**
- ❑ **Mutational Panels include 10 models expressing either KRAS, BRAF, BRCA1, or EGFR (8 mutant, 2 wildtype).**
- ❑ **Assay endpoints include tumor size reduction and tumor cell death via high content image analysis.**
- ❑ **Responders enabled indication and model selection for subsequent preclinical studies.**

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