Patient-derived organoid drug responses corroborate known target–drug interactions for selected anticancer agents

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Abstract:

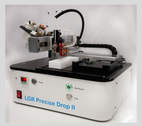
Patient-derived organoids (PDOrgs) are heterogeneous three-dimensional cellular clusters that have been shown to recapitulate the tumor histology and genetic alterations of their originating tissue. Numerous studies suggest the *in vitro* drug responses of tumor organoids align with *in vivo* responses. In this study, we evaluated fourteen anticancer agents against a cohort of PDOrgs from three disease histologies: colon, pancreatic, and non-small cell lung adenocarcinoma. The PDOrgs were obtained from the National Cancer Institute's Patient-Derived Models Repository (https://pdmr.cancer.gov), a resource that offers clinically annotated and molecularly characterized models. The PDOrg models were selected for specific genetic variants of KRAS and BRAF or different RNA levels of ABCB1, an ATP-dependent efflux pump. The approved and investigational agents were selected to target specific genetic variants and pathways: KRAS G12C covalent inhibitors (sotorasib and MRTX-1257), RAS pathway inhibitors (BAY-293, BI-3406, and TNO-155), BRAF V600E/K inhibitors (dabrafenib and encorafenib), ABCB1 substrates (paclitaxel, doxorubicin, 5-FU, AZD-1775, and SN-38), and ABCB1 non-substrates (gemcitabine and trametinib). The goal of the study was to assess whether the sensitivities of PDOrgs to therapeutic agents matched these genetic profiles under standard in vitro conditions. PDOrgs were seeded into 384-well microplates in a semi-automated fashion and exposed to nine concentrations of each anticancer agent for six days, followed by cell viability assessment by CellTiter-Glo 3D. Data analysis was performed using GRmetrics (https://git.bioconductor.org/packages/GRmetrics), an R package for calculation and visualization of concentration-response metrics based on growth rate inhibition. These data demonstrated that PDOrgs harboring a KRAS G12C variant were uniquely sensitive to sotorasib and MRTX-1257 and were, overall, more sensitive to the other RAS-pathway targeting agents. Conversely, PDOrgs harboring wild-type RAS and other KRAS variants were largely unresponsive to these targeted agents. Likewise, only PDOrgs harboring the BRAF V600E variant were sensitive to dabrafenib and encorafenib. For the majority of PDOrgs, the pharmacological responses to agents that are ABCB1 substrates inversely correlated with ABCB1 RNA expression. This study demonstrates the ability of organoids to serve as useful models for evaluating therapeutic responses to anticancer agents, including identifying known target–drug associations. Moreover, the technical conditions, as well as the selected PDOrgs and therapeutic agents, may be used as a reference set for the validation of a fully automated PDOrg screening system. This project was funded in part with federal funds from the NCI, NIH, under contract no. HHSN261201500003I.

Methods:

Organoid Growth and Preparation:

Organoids were grown in 100% BME domes either in 6-well plates or 100 mm dishes. On the day of plate inoculation, organoids were released from domes using dispase to digest the BME and mechanical disruption. Organoid pellets were washed with media to remove residual BME, counted and resuspended in 5% BME at a density that was predetermined by measuring growth over 7 days at different organoid densities.

Organoid Inoculation onto 384-Well Plates:



The organoid suspension was inoculated into black, 384, clear-bottom ULA plates (Corning #4588) using the Precise Drop II micro dispensing system (Let's Go Robotics, Carlsbad CA), which simultaneously dispensed 40 µL of media to the outer rows and columns of the plate and 40 µL of organoid suspension to all other wells of the plate (308 wells). After inoculation, the plates were incubated at 37 ºC, 5% CO_2 for 24 h.

Drug and Vehicle Addition:

Approximately 24 h after inoculation, vehicle and drugs were added to the plates using the Echo Acoustic Liquid Handler (Beckman Coulter, Indianapolis, IN). Drugs were made up in DMSO at a 400x concentration, diluted into 9, ½ log dilutions and plated onto polypropylene plates designed for use in the Echo Acoustic Dispenser. For each vehicle/drug well on the acoustic plate, 100 nL of drug was added to each well of the test plates containing organoids. After drug addition, plates were incubated at 37 ºC, 5% CO_2 for 6 days.

Measurement of Organoid Viability:

Organoid viability was measured at two different time points. Measurements were taken at time zero (TZ, the time of drug addition) and at 6 days after drug addition to assess the viability of organoids in the vehicle control and drug-treated wells. The viability of organoids was measured using CellTiter Glo 3D (CTG3D) (Promega, Madison WI). In brief, CTG3D and test plates were equilibrated to room temperature, after which an equivalent volume of CTG3D was added to the organoid suspension in each well of the 384-well plate. The plates were then placed on a BioShake XP (QInstruments, Germany) and shaken for 5 min at 1,200 rpm followed by a 25 min RT incubation. Plate luminescence was measured using a Pherastar FSX (BMG LABTECH Inc., Cary, NC).

Data Analysis:

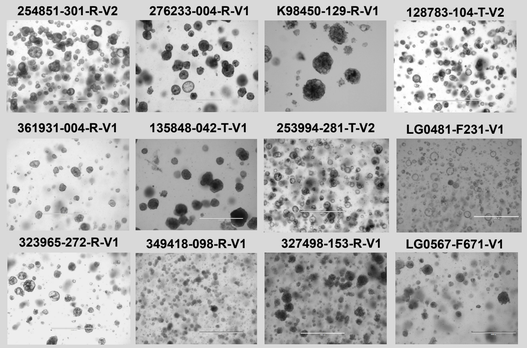
Excel was used to get the raw luminescence data in a format that could be imported and analyzed in R using the GRmetrics package. Normalized growth rate inhibition (GR) values are based on the ratio of growth rates in the presence and absence of perturbagen, calculated by GRMetrics. This parameter is part of a growth rate correcting strategy proposed by Hafner et al. (*Nature Methods,* 13(6) June 2016) to calculate and score dose-response curves. The method adjusts cell viability using a log2 fold change estimator and allows comparison of drug responses between models even when growth rates are different.

Organoid Models, plating densities and status/expression of genes of interest

Organoid Model	Disease	RAS STATUS	RAF STATUS	ABCB1 EXPRESSION (TPM)	DENSITY (orgs/well)
349418-098-R-V1	Non small cell lung cancer	WT	V600E	0.00	125
361931-004-R-V1	Adenocarcinoma - colon	WT	V600E	0.91	250
LG0567-F671-V1	Non small cell lung cancer	KRAS G12C	WT	0.00	250
323965-272-R-V1	Adenocarcinoma-pancreas	KRAS G12C	WT	0.08	125
LG0481-F231-V1	Non small cell lung cancer	KRAS G12C	WT	0.22	125
327498-153-R-V1	Carcinosarcoma - uterus	KRAS G12C	WT	18.65	250
135848-042-T-V1	Adenocarcinoma - colon	KRAS G12C	WT	458.67	250
K98450-129-R-V1	Adenocarcinoma - colon	KRAS G12C	WT	590.03	125
253994-281-T-V2	Colorectal cancer, NOS	KRAS G12V	WT	602.31	125
128783-104-T-V2	Adenocarcinoma - colon	KRAS G13D	WT	45.46	125
254851-301-R-V2	Adenocarcinoma - colon	KRAS G12D	WT	51.88	125

Organoid models LG0481-F231-V1 and LG0567-F671-V1 were developed, under agreement, from PDX models available from The Jackson Laboratory.

Morphology of Organoid Models in 100% BME2



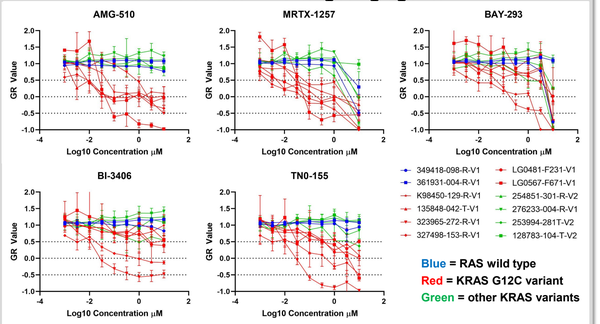
Morphology of organoids used for the experiment. Pictures were taken at 4X magnification (bar indicates 1000 µm).

Agents used and known molecular targets

DRUG or AGENT	NSC #	HIGH TEST (µM)	TARGET	ABCB1 SUBSTRATE
Sotorasib (AMG-510)	818433	10	KRAS G12C	
MRTX-1257	819558	10	KRAS G12C	
BAY-293	824723	10	SOS-1	
BI-3406	825286	10	SOS-1	
TNO-155	825523	10	SHP-2	
Gemcitabine	613327	3.16	DNA synthesis inhibitor	No
5-FU	19893	100	Thymidylate synthase	No
Encorafenib	778304	10	BRAF V600E	+/-
Dabrafenib	764134	10	BRAF V600E	Yes
Paclitaxel	125973	1	Tubulin	Yes
Doxorubicin	123127	10	DNA intercalator; Topo 2	Yes
AZD1775	754352	10	Wee-1	Yes
SN-38	673596	1	Topoisomerase I	Yes
Trametinib	758246	0.316	MEK	Yes*

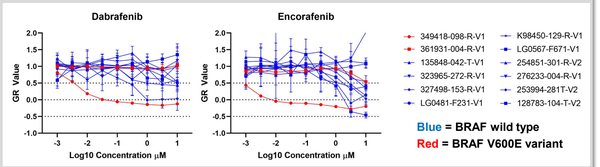
* Trametinib has been shown to be a ABCB1 substrate (Vaidhyanathan S. et al. Drug Metab Dispos. 2014 Aug;42(8):1292-300.) but also to inhibit ABCB1 function (Qiu J et al. Oncotarget. 2015 Jun 20; 6(17): 15494–15509)

Organoid models harboring KRAS G12C are, overall, more sensitive to RAS-targeting agents



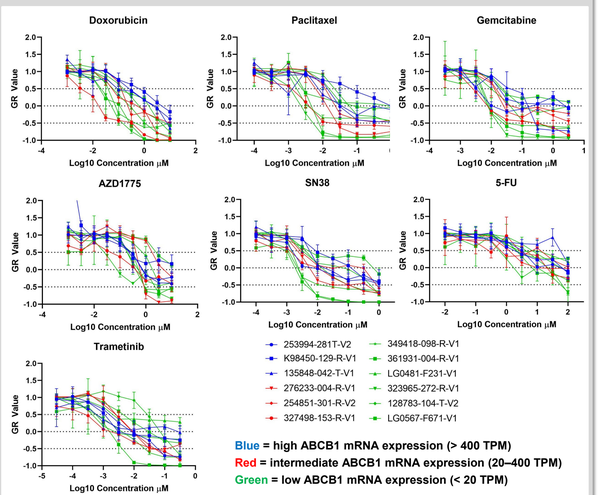
PDOrgs expressing the KRAS G12C variant are, overall, more sensitive to covalent KRAS G12C inhibitors (sotorasib and low doses of MRTX-1257). PDOrgs 323965-272-R-V1 and 327498-153-R-V1, KRAS G12C models, are more sensitive to RAS pathway inhibitors (SOS1 inhibitors: BAY-293 and BI-3406; SHP2 inhibitor: TNO-155).

Dabrafenib and encorafenib induce cytotoxicity in 349418-098-R-V1 but not in 361931-004-R-V1



Dabrafenib and encorafenib profoundly affect growth of the 349418-098-R-V1 organoid model (NSCLC, BRAF V600E) but not of 361931-004-R-V1 (colon adenocarcinoma, BRAF V600E).

High levels of ABCB1 mRNA in PDOrgs confer resistance to doxorubicin but not to other ABCB1 substrates

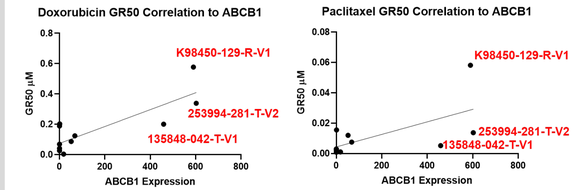


All PDOrgs expressing high levels of ABCB1 are more resistant to doxorubicin, while only one model expressing high levels of ABCB1 is less responsive to paclitaxel. TPM = transcripts per million (RNASeq data)

Good correlation between ABCB1 mRNA expression and doxorubicin responses in PDOrgs

DRUG or AGENT	NSC #	HIGH TEST DOSE (µM)	ABCB1 SUBSTRATE	CORRELATION ABCB1 and GR50
Gemcitabine	613327	3.16	No	No
5-FU	19893	100	No	No
Dabrafenib	764134	10	Yes	No
Paclitaxel	125973	1	Yes	Yes (0.037*)
Doxorubicin	123127	10	Yes	Yes (0.001)
AZD1775	754352	10	Yes	No
SN-38	673596	1	Yes	No
Trametinib	758246	0.316	Yes	No

* Correlation driven by one model



Summary

- The assay was able to identify sensitivities to targeted agents (KRAS G21C covalent inhibitors and BRAF inhibitors) in 7 of the 8 models selected based on KRAS G12C and BRAF V600E variant expression.
- KRAS G12C covalent inhibitors preferentially decreased growth and induced cytotoxicity in organoid models harboring KRAS G12C.
- 323965-272-R-V1 and 327498-153-R-V1, pancreatic adenocarcinoma and uterine carcinosarcoma organoid models harboring KRAS G12C, were particularly sensitive to RAS pathway inhibitors (agents targeting SOS1 and SHIP2).
- Dabrafenib and encorafenib profoundly affected growth of the 349418-098-R-V1 organoid model (NSCLC, BRAF V600E) but not of 361931-004-R-V1 (colon adenocarcinoma, BRAF V600E).
- Correlations between ABCB1 mRNA expression and resistance to doxorubicin and paclitaxel both reached statistical significance; however, the result with paclitaxel was driven by only one model. No correlation was observed for the other ABCB1 substrates (AZD1775, trametinib, SN-38, and dabrafenib). No correlation was observed between gemcitabine or 5-FU responses and ABCB1 expression (non-ABCB1 substrates).

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