#2358: Evaluation of patient-derived cell lines and cancer organoids for the prediction of drug responses in patient-derived xenograft models

Petreena Campbell[1], Curtis Hose[1], Lara El Touny[1], Erik Harris[1], John Connelly[1], Carrie Bonomi[2], Kelly Dougherty[2], Savanna Styers[2], Abigail Walke[2], Jenna Moyer[2], Mariah Baldwin[2], Anna Wade[2], Michael Mullendore[2], Kaitlyn Arthur[2], Matthew Murphy[2], Kevin Plater[2], Marion Gibson[1], Joseph Geraghty[2], Michelle Gottholm-Ahalt, Tara Grinnage-Pulley[2], Tiffanie Chase[2], John Carter[2], Howard Stotler[2], Debbie Trail[2], Luke Stockwin[2], Dianne Newton[2], Yvonne Evrard[2], Melinda Hollingshead[3], Ralph E. Parchment[4], Nathan P. Coussens[1], Beverly A. Teicher[5], James H. Doroshow[6], Annamaria Rapisarda[1].

[1]Molecular Pharmacology Laboratory, [2]Biological Testing Branch, [4]Clinical Pharmacodynamic Biomarkers Program, Applied/Developmental Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, Maryland, 21702; [3]Biological Testing Branch, Developmental Therapeutics Program, National Cancer Institute-Frederick, Frederick, Maryland, 21702; [5]Molecular Pharmacology Branch, Developmental Therapeutics Program, National Cancer Institute, Rockville, MD 20852; [6]Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland 20892.

Abstract

Cancer organoids are heterogeneous 3D cellular clusters with complexities that mimic some characteristics of tumors in situ. Thus, assays performed with cancer organoids might enable better predictions of in vivo drug responses than those performed with cell monolayers. The National Cancer Institute (NCI) is developing a national repository of Patient-Derived (PD) models comprised of clinically annotated and molecularly characterized PD xenografts (PDXs) and PD/PDX-derived tumor cell lines (PDCs), and cancer organoids (PDOrgs) (https://pdmr.cancer.gov/). We evaluated the therapeutic activity of a panel of FDA-approved and investigational anticancer agents, including gemcitabine, paclitaxel, SN-38, 5-FU, adavosertib, erlotinib, trametinib, and vemurafenib, against a cohort of PDCs, PDOrgs, and PDXs from solid tumors including colon, gastroesophageal, head and neck, NSCLC, pancreatic, bladder, and uterine cancers. Our goal was to investigate whether drug sensitivities determined using PDCs and PDOrgs correlate with responses observed in the matching PDXs. Cultures were exposed to anticancer agents at concentrations ranging from 1 pM to 100 μM for periods of 4 or 6 days. The data indicated that the GI50 values for PDOrgs were in overall agreement with in vivo PDX drug responses measured as relative median to event free survival (RMEFS), where an event is the median time (days) from treatment initiation to tumor volume quadrupling, calculated as median time to tumor volume quadrupling for treated animals/median time to tumor volume quadrupling for control animals. For both paclitaxel and trametinib, responses in PDOrgs, from most sensitive to most resistant, were similar to the corresponding PDXs. Drug sensitivities determined in PDC monolayers were less clearly related to in vivo PDX responses; particularly for PDCs treated with carboplatin, gemcitabine, and SN-38. This work is part of a larger effort to provide a rigorous comparison between fully characterized and annotated PDCs-PDOrgs-PDXs to assess the value of different in vitro model systems for the prediction of PDX drug responses.

Experimental conditions for in vitro pharmacological profiling

Compound	Drug Target/MOA	Clinical C_{max} (μM)
Gemcitabine	nucleoside analog	89.29
Paclitaxel	mitotic inhibitor	4.27
SN-38	topo-I inhibitor	0.14
5-FU	nucleoside analog	425.6
Adavosertib	WEE1 inhibitor	3.7
Erlotinib	EGFR inhibitor	3.15
Trametinib	MEK inhibitor	0.02
Etoposide	topo-II inhibitor	33.4
Vemurafenib	BRAF inhibitor	126.55

- **PDOrgs**
  - Fully formed organoids seeded 24 h before (T0) time zero (~100-150 μm)
  - Organoid density/well (~30-50)
  - Basement membrane extract (BME) used (7.2 mg/ml)
- **PDCs**
  - Single cells seeded 24h before T0
  - Cells density/well (~1000)
  - No BME
- Endpoint (PrestoBlue). Collected data at T0 (baseline) and early (96h) and late (144 h) time points post test agents addition
- Four technical replicates; 2 biological replicates
- Automated dispensing into 384 well plates
- Automated drug addition into 384 well plates

Acknowledgments

We are grateful to Stephen White, Alex Martinkosky and John Britt for supply and preparation of the drugs.

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. This research was supported, in part, by the Developmental Therapeutics Program in the Division of Cancer Treatment and Diagnosis of the National Cancer Institute. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government

Leidos Biomedical Research, Inc.

Description of in vitro and in vivo models

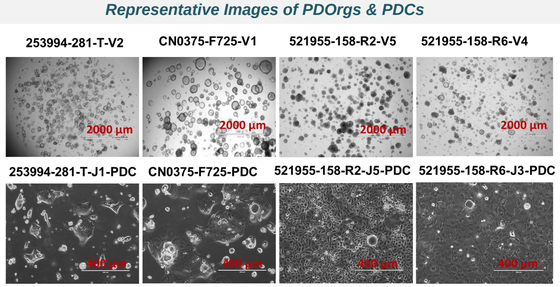
Tumor type	PDXs	PDCs	PDOrgs
Colon adenocarcinoma	135848-042-T		135848-042-T-V1
	199195-117-R		199195-117-R-V2
	CN0375-F725*†	CN0375-F725-PDC	CN0375-F725-V1
Colorectal cancer	253994-281-T	253994-281-T-J1-PDC	253994-281-T-V2
Rectal adenocarcinoma	945468-187-R	945468-187-R-J2-PDC	945468-187-R-V1
Pancreas adenocarcinoma	292921-168-R	292921-168-R-J2-PDC	292921-168-R-V1
	521955-158-R2	521955-158-R2-J5-PDC	521955-158-R2-V5
	521955-158-R6	521955-158-R6-J3-PDC	521955-158-R6-V4
	K24384-001-R*	K24384-001-R-PDC	K24384-001-R-V2
Uterine carcinosarcoma	327498-153-R		327498-153-R-V1
Non small cell lung cancer	349418-098-R	349418-098-R-PDC	349418-098-R-V2
	LG0567-F671*†	LG0567-F671-PDC	LG0567-F671-V1
Lung adenocarcinoma	952719-076-R		952719-076-R-V1
Head and Neck Cancer - Squamous	945586-337-R		945586-337-R-V1
Urothelial/Bladder carcinoma	BL0293-F563*	BL0293-F563-PDC	BL0293-F563-V1
	BL0382-F1232*		BL0382-F1232-V1

Patient-derived organoid models (PDOrgs) and in vitro patient-derived tumor cell cultures (PDCs) were derived from either patient or PDX material, with at least one of the PDCs derived from PDOrg material. PDCs were cultured as adherent cells, while PDOrgs were grown embedded in basement membrane extract (BME). Information on these models can be found on the **NCI Patient-Derived Models Repository (PDMR) website** https://pdmr.cancer.gov. *These PDX models originally developed by Jackson Laboratories. †These PDX models only available from Jackson Laboratories.

Parameters Evaluated In vitro

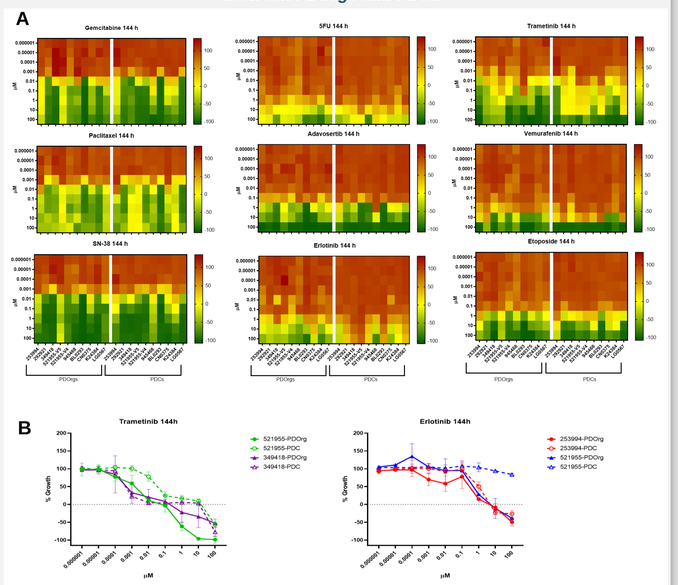
- **% Growth (% G):** evaluates the effect of test agents on models' growth, compared to controls and including time zero values.
  - If T0 values < Treated values
    - $(Mean\ Treated - Bkg) - (Mean\ T0 - Bkg)/(Mean\ Ctrl - Bkg) - (Mean\ T0 - Bkg)$
  - If T0 values > Treated values
    - $(Mean\ Treated - Bkg) - (Mean\ T0 - Bkg)/\ (Mean\ T0 - Bkg)$
- **Fifty Percent Growth inhibition (GI50)**: concentration of test agent resulting in 50% growth inhibition ➔ $100\ x\frac{Mean\ Treated - Mean\ T0}{Mean\ Ctrl - Mean\ T0} = 50$
- **Total Growth Inhibition (TGI)**: concentration of test agent resulting in 100% growth inhibition (cytostatic effect) ➔ $100x\frac{Mean\ Treated - Mean\ T0}{Mean\ Ctrl - Mean\ T0} = 0$
- **Area Under the Curve (AUC)** ➔ $\sum_{1\ \_\ n} = \left\{\frac{\%G1+\%G2}{2} * (Conc2 - Conc1)\right\} + \left\{\frac{\%G2+\%G3}{2} * (Conc3 - Conc2)\right\} + \ldots.$

Representative Images of PDOrgs & PDCs



Brightfield images of representative PDOrgs and phase-contrast images of corresponding PDCs (EVOS® FL Cell Imaging System)

Pharmacological evaluation shows similar overall drug response profiles between PDOrgs and PDCs



PDOrgs and PDCs were exposed to the indicated compounds for 144 hours. Fig. A. Heat maps represent mean percentage growth of at least 2 independent experiments (normalized by T0 and vehicle-treated controls). In most cases, both PDOrgs and PDCs had similar drug response profiles to individual compounds, with the exception of PDOrgs showing overall increased sensitivity to trametinib and erlotinib compared to PDCs.
Fig. B. Representative dose response curves (Average +/- SD), of at least 2 independent experiments, demonstrate differential sensitivity to trametinib and erlotinib.

Significant correlation observed between drug response profiles of 6/10 paired PDC and PDOrg models independent of drug tested

A

Models (PDOrgs vs PDCs)	R Squared	P value
253994-281-T	0.7453*	0.0027*
292921-168-R	0.6926*	0.0054*
349418-098-R	0.8139*	0.0009*
521955-158-R2	0.1694	0.2711
521955-158-R6	0.5425	0.4625
945468-187-R	0.5347*	0.0252*
BL0293-F563	0.5267*	0.0269*
CN0375-F725	0.395	0.0699
K24384-001-R	0.5248*	0.0273*
LG0567-F671	0.3711	0.0816

B

	Metrix Compared (PDOrgs vs PDCs_144h)	Gemcitabine	Paclitaxel	SN-30	5-FU	Adavosertib	Erlotinib	Trametinib	Etoposide	Vemurafenib
R squared	GI50	0.173	0.8903*	0.4633	0.1665	0.2371	0.02172	0.0003	0.0133	0.1114
	TGI	0.03036	0.8224*	0.0353	0.2741	0.0699	0.01251	0.1768	0.8224*	0.1063
	AUC	0. 1342	0.0288	0.0429	0.0295	0.0054	0.015	0.1196	0.0288	0.0211
P Value	GI50	0.2301	<0.0001*	0.0148	0.1879	0.1084	0.6476	0.7508	0.7513	0.3459
	TGI	0.2504	0.0003*	0.5585	0.0806	0.4063	0.7293	0.1736	0.0003*	0.358
	AUC	0.2978	0.639	0.5179	0.5933	0.8213	0.7008	0.2708	0.639	0.6886

Fig A. Regression analysis was used to compare the AUC of matched PDCs and PDOrgs, revealing correlation between 6/10 paired models. Significance *p <0.05.
Fig. B. GI-50, TGI, and AUC were evaluated. Grouping models as PDCs and PDOrgs and evaluating drug response to a single agent, showed no correlation between the 2 groups, except for paclitaxel and etoposide. Significance *p <0.05, where correlation is driven by a single data point.
Data suggest that overall similarities in drug response profiles between the panel of PDCs and PDOrgs (as noted in heat maps above) may be driven by inherent similarities in the pharmacological profile of paired models.

Parameters Evaluated In vivo

Bin 1	Complete Regression Achieved >1 timepoint (<60mg)
Bin 2	Tumor regressed ~30%, >1 timepoint, durable response (0.5-1 cycle)
Bin 3	Tumor regressed ~30%, >1 timepoint, regrew at drug removal
Bin 4	Stable, durable response (0.5-1 cycle)
Bin 5	Stable, regrew at drug removal
Bin 6	Slowed but progressive growth
Bin 7	Grew at Same Rate as Control

- **Optimal % Delta Treated/Control (Opt. % T/C)** ➔ $\left[\frac{Treated\ Tn - Treated\ T0}{Control\ Tn - Control\ T0}\right] * 100$
- **Percentage growth inhibition compared to control** ➔ $100 - \left[\frac{AUC\ Treated}{AUC\ Ctrl} * 100\right]$
- **Relative median to event free [tumor volume quadrupling] survival (RMEFS)**➔ ratio of the median time to event between treated and control groups, where an event is the quadrupling of tumor volume

PDX responses were ranked based on qualitative assessment of treatment responses (visual binning) as defined above, Opt. %T/C calculated as median treated tumor weight divided by median control tumor weight obtained while at least 50% of the tumored animals were alive in each group expressed as a percentage, % growth inhibition (%GI) calculated as percent area under the fitted tumor volume-time curve (AUC) for each treatment group relative to control animals out to timepoint where control animals were terminated, and relative median to event free survival (RMEFS).

Ranking PDX Responses

Paclitaxel

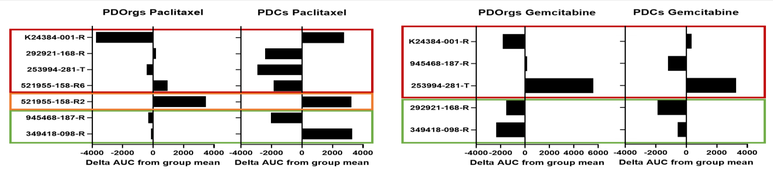
Model	Binning	RMEFS	Optimal % Delta T/C (Day)	Growth Inhibition (%)	Code
349418-098-R	1	1.6	-32% (22)	70.6	R
945468-187-R	4	2.2	5% (18)	58.9	R
521955-158-R2	4	1.2	-27% (21)	27.2	IR
521955-158-R6	6	1.4	33% (7)	41.8	NR
253994-281-T	7	1.2	57% (8)	42	NR
292921-168-R	7	1.3	56% (3)	21.6	NR
K24384-001-R	7	1	84% (29)	4.9	NR

Gemcitabine

Model	Binning	RMEFS	Optimal % Delta T/C (Day)	Growth Inhibition (%)	Code
349418-098-R	2	1.3	-33% (19)	68.8	R
292921-168-R	4	1.7	-22% (3)	60.5	R
253994-281-T	6	1.8	21% (8)	47.2	NR
945468-187-R	6	1.5	24% (11)	58.7	NR
K24384-001-R	7	1.2	54% (12)	23.1	NR

PDX responses were ranked based on visual binning, RMEFS, best Opt.% T/C (day it was achieved), and %GI. Cut offs are: binning 1-4, RMEFS>1.5, Opt.% T/C<0, and % GI >50. Models displaying at least 3 parameters above cut offs are considered responsive (R). Models displaying 2/4 parameters above cut offs are considered intermediate responders (IR). All other models are considered non-responders (NR). Green highlights indicate parameters above the cut offs. Paclitaxel and Gemcitabine had the largest number of matched models (PDX-PDOrg-PDC), thus allowing for comparisons.

Pharmacological profiles of PDOrgs and PDCs are partially in line with in vivo data



Mean graphs were plotted using AUC, 144h values from dose response data available for PDOrgs and PDCs. The vertical line represents the mean response of all the models in the panel to a given agent. Negative and positive numbers represent increased and decreased sensitivity respectively, compared to the mean of the group. Green, orange and red boxes highlight PDOrgs and PDCs with corresponding PDXs defined as responders, intermediate responders or non-responders, respectively.

Conclusions

- Pharmacological profiles were assessed in 10 paired PDOrgs and PDCs.
- 6/10 paired PDOrgs and PDCs had significantly correlated drug responses.
- Overall, PDOrgs and PDCs had similar drug response profiles, with the exception of PDOrgs being generally more sensitive to trametinib and erlotinib.
- However, unlike paired samples, grouping models as PDOrgs and PDCs showed little correlation between groups in response to drug tested. This suggests that the overall congruence between PDC and PDOrg drug response profiles is due to similarities between paired models and that a more statistically powerful dataset is needed to observe drug-dependent correlation between PDOrgs and PDCs.
- Both PDOrgs and PDCs partially recapitulated PDX responses to paclitaxel and gemcitabine.