Enhancing Drug Discovery & Development Throughput Without Sacrificing Predictivity: ex vivo 3D Drug Response Profiling Using PDX

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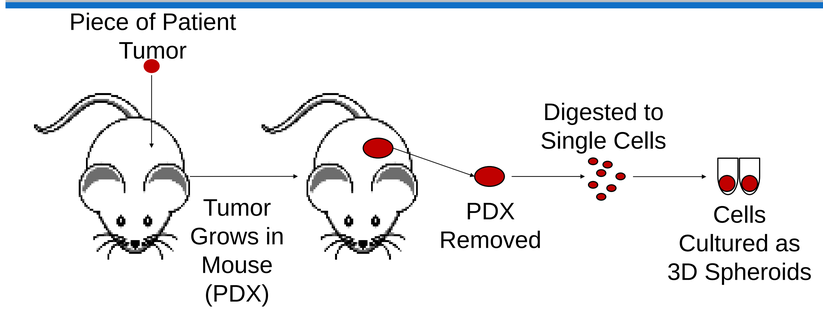
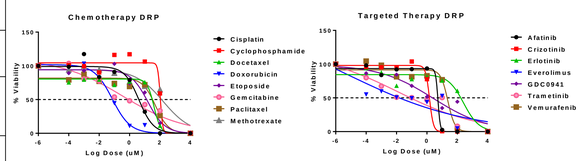
Background

Patient-derived xenografts (PDX) have become critical elements of preclinical drug development as they better reflect the heterogeneity, molecular and histopathologic signatures of the original tumor than cell lines or genetically engineered mouse models, and their drug response profiles (DRP) correlate with clinical response. While PDX models have become a powerful tool in drug discovery and development, limitations include low throughput for broad drug screening, lack of dose-response curves, high cost and progressive loss of human-derived stromal elements over serial passages, restricting utility for certain therapeutic classes. A potential mechanism to overcome the low throughput and high cost of PDX models is the incorporation of ex vivo 3D (EV3D) DRP on cells isolated from early passage PDX models. Thus, we correlated DRP results using PDX with genetic mutations and drug response of PDX tested in vivo.

Methods



Study Metrics	2D	EV3D DRP	In Vivo PDX
# Models*	6	6	6
Total # Drugs Tested	12	90	18
Assay Success**	6/12	87/90	N/A



Pieces of tumors removed from patients were grafted onto mice and allowed to grow. When tumors reached a specific size, they were removed, cut into small pieces and grafted onto a new mouse for propagation of the tumor. After reaching a specified size, these tumors were removed, enzymatically digested into single cells and cultured in KIYATEC's EV3D™ DRP platform. Ex vivo PDX cultures were treated with 15 clinically relevant chemotherapy and targeted agents. Viability was assessed and dose-response curves were generated using nonlinear regression. Relative IC_{50} (Concentration at which 50% of cells were viable) were generated for all compounds and results were compared to tumor volume measurements of matched PDX and compared with genetic mutations of primary tumor tissue and clinical outcomes after targeted therapy. *2 breast, 2 bladder, and 2 lung. **Assay success defined as statistically significant differences between positive and negative controls.

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EV3D DRP Mimics In Vivo Response

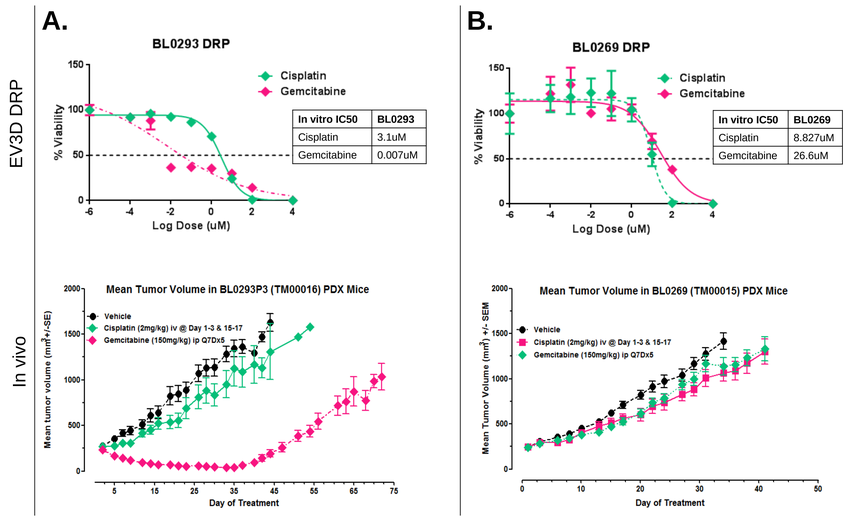


Figure 1: EV3D DRP predicts cisplatin and gemcitabine response *in vivo* for bladder PDX. Cells were isolated from 2 bladder PDX (BL0293 and BL0269) and treated with cisplatin or gemcitabine for 96 hours. (**A**) IC_{50} for BL0293 predicted response to gemcitabine over cisplatin. *In vivo* results correlated to DRP with response to gemcitabine while cisplatin showed no response compared to vehicle. (**B**) IC_{50} for BL0269 predicted no significant differences in response to cisplatin or gemcitabine which was also seen *in vivo*.

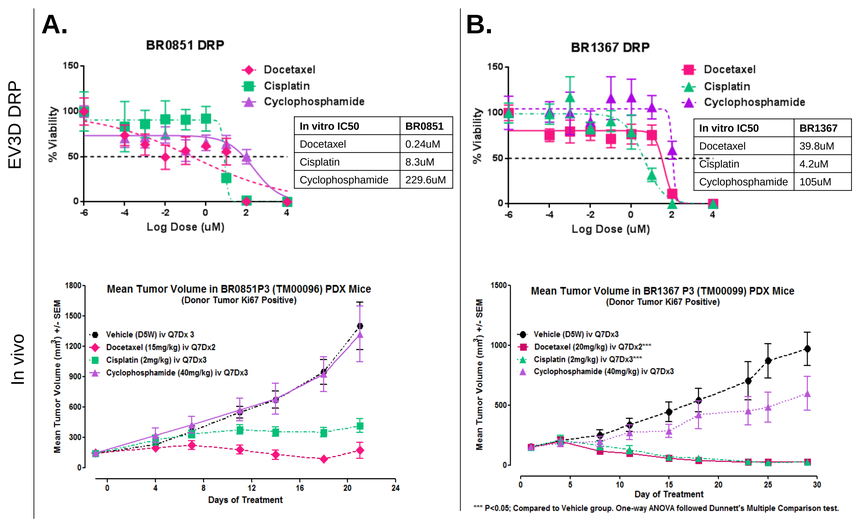
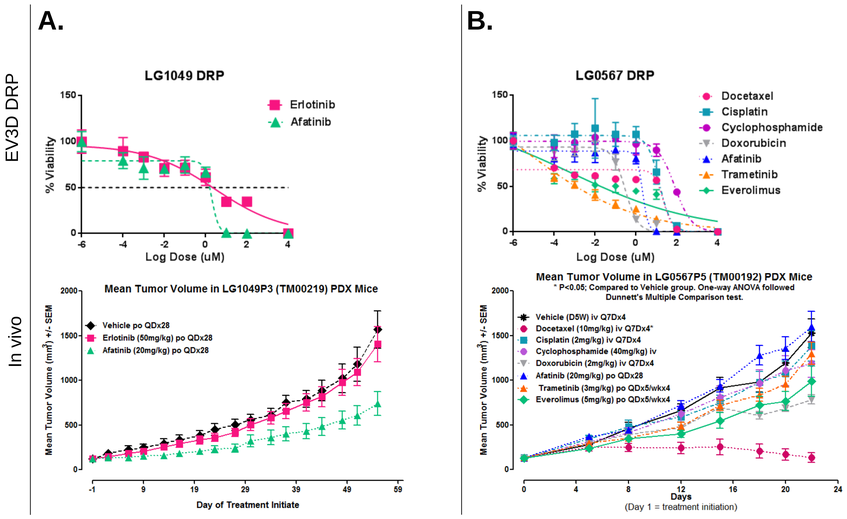


Figure 2: EV3D DRP predicts docetaxol, cisplatin, and cyclophosphamide response *in vivo* for breast PDX. Cells were isolated from 2 breast PDX (BR0851 and BR1367) and treated with docetaxol, cisplatin, or cyclophosphamide for 96 hours. (**A**) IC_{50} for BR0851 predicted response to docetaxol and cisplatin, but not cyclophosphamide. *In vivo* results correlated to DRP with greater response to docetaxol than cisplatin and no response to cyclophosphamide compared to vehicle. (**B**) IC_{50} for BR1367 predicted response to cisplatin and a better response to cyclophosphamide. *In vivo* results correlated with the cisplatin and cyclophosphamide results.

EV3D DRP Correlates with Genetics



EGFR e19del, T790M

KRAS G12C

	IC50 (uM)	
	Erlotinib	Trametinib
LG1049 (EGFR e19del, T790M)	2.869	.00011
LG0567 (KRAS G12C)	38.31	6.341×10^{-6}

Figure 3: EV3D DRP predicts erlotinib resistance and trametinib sensitivity for lung tumors. Cells were isolated from 2 lung PDX (LG1049 and LG0567) and treated with erlotinib or trametinib for 96 hours. (**A**) IC_{50} for LG1049 predicted resistance to erlotinib. *In vivo* results correlated to EV3D DRP with no response to erlotinib compared to vehicle. Importantly, LG1049 harbors an EGFR T790M mutation that often confers resistance to erlotinib. ***This lack of response correlated with the patient's resistance to erlotinib***. (**B**) IC_{50} for LG0567 predicted response to trametinib. However, in vivo PDX treatment did not. LG0567 harbors a KRAS mutation, G12C that may confer sensitivity to trametinib.

Conclusions

- EV3D cultures can be readily generated from primary PDX tumors with a 98% success rate (as compared with 50% in 2D)
- Ex vivo Drug Response Profiling (EV3D™ DRP) correlated with in vivo PDX drug testing for approximately 79% of drugs tested indicating the feasibility and reliability of the DRP and the ability to generate larger data sets over a shorter time period
- EV3D DRP using PDX may be a powerful pre-screening tool to identify the most active therapeutic or combination with *in vivo* confirmation of activity

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