3202. Reconstructed Plate-Based 3D Screening Assays That Predict *In Vivo* Responses

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Introduction

- Labcorp has employed 3D model systems co-developed with zPREDICTA® to create preclinical models to address the increasing relevance of 3D *in vitro* models.
- These organ-specific models support tumor cell growth and mimic the composition and architecture of both solid tumors and hematological malignancies.
- While 2D *in vitro* assays are preferred for screening efforts of chemical libraries, mainly because of cost, they do not recapitulate the tumor architecture and the microenvironment that is more comparable to *in vivo* models. This results in differences in pharmacological potencies of standard of care (SoC) agents when compared to the 3D and *in vivo* models.
- Here we have used zPREDICTA® 3D culture models as a tool for *in vitro* screening of tumor cell lines and compare the response of the model in 2D, 3D and *in vivo* to standard of care agents. Specifically, the 3D *in vitro* system correlates well with breast and lung carcinoma *in vivo* models when matching rank order of potencies of known SoC agents.
- These 3D platforms are predictive and relevant at the same time.

Methods

- MDA-MB-231 or 4T1-Luc or NCI-H460 were implanted subcutaneously into the right axilla of female nude mice (Envigo). Standard of care agents were procured from approved vendors and administered as indicated (dosed daily or twice per week for two or three weeks starting the day of staging). Tumor progression was monitored by digital caliper.
- All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria on all studies.

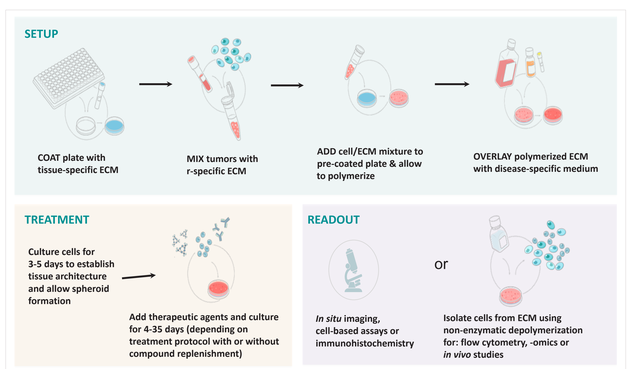
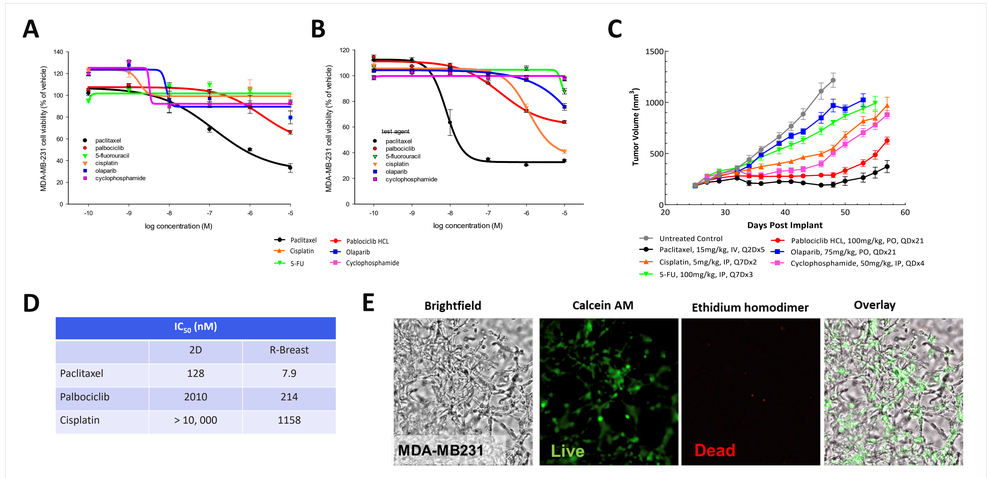
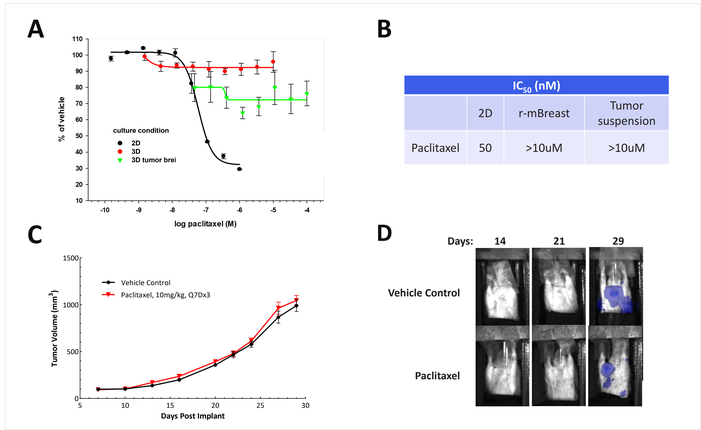


Figure 1. Overview of 3D culture workflow. Schematic representation of 3D culture plate setup, treatment and readout capabilities.



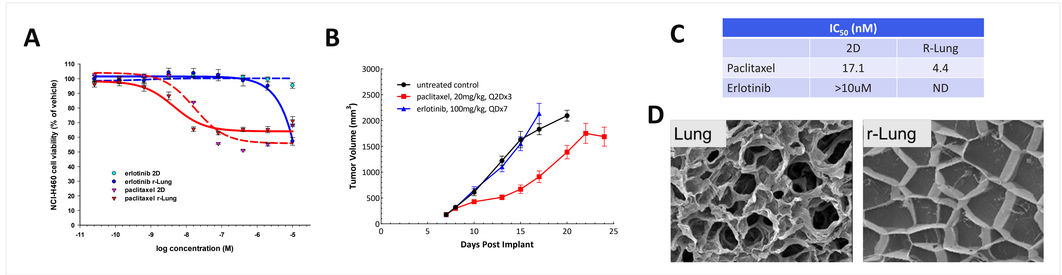
IC_{50} (nM)	2D	R-Breast
Paclitaxel	128	7.9
Palbociclib	2010	214
Cisplatin	> 10, 000	1158

Figure 2. Response to the standard of care drugs in the human r-Breast cultures correlates with *in vivo* models. CellTiter-Glo analysis for standard of care agents against MDA-MB-231 cells in 2D (A), 3D r-Breast matrix (B) and *in vivo* response in NSG mice (C) is shown. The 3D response for all the tested compounds compares to the *in vivo* response. The IC_{50} values for the 2D and 3D are shown in Table D. Micrograph of MDA-MB231 untreated cells grown in 3D r-Breast matrix stained at 13 days.



IC_{50} (nM)	2D	r-mBreast	Tumor suspension
Paclitaxel	50	>10uM	>10uM

Figure 3. Response to the SoC in the mouse r-mBreast cultures correlates with *in vivo* models. CellTiter-Glo analysis for paclitaxel (A) against murine breast cancer 4T1-Luc cells in 2D, 3D r-mBreast matrix and with tumor cell suspension is shown. Paclitaxel treatment in 2D shows cytotoxicity, but in r-mBreast 3D matrix when cells or tumor suspension is used, there is minimal cytotoxic effect. *In vivo*, treatment with paclitaxel as a single agent shows to be ineffective in decreasing 4T1 tumor growth in Balb/c mice (C). Shielded Bioluminescence imaging (BLI) of animals shows metastasis of 4T1-luc tumors to the lungs (D).



IC_{50} (nM)	2D	R-Lung
Paclitaxel	17.1	4.4
Erlotinib	>10uM	ND

Figure 4. NCI-H460 response to the standard of care drugs in the human r-Lung cultures correlates with *in vivo* models. CellTiter-Glo analysis of paclitaxel and erlotinib cytotoxicity on NCI-H460 human NSCLC cells assayed in 2D and 3D (A) and *in vivo* tumor burden (B). Paclitaxel treatment of NCI-H460 in 2D and 3D r-Lung (A) showed similar cytotoxic effect that was also recapitulated in the *in vivo* tumors in female nude mice (B). Erlotinib in 2D had no cytotoxic effect at tested concentration and in 3D on r-Lung matrix had a cytotoxic effect at a higher concentration. *In vivo*, erlatonib at 100mg/kg did not show much of a response; however, 2/10 mice exited the study due to body weigh loss >20%. Control animals on the other hand showed progressive body weight increase with tumor progression. IC_{50} values are shown in Table C. Scanning cryo-electron micrograph of human lung matrix (left) and 3D matrix (right) show similar architecture.

Results and Conclusions

- zPREDICTA® tumor-specific 3D systems as well as 2D cultures and *in vivo* models were used to compare and contrast the activity of a set of anticancer agents.
- Human MDA-MB-231 cells were sensitive to paclitaxel both in 2D and in the 3D r-Breast as well as *in vivo*, with complete growth inhibition at the dose of 10mg/kg and 15mg/kg.
- Paclitaxel was cytotoxic in 2D cultures of murine 4T1-Luc breast tumor cells (IC_{50}=59nM), but not in the 3D reconstructed mouse breast model (r-mBreast) or *in vivo*, where the syngeneic 4T1 model was refractory to paclitaxel at 10mg/kg and 15mg/kg (not shown).
- Similarly, NCI-H460 cells were sensitive to paclitaxel in 2D (IC_{50}=2.3nM), while minimal response was seen in the 3D r-Lung model, and *in vivo* there was minimal response seen at a 20mg/kg treatment. These same cells were refractory to erlotinib in 2D, 3D and *in vivo*.
- Additional SoC agents are being profiled in these and other models. Taken together, these data demonstrate that zPREDICTA's organ-specific 3D models mimic *in vivo* response with a higher fidelity than the standard 2D cultures.
- Additional *in vitro* reconstructed 3D histotype models are being developed and validated with the goal of providing faster and more predictive *in vivo* results.
- Labcorp is now offering human and exclusively developed mouse 3D models in collaboration with zPREDICTA.
- Multiple types of therapeutic agents can be screened in 3D models, including small molecules, antibodies and CAR T cells. Multiple endpoints can be measured in these *in vitro* assays.

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