

Summary

We develop patient-derived organoid (PDO) models that represent the large spectrum of patient tumors. We describe here a breast cancer organoid model that is HER2 and progesterone negative and estrogen receptor positive that we use to test and estimate the IC₅₀ values of the various known and novel drugs. In separate experiments we administered the drugs for 2, 3 or 4 days. A Glow 3D ATP assay was used to assess cell viability post treatment with drug. The size and condition of the organoids were monitored daily by phase contrast microscopy prior to and during the cytotoxicity assays. The assays were repeated at 2 or 3 different times in which either smaller or larger sized organoids were tested. We observe that the IC₅₀ of Doxorubicin (DOX) was about 5-fold lower than Taxol (TAX) when smaller sized organoids were tested. This correlates well with the clinical standard practice where Taxol is used at several-fold higher doses compared to DOX. As expected, Anastrozole (an aromatase inhibitor that converts testosterone into the estrogen) that was used as a negative control, did not show any cytotoxicity. In the subsequent experiments we tested the same drugs and additional known and novel drugs at 9 concentrations per drug. The nano-formulated DOX showed exactly the same cytotoxic activity as DOX alone. Fulvestrant (an estrogen inhibitor) showed little or no cytotoxic activity. A novel antibody-drug conjugate (ADC) also showed low cytotoxic activity despite the fact the cells expressed the target molecule and tumor inhibition was observed in vivo in xenografts reflecting that either higher concentrations were needed in vitro or that additional immune components such as engineered T cells and macrophages may enhance the in vitro activity of this ADC. Our comparative study with various known and novel drugs reveal that organoids can be used for screening libraries of drugs and anti-cancer biologicals. The ability to derive PDO's from patient tumors and the availability of a large array of PDO's that represent the several classes and subtypes of cancers will enhance both drug development and will be key in personalized medicine and is our quest.

Methods

Organoids were plated in InSphero AkuraTM 96 Spheroid Microplates at 2000-2500 cells per well. The drugs were administered in 3 separate experiments for 2, 3 or 4 days. A Glow 3D ATP assay was used to assess cell viability post treatment with each drug. The size and condition of the organoids were monitored daily by phase contrast microscopy prior to and during the cytotoxicity assays. The assays were repeated at 2 or 3 different times in which either smaller or larger sized organoids were tested. The luminescence was measured with the Molecular Devices SpectraMax M4 plate reader.

Results

Comparison of the Sizes of Organoids

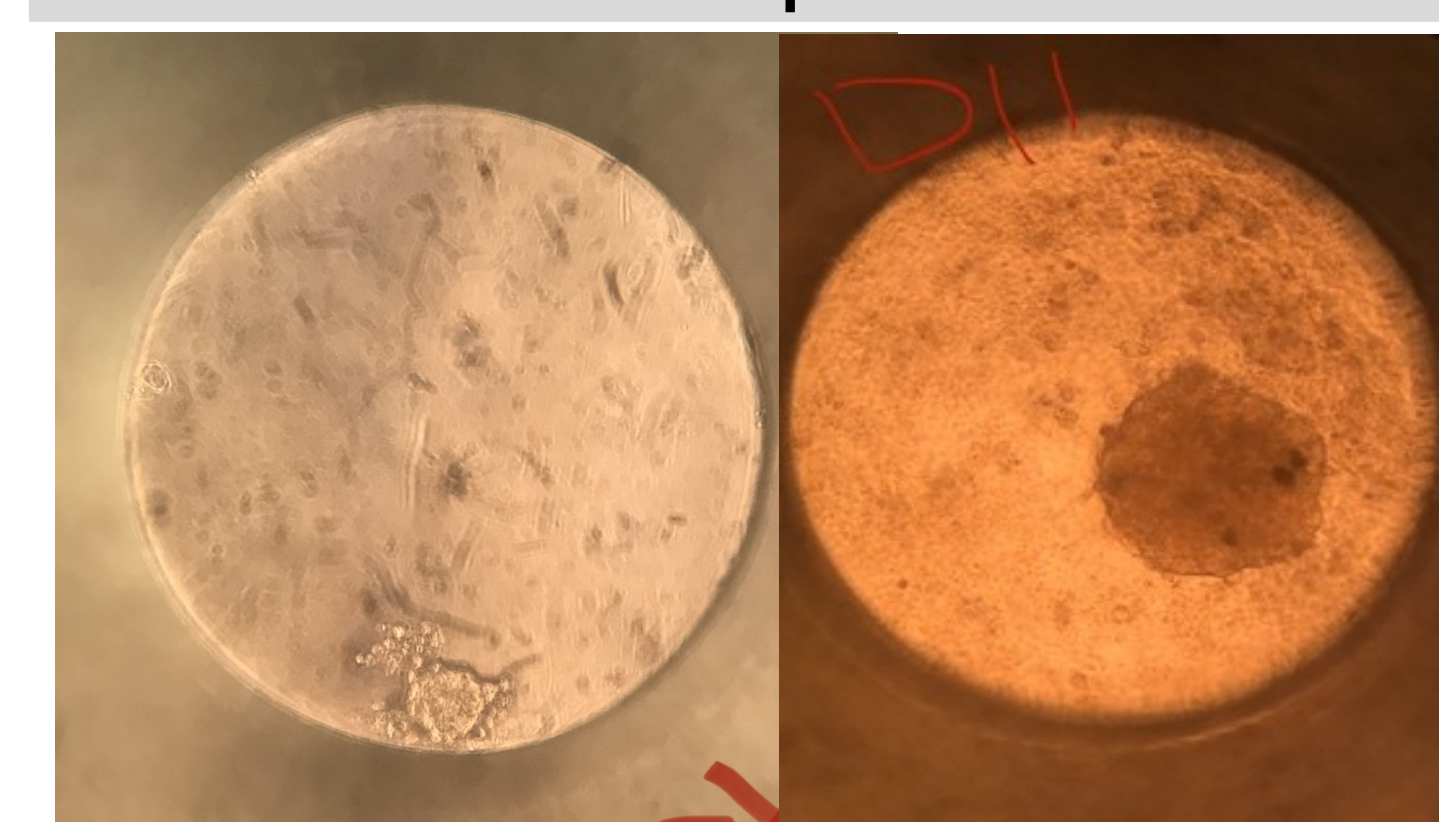


Figure 1. Comparison of the sizes of organoids in which 2 different organoid sizes were tested. The size (measured by area, not volume) of the later tested organoids was about 9-fold larger. IC₅₀ for Dox was about 5-fold lower with a smaller organoid size.

Viability

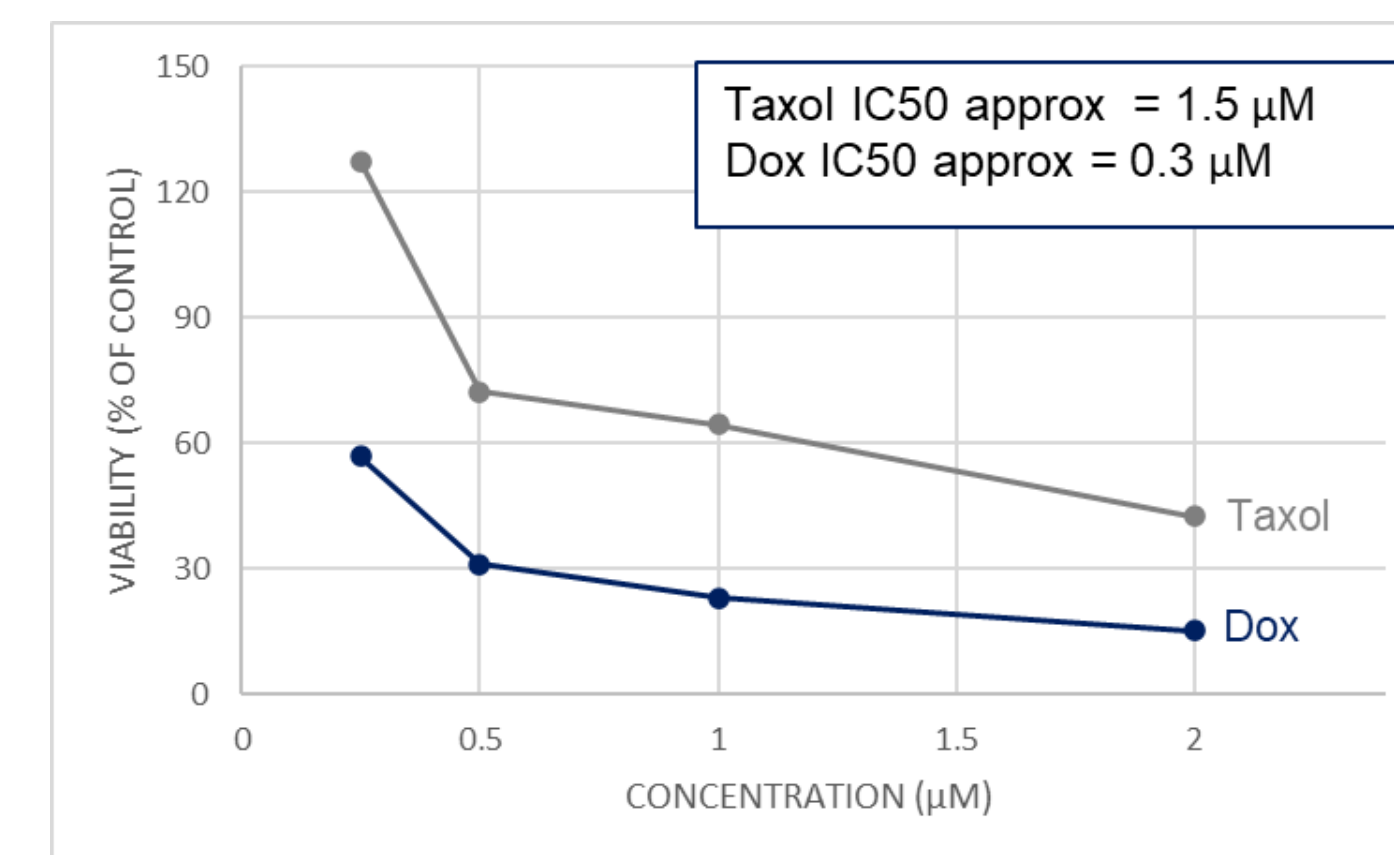


Figure 2. Experiment 1, N=1
 Comparison of cell viability post treatment with Taxol and Doxorubicin. IC₅₀ for Doxorubicin was about 5-fold lower compared to Taxol.

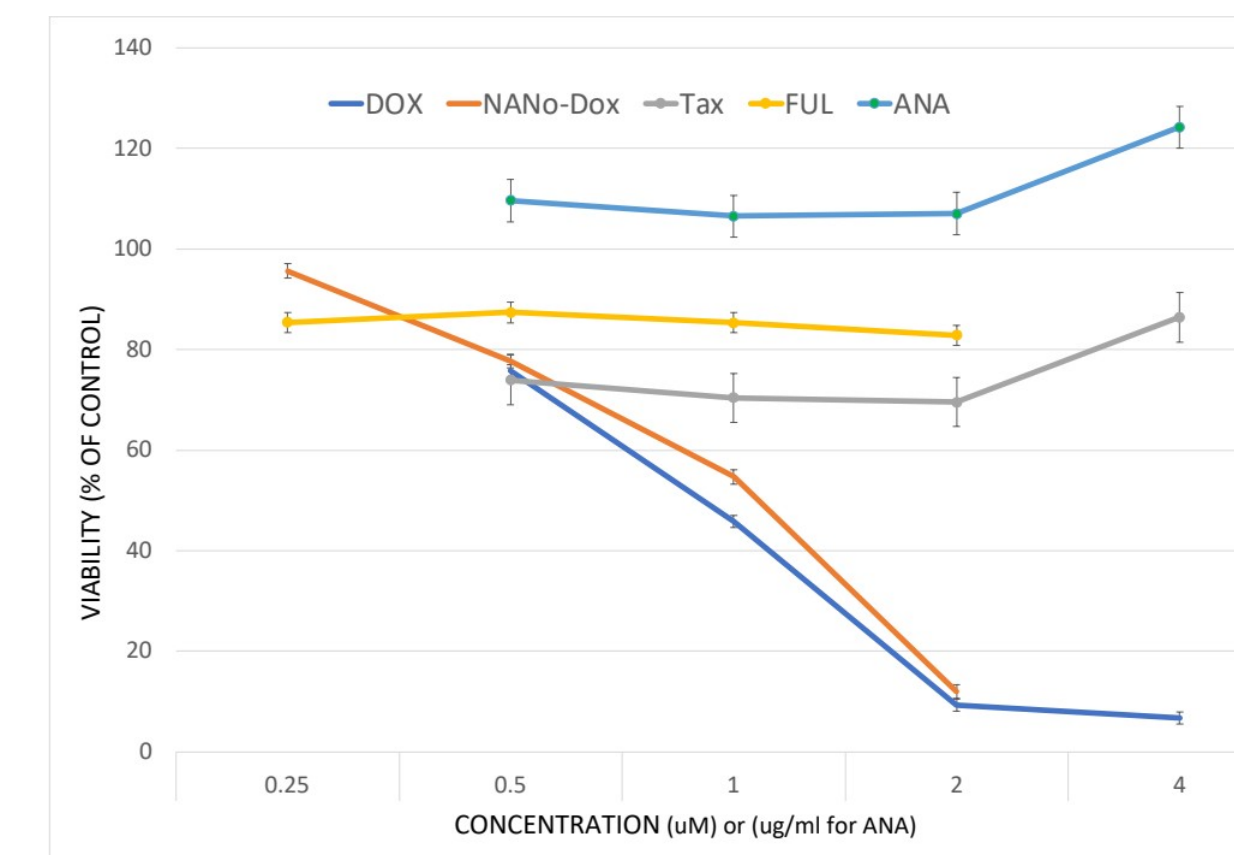


Figure 3. Experiment 2, N=2
 Comparison of cell viability of Anastrozole, Doxorubicin, nano-Doxorubicin, Taxol, and Fulvestrant (FUL). DOX and nano-DOX showed equal activity and both were more cytotoxic than Taxol followed by Anastrozole and Fulvestrant.

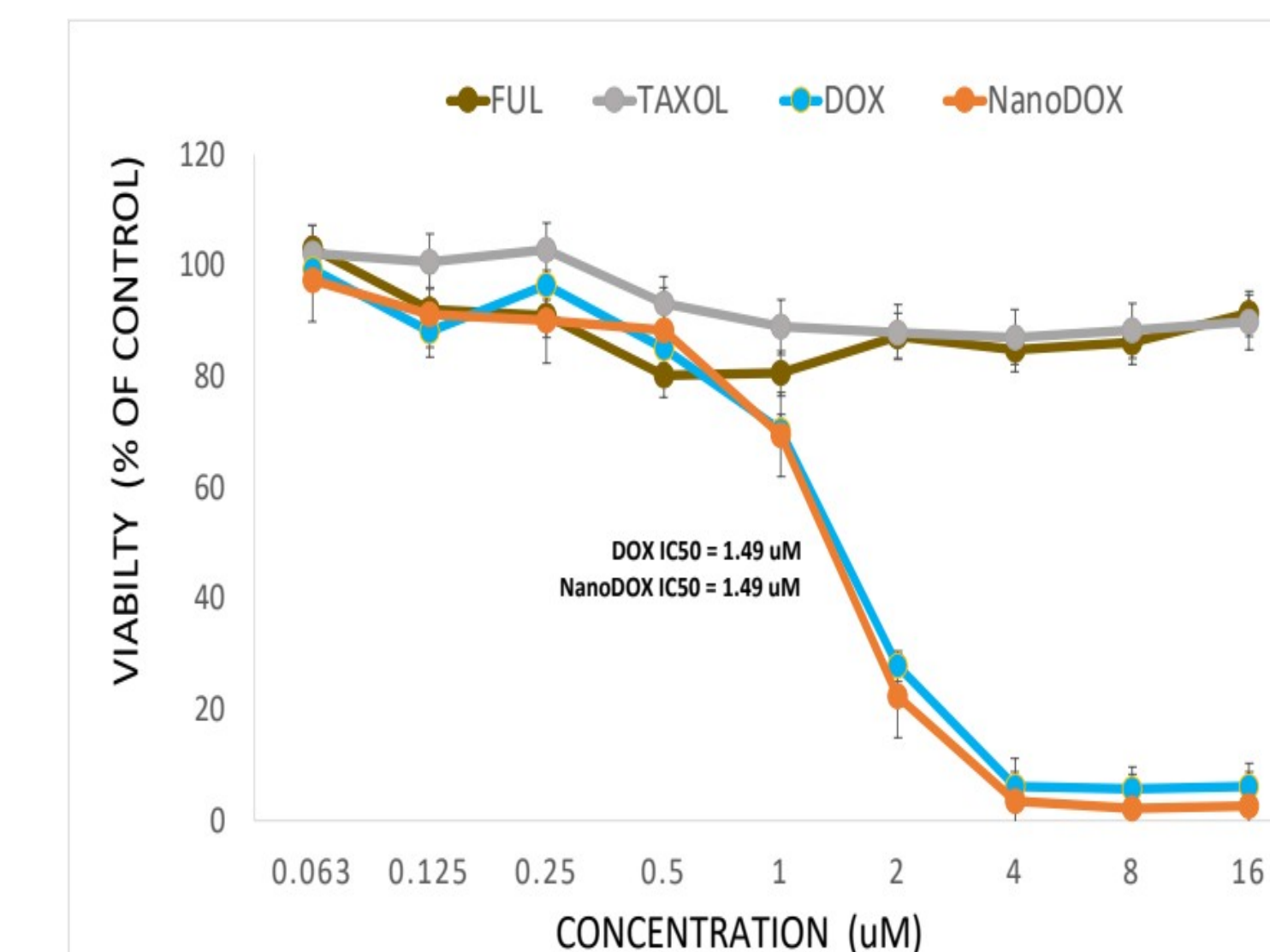


Figure 4. Experiment 3, N=3.
 Comparison of Dox, Nano-Dox, FUL and Taxol Organoid cell viability. The IC₅₀ was identical for Dox and NanoDOX drugs. Even though there is no increased cytotoxicity of the Nano-Dox over the conventional Dox drug, this formulation was designed to increase a passive targeting of the drug to the tumor and thus should be still superior over the non formulated DOX. Fulvestrant and Taxol did not show an evident dose-response compared to Doxorubicin.

Breast Cancer Organoid Model

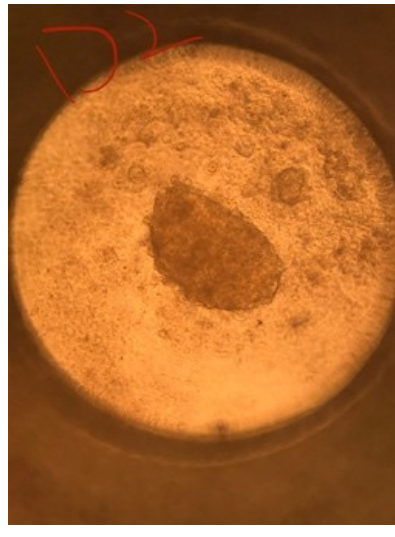
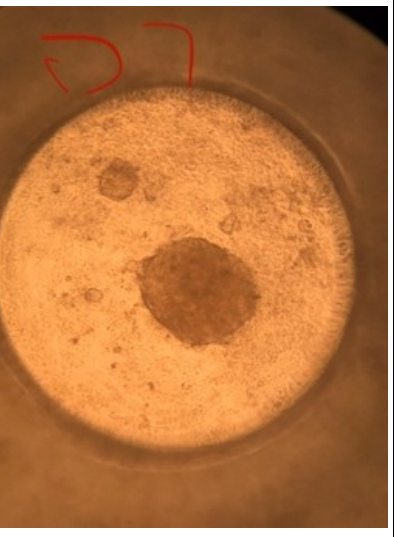
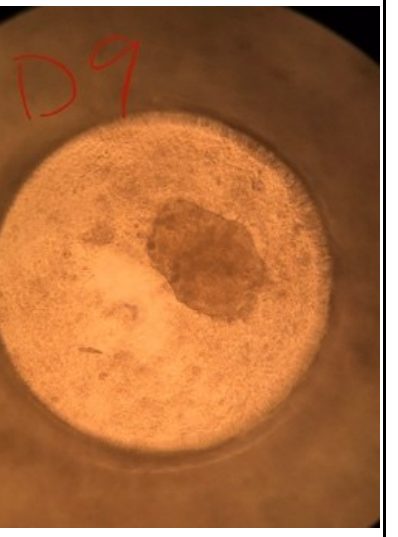
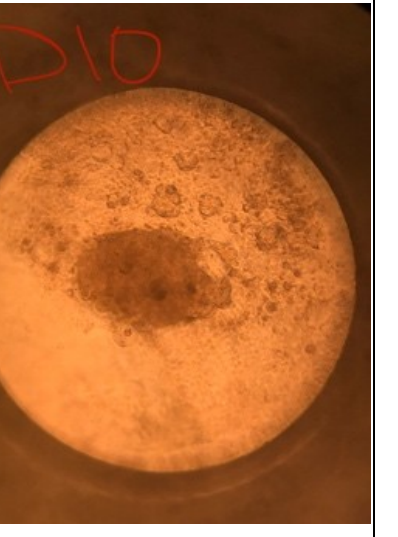
Drug:		Day 3 (Before 1st Dosing)				
		Concentrations Per Well (uM)				
		0	0.5	1	2	4
Dox alone						
		Viability 100%	Viability 67.77	Viability 39.7%	Viability 13.2%	Viability 7.8%

Figure 5. Images of organoids treated with Dox. Loss of the organoid membrane integrity and organoid size shrinkage is observed at the higher concentrations on Day 4 of drug treatment.

Organoid Cells Immunohistochemistry

The novel ADC did not show significant cytotoxicity effect whereby highest dose tested (2uM) caused only 20% decrease in cell viability. The antibody alone did not show any cytotoxicity whereas the drug alone only showed a 20% overall decrease in cell viability (data not shown).

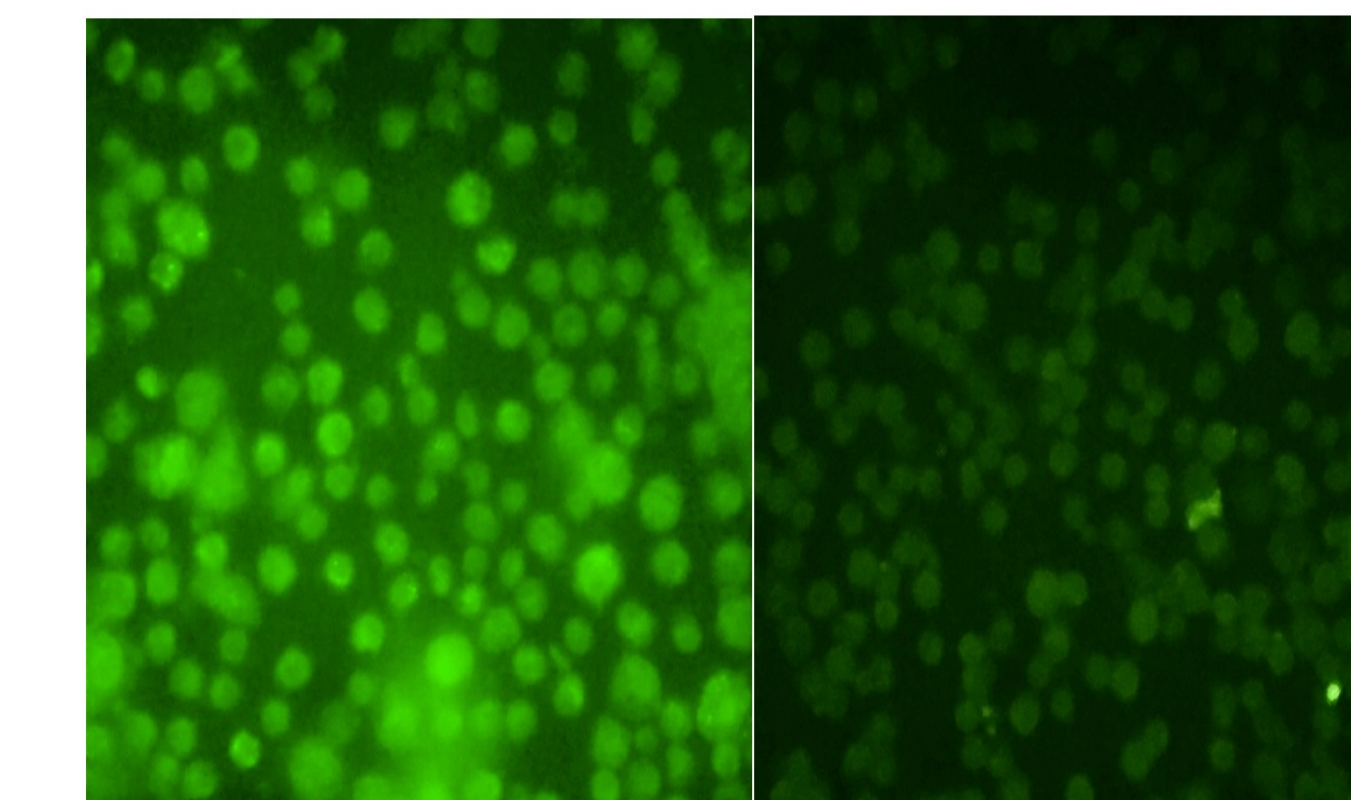


Figure 6. Breast cancer organoid cells stained with antibodies against the target molecule of Antibody-Drug Conjugate (ADC) by indirect immunofluorescence staining with FITC conjugated anti-mouse IgG1 secondary antibody (Left Panel). Right panel shows the breast cancer organoid cells stained with FITC conjugated to CD45 (a marker for all nucleated hematopoietic cells). Identical lack of staining similar to this image were seen with an isotype control IgG1 antibody (negative control), FITC- anti CD3, CD4 and CD8. 40X Magnification.

Conclusions

- 1) We have developed and standardized assays for testing anti-cancer agents. This assay can be used for the high throughput testing of novel and established drugs and combinations of drugs and may also be used for selection of the most effective drugs for the individual patients.
- 2) Smaller sized organoids may be useful for quick screening and high throughput testing of libraries of small amounts of drugs to select lead candidates.
- 3) Larger sized PDOs can be used for more precise selection of the drug sensitivity/resistance for a particular patient.
- 4) The new models of organoids supplemented with blood and lymphatic vessels will allow to adopt the model to the enhanced delivery anti-cancer drug testing (Bogseth et al., 2022).
- 5) Doxorubicin consistently showed cytotoxicity and can be used as a positive control in organoid studies.
- 6) Our data using an Fulvestrant against an estrogen receptor positive PDO reveals that identification of genetic tumor biomarkers maybe not sufficient for selection of appropriate drugs or drug-combinations for the treatment of patients as the drug resistance can be developed in a patient even with a marker present.

Future Directions

1. We continually develop novel PDO models representing a large array of tumor classes and subclasses. In particular, we will develop PDO models that carry genetic markers that are found in human cancers and those that show different drug sensitivities/resistances.
2. We will develop more complex models where the tumor cells can be supplemented with immune cells (e.g., T-cells, macrophages, endothelial cells, growth factors, etc.).
3. We will supplement culture medium models with testosterone to create a model for testing of aromatase inhibitors.

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- (1) Bogseth A, et al. (2022). In vitro models of blood and lymphatic vessels—Connecting tissues and immunity. *Advanced Biology*. 41, 1-19.
 (2) Yao Y, et al. (2020). Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. *Front Mol Biosci*, 7, 193.