

Ultra-High Throughput Screening to Assess Oncologic Drug Effects in 3D Primary Melanoma and Renal Cell Carcinoma

Luis M. Ortiz Jordan¹; Virneliz Fernández Vega¹; Justin Shumate¹; Adam Peles¹; Jordan Zeiger¹; Louis Scampavia¹, and Timothy P. Spicer¹.

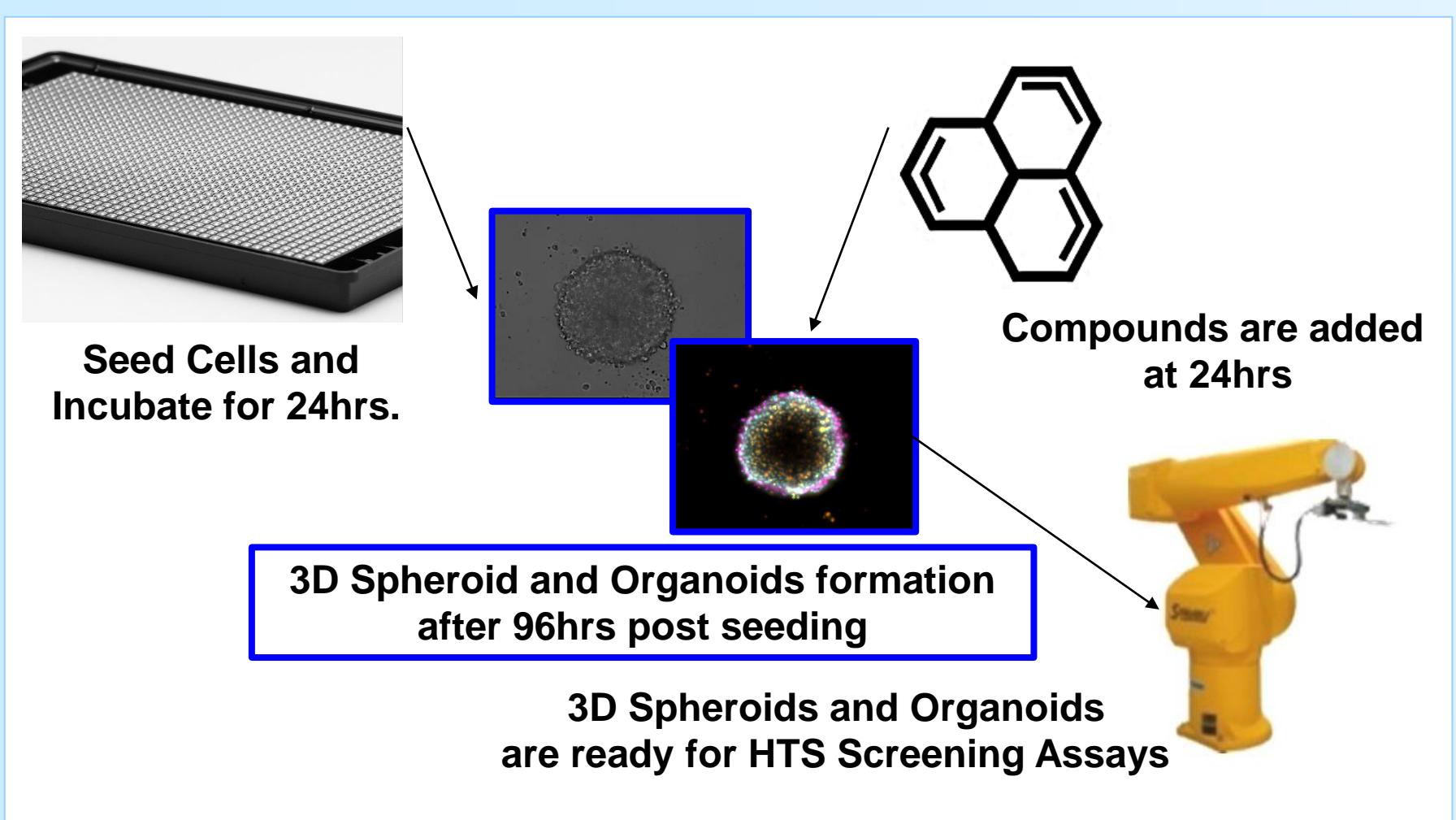
The Herbert Wertheim UF Scripps Research Institute for Biomedical Innovation & Technology High-Throughput Molecular Screening Center,
Department of Molecular Medicine Jupiter; 33458, USA



Abstract

High Throughput Screening (HTS) with 3D cell models is possible thanks to the recent progress and development in 3D cell culture technologies. Results from multiple studies, including some from our lab, have demonstrated different drug responses between 2D and 3D cell culture. It is now widely accepted that 3D cell models more accurately represent the physiologic conditions of tumors than in 2D cell models. Here, we describe an ultrahigh throughput 3D model of drug response profiling in primary melanoma and renal cell carcinoma tested against the NCI oncologic set of FDA approved drugs. In addition to cancer associated fibroblasts, we also tested varying Melanoma and RCC cells grown Matrigel domes vs matrix free 3D vs 2D rendered cancer cells. The result of which represent heterologous response to the drugs based on their WT vs mutant background but not on their maintenance condition. Here, we will present the results of the HTS screening efforts using the 3D models for Melanoma and RCC using organoids derived from patients. Cells were screened against well-known anti-cancer agents from the NCI Pilot Screening Library. This solid tumor research demonstrated yet again the possibility of using 3D cell models for HTS, which opens the window to expand the screening strategy to test on other types of cancer models with the utilization of other anti-cancer compound libraries to find drug for potential off label use or to identify first in class cancer specific inhibitors.

Goal



3D Technologies

Greiner 45 Angle Technology

- Greiner cell repellent plates were used for the test of this method.
- This method uses a 45 Angle device designed by Greiner and created using a 3D printer
- This will allow the formation of the spheres uniformly across the plate.
- Aiming to have a homogenous format that will help with the spheroid formation.

Corning ULA spheroid Microplates

- Single Spheroid per well and centrally located within the well
- Well-to-well uniformity of spheroid size and morphology
- Homogenous format (no transfer of spheroids or aspiration step required)

Panel of 5 PDMR Cell Lines Used

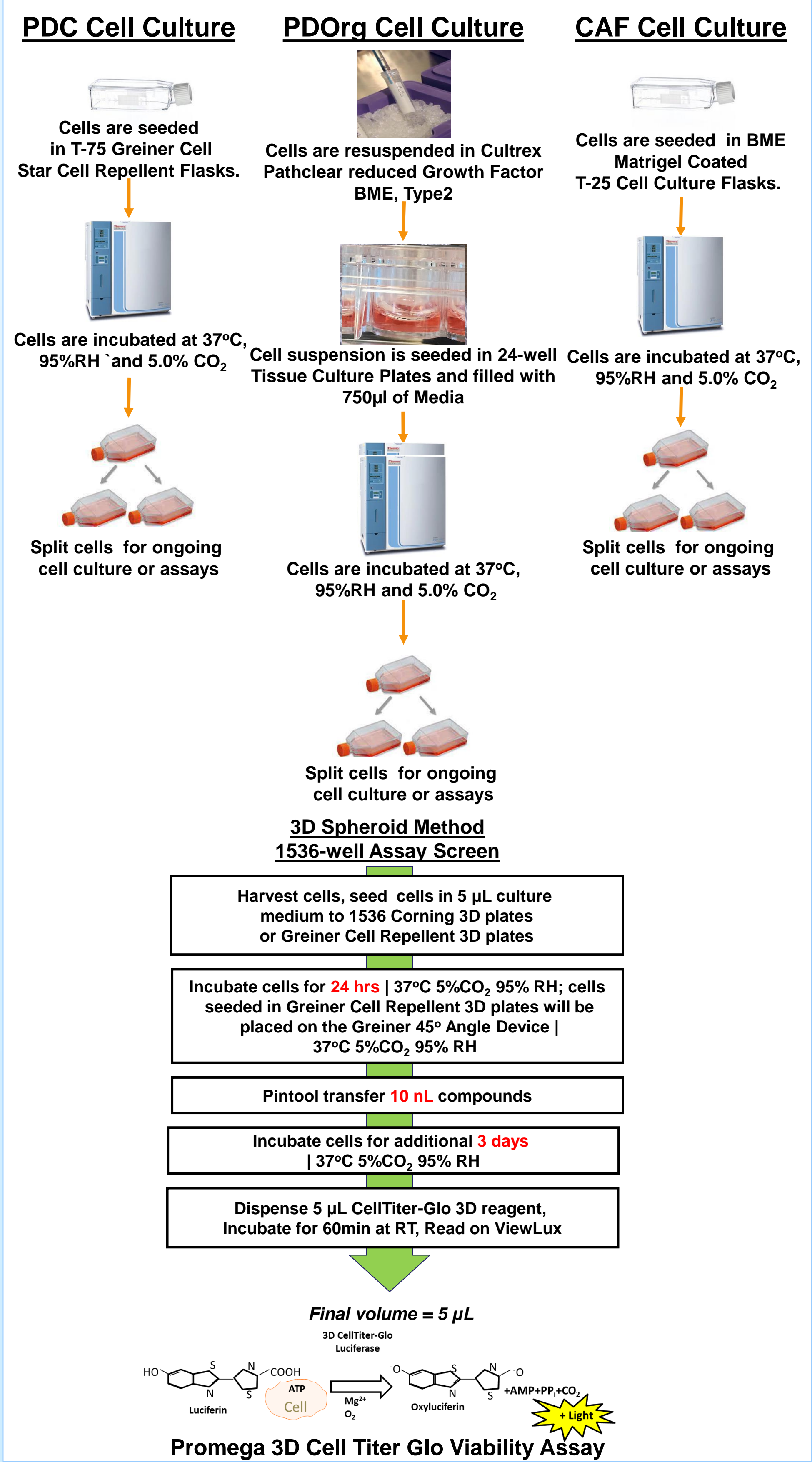
- Melanoma 251568-266-R-J2-PDC (Patient-Derived Mixed Tumor Culture)
- Melanoma 251568-266-R-V1-organoid (Patient-Derived Organoid Culture)
- Melanoma 182917-245-R-J1-CAF (Cancer Associated Fibroblasts)
- Renal Cell Carcinoma 275375-350-R-V1-organoid (Patient-Derived Organoid Culture)
- Renal Cell Carcinoma 275876-140-R-J1-CAF (Cancer Associated Fibroblasts)

Cell Culture Conditions

PDMR Cell Line	Cell Media Reagents				
	CAF Melanoma	CAF RCC	PDC Melanoma	PDORg Melanoma 2D/3D	PDORg RCC 2D/3D
Media Used	Advanced DMEM/F12	Advanced DMEM/F12	Advanced DMEM/F12	Advanced DMEM/F12	Advanced DMEM/F12
HI FBS	X	X	X		
Hydrocortizone	X	X	X		
Adenine	X	X	X		
EGF	X	X	X		
Y-27632	X	X	X	X	X
N-acetylcystein				X	X
Nicotinamide				X	X
Primocin/ Pen/Strep	X	X	X	X	X
L-Glutamine	X	X	X		
HEPES				X	X
Glutamax				X	X
LWRN Conditioned Media				X	X

PDMR Cell Line	Cell Culture Vessels and BME Matrigels				
	CAF Melanoma	CAF RCC	PDC Melanoma	PDORg Melanoma 2D/3D	PDORg RCC 2D/3D
T-25 Flask	X	X		2D	2D
T-75 ULA Flask			X		
T-75 Cell Culture Flask					3D
24 well Cell Culture Plate				3D	
Cultrex BME	X	X			
Cultrex Pathclear Reduced GF Type 2				3D	2D and 3D

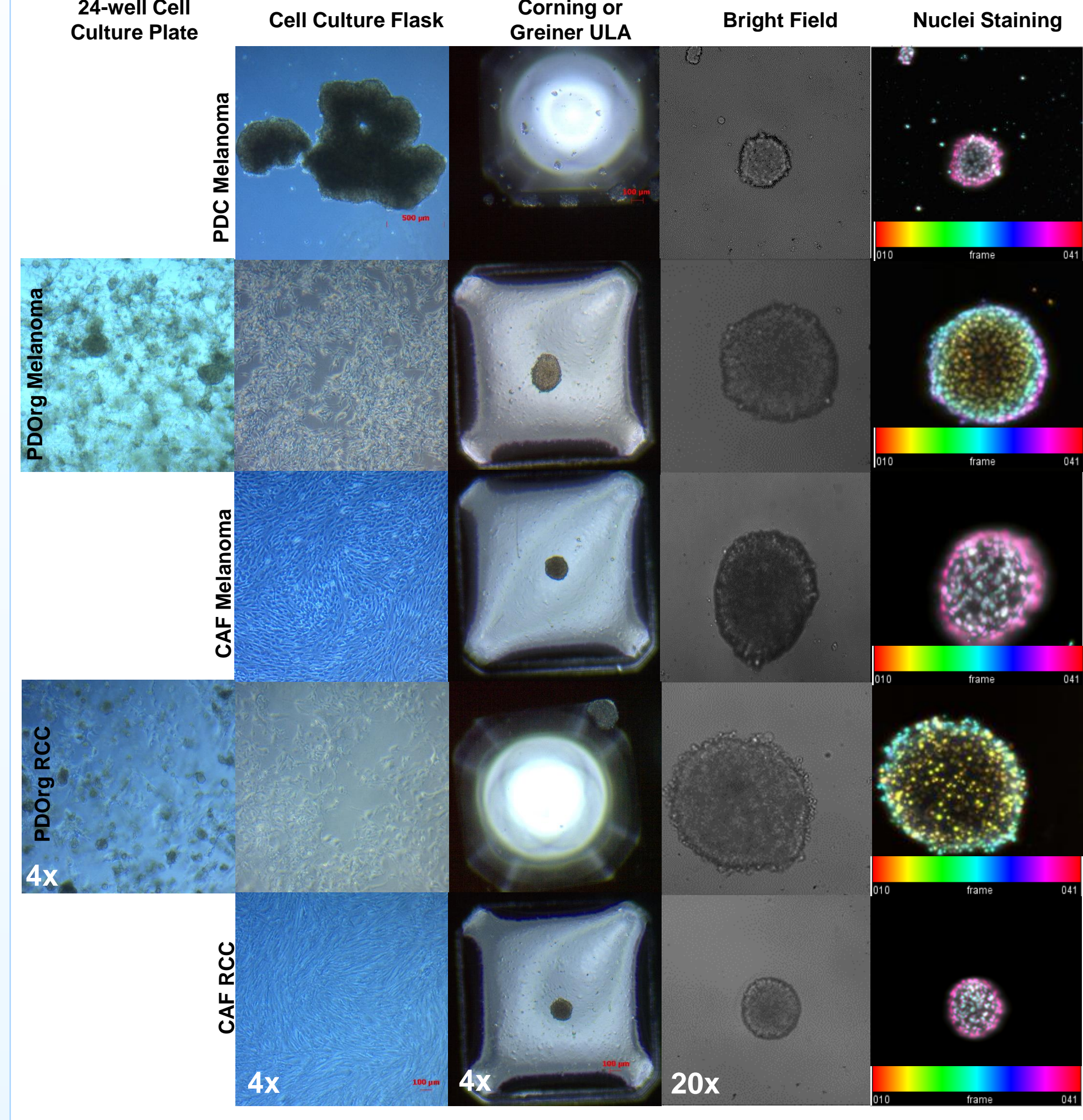
Assay Method



QR Code

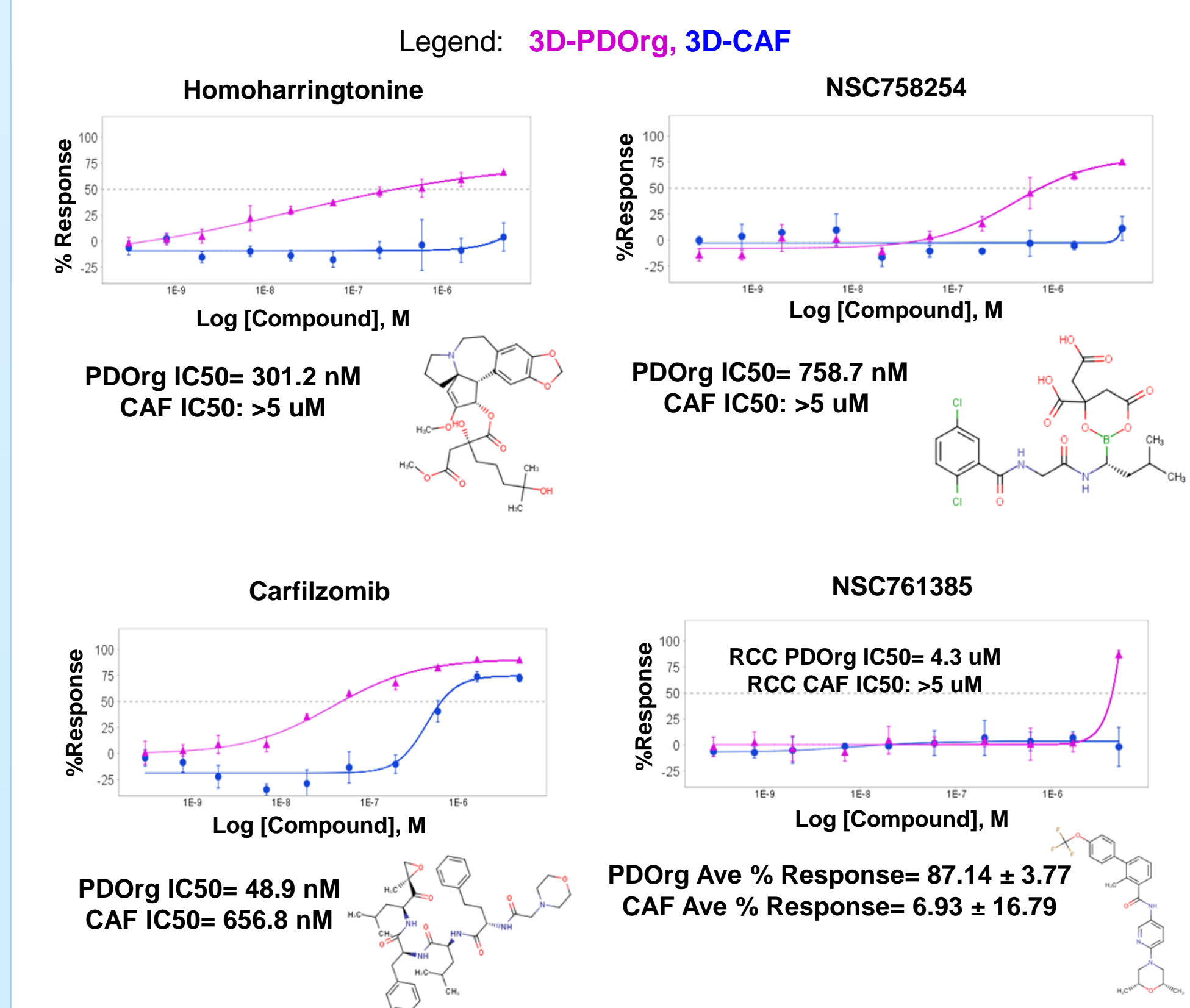
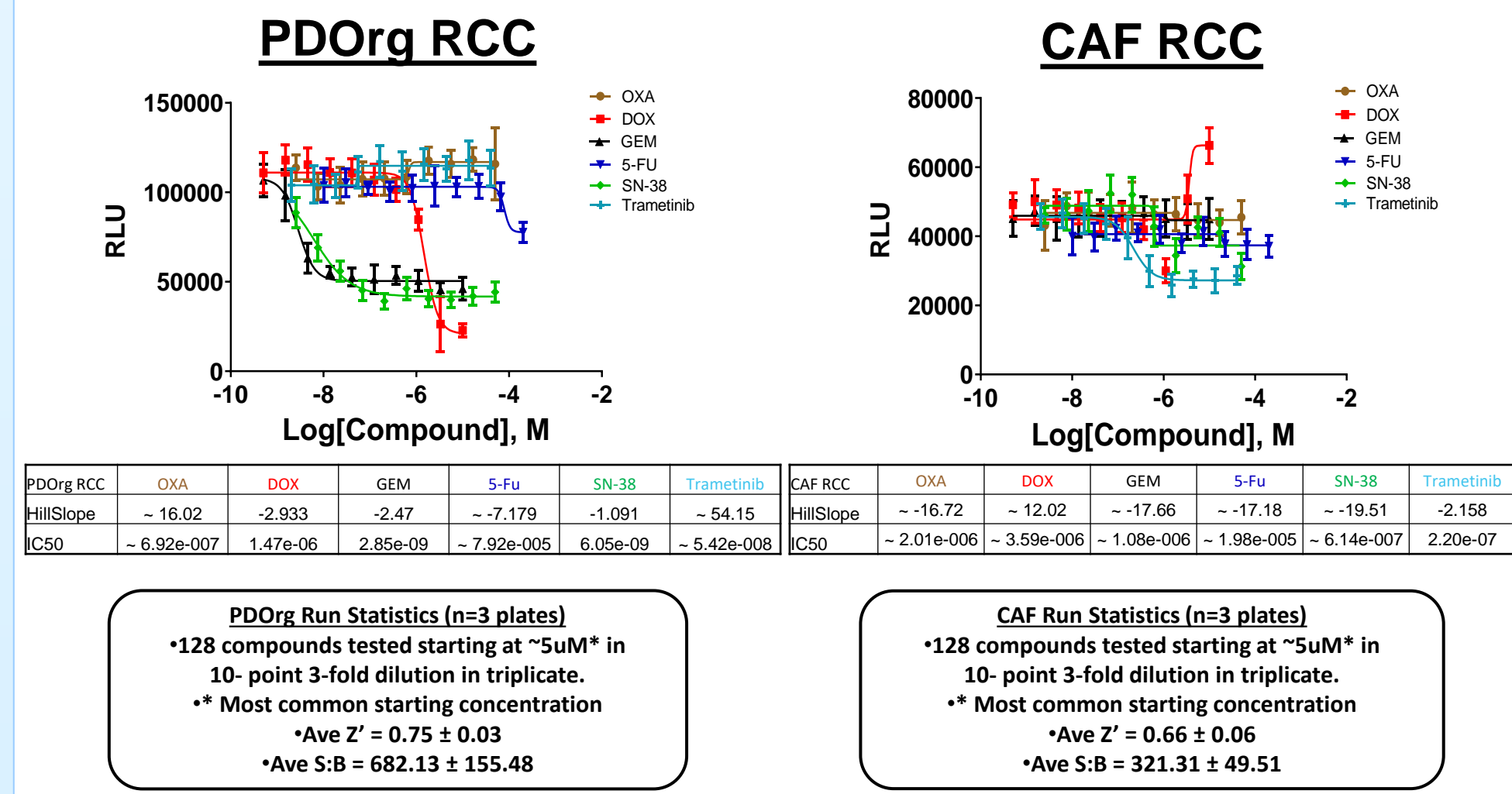


Z-Stack Confocal Images



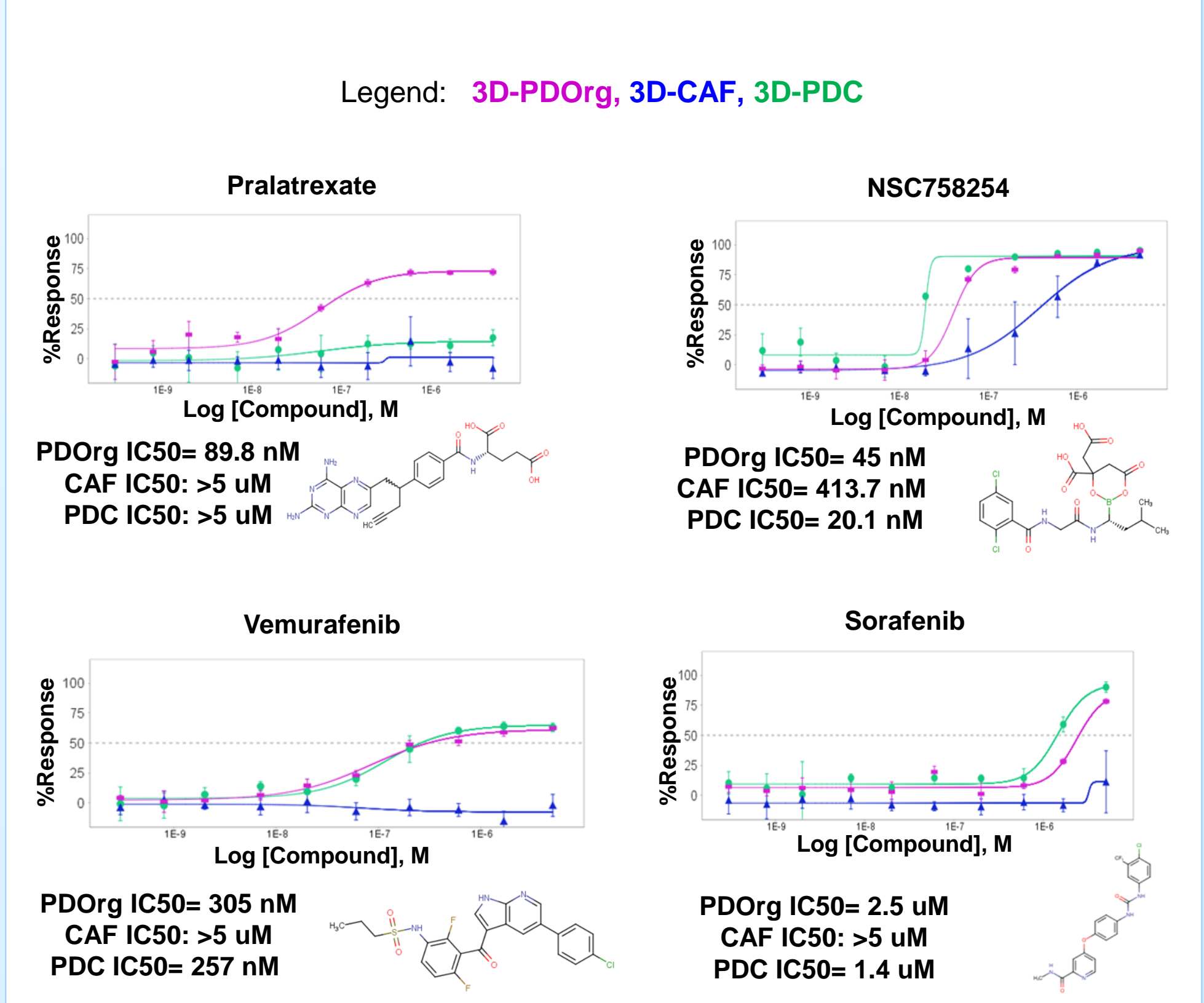
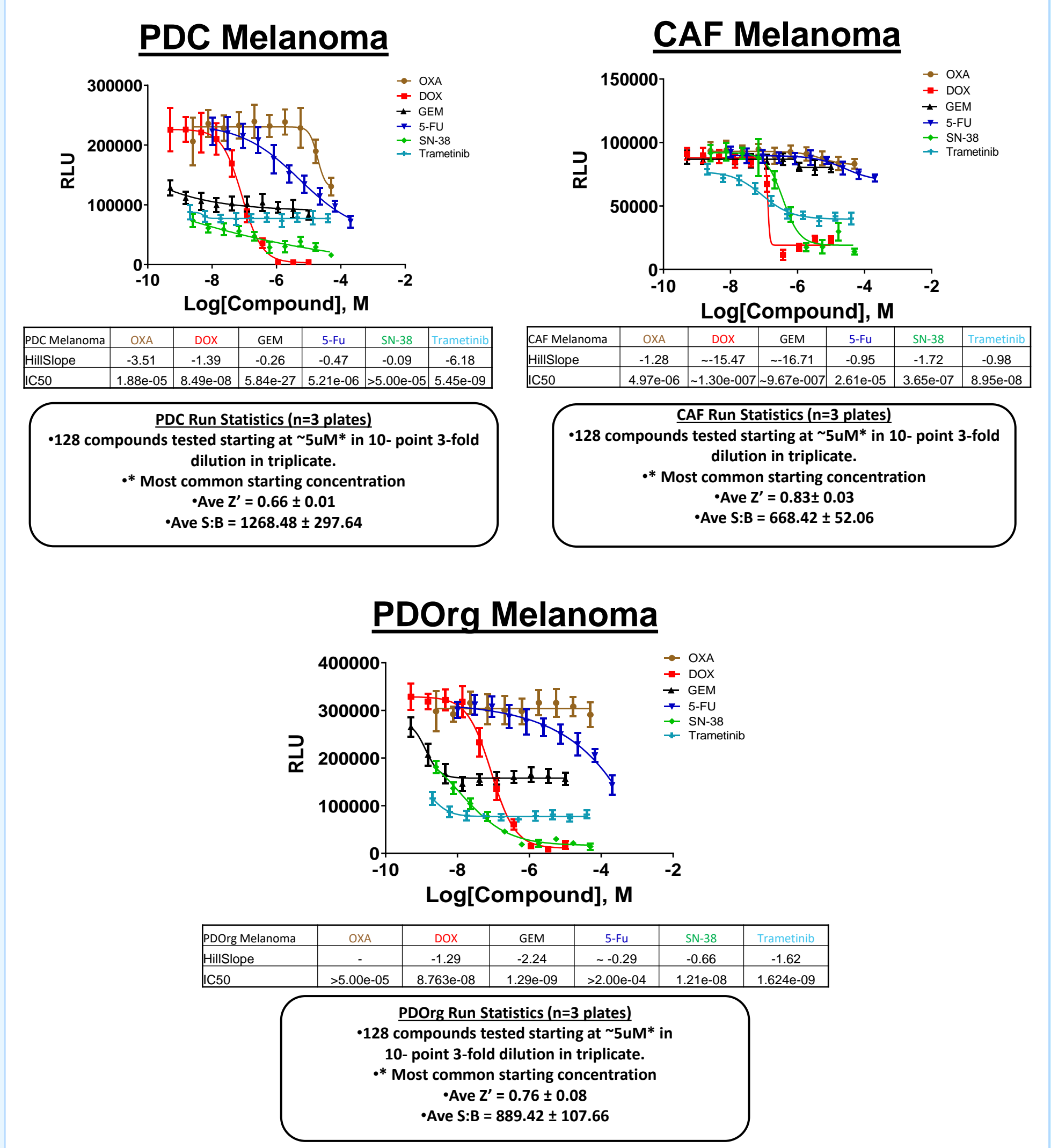
PDORg Melanoma, CAF Melanoma and CAF RCC were seeded in Corning ULA plates while PDC Melanoma and PDORg RCC were seeded in Greiner cell repellent plates. All cultures were monitored across 5 days to allow cell aggregation and the formation of spheroids/organoids. As expected, the organoids in the Corning plates formed in the center while the organoids in the Greiner plates formed in the corners due to the 45° angle position in which the plates were incubated for 24 hours. Z-Stack confocal images evidence the formation of true 3D spheroids. Nuclei staining with Hoechst using 2 micron image slices

Renal Cell Carcinoma



PDORg Renal Cell Carcinoma and CAF did not come from the same patient. CAF RCC cells were grown in a 2D monolayer in the presence of BME gel Matrix. PDORg RCC cells started out as 3D spheroids grown in BME Matrigel domes. The first intent of growing the PDORg cells in 2D methods without the presence of a matrix did not work, and cells grew very slowly. These same cells were then reseeded in the presence of the BME gel in the flask and started to grow faster and easier. PDORg were scaled as 2D cells which were used for the screening. Oxaliplatin, Doxorubicin, Gemcitabine, Fluorouracil, SN-38 and Trametinib were used as pharmacologic controls. For Renal Cell Carcinoma, there were 3 compounds with a IC50 of 5-fold window or greater for Renal PDORg compared to CAF; 2 of the compounds had a maximum response of >50% in PDORg while for CAF its 50% lower. There were 2 additional compounds with a IC50 around 5µM but with a significant delta among the % response between both assays.

Melanoma



PDC and PDORg Melanoma cell lines originated from the same patient, while the CAF was from another patient. PDC cells were grown as matrix free 3D organoids. CAF were grown in a monolayer in the presence of BME gel matrix. PDORg originated as 3D organoids in BME Matrigel domes but were easily adapted to 2D monolayer growth without the need of BME matrix. Both models were successfully used in the screening of compounds. Oxaliplatin, Doxorubicin, Gemcitabine, Fluorouracil, SN-38 and Trametinib were used as pharmacologic controls. The most active compounds of the screening are reproducible across the replicates and the compounds with an activity > 25% show a good correlation between plates. From the screening, there were about 20 compounds with a IC50 of 2-fold window or greater for melanoma and/or PDC compared to CAF.

Conclusions

- Using 3D Cell models in combination with 3D technologies allowed for a successful screening of the NCI oncologic library against a panel of five PDMR Cell Lines.
- 3D PDORg melanoma in Matrigel domes demonstrated an easy and smooth transition to 2D cell culture without the need for BME Matrigel. Both cell models grew vigorously in cell culture and were used successfully for the compound screening.
- 3D PDORg RCC in Matrigel domes initially grew very slow and failed to be grown in 2D cell culture without the presence of a BME matrix. Once reseeded in 2D format with Matrigel the cells start to grow slowly but steadily. Thus, only the PDORg RCC cells were scaled in 2D and reseeded for 3D for screening.
- We again validate our previous research in the use of 3D technologies for the formation and use of spheroids and organoids within HTS.
- This solid tumor research will help pave the way toward new advancements testing compound libraries on different cancer cell lines.

References

- Fernández Vega, V., Yang, D., Ortiz Jordan, L., Ye, F., Conway, L., Chen, L., Shumate, J., Baillargeon, P., Scampavia, L., Parker, C., Shen, B., Spicer, T. Protocol for 3D Screening of lung cancer spheroids using natural products. *SLAS Discovery*, 2023 Jan.
- Li, Z., Xu, H., Yu, L., Wang, J., Meng, Q., Mei, H., Cai, Z., Chen, W., & Huang, W. Patient-derived renal cell carcinoma organoids for personalized cancer research. *Cancer and Translational Medicine*, 2022 Jul; 12(7): e970.