# A Metastatic Phenotype is Reproduced in Spheroids Containing Patient-Specific Cancer Cells and Mesenchymal Stem Cells

Dmitry Shvartsman, Amit Shahar, Liam Deems, Amy Pepicelli, David Deems, and Elin Agoston | *Cellaria Inc, Wakefield, MA*

## ABSTRACT

Reproducible, scalable, and patient-specific 3D models of the tumor microenvironment are required to study tumor growth, metastasis and dormancy. In this study, patient-derived cancer cells, mesenchymal stem cells (MSCs), and optimization of the oxygen level and extracellular matrix (ECM) were incorporated in the development of a spheroid system using 5 cancer cell models (pancreatic [Panc], lung adenocarcinoma [LA], colon adenocarcinoma [CA], endometrioid ovarian [EO] and high-grade serous carcinoma [HGSC]).

The cell models were isolated and scaled up using a feeder-free culture medium in either ambient oxygen (21% $O_2$) or hypoxic (5% $O_2$) conditions, depending on the individual model requirements. Cell migration was assessed via monolayer scratch assays, and the relative rates were compared to the T stage of the tumor from which the models were derived. To assess spheroid formation, the 5 models were cultured in ECM-containing medium. The Panc and HGSC spheroids were also generated with the addition of MSCs at ratios of 1-100 MSCs per 100 cancer cells.

The relative rates of migration of the cell models in ambient oxygen (CA > EO ≈ Panc > HGSC ≈ LA) differed significantly from that in 5% $O_2$ (CA = Panc = HGSC > EO > LA). Interestingly, the T stage of the original tumors (CA = Panc = HGSC > LA > EO) correlated more closely to the relative rate of cell migration when the cell models were cultured in 5% compared to 21% $O_2$.

When cultured in a 3D (spheroid) format containing ECM, the Panc and EO models demonstrated increased invasion into the surrounding ECM compared to the LA, HGSC, and CA models. The addition of MSCs into the LA model dramatically increased the invasive phenotype of the LA model. A seeding ratio of 1:100 MSC:LA cells enabled spheroid formation and invasion into the ECM, previously unobserved for the LA model in this format. The Panc model became extremely aggressive with respect to invasion into the ECM, at a ratio as low as 1:100 MSCs to cancer cells. In pilot studies with the Panc and HGSC models, spheroid size and invasion into the ECM increased upon adding the MSCs. Spheroid diameter increased by approximately 30%, and there was a notable increase in invasiveness of the already mobile and invasive Panc cancer cells into the surrounding ECM.

We present here an efficient, patient-specific, spheroid model system representing dormant and metastatic tumor states that is suitable for studying antitumor drug response, personalized therapy, and disease mechanisms. The relative rates of cell migration positively correlated with the T stage of the original tumors in the more physiologically-relevant oxygen condition (5%), underscoring the need to optimize this parameter during model development. Continued optimization will include incorporating tissue-specific ECMs from lung, liver and pancreas, and assessing the robustness and scalability of these models for high-throughput drug screens.

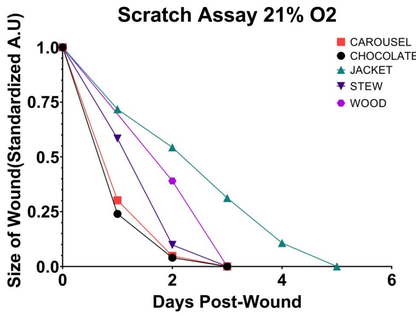
## METHODS

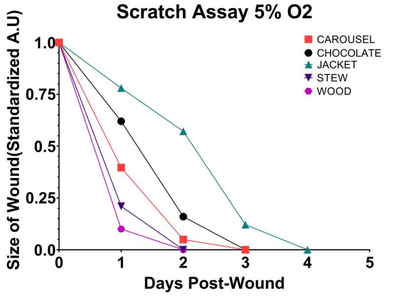
Cell line of interest is passaged according to each specific cell line protocol. Spheroids are seeded into an ultra-low-attachment (ULA) plate at a density of 5000 cells/well after being suspended in Renaissance Essential Tumor Medium. The seeding medium contains 2-5% Matrigel, depending on the cell line utilized. If staining is desired, MSC's and Cancer cells are labelled individually before being seeded into wells as a co-culture. Spheroids are incubated in 37°C for 72 hours, or until they are at least 200µM in size.

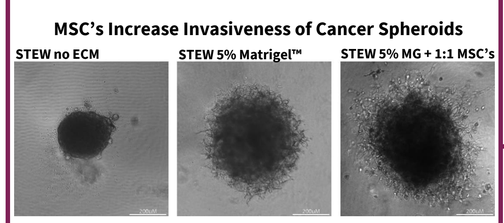
100 µL of a drug concentration suspended in cell culture media is added to each well. Fresh media is added to controls.

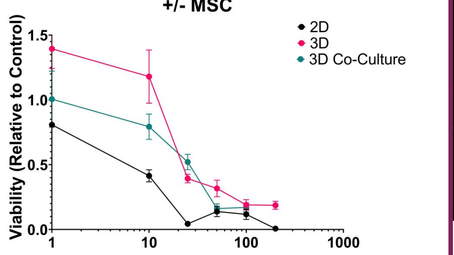
Following 72 hours of incubation with the drug, 100 µL are removed from each well, and replaced by 50 µL of XTT reagent.

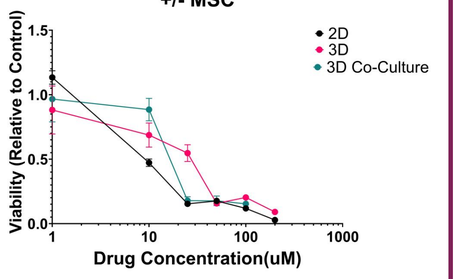
Following a 4-hour incubation with the XTT reagent, the wells are scanned for a colorimetric response. Results have the background subtracted and are normalized by the average of the no-drug control for each individual plate.



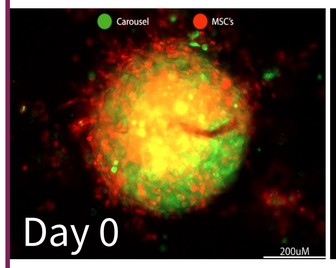



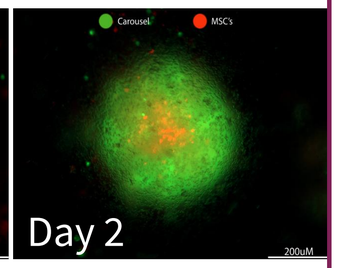



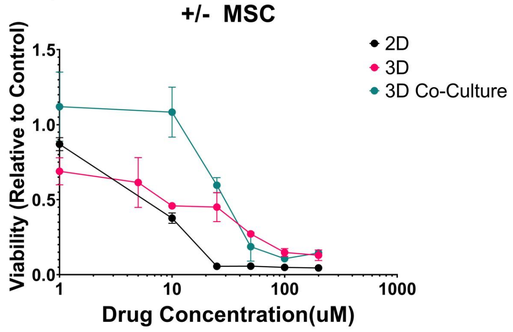




## RESULTS

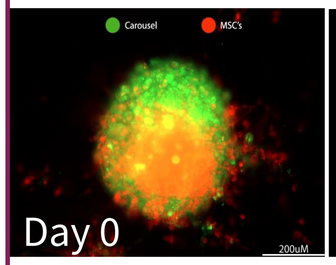
### Cisplatin

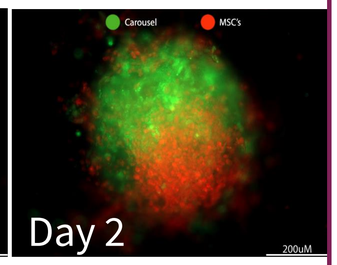


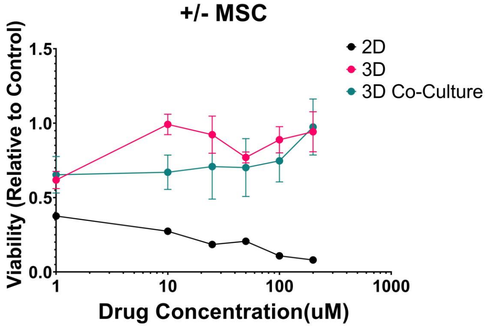



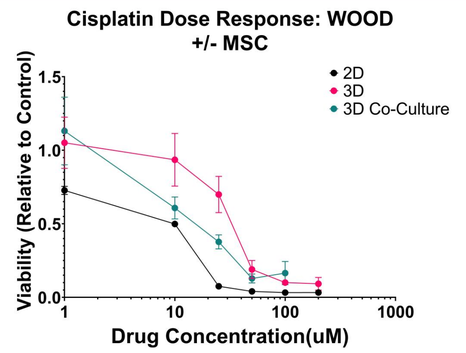



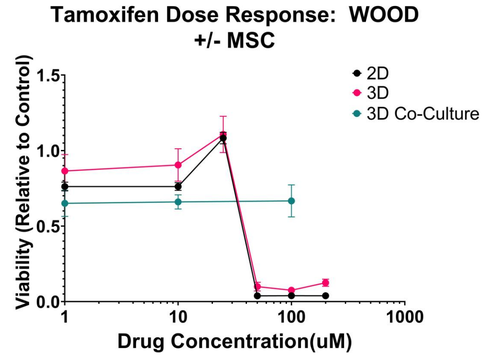

### Paclitaxel

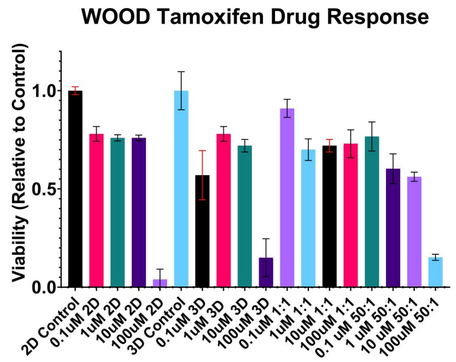


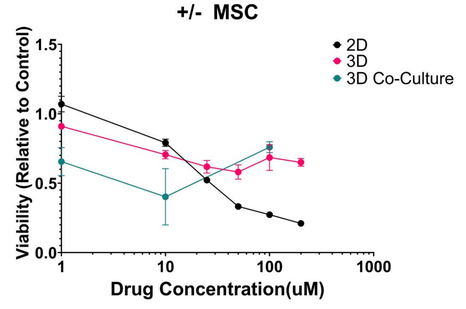




## KEY FINDINGS

- The T stage of the original tumors (CA = Panc = HGSC > LA > EO) correlated more closely to the relative rate of cell migration when the cell models were cultured in 5% compared to 21% O2.
- Drug sensitivity was attenuated in several models when switched from a 2D to a 3D spheroid format. Upon adding MSCs, drug sensitivity in 3D was restored in some models, at higher drug concentrations with a different effect, and increased invasion into the surrounding ECM was observed.
- Two pancreatic cancer models, Chocolate and Stew, exhibit different responses to cisplatin treatment when compared in 2D and 3D formats. Sensitivity was attenuated in the 3D format in the Chocolate model, but not the Stew model. Addition of MSCs to the Chocolate spheroids increased drug sensitivity at the lower concentrations, reducing the difference in drug response between 2D vs. 3D cultures. This was not observed for Stew, which had similar cisplatin sensitivity for all three culture types.
- Endometrioid ovarian cancer cell line Carousel was sensitive to cisplatin and paclitaxel in 2D. However, increased drug resistance was observed when grown in 3D spheroids with a 1:1 ratio of MSC's.
- Expectedly, ER+ breast cancer cell line Wood was not sensitive to the prodrug tamoxifen at concentrations less than 50uM. Unexpectedly, at concentrations of 50uM and greater the cells were sensitive to tamoxifen in both 2D and 3D cultures. The reduction in viability was significantly attenuated by 3D co-culture with MSCs at 100 uM.
- The cisplatin sensitivity of breast cancer cell line Wood, and the erlotinib sensitivity of lung cancer cell line Jacket increased in 3D culture compared to 2D.
- Cellaria models in 2D and 3D, with and without the addition of MSCs, are valuable tools for building dormant and metastatic niche models for researchers in basic and applied areas.

## REFERENCES

Brady, Samuel W., et al. "Combating subclonal evolution of resistant cancer phenotypes." Nature communications 8.1 (2017): 1-15.

Coffman, Lan G et al. "Ovarian Carcinoma-Associated Mesenchymal Stem Cells Arise from Tissue-Specific Normal Stroma." Stem cells (Dayton, Ohio) vol. 37,2 (2019): 257-269. doi:10.1002/stem.2932

Feldman, Leonardo Julio, Sr., et al. "Bone Marrow Mesenchymal Stem Cells: Pre-Metastatic Niche For Breast Cancer." Blood, vol. 122, no. 21, American Society of Hematology, Nov. 2013, pp. 4859–4859. Crossref, doi:10.1182/blood.v122.21.4859.4859.

Hurst, Douglas R., and Danny R. Welch. "A MSC-ing link in metastasis?." Nature medicine 13.11 (2007): 1289-1291.

Karnoub, Antoine E., et al. "Mesenchymal stem cells within tumour stroma promote breast cancer metastasis." Nature 449.7162 (2007): 557-563.

Melzer, Catharina et al. "Cancer stem cell niche models and contribution by mesenchymal stroma/stem cells." Molecular cancer vol. 16,1 28. 1 Feb. 2017, doi:10.1186/s12943-017-0595-x