# Circulating Tumor Cells from in Vitro 3D Culture Systems have Tumor-Initiating Capacity in Vivo

ABSTRACT
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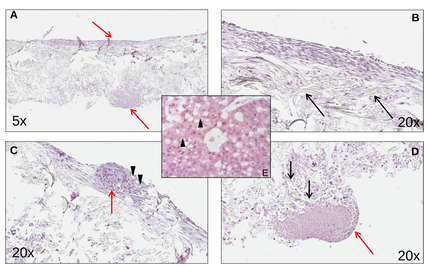
## Abstract

Circulating tumor cells (CTCs) are cells that have detached from the primary tumor and entered the bloodstream with the potential to seed metastatic tumors in distal sites. High CTC numbers correlate with aggressive disease, increased metastasis, and decreased time to relapse. It has also been shown that cancer stem cells (CSCs) represent a proportion of the CTCs present in patients. Given that CSCs are resistant to chemotherapy and are responsible for tumor initiation, it is posited that the CSCs are the seeds of metastasis. However, direct evidence for this hypothesis is limited because there are few methods for culturing and studying these rare cells. We are using a 3D culture chamber system (RealBio D4™) to establish long-term cultures of human-derived breast and pancreatic tumors. We observed that the system's 3D matrix supported culture development and incorporation of tissue organization and microenvironment. Further, the chamber design allowed CTCs generated within the cultures to migrate out of the cell mass into the circulating nutrient medium where they were collected for characterization. The isolated CTCs displayed CSC properties via CellSearch® (CK+,CD44+,CD24-; CK+, CD44+,CD24+ for breast and pancreas respectively). In addition, these isolated CTCs displayed tumor-initiating capacity when implanted into mice. Future studies will compare CTCs isolated from 3D culture with cells from tumors, blood, and tissue grown on scaffold through RT-PCR, histology, and gene expression assays.

## Results

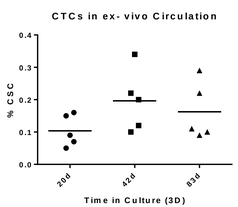
### Establishing Ex-Vivo "D4" Model

Figure 1. Long term "D4" in vitro culture of pancreas PDX tumor model shows histological similarities to in vivo cohort.




Representative H&E sections illustrate morphological structures in D4 cultures of PA0143 pancreas tumor xenograft model in culture for 85 days. A. Cross section of 3D matrix scaffold seeded with PA0143 tumor cells. B-D Multilayered cell structures growing throughout scaffold with areas of fibrosis displaying multiple cell types. E. H&E section from in vivo tumor of PA0143 PDX. Red arrows point to structural components; black arrows point to polyester matrix fibers; black arrow heads point to adipocytes.

Figure 2. Circulating tumor cells from in vitro "D4" pancreas PDX tumor model displayed cancer stem cell markers

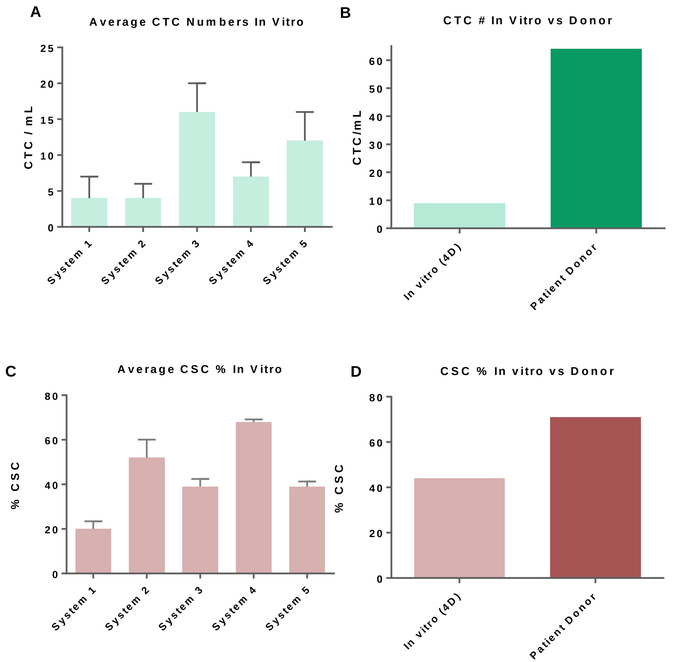



CTCs generated from tumor tissue grown on the 3D matrix migrated out of the cell mass and into the circulating nutrient medium. CTCs were collected at days 20, 42, 83 and assessed using flow cytometry for CSC markers Epcam+, CD24+, CD44+. At each timepoint, a percentage of CTCs displayed CSC characteristics.

## Study Design

### CTC174 Breast Cancer Model

Figure 3. Comparison of CTC numbers and CSC % between long-term "D4" in vitro culture of breast PDX tumor model and human donor




Five tumors from PDX CTC174 breast carcinoma were seeded into Realbio D4 chambers and maintained in culture for 223 days. CTCs generated from tumor tissue grown on the 3D matrix migrated out of the cell mass and into the circulating nutrient medium. CTCs were collected at days 218, 222, 223 and assessed using CELLSEARCH®. A. 2-21 CTCs per mL of media were collected from the systems. B. The total average CTC number per mL of media was 9 cells compared to 64 cells in patient donor. C. 20-70% of CTCs from in vitro D4 cultures displayed CSC characteristics (CK+, CD44+, CD24-) via CELLSEARCH®. D. The total average CSC % from in vitro D4 cultures was 42% compared to 71% in patient donor.

## Results

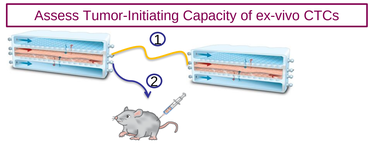
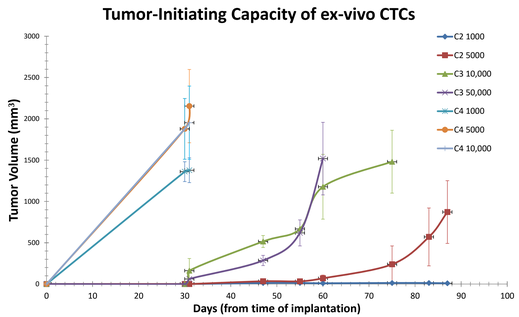
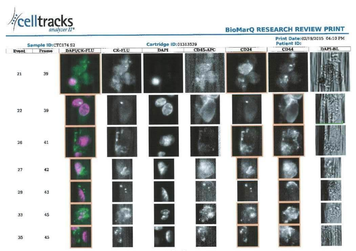



Figure 4. CTCs isolated from circulation of RealBio "D4" systems displayed tumor-initiating capacity in vivo




CTCs isolated from circulation of RealBio D4 systems displayed tumor-initiating capacity in vivo. CTCs were collected from 3 different chambers (C2, C3, C4) at day 280 and injected in Matrigel into the mammary fat pad of XID mice in the following dilutions: 1000, 5000, 10000, 50000. Tumors were observed as early as day 30 post implantation. By day 47, all groups displayed tumors. (n=1-3 per group)

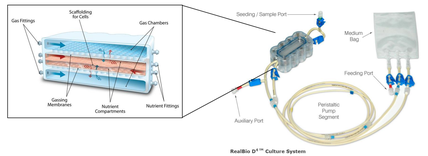
Figure 5. Representative image of CTCs displaying CSC markers (CK+, CD44+, CD24-) captured by CellSearch

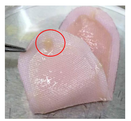



## Techniques

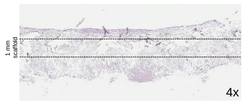
### Long-term Realbio "D4" Cell Culture

Support biologically-relevant three-dimensional structures that mimic the natural composition and architecture of normal or tumor tissue



Tumor mass grown through the 3-D Scaffold.
PA0143 Tumor 100 days in culture



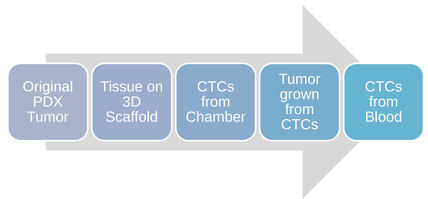

H&E of 3D scaffold displaying a multilayered tissue mass with tumor cells migrated through the scaffold. PA0143 tumor 85 days in culture

## Conclusions

- We established that Realbio D4 ex-vivo model could successfully recapitulate a pancreas in vivo tumor model through histology and functional CSC enrichment assays.
- We successfully seeded a breast CTC tumor model in the D4 system with CTCs that migrate into "circulation"
- CTCs isolated from breast D4 CTC174 model displayed CSC markers (CK+, CD44+, CD24-)
- Ex-vivo CTCs from D4 model showed tumor-initiating capacity when implanted into mice

## Future Directions

Gene Expression Profiles will be compared from CTCs isolated from all models at different timepoints

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