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Patient-Derived Organoids for Triple Negative Breast Cancer: Characterization and Functional Applications

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Introduction

Triple-negative breast cancer (TNBC), characterized by the absence of ER, PR, and HER2 expression, is among the most aggressive breast cancer subtypes. The lack of therapeutic targets severely limits treatment options, resulting in persistently poor patient prognoses. Standard treatment relies on chemotherapy, which often results in resistance and relapse, while TNBC heterogeneity and the presence of multiple molecular subtypes further complicate therapeutic strategies. Recent advances in three-dimensional (3-D) culture systems, particularly patient-derived organoids (PDOs), offer a physiologically relevant platform to model tumor biology, drug response, and immune interactions. As part of the Human Cancer Models Initiative (HCMI), rare PDOs that recapitulate TNBC architecture and signaling have been developed, enabling precision oncology and translational research. PDOs retained key histological and molecular features, including subtype-specific HER2, PR, and ER expression, and were characterized using immunofluorescence, histology, and drug-sensitivity profiling. Whole-exome sequencing confirmed genetic integrity and clinical concordance with reference datasets from The Cancer Genome Atlas, while drug screening incorporated both standard-of-care and targeted therapies to evaluate combination strategies. These findings underscore the ability of PDOs to more accurately model tumor heterogeneity and translational clinical concordance compared with conventional two-dimensional systems. The inclusion of three expanded breast cancer models (ATCC® PDM-92™, PDM-523™, and PDM-411™) within the HCMI portfolio, spanning HER2$^+$, ER$^+$/PR$^+$, and triple-negative subtypes, provides a robust resource for comparative studies. The results demonstrate that patient-derived TNBC organoid models faithfully recapitulate the genomic and molecular features of the originating tumors and enable the identification of mutation- and subtype-specific drug-response patterns, supporting functional precision oncology and the development of personalized therapeutic strategies for TNBC.

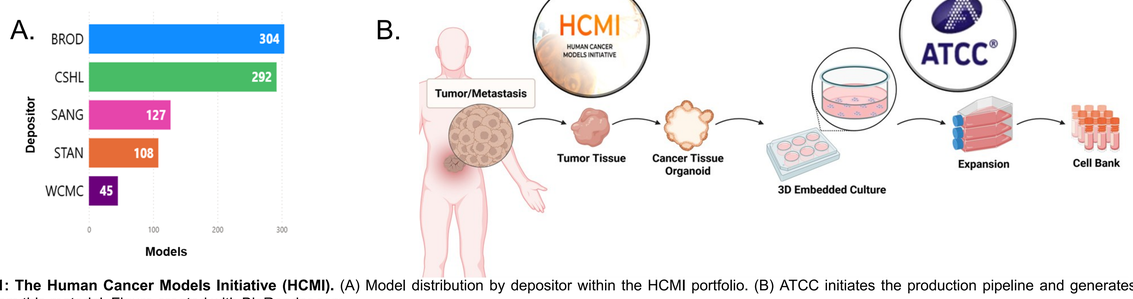


Figure 1: The Human Cancer Models Initiative (HCMI). (A) Model distribution by depositor within the HCMI portfolio. (B) ATCC initiates the production pipeline and generates cell banks from this material. Figure created with BioRender.com.

Materials & Methods

Organoid culture and expansion: Human primary tissue–derived organoids are established by mechanically and enzymatically dissociating fresh tissue into small epithelial fragments, which are then embedded in a supportive extracellular matrix such as Matrigel and cultured as three-dimensional domes. These structures are maintained in defined, tissue-specific media supplemented with key niche factors (e.g., Wnt agonists, R-spondin, Noggin, EGF, and pathway inhibitors) to promote stem cell maintenance and growth, with ROCK inhibitor added during early stages to enhance cell survival. Organoids typically emerge within days and are expanded through periodic passaging by mechanical or enzymatic disruption into small multicellular clusters followed by re-embedding in fresh matrix. Successful long-term propagation depends on maintaining optimal fragment size, consistent extracellular matrix handling, and precise timing of passaging to avoid overgrowth and loss of viability. Cryopreservation is achieved by freezing organoid fragments in DMSO-containing media using controlled-rate cooling, enabling the establishment of stable biobanks, with efficient recovery following rapid thawing and re-culture. Overall, robust organoid growth relies on preserving tissue heterogeneity, optimizing culture conditions, and minimizing stress during early establishment and expansion to maintain physiological relevance.

Culture conditions: Organoids were sourced from ATCC® and cultured in standard domed conditions with Complete Organoid Media using ATCC®'s Growth Kit F1 (ATCC® ACS-7105™). Culture was supplemented with Cell Basement Membrane (ATCC® ACS-3035 ™), Rock Inhibitor Y27632 (ATCC® ACS-3030™), and HA-R-Spondin1-Fc 293T (RSPO1) Conditioned Media.

Drug sensitivity testing: Models were passaged as single cells and seeded at the equivalent of 2.5x10^3 cells/well in 100 µL Complete Organoid Media then left undisturbed for 72 hours prior to dosing within 96-well U-bottom plates (Corning 3474) (Figure 2). The organoids were tested for sensitivity to a custom panel of 7 chemotherapeutic compounds with varying mechanisms of action and molecular targets reconstituted in either D-PBS (ATCC® 30-2200™) or DMSO (ATCC® 4-X™) and treated with an 8-point, half-log curve in triplicate. After 5 days exposure, the organoids were stained using Hoechst 33342 (Thermo Fisher Scientific 62249), Calcein AM (Thermo Fisher Scientific C1430), and Ethidium Homodimer-1 (Thermo Fisher Scientific E1169) then imaged in brightfield and using fluorescence for visualization. Lastly viability was measured using a luminescent ATP viability assay, CellTiter-Glo 3D (Promega G9681).

Data analysis: Responses were normalized to vehicle treatment condition and expressed as percent viability. Figures were plotted, non-linear curves generated, and IC_{50} was calculated in GraphPad Prism.

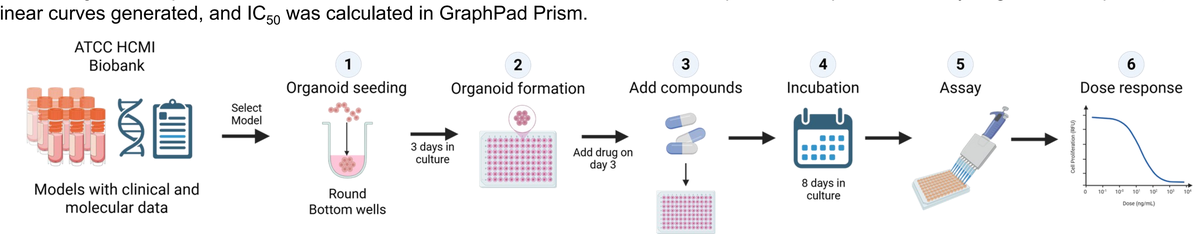


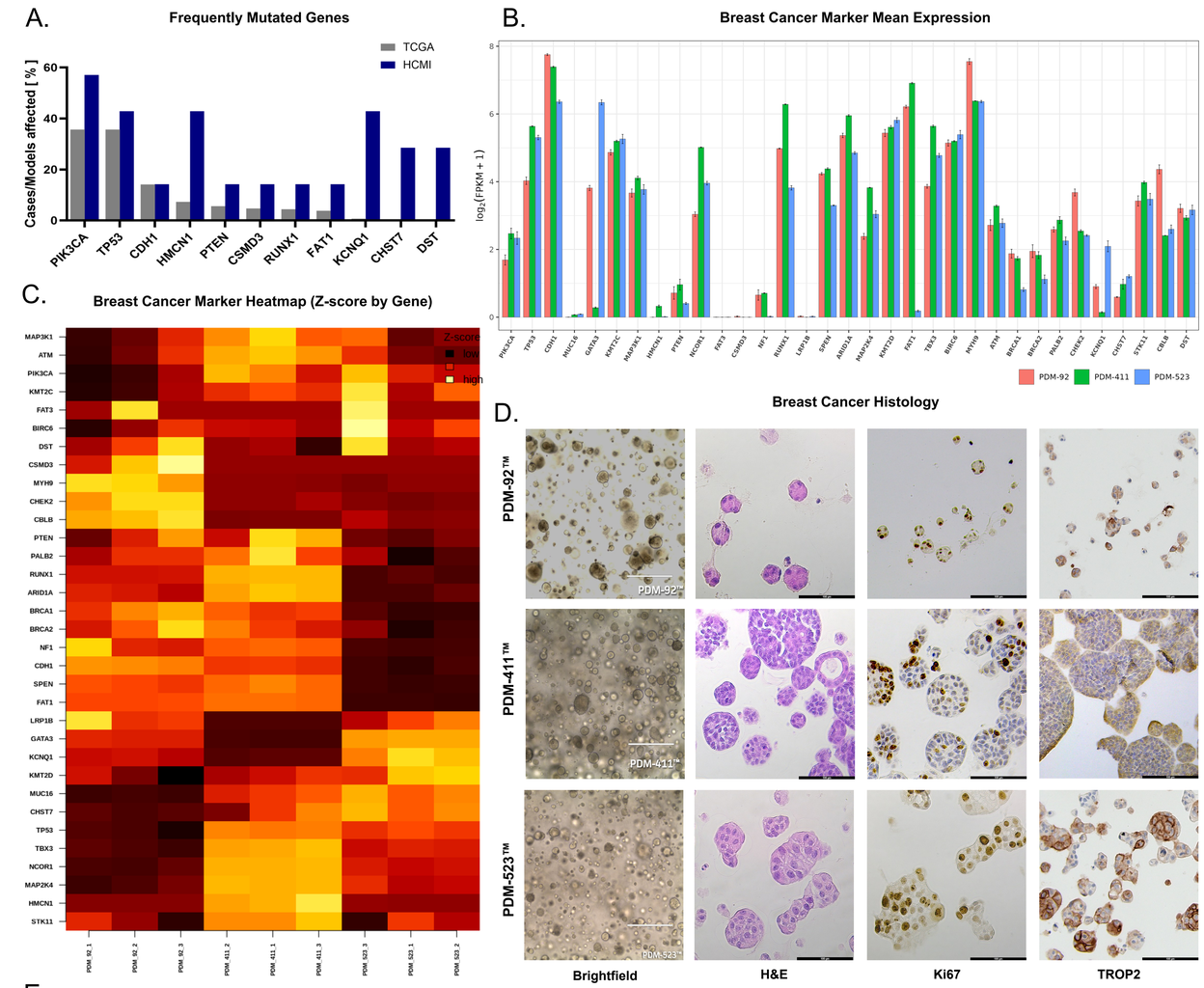
Figure 2: Integrated workflows for organoid culture and drug sensitivity testing. (A) Drug sensitivity testing workflow for breast cancer organoids. Figure created with BioRender.com.

Results

Table 1: Clinical characteristics of subset of breast cancer. Breast cancer models derived from a variety of acquisition sites, gender, races, age, and clinical stage.

ATCC® Part	Primary Site	Tissue Status	Disease Type	HER2	PR	ER	Sex	Race	Culture Format
PDM-92™	Breast	Primary	Epithelial Neoplasms	+	—	—	F	Asian	3-D organoid
PDM-411™	Breast	Metastasis	Ductal and Lobular Neoplasms	—	—	—	F	Black/African American	3-D organoid
PDM-523™	Breast	Metastasis	N/A	—	+	+	F	White	3-D organoid
PDM-195™	Breast	Primary	Complex Epithelial Neoplasms	—	—	+	F	White	3-D organoid
PDM-250™	Breast	Primary	Epithelial Neoplasms	—	+	+	F	White	3-D organoid
PDM-350™	Breast	Primary	Ductal and Lobular Neoplasms	—	+	N/A	F	N/A	3-D organoid
PDM-520™	Breast	Primary	N/A	—	+	+	F	Black/African American	3-D organoid

Results



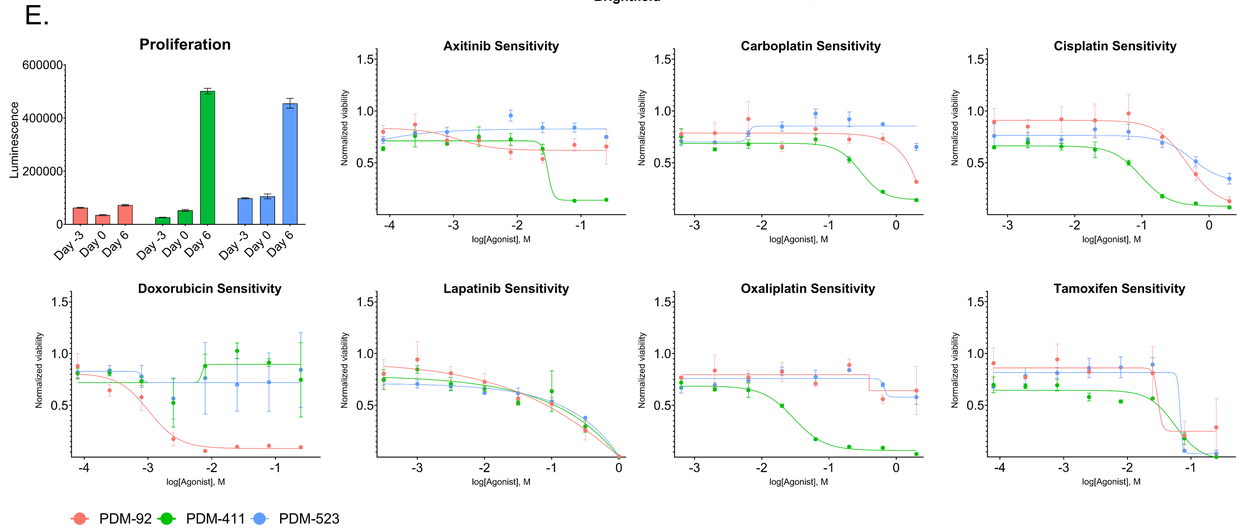


Figure 3: Genomic, transcriptomic, histopathologic, and pharmacologic profiling of breast cancer organoid models. (A) Frequently mutated genes associated with breast cancer. (B) RNA-seq data for breast cancer models showing mean expression of frequently mutated genes. (C) Breast cancer heatmap showing z-score by gene. (D) Histopathology showing the different markers for H&E, TROP2, and Ki67 staining in a breast cancer cohort. (E) These models were tested against standard-of-care (SOC) drugs recommended for breast cancer treatment. The tested cohort of models exhibited marked sensitivity to the PARP inhibitors. Interestingly, PDM-411™, which is a triple-negative breast cancer (TNBC) model, demonstrated a pronounced cytotoxic response towards the VEGF inhibitor, Axitinib, suggesting a new therapeutic avenue to explore for TNBC breast cancer treatment.

Conclusion

- Patient-derived breast cancer models recapitulate key disease-defining genomic alterations, including HER2, ER, PR, TROP2, etc., and demonstrate strong concordance with patient tumors.
- Models retain clinically relevant molecular features and tumor-specific phenotypes.
- Drug-response profiling revealed heterogeneous sensitivities across models, with ATP-based viability assays enabling robust, reproducible comparisons.
- Differential responses to standard-of-care and targeted agents highlight the influence of underlying genotype on therapeutic sensitivity.
- Together, these data support the use of HCMI-derived organoids as high-fidelity, clinically relevant platforms for drug discovery, biomarker development, and precision oncology research.

Explore HCMI Models

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