Clinical, molecular, and functional characterization of a diverse collection of patient-derived colorectal cancer organoids from the Human Cancer Models Initiative

Abstract # 5202

Matthew Graziano, BS; Stephen Friend, MS; James Clinton, PhD; Changsuk Moon, PhD; and Carolina Lucchesi, PhD
ATCC®, Manassas, VA 20110

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide. There is a need for clinically representative, in vitro CRC models that more accurately reflect the genomic and phenotypic diversity of disease seen in patient populations. The Human Cancer Models Initiative (HCMI) is a collaboration between the National Cancer Institute, Cancer Research UK, Wellcome Sanger Institute, Hubrecht Organoid Technology, and NCI-funded model development institutions like the Broad Institute and the Broad Institute and the Cold Spring Harbor Laboratory that develop next-generation cancer models for the research community. ATCC® is the sole distributor of the HCMI portfolio with over 300 next-generation cancer models derived from over 18 tissue types that have been manufactured, characterized, and validated. These models are available for academic and commercial use to support cancer research and drug discovery.

HCMI models within the ATCC portfolio:

- Human patient-derived
- Next-generation cancer models; including organoids, neurospheres, spheroids and adherent lines
- Diverse genetic backgrounds
- Models from primary, metastatic, and recurrent cancer
- Rare and pediatric cancers included
- Clinical and sequencing data available
- Model-specific, easy-to-follow culturing protocols

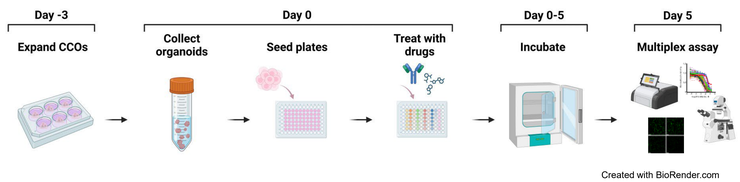
There are currently 78 expanded colorectal cancer organoids (CCOs) available in the ATCC portfolio, a subset of 28 have been used herein for testing against a customized panel of compounds. CCOs were tested with a 12-point, half log dilution dose curve and drug sensitivity was calculated as the half-maximal inhibitory concentration. Post-treatment cultures were stained with live-cell fluorescent dye and imaged followed by a luminescent ATP viability assay to assess response. The data indicate that CCOs in the cohort mostly preserve the heterogeneity and complexity of the original patient tumor, including the genetic, physiological, and histological characteristics. The results support the potential of utilizing ATCC provided HCMI models to be utilized for personalized medicine, drug discovery, mechanism of action studies, cancer stem cell research, and tumor microenvironment modeling. To enhance their clinical relevance, the sequencing data and patient clinical information for each model is available to the research community via the HCMI portal.

Methods

Organoid culture and seeding: CCOs were sourced from ATCC and cultured in standard 3-D extracellular matrix embedded conditions using Organoid Growth Kits (ATCC® ACS-7100™ and ATCC® ACS-7103™), Cell Basement Membrane (ATCC® ACS-3035™), and Rock Inhibitor Y27632 (ATCC® ACS-3030™). The organoids were passaged as single cells and cultured for 72 hours to develop into uniform, small organoids prior to seeding and dosing. The organoids were collected and seeded as intact organoids into 96-well plates at the equivalent of 2.5x10^3 cells/well in 100 µL organoid growth media supplemented with 0.5 mg/mL Cell Basement Membrane.

Drug sensitivity assays: CCOs were tested for sensitivity to a custom panel of 24 different chemotherapeutic compounds with varying mechanisms of action and molecular targets reconstituted in either D-PBS (ATCC® 30-2200™) or DMSO (ATCC® 4-X™). An initial medium-throughput, 12-point half log dilution curve was used for screening, followed by an optimized 8-point, half-log curve in triplicate for models and compounds of interest. After 5 days, the organoids were treated with 12.5 µM of Calcein AM (Thermo Fisher Scientific, C1430) to quantify fluorescence intensity readings followed by measuring ATP using the CellTiter-Glo 3-D Cell Viability Assay (Promega, G9681).

Data analysis: Responses were normalized to vehicle treatment condition and expressed as percent viability. Figures were plotted, non-linear curves generated, and IC50s and AUCs calculated in GraphPad Prism. Z' Factor scores were determined using vehicle control and positive control.



Results

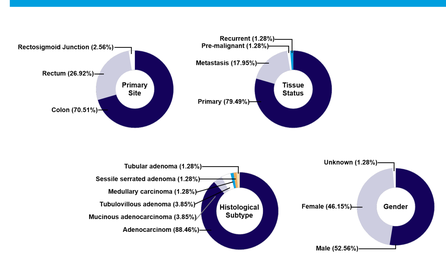


Figure 1: Clinical characteristics of established human CCOs from the HCMI portfolio. ATCC's HCMI portfolio has a diverse collection of over 78 patient-derived CCOs.

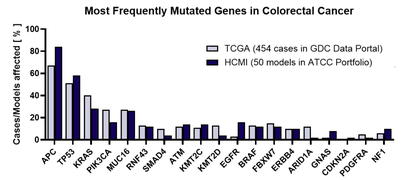


Figure 2: Comparison of the mutation frequency of CRC genes in patient tissue from GDC Data Portfolio and CCOs from ATCC. Frequency of mutation is closely correlated between ATCC CRC models and clinical patient tissues.

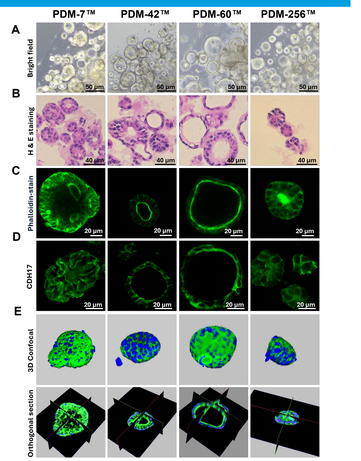


Figure 3: Characterization of CCOs of varying morphology. (A) Live culture in embedded conditions. (B) H&E-stained samples. CCOs preserve histological structure and morphology of in-vivo CRC tumors. (C) Phalloidin staining demonstrates the polarization of epithelial cells. (D) Cadherin-17; CCOs display expression of a colorectal marker commonly used as a diagnostic tool. (E) Confocal images display dynamic 3-D structure and morphological variation amongst models.

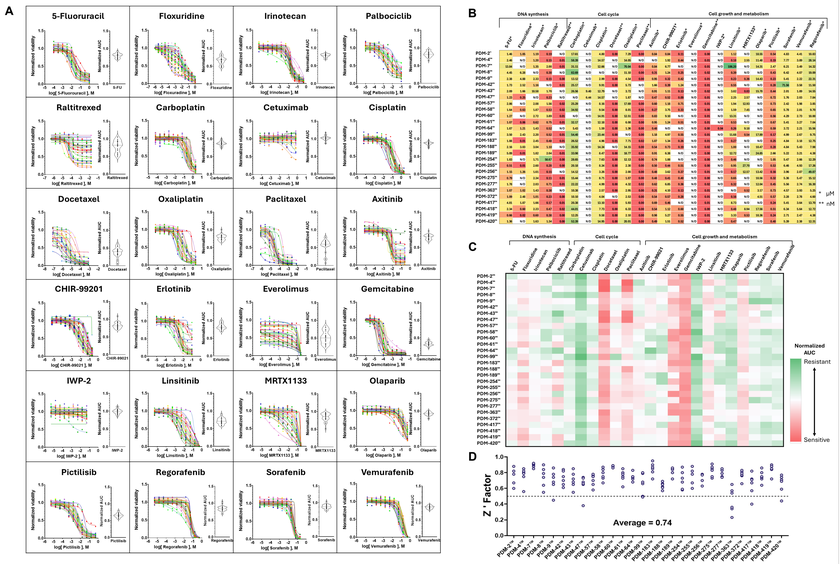


Figure 4: Compound screening of CCOs using a panel of chemotherapeutics. (A) Cell viability assays indicate chemotherapeutic drug sensitivity in a dose-dependent manner with AUC displayed as a violin plot on the right. (B) IC50s were determined by the concentration corresponding to 50% inhibition on the dose-response curves. (C) Heatmap of AUC values displays relative efficacy of drug compounds in individual CCOs. (D) Z' factors assess quality of high-throughput assay, displayed with points from each assay plate (6 per model).

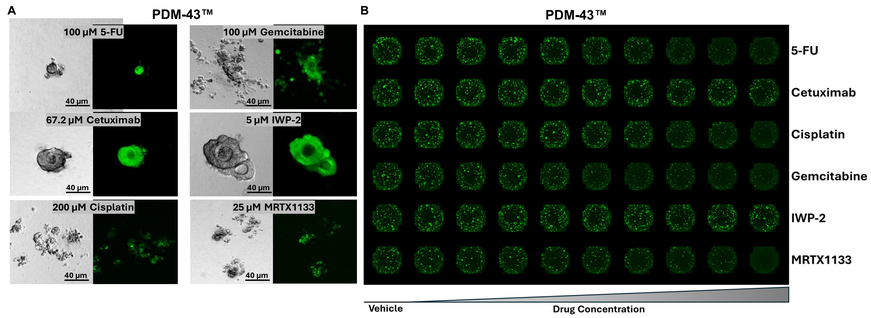


Figure 5: Calcein-AM fluorescence staining to display drug sensitivity. (A) Representative close-up images of PDM-43™ show different inhibitory mechanisms by different compounds. (B) The entire 96-well plate image presents capabilities of quantitative/qualitative image-based drug sensitive assay.

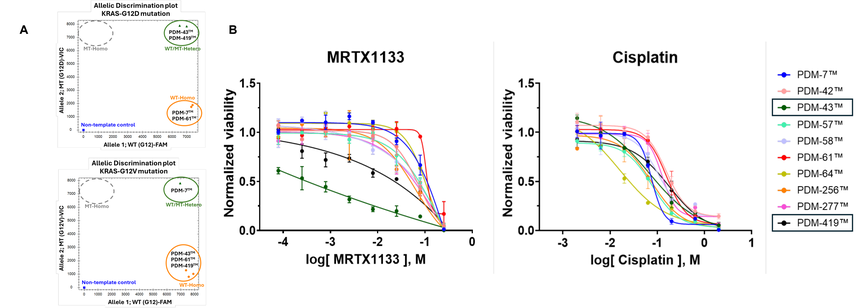


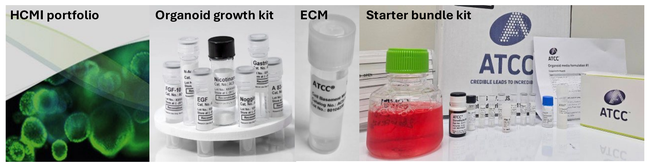
Figure 6: Evaluating the mechanism of action of MRTX1133 as a selective KRAS-G12D inhibitor. (A) The allelic discrimination plots confirm KRAS-G12D and KRAS-G12V mutation in selected CCOs. (B) KRAS-G12D mutated CCOs (PDM-43™ and PDM-419™) are more sensitive to MRTX1133.

Conclusions

- The HCMI portfolio contains next-generation cancer models from a diverse collection of patients and disease indications, including over 78 models derived from colorectal cancer tissue.
- CCOs from the HCMI have canonical mutations in key genes (APC, TP53, KRAS) seen in patient populations at comparable rates to existing large cancer datasets such as TCGA, as well as uncanonical mutations of potential therapeutic interest.
- Initial high-throughput compound screening of 28 models with 24 compounds is expanded upon with a subset of 12 compounds across 12 models to demonstrate repeatability and to further illicit variability in response.
- CCOs respond to therapeutic treatment in vitro and exhibit variable drug toxicity that may be in part a consequence of genotype. CCOs are amenable to a variety of endpoint assays used to assess response.
- There is great potential for further study of alternative seeding conditions such as seeding in embedded format, on fluidic systems, and with co-culture to further capture the in-vivo tumor micro-environment (TME).

ATCC – Science Innovation for Cancer Research

HCMI portfolio and ATCC Organoid Kits to simplify and standardize culture



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ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** sales@atcc.org **Web:** www.atcc.org