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Modeling Tumor–Immune Dynamics: Patient-Derived Cancer Based Fluidic System for In Vitro Immunotherapy

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Introduction

Lung cancer is the second most common cancer among both men and women in the United States, yet it remains the leading cause of cancer-related mortality, accounting for nearly one in five cancer deaths nationwide. Lung cancer is broadly classified into two major types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is the most common form, accounting for approximately 70-85% of lung cancer diagnoses, whereas SCLC represents about 15% of cases. Notably, the incidence of lung cancer is rising among nonsmokers and younger individuals, underscoring the critical need for continued research to better understand disease etiology and progression. Lung cancer also serves as an important model for the development of molecularly targeted therapies, given its identifiable genetic diversity and the growing role of immunotherapy in treatment.

Immunotherapy is emerging as a transformative approach in cancer treatment, offering targeted and personalized strategies that minimize damage to surrounding healthy tissues. In vitro cancer models play a critical role in advancing cancer treatments by enabling identification of drug resistance mechanisms and exploration of novel therapeutic targets. Using in vitro cancer models can help evaluate immune cell function and therapeutic efficacy, allowing for further research to be done before clinical trials. Patient-derived cancer organoid models offer enhanced physiological relevance by closely mimicking structures of tumors in vivo, including cell-cell and cell-matrix interactions.

Recent advances in 3-D in vitro modeling further enhance the physiological relevance of cancer research by better reflecting human tumor biology than traditional 2-D cultures and animal models. Three-dimensional systems capture complex tissue architecture and multicellular interactions that are critical for understanding tumor progression and immune engagement. Emerging 3-D microfluidic platforms introduce dynamic, flow-based environments that mimic key aspects of blood circulation. These systems help enable controlled immune cells trafficking and real-time tumor-immune interactions, providing a deeper understanding of the mechanisms of immunotherapy response and resistance.

ATCC® provides patient-derived cancer organoid models deposited by the Human Cancer Model Initiative (HCMI). HCMI is an international consortium dedicated to the generation of novel human tumor-derived culture models with associated genomic and clinical data available. ATCC®'s portfolio includes common and rare understudied examples of cancers from numerous tissues.

In this study, we utilized lung cancer HCMI models to establish a physiologically relevant tumor microenvironment (TME) using 3-D co-culture and microfluidic platforms to evaluate cell-based immunotherapies in vitro. This work demonstrated proof of concept for immunotherapy, with Jurkat T cells actively infiltrating the tumor microenvironment and exerting cytotoxic effects on tumor cells. Collectively, these findings highlight the suitability of HCMI models for studying cell-based immunotherapies and performing drug screening in advanced 3-D co-culture and microfluidic systems. The complex culture platforms offered by ATCC® closely recapitulate key aspects of the in vitro cellular microenvironment, advancing efforts to model physiologically relevant conditions for therapeutic development.

The Human Cancer Models Initiative

The Human Cancer Models Initiative (HCMI) is an international consortium that is dedicated to generating novel human tumor-derived culture models with associated genomic and clinical data. The HCMI consortium comprises funding agencies and cancer model development institutions. The consortium's funding agencies include the National Cancer Institute (NCI), Cancer Research UK (CRUK), Hubrecht Organoid Technology (HUB), and Wellcome Sanger Institute (WSI). NCI-funded model development institutions include the Broad Institute and the Cold Spring Harbor Laboratory. CRUK and WSI co-fund organoid development in the United Kingdom; CRUK provides the patient samples, while WSI derives and sequences the organoid models. The foundation HUB is a Netherlands-based not-for-profit organization that derives and sequences organoid models. ATCC® was selected as the sole distributor for the HCMI models. The generating institutions deposit the models into ATCC®, where they are authenticated, expanded, preserved, and made available for global distribution. The HCMI model data are available from the NCI as a resource to the research community.

Learn more about HCMI models

Materials and Methods

Cell Culture: The lung cancer HCMI model HCM-WCMC-0789-C34 (ATCC® PDM-685™) was cultured using Organoid Media Formulation #10 (ATCC®) and embedded in Cellular Basement Membrane (ATCC® ACS-3035™). Organoids were passaged every 5–7 days. Jurkat, Clone E6-1 T cells (ATCC® TIB-152™) were maintained in RPMI-1640 medium (ATCC® 30-2001™) supplemented with 10% fetal bovine serum (FBS; ATCC® 30-2020™). Primary normal human lung fibroblasts (ATCC® PCS-201-013™) were cultured using Fibroblast Basal Medium (ATCC® PCS-201-030™) supplemented with the Fibroblast Growth Kit–Low Serum (ATCC® PCS-201-041™). Human lung microvascular endothelial cells (HULEC-5a, ATCC® CRL-3244™) were cultured in ATCC Vascular Cell Basal Medium (ATCC® PCS-100-030™) supplemented with the Microvascular Endothelial Cell Growth Kit–VEGF (ATCC® PCS-110-041™).

3-D Tumor Microenvironment Architecture: To generate a 3-D static tumor microenvironment (TME), HCM-WCMC-0789-C34 lung cancer organoids were combined with primary lung fibroblasts at a concentration of 2 × 10^6 cells/mL and embedded in 5 mg/mL Cellular Basement Membrane prior to culture.

Microfluidic Culture Operation: Two PDMS (polydimethylsiloxane) microfluidic platforms (SynVivo VIV102012STU3 and Emulate S1) were prepared according to the manufacturers' protocols. Devices were coated overnight with Collagen I (Corning, 354236) and Fibronectin (Corning, 354008). Pulmonary endothelial cells (ATCC® CRL-3244™) were first seeded into the channels and maintained overnight. HCM-WCMC-0789-C34/ primary normal human lung fibroblasts matrices were introduced the following day, and GFP-overexpressing Jurkat, Clone E6-1 T cells were added on the subsequent day to establish immune co-culture conditions.

Results

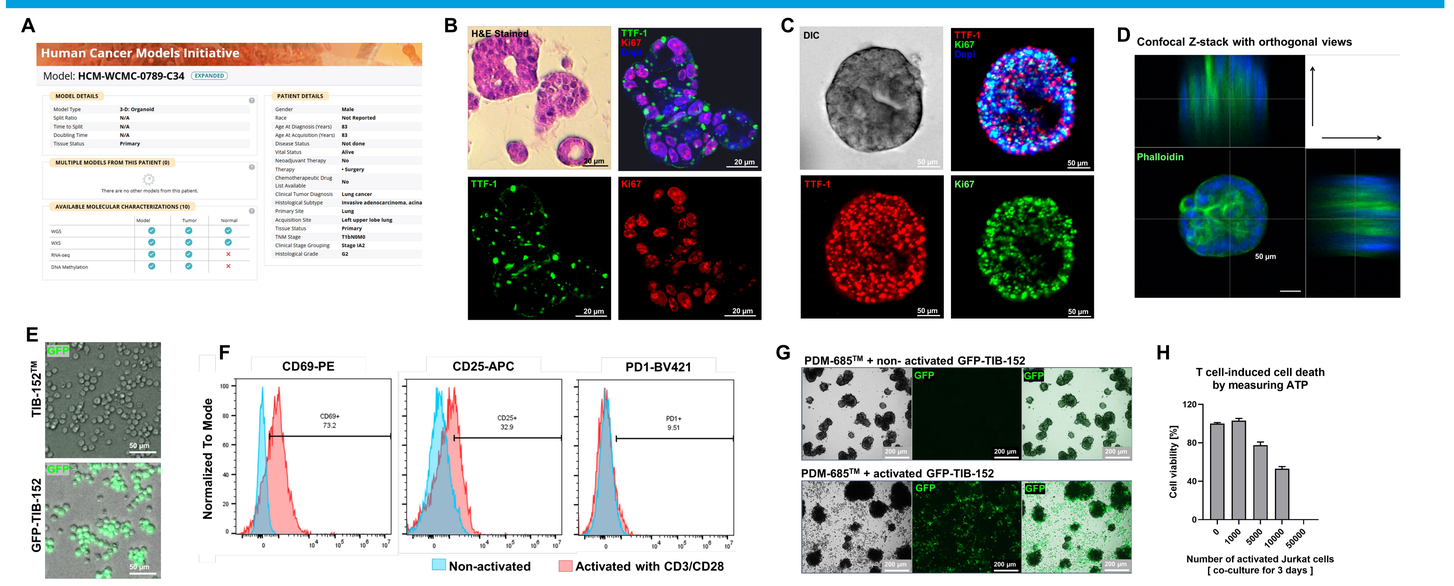

Figure 1: Characterization of HCM-WCMC-0789-C34 (ATCC® PDM-685™) lung cancer organoids and functional interaction with GFP-expressing Jurkat, Clone E6-1 T cells (GFP-TIB-152). (A) Information and corresponding clinical data for HCM-WCMC-0789-C34 organoids are available through the HCMI Searchable Catalog (https://hcmi-searchable-catalog.nci.nih.gov/). (B and C) H&E staining and DIC image demonstrate the morphological features of HCM-WCMC-0789-C34 organoids.HCM-WCMC-0789-C34 organoids expressed TTF-1 (Invitrogen, MA5-31938), a lung adenocarcinoma marker, and Ki-67 (R&D Systems, MAB7617-SP), a marker of cellular proliferation, thereby characterizing HCM-WCMC-0789-C34 organoids as a lung adenocarcinoma model. (D) Representative confocal z-stack images of HCM-WCMC-0789-C34 organoids stained with phalloidin, illustrating the three-dimensional organization of the organoid structure. (E) Representative brightfield and fluorescence microscopy images of parental Jurkat, Clone E6-1 cells and GFP-expressing Jurkat, Clone E6-1 (GFP-TIB-152) cells, demonstrating stable GFP expression in the labeled cells. (F) Flow cytometry analysis of GFP-TIB-152 comparing non-activated and activated conditions. Activation resulted in increased expression (rightward shift) of CD69 (BD Biosciences, 560968) and CD25 (BD Biosciences, 565107). PD-1 (BD Biosciences, 569466) expression was not detected under either condition. (G) HCM-WCMC-0789-C34 organoids co-cultured with non-activated or activated GFP-TIB-152. Activated GFP-TIB-152 shows increased aggregation around HCM-WCMC-0789-C34 organoids as compared to non-activated cells. (H) T cell–mediated cytotoxicity assay showing decreased viability of HCM-WCMC-0789-C34 organoids with increasing numbers of activated GFP-TIB-152 following 3 days of co-culture.

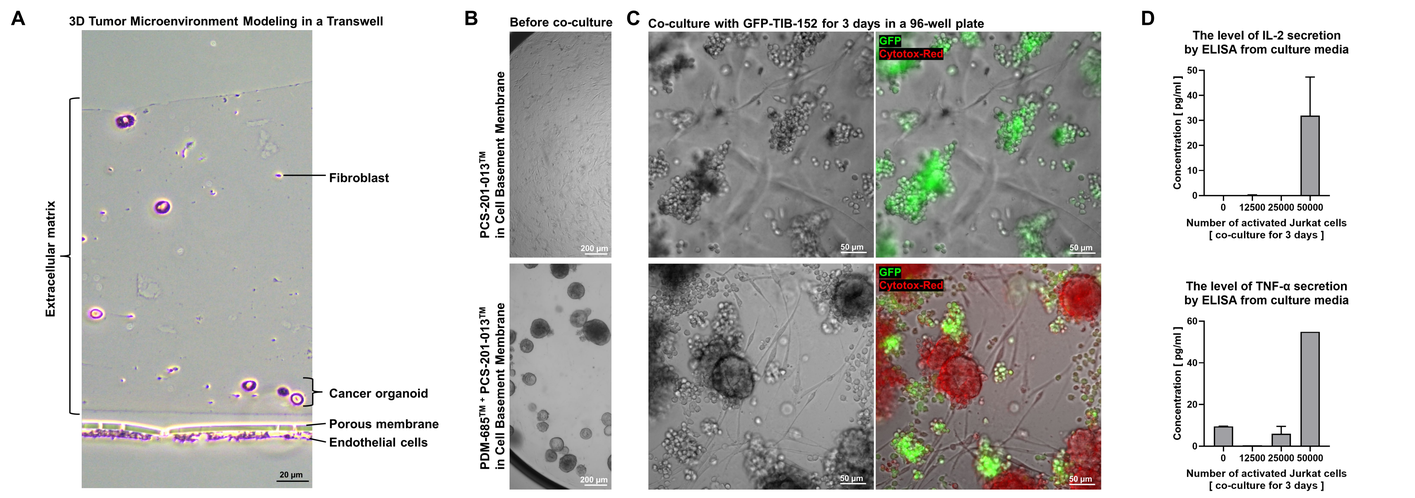

Figure 2: Establishing a fibroblast-based tumor microenvironment and T-cell interactions with HCM-WCMC-0789-C34 (ATCC® PDM-685™) lung cancer organoids. (A) H&E staining of cross-sections from transwell inserts containing collagen I–embedded primary human lung fibroblasts (ATCC® PCS-201-013™) cultured alone or co-embedded with HCM-WCMC-0789-C34 organoids (ATCC® PDM-685™), modeling the tumor microenvironment (TME). (B) 96-well plates (STEMCELL Technologies, 200-0562) containing cell basement membrane–embedded primary human lung fibroblasts cultured alone or co-embedded with HCM-WCMC-0789-C34 organoids and prepared for downstream assays. Brightfield images of extracellular matrix–embedded primary human fibroblasts alone or co-cultured with HCM-WCMC-0789-C34 organoids in 96-well plates prior to the addition of GFP-TIB-152 T cells. (C) A three-day co-culture of HCM-WCMC-0789-C34 organoids within a fibroblast-based TME in a 96-well plate with GFP-TIB-152 cells demonstrates T-cell binding to organoids and Incucyte Cytotox Red (Sartorius, 4632)–positive cell death. (D) ELISA analysis demonstrated increased IL-2 and TNF-α secretion (R&D systems, DY202 and DY210) with higher Jurkat T cell numbers and TNF-α levels exceeding IL-2 (n=3).

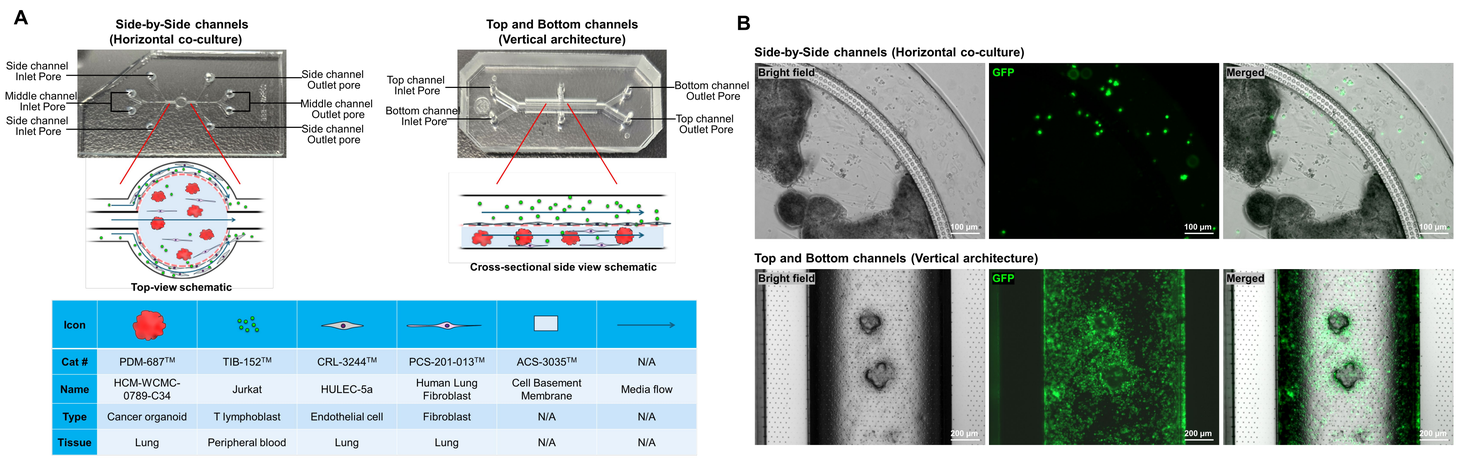

Figure 3: Two different fluidic platforms for immune cancer cell therapy. (A) Schematic overview of the side-by-side channel microfluidic chip (SynVivo) and the top-and-bottom channel microfluidic chip (Emulate), illustrating device architecture and fluidic functionality. A summary table accompanies the schematics, detailing the cell types and cellular basement membrane used in each system. In the side-by-side channel model, HCM-WCMC-0789-C34 organoids (ATCC® PDM-685™)/primary human lung fibroblasts (ATCC® PCS-201-013™) matrices were seeded in the central channel, while Jurkat, Clone E6-1 T cells (ATCC® TIB-152™) were circulated through the two outer channels lined with the endothelial cells. In the top-and-bottom channel system, lung cancer organoids with fibroblast matrices were placed in the bottom channel, the endothelial cells were seeded in the top channel, and Jurkat T cells were introduced under flow through the top channel. (B) Brightfield and fluorescence microscopy images of both fluidic platforms containing WCMC-0789-C34 organoids and GFP-expressing Jurkat T cells demonstrating immune cell movement and membrane traversal driven by fluidic flow.

Conclusions

- ATCC® provides patient-derived cancer organoids through the HCMI, along with optimized culture media to support consistent and reproducible data for cancer research applications such as drug screening and mechanism-of-action studies.
- Using ATCC® cell models and biomaterials, we established 3-D static and fluidic tumor microenvironment models incorporating Jurkat T cells to visualize and assess tumor–immune interactions during co-culture.
- ATCC®-qualified biomaterials enable advanced, physiologically relevant 3-D architectures, including distinct fluidic platform approaches that support diverse experimental applications in cancer immunology research.

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