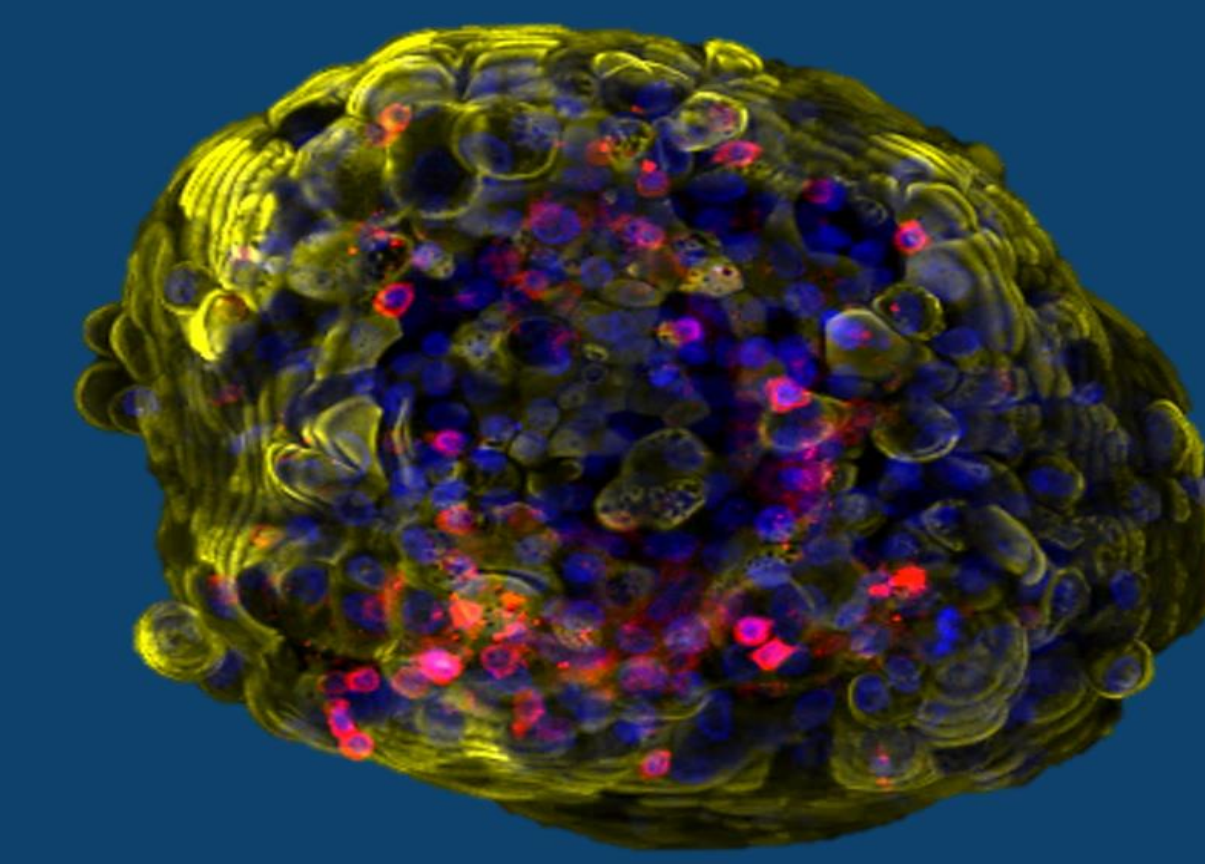


Ex vivo evaluation of a MUC1-targeted bispecific T-cell engager in ovarian tumoroids using Nilogen's 3D-EXpress platform



Background

- Mucin 1 (MUC1) is a clinically relevant target for bispecific T-cell engagers due to its widespread expression across epithelial malignancies.
- Nilogen Oncosystems' 3D-EXpress ex vivo tumoroid platform enables direct evaluation of patient-specific responses to immunotherapies within a physiologically preserved tumor-immune microenvironment.
- This study evaluated the activity of a commercially available recombinant Anti-CD3 x Anti-MUC1 bispecific T cell engager antibody (MUC1-TCE) in cryopreserved ovarian cancer-derived tumoroids to characterize immune activation using Nilogen's integrated platform combining flow cytometry, cytokine profiling, and tumor cell killing assays.

Methods

- Ovarian cancer (OC) tissues, including high-grade serous carcinoma and granulosa cell tumors, were assessed for MUC1 expression, spanning low to high levels. In addition, multiparametric flow cytometry was used to characterize immune cell populations at baseline.
- Tumoroids were generated via mechanical dissociation without enzymatic digestion or in vitro expansion, preserving the native tumor microenvironment, and were cryopreserved for subsequent use. Following thawing, tumoroids were treated with MUC1-TCE, isotype control, or assay positive control for 48 hours.
- T-cell activation and phenotypic changes were assessed by multiparametric flow cytometry following tumoroid enzymatic digestion, enabling characterization of major immune subsets and activation markers.
- In parallel, culture supernatants were collected and analyzed using multiplex cytokine assays to quantify soluble mediators associated with immune activation and cytotoxic responses.
- Tumor cell killing (TCK) was quantified using high-content confocal imaging. Live tumoroids were stained with viability fluorescent dyes. Following 3D image acquisition and segmentation, TCK was calculated as the percentage of dead cells relative to the initial viable cell population.

Ovarian Tumor Histology & Pathology

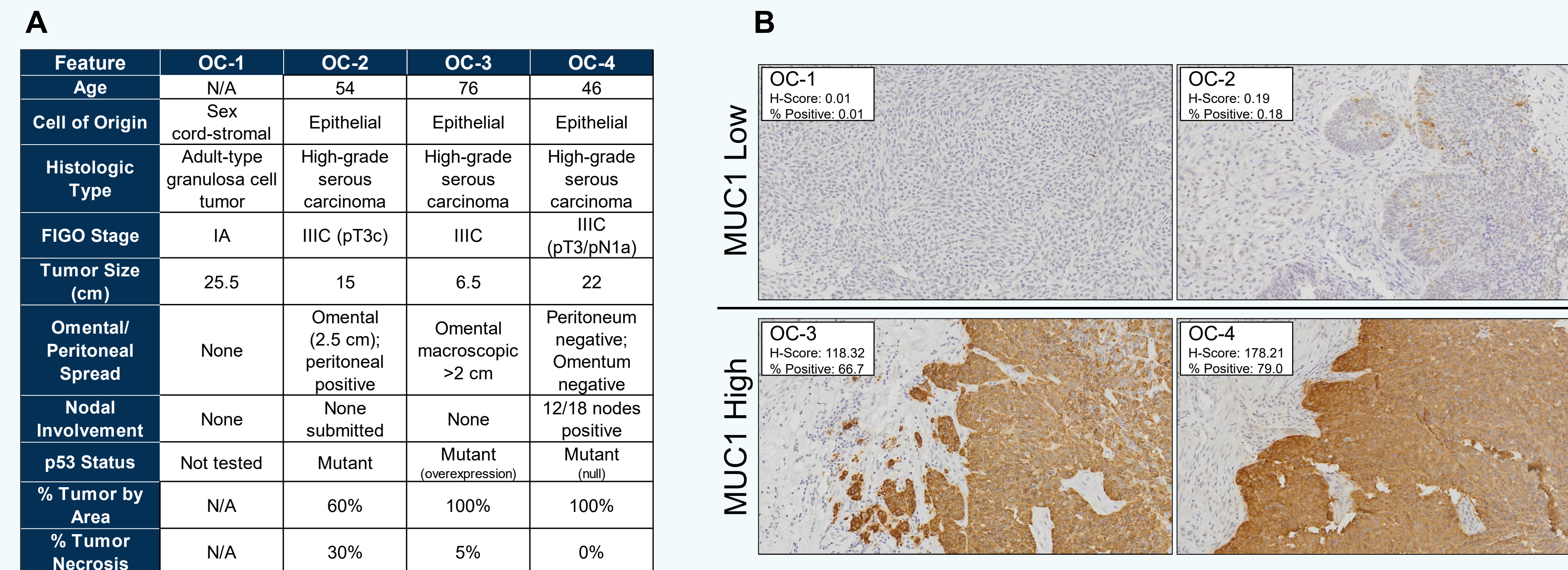


Figure 3. MUC1 expression and clinical/pathological characteristics across ovarian cancer tissues. A. Clinical and pathological characteristics of ovarian cancer patient samples used for tumoroid generation. These samples encompass distinct ovarian cancer subtypes and disease stages, providing a diverse set of tumor microenvironments for downstream functional analyses. B. Representative MUC1 immunohistochemistry (IHC) images from four ovarian cancer tumoroid samples, illustrating heterogeneous levels of MUC1 expression across the cohort. MUC1 staining intensity and distribution varied across samples, enabling stratification into MUC1-low and MUC1-high groups. H-scores were quantified using QuPath and calculated as: (% cells with weak staining × 1) + (% cells with moderate staining × 2) + (% cells with strong staining × 3), yielding a total score ranging from 0 to 300. The percentage of MUC1-positive cells was determined by dividing the number of MUC1-positive cells by the total number of cells within each tissue section. Together, these metrics provide a quantitative assessment of both staining intensity and tumor cell positivity, supporting the classification of samples based on target expression levels for downstream analyses.

High-Content 3D Tumor Cell Killing

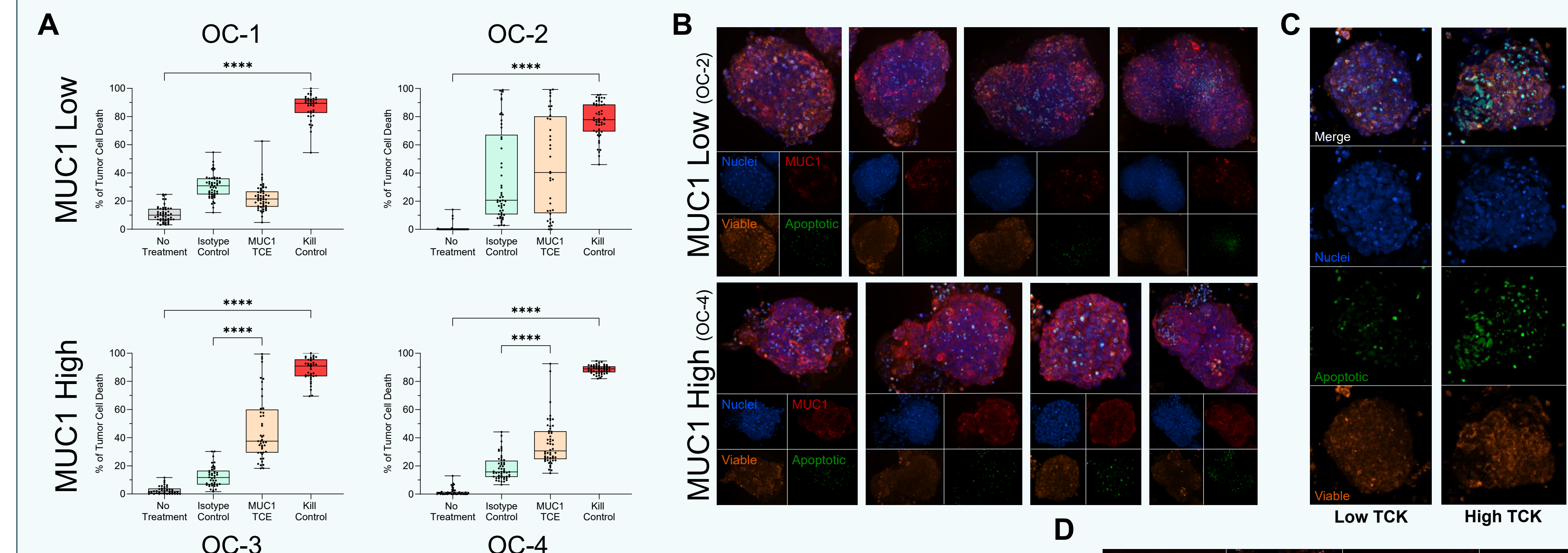


Figure 4. Ovarian tumoroids with high MUC1 expression undergo significant tumor cell killing when treated with MUC1-TCE. A. Ovarian samples with low MUC1 expression (OC-1 and -2; top) did not achieve significant tumor cell killing when treated with MUC1-TCE compared to isotype control (MUC1-TCE vs. Isotype Control). Ovarian samples with high MUC1 expression (OC-3 and -4; bottom) achieved significant tumor cell killing when treated with MUC1-TCE compared to isotype control (MUC1-TCE vs. Isotype Control). B. Representative images of tumoroids stained with MUC1 antibody, with MUC1 Low on top (OC-2) and MUC1 High on the bottom (OC-4). C. Representative images of ovarian cancer tumoroids with low TCK (few green cells) and high TCK (many green cells). D. Representative images of ovarian cancer tumoroids stained with tumor markers EpCAM or Lamin A/C antibodies.

3D-EXpress Tumoroid Platform

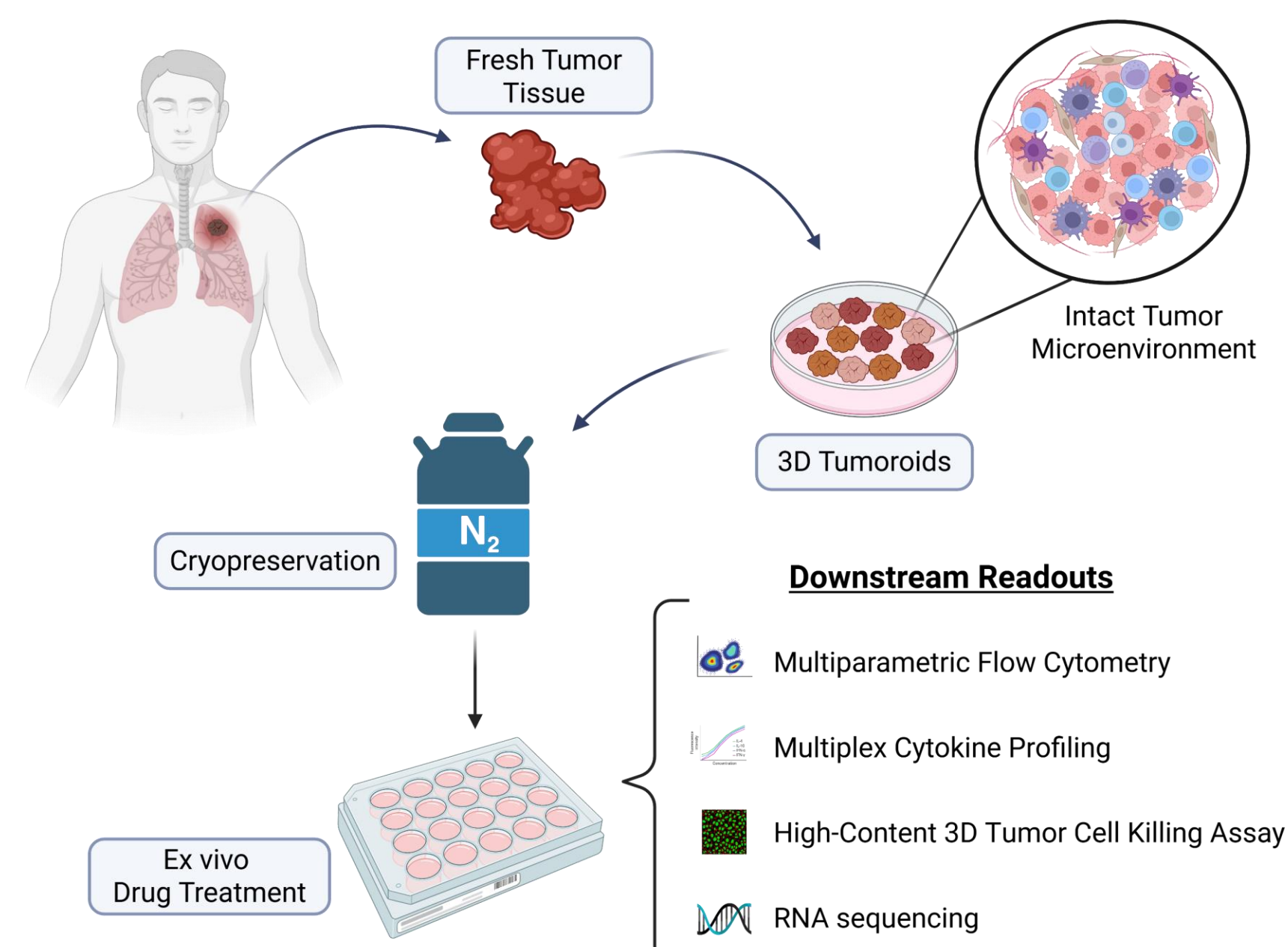


Figure 1. Workflow for generation and ex vivo testing of patient-derived 3D tumoroids. Fresh tumor tissue obtained from patients is mechanically processed to generate multicellular 3D tumoroids that preserve the native tumor microenvironment, including tumor and immune cell components. Tumoroids can be used immediately or cryopreserved for future studies. For functional assays, tumoroids are plated in multiwell formats and subjected to ex vivo drug treatments. This platform enables evaluation of therapeutic responses within a physiologically relevant, patient-specific tumor context.

Flow Cytometry Immune Profiling



Figure 5. Baseline immune cell profile of ovarian cancer (OC) samples prior to treatment. Multiparametric flow cytometry analysis of untreated tumoroids reveals heterogeneous immune composition across samples. Baseline T cells exhibit expression of activation and inhibitory markers, including CD69, HLA-DR, and PD-1, indicating a partially activated/exhausted phenotype.

Figure 6. Enhanced T-cell Activation in Response to MUC1-TCE in Ovarian Cancer Tumoroids. MUC1-TCE treatment induced increased expression of activation markers (CD25, CD69, 4-1BB, OX40) and cytotoxic marker GRZB, with more pronounced effects observed in MUC1 High samples (OC-3 and OC-4). Upregulation of inhibitory/exhaustion-associated markers (PD-1 and TOX) was also observed, consistent with T-cell activation. The Immune Activator control elicited robust activation across all markers, confirming assay responsiveness. Heatmaps summarize the frequency (%) of activation and inhibitory markers on CD4+ and CD8+ T cells.

Multiplex Cytokine Profiling

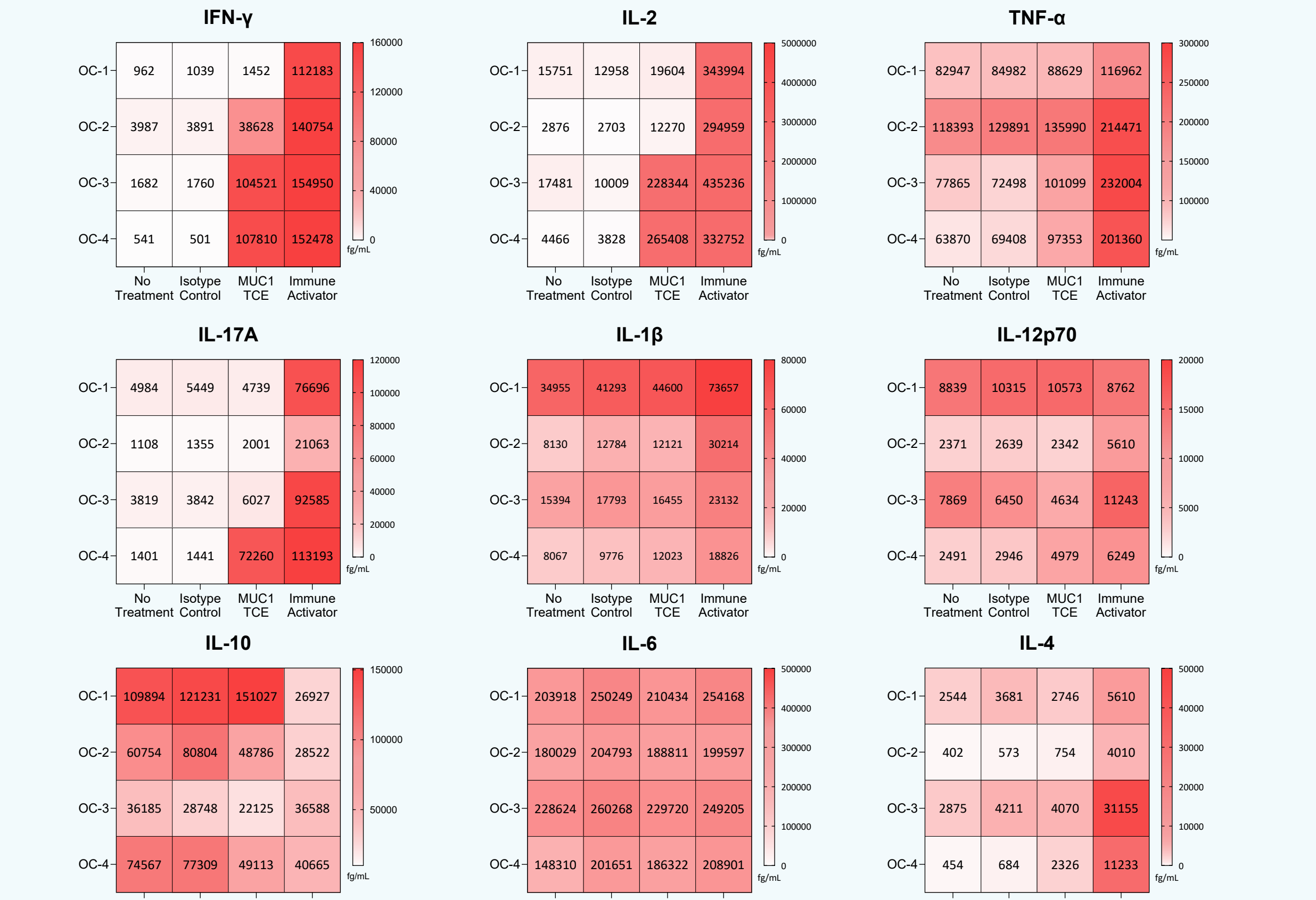


Figure 7. Treatment-induced modulation in cytokine profiles from ovarian cancer-derived tumoroids. MUC1-TCE treatment induced increased secretion of pro-inflammatory and effector cytokines, including IFN- γ , TNF- α , and IL-2, with more pronounced responses observed in MUC1-high samples (OC-3 and OC-4). OC-2 (low MUC1) showed measurable cytokine induction (e.g., IFN- γ and IL-2), though to a lesser extent. The Immune Activator control elicited robust cytokine production across samples, confirming functional responsiveness of the system.

3D Tumoroids Preserve Native Tumor Architecture and Cellular Diversity

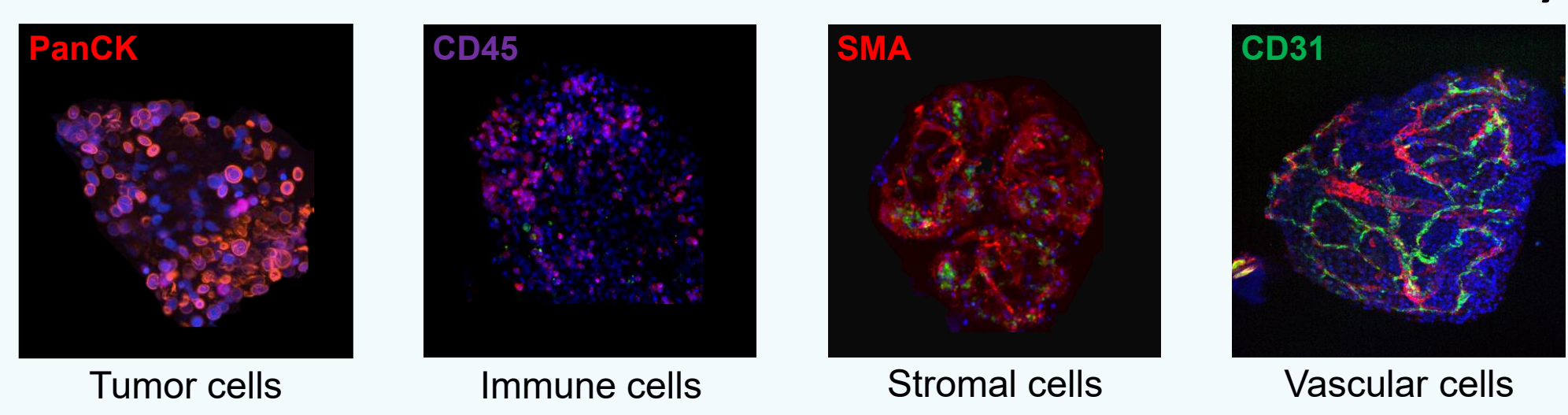


Figure 2. Preservation of tumor microenvironment components in 3D tumoroids. Immunofluorescence staining demonstrates retention of key cellular compartments within tumoroids, including tumor cells (PanCK), immune cells (CD45), stromal cells (SMA+ fibroblasts), and vascular elements (CD31). Nuclei are shown in blue. These results highlight the ability of the 3D tumoroid platform to maintain cellular heterogeneity and spatial organization of the native tumor microenvironment.

Conclusions

- These findings demonstrate the feasibility of using cryopreserved patient-derived tumoroids for ex vivo testing of bispecific T-cell engagers while preserving the native tumor-immune microenvironment.
- Integration of cytokine measurements, immune phenotyping, and tumor cell killing assay provide a scalable approach for characterizing mechanisms of action of MUC1-targeted agents and supports continued development of bispecific immunotherapies for solid tumors.