3D-EXpress *Ex Vivo* Platform Using Viable Cryopreserved Tumoroids for Rapid Assessment of Targeted Therapeutic Outcomes

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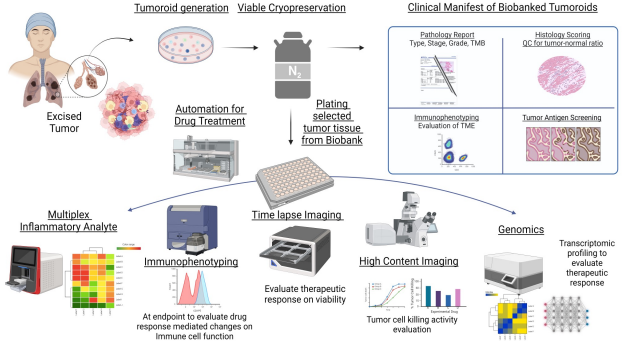
Background

Selecting, integrating, and arranging multiple FDA-approved therapies and drugs have posed significant challenges, underscoring the necessity for a precision oncology platform to explore innovative combination treatments. Enter 3D-EXpress, a distinctive ex vivo therapeutic testing platform utilizing a biorepository of fresh patient tumoroids that have never been dissociated, propagated, or reassembled, thus preserving their intact microenvironment. Consequently, these samples offer an exceptional ex vivo setting to assess the effectiveness of therapeutics aimed at the tumor environment. In this study, we utilized the 3D-EXpress platform to compare the efficacy of various therapeutic modalities targeting tumor antigens and immune checkpoint inhibitors ex vivo.

3D-Express Platform



(A)

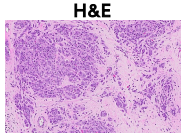
Tissue Code	Tissue Type	PR-Pathology Report						
		Age	Gender	Site	Sub Type	Grade	Stage (pT)	Mutation Information
S1	Endometrial	79	Female	Uterus	Endometrioid	III	pT1b pN0	MSS; p16 (strong), p53 (mutant pattern)
S2	Endometrial	71	Female	Endometrium	Endometrioid	III	pT3b pN1a	MSI-h instable; loss of MLH1/PMS2
S3	Endometrial	57	Female	Endometrium	Endometrioid	I	pT1a pN0	ER+, PR+, HER2+ (3+)
S4	Colorectal	68	Male	Colon	Adenocarcinoma	III	pT3b pN1a	MSI-h instable; loss of MLH1/PMS2
S5	Colorectal	75	Male	Right Colon	Adenocarcinoma	II	pT2 pN0	MSS
S6	Colorectal	86	Female	Right Colon	Adenocarcinoma	III	pT3pN0	MSI-h; Loss of MLH1/PMS2

(B)

Tissue ID	(% Live Singlet)									
			(% CD45+)							
			(%CD3-CD56-)						(%CD3)	
	CD45-	CD45+	CD11b+	CD11c_hi	CD14+	CD163+	CD56+	CD3+	CD4+	CD8+
S1	36.5	63.5	10.8	8.63	10.7	9.36	7.36	78.6	33.7	48.9
S2	46.2	53.7	22.9	20.77	18.7	6.06	10.7	58.8	47.5	35.3
S3	28.5	71.3	22.2	13.7	14.8	10.2	14.3	51.7	45.3	26.3
S4	53.6	44.9	19.6	9.48	10.1	9.24	16.3	55.5	30.6	52.6
S5	23.6	76.1	19.3	13.7	8.69	9.15	27.7	39.5	37.7	49.1
S6	32.4	65.2	12.5	10.1	9.27	7.56	18.4	32.6	29.8	38.4

(C)

H&E



(D)

PD-L1

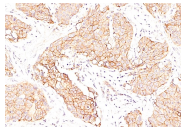


Figure 1. Clinical manifest of selected tissues from the biobank. (A) Pathological information of selected patients. **(B)** Analysis of immune cell populations at T_0 (pre-cryopreservation). **(C)** A representative H&E image depicting the diverse microenvironment (tumor cells present with fibrous stromal components) within the parent endometrial tumor tissue. **(D)** A representative image depicting PD-L1 expression level assessed by IHC on endometrial TMA slides.

Results

(A)

Tissue ID	(% Live Singlet)									
			(% CD45+)							
			(%CD3-CD56-)						(%CD3)	
	CD45-	CD45+	CD11b+	CD11c_hi	CD14+	CD163+	CD56+	CD3+	CD4+	CD8+
S1	60.3	38.9	10.9	2.33	9.67	8.09	8.28	76	32.7	41.8
S2	49.4	49.9	12.7	5.38	10.2	8.3	10.5	62.6	49.9	40.2
S3	30.4	68.4	18.4	7.31	18.3	13.7	24	46.6	41.2	33.4
S4	75.2	24.4	14.2	9.59	6.8	4.01	12.5	42.7	44.5	33.3
S5	60.2	38.9	29.6	13.8	7.71	4.31	22	31.4	50.8	33.1
S6	51.6	42.3	15.4	10.7	11.21	8.18	21.9	38.7	34.1	42.5

(B) (C) (D) (E) (F)

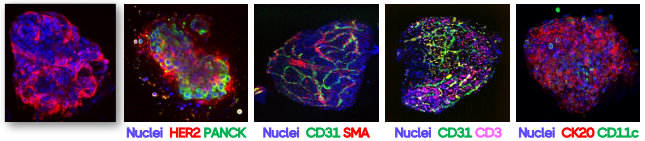


Figure 2. Cryopreserved 3D tumoroids derived from patients' tumors were assessed for viability and flow cytometry-based immune cell phenotyping. (A) Post-thaw, the 3D tumoroids retained the unique immune cell heterogeneity observed in lymphoid and myeloid populations. **(B-F)** Immunofluorescent characterization of the unique and diverse tumor microenvironment retained in the 3D tumoroids. Specific markers used to identify tumor cell, immune cell, and stromal cell populations (cancer-associated fibroblast and vascular components) within 3D tumoroids from patient S3 are indicated.

(A) Viability Identifier (B) Tumor Cell & Immune Cell Identifier

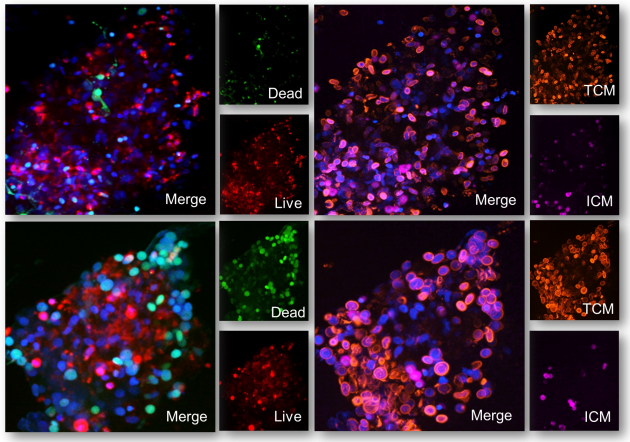


Figure 3. Cryopreserved 3D tumoroids were profiled for viability (A), and tumor and immune cell (B) phenotyping by high content imaging. Tumor and immune cell identifier parameters were used along with the viability parameter to identify therapeutic response. Images were analyzed by Nilogen's proprietary AI-algorithm for quantitative measurements.

(A) (B)

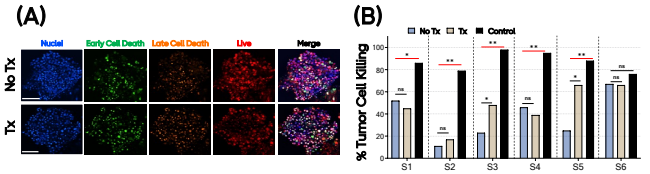


Figure 4. Drug-mediated tumor cell killing. Nilogen's tumor cell killing (TCK) assay was performed using high-content confocal imaging to visualize treatment-mediated changes in viability of tumor cells within the live tumoroids. **(A)** Images show increased tumor cell death in tumoroids treated with Nivolumab for 72 hours. Scale bar, 100 µm. **(B)** Quantification of relative TCK observed in tumoroids from the 6 patients under the indicated treatment conditions.

(A) (B)

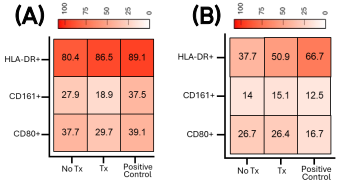


Figure 5. Treatment-mediated immune cell activation was observed in 3D tumoroids. (A) (B) Heat maps depicting the percentage of cells expressing functional markers revealing changes in T cell subsets CD4+ **(A)** and CD8+ **(B)** following indicated treatments.

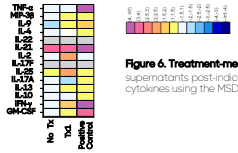


Figure 6. Treatment-mediated immune cell activation was observed in the 3D tumoroids. Culture supernatants post-indicated treatments of the 3D tumoroids were assayed for expression of cytokines using the MSD Multiplex Assay.

Conclusions

- The 3D-EXpress platform using cryopreserved 3D tumoroids with intact tumor microenvironment is an effective tool for the pre-clinical assessment of rational drug combinations for treatment of solid tumors.
- The 3D-EXpress platform provides unique functional insight into the microenvironment of both treatment responsive and non-responsive tumors and can aid in the development of patient-centered therapeutic regimens.

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