#3086 Organoids Standardized to a Clinically Validated Drug Response Assay for Truly Predictive In Vitro Drug Response Profiling

Abstract **High-Grade Glioma Organoids** Unlike cell lines, organoids maintain most of the biological properties of the parental tissue from which the starting cells were isolated including the histology and gene expression. When organoids include clinical Tissue 1 Tissue 2 Tissue 3 Tissue 4 annotation and follow-up, they become a useful, renewable tool for clinical correlation studies, but to be truly predictive the drug profiling assays utilized to screen organoid response must have measurable correlation with patient response. 3D Predict[™] is a highly accurate assay that is 89% and 85% predictive of response in first-line ovarian cancer and high-grade gliomas (HGG) respectively. We have developed a panel of organoids that are clinically annotated, include correlative primary tissue 3D Predict[™] drug response data, and have been assessed for the recapitulation of primary tissue histology and genomics. Additionally, our organoid models incorporate matched immune cells, a key component of the tumor microenvironment, making them an ideal model for immune-oncology studies. Here we present data on 15 available organoid models across HGG, breast, colorectal Tissue 3 **Tissue 4** and bladder cancer. We have applied these models to drug response studies, including checkpoint inhibitors and shown correlation to primary patient response. The assurance of predictive capacity and the inclusion of clinical Sample ID **Tumor Type** annotation and follow-up is unique to KIYATEC's organoids and is significant because it avoids the pitfalls of High-grade Glioma Tissue 1 comparing drug responses across non-concordant assay platforms while providing assurance that the models are Glioblastoma Tissue 2 reflective of individual patient response and outcomes. High-grade Glioma Tissue 3 High-grade Glioma Tissue 4 Methods D. Tissue 2 **Tissue 1** Tissue 4 **Tissue 3** Samples taken for Drug Response Tumor 150 150-150-150-Excised & DNA/RNA and **Data Collection** Shipped to histology 100 100 KIYATEC Log Dose (µM) Log Dose (µM) Log Dose (µM) Log Dose (µM) Abemaciclib Trametinih **Everolimus** Carboplatin Irinotecan Etoposide Lomustine KIYA-PREDICT™ Assay, Day 0 — Procarbazine — Dabrafenib TMZ — Rucaparib Figure 2. High-grade Glioma Organoids (HGG) (A) Representative Bright-field images of established HGG Organoids, 10x magnification. Inset: 4x Bright field images of organoid ECM domes. (B) Table of tumor organoid subtypes. (C) Hematoxylin and eosin (H&E) stained organoids, 40x magnification. (D) HGG organoid drug response Primary **Dissociated Primary** KIYA-PREDICT™ profiling using KIYAYEC's HGG KIYA-PREDICT[™] assay platform. Tumor **Tumor Cells** Platform **Breast / Ovarian / CRC Tumor Organoids DAY 0:** Organoid Biobanking DTCs Seeded into ECM for Organoid Culture Β. Viably Cryo-Preserved OV Tissue А. **OV Tissue 2** OV Tissue 3 Bulk and/or CD3 depleted DTCs Drug Response Profiling Bioinformatics Analyses D. $\bigcirc \bigcirc$ **Organoid Expansion Tumor Cells** • Expanded for 10 Passages • Mycoplasma Screened Primary , H&E Organoids, H&E BC Tissue **L**. CD3+ positive selection .00 F. **Autologous** CD3+ TILs **Expanded CD3+ TILs Banked** E. CRC Tissue 2 **CRC** Tissue 1 • Characterized by FCM **Day 0:** Figure 4. Additional organoids representing breast (BC), ovarian Isolation/Expansion of CD3+ TILs • Viably-Cryopreserved (OV) and colorectal (CRC) cancers. (A) Representative bright-field images of ovarian cancer organoids, 10x Magnification. (B) Table listing ovarian cancer subtypes of organoids. (C) Breast cancer Tissue Representative images Left- Brightfield image of established **CRC** Tissue 1 G. CRC Tissue 2 10² 10³ 10⁴ 10⁵ 10⁶ organoids, 10x magnification. Middle- H&E stained tissue section of primary tumor, 40x magnification. Right- H&E-stained section of Figure 1. Workflow Schema of Organoid Establishment and Autologous T-cell Isolation and 100corresponding established organoid. (D) Table listing breast organoid Expansion from Primary Tissue Samples. (A) Sample acquisition and processing for KIYA-50subtypes. (E) Representative brightfield images of CRC organoids, 10x PREDICT[™] Drug Response Profiling Assay. (B) Tissue samples designated for organoid magnification. (F) Table of CRC organoid subtypes. (G) CRC organoid development, including the isolation and expansion of autologous CD3+ T-cells undergo a Log Dose (µM) Log Dose (µM) drug response curves. rigorous process of expansion and characterization. Irinoteca



Melissa Millard, Natalie Williams, Ashley K. Elrod, Teresa M. DesRochers KIYATEC Inc., Greenville, SC 29605 USA











Sample ID	Tumor Type	
OV Tissue 1	High-grade Serous Carcinoma	
OV Tissue 2	High-grade Serous Carcinoma	
OV Tissue 3	High-grade Serous Carcinoma	
Sample ID	Tumor Type	
BC Tissue 1	TNBC	
BC Tissue 2	HER2+	
BC Tissue 3	HER2+	
Sample ID	Tumor Type	
CRC Tissue 1	Adenocarcinoma	
CRC Tissue 2	Adenocarcinoma	



Figure 3. Bladder Tumor Organoids (A) Representative Bright-field image of established bladder tumor organoids derived from Tissue 1, 10x magnification. (B) On the left, H&E-stained tissue section of primary bladder tumor, Tissue 1 and (right) corresponding H&E-stained organoids. (C) Table of established bladder tumor organoid samples. (D) Bladder tumor organoid drug response curves profiled using KIYATEC's KIYA-PREDICT EV3D platform.

Gemcitibine

— Vinblastine

Matched Organoid-Immune Cell Models

С.





Bladder Tumor Organoids

		C	
rimary	Organoids	Sample ID	Tumor Type
		Tissue 1	Urothelial carcinoma
		Tissue 2	Urothelial carcinoma
Tissue 2	Tissue 3	Tissue 3	Transitional Cell Carcinoma (Papillary)
	% Viability	Tissue 4	Transitional Cell Carcinoma (Papillary)
og Dose (uM)	Log Dose (uM)	Tissue 5	Urothelial carcinoma
Tissue 5	Tissue 6 .ᡓ ¹⁵⁰]	Tissue 6	Urothelial carcinoma
	liqpi 100 50	Tissue 7	Urothelial carcinoma
og Dose (uM) Tissue 8	- 0 Log Dose (uM) Tissue 9	– Tissue 8	Papillary- urothelial carcinoma low grade
_og Dose (uM)	Vigpility 50- 0 Log Dose (uM)		Invasive papillary- urothelial carcinoma, high grade



Organoid & Autologous T-cell Sets			
Tumor Type	# of Models		
HGG	5		
Ovarian	3		
Bladder	2		

Figure 5. CD3-based selection and expansion of autologous T-cells from bulk dissociated, primary tumors. A small-fraction of CD3+ immune cells were detected, isolated, and expanded from bulk primary tumor samples prior to organoid development including (A) HGG, (B) Ovarian, and Bladder tissues (data not shown). Characterization has included Tcell analysis by flow cytometry before and after expansion. CD3+ T-cells are preferentially expanded and both CD4+ and CD8+ T-cells are detected following expansion. (C) Table listing available organoid/autologous T-cell models currently established by KIYATEC.