Organoid Models Derived from Primary Tumors and Patient-Derive Xenograft Tumors Reflect Platinum Sensitivity of Ovarian Cancer Patients

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Abstract

Ovarian cancer (OC) remains the deadliest gynecological cancer, primarily due to late stage diagnosis and high rates of chemotherapy resistance and recurrence. Lack of representative preclinical models exacerbates the challenges of discovering effective therapies to treat platinum-resistant OC. Patient-derived xenograft (PDX) models maintain the genetic characteristics of the original tumor, but require considerable development time and have limited screening capabilities. Organoid models mimic the tumor's 3D structure and preserve intra-tumoral heterogeneity, so are ideal for drug screening. The purpose of this study was to determine if PDX-derived organoids (PDXOs) can reflect patient responses to chemotherapy similar to organoids derived from primary patient tumors (PDOs). In drug response assays, PDXOs and PDOs demonstrated similar sensitivity to standard chemotherapy. Furthermore, both PDXOs and PDOs reliably reflected patient response based on the clinical designation of platinum sensitivity. Seven out of eight models derived from six platinum-sensitive cases showed a significant reduction in cell viability when treated with carboplatin, paclitaxel, or the combination therapy. Six of seven organoid models derived from four platinum-resistant or refractory patients demonstrated little to no reduction in cell viability with carboplatin or combination treatment. In these platinum-resistant models, response to single agent paclitaxel was mixed, suggesting that organoid models could predict response to second-line paclitaxel. This study demonstrates that PDXOs can effectively mirror patient responses to chemotherapy, underscoring their potential as valuable, renewable models for screening novel therapies and developing personalized treatment strategies in OC.

Introduction

Ovarian cancer (OC) has one of the highest mortality rates among gynecological cancers, largely due to its late-stage diagnosis, resistance to chemotherapy and recurrence after first line treatment (1). OC is a highly heterogeneous disease and there is a shortage of preclinical models that accurately reflect this variability among patients (2). Previous studies demonstrate that patient-derived xenograft (PDX) models preserve the genetic diversity of the original tumor and accurately reproduce its morphology (3-6). However, PDXs are challenging to establish due to the time-intensive process to establish them and their limited screening capabilities due to the high costs of preclinical studies (7, 8). Organoid cultures offer new opportunities for therapeutic testing and development in pre-clinical settings. Initial drug testing has historically been performed on OC cell lines grown as monolayers (9, 10). 3D organoids closely recapitulate the tumor morphology and preserve intra-tumoral heterogeneity (11), unlike traditional two-dimensional (2D) cultures of immortalized cancer cell lines that lose their heterogeneity due to rapid clonal selection (12). In addition, response to targeted therapies correlates with genomic aberrations in the target gene in OC PDOs but not in 2D OC cell cultures, meaning that organoids better replicate the tumor microenvironment and architecture, leading to more accurate predictions of drug efficacy

based on specific genetic mutations (13). In order to use organoid models to guide the selection of clinical treatment strategies, it is essential to confirm that drug response patterns in these organoids align with real-world clinical results. Clinical trials directly comparing responses in colorectal cancer organoids to patient response have shown that these *in vitro* models can effectively reflect patient responses to both chemotherapy and targeted therapies (14, 15). Similarly, initial findings in high throughput organoid drug response studies showed a correlation between responses in OC patient-derived organoids and clinical outcomes (16-18).

In this study, we examined a cohort of high-grade serous OC cases where we have collected the clinical response data for first line standard chemotherapy combination carboplatin (CBDCA) and paclitaxel (PTX). These models included platinum-sensitive cases, which exhibited reduced tumor growth, resistant cases, where tumors regrew within six months, and refractory cases that showed no response to treatment. To establish PDX models, cancer spheroids isolated from OC primary malignant ascites was engrafted intraperitoneally (IP) into NSG mice to simulate peritoneal dissemination. Subsequently, we generated organoids from solid tumor and ascites samples obtained from PDXs (PDXOs) and from primary patient samples (PDOs), to determine if the PDXOs could accurately replicate patient responses to chemotherapy. Our results indicate that PDXOs, similar to primary PDOs, consistently reflect the clinical response to standard first-line chemotherapy based on the clinical designation of platinum sensitivity. Thus, 3D *in vitro* OC organoids derived from PDXs offers a renewable alternative to PDOs that supports the growth of patient-derived cancer cells in a manner that closely mimics the *in vivo* environment.

Material and Methods

Isolation of cancer cells from patient samples. Malignant ascites fluid and fresh primary OC tumor pieces were collected from consenting OC patients undergoing cytoreductive surgery at the University of New Mexico Comprehensive Cancer Center as detailed in Steinkamp et al, 2023. Acquisition of patient samples was approved by the UNM Health Science Center Institutional Review Board (protocol no. INST1509) and studies were conducted in accordance with the U.S. Common Rule. Red blood cells were lysed using Ammonium Chloride Solution (STEMCELL Technologies). Live cancer cell spheroids and associated immune cells were isolated from the ascites fluid, cryopreserved in freezing media (5% DMSO/95% FBS, Gibco, Thermo Fisher Scientific), and stored at -80° C prior to processing for organoid development. Primary OC tumors were cut into 1 mm x 2 mm pieces and cryopreserved in freezing media.

Engraftment of patient samples into the mice or preparation of PDX mice. In samples that yielded a large number of cancer cells from the ascites fluid (minimum 3 x 10^6, maximum 20 x 10^6 cancer cells as spheroids), fresh or cryopreserved spheroids were injected into the peritoneal cavity of in-house bred NSG mice (RRID: IMSR_JAX:005557) to establish orthotopic PDX models of disseminated OC as detailed in Steinkamp et al, 2023. PDX-engrafted mice were euthanized at a humane endpoint when ascites fluid accumulation caused abdominal distention or when mice showed signs of wasting. At necropsy, solid tumors and ascites fluid was collected and passaged directly into new donor NSG mice, or cryopreserved in 5% DMS/95% FBS. PDXOs were generated from fresh or cryopreserved PDX solid tumor or ascites fluid as indicated in the figures.

Organoid Formation. Development of patient-derived organoids (PDOs) or PDX organoids (PDXOs) followed protocols based on those developed in the Leslie (University of New Mexico) and Thiel (University of Iowa) labs (19). Briefly, for solid tumors, tumors were finely minced then incubated in digestion buffer (2 U/mL dispase, 1 mg/mL collagenase P, and 2 µL/ml DNAse in human wash buffer) for 30 minutes at 37°C on a tube rotator. The digested tissue was strained through a 40 µm strainer into a 15 mL tube to obtain a single cell suspension, then washed twice with human wash buffer (DMEM/F12 (Gibco), 10 mM HEPES, 1X GlutaMAX, 100 µg/ml primocin, and 0.1% BSA), and centrifuged at 500 x g for 10 minutes after each wash. For cancer spheroids, spheroids were centrifuged at 500 x g and resuspended in digestion buffer after which point the same protocol was followed as for solid tumor tissue. To establish organoid cultures, 50,000 live cancer cells/well were embedded in 100% ultimatrix gel (Cultrex) in 50 µL domes in a 24-well plate. After solidification, domes were overlaid with 450 µL of organoid media (Advanced DMEM/F12 (Gibco) supplemented with 1X GlutaMAX, 10 mM HEPES, and Pen/Strep and enriched with 100 ng/ml Noggin, 250 ng/ml R-spondin-1, 100 ng/ml FGF10, 37.5 ng/ml Heregulin B-1, 50 ng/ml EGF, B27 supplement (1:50), 1.25 mM N-Acetylcysteine, 100 nM estradiol, 2% Primocin , 5 mM Nicotinamide, 5 µM A83-01, 10 µM Y-27632, a TGF-beta inhibitor and ROCK inhibitor, 10 µM Forskolin, and 250 µg/ml Hydrocortisone (**Figure 1**). Initial organoid formation from a single -cell suspension took from 7-14 days for PDOs and from 6-9 days for PDXOs.

Drug Screening. Drug screening was performed in 96-well white plates (CELLSTAR) with µClear® bottoms. Each well was seeded with 20-30 organoids following the Thiel lab's protocol (20). PDOs or PDXOs cultured in 24-well plates were harvested, washed with cold buffer, and resuspended in cold media. The PDO suspension was mixed with ultimatrix in a 1:1 ratio and seeded as 5 µL domes. After dome solidification, 200 µL of prewarmed organoid culture media was added to each well. The following day, the organoids were treated with standard chemotherapy (Carboplatin (CBDCA), Paclitaxel (PTX), or a combination of CBDCA and PTX) at concentrations specified in the figures. 0.1% DMSO diluted in organoid media served as the vehicle control. After 72 hours, organoid cell viability was determined using the CellTiter-Glo® 3D Cell Viability Assay (Promega), which measures ATP as an indicator of active cell metabolism. An equal amount of CellTiter-Glo® 3D reagent was added to the media in each well to promote cell lysis and release ATP. Plates were shaken vigorously in a microplate reader for 5 minutes and allowed to sit at room temperature for 25 minutes to stabilize the luminescence signal. Luminescence was measured using a BioTek Synery Neo2 Multi-Mode Plate Reader high-sensitivity plate reader to assess cell viability (**Figure 1**).

Statistical Analyses. Statistical analysis was performed using Graphpad Prism. One-way ANOVA examined differences between groups. A p-value of $p<0.05$ was considered significant.

Data Availability. The data generated in this study are available within the article and its Supplementary Data.

Results

Characterization of chemotherapy sensitivity and growth dynamics in Patient-Derived and PDX-Derived Ovarian Cancer organoids.

In order to develop patient-derived OC models to test between-patient variation in response to standard and targeted treatments, cancer cells isolated from primary OC solid tumors and malignant ascites or from PDX solid tumor or malignant ascites were seeded in matrix domes to form patient-derived organoids (PDOs) or PDX-derived organoids (PDXOs) respectively (**Figure 1**). These organoid models preserve the genetic and phenotypic characteristics of the original patient tumor and, when seeded in a 96-well format, allow for rapid high-throughput testing of single agents and combination treatments *in vitro*.

For this study, seven PDOs and eight PDXOs were established from ten high grade OC patients (**Table 1**). Paired PDOs and PDXOs were established from five of the ten patients. The clinical designation of platinum sensitivity was collected for each patient and is defined as: platinum-sensitive (partial or complete response to standard CBDCA/PTX treatment with recurrence >6 months after treatment), platinum-resistant (disease progression on therapy or recurrence within 6 months), or platinum-refractory (no response to initial treatment) (21-23).

Over all, comparing the growth dynamics of PDOs and PDXOs, PDXOs were distinct from PDOs in that PDXOs established well-formed organoids faster than the PDOs. PDOs from ascites samples typically exhibited slower growth, forming smaller organoids within 7-14 days (**Figure 2A**). In contrast, PDOs from a solid tumor sample taken from one patient (PT 90) showed notably slower growth, taking approximately 20 days to develop into larger organoids (**Figure 2B**). Similar to PDOs, PDXOs derived from ascites samples grew slowly, forming small organoids within 7-10 days (**Figure 3**). PDXOs derived from PDX solid tumor samples generally demonstrated faster growth than the PDXOs derived from ascites samples and formed large organoids within 6-9 days (**Figure 4**). Over all, both PDOs and PDXOs from ascites form smaller organoids, whereas those derived from solid tumors form larger organoids.

PDO response aligns with the clinical designation of platinum sensitivity.

PDOs were plated in 5 ul domes in 96 well plates and treated with 1 µM CBDCA, 10 nM PTX, or the combination for 72 hours. Cell viability was assessed using a 3D bioluminescent viability assay and viability of treated organoids was normalized to that of untreated controls. Representative images of organoid response after 72 hours are shown in **Supplemental Figure 1**. All PDOs derived from platinum-sensitive OC patients demonstrated a sensitivity to standard chemotherapeutic agents (**Figure 5A**). PDO18 and PDO20, derived from malignant ascites (**Figure 5A**) and PDO90 (**Figure 5D**), derived from an omentum metastatic solid tumor, exhibited a significant reduction in cell viability when treated with 1 µM CBDCA, 10 nM PTX, and the combination, indicating a consistent pattern of sensitivity with ascites-derived and solid tumor-derived PDOs. Platinum-sensitive PDO92, developed from malignant ascites, was resistant to single agent CBDCA. However, when treated with single agent PTX or the combination of CBDCA and PTX, a significant reduction in viability was seen compared to the control (**Figure 5A**). This suggests that response to standard combination treatment in this patient may be due to sensitivity to PTX. In all platinum-sensitive cases, the standard combination therapy significantly reduced cell viability.

All PDOs derived from platinum-resistant or refractory cancers exhibited resistance to combination CBDCA plus PTX. However, their response to single agent chemotherapies was varied. Platinum-resistant PDO2 and platinum-refractory PDO1 displayed a small but significant reduction in cell viability when treated with PTX alone, while no significant reduction in viability was seen with single agent CBDCA (**Figure 5B, C**). PDO80 demonstrated no significant reduction in viability when treated with CBDCA or PTX. Surprisingly, CBDCA + PTX treatment led to a slight, but significant increase in PDO80 organoid viability above that of control (**Figure 5B**).

Results from all seven PDOs suggest that PDO models are a good indicator of patient clinical response to standard chemotherapy. Furthermore, differences in response to single-agent PTX might further subdivide platinum-resistant patients, potentially predicting their responsiveness to second-line PTX therapy. This capability to forecast reactions to subsequent treatments could enhance therapeutic decision-making and optimize treatment sequences for resistant cancer cases (15, 24).

Similarities in Response Between PDXOs and Primary PDOs.

Similar to PDOs, PDXOs derived from platinum-sensitive patient samples demonstrated significant reductions in organoid viability with single agent CBDCA, PTX, and the combination. Representative images of organoid response after 72 hours in platinum-sensitive, resistant or refractory PDXOs are shown in **Supplemental Figure 2**. PDXOs established from PDX malignant ascites (**Figure 6**) or PDX solid tumors (**Figure 7**) displayed significant reduction in viability when treated with 1 µM CBDCA, 10 nM PTX, or the combination. This suggests that organoids developed from PDXs, whether derived from ascites or solid tumors, effectively mimic the response of PDOs and the patient's response to standard chemotherapy.

All but one PDXO derived from PDXs engrafted with platinum-resistant or refractory patient samples demonstrated resistance to standard chemotherapy (**Figure 7**). PDXO9, PDXO80 and PDXO1 were resistant to combination therapy with no significant reduction in organoid viability (**Figure 7B**). PDXO1 and PDXO9 developed from PDX malignant ascites (**Figure 6B, C**) or PDX solid tumor (**Figure 7B, C**) showed similar resistance to treatment demonstrating that either source of cancer cells responds similarly to standard therapy. Surprisingly, the PDXO2 organoids, developed from a PDX solid tumor, exhibited minimal response to CBDCA, but demonstrated a significant reduction in viability when treated with PTX or the combination treatment (**Figure 7B**). The paired PDO2 derived from primary malignant ascites also demonstrated a sensitivity to PTX that may have been further selected in the PDX (**Figure 5B**).

In conclusion, this study's findings demonstrate that patient-derived organoid models effectively reflect the varied responses of patients to chemotherapy, including significant reductions in viability for platinum-sensitive cases and CBDCA resistance in platinum-refractory or resistant cases. This ability to indicate clinical outcomes highlights the value of PDO models in cancer research. They prove effective not only for assessing existing treatments, but they can also be adapted for high-throughout screening of novel therapies. Because PDX provide a renewable source for patient-derived cancer cells, using PDXOs for drug screening would extend the lifetime of each patient sample. The reliable performance of PDXOs makes organoids essential tools for developing and

refining personalized treatment strategies, extending their utility across different therapeutic investigations in oncology.

Discussion

Developing effective precision-targeted therapies necessitates preclinical models that represent the morphological and molecular profiles of patient tumors. Traditional models, particularly cancer cell lines grown as a monolayer, fall short in replicating the genetic heterogeneity, morphology and growth characteristics of human tumors, leading to limited predictive power for clinical outcomes (25). Rapid growth of human cancer cells as a monolayer provides a strong selection for clonal outgrowth (26). PDXs grown in immunocompromised mice better maintain the heterogeneity of the primary tumor and model 3D tumor growth, vascularization and metastasis (6, 27). However, they are slow to develop, often require a large number of primary cancer cells, and are expensive to maintain (28). Our study highlights the potential of PDXOs as suitable preclinical models that can be used to screen a large number of drugs across a heterogeneous cohort to identify promising candidate therapies.

PDOs have been successfully cultured from many primary tumors' types including breast cancer (9), lung (29), colon (30, 31), gastrointestinal (15), prostate (20, 32, 33), bladder (34, 35), ovarian (19, 36), endometrial (19, 37), and liver cancer (38). These PDOs have shown promise in predicting patient responses to treatment. For example, the response of PDOs from patients with metastatic gastrointestinal cancer and colorectal cancer correlated with patient responses to anticancer agents observed in clinical trials (15, 39). The development of PDXOs from PDX solid tumor have only recently been reported in a study on endometrial cancer where they were used to predict response to FGFR inhibitors (40).

Uniquely, in our study, we developed organoids from both primary patient samples and PDX samples, with cancer cells originating from ascites fluid and solid tumors, to compare the drug response between these models and correlate the *in vitro* responses to patient clinical response. Our findings demonstrate that both PDOs and PDXOs derived from platinum-sensitive OC patients exhibited a significant reduction in cell viability, generally more than 50%, when treated with CBDCA, PTX, and their combination. This consistent response across different types of organoids mirrors clinical observations, where a reduction in cell viability correlates with a platinum-sensitive response (17, 41, 42). PDOs and PDXOs from platinum-resistant and refractory cases were all resistant to CBDCA, and the combination therapy, but varied in response to single agent PTX, suggesting that the platinum-resistant group could be subdivided further based on PTX response. Intriguingly, it would be valuable to investigate whether patients whose organoid models are sensitive to PTX also exhibit enhanced response to second-line therapies. Such findings could help tailor more effective treatment strategies for those not responding to first-line platinum-based therapies (42). PDOs and PDXOs showed a similarity in response to treatments, indicating that sensitivity to standard chemotherapy of the primary cancer cells were maintained in the PDX, in agreement with other PDX studies (5, 43). We were able to establish paired PDOs and PDXOs from five patient samples, two platinum-sensitive, two platinum-resistant, and one platinum-refractory. PDXO20 was less sensitive to single agent CBDCA than its paired PDO, but still showed significant reduction in viability with CBDCA treatment and remained sensitive to the combination. PDXO1 was equally resistant to CBDCA and the

combination therapy, but unlike PDO1, showed a small reduction in viability with PTX treatment. The two platinum-resistant PDXOs, PDXO2, and PDXO80 exhibited the most altered responses to treatment compared to their paired PDOs PDXO2 and PDXO80 remained resistant to CBDCA, but PDXO80 was more sensitive to PTX, and PDXO2 was more sensitive to both PTX and the combination treatment. Overall, PDXOs retained their platinum-sensitivity or resistance supporting the utility of these models for preclinical testing.

Here we directly compared PDOs derived from primary patient cancer cells and PDXOs derived from PDXs to demonstrate that these two patient-derived models show similar responses to standard chemotherapy. Both models can be valuable in validating candidate therapies. PDOs require less starting material and so can be established for a larger number of patients and are ready to assay for drug response in less than a month. However, a limited supply of primary tumor sample can be depleted and PDOs are often difficult to cryopreserve with reduced viability in thawed organoids. PDX tumor tissue serves as a renewable source for PDXOs in that more tissue can be collected by passaging the PDX tumors into new NSG mice. Thus, the same PDXOs can be used in subsequent drug response studies allowing comparisons of drug response across many studies. PDXOs can also be used as an initial *in vitro* screen to 1) identify candidate drugs that are more likely to have an effect in preclinical *in vivo* studies and 2) predict treatment response of PDX models as in the Colling et al. study (20) to be able to choose appropriate PDX with diverse responses to test *in vivo*. Being able to use PDX cancer cells grown in mice to establish organoid models provides platform flexibility. PDX-derived organoids can be used to pre-screen larger drug libraries to identify candidate treatments that can then be tested *in vivo* in PDX models.

While PDOs and PDXOs are a considerable improvement over cancer cell lines, PDOs still do not fully replicate the tumor microenvironment (TME). Enhancing organoid cultures with additional TME components, such as immune cells or stromal elements, could improve their representational accuracy (44). Additionally, while organoids are valuable for short-term drug screening, they cannot capture long-term treatment responses, such as the development of drug resistance. Future studies will aim to integrate promising therapies identified in organoid models into *in vivo* testing using our humanized PDX (huPDX) models (6) to correlate with organoid results.

In conclusion, this study demonstrates that organoid viability in patient-derived organoid models, either PDOs or PDXOs, treated with chemotherapy correlates with patient clinical responses. Patient derived organoid models represent the heterogeneity of the OC patient populations and provide a way to examine the variability in response to existing treatments and screen for new therapeutic strategies that can have broad efficacy across patients or increased efficacy in a subpopulation of patients. Thus, PDOs are essential for the advancement of personalized medicine in oncology. Their reliable performance across various treatments and patient-derived samples highlights their utility in developing and refining precision-targeted therapies.

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Organoid Models Derived from Primary Tumors and Patient-Derive Xenograft Tumors Reflect Platinum Sensitivity of Ovarian Cancer Patients

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Figure 1. Comprehensive Workflow for Developing and Evaluating Chemotherapy Responses in Patient-Derived and PDX-Derived Ovarian Cancer Organoids.

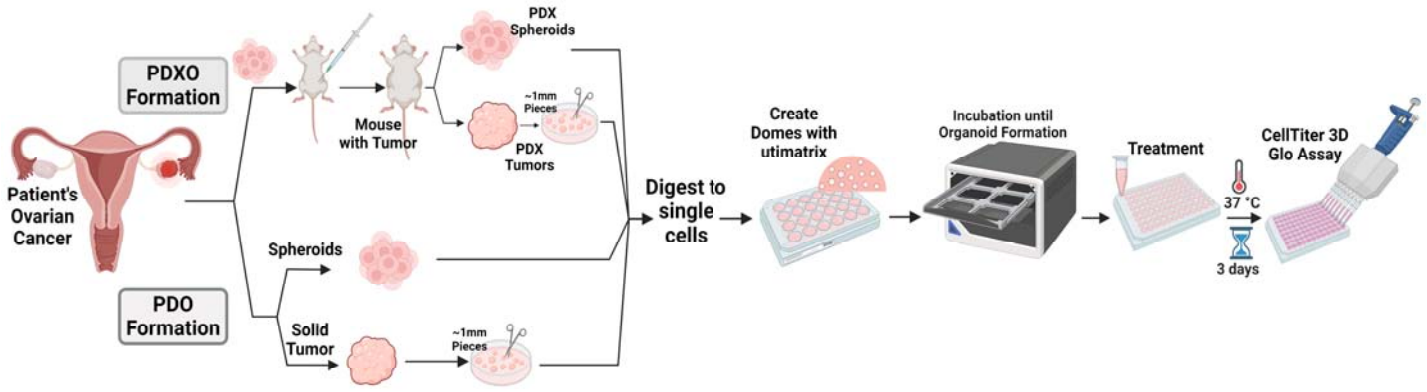

Figure 1. Comprehensive workflow for developing and evaluating chemotherapy responses in Patient-Derived and PDX-Derived ovarian cancer organoids. A schematic of the generation and treatment of patient-derived organoids (PDOs) and patient-derived xenograft organoids (PDXOs) from ovarian cancer samples. Fresh ovarian cancer samples are first collected from patients. Cancer cells from ascites fluid or solid tumor are engrafted into NSG mice to establish PDXs. Once tumors develop, the resulting ascites or solid tumors can be used for PDXO formation. For PDO formation, primary ascites or solid tumors are processed directly. To seed organoids, soli tumors are cut into ~1 mm pieces and all samples are enzymatically digested into single-cell suspensions. Cancer cells are subsequently embedded in ultimatrix, an organic extracellular matrix to form organoids. Once formed, organoids are harvested and seeded in 5 µL ultimatrix domes in 96-well plates. One day later, they are treated with chemotherapeutic agents CBDCA, PTX, or their combination. After three days of treatment, organoid viability is assessed using the CellTiter-Glo® 3D Cell Viability Assay. This figure was created with BioRender.com.

Table 1. Clinical designation of ovarian cancer patients who donated the samples used in this study.

Platinum sensitivity	Patient #	Established models
Sensitive	Pt 3	PDXO
	Pt 18	PDO PDXO
	Pt 20	PDO PDXO
	Pt 33	PDXO
	Pt 90	PDO
	Pt 92	PDO
Resistant	Pt 2	PDO PDXO
	Pt 9	PDXO
	Pt 80	PDO PDXO
Refractory	Pt 1	PDO PDXO

Table 1. Clinical designation of ovarian cancer patients who donated the samples used in this study. Ovarian cancer patients are arranged by their clinical designation of their response to platinum-based chemotherapy. Patients are classified as platinum-sensitive (experiencing partial or complete response to standard CBDCA/PTX treatment with recurrence more than six months after treatment), platinum-resistant (showing disease progression on therapy or recurrence within six months), or platinum-refractory (no response to initial treatment). Depending on the amount of available tissue, samples were used to establish PDOs, PDXOs, or both.

Figure 2. Growth of PDOs derived from high grade OC primary patient samples.

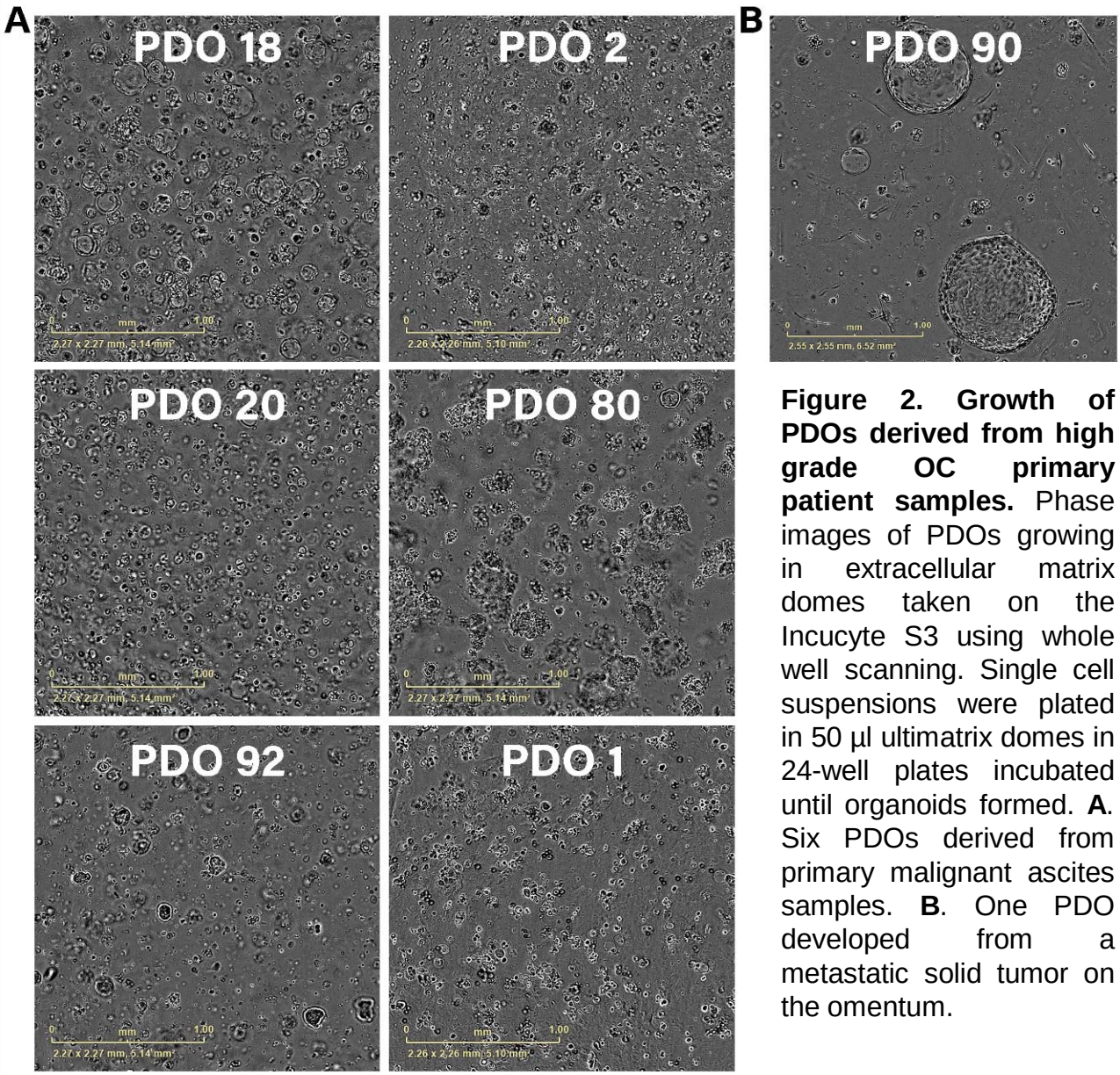

Figure 2. Growth of PDOs derived from high grade OC primary patient samples. Phase images of PDOs growing in extracellular matrix domes taken on the Incucyte S3 using whole well scanning. Single cell suspensions were plated in 50 µl ultimatrix domes in 24-well plates incubated until organoids formed. **A**. Six PDOs derived from primary malignant ascites samples. **B**. One PDO developed from a metastatic solid tumor on the omentum.

Figure 3. Growth of PDXOs derived from PDX ascites samples shows similarities to PDOs formed from primary ascites.

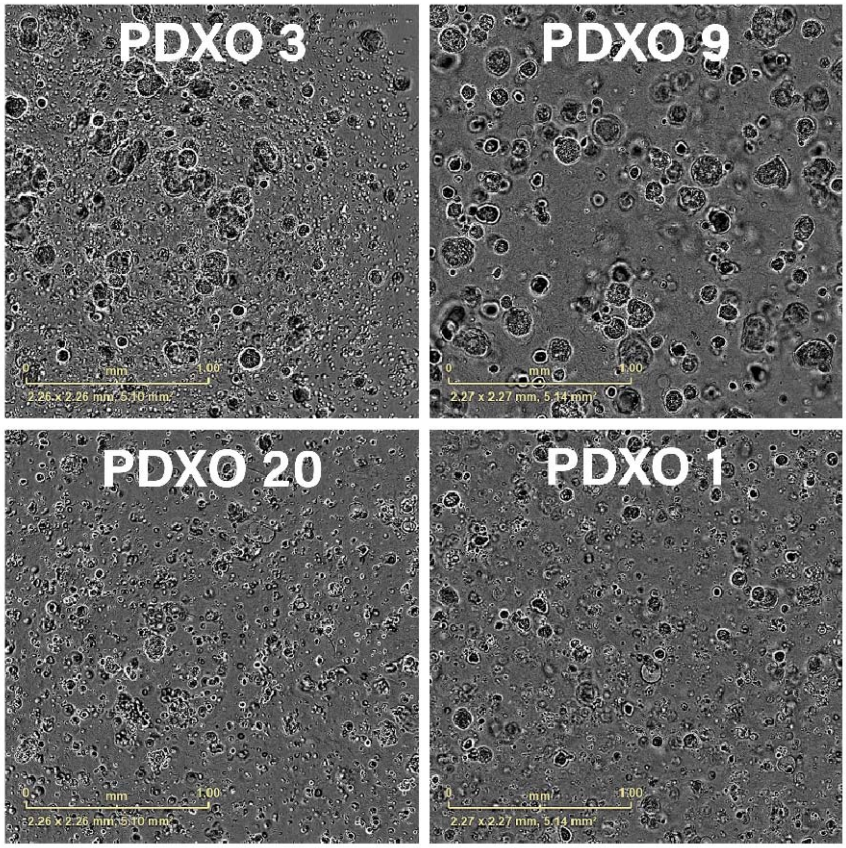

Figure 3. Growth of PDXOs derived from PDX ascites samples shows similarities to PDOs formed from primary ascites. Phase images of four ascites-derived PDXOs growing in ultimatrix domes. Single cell suspensions were plated in 50 µl ultimatrix domes in 24-well plates incubated until organoids formed.

Figure 4. PDXOs derived from PDX solid tumors develop large organoids similar to the PDO developed from primary solid tumor

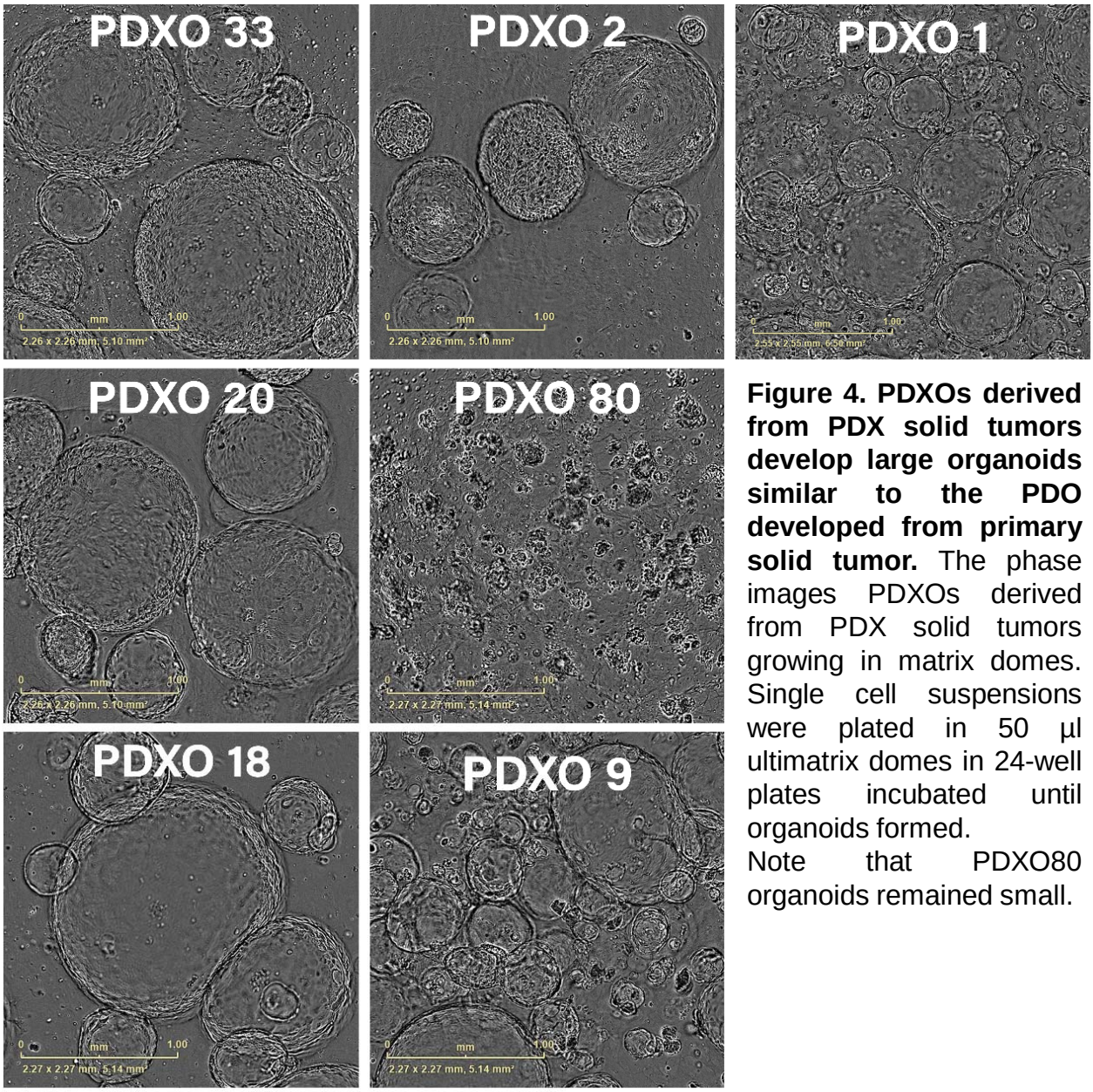

Figure 4. PDXOs derived from PDX solid tumors develop large organoids similar to the PDO developed from primary solid tumor. The phase images PDXOs derived from PDX solid tumors growing in matrix domes. Single cell suspensions were plated in 50 µl ultimatrix domes in 24-well plates incubated until organoids formed.
Note that PDXO80 organoids remained small.

Figure 5. PDO response aligns with the clinical designation of platinum sensitivity.

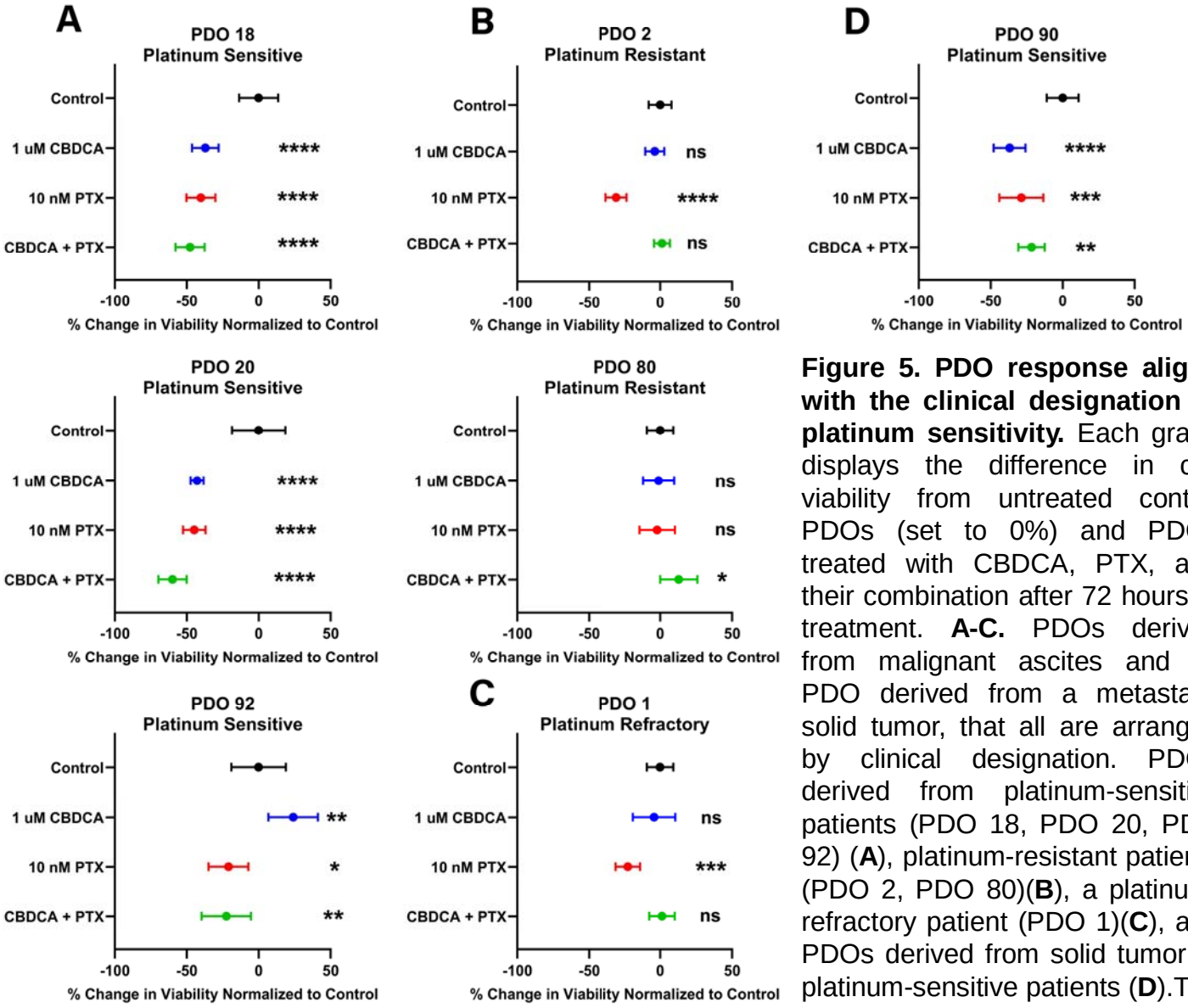

Figure 5. PDO response aligns with the clinical designation of platinum sensitivity. Each graph displays the difference in cell viability from untreated control PDOs (set to 0%) and PDOs treated with CBDCA, PTX, and their combination after 72 hours of treatment. **A-C.** PDOs derived from malignant ascites and **D.** PDO derived from a metastatic solid tumor, that all are arranged by clinical designation. PDOs derived from platinum-sensitive patients (PDO 18, PDO 20, PDO 92) (**A**), platinum-resistant patients (PDO 2, PDO 80)(**B**), a platinum-refractory patient (PDO 1)(**C**), and PDOs derived from solid tumor of platinum-sensitive patients (**D**).The graphs display the difference in organoid viability, assessed by the CellTiter 3D Glo Assay, normalized to untreated control. More negative values indicate response to treatment. Error bars designate standard deviation. Statistical significance is marked by asterisks (ns: not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$), assessed via one-way ANOVA.

Figure 6. Response of PDXOs established from PDX ascites samples.

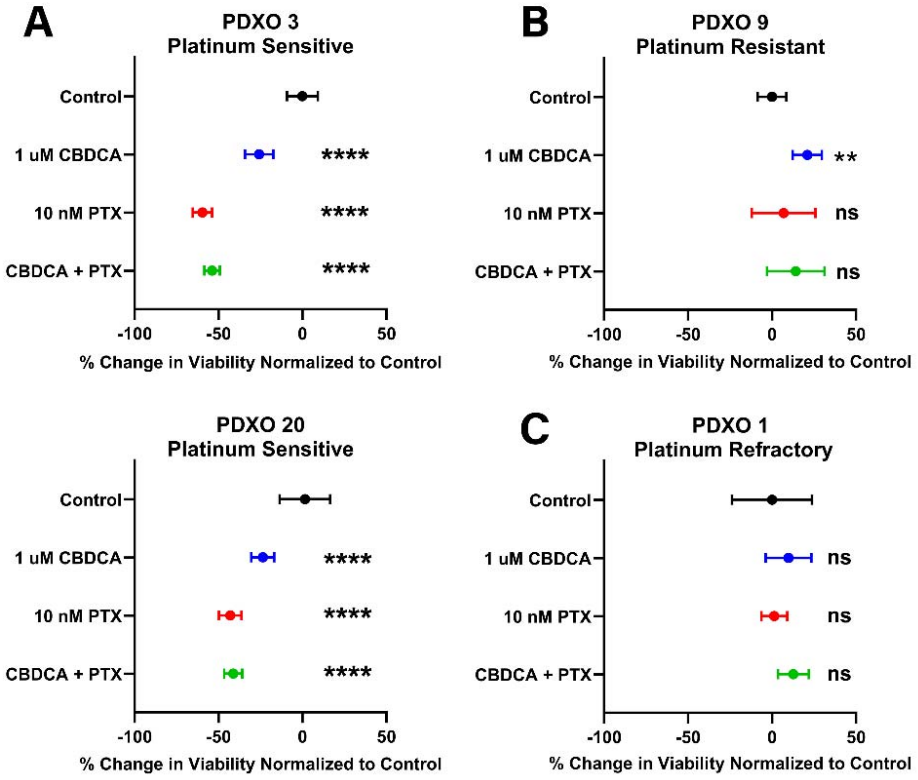

Figure 6. Response of PDXOs derived from PDX ascites samples. Graphs display the difference in cell viability from untreated control PDXOs (set to 0%) and PDXOs treated with CBDCA, PTX, and their combination after 72 hours of treatment. PDXOs are arranged by clinical designation. PDXOs derived from platinum-sensitive patients (PDXO 3, PDXO 20) (**A**), platinum-resistant patients (PDXO 9)(**B**), and a platinum-refractory patient (PDXO 1)(**C**). The differences in viability assessed by the CellTiter 3D Glo Assay, are presented as percentage changes normalized to untreated controls. More negative values indicate response to treatment. Error bars designate standard deviation. Statistical significance is marked by asterisks (ns: not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$), assessed via one-way ANOVA.

Figure 7. PDXOs derived from PDX solid tumors.

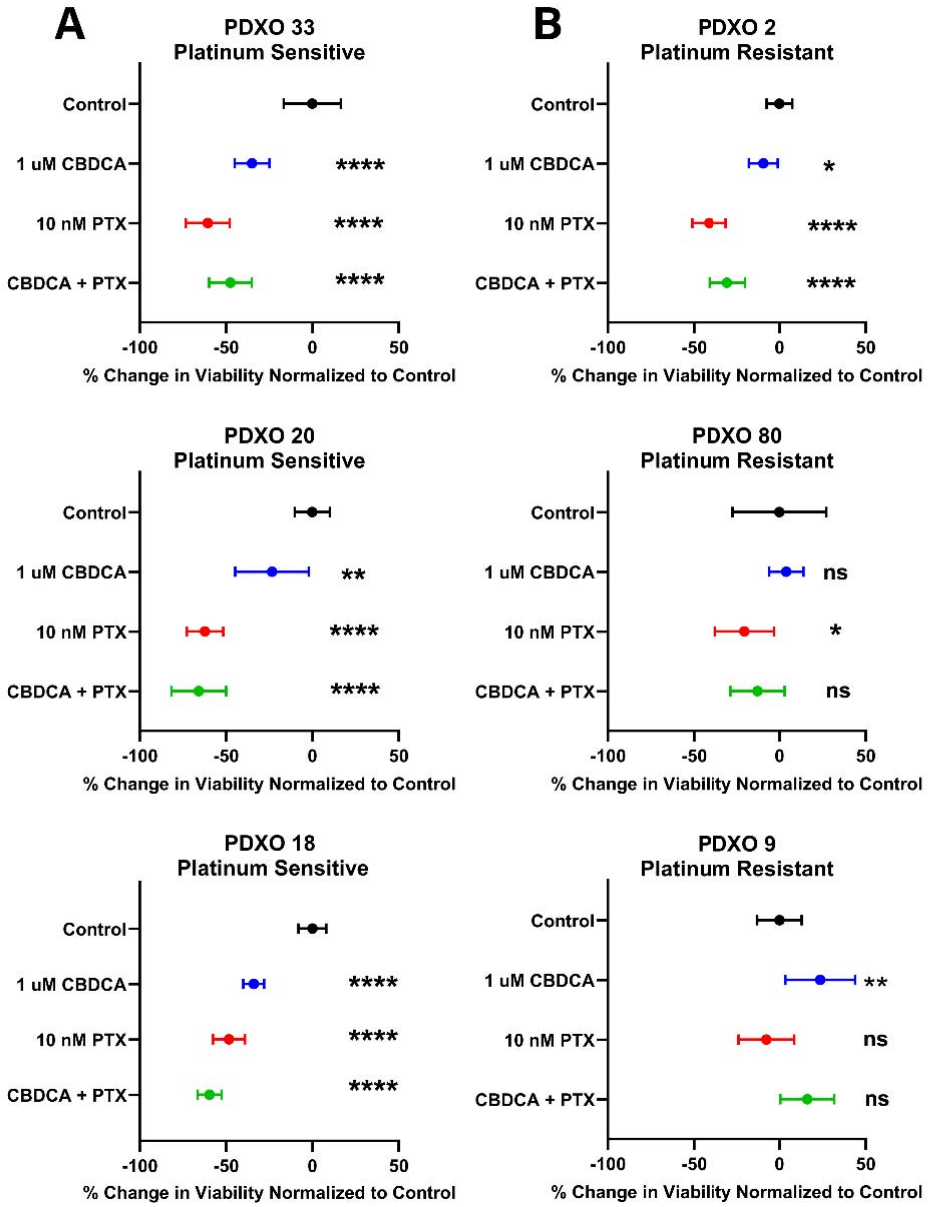

Figure 7. PDXOs derived from PDX solid tumors. Each graph displays the difference in cell viability from untreated control PDOs (set to 0%) and PDOs treated with CBDCA, PTX, and their combination after 72 hours of treatment. PDXOs are arranged by clinical designation. PDXOs derived from platinum-sensitive patients (PDXO 33, PDXO 20, PDXO 18) (**A**), platinum-resistant patients (PDXO 2, PDXO 80, PDXO 9)(**B**), and a platinum-refractory patient (PDXO 1)(**C**). Viability changes, assessed by the CellTiter 3D Glo Assay, are expressed as a percentage change normalized to untreated control.

Error bars represent standard deviation. Statistical significance is indicated by asterisks (ns: not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$), determined through one-way ANOVA.