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Original Article

Ex vivo platform *en route* to functional precision medicine: clinical relevance in gynecological cancers

Raed Sulaiman[1], Jennifer C Aske[2], Luis Rojas Espaillat[2], Xiaoqian Lin[2], David Starks[2], Adam Dale[2], Kris Gaster[3], Pradip De[2], Nandini Dey[2]

[1]Avera McKennan, Sioux Falls, SD, USA; [2]Avera Cancer Institute, Sioux Falls, SD, USA; [3]Outpatient Cancer Clinics, Avera Cancer Institute, Sioux Falls, SD, USA

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Abstract: A scientific interrogation-driven approach to the clinical management of cancer patients is based on molecular profiling of the tumor. Empowered by the knowledge of oncogenic drivers and biomarkers, oncologists chart an optimal treatment path toward increasing the mathematical probability of a positive outcome. In this entire chain of events, an experimental proof of logical interrogation has never been incorporated before. Here, we provide the first evidence that the result of ex vivo testing of a drug matched to the genomic profiling of an N-of-1 tumor can deliver meaningful insight connecting scientific interrogation and a clinical event. Using resected tissues from endometrial (EC) and ovarian (OC) cancer patients, we designed a personalized ex vivo platform to test combinations of drugs in the default histological architecture of the individual tumors. Following the CART-T cells' principle, we co-cultured with autologous T-cells to test targeted drugs and immune checkpoint inhibitors. The study was designed with a limited clinical information window from patient registration/consent to obtaining the tumor tissues, and adjuvant treatment/post-surgery event (PSE) data were accessed retrospectively. Using a checkerboard analysis, we found that PSE-free survival time was longer in patients whose therapy "matched" the effective drug combination in ex vivo culture/co-cultures compared to those with no effect. Specifically, out of 32 EC patients in the "test & treatment-matched" category whose tumor cells failed to respond to ex vivo drug testing, none achieved > 4 and > 3 years of PSE-free survival. In contrast, out of 38 EC patients in the "test & treatment-matched" category, 4 and 6 patients, whose tumor cells responded to drugs in ex vivo culture, achieved > 4 and > 3 years of PSE-free survival, respectively. Cases with genomically-guided ex vivo testing showed that a "match" between an effective ex vivo drug combination and therapy resulted in late PSE, whereas a "match" between prescribed treatment and an ineffective drug combination in ex vivo testing led to early PSE. Our study demonstrates that integrating genomic data with personalized drug testing on an ex vivo culture/co-culture platform is an *effective tool for modeling functional precision medicine* in gynecological cancers. This approach bridges the gap between next-generation drug testing in translational research and patient care, providing insight for improved treatment outcomes.

Keywords: Ex vivo, co-culture, drug combination(s), post surgical event, genomics

Introduction

The success of personalized precision medicine demands a tailored treatment strategy based on knowledge regarding the molecular profiling of the tumor, including its microenvironment. The molecular profiling of a patient's tumor provides the valuable option to design a genomics-interrogation-driven drug combination(s), which is mathematically expected to have the best antitumor effect on the patient's tumor cells in real-time. However, the era of functional precision medicine in cancer research faces challenges in achieving a zero-error prediction of the clinical efficacy of a particular drug combination. Since precision medicine is an approach to treating cancer that aims to identify effective therapeutic strategies for individual patients, a successful prediction of clinical response to anticancer drugs remains a critical challenge, and predicting response to chosen combination therapy in a patient-by-patient manner remains incomplete.

In translational research, there remains a discordance between preclinical data and actual

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clinical outcomes. Although genomic-guided therapy incorporates the best logical prediction, not all genomic alterations are tumor-agnostic, nor are all genomics-guided matched drug combinations experimentally tested/proven on tumor cells in every patient. Even in the era of knowledge-driven treatment strategy, a significant number of cases persist where genomic analysis fails to identify effective drug combinations [1]. The instances where genomic analysis fails to (1) identify effective drug combinations and/or (2) predict clinical response to a drug combination point to the complexities of tumor heterogeneity in solid tumors. Tumor heterogeneity originating from variability in the organ of origin, histology, pathological stage, and genetic background makes it more complex, especially in the context of drug response and clinical outcome. Published literature indicates that the tumor heterogeneity and microenvironment can limit the predictive command of current biomarker-guided strategies for chemotherapy, targeted therapy, and immunotherapy [2, 3]. Biomarker-driven precision cancer therapy has emerged as a powerful concept. However, the mere presence of a biomarker in a cancer cell may not translate into clinical efficacy [4, 5]. For example, trastuzumab deruxtecan is highly effective even in HER2 low-expressed breast cancer patients [6], but no responses were observed in medium to low HER2-expressing colorectal cancer patients [7]. Although tissue-agnostic drug approvals represent a paradigm shift in drug development, and several drugs are approved based on tumor-agnostic biomarkers (e.g., NTRK, RET, dMMR), BRCA mutations (among others) are not tumor-agnostic biomarkers for PARP inhibitor therapy [8]. Thus, a successful functional testing platform for precision medicine in patient-centric clinical settings is demanding and remains unmet.

One challenge that complicates tissue-agnostic drug development is the lack of appropriate models in the translational research space. Since experimental evidence is the most scientific way to test a logically driven conclusion, a need for a patient-specific testing platform for functional precision medicine is pressing. To address this issue, we developed a personalized ex vivo platform for drug testing using 152 resected tumor tissues cultured or co-cultured with autologous T-cells. The value of our N-of-1 platform was tested to answer two questions; First, whether genomics-driven drug combination matches drug response in tumor cells within tumor tissue without affecting stroma. Second, whether a clinical validation of drug effect is possible retrospectively in terms of patient outcome; post-surgery event (PSE) free survival. Our *platform of functional precision medicine gives an exclusive algorithmic advantage* as it provides *experimental proof-of-testing* to reinforce the logical concept of drug-matching and put to the test the matched drug in action on the individual patient's tumor cells in their histological microenvironment. The platform experimentally tests the predictability of genomically matched drugs' response regarding PSE-free survival in patients with gynecological malignancies.

Methods and materials

Tissue collection at the time of surgery

All experimental protocols were approved by the institutional and/or licensing committee(s). Informed consent (IRB approved: Protocol Number Study: 2017.053-100399_ExVivo001) was obtained from a total of 172 patients. Patients were de-identified. The resected tumor (T) and tumor-adjacent normal (N) tissues were collected during surgery in designated collection media as per the guidelines and relevant regulations provided by the pathologist, depending upon the availability of the tissue on a case-to-case basis. We included samples from consecutive consented patients with endometrial and ovarian tumors at any stage/grade of the disease undergoing surgery with or without pre-treatment/history of any previous carcinoma. Tonsil tissues and gynecological tumor tissues were obtained from the pathology department for validation of IHC staining. Blood for the isolation of CD3+ T-cells was obtained on the day of the surgery, as reported elsewhere [9].

Ex vivo culture of resected tumor tissues and tumor-adjacent normal tissues

The tissues were set into cultures within an hour of resection in 3D matrigel on separate γ-irradiated sterile cloning discs (Scienceware® cloning discs diameter 3.2 mm/4.8 mm) in complete medium (DMEM/F-12 + Glutamax 500 mL + 10% HyClone Fetal Bovine Serum 0.1

uM Sterile Filtered 500 mL + 1% HyClone Penicillin-Streptomycin 100X 100 mL + 3% Bovine Serum Albumin + 1% HyClone HEPES Buffer). The culture was continued for 3 consecutive days (depending on the availability of the resected tissues). The cultures were terminated by fixing the tissue in Expredia 10% Neutral Buffered Formalin and processed for FFPE sections for H&E and IHC stains for further evaluation of the effect of the drugs following standard histological processing.

Ex vivo co-culture of resected tumor tissues

In parallel to the setup of the ex vivo culture, the resected tumor tissues were set in an ex vivo 3D matrigel format co-cultured with the DiI-stained CD3+ T-cells isolated from the same patient on the same day of the surgery in the presence of pembrolizumab. For isolation, WBCs from whole blood were stained with corresponding extracellular antibodies, CD3+, in FACS buffer (RPMI phenol red free + 1% FBS) for 20 minutes at 4°C. Cells were then rinsed with FBS. The cells were fixed using the FOXP3 cell fixation kit (Miltenyi Biotech) for 30 minutes at 4°C, followed by the addition of a permeabilization buffer. Cells were blocked for 5 minutes with Fc block, and intracellular antibody was added for 30 minutes at 4°C. Cells were rinsed 1× with FACS buffer and resuspended in FACS buffer for analysis. Cells were run and analyzed on an Accuri C6. Following magnetic isolation/purification on a whole blood column with CD3-bead, the sample was over 99% CD3+ cells. The cells were stained with DiI-stain before using them for co-culture setup. Figure S2 shows a representative presentation of "in-co-culture" whole mounts, DAPI-stained fresh frozen sections, and DiI-stained T-cells from the ex vivo co-culture of tumor tissue (T) or tumor-adjacent normal tissue (N) and isolated CD3+ T-cells from the peripheral blood of patients on the day of surgery is presented.

IHC expression of proliferative, apoptotic, angiogenic, and immune markers on FFPE sections from resected and ex vivo cultured tumor tissues

Details of IHC expression of Ki67, cleaved caspase3 (cIC3), cleaved PARP(cl-PARP), and pERK of FFPE Sections from tumor tissues at day zero (D0), day 1 (D1), day 2 (D2), and day 3 (D3) of ex vivo cultures were carried out using the IHC detection kits that were procured from Dako (Envisioin+ Dual-link system-HRP (DAB+)) as mentioned elsewhere [10]. For IHC expression kits were procured from Dako (Envision+ Dual-link system-HRP (DAB+), code K4065; Envision GI2 Doublestain system, Rabbit/Mouse (DAB+/Permanent Red), code K5361), and Abcam (ab210059 DoubleStain IHC Kit: M&R on human tissue (DAB and AP/Red)). The validation of the protein expression was carried out in FFPEs of tonsil and tumor tissues. A board-certified pathologist evaluated the morphology of the proliferating and apoptotic cells, their staining intensities, and the distribution pattern of expression of proteins.

Retrospective access to patients' pre-treatment history, adjuvant treatment, post-surgery events & imaging studies

We tested the clinical relevance of the ex vivo platform by determining whether the result of drug testing corroborated with the PSE in patients. To evaluate whether the result of the drug testing has any meaningful impact at the clinical level, we retrospectively accessed individual patients' pre-treatment history, adjuvant treatment(s), PSEs, and PET-CT images. The occurrence of PSEs in patients with endometrial and ovarian cancers was obtained from the patient's information (Electronic Medical Records, EMR) in accordance with the IRB approval of the Avera Cancer Institute. The PSE included (1) the metastasis/recurrence of the disease detected radiologically or pathologically, (2) clinical worsening symptomatically, such as ascites, and (3) death of a patient.

Results

Study hypothesis

To establish our hypothesis, we presented 6 representative cases, including 4 primary tumor(s) from patients with endometrial and ovarian cancers, a primary and metastatic tumor pair, and a tumor biopsy from a patient with recurrent ovarian cancer. Summarizing our data from all the tumor samples obtained from patients tested ex vivo in the context of their adjuvant therapy and PSE, we have 4 possible options in the checkerboard pattern between effective/ineffective drug(s) combinations in the ex vivo laboratory testing in one hand and matched/unmatched adjuvant therapy re-

Table 1. PSE free survival of patients with endometrial cancers whose treatment in clinics matched (fully/partially) with *Ex vivo* drug testing in the laboratory

Post Surgery Event (PSE) Free Survival				
Post Surgery Event (PSE) Free Survival (From the Surgery Date Till Sept.'22)	The Ineffective Drug(s): Drug (Single/Combination), When Tested In *Ex Vivo* Cultures, Had No Effect On Tumor Cells		The Effective Drug(s): Drug (Single/Combination), When Tested In *Ex Vivo* Cultures, Caused an Increase in Apoptosis (cl-PARP & cl-Caspase3) and/or Decrease in Proliferation (Ki67) of Tumor Cells	
< 1 Year PSE Free Survival	6 Patients	Total 19% (6 out of 32)	5 Patients	Total 13% (5 out of 38)
1-2 Years PSE Free Survival	14 Patients	Total 44% (14 out of 32)	14 Patients	Total 37% (14 out of 38)
> 2 Years PSE Free Survival	12 Patients	Total 37% (12 out of 32)	13 Patients	Total 34% (16 out of 38)
> 3 Years PSE Free Survival	0 Patient	0%	2 Patients	Total 5% (6 out of 38)
> 4 Years PSE Free Survival	0 Patient	0%	4 Patients	Total 11% (4 out of 38)

Table 2. PSE free survival of patients with ovarian cancers whose treatment in clinics matched (fully/partially) with *Ex vivo* drug testing in the laboratory

PSE Free Survival of Patients		
Post Surgery Event (PSE) Free Survival (From the Surgery Date Till Sept.'22)	The Ineffective Drug(s): Drug (Single/Combination), When Tested In *Ex Vivo* Cultures, Had No Effect On Tumor Cells	The Effective Drug(s): Drug (Single/Combination), When Tested In *Ex Vivo* Cultures, Caused an Increase in Apoptosis (cl-PARP & cl-Caspase3) and/or Decrease in Proliferation (Ki67) of Tumor Cells
< 1 Year PSE Free Survival	3 Patients	2 Patients
1-2 Years PSE Free Survival	4 Patients	3 Patients
> 2 Years PSE Free Survival	0 Patient	0 Patient
> 3 Years PSE Free Survival	0 Patient	1 Patient
> 4 Years PSE Free Survival	0 Patient	1 Patient

ceived by patients in the clinics on the other (**Tables 1** and **2**). Taking the combination of ineffective-unmatched options out of the scenario, we have 3 possible options, (1) ineffective drug combinations matched the adjuvant therapy, (2) effective drug combinations did not match the adjuvant therapy, and (3) effective drug combinations matched the adjuvant therapy. In our study, the first two options led to an early PSE. In contrast, the last one led to a delayed PSE, demonstrating the ex vivo drug testing platform's clinical relevance as a functional precision medicine model.

Study design

The study was designed to test the clinical relevance of genomics-driven ex vivo testing of chemotherapy, targeted therapy, and immune therapy drugs in surgically resected tumor/biopsy samples from consented patients with endometrial and ovarian cancers undergoing treatment at Avera Cancer Institute. The culture and co-culture experiments were set up following the availability of the tumor tissue, tumor-adjacent normal tissue, and blood on the day of surgery in accordance with scientific interrogation of the available genomics data. Depending on the quantity of the available tissues received from the pathology department, cultures/co-cultures were set up for 3 consecutive days (Day 1, D1: Day 2, D2; and Day 3, D3). Table S1 presents the list of drugs (single/combinations) tested in ex vivo culture on tumor and tumor-adjacent normal tissues from patients with endometrial and ovarian cancers. A pathologist performed initial evaluations of the drug(s) effect in a blinded fashion. Confirmatory immunohistochemistry (IHC) staining was then conducted on serial sections of the formalin-fixed cultured/co-cultured tissues to examine the effect of drugs (chemotherapy/targeted therapy/immune therapy) on proliferation (Ki67), apoptosis (cl-PARP & cl-Caspase3), and cell survival (phospho-S6 Ribosomal Protein, pS6RP, and phospho-ERK, pERK) markers. The entire cohort of patients was analyzed based on (1) the effectiveness of the tested drug in ex vivo culture, (2) retrospective matching/similarity of the adjuvant treatment received by the patient with the laboratory-tested ex vivo drug/combo, and (3) the time of PSE (Early PSE: < 16 months; Late/Delayed PSE: > 16 months) based clinical determination and the PET-CT images. We retrospectively evaluat-

ed the PSE of the patients with endometrial cancers (along with their age at surgery, pathological parameters, and treatment details), whose tumor cells neither exhibited increased apoptosis (cl-PARP & cl-Caspase3) nor had decreased proliferation (Ki67) following drug treatment in the *ex vivo* cultures as presented in Table S2. In contrast, Table S3 presents the PSE of the patients with endometrial cancers whose tumor cells exhibited increased apoptosis (cl-PARP & cl-Caspase3) and/or had decreased proliferation (Ki67) following drug treatment. Analyzing the PSE-free survival of patients with endometrial cancers whose treatment in clinics matched (fully/partially) with ex vivo drug testing in the laboratory, we observed that the number of patients whose therapy matched with the ineffective drug in ex vivo laboratory tests was higher in the category of "early PSE" and exhibited lower PSE-free survival as presented in the table (**Table 1**). In **Table 1**, we organized the PSE of patients whose treatment in clinics matched (fully/partially) with ex vivo drug testing in the laboratory into (1) patients (n=32) whose tumor cells did not respond to drugs in culture and (2) patients (n=38) whose tumor cells responded to drugs in culture. Out of 32 patients, 6 patients had < 1 year of PSE-free survival (19%), 14 patients had 1-2 years of PSE-free survival (44%), and 12 patients had > 2 years of PSE-free survival (37%). More strikingly, none of the patients had > 3-4 years of PSE-free survival. In contrast, 16% (6 out of 38) of the patients in the category whose therapy matched with the effective drug in ex vivo laboratory tests exhibited > 3-4 years of PSE-free survival (**Table 1**). We similarly evaluated the PSE of the list of patients with ovarian cancers tested whose tumor cells were not affected by drug treatment in culture (Table S4) as compared to patients whose tumor cells responded to drug treatment in culture (Table S5). The PSE-free survival of these patients whose treatment in clinics matched (fully/partially) with *ex vivo* drug testing in the laboratory demonstrated a comparable pattern to that observed in patients with endometrial cancers (**Table 2**). However, the data remains inconclusive due to the insignificant patient number. Although the sample size is small, the PSE-free survival of ovarian cancer patients whose clinical treatment matched (fully/partially) with ex vivo laboratory drug testing demonstrated similar findings to that was observed in endometrial cancers.

The results suggest that the ex vivo drug testing platform can help identify drug combinations that positively impact PSE-free survival in gynecological cancers. Patients whose therapy matched with effective drug combinations in ex vivo laboratory tests showed a trend towards longer PSE-free survival compared to those with ineffective drug combinations. The patients whose therapy matched with the ineffective ex vivo laboratory drug testing had a higher percentage of early PSE and lower PSE-free survival.

Paclitaxel plus pembrolizumab was an effective in ex vivo culture that matched adjuvant treatment & recorded delayed PSE

We tested the effect of paclitaxel and pembrolizumab in ex vivo culture and co-culture, respectively, on tumor tissues, from a patient with lymph node-positive superficially invasive endometrial serous carcinoma, grade 3, stage IIIC1 disease whose adjuvant therapy and PSE were recorded retrospectively. The drug combination was decided based on the scientific interrogation of the genomic alterations observed in the tumor (as presented in **Table 3**; AC-1-37). **Figure 1A** presents H&E, Ki67-clC3, and cl-PARP stained FFPE section from D1 treated with paclitaxel as compared to vehicle-treated control. The number of Ki67 stained tumor cells was markedly abrogated along with the enhanced staining of clC3 stains in the same section, as shown in the microphotographs from the double-stained section of FFPE tissue blocks (middle panel). In line with the result, there was a marked increase in the cl-PARP stains in the tumor cells in the treated samples as compared to the control. Apoptotic bodies are labeled as green circles. This pattern of staining continued in D2 (**Figure 1B**) and D3 (**Figure 1C**). However, on D3, although there was an increase in the baseline clC3 staining in the control sample, the Ki67 stains were totally replaced by clC3 stains in treated samples confirming drug-induced apoptosis (green circles). **Figure 1D** presents apoptotic bodies (green circles) in the pembrolizumab-treated sample as compared to vehicle-treated control in H&E stained FFPE sections from D1, D2, and D3 ex vivo tumor tissue co-cultured

Table 3. Clinical relevance of *Ex Vivo* testing of genomics-guided drug combos on tumor tissues from surgically resected samples obtained from patients with endometrial and ovarian cancers

Patients with Endometrial Cancers						
Patient ID		AC-1-37		AC-1-40		
Pathological Parameters	Tumor Histology	Superficially invasive serous carcinoma		Endometrioid adenocarcinoma		
	Tumor Grade	3		1		
	Tumor Stage	IIIC1		IB		
	LVI	Absent		Absent		
	Myometrial Invasion (%)	8		58		
	LN Status	Present (3/11)		Absent (0/6)		
	Uterine Serosa & Cervical Stroma Involvement	Absent		Invades cervical stroma, uterine serosa absent		
	TMN	pT1aN1a		pT2 pN0		
	MSI	Stable		Stable		
	MMR	Normal	Lynch Syndrome is not entirely ruled out; HER2 negative	Normal	Lynch Syndrome is not entirely ruled out	
	CA-125 (Units per millimeter (U/mL) Pre & Post [Months (M) & Weeks (W)] Surgery	1 W Pre	109.3	Not Available		
		3 W Post	496.6			
		1 M Post	197.7			
		2 M Post	88.3			
		3 M Post	46.6			
		4 M Post	32.4			
Genomic Alterations In Cell Signal Pathways (FOUNDATIONONE CDx)		AKT L52H	PIK3CA V344A	ARID1A G276fs*87	ARID1A T294fs*69	
		NF2 R424C	CCNE1 amplification	ATM E2975fs*10	DNMT3A R771*	
		MYC amplification	MYCL1 amplification	PIK3R1 E458_E462del	PTEN R130Q	
		AR amplification	TP53 G245D	TSC2 A1778fs*12		
				MLL2 P2354fs*30	MUTYH G382D	
		KDMSA amplification		TP53 splice site 96+1G > T	TP53 R273C	
		KEAP1 KEAP1(NM_012289) rearrangement exon 4		RB1 A74fs*4	RB1 R320*	
		Tumor Mutational Burden 1 Muts/Mb		Tumor Mutational Burden 20 Muts/Mb		
Pretreatment History		None		None		
Ex Vivo Effect of Drug Combination (s) On Tumor Tissue		Paclitaxel (P)	Effect	Paclitaxel (P)	No Effect	
		P+ Trametinib	Effect	P+ Trametinib	No Effect	
		P+ TAK228	Effect	P+ Lemvatinib	No Effect	
		P+ Lenvatinib	No Effect	P+ TAK228	Effect	
		P+ BKM120	No Effect	P+ Copanlisib	Effect	

Adjuvant Treatment: Treatments - Surgery (S), ChemoT (C), Radiation (R), Immune T (I)		S+C+R+T+I	Carboplatin/Paclitaxel, Bevacizumab (9/9/20-03/21); started Pembrolizumab (4/13/2021); Whole pelvic radiation with vaginal cuff brachytherapy	S+R	Whole pelvic radiation with vaginal cuff brachytherapy				
Outcome Data	PSE (Post Surgery Event) (Number of Months) (Sept.'22)	22		11					
Summary Of Events	Did the treatment received by the patient match with the drug combo tested on the patient's tumor, *Ex Vivo,* in the laboratory	Partial Match		Not Matched	Not Matched				
	Effectiveness of the drug-combo in the *Ex Vivo* culture	Effective combo (Paclitaxel)		Effective combo(s) (Paclitaxel + Copanlisib) (Paclitaxel + TAK228)	Ineffective (Paclitaxel)				
	Time of PSE (Early PSE: < 16 months; Late/Delayed PSE: > 16 months)	Delayed PSE		Early PSE					
	PET-CT	Several mildly avid pulmonary nodules in both lungs consistent with metastatic disease		Intraperitoneal peripheral enhancing masses (particularly anterior mid abdomen) compatible with recurrent disease					

Patients with Ovarian Cancers

Patient ID		AC-1-26		AC-1-30		AC-1-B091		AC-1-94	
Pathological Parameters	Tumor Histology	High-grade serous carcinoma		Poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor		Adenocarcinoma consistent with a history of high-grade recurrent ovarian papillary serous carcinoma		Serous carcinoma	
	Tumor Grade	3		3		X		1	
	Tumor Stage	IIIC		IIIA1		IIIC/IV		IIIC	
	LVI	Present		Present		X		Present	
	LN Status	Present (1/1)		Present (1/1)		X		Not Submitted	
	Uterine Serosa & Cervical Stroma Involvement	Present		Absent		X		Absent	
	TMN	ypT3c ypN1a		pT3 pN1b				(y)pT3c pNX pMX	
	MSI	Stable		Stable		Stable		Cannot Be Determined	
	MMR	N/A	PD-L1 negative, PD-1 low positive; No record of IHC MMR	N/A	PD-L1 negative, PD-1 low positive; No record of IHC MMR	N/A	PD-L1 negative, PD-1 low positive; No record of IHC MMR	N/A	PD-L1 negative, PD-1 low positive; No record of IHC MMR

	CA-125 (Units per millimeter (U/mL) Pre & Post [Months (M) & Weeks (W) & Days (D)] Surgery	3 M Pre	1442.9	2 D Post	94.6	1 M Pre	57.3	5 M Pre	666.7
		2 M Pre	812.7	3 W Post	142.6	1 M Post	218.2	4 M Pre	781.2
		1 M Pre	615	1 M Post	30.3	2 M Post	530	3 M Pre	380.8
		2 W Post	215.2	2 M Post	19.7	3 M Post	255.5	2 M Pre	539.8
		2 M Post	116.4	3 M Post	71	5 M Post	434	2 M Pre	487.6
		12 M Post	86.5	4 M Post	43.4	6 M Post	2929.3	1 M Pre	594.9
		24 M Post	68.4			7 M Post	391	1 M Post	70.6
		36 M Post	118.1			8 M Post	815.8	3 M Post	49.9
		39 M Post	154			9 M Post	412.6	6 M Post	43
						10 M Post	262.4	9 M Post	21
						12 M Post	111	12 M Post	23.1
						14 M Post	186.1	15 M Post	33.4
						16 M Post	777.5	18 M Post	28.4
	Genomic Alterations In Cell Signal Pathways (FOUNDATIONONE CDx)	KRAS G12D		CCNE1 amplification	FAS loss	BRCA2 E2846fs*22	NF1 loss exons 23-37	BRAF G464V	
		Tumor Mutational Burden 5 Muts/Mb		FOXL2 C134W	PTEN loss	TSC1 loss exons 9-12	TP53 R337L	NRAS Q61R	
				TP53 1255T		CDKN2A/B loss			
				RPTOR amplification				Loss of Heterozygosity (LOH) score Cannot Be Determined	
				MYC amplification		AR/D18 splice site 3865_3974+19del129			
				Tumor Mutational Burden 3 Muts/Mb		Tumor Mutation Burden TMB-intermediate; 11 Muts/Mb		Tumor Mutational Burden Cannot Be Determined	
	Pretreatment History	Carboplatin/Paclitaxel		None		Cisplatinum & Paclitaxel (July-Oct 2007); unknown agents (January 2010 treated); Carboplatin AUC5, Paclitaxel, and Veliparib (1/22/2015-4/7/2015); Anastrozole (1/14/2016); Carboplatin AUC5, Olaparib (2/11/2016-6/21/2016); single agent Olaparib (6/21/2016 started); Rucaparib 12/26/2017 started); Rubraca at lower dose (Aug 2019)		Carboplatin/Paclitaxel/Bevacizumab (2019)	
	Effect of Drug Combination (s) On Tumor Tissue In *Ex Vivo* Culture	Paclitaxel (P) + Carboplatin (C)	No effect	Paclitaxel (P) + Carboplatin (C)	No Effect	Carboplatin + BMN673 + Trametinib	Effect	Paclitaxel (P) + Carboplatin (C)	Effect
		P+C+ Rucaparib	No effect	P+C+ Rucaparib	Effect			P+C+ Trametinib	Effect
		P+C+ Copanlisib	No effect	P+C+ Copanlisib	Effect			P+C+ Trametinib + Copanlisib	Effect
		P+C+ TAK228	No effect	P+C+ TAK228	Effect				
		P+C+ Lenvatinib	No effect	P+C+ Lenvatinib	No effect				

Adjuvant Treatment: Treatments - Surgery (S), Chemo T (C), Radiation (R), Immune T (I)		S+C	Carboplatin Taxol, Bevacizumab: Mekinist and Letrozole upon recurrence	S+C	Paclitaxel/Carboplatin, Bleomycin, Etoposide, Cisplatin.	S+I+C+T	Mekinist and Niraparib, the patient could not tolerate (March-April 2020); Pembrolizumab (04/28/2020-06/9/2020); started Avastin and oral Cyclo-phosphamide (07/21/2020)	S+C+T	Carboplatin/Paclitaxel/Bevacizumab (10/8/2019); started carboplatin/Paclitaxel for second time (3/10/2020); started Letrozole (5/20/2020)
Outcome Data	PSE (Post Surgery Event) (Number of Months) (Sept.'22)	16 (Sept.2022)		2		6		23 (1/24/2022)	
Summary Of Events	Did the patient's treatment match the drug combo tested on the patient's tumor, *Ex Vivo,* in the laboratory	Matched		Matched		Not Matched		Matched	
	Effectiveness of the drug-combo in the *Ex Vivo* culture	Ineffective Drug-Combo		Ineffective Drug-Combo		Effective Drug-Combo		Effective Drug-Combo	
	Time of PSE (Early PSE: < 16 months; Late/Delayed PSE: > 16 months)	Early PSE		Early PSE		Early PSE		Delayed PSE	
	PET-CT	Mild retroperitoneal adenopathy and multiple pulmonary nodules		Markedly increased hepatic metastatic disease, with innumerable lesions now present. Increased retroperitoneal adenopathy. There are also increased nodal or soft tissue masses within the bilateral obturator chains in the pelvis		2 foci of abnormal uptake in the right psoas muscle, likely metastatic disease		Not Available	

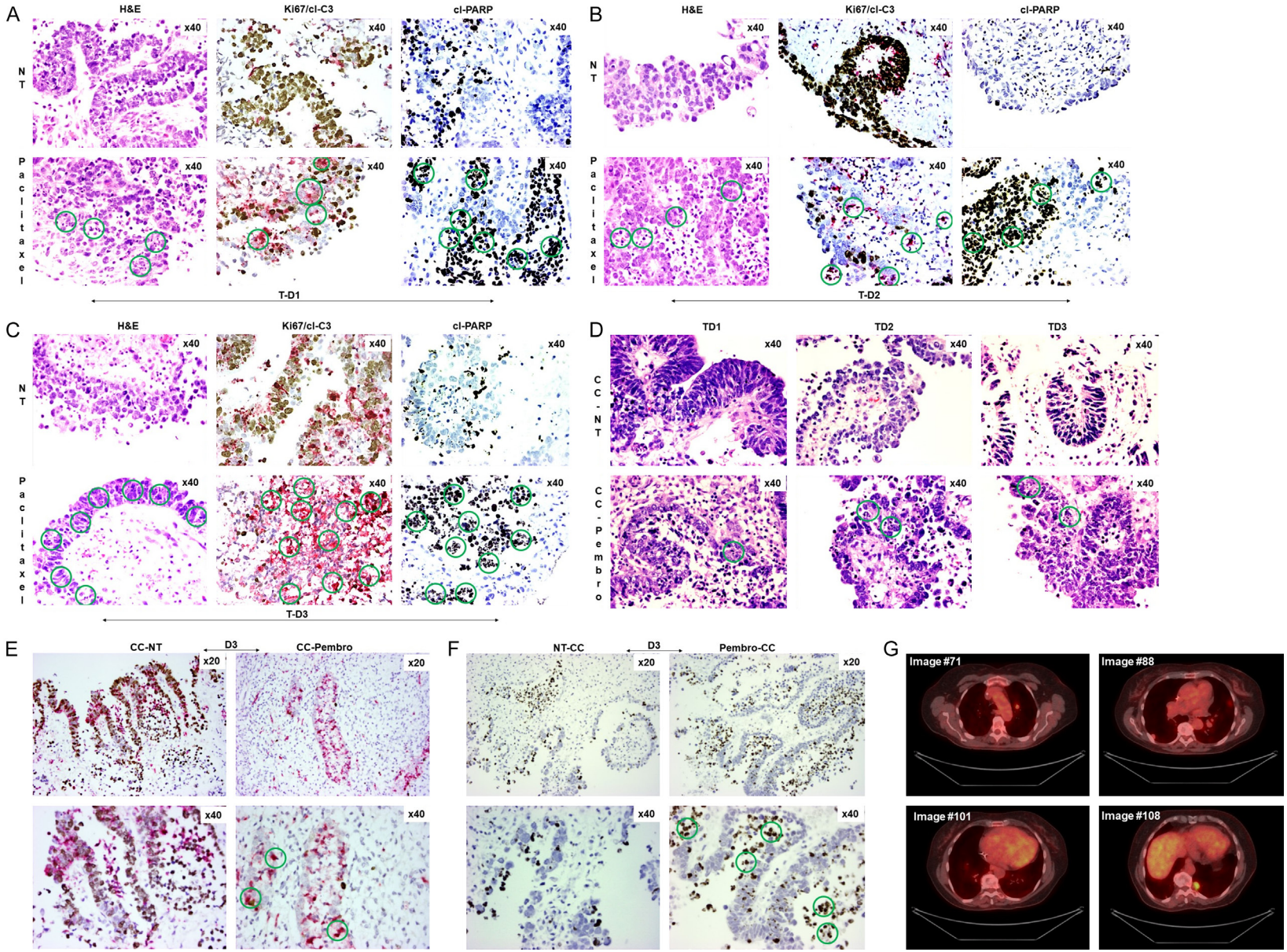

A
H&E
Ki67/cl-C3
cl-PARP
NT
Paclitaxel
x40
T-D1
B
H&E
Ki67/cl-C3
cl-PARP
NT
Paclitaxel
x40
T-D2
C
H&E
Ki67/cl-C3
cl-PARP
NT
Paclitaxel
x40
T-D3
D
TD1
TD2
TD3
CC-NT
CC-Pembro
x40
E
CC-NT
D3
CC-Pembro
x20
x40
F
NT-CC
D3
Pembro-CC
x20
x40
G
Image #71
Image #88
Image #101
Image #108

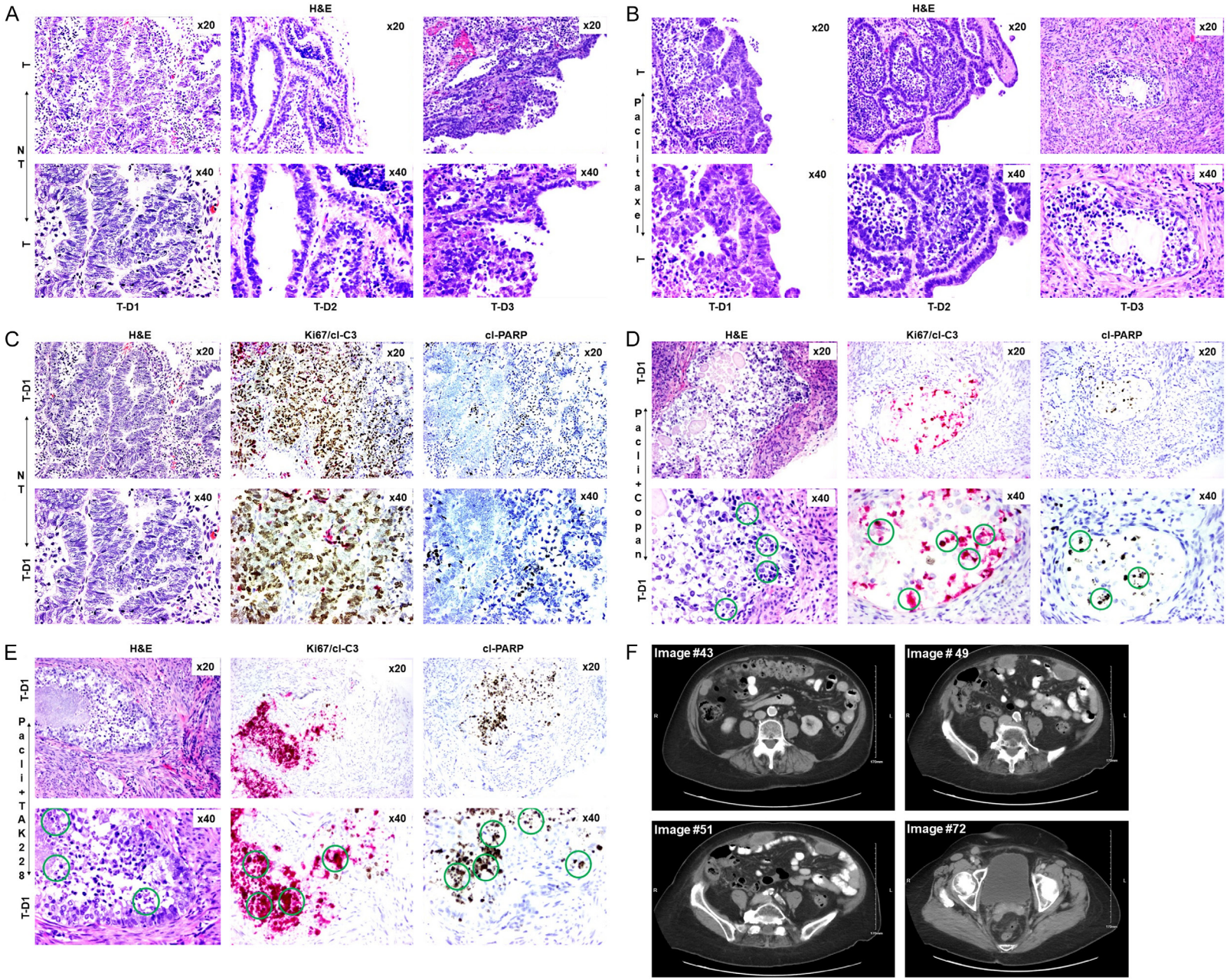
Figure 1. Effect of paclitaxel and pembrolizumab in ex vivo culture and co-culture respectively on tumor tissues, respectively from a patient with lymph node-positive superficially invasive endometrial serous carcinoma, grade 3, stage IIIC1 disease whose adjuvant therapy and post-surgery event (PSE) were recorded retrospectively. A: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 1 (D1) ex vivo cultured tumor tissue (T) from a patient with high-grade serous endometrial carcinoma (Original Mag. X40 obj.) treated with paclitaxel as compared to vehicle-treated control (NT). Apoptotic bodies are labeled as green circles. B: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 2 (D2) ex vivo cultured tumor tissue (T) from a patient with high-grade serous endometrial carcinoma (Original Mag. X40 obj.) treated with paclitaxel as compared to vehicle-treated control (NT). Apoptotic bodies are labeled as green circles. C: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 3 (D3) ex vivo cultured tumor tissue (T) from a patient with high-grade serous endometrial carcinoma (Original Mag. X40 obj.) treated with paclitaxel as compared to vehicle-treated control (NT). Apoptotic bodies are labeled as green circles. D: H&E stained FFPE section from D1, D2, and D3 ex vivo co-cultured tumor tissue (T) with isolated CD3+ DiI-stained T-cells from a patient with high-grade serous endometrial carcinoma (Original Mag. X40 obj.) treated with pembrolizumab (CC-Pembro) as compared to vehicle-treated control (CC-NT). Apoptotic bodies are labeled as green circles. E: Ki67-cleaved Caspase3 (cIC3) double-stained FFPE section from D3 ex vivo co-cultured tumor tissue (T) with isolated CD3+ DiI-stained T-cells from the same patient with high-grade serous endometrial carcinoma (Original Mag. X40 obj. & X20 obj.) treated with Pembrolizumab (CC-Pembro) as compared to vehicle-treated control (CC-NT). Apoptotic bodies are labeled as green circles. F: Cleaved-PARP (cl-PARP) stained FFPE section from D3 ex vivo co-cultured tumor tissue (T) with isolated CD3+ DiI-stained T-cells from the same patient with high-grade serous endometrial carcinoma (Original Mag. X40 obj. & X20 obj.) treated with Pembrolizumab (CC-Pembro) as compared to vehicle-treated control (CC-NT). Apoptotic bodies are labeled as green circles. G: Images from PET-CT showing several mildly avid pulmonary nodules in both lungs consistent with metastatic disease.

with isolated CD3+ DiI-stained T-cells from the same patient. **Figure 1E** presents Ki67-cIC3 double-stained FFPE section from D3 ex vivo co-cultured tumor tissue treated with pembrolizumab as compared to vehicle-treated control, demonstrating a simultaneous decrease of Ki67 staining and an increase of cIC3 staining (green circles), which concurred with the increase of another apoptotic (green circles) marker, cl-PARP in the treated sample (**Figure 1F**). The adjuvant treatment of the patient included both paclitaxel and pembrolizumab (**Table 3**). A delayed PSE was recorded in the 22nd month following the surgery. **Figure 1G** presents images from PET-CT showing several mildly avid pulmonary nodules in both lungs consistent with metastatic disease.

In summary, effective drug treatments with paclitaxel and pembrolizumab in ex vivo culture and co-culture were observed on tumor tissues. We recorded *retrospectively that the patient had received adjuvant therapy, which matched/similar to the effective drug tested in ex vivo cultures. This patient had a delayed post-surgery event (PSE).*

Paclitaxel in combination(s) with copanlisib/ TAK228 was an ineffective in ex vivo culture that matched adjuvant treatment & recorded early PSE

We tested the effect of paclitaxel and its combination with copanlisib (pan PI3K inhibitor)/ TAK228 (mTORC1/C2 kinase inhibitor) in ex vivo culture on tumor tissues from a patient with lymph node-negative endometrioid endometrial adenocarcinoma, grade 1, stage IB disease whose adjuvant therapy and post-surgery event (PSE) were recorded retrospectively. The drug combination was decided based on the scientific interrogation of the genomic alterations observed in the tumor (**Table 3**; AC-1-40). **Figure 2A** shows H&E stained FFPE sections from D1, D2, and D3 ex vivo cultured tumor tissue from the patient treated with the vehicle, while **Figure 2B** shows H&E stained FFPE sections from D1, D2, and D3 ex vivo cultured tumor tissue treated with paclitaxel. **Figure 2C** presents H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 1 ex vivo cultured tumor tissue from the same patient. In contrast to no effect as observed following paclitaxel, **Figure 2D** and **2E** showed both decrease of Ki67 and an increase of staining of cIC3 as well as cl-PARP with apoptotic bodies (green circles) at day 1 in the same tumor tissue, only this time treated with two effective combinations of paclitaxel plus copanlisib and paclitaxel plus TAK228, respectively. The adjuvant treatment of the patient included whole pelvic radiation with vaginal cuff brachytherapy (**Table 3**). An early PSE was recorded during the 11th month following the surgery. **Figure 2F** PET-CT images showed intraperitoneal peripheral enhancing masses (particularly anterior mid abdomen) compatible with recurrent disease.


A
H&E
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B
H&E
Paclitaxel
C
H&E
Ki67/cl-C3
cl-PARP
D
Paci+Copan
E
Paci+TAK228
F
Image #43
Image # 49
Image #51
Image #72

Figure 2. Effect of paclitaxel and its combination with copanlisib/TAK228 in ex vivo culture on tumor tissues from a patient with lymph node-negative endometrioid endometrial adenocarcinoma, grade 1, stage IB disease whose adjuvant therapy and post-surgery event (PSE) were recorded retrospectively. A: H&E stained FFPE section from D1, D2, and D3 ex vivo cultured (NT) tumor tissue (T) from a patient with lymph node-negative endometrioid endometrial adenocarcinoma, grade 1, stage IB disease (Original Mag. X40 obj. & X20 obj.). B: H&E stained FFPE section from D1, D2, and D3 ex vivo cultured tumor tissue (T) from a patient with lymph node-negative endometrioid endometrial adenocarcinoma, grade 1, stage IB disease treated with paclitaxel (Original Mag. X40 obj. & X20 obj.). C: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 1 (D1) ex vivo cultured tumor tissue (T) from the same patient (Original Mag. X40 obj. & X20 obj.) treated with vehicle control (NT). D: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 1 (D1) ex vivo cultured tumor tissue (T) from the same patient (Original Mag. X40 obj and X20 obj.) treated with paclitaxel plus copanlisib (Pacli+Copan). Apoptotic bodies are labeled as green circles. E: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 1 (D1) ex vivo cultured tumor tissue (T) from the same patient (Original Mag. X40 obj and X20 obj.) treated with paclitaxel plus TAK228 (Pacli+TAK228). Apoptotic bodies are labeled as green circles. F: Images from CT showing intraperitoneal peripheral enhancing masses (particularly anterior mid abdomen) compatible with recurrent disease.

In summary, ineffective drug treatment with paclitaxel in ex vivo culture was recorded on tumor tissues from the patient. The paclitaxel plus copanlisib or paclitaxel plus TAK228 combinations were effective in ex vivo culture but were not administered clinically. The patient had received *adjuvant therapy, which did not match the effective drug (on the contrary, matched/similar to the ineffective drug) as tested in ex vivo culture,* and had an *early post-surgery event (PSE).*

Paclitaxel plus carboplatin with rucaparib/copanlisib/TAK228/lenvatinib as an ineffective in ex vivo culture that matched adjuvant treatment & recorded early PSE

We tested the effect of combinations of paclitaxel plus carboplatin with rucaparib (PARP inhibitor)/copanlisib/TAK228/lenvatinib (multi-tyrosine kinase inhibitor) of a patient with lymph node-positive grade 3 serous carcinoma of ovary, stage IIIC, both primary and metastatic disease whose adjuvant therapy and PSE were recorded retrospectively. **Figure 3A** and **3B** showed no effect of the drug in H&E stained FFPE section from day 3 ex vivo cultured primary tumor tissue and metastatic tumor tissue from the same patient treated with combinations of paclitaxel plus carboplatin with rucaparib or copanlisib or TAK228 or lenvatinib as compared to vehicle-treated controls, respectively. *The patient had received adjuvant therapy, which matched/similar to the ineffective ex vivo combinations, and retrospectively we recorded that she had an early PSE.* PET-CT images showed mild retroperitoneal adenopathy and multiple pulmonary nodules (**Figure 3C**).

In summary, ineffective drug treatment with combinations of 5 different combinations in ex vivo culture on primary and metastatic tumor tissues from the same patient is recorded. The adjuvant treatment of the patient included carboplatin, paclitaxel, bevacizumab, followed by mekinist and letrozole upon recurrence (**Table 3**; AC-1-26). An early PSE was recorded during the 16th month following the surgery.

Paclitaxel plus carboplatin was an ineffective combination in ex vivo culture that matched adjuvant treatment & recorded early PSE

We tested the effect of combinations of paclitaxel plus carboplatin in ex vivo culture of tumor tissues of a lymph node-positive patient with poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor of ovary, grade 3, stage IIIA1 disease whose adjuvant therapy and PSE were recorded retrospectively. **Figure 4A-C** show H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 1, day 2, and day 3 ex vivo cultured tumor tissue, vehicle control from the patient, as compared to the paclitaxel plus carboplatin treated tissue (**Figure 4D**), respectively. Cyan rectangles indicated some of the prominent mitotic bodies in both control and treated samples. The drug combination was decided based on the scientific interrogation of the genomic alterations observed in the tumor (**Table 3**; AC-1-30). An early PSE was recorded during the 2nd month following the surgery. PET-CT images indicate markedly increased hepatic metastatic disease, with innumerable lesions now present. Increased retroperitoneal adenopathy was observed. There are also increased nodal or soft tissue masses within the bilateral obturator chains in the pelvis (**Figure 4E**).

In summary, ineffective drug combinations were recorded in the ex vivo tests. The adjuvant

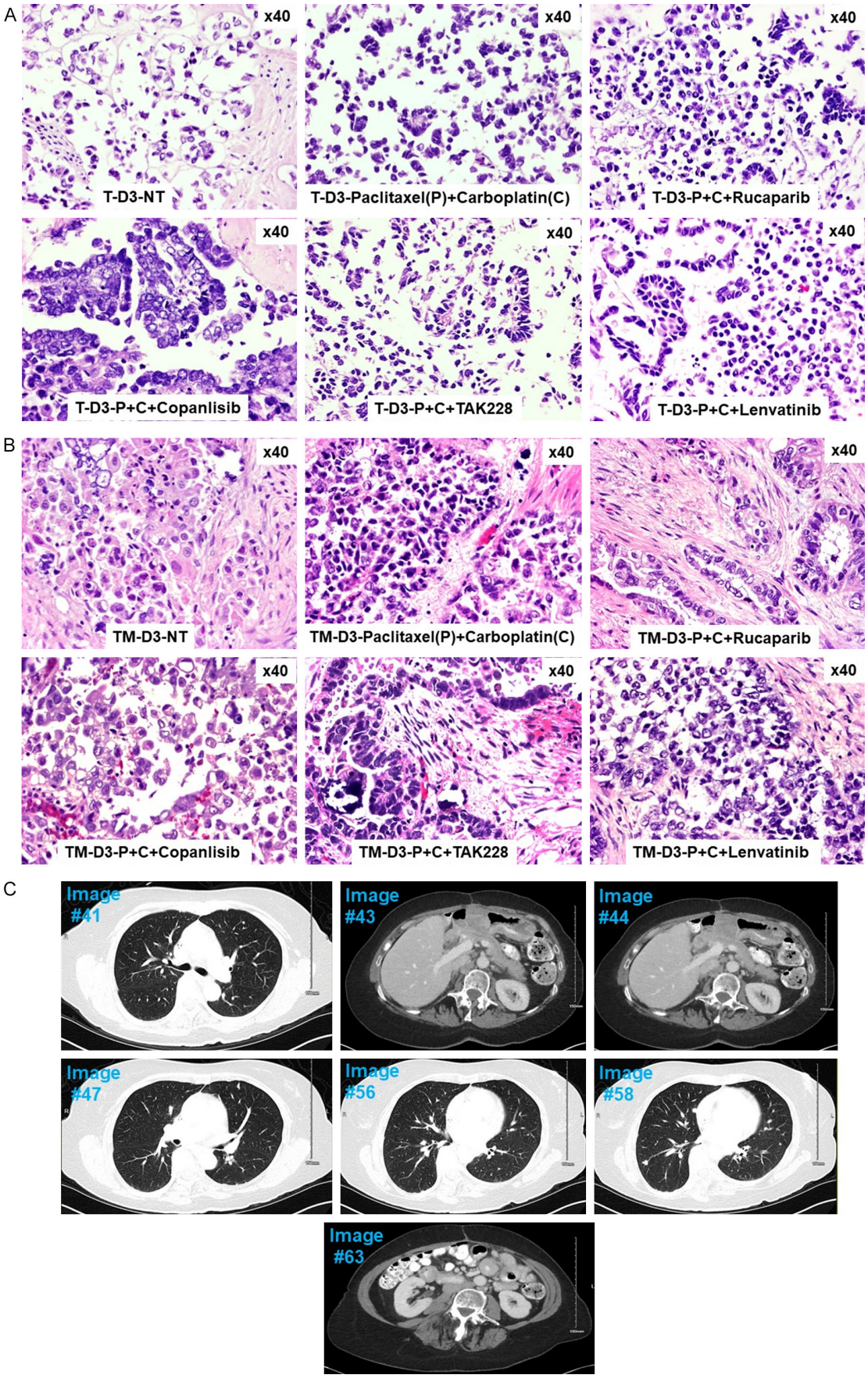

A
x40
T-D3-NT
T-D3-Paclitaxel(P)+Carboplatin(C)
T-D3-P+C+Rucaparib
T-D3-P+C+Copanlisib
T-D3-P+C+TAK228
T-D3-P+C+Lenvatinib
B
TM-D3-NT
TM-D3-Paclitaxel(P)+Carboplatin(C)
TM-D3-P+C+Rucaparib
TM-D3-P+C+Copanlisib
TM-D3-P+C+TAK228
TM-D3-P+C+Lenvatinib
C
Image #41
Image #43
Image #44
Image #47
Image #56
Image #58
Image #63

Figure 3. Effect of combinations of paclitaxel plus carboplatin with rucaparib/copanlisib/TAK228/lenvatinib in ex vivo culture on primary tumor tissues and metastatic tumor tissues of a patient with lymph node-positive grade 3 serous carcinoma, stage IIIC, whose adjuvant therapy and post-surgery event (PSE) were recorded retrospectively. A: H&E stained FFPE section from day 3 (D3) ex vivo cultured primary tumor tissue (T) from a patient with lymph node-positive grade 3 serous carcinoma, stage IIIC disease (Original Mag. X40 obj.) treated with combinations of paclitaxel plus carboplatin (P+C) and rucaparib (P+C+rucaparib) or copanlisib (P+C+copanlisib) or TAK228 (P+C+TAK228) or lenvatinib (P+C+lenvatinib) as compared to vehicle-treated control (NT). B: H&E stained FFPE section from day 3 (D3) ex vivo cultured metastatic tumor tissue (TM) from the same patient with lymph node-positive grade 3 serous carcinoma, stage IIIC disease (Original Mag. X40 obj.) treated with combinations of paclitaxel plus carboplatin (P+C) and rucaparib (P+C+rucaparib) or copanlisib (P+C+copanlisib) or TAK228 (P+C+TAK228) or lenvatinib (P+C+lenvatinib) as compared to vehicle-treated control (NT). C: Images from CT showing mild retroperitoneal adenopathy and multiple pulmonary nodules.

treatment of the patient included paclitaxel, carboplatin, bleomycin, etoposide, and cisplatin (**Table 3**). *The patient had received adjuvant therapy, which matched/was similar to the ineffective ex vivo combinations, and retrospectively had an early PSE.*

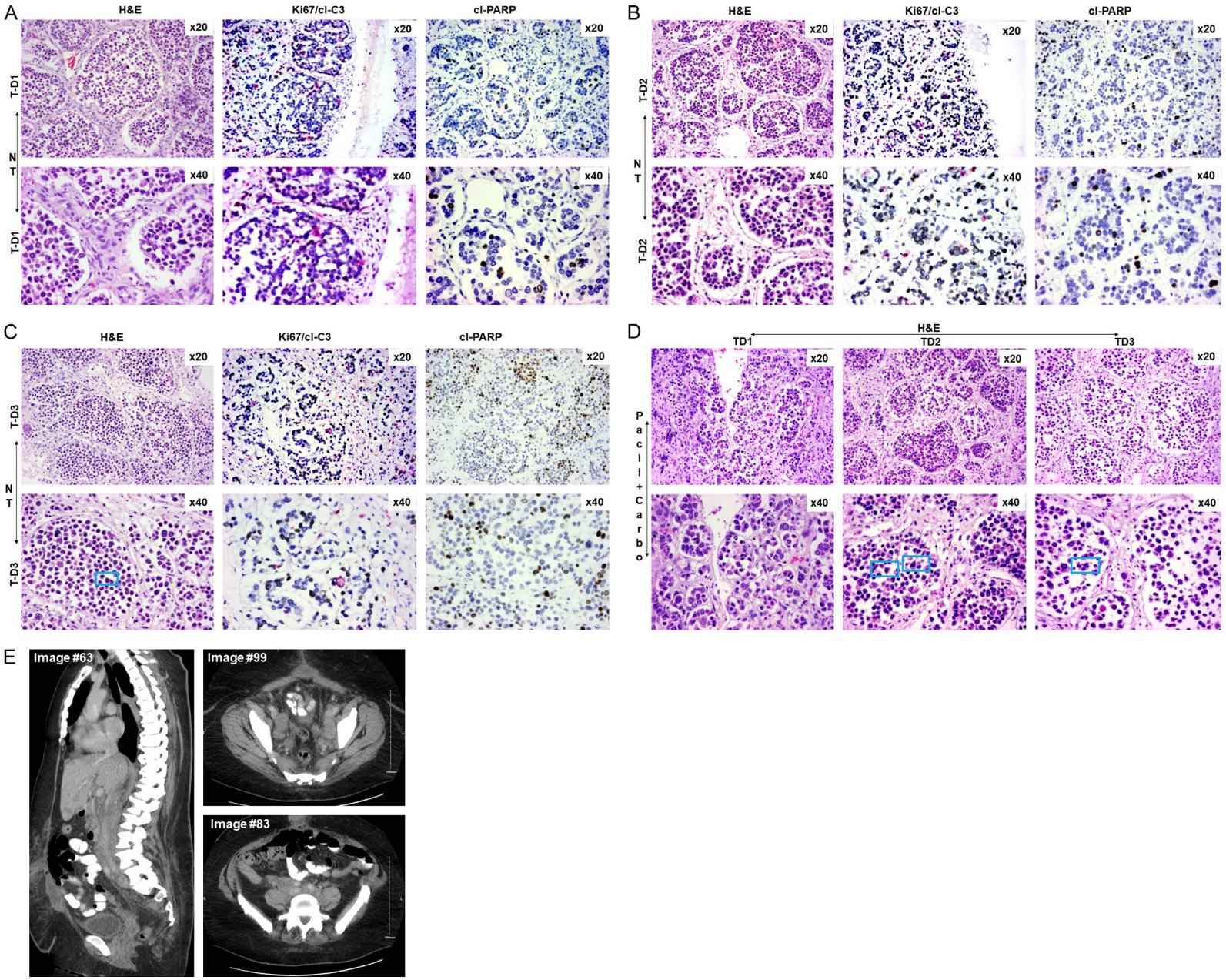
Carboplatin, BMN673 plus trametinib was an effective combination in ex vivo culture that did not match the adjuvant treatment & recorded early PSE

We tested the effect of combinations of carboplatin, BMN673 (Talazoparib, PARP inhibitor) plus trametinib (MEK1/2 inhibitor) in ex vivo culture of tumor biopsy tissue of a patient with recurrent ovarian carcinoma, stage IIIC/IV disease, whose adjuvant therapy and PSE were recorded retrospectively. The drug combination was decided based on the scientific interrogation of the genomic alterations observed in the tumor (as presented in **Table 3**; AC-1-B091). **Figure 5A** and **5B** present H&E, Ki67/cIC3, and pERK stained FFPE section from day 3 ex vivo cultured tumor biopsy tissue, vehicle control, and treated with carboplatin, BMN673 plus trametinib, respectively. The drug-treated biopsy tissue expressed significantly higher levels of cIC3, although the expression of Ki67 expression remained comparable. On the contrary, the expression of pERK was obliterated following the drug indicating the loss of the RAS-MAPK-signal mediated proliferation signals in the tumor cells. Prominent apoptotic bodies were marked in green circles. The adjuvant treatment of the patient included Mekinist and Niraparib (March-April 2020); Pembrolizumab (04/28/2020-06/9/2020); Avastin and oral cyclophosphamide (07/21/2020) (**Table 3**). An early PSE was recorded during the 6th month following the surgery. Images from PET-CT showed 2 foci of abnormal uptake in the right psoas muscle, likely metastatic disease (**Figure 5C**).

In summary, effective drug treatment with combinations of carboplatin, BMN673, plus trametinib in ex vivo culture on biopsy of tumor tissues was recorded. *The patient had received therapy that did not match the effective ex vivo combinations, and retrospectively, an early PSE was recorded.* Although the patient received Neraparib (PARP inhibitor) in the course of her treatment, current literature showed that single-agent PARP inhibitor had limited efficacy in the presence of upregulation of the RAS-MAPK pathway in the tumor [11-13].

Paclitaxel plus carboplatin was an effective combination in ex vivo culture that matched/similar to the adjuvant treatment & recorded delayed PSE

We tested the effect of combinations of paclitaxel plus carboplatin in tumor tissues of a patient with serous carcinoma of the ovary, stage IIIC disease, whose adjuvant therapy and PSE were recorded retrospectively. The drug combination was decided based on the scientific interrogation of the genomic alterations observed in the tumor (as presented in **Table 3**; AC-1-94). **Figure 6A** and **6B** present H&E, and Ki67/cIC3, stained FFPE section from day 3 ex vivo cultured tumor tissue, vehicle control from a patient treated with paclitaxel plus carboplatin from the same patient, respectively. The adjuvant therapy included carboplatin, paclitaxel, and bevacizumab (10/8/2019); started carboplatin, paclitaxel for the second time (3/10/2020); started letrozole (5/20/2020) as mentioned in **Table 3**. The patient had a delayed PSE of 23 months following the surgery (PET-CT was not available).


A
H&E
Ki67/cl-C3
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T-D1
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TD1
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TD3
Paci+Carbo
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Image #63
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Image #83

Figure 4. Effect of combinations of paclitaxel plus carboplatin in ex vivo culture of tumor tissues of a lymph node-positive patient with poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor, grade 3, stage IIIA1 disease whose adjuvant therapy and post-surgery event (PSE) were recorded retrospectively. A: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 1 (D1) ex vivo cultured tumor tissue (T), vehicle control (NT) from a patient with poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor, grade 3, stage IIIA1 (Original Mag. X40 obj and X20 obj.). B: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 2 (D2) ex vivo cultured tumor tissue (T) as compared to vehicle control (NT) from a patient with poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor, grade 3, stage IIIA1 (Original Mag. X40 obj and X20 obj.). C: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from day 3 (D3) ex vivo cultured tumor tissue (T) with vehicle control (NT) from a patient with poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor, grade 3, stage IIIA1 (Original Mag. X40 obj and X20 obj.). Mitotic figures are labeled as cyan rectangles. D: H&E stained FFPE section from day 1 (D1), day 2 (D2), day 3 (D3) ex vivo cultured tumor tissue (T) from the same patient with poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor, grade 3, stage IIIA1 treated with paclitaxel plus carboplatin (Pacli + Carbo) (Original Mag. X40 obj and X20 obj.). Mitotic figures are labeled as cyan rectangles. E: Images from CT showing markedly increased hepatic metastatic disease, with innumerable lesions now present. Images show increased retroperitoneal adenopathy. There are also increased nodal or soft tissue masses within the bilateral obturator chains in the pelvis.

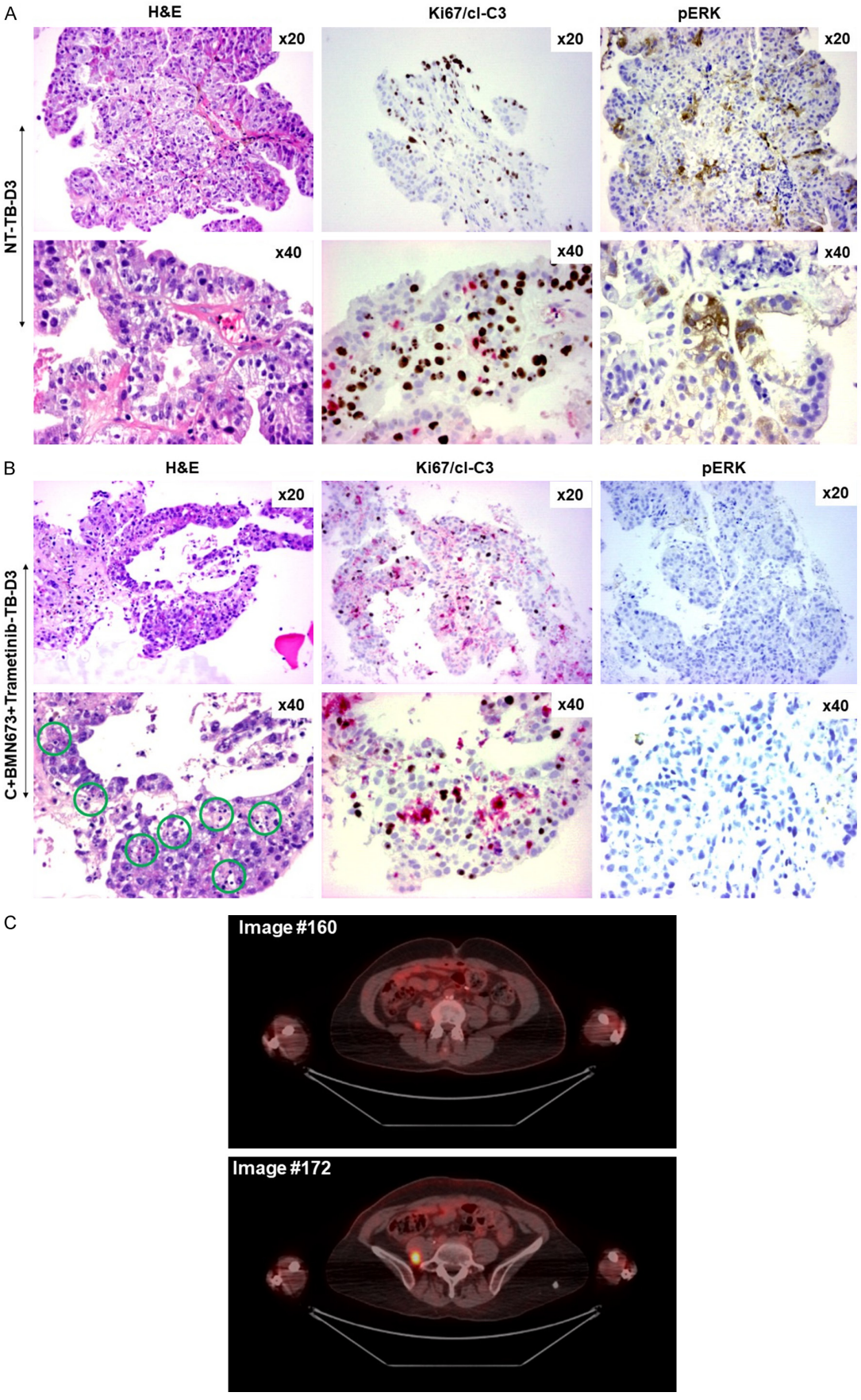
In summary, effective drug treatment with combinations of paclitaxel plus carboplatin in ex vivo culture of resected tumor tissues from the patient was recorded. *The patient had received therapy matched/similar to the effective ex vivo combinations and had retrospectively delayed PSE.*

Discussion

Our ex vivo drug testing platform was designed to model functional precision medicine based on its power of clinical relevance. First, we tested genomics-driven drug combinations to establish their effectiveness in contrast to ineffective ones. Once we determined the patient-wise effectiveness/ineffectiveness of the genomic-alteration-driven drug combination(s), we retrospectively accessed patients' adjuvant treatment details and PSE. The clinical relevance of the platform was tested based on the hypothesis that if the *effective drug combination matched/was similar to the adjuvant treatment received by the patient, the PSE would be delayed.* On the contrary, if the *ineffective drug combination matched/was similar to the adjuvant treatment, an early PSE would be encountered in the clinics.* As an extension of the above premise, if the *effective drug combination did not match/was similar to the adjuvant treatment received by the patient, the PSE would occur early.*

The ex vivo drug testing platform developed in this study aimed to model functional precision medicine by testing genomics-driven drug combinations for their effectiveness in individual patients with endometrial and ovarian cancers. The platform correlated the ex vivo drug testing results with the patients' adjuvant treatment and subsequent PSE outcomes. The hypothesis was that a match between the effective drug combination in ex vivo testing and the adjuvant therapy received by the patient would result in delayed PSE, while a match between the ineffective drug combination in ex vivo testing and the adjuvant therapy would lead to early PSE. In support of our hypothesis, the results demonstrated that patients whose therapy matched with ineffective drug combinations in ex vivo testing experienced early PSE, while patients whose therapy matched with effective drug combinations in ex vivo testing showed delayed PSE. This finding highlights the ex vivo drug testing platform's clinical relevance as a functional precision medicine model.

The strength of our platform is that once tested in a prospective clinical trial, it can be viewed as a patient-specific laboratory in an oncologist's pocket. We received tumor tissues, interrogated their genome, cultured them in the laboratory with genomics-wise matched drug combinations, and retrospectively correlated ex vivo data to the adjuvant therapy that an individual patient received and the patient's PSE following the adjuvant therapy. Thus, we enriched the logistics of genomics-driven drug-matching with experimental evidence, which translated into therapy. Our platform of functional precision medicine possesses an advantage built-in in its inherent development in a community-based cancer center; the platform is cost-effective, time-sensitive (5 working days from the day of surgery to pathological report-


A
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C+BMN673+Trametinib-TB-D3
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Image #160
Image #172

Figure 5. Effect of combinations of carboplatin, BMN673 plus trametinib in ex vivo culture of tumor biopsy tissue of a patient with adenocarcinoma consistent with a history of ovarian carcinoma, stage IIIC/IV disease, whose adjuvant therapy and post-surgery event (PSE) were recorded retrospectively. A: H&E, Ki67-cleaved-Caspase3 (Ki67/cl-C3), and phospho-ERK (pERK) stained FFPE section from day 3 (D3) ex vivo cultured tumor biopsy tissue (TB), vehicle control (NT) from a patient with adenocarcinoma consistent with a history of high-grade, recurrent ovarian papillary serous carcinoma, stage IIIC/IV disease (Original Mag. X40 obj and X20 obj.). B: H&E, Ki67-cleaved-Caspase3 (Ki67/cl-C3), and phospho-ERK (pERK) stained FFPE section from day 3 (D3) ex vivo cultured tumor biopsy tissue (TB), treated with carboplatin, BMN673 plus Trametinib (Carbo+BMN673+Trametinib) from the same patient (Original Mag. X40 obj and X20 obj.). Apoptotic bodies are labeled as green circles. C: Images from PET-CT showing 2 foci of abnormal uptake in the right psoas muscle, likely metastatic disease.

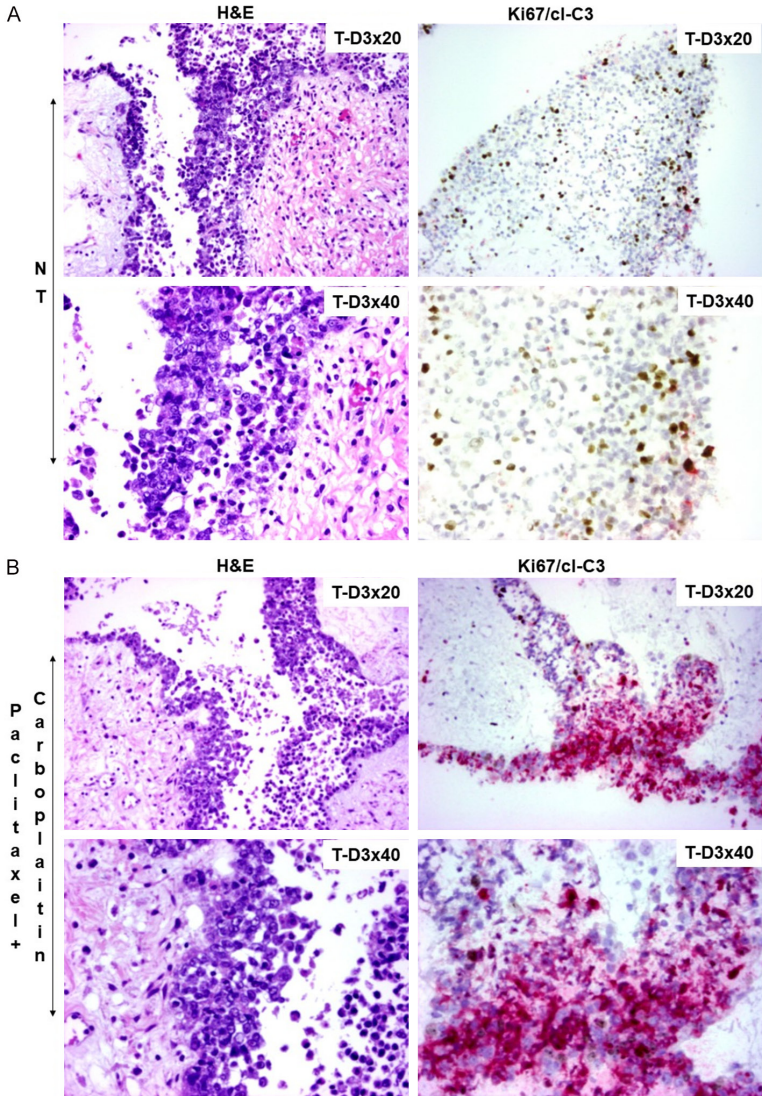

Figure 6. Effect of combinations of paclitaxel plus carboplatin in ex vivo culture of tumor tissues of a patient with serous carcinoma of the ovary, grade 1, stage IIIC disease whose adjuvant therapy and post-surgery event (PSE) were recorded retrospectively. A: H&E, and Ki67-cleaved-Caspase3 (Ki67/cl-C3), stained FFPE section from day 3 (D3) ex vivo cultured tumor tissue (T), vehicle control (NT) from a patient with serous carcinoma of the ovary, grade 1, stage IIIC disease (Original Mag. X40 obj and X20 obj.). B: H&E, and Ki67-cleaved-Caspase3 (Ki67/cl-C3), stained FFPE section from day 3 (D3) ex vivo cultured tumor tissue (T), treated with paclitaxel plus carboplatin from the same patient (Original Mag. X40 obj and X20 obj.).

ing), laboratory-friendly, and needs a single full-time employee. Unlike organoid cultures, our culture/co-culture platform test the effect of drug(s) on tumor cells in their default histological microenvironment. Our autologous co-culture of tumor cells and CD3+ T-cells provides a unique opportunity to test immune-checkpoint inhibitors.

We used day zero tumor samples as our starting point of reference. The photomicrographs of day zero (D0) cultured tumor tissues from 6 representative patients with endometrial and ovarian cancers are presented in Figure S1. Figure S2 illustrates a representative photomicrograph of in-co-culture whole mounts, DAPI-stained fresh frozen sections, and DiI-stained T-cells from the ex vivo co-culture of tumor tissue or tumor-adjacent normal tissue and isolated CD3+ T-cells from the peripheral blood of patients on the day of surgery. In co-culture, we observe that CD3+ T-cells tend to form aggregates. Interestingly, we observed a distinct pattern of CD3+ T-cell engagement with tumor samples in co-culture versus tumor-adjacent normal tissues (Figure S2B and S2C). The CD3+ T-cell engagement with tumor tissues followed a uniform dispersion, while

CD3+ T-cell engagement was closely aggregated in the tumor-adjacent normal sample. Whether or not it is a tumor-specific event or related to the tumor's genomic alteration status is beyond our study's scope. In line with our proposition, on the 19th February this year, the Food and Drug Administration (FDA) has granted accelerated approval to lifileucel (Amtagvi, Iovance Biotherapeutics), an autologous T cell immunotherapy, for adult patients with unresectable or metastatic melanoma.

We tested the specificity of the drug(s) targeted to the tumor compartment of the tissue. For this purpose, we evaluated the effect of the same drugs (1) on the tumor-adjacent normal tissue from the same patient in a parallel set of ex vivo cultures and (2) on the cells of the tumor micro-environment from the same tumor sample. In the samples showing the antitumor effects following the drug, no effect of the drug was noticed either in the cells of the tumor micro-environment next to the tumor cells or in the tumor-adjacent normal tissue from the same patient highlighting the specificity of the effect in the tumor compartment.

The intrinsic limitation of our platform is its built-in development in a community-based cancer center. As of now, it has not been tested in a prospective clinical trial, and the results are based on retrospective data analysis. The availability of tissue samples and the consecutive 3-day ex vivo culture are also logistical challenges that need to be addressed to ensure wider applicability. Our cohort has a significantly fewer number of biopsy and primary-metastatic paired samples. One limitation of the platform is the amount of the resected tissue obtained within 60 minutes of the surgery. It is imperative to get the pathologically determined tumor tissue in the culture media under aseptic conditions. The ex vivo culture is a terminating culture (witnin 72 hours). The study is limited by the availability of tissue which restricts a consecutive 3-day ex vivo culture, especially in the case of tumor biopsy as in the case of the patient with adenocarcinoma with a history of high-grade, recurrent ovarian papillary serous carcinoma. To overcome this limitation, we restricted (1) the ex vivo culture to day 3 only and/or (2) the IHC staining only to the samples, which according to the pathologist's evaluations, showed apoptotic changes. The number of consents we received from ovarian cancer patients is less than that of the patients with endometrial cancers. Despite its limitations, the functional precision medicine platform provides a promising patient-centric approach to decision-making in neoadjuvant/adjuvant therapy.

Our functional precision medicine platform allows clinicians to review the effect of genomics-driven matched drug combinations. It experimentally validates and reinforces the logical concept of drug-matching *for each patient, outside the body, before the treatment*.

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Informed (IRB approved: Protocol Number Study: 2017.053-100399_ExVivo001) consents for receiving resected tissue and blood from a total of 172 enrolled patients with endometrial and ovarian cancers.

Disclosure of conflict of interest

None.

Address correspondence to: Nandini Dey, Translational Oncology Laboratory, Avera Research Institute, 1000 E 23rd Street, Sioux Falls, SD 57105, USA. Tel: 605-322-3298; E-mail: Nandini.Dey@avera.org

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Clinical relevance of N-of-1 drug testing platform

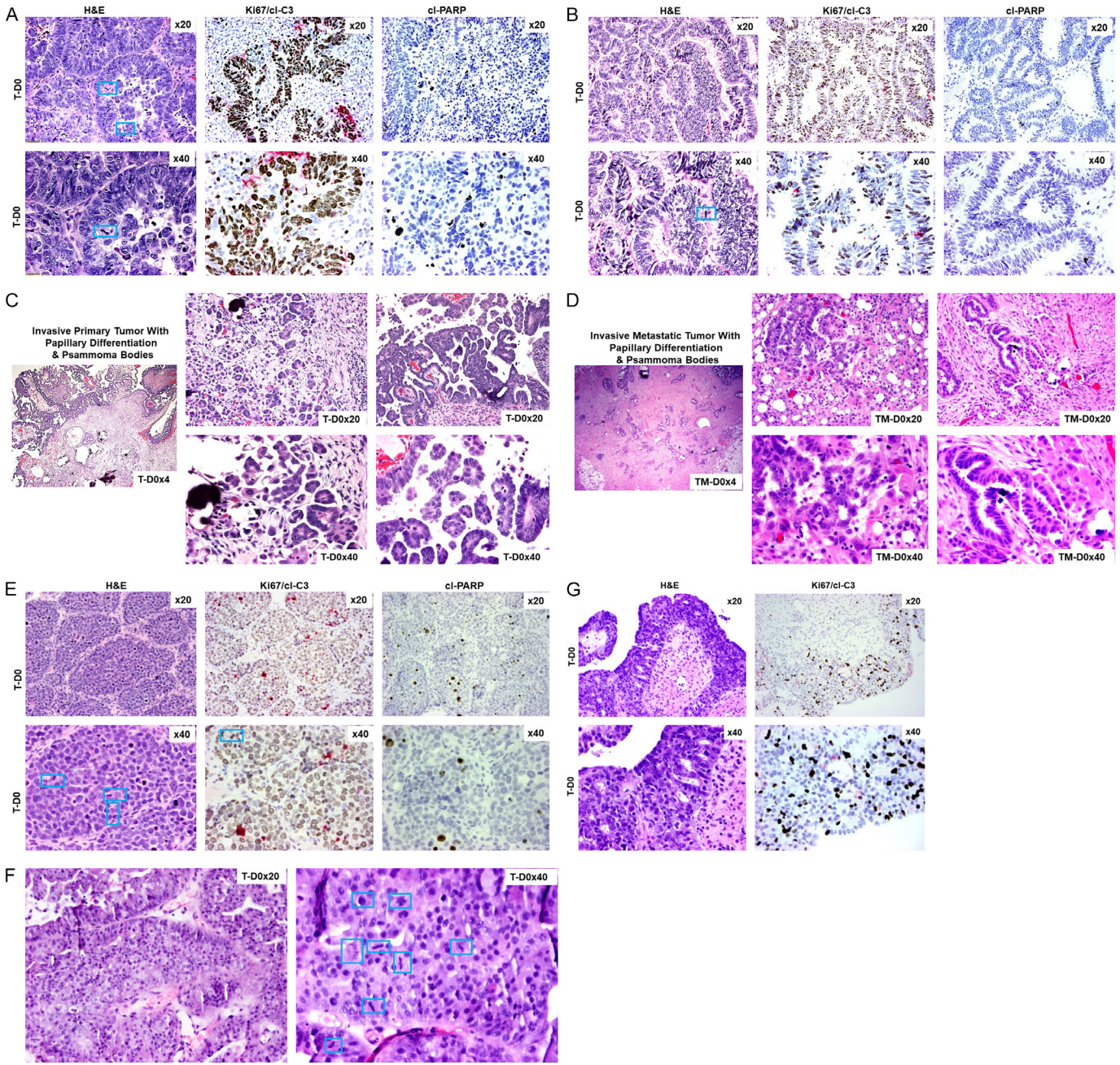
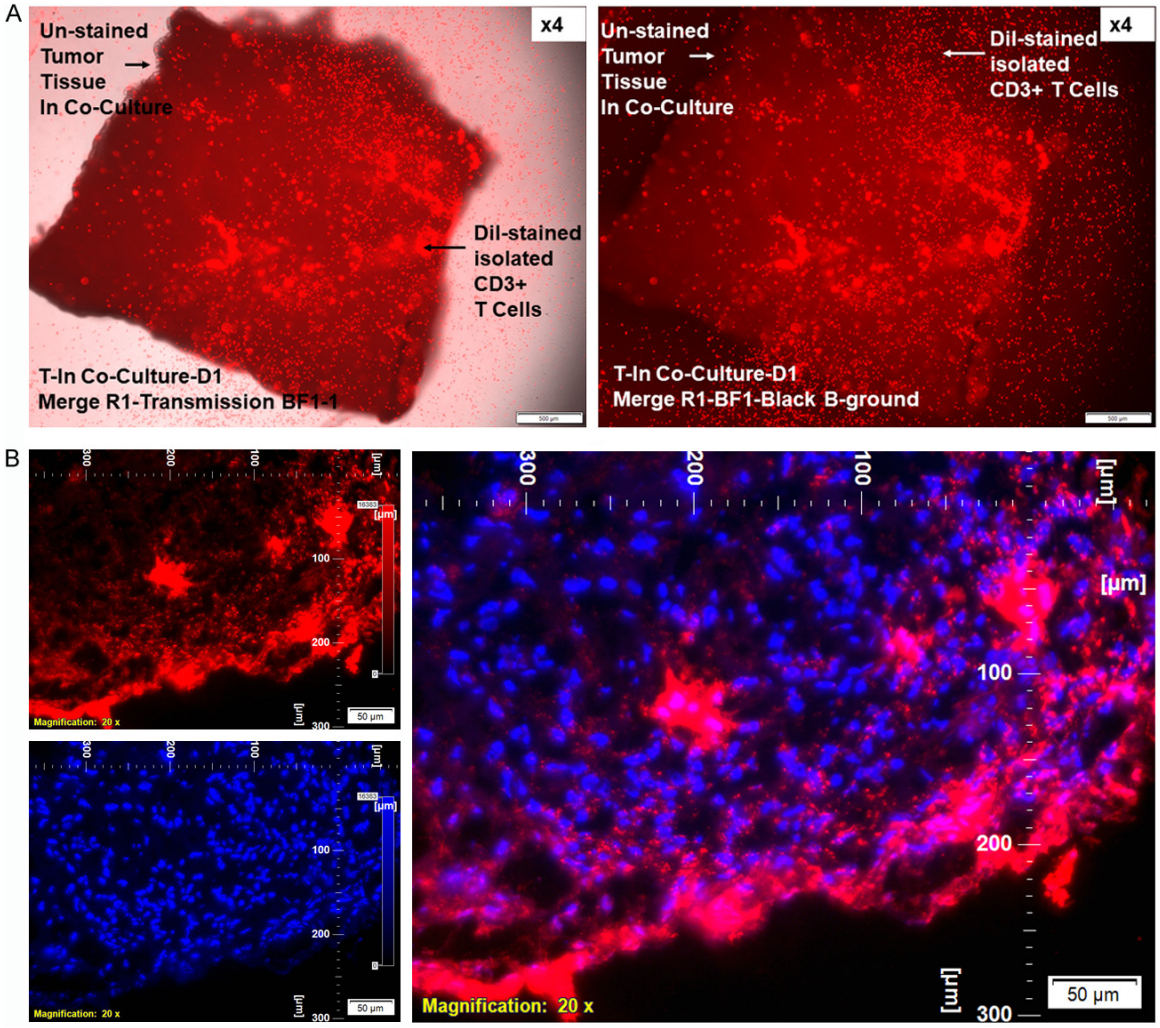


Figure S1. Photomicrographs of the Day Zero (D0) cultured tumor tissues from representative patients with endometrial and ovarian cancers. A: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from D0 cultured tumor tissue from patients with high-grade serous endometrial carcinoma (Original Mag. X40 obj. & X20 obj.). Mitotic figures are labeled as cyan rectangles. B: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from D0 cultured tumor tissue from patients with endometrioid endometrial adenocarcinoma, FIGO 2 (Original Mag. X40 obj. & X20 obj.). Mitotic figures are labeled as cyan rectangles. C: H&E stained FFPE section from D0 cultured primary tumor tissue from patients with high-grade serous carcinoma with papillary growth pattern and psammoma bodies (as shown in the left and middle panel) (Original Mag. X40 obj., X20 obj. & X4 obj.). D: H&E stained FFPE section from D0 cultured metastatic tumor tissue from patients with high-grade serous carcinoma with papillary growth pattern and psammoma bodies (as shown in the left and middle panel) (Original Mag. X40 obj., X20 obj. & X4 obj.). E: H&E, Ki67-cIC3, and cl-PARP stained FFPE section from D0 cultured tumor tissue from patients with poorly differentiated carcinoma within adult granulosa cell tumor (Original Mag. X40 obj. & X20 obj.). Mitotic figures are labeled as cyan rectangles. F: H&E stained FFPE section from D0 cultured biopsy tumor tissue from patients with (Original Mag. X40 obj., and X20 obj.). Mitotic figures are labeled as cyan rectangles. G: H&E and Ki67-cIC3 stained FFPE section from D0 cultured tumor tissue from patients with Low-grade papillary serous carcinoma (right ovary and omentum) (Original Mag. X40 obj. & X20 obj.).



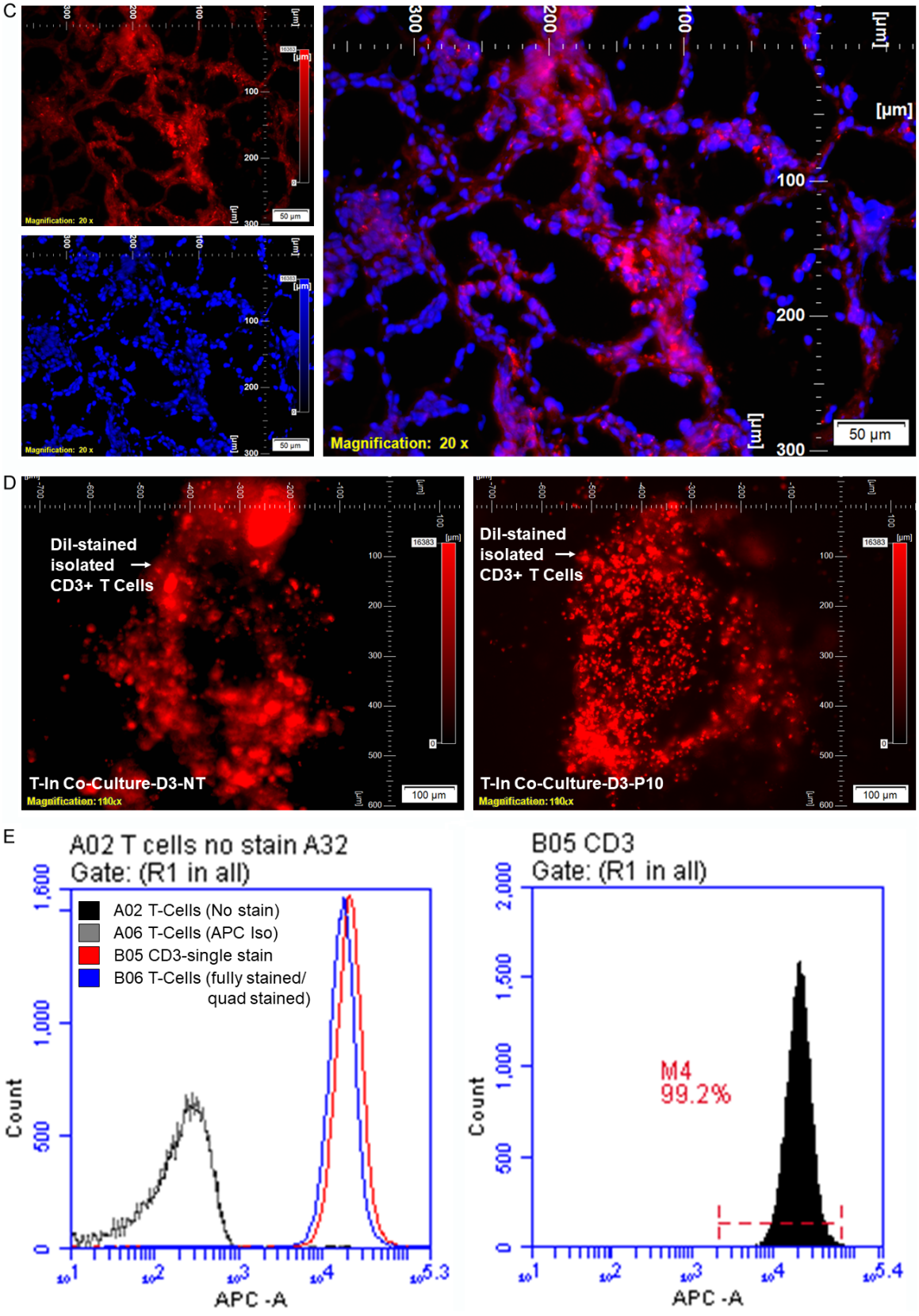


Figure S2. Representative in-co-culture whole mounts, DAPI-stained fresh frozen sections, and Dil-stained T-cells from the ex vivo co-culture of tumor tissue (T) or tumor-adjacent normal tissue (N) and isolated CD3+ T-cells from

the peripheral blood of patients on the day of surgery is presented. A: Unstained tumor tissue on day 1 (D1) in co-culture with isolated DiI-stained CD3+ T-cells (R1) shows a merge of R1 and Transmission image (BF1-1) (x4 Obj.) (left panel image). Unstained tumor tissue in co-culture with isolated DiI-stained CD3+ T-cells (R1) shows a merge of R1 and image (BF1-1) in a black background (x4 Obj.) (left panel image). B: DAPI-stained tumor-adjacent normal tissue (Blue nucleus) on day 3 (D3) in co-culture with isolated DiI-stained CD3+ T-cells (Red) (left panel image) and a merge of DAPI stain and DiI stain (right panel image) is presented. C: Dapi-stained tumor tissue (Blue nucleus) on day 3 (D3) in co-culture with isolated DiI-stained CD3+ T-cells (Red) (left panel image), and a merge of DAPI stain and DiI stain (right panel image) is presented. D: Unstained tumor tissue on day 3 (D3) in co-culture with isolated DiI-stained CD3+ T-cells (Red fluorescence) is presented. The left image represents vehicle-treated co-culture, and the right image represents pembrolizumab-treated co-culture. E: Following isolation/purification on a whole blood column with CD3-bead, the sample is over 99% CD3+ cells.

Table S1. List of drugs (single/combinations) tested in ex vivo culture on tumor and tumor-adjacent normal tissues from patients with endometrial and ovarian cancers

List of drugs (Single/Combinations) Tested on Resected Tumor Tissue Samples from Patients with Endometrial Cancers in *Ex Vivo* Cultures	
35 Endo Combinations	Copanlisib
	Paclitaxel
	Trametinib
	Carboplatin + Paclitaxel
	Carboplatin + Paclitaxel + Cabozantinib
	Carboplatin + Paclitaxel + Lenvatinib
	Carboplatin + Paclitaxel + Ripretinib
	Carboplatin + Paclitaxel + TAK228
	Carboplatin + Paclitaxel + Trametinib
	Carboplatin + Paclitaxel + Lenvatinib + Alpelisib
	Carboplatin + Paclitaxel + Lenvatinib + Ripretinib
	Carboplatin + Paclitaxel + TAK228 + Ripretenib
	Carboplatin + Paclitaxel + TAK228 + Lenvatinib
	Carboplatin + Paclitaxel + Trametinib + Lenvatinib
	Paclitaxel + AZD6482
	Paclitaxel + Buparlisib
	Paclitaxel + Cabozantinib
	Paclitaxel + Copanlisib
	Paclitaxel + Erdafitinib
	Paclitaxel + Everolimus
	Paclitaxel + Lenvatinib
	Paclitaxel + Panobinostat
	Paclitaxel + TAK228
	Paclitaxel + Trametinib
	Paclitaxel + Cabozantinib + Everolimus
	Paclitaxel + Cabozantinib + TAK228
	Paclitaxel + Copanlisib + Panobinostat
	Paclitaxel + Lenvatinib + Trametinib
	Paclitaxel + Panobinostat + Trametinib
	Paclitaxel + TAK228 + Erdafitinib
	Paclitaxel + TAK228 + Lenvatinib
	Paclitaxel + TAK228 + Panobinostat
	Paclitaxel + TAK228 + Tazemetostat
	Paclitaxel + TAK228 + Trametinib
	Paclitaxel + Trametinib + Copanlisib

List of drugs (Single/Combinations) Tested on Resected Tumor Tissue Samples from Patients with Ovarian Cancers in *Ex Vivo* Cultures	
20 Combinations Tested on Ovarian Tumor Tissues	Carboplatin + Paclitaxel
	Carboplatin + Paclitaxel + Everolimus
	Carboplatin + Paclitaxel + Lenvatinib
	Carboplatin + Paclitaxel + Rucaparib + Talazoparib
	Carboplatin + Paclitaxel + Trametinib
	Carboplatin + Paclitaxel + Rucaparib
	Carboplatin + Paclitaxel + Copanlisib
	Carboplatin + Paclitaxel + TAK228
	Carboplatin + Talazoparib + Trametinib
	Carboplatin + Paclitaxel + TAK228 + Talazoparib
	Carboplatin + Paclitaxel + Talazoparib
	Carboplatin + Paclitaxel + Lurbinectedin
	Talazoparib
	Paclitaxel + Talazoparib
	Paclitaxel + Talazoparib + Lenvatinib
	Talazoparib + Lenvatinib
	Lenvatinib + Lurbinectedin
	Paclitaxel + TAK228 + Talazoparib
	Paclitaxel + Lenvatinib
	Paclitaxel + Lurbinectedin

Table S2. The PSE of the list of patients with endometrial cancers whose tumor cells within the tumor samples neither exhibited increased apoptosis (cl-PARP & cl-Caspase3) nor had decreased proliferation (Ki67) following drug treatment in the *Ex Vivo* cultures

Patient ID	Surgery Info.		Pathological Parameters						Treatment Details		Outcome
Ex Vivo: AC-1-Series	DOS	Age at Surgery (Rounded to Years)	Tumor Type - Histology	Grade	Stage	LVI	Myometrial Invasion (%)	MSI Status: Stable (S), Microsatellite instability "High" (H)	Treatment In Clinics: Surgery (S), ChemoT (C), Radiation (R), ImmuneT (I)	Adjuvant Treatment Details	PSE (Mo)
AC-1-45	Oct.'18	66	Invasive endometrioid adenocarcinoma	2	IIIC1	Present	100	NA	S+C	Paclitaxel/Carboplatin	4
AC-1-47	Oct.'18	72	Endometrioid adenocarcinoma	1	IA	Absent	46	NA	S	No adjuvant therapy recorded	4
AC-1-58	Jan.'19	76	Endometrioid adenocarcinoma	1	IB	Absent	64	NA	S+R	Vaginal cuff brachytherapy 1410 cGy in 3fx, vaginal cuff brachytherapy revised 940 cGy in 2fx	9
AC-1-86	Nov.'19	68	Carcinosarcoma	3	IIIC1	Present	72	S	S+C+R	Paclitaxel/Carboplatin ×6 cycles. Whole pelvic radiation, vaginal cuff brachytherapy	10
AC-1-96	Feb.'20	79	Noninvasive endometrioid adenocarcinoma	1	I	Absent	0	NA	S	No adjuvant therapy recorded	31
AC-1-97	Feb.'20	70	Endometrioid adenocarcinoma	1	IA	Cannot be determined	25	NA	S+R	Vaginal cuff HDR brachytherapy, 2100 cGy in 3fx (700 cGy per visit)	5
AC-1-98	Mar.'20	63	Superficially invasive endometrioid adenocarcinoma	1	I	Absent	17	NA	S	No adjuvant therapy recorded	30
AC-1-99	Mar.'20	65	Invasive endometrioid adenocarcinoma	3	IB	Absent	95	NA	S+R	Whole pelvic radiation therapy to 4500 cGy in 25fx, HDR brachytherapy to 1200 cGy in 3fx (400 cGy per visit)	27
AC-1-101	Mar.'20	62	Endometrioid adenocarcinoma	2	I	Absent	11	H	S	No adjuvant therapy recorded	27
AC-1-102	Mar.'20	68	Invasive endometrioid adenocarcinoma	1	IA	Absent	29	NA	S	No adjuvant therapy recorded	27
AC-1-104	Mar.'20	65	Endometrioid adenocarcinoma	1	IA	Present	34	NA	S+R	HDR brachytherapy to 2100 cGy in 3fx (700 cGy per visit)	27
AC-1-106	June'20	74	Endometrioid adenocarcinoma	1	IA	Absent	17	NA	S	No adjuvant therapy recorded	27
AC-1-107	June'20	43	Invasive endometrioid adenocarcinoma	1	IA	Absent	34	NA	S	No adjuvant therapy recorded	26
AC-1-108	June'20	65	Invasive endometrioid adenocarcinoma	1	IA	Absent	13	NA	S	No adjuvant therapy recorded	26
AC-1-109	June'20	66	Invasive endometrioid adenocarcinoma	2	IA	Present	25	NA	S	No adjuvant therapy recorded	27
AC-1-110	July'20	79	Endometrioid adenocarcinoma	1	IA	Absent	6	NA	S	No adjuvant therapy recorded	26

AC-1-111	July'20	77	Invasive endometrioid adenocarcinoma	3	IA	Absent	37	NA	S+R	Vaginal brachytherapy, 5.5 Gy in 4fx (550 cGy per visit)	24
AC-1-113	July'20	74	Endometrioid adenocarcinoma	1	IA	Absent	< 50%	NA	S	No adjuvant therapy recorded	26
AC-1-115	Aug.'20	65	Endometrioid adenocarcinoma	2	IA	Absent	15	NA	S+R	HDR vaginal cuff brachytherapy, 5.5 Gy in 4fx (550 cGy per visit)	24
AC-1-117	Aug.'20	46	Endometrioid adenocarcinoma	2	IA	Absent	8	H	S+C	Carboplatin AUC 6, Paclitaxel 175 mg/m^2	24
AC-1-119	Sept.'20	65	Endometrial carcinoma, NOS	1	IA	Absent	0	NA	S	No adjuvant therapy recorded	23
AC-1-121	Oct.'20	44	Endometrioid adenocarcinoma	1	IA	Absent	22	NA	S	No adjuvant therapy recorded	23
AC-1-124	Oct.'20	68	Endometrial adenocarcinoma	1	IA	Absent	38	NA	S+R	HDR vaginal cuff brachytherapy, 5.5 Gy in 4fx (550 cGy per visit)	20
AC-1-125	Dec.'20	60	Endometrioid adenocarcinoma, noninvasive	1	IA	Absent	0	NA	S	No adjuvant therapy recorded	20
AC-1-126	Dec.'20	62	Endometrioid carcinoma	1	IA	Absent	0	NA	S	No adjuvant therapy recorded	21
AC-1-127	Feb.'21	71	Endometrioid adenocarcinoma	1	IA	Absent	41	NA	S	No adjuvant therapy recorded	19
AC-1-128	Mar.'21	71	Endometrioid adenocarcinoma	1	IA	Absent	23	NA	S	No adjuvant therapy recorded	18
AC-1-130	Mar.'21	84	High-grade mixed adenocarcinoma, clear cell and serous 50% each	3	IVB	Absent	0	NA	S+C	DUO-E clinical trial, Paclitaxel/Carboplatin with Durvalumab vs. placebo	17
AC-1-132	Mar.'21	68	Uterine carcinosarcoma	X	IA	Absent	13	NA	S	No adjuvant therapy recorded	18
AC-1-133	April'21	62	Mixed cell carcinoma, high-grade (10% serous carcinoma, 90% endometrioid carcinoma)	3	IA	Absent	15	NA	S+C	Paclitaxel/Carboplatin	17
AC-1-143	July'21	68	Endometrioid adenocarcinoma	1	IIIC1	Absent	46	NA	S+C	Carboplatin/Paclitaxel 6 cycles	14
AC-1-147	Sept.'21	53	Carcinosarcoma (predominantly endometrioid adenocarcinoma FIGO grade III)	3	IA	Present	48	H	S+C	9/30/2021 - Present Carboplatin/Paclitaxel, calc. end date of 2/2/2022	11

Table S3. The PSE of the list of patients with endometrial cancers whose tumor cells of the tumor tissue samples exhibited increased apoptosis (cl-PARP & cl-Caspase3) and/or had decreased proliferation (Ki67) following drug treatment in the *Ex Vivo* cultures

Patient ID	Surgery Info.		Pathological Parameters						Effective Drug (Single & Combination) tested in *Ex Vivo* culture	Treatment Details		Match/Partial Match of *Ex Vivo* Drug Tests with the Treatment the Patient Received In Clinic	Outcome
Ex Vivo: AC-1-Series	DOS	Age at Surgery (Rounded to Years)	Tumor Type - Histology	Grade	Stage	LVI	Myometrial Invasion (%)	MSI Status: Stable (S), Microsatellite instability "High" (H)		Treatment in Clinics: Surgery (S), Chemo T (C), Radiation (R), Targeted T (T), ImmuneT (I)	Adjuvant Treatment Details (EMR)		PSE (Months)
AC-1-28	May'18	70	Carcinosarcoma	Not applicable	IA	Absent	25	NA	Paclitaxel (Single & Combination)	S+C+R	Paclitaxel/Carboplatin ×6 cycles, Vaginal cuff brachytherapy to 2350 cGy in 5fx	Partial Match with the Treatment	52
AC-1-32	June'18	62	Endometrioid adenocarcinoma	1	IA	Absent	0	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		51
AC-1-33	July'18	55	Endometrioid adenocarcinoma	1	IA	Absent	15	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		50
AC-1-37	Sept.'18	66	Superficially invasive serous carcinoma	3	IIIC1	Absent	8	S	Paclitaxel (Single & Combination)	S+C+R+T+I	Paclitaxel/Carboplatin ×6 cycles, bevacizumab 9/9/20-03/21, pembrolizumab start 4/13/2021. Whole pelvic radiation with vaginal cuff brachytherapy	Partial Match with the Treatment	22
AC-1-39	Sept.'18	67	Endometrioid adenocarcinoma	2	IB	Absent	65	NA	Paclitaxel (Single & Combination)	S+R	Vaginal cuff HDR, 2350 cGy in 5fx	Partial Match with the Treatment	48
AC-1-40	Sept.'18	85	Endometrioid adenocarcinoma	1	IB	Absent	58	S	Paclitaxel (Single & Combination)	S+R	Whole pelvic radiation with vaginal cuff brachytherapy, 2400 cGy in 5fx	Partial Match with the Treatment	11
AC-1-42	Sept.'18	75	Endometrioid adenocarcinoma	2	IB	Present	72	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		35
AC-1-48	Oct.'18	66	Invasive endometrioid adenocarcinoma	2	IA	Absent	22	NA	Paclitaxel	S	No adjuvant therapy recorded		47
AC-1-50	Nov.'18	56	Endometrioid adenocarcinoma	1	IB	Absent	68	NA	Copanlisib	S	No adjuvant therapy recorded		46
AC-1-52	Dec.'18	75	Endometrioid adenocarcinoma	1	IA	Absent	0	NA	Copanlisib	S	No adjuvant therapy recorded		22
AC-1-66	May'19	70	Invasive endometrioid adenocarcinoma	1	IA	Absent	25	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		16

AC-1-75	Aug.'19	49	Endometrioid adenocarcinoma	1	IA	Absent	0	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		35
AC-1-78	Sept.'19	68	High-grade papillary serous carcinoma	3	IIIB	Absent	100	NA	Paclitaxel (Single & Combination)	S+C+R	Paclitaxel/Carboplatin ×6 cycles. External beam radiation therapy to 4500 cGy, 25fx with a pelvic boost to 540 cGy. HDR brachytherapy 1200 cGy 3fx	Partial Match with the Treatment	32
AC-1-79	Sept.'19	56	Endometrioid adenocarcinoma	1	IA	Absent	9	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		25
AC-1-80	Sept.'19	76	Invasive endometrioid adenocarcinoma	1	IA	Absent	14	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		34
AC-1-85	Nov.'19	72	Endometrioid adenocarcinoma	2	II	Present	87	NA	Paclitaxel (Single & Combination)	S+R	External beam radiation therapy 4500 cGy 25fx, vaginal cuff brachytherapy 1200 cGy 3 fx	Partial Match with the Treatment	33
AC-1-87	Nov.'19	52	Endometrioid adenocarcinoma	1	IA	Absent	0	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		33
AC-1-88	Nov.'19	59	Endometrial adenocarcinoma	1	IA	Absent	0	H	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		33
AC-1-89	Nov.'19	63	Carcinosarcoma with high-grade serous carcinoma and rhabdomyoma sarcomatous differentiation	3	IIIC1	Absent	38	S	Paclitaxel (Single & Combination)	S+C+R+I	Paclitaxel/Carboplatin ×6 cycles. Whole pelvic radiation therapy, vaginal cuff brachytherapy. Therapy under DUO-E clinical trial, carboplatin/Paclitaxel with durvalumab vs. placebo	Partial Match with the Treatment	19
AC-1-90	Dec.'19	83	Invasive endometrioid adenocarcinoma (metastatic)	3	IV	Absent	50	S	Paclitaxel (Single & Combination)	S+C	Paclitaxel/Carboplatin ×6 cycles	Partial Match with the Treatment	10
AC-1-92	Jan.'20	77	Invasive endometrioid adenocarcinoma	2	IA	Absent	44	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		11
AC-1-95	Jan.'20	71	Endometrioid adenocarcinoma with squamous cell differentiation	1	IA	Absent	0	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		30

AC-1-103	Mar.'20	56	Endometrioid adenocarcinoma	3	IA	Absent	43	NA	Paclitaxel (Single & Combination)	S+R	Vaginal cuff brachytherapy, 5.5 Gy in 4fx (550 cGy per visit)	Partial Match with the Treatment	27
AC-1-112	July'20	66	Invasive endometrioid adenocarcinoma	1	IA	Absent	35	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		24
AC-1-114	Aug.'20	62	Endometrioid adenocarcinoma	3	IB	Absent	90	NA	Paclitaxel (Single & Combination)	S+C+R	Carboplatin AUC 6, Paclitaxel 175 mg/m^2. External beam radiotherapy to pelvis to 45 Gy in 25fx	Partial Match with the Treatment	24
AC-1-116	Aug.'20	65	Invasive endometrioid adenocarcinoma	2	IA	Absent	32	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		24
AC-1-118	Sept.'20	56	High-grade serous adenocarcinoma	3	IIIC2	Present	46	NA	Paclitaxel (Single & Combination)	S+C+R	Carboplatin/Paclitaxel ×6 cycles. Whole pelvic radiation to 4500 cGy	Partial Match with the Treatment	23
AC-1-120	Sept.'20	46	Invasive endometrioid adenocarcinoma	2	IIIC1	Present	57	NA	Paclitaxel (Single & Combination)	S+C	Portec 3 trial. Cisplatin + XRT followed by Carboplatin/Paclitaxel ×4 cycles	Partial Match with the Treatment	23
AC-1-122	Oct.'20	68	Endometrioid adenocarcinoma	1	IA	Absent	19	H	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		22
AC-1-123	Nov.'20	79	Invasive endometrioid adenocarcinoma	1	IA	Absent	30	NA	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		22
AC-1-129	Mar.'21	67	Invasive high-grade serous adenocarcinoma	3	IIIC2	Present	87	NA	Paclitaxel (Single & Combination)	S+C+R+T	Carboplatin, paclitaxel, Trastuzumab ×4 cycles. External beam radiation + Vaginal cuff HDR brachytherapy	Partial Match with the Treatment	17
AC-1-131	Mar.'21	59	High-grade serous adenocarcinoma	3	IVB	Present	0	NA	Paclitaxel (Single & Combination)	S+C+I	4/23/2021 - 8/5/2021 DUO-E clinical trial; Carboplatin/Paclitaxel with Durvalumab vs. placebo	Partial Match with the Treatment	17
AC-1-134	April'21	75	Endometrioid adenocarcinoma	2	IA	Absent	6	NA	Paclitaxel (Single & Combination)	S+R	6/8/2021 - 6/18/2021 4fx HDR vaginal cuff brachytherapy, 2200 cGy	Partial Match with the Treatment	8
AC-1-135	May'21	60	Mixed cell carcinoma, high-grade (90% high-grade serous carcinoma, 10% endometrioid adenocarcinoma)	3	IA	Absent	38	NA	Paclitaxel (Single & Combination)	S+C+R	6/17/2021 - 10/20/2021 Carboplatin/Paclitaxel ×6 cycles. Vaginal cuff HDR brachytherapy	Partial Match with the Treatment	15

AC-1-138	July'21	70	Endometrioid carcinoma	1	IB	Absent	64	H	Paclitaxel (Single & Combination)	S+R	8/20/2021 - 9/3/2021 vaginal cuff brachytherapy 2350 cGy 5fx	Partial Match with the Treatment	12
AC-1-140	Aug.'21	71	Endometrioid carcinoma arising in a background of complex atypical hyperplasia	1	IA	Absent	35	H	Paclitaxel (Single & Combination)	S	No adjuvant therapy recorded		12
AC-1-142	Aug.'21	73	Endometrioid adenocarcinoma	2	IA	Absent	10	NA	Paclitaxel (Single & Combination)	S+R	9/27/2021 - 10/8/2021 vaginal cuff HDR brachytherapy 2200 cGy 4fx	Partial Match with the Treatment	12
AC-1-145	Aug.'21	74	Endometrioid adenocarcinoma	2	I	Absent	25	NA	Paclitaxel (Single & Combination)	S+R	HDR Brachytherapy, 4fx	Partial Match with the Treatment	11

Table S4. The PSE of the list of patients with ovarian cancers whose tumor cells within the tumor tissue samples neither exhibited increased apoptosis (cl-PARP & cl-Caspase3) nor had decreased proliferation (Ki67) following drug treatment in the *Ex Vivo* cultures

Patient ID	Surgery Info.		Pathological Parameters					Treatment Details		Outcome
Ex Vivo Series	DOS	Age at Surgery (Rounded to years)	Tumor Type - Histology	Grade	Stage	LVI	MSI	Treatment: Surgery (S), ChemoT (C), Radiation (R), ImmuneT, (I), TargtedT (T)	Treatment Details	EFS (Months)
A26	May'18	62	High-grade serous carcinoma, right and left ovarian	3	IIIC	Present	S	S+C+T	Carboplatin + Paclitaxel neoadjuvant. Carboplatin, Paclitaxel, and bevacizumab adjuvantly. Mekinist and letrozole upon recurrence	16
A30	June'18	59	Poorly differentiated carcinoma of sex cord derivation arising out of adult granulosa cell tumor - right ovary	3	IIIA1	Present	S	S+C	Paclitaxel/Carboplatin. Bleomycin, Etoposide, Cisplatin	2
A137	July'21	58	Omentum: Low-grade serous carcinoma with abundant psammoma bodies	1	IIIA2	NA	S	S+T	NRG-GY019 trial. Oral letrozole	13
A141	Aug.'21	44	Ovarian mucinous cystadenoma	X	X	Absent	NA	S	No adjuvant therapy recorded	12
A144	Aug.'21	64	Low-grade serous borderline tumor with psammoma bodies	1	IIIA	NA	NA	S+C	NRG-GY019 trial, study arm #1. Carboplatin/Paclitaxel	12
A149	Dec.'21	82	Low-grade serous carcinoma	1	IB	Absent		S	No adjuvant therapy recorded	8
A150	Dec.'21	19	Mucinous borderline tumor		1A	NA		S	No adjuvant therapy recorded	8

Table S5. The PSE of the list of patients with ovarian cancers whose tumor cells of the tumor tissue samples exhibited increased apoptosis (cl-PARP & cl-Caspase3) and/or had decreased proliferation (Ki67) following drug treatment in the *Ex Vivo* cultures

Patient ID	Pathological Parameters							Effective Drug (Single & Combination) tested in *Ex Vivo* culture	Treatment Details		Match/Partial Match of *Ex Vivo* Drug Tests with the Treatment In Clinics	Outcome
Ex Vivo Series	DOS	Age at Surgery (Rounded to years)	Tumor Type - Histology	Grade	Stage	LVI	MSI		Treatment: Surgery (S), ChemoT (C), Radiation (R), ImmuneT, (I), TargtedT (T)	Treatment Details		EFS (Months)
A35	Aug.'18	42	Mucinous borderline tumor/atypical proliferative mucinous tumor - Left ovary	1	IA	Absent	NA	Carboplatin + Paclitaxel + Copanlisib/ TAK228/Lenvatinib	S	No adjuvant therapy recorded	?	48
A49	Oct.'18	59	Mucinous borderline tumor with intraepithelial carcinoma	1	IA	?	NA	Carboplatin + Paclitaxel	S	No adjuvant therapy recorded	?	46
AB91	Jan.'20	62	Adenocarcinoma consistent with a history of ovarian carcinoma	X	IIIC/IV	NA	S	Carboplatin + BMN673 + Trametinib	S+C+T+I	July-Oct 2007, seven cycles intraperitoneal Cisplatinum and intravenous and intraperitoneal Paclitaxel. January 2010, treated with unknown agents. 1/22/2015-4/7/2015 Carboplatin AUC5, Paclitaxel 80 mg/m^2, and veliparib 3 cycles. 1/14/2016 started Anastrozole. 2/11/2016-6/21/2016 Carboplatin AUC5, olaparib 200 mg twice daily, 4 cycles. 6/21/2016 single agent olaparib 200 mg twice/day, stabilized at 300 twice/day. 12/26/2017 started rucaparib 600 mg twice/day. Aug 2019 decreased Rubraca to 250 mg twice/day. March-April 2020: Mekinist and niraparib, patient could not tolerate, 04/28/2020-06/9/2020: Pembrolizumab 3 cycles every 6 weeks at 400 mg, 07/21/2020: started Avastin and oral Cyclophosphamide	Partial Match	6
A94	Feb.'20	58	Serous carcinoma	1	IIIC	Present	CBD	Carboplatin + Paclitaxel + Trametinib	S+C+T	10/8/2019 Carboplatin/Paclitaxel/ Bevacizumab ×4 cycles. 3/10/2020 started Carboplatin/Paclitaxel for the second time, 3 cycles. 5/20/2020 started letrozole 2.5 mg daily	Partial Match	23

A136	May'21	52	Adult granulosa cell tumor, 13.5 cm, confined to the right ovary	X	IA	Absent	NA	Carboplatin + Paclitaxel + Talazoparib/ TAK228/Lenvatinib/Lubrinectidine	S	No adjuvant therapy recorded	?	15
A139	July'21	62	High-grade serous carcinoma (FIGO grade III) with capsular disruption. Fallopian tube involvement by high-grade serous carcinoma	3	IIB	Absent	NA	Carboplatin + Paclitaxel + Talazoparib/ TAK228/Lenvatinib/Lubrinectidine	S+C	Carboplatin + Paclitaxel	Partial Match	13
A148	Sept.'21	78	Low-grade appendiceal mucinous neoplasm	1	IVA	Absent	NA	Paclitaxel + Talazoparib/Lenvatinib/ Lubrinectidine	S	No adjuvant therapy recorded	?	11