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Patient-Derived Tumor Organoids Can Predict the Progression-Free Survival of Patients With Stage IV Colorectal Cancer After Surgery

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BACKGROUND: Recent studies have shown patient-derived tumor organoids can predict the drug response of patients with cancer. However, the prognostic value of patient-derived tumor organoid–based drug tests in predicting the progression-free survival of patients with stage IV colorectal cancer after surgery remains unknown.

OBJECTIVE: This study aimed to explore the prognostic value of patient-derived tumor organoid–based drug tests in patients with stage IV colorectal cancer after surgery.

DESIGN: Retrospective cohort study.

SETTINGS: Surgical samples were obtained from patients with stage IV colorectal cancer at the Nanfang Hospital.

PATIENTS: A total of 108 patients who underwent surgery with successful patient-derived tumor organoid culture and drug testing were recruited between June 2018 and June 2019.

INTERVENTIONS: Patient-derived tumor organoid culture and chemotherapeutic drug testing.

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MAIN OUTCOMES MEASURES: Progression-free survival.

RESULTS: According to the patient-derived tumor organoid-based drug test, 38 patients were drug sensitive and 76 patients were drug resistant. The median progression-free survival was 16.0 months in the drug-sensitive group and 9.0 months in the drug resistant group ($p < 0.001$). Multivariate analyses showed that drug resistance (HR, 3.38; 95% CI, 1.84–6.21; $p < 0.001$), right-sided colon (HR, 3.50; 95% CI, 1.71–7.15; $p < 0.001$), mucinous adenocarcinoma (HR, 2.47; 95% CI, 1.34–4.55; $p = 0.004$), and non-R0 resection (HR, 2.70; 95% CI, 1.61–4.54; $p < 0.001$) were independent predictors of progression-free survival. The new patient-derived tumor organoid–based drug test model, which includes the patient-derived tumor organoid–based drug test, primary tumor location, histological type, and R0 resection, was more accurate than the traditional clinicopathological model in predicting progression-free survival ($p = 0.001$).

LIMITATIONS: A single-center cohort study.

CONCLUSIONS: Patient-derived tumor organoids can predict progression-free survival in patients with stage IV colorectal cancer after surgery. Patient-derived tumor organoid drug resistance is associated with shorter progression-free survival, and the addition of patient-derived tumor organoid drug tests to existing clinicopathological models improves the ability to predict progression-free survival.

KEY WORDS: Drug tests; Patient-derived tumor organoid; Prognostic value; Progression-free survival; Predictive model; Stage IV colorectal cancer.

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related death globally, with an estimated 1.9 million new cases and an estimated 0.94 million deaths worldwide in 2020.[1] The prognosis of CRC is heterogeneous, with 20% to 25% of patients presenting with metastases at diagnosis and 50% to 60% of the remaining patients eventually developing disseminating disease.[2] Postoperative chemotherapy improves the outcome of patients with CRC after surgery. Standard first-line treatments include capecitabine plus oxaliplatin (XELOX) and fluorouracil with leucovorin and irinotecan (FOLFIRI) or oxaliplatin (FOLFOX).[3–5] However, the recurrence rate after surgery for patients with stage IV CRC is high, with a 1-year progression-free survival (PFS) rate of 42.2% to 50.0%.[6,7] Therefore, there is a pressing need for a reliable model or biomarker that enables better prediction of PFS in patients with stage IV CRC after surgery. Currently, the TNM staging system and some clinicopathological factors, such as histological type, tumor location, lymphovascular invasion, and carcinoembryonic antigen level, are used to predict prognosis, but their accuracy is limited.[8,9]

Commonly used preclinical models, such as cancer cell lines or patient-derived xenografts (PDXs), have limited success in predicting the response to chemotherapy because of the poor representation of features of the original tumors, the high economic cost, or the long turnaround time.[10–12] Patient-derived tumor organoids (PDTOs) are cultures of tumor cells that can be derived from individual patients, and they are much cheaper and have a shorter propagation time than PDXs while preserving intratumoral heterogeneity.[12,13] PDTOs provide a preclinical platform for drug testing and have the potential to predict treatment response.[13] Recent studies have shown that PDTO faithfully predicts the drug response of patients with different kinds of cancers,[14–17] including CRC.[18–20] However, the prognostic value of PDTO-based drug tests in predicting PFS remains unknown. Therefore, we designed a cohort study to investigate the prognostic value of PDTO-based drug tests in predicting PFS in patients with stage IV CRC after surgery.

MATERIALS AND METHODS

Study Design

This study was a cohort study designed to investigate whether PDTO-based drug tests can predict the PFS of patients with stage IV CRC after surgery. In our previous study,[21] we generated PDTOs from 30 samples from patients with stage IV CRC who received chemotherapy, which enabled us to correlate drug sensitivity in PDTOs with chemosensitivity in the clinic. Then, we constructed a receiver operating characteristic (ROC) curve analysis to determine the best cutoff value for the IC_{50} of the PDTO drug test for predicting patient responses. The results showed that 10 μmol/L was the threshold of sensitivity for the PDTO drug test: ≤10 μmol/L was determined to be sensitive and >10 μmol/L was defined as resistant. According to the PDTO-based drug test, patients were divided into 2 groups: the drug-sensitive PDTO group and the drug-resistant PDTO group. In this study, surgical samples were collected from patients with CRC who underwent PDTO culture. PDTOs were tested with first-line chemotherapy regimens, including XLEOX/FOLFOX, FOLFIRI, and FOLFOXIRI. Four weeks after surgery, patients received designated first-line chemotherapy regimens, such as FOLFOX or FOLFIRI, under the guidance of National Comprehensive Cancer Network guidelines. Oncologists did not know the results of PDTOs test. This study was an observational study rather than an interventional study. We followed up with patients and analyzed the correlations between PDTO results and patient PFS. In short, the PDTO test included all first-line chemotherapy regimens, such as FOLFOX, FOLFIRI, and FOLFOXIRI. We observed and analyzed the outcome

according to the chemotherapy regimen that the patient actually received, regardless of whether the PDTO test was sensitive or resistant.

This study was approved by the Institutional Review Board at the Nanfang Hospital of Southern Medical University. All procedures performed in the study involving human participants were in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

Inclusion and Exclusion Criteria

Patients who were treated at the Nanfang Hospital of Southern Medical University from June 2018 to June 2019 were recruited for this study. The inclusion criteria were as follows: 1) patients aged between 18 and 80 years; 2) patients with histologically confirmed CRC; 3) patients with ASA scores of 3 or less; and 4) patients with stage IV CRC. The exclusion criteria were as follows: 1) patients refusing chemotherapy; 2) patients with chemotherapy contraindications; 3) patients with other cancers; and 4) pregnant or breastfeeding women.

Sample Processing, Organoid Culture, and Drug Test

Samples were obtained from patients who underwent surgery. These samples included primary tumors, liver metastases, lung metastases, and peritoneal metastases. Once collected by surgery, samples were collected in RPMI 1690 (Basalmedia) supplemented with 5% penicillin–streptomycin (Biosharp) and stored for a maximum of 10 hours at 4°C before being minced with scissors. Then, organoids were generated according to a previously described protocol with modifications.[22,23] Briefly, the tumor tissue was washed in Hank's balanced salt solution (STEMCELL Technologies) containing antibiotics, minced into tiny pieces, and digested with 5 mL DMEM/F12 containing collagenase type II (Invitrogen) on a shaker for approximately 4 hours at 37°C. To eliminate erythrocytes, the digested tissue suspension was incubated with lysis buffer (Invitrogen) and centrifuged at 300*g* for 5 minutes. Tumor cells were collected, washed, counted, and resuspended in Matrigel basement membrane matrix (Corning), which was dispended as 30-μL drops into prewarmed 60-mm culture plates and allowed to solidify for 30 minutes at 37°C. The cells were then overlaid with organoid culture medium and incubated at 37°C and 5% CO_2. Organoid culture medium was refreshed every 4 days. Organoids were cultured in advanced DMEM/F12 (Thermo Fisher Scientific) containing *N*-acetylcysteine (Sigma), EGF (PeproTech), FGF-10 (PeproTech), FGF-basic (PeproTech), Y-27632 (Selleck), A-83-01 (Sigma), SB202190 (Selleck), nicotinamide (Sigma), Noggin (homemade), R-spondin (homemade), Wnt3a (homemade), HEPES (Sigma), GlutaMAX (Gibco), B27 (Gibco), and N2 (Gibco). PDTOs were periodically checked for mycoplasma contamination using the MycoAlert Mycoplasma Detection Assay (Lonza) and authenticated by short tandem repeat profiling using a multi-amplification kit (PowerPlex 21 System, Promega). A PDTO video is at https://links.lww.com/DCR/C183.

PDTO-based drug tests were performed as previously described.[17,18] All drug tests were performed 3×, with each test including 2 technical replicates. In short, PDTOs were exposed to drugs in a 6-point 5-fold dilution series of concentrations, ranging from 50 to 0.016 μmol/L. All drug tests were performed in technical triplicate. Cell viability was evaluated using CellTiter-Glo 3D (Promega) according to the manufacturer's instructions every 3 days. The data were analyzed using GraphPad Prism 7.0, and the half-maximal inhibitory concentration (IC50) values were calculated by the dose–response curve produced using nonlinear regression (curve fit). We tested CRC PDTOs with combinations of fluorouracil plus oxaliplatin and fluorouracil plus irinotecan at a ratio of 1:1, representing the chemotherapy regimens of XELOX/FOLFOX and FOLFIRI, respectively.

Treatment and Follow-up

Patients with stage IV CRC received multidisciplinary team (MDT) consultations after hospitalization. If primary and metastatic lesions were determined to be suitable for R0 resection after the MDT consultation, patients received simultaneous resection. Asymptomatic patients with potentially resectable metastases received neoadjuvant chemotherapy and subsequent simultaneous resection. Patients with symptomatic primary tumors (obstruction, perforation, abscess, etc) and synchronous metastases received primary lesion resection. For patients with metachronous metastases, if metastatic lesions were suitable for R0 resection after the MDT consultation, the patients underwent metastasectomy. For obstructive left-sided colon cancer that was not suitable for primary anastomosis after assessment, patients received diversion colostomy. Patients with low rectal cancer who were assessed as having a high risk of anastomotic leakage underwent diversion ileostomy.

All patients received adjuvant chemotherapy, including XELOX, FOLFOX, and FOLFIRI. The XELOX regimen consisted of a 2-hour intravenous infusion of oxaliplatin 130 mg/m^2 on day 1 plus oral capecitabine 1000 mg/m^2 2× daily for 2 weeks as a 3-week cycle. The first dose of capecitabine was given in the evening on day 1, and the last dose was given in the morning on day 15. The mFOLFOX6 regimen was administered as 85 mg/m^2 oxaliplatin over 120 minutes and 400 mg/m^2 leucovorin over 2 hours followed by a 400 mg/m^2 bolus of fluorouracil and then a 2400 mg/m^2 bolus of fluorouracil by a 46- to 48-hour infusion repeated every 2 weeks. The FOLFIRI regimen was administered as 180 mg/m^2 irinotecan over 90 minutes and 400 mg/m^2 leucovorin over 2 hours followed by a 400 mg/m^2 bolus of fluorouracil and then a

2400 mg/m^2 bolus of fluorouracil by a 48-hour infusion repeated every 2 weeks.

The follow-up duration was measured from the time of administration of chemotherapy to the last follow-up date. Blood CEA levels were measured and CT scans obtained 1 month after the surgery as the baseline data. CT (or MR or PET) was performed every 3 months in the first 2 years, and blood CEA levels were measured every month in the first 6 months and every 3 months after adjuvant chemotherapy according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (RECIST 1.1) through CT/MR/PET imaging.[24] The RECIST 1.1 outcome options were as follows: complete response, partial response, progressive disease (PD), or stable disease.[24] The progression of disease was evaluated and diagnosed through periodically performed CT (MR or PET) imaging and blood CEA levels. For R0 and R1 patients, local recurrence and new distant metastases detected by CT (MR or PET) imaging or elevated blood CEA levels were diagnosed as PD. For R2 patients, the evaluation of disease progression was performed according to the RECIST version 1.1 (RECIST 1.1) through CT (MR or PET) imaging. Specifically, PDs were defined as at least a 20% increase in the sum of diameters of target lesions compared with the smallest sum on the study (including the baseline data). In addition, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of 1 or more new lesions was also considered progression.

Statistical Analyses

The Kaplan-Meier method was used to estimate PFS for patients with different drug test results, which was compared using the log-rank test. Univariate and multivariate Cox regression analyses were performed to identify the independent predictors for PFS. The area under the curves (AUCs) of the time-dependent ROC curves of different models were calculated to compare the prognostic value of these models. Statistical analyses were performed using SPSS 24.0 software (SPSS, Inc., Chicago, IL) and R Studio version 4.0.3 (R Project for Statistical Computing, Vienna, Austria, www.r-project.org). Differences with a 2-sided $p < 0.05$ were considered statistically significant.

RESULTS

Patient Demographics and Tumor Characteristics

In total, 108 patients were enrolled in this study. According to the PDTO-based drug test, 32 patients were included in the drug-sensitive PDTO group and 76 patients were included in the drug-resistant PDTO group. The median follow-up time for the patient cohort was 18 months (interquartile range, 15–20 mo). The patient demographics and tumor characteristics are shown in Table 1. The flowchart of this study is shown in Figure 1.

PDTO Culture

We successfully cultured 108 PDTOs from 136 surgical samples with an overall success rate of 79.41%, which was similar to other studies.[25,26] Organoids derived from these patients demonstrated a great diversity in morphology under brightfield microscopy, such as tubular structure, cystic structure, and solid structure. Subsequent hematoxylin and eosin staining of the organoids showed that the organoids we cultured retained the histological features of their original tumors (Fig. 2). Short tandem repeat testing results also confirmed that the organoids we cultured were highly consistent with the original tumor. Mycoplasma detection was performed to ensure that organoids cultured for drug testing were free from mycoplasma contamination.

Prognostic Value of the PDTO-Based Drug Test

Kaplan-Meier curve analysis was performed to explore the prognostic value of the PDTO-based drug test. The results showed that patients in the drug-sensitive PDTO group had a significantly longer PFS than those in the drug-resistant PDTO group ($p < 0.001$) (Fig. 3). The median PFS was 16.0 months in the drug-sensitive PDTO group and 9.0 months in the drug-resistant PDTO group. Then, we compared PFS for the different chemotherapeutic regimens in this study. We found that there was no significant difference in PFS between patients who received FOLFOX or FOLFIRI (Fig. 4).

Construction of a Predictive Nomogram

The univariate and multivariate Cox regression analyses of the associations of the drug test and clinicopathological characteristics in the whole cohort with PFS are presented in Table 2. Univariate analyses demonstrated that right-sided colon cancer, mucinous adenocarcinoma, poor differentiation, oligometastatic diseases, non-R0 resection, and resistance detected by the PDTO-based drug test were risk factors for poor PFS. Given the high correlation between histological type and tumor differentiation (Kendall $r = 0.78$, $p < 0.001$) and that the HR of histological type was higher than that of tumor differentiation, histological type was subjected to subsequent multivariate Cox regression analysis. In the multivariate analyses, resistance detected by the PDTO-based test (HR, 3.38; 95% CI, 1.84–6.21; $p < 0.001$), right-sided colon (HR, 3.50; 95% CI, 1.71–7.15; $p < 0.001$), mucinous adenocarcinoma (HR, 2.47; 95% CI, 1.34–4.55; $p = 0.004$), and non-R0 resection (HR, 2.70; 95% CI, 1.61–4.54; $p < 0.001$) were shown to be independent risk factors for PFS. The nomogram for the prediction of PFS was generated on the basis of the PDTO-based drug test (resistant and sensitive), primary tumor location (right-sided colon and left-sided colorectum), histological type (mucinous adenocarcinoma and adenocarcinoma), and

TABLE 1. Patient demographics and tumor characteristics

Characteristics	*Drug-sensitive PDTO group (N = 32)*	*Drug-resistant PDTO group (N = 7 6)*	*p value*
Age, y, median (IQR)	59.5 (51.0–62.0)	54.0 (43.0–62.0)	0.419
Sex, n (%)			0.651
Male	25 (78.1)	52 (68.4)	
Female	7 (21.9)	24 (31.6)	
BMI, median (IQR)	23.9 (22.6–25.2)	24.1 (22.1–25.9)	0.777
Primary tumor location, n (%)			0.141
Left-sided colorectum	30 (93.8)	62 (81.6)	
Right-sided colorectum	2 (6.25)	14 (18.4)	
Histological type, n (%)			0.114
Adenocarcinoma	28 (87.5)	54 (71.1)	
Mucinous adenocarcinoma	4 (12.5)	22 (28.9)	
Tumor differentiation, n (%)			0.124
Well or moderate	25 (78.1)	46 (60.5)	
Poor	7 (21.9)	30 (39.5)	
Oligometastatic diseases,[a] n (%)			0.179
Yes	27 (84.4)	53 (69.7)	
No	5 (15.6)	23 (30.3)	
Metastases, n (%)			0.154
Synchronous	24 (75.0)	67 (88.2)	
Metachronous	8 (25.0)	9 (11.8)	
Location of distant diseases, n (%)			0.342
Liver	19 (59.4)	37 (48.7)	
Lung	8 (25.0)	13 (17.1)	
Peritoneal	2 (6.3)	11 (14.5)	
Multiple metastases	1 (3.1)	10 (13.2)	
Other	2 (6.2)	5 (6.5)	
R0 resection, n (%)			0.084
R0 resection	20 (60.3)	32 (42.1)	
Simultaneous resection	12 (37.5)	23 (30.3)	
Metastatectomy	8 (25.0)	9 (11.8)	
Non-R0 resection	12 (37.5)	44 (57.9)	
Primary lesion resection only	12 (37.5)	44 (57.9)	
Neoadjuvant chemotherapy, n (%)			0.144
No	23 (71.8)	44 (57.9)	
Yes	9 (28.2)	32 (42.1)	
Lymphovascular invasion, n (%)			0.772
No	17 (53.1)	41 (52.6)	
Yes	15 (46.9)	35 (47.4)	
Preoperative CEA, n (%)			0.068
Normal	22 (68.8)	35 (46.1)	
Elevated	10 (31.2)	41 (53.9)	

IQR = interquartile range.
[a]Oligometastatic disease is defined as the existence of metastases at up to 3 sites and 5 lesions.

R0 resection (no and yes) (Fig. 5). The nomogram indicates the risk of progression in patients with stage IV CRC. For clinical use, the PDTO drug test is determined by drawing a line straight up to the point axis to establish the score associated with the PDTO drug test. Then, this process was repeated for the other 3 variables (histological type, R0 resection, and primary tumor location). The score of each variable is added, and the total score is located on the total score points axis. Finally, a line is drawn straight down to the 12-month PFS probability axis to obtain the PFS probability.

For example, when patient A presented with left-sided colon adenocarcinoma and non-R0 resection and the PDTO-based drug test showed sensitivity, the points for each factor were 55, 0, 52, and 100 according to the nomogram. The total score for patient A was 207, and the probability of 12-month PFS was approximately 60%.

The new PDTO-based drug test model showed good discrimination with an AUC of 0.901 (95% CI, 0.844–0.959) (Fig. 6). The C-index of the nomogram was 0.777 (95% CI, 0.731–0.823).

Comparison Between the New PDTO-Based Drug Test Prediction Model and the Traditional Clinicopathological Prediction Model

The new PDTO-based drug test prediction model, which combines the PDTO-based drug test, primary tumor location, histological type, and R0 resection, was compared

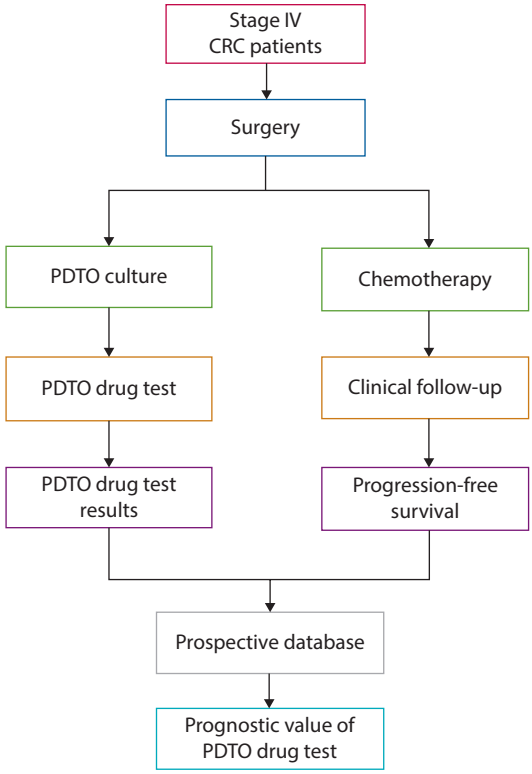

FIGURE 1. Flowchart of this study. CRC = colorectal cancer; PDTO = patient-derived tumor organoid.

with the traditional clinicopathological prediction model that includes only primary tumor location, histological type, and R0 resection. The ROC curves of the new PDTO-based drug test prediction model and the traditional clinicopathological prediction model were generated for disease status at 12 months of follow-up evaluation (Fig. 6). The AUC value of the new PDTO-based drug test prediction model was 0.901 (95% CI, 0.844–0.959), of the traditional clinicopathological prediction model was 0.828 (95% CI, 0.755–0.901), of the drug test alone was 0.731 (95% CI, 0.637–0.825), of the primary tumor location alone was 0.595 (95% CI, 0.546–0.644), of the histological type alone was 0.692 (95% CI, 0.631–0.753), and of R0 resection alone was 0.729 (95% CI, 0.634–0.823). The AUC value of the new PDTO drug test prediction model was higher than that of the clinicopathological model ($p = 0.001$), the drug test alone ($p < 0.001$), the primary tumor location ($p < 0.001$), the histological type alone ($p < 0.001$), and R0 resection alone ($p < 0.001$).

DISCUSSION

Patients with stage IV CRC exhibit large variations in prognosis after surgery.[9] Identifying patients at a high risk of recurrence remains a clinical dilemma. Although multiple clinicopathological factors are used and can be combined

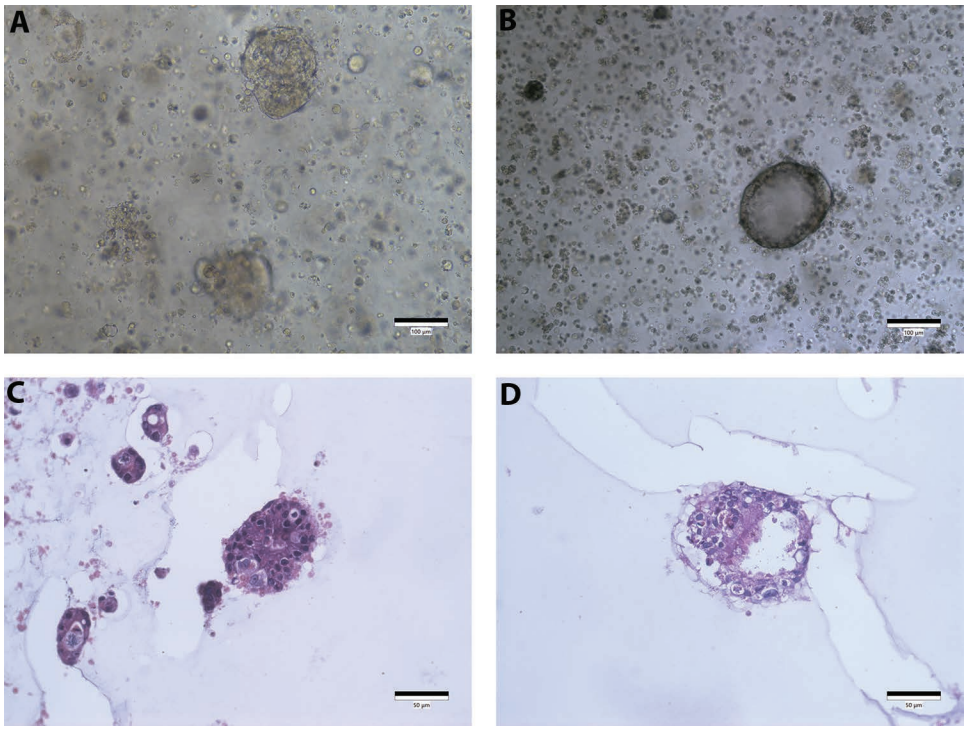

FIGURE 2. Organoids derived from patients with different pathological types. A, Brightfield image and hematoxylin and, B, eosin staining of organoids derived from adenocarcinoma. C, Brightfield image and, D, hematoxylin and eosin staining of organoids derived from mucinous adenocarcinoma.

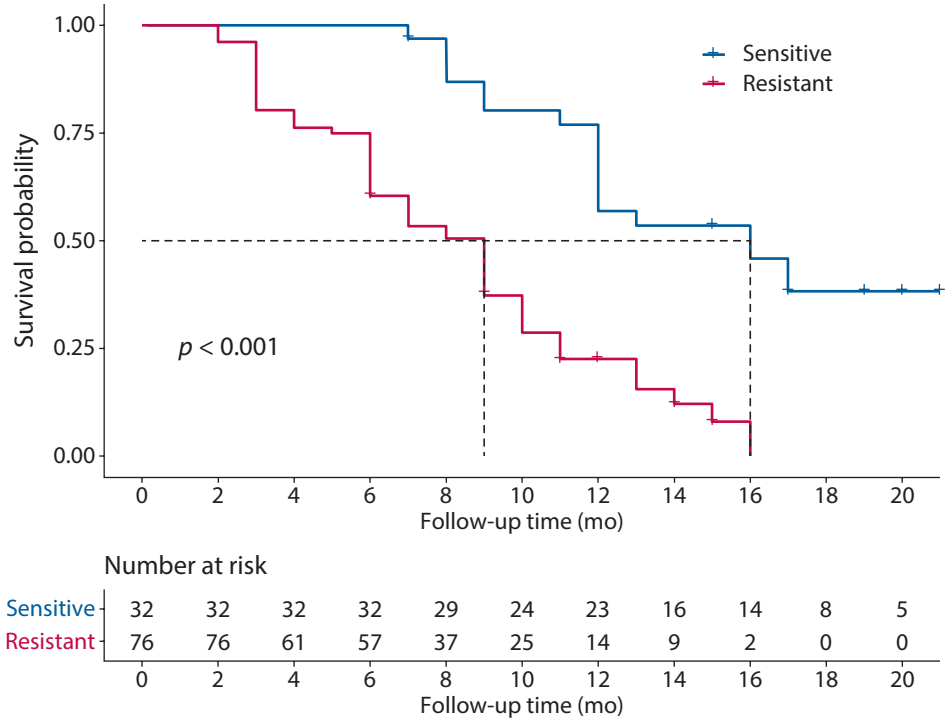

FIGURE 3. Comparison of PFS between the drug-sensitive PDTO group and drug-resistant PDTO group. Kaplan-Meier graph of the PFS of the drug-sensitive PDTO group and drug-resistant PDTO group showed that the median PFS was 16.0 months in the drug-sensitive PDTO group and 9.0 months in the drug-resistant PDTO group. Patients in the drug-sensitive PDTO group showed a significantly longer PFS than those in the drug-resistant PDTO group ($p < 0.001$). PDTO = patient-derived tumor organoid; PFS = progression-free survival.

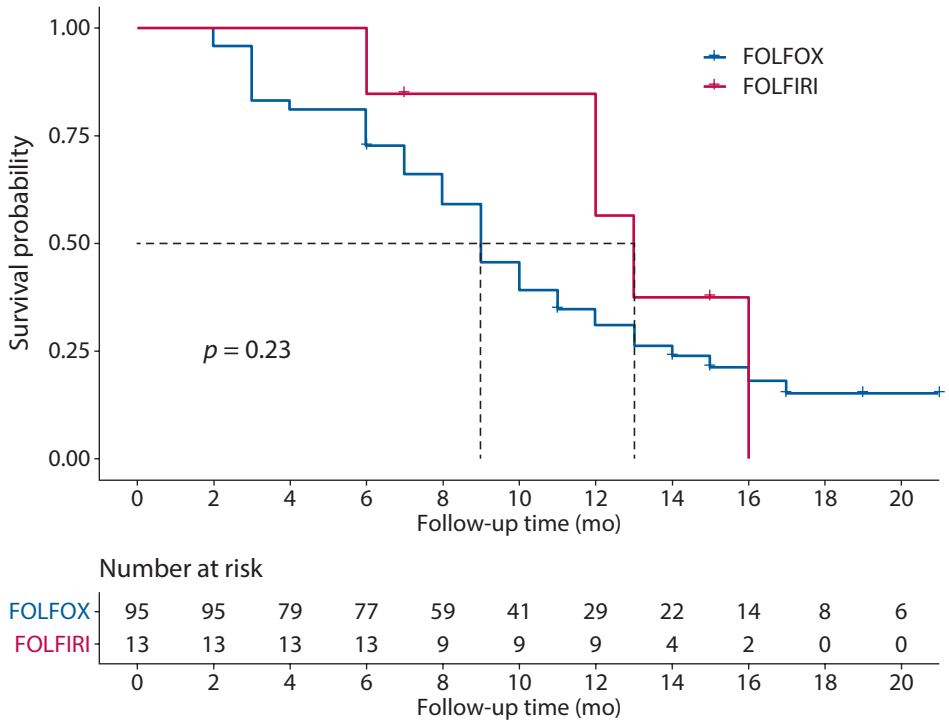

FIGURE 4. Comparison of progression-free survival between patients who received FOLFOX or FOLFIRI. The Kaplan-Meier graph of PFS showed that there was no significant difference in PFS between patients who received FOLFOX or FOLFIRI ($p = 0.23$). FOLFIRI = fluorouracil with leucovorin and irinotecan; FOLFOX = fluorouracil with oxaliplatin; PFS = progression-free survival.

TABLE 2. Univariate and multivariate Cox regression of PFS

	Univariate		*Multivariate*	
Variables	*HR (95% CI)*	*p value*	*HR (95% CI)*	*p value*
PDTO drug test				
Sensitive	Reference		Reference	
Resistant	4.35 (2.43–7.79)	**<0.001**	3.38 (1.84–6.21)	**<0.001**
Age (y)				
≤65	Reference	0.09		
>65	0.99 (0.97–1.00)			
Sex				
Male	Reference	0.70		
Female	0.91 (0.57–1.46)			
BMI (kg/m^2)	1.02 (0.95–1.10)	0.59		
Primary tumor location				
Left-sided colorectum	Reference		Reference	
Right-sided colon	2.60 (1.37–4.93)	**0.004**	3.50 (1.71–7.15)	**<0.001**
Histological type				
Adenocarcinoma	Reference	**<0.001**	Reference	**0.004**
Mucinous adenocarcinoma	5.02 (2.88–8.76)		2.47 (1.34–4.55)	
Tumor differentiation				
Well or moderate	Reference			
Poor	4.24 (3.69–4.79)	**<0.001**		
Oligometastatic diseases[a]				
No	Reference	**0.012**	Reference	0.59
Yes	1.87 (1.14–3.05)		1.16 (0.68–1.97)	
Metastases				
Synchronous	Reference			
Metachronous	0.86 (0.49–1.51)	0.611		
Location of distant disease				
Liver	Reference	0.27		
Lung	0.67 (0.36–1.24)	0.20		
Peritoneal	1.61 (0.83–3.11)	0.16		
Multiple metastases	2.14 (0.99–4.58)	0.06		
Other	0.93 (0.37–2.36)	0.89		
R0 resection				
Yes	Reference	**<0.001**	Reference	**<0.001**
No	3.08 (1.96–4.84)		2.70 (1.61–4.54)	
Neoadjuvant chemotherapy				
No	Reference	**0.045**	Reference	0.13
Yes	1.5 (1.01–2.45)		1.51 (0.89–2.55)	
Lymphovascular invasion				
No	Reference	0.49		
Yes	1.16 (0.76–1.79)			
CEA (ng/mL)				
Normal	Reference	0.21		
Elevated	1.31 (0.85–2.02)			

Boldface indicates statistical significance.
PDTO = patient-derived tumor organoid; PFS = progression-free survival.
[a]Oligometastatic disease is defined as the existence of metastases at up to 3 sites and 5 lesions.

to predict PFS in patients with CRC, their accuracy is limited.[8,9] Thus, a robust prediction model is critically needed. In this study, we found that the PDTO drug test was an independent prognostic factor for PFS in patients with stage IV CRC ($p < 0.001$). Then, we generated a new prediction model combining the PDTO drug test with other clinicopathological factors, which performed better in predicting PFS than the clinicopathological model.

PDTOs hold great promise for precision cancer medicine because of their great advantages over cancer cell lines and PDXs: they can be cultured with a high success rate, can faithfully recapitulate the biological characteristics of the original tumors, are amenable to high-throughput drug screening, and are cost-effective.[12] PDTO culture models have been established from various cancer types, including CRC,[19] gastric cancer,[27] liver cancer,[28] lung cancer,[29] prostate cancer,[30] bladder cancer,[31] and ovarian cancer.[32] Some studies have shown that PDTO can mirror the clinical responses of individual patients to chemotherapy.[18,19,27] In our study, we performed drug tests in PDTOs derived from the surgical samples of patients with stage IV CRC and investigated whether the chemosensitivity

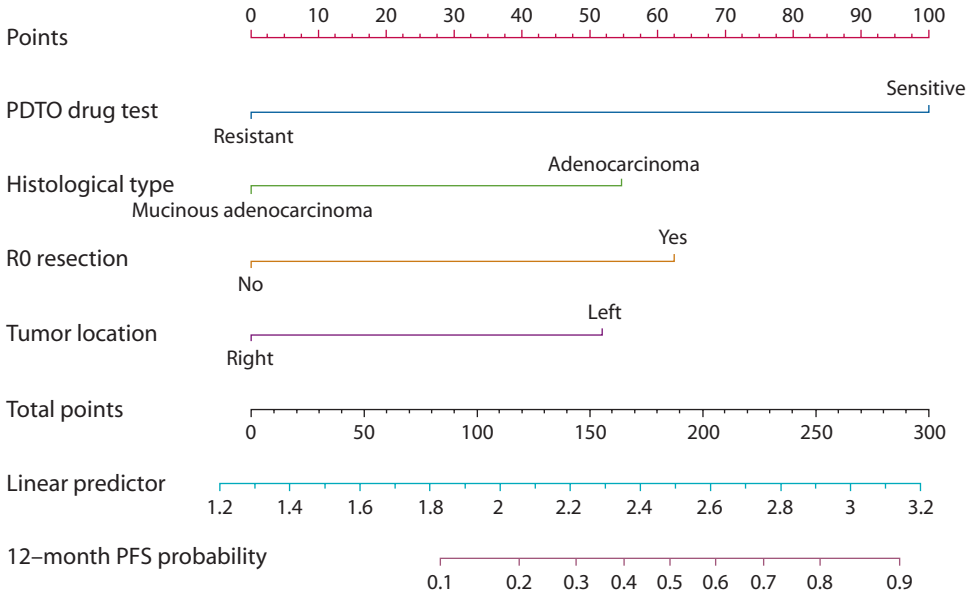

FIGURE 5. Nomogram for the prediction of PFS. The nomogram for the prediction of PFS was generated on the basis of the PDTO-based drug test, histological type, and R0 resection. PDTO = patient-derived tumor organoid; PFS = progression-free survival.

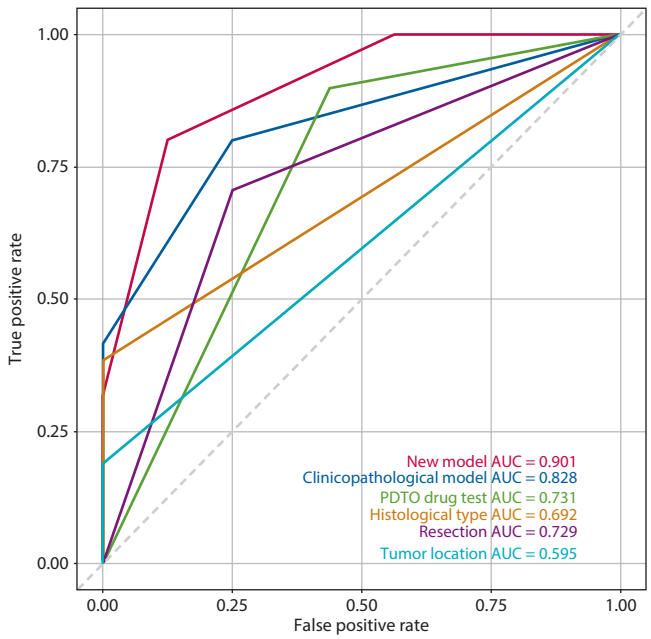

FIGURE 6. Comparison of the value of the new PDTO drug test model and the traditional clinicopathological model in predicting 1-y PFS. The AUC value of the new PDTO-based drug test prediction model was 0.901 (95% CI, 0.844–0.959), of the traditional clinicopathological prediction model was 0.828 (95% CI, 0.755–0.901), of the drug test alone was 0.731 (95% CI, 0.637–0.825), of the primary tumor location alone was 0.595 (95% CI, 0.546–0.644), of the histological type alone was 0.692 (95% CI, 0.631–0.753), and of R0 resection alone was 0.729 (95% CI, 0.634–0.823). The AUC value of the new PDTO drug test prediction model was higher than that of the clinicopathological model, the drug test alone, the histological type alone, and R0 resection alone ($p = 0.031$, $p = 0.012$, $p < 0.001$, $p = 0.001$, respectively). AUC = area under the curve; PDTO = patient-derived tumor organoid; PFS = progression-free survival.

determined by the PDTO drug test could be used to identify patients at risk of disease progression. To the best of our knowledge, this was the first cohort study to explore the prognostic value of the PDTO drug test in predicting PFS in stage IV CRC. Our study showed that patients who received sensitive chemotherapy regimens revealed by PDTO drug tests had a longer PFS than patients who received resistant chemotherapy. In clinical practice, we suggest that patients receive the most sensitive chemotherapy regimens revealed by PDTO drug tests. For patients who receive resistant chemotherapy, a closer follow-up or other treatment should be considered.

To develop a clinically practicable prediction model, we used univariate and multivariate analyses to identify the associations of the drug test and clinicopathologic characteristics with PFS. Our results demonstrated that PDTO-based drug resistance (HR, 3.38; 95% CI, 1.84–6.21; $p < 0.001$), right-sided colon (HR, 3.50; 95% CI, 1.71-7.15; $p < 0.001$), mucinous adenocarcinoma (HR, 2.47; 95% CI, 1.34–4.55; $p = 0.004$), and non-R0 resection (HR, 2.70; 95% CI, 1.61–4.54; $p < 0.001$) were independent risk factors for patients with stage IV CRC. The drug test result, tumor location, histological type, and R0 resection contributed to the risk of disease progression. Our nomogram with good discrimination was built by incorporating these 4 independent predictors. For example, in a patient with right-sided colon cancer who underwent R0 resection and had adenocarcinoma, the probability of 12-month PFS for a patient with resistance indicated by the PDTO-based drug test was approximately 20%, and for patients with sensitivity indicated by the PDTO-based drug test, the probability increased to approximately 65%. As histological type is

routinely assessed in clinical practice and the results of drug tests are available by obtaining tumor samples and generating PDTO, the individual risk of disease progression could be conveniently estimated by the nomogram and could help clinicians make treatment decisions. In this study, the new PDTO drug test model showed a better prognostic value with an AUC of 0.901 (95% CI, 0.844–0.959) than the traditional clinicopathological model with an AUC of 0.828 (95% CI, 0.755–0.901), and the difference between the new PDTO drug test model and the traditional clinicopathological model was significant ($p = 0.001$).

In summary, this study took a different approach by conducting a PDTO-based drug test to predict PFS in patients with stage IV CRC after surgery. This newly developed model combining PDTO-based drug tests and clinicopathological characteristics is superior to the clinicopathological model in predicting the PFS of patients with stage IV CRC.

Limitations

This study had some limitations. First, it was a single-center study, which might result in a potential selection bias. Thus, a multicenter prospective clinical trial is required. Second, we did not test PDTOs with epidermal growth factor receptor inhibitors (eg, cetuximab), B-raf inhibitors (eg, trametinib), or immune checkpoint blockade in this study. In our future studies, we will test PDTOs with epidermal growth factor receptor inhibitors, B-raf inhibitors, and immune checkpoint blockade.

CONCLUSIONS

The PDTO-based drug test can serve as a promising prognostic factor to predict the PFS of patients with stage IV CRC after surgery.

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