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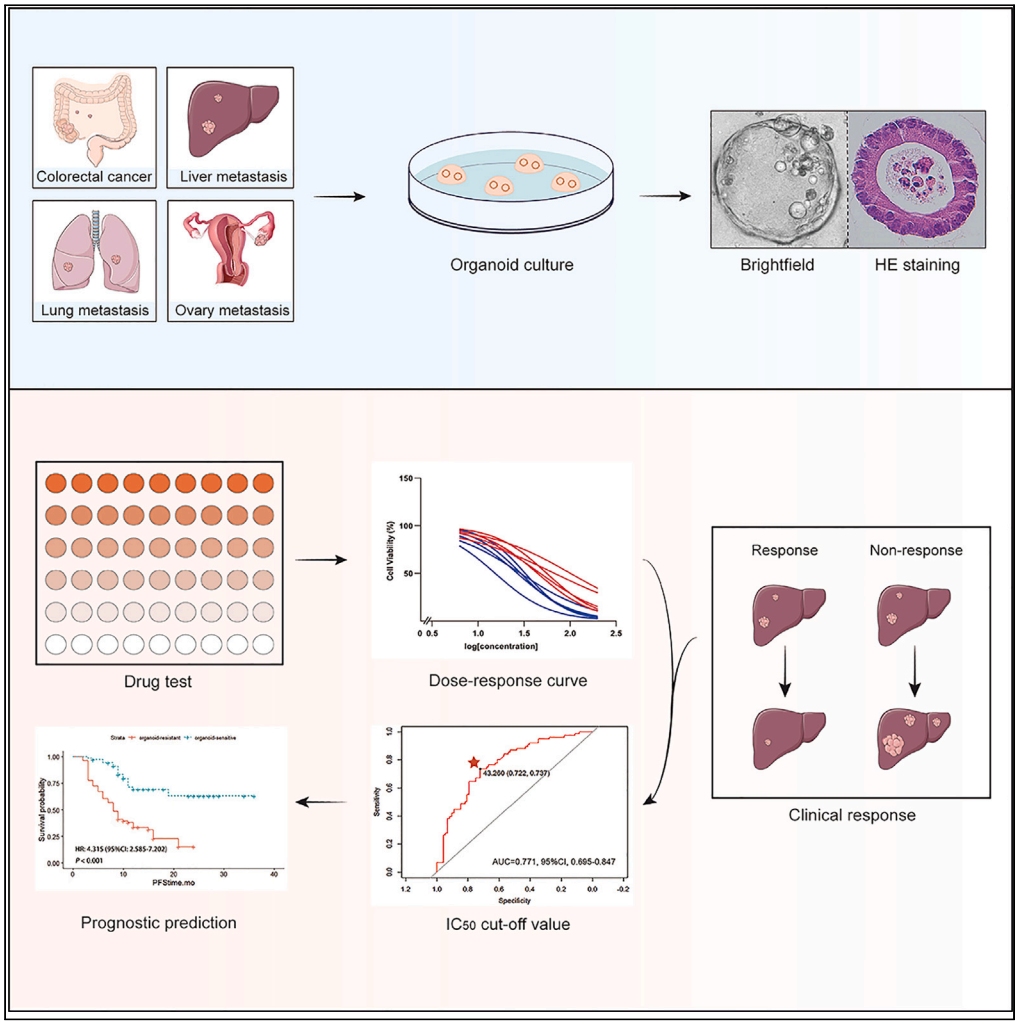

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Highlights

Generation of PDTOs of CRC with different lesions and biological characteristics

Definition of the IC_{50} cutoff value for PDTO drug sensitivity based on clinical responses

The defined cutoff value can predict the chemotherapy efficacy and prognosis

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Cutoff value of IC_{50} for drug sensitivity in patient-derived tumor organoids in colorectal cancer

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SUMMARY

Patient-derived tumor organoids (PDTOs) have the potential to be used to predict the patient response to chemotherapy. However, the cutoff value of the half-maximal inhibition concentration (IC_{50}) for PDTO drug sensitivity has not been validated with clinical cohort data. We established PDTOs and performed a drug test in 277 samples from 242 CRC patients who received FOLFOX or XELOX chemotherapy. After follow-up and comparison of the PDTO drug test and final clinical outcome results, the optimal IC_{50} cutoff value for PDTO drug sensitivity was 43.26 μmol/L. This PDTO drug test-defined cutoff value could predict patient response with 75.36% sensitivity, 74.68% specificity, and 75% accuracy. Moreover, this value distinguished groups of patients with significant differences in survival benefit. Our study is the first to define the IC_{50} cutoff value for the PDTO drug test to effectively distinguish CRC patients with chemosensitivity or nonsensitivity and predict survival benefits.

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer-related death globally,[1] with a 5-year relative survival rate of 65%.[2] Resistance to existing therapeutics remains the main cause of disease progression and CRC-associated mortality.[3] Currently, the fluorouracil combined with oxaliplatin (FOLFOX or XELOX) regimen is the first-line treatment with objective response rates of 20–40%.[4–7] However, there is no drug test to predict the therapeutic efficacy in individual patients in the clinic. Therefore, a reliable drug-sensitivity test performed before treatment is urgently needed, with individualized therapy being the final goal.

Currently, the commonly used disease models mainly include cell lines or patient-derived tumor xenografts (PDTXs). Cell lines cannot faithfully represent the original tumors, PDTXs take a long time to establish, and the success rate is low.[8] In particular, they cannot be used to perform individual drug tests.[9] Patient-derived tumor organoids (PDTOs), however, are an emerging three-dimensional *in vitro* system derived from stem cells.[10] Compared with cell lines or PDTXs, PDTOs require only a small amount of tissue for establishment and can be generated in a shorter propagation time while faithfully capturing the histopathology and genome stability of the tumor tissue from which they are derived; these features are especially important when applied to high-throughput drug screening.[11,12] In recent years, organoids have been generated from many kinds of tumors and have been successfully used in drug sensitivity tests.[13–16] Some studies have shown that PDTOs have the potential to be used to predict patient response to chemotherapy, and the preliminary implications of this finding in terms of precision medicine in CRC patients have been reported.[13,17] Nevertheless, the small number of patients analyzed as well as the lack of validated clinical responses, were insufficient to confirm the accuracy of using PDTOs to predict efficacy.[18] More importantly, the specific half-maximal inhibitory concentration (IC_{50}) cutoff value for determining drug sensitivity or drug resistance is unknown because of the lack of clinical cohort data. Therefore, we designed this study using a clinical cohort to define the IC_{50} cutoff value for the PDTO drug sensitivity test based on clinical responses and evaluate its association with prognosis in CRC patients. To the best of our knowledge, this is the first study to define the IC_{50} cutoff value for the PDTO drug sensitivity test by comparing the results of the PDTO drug test and the final clinical response.

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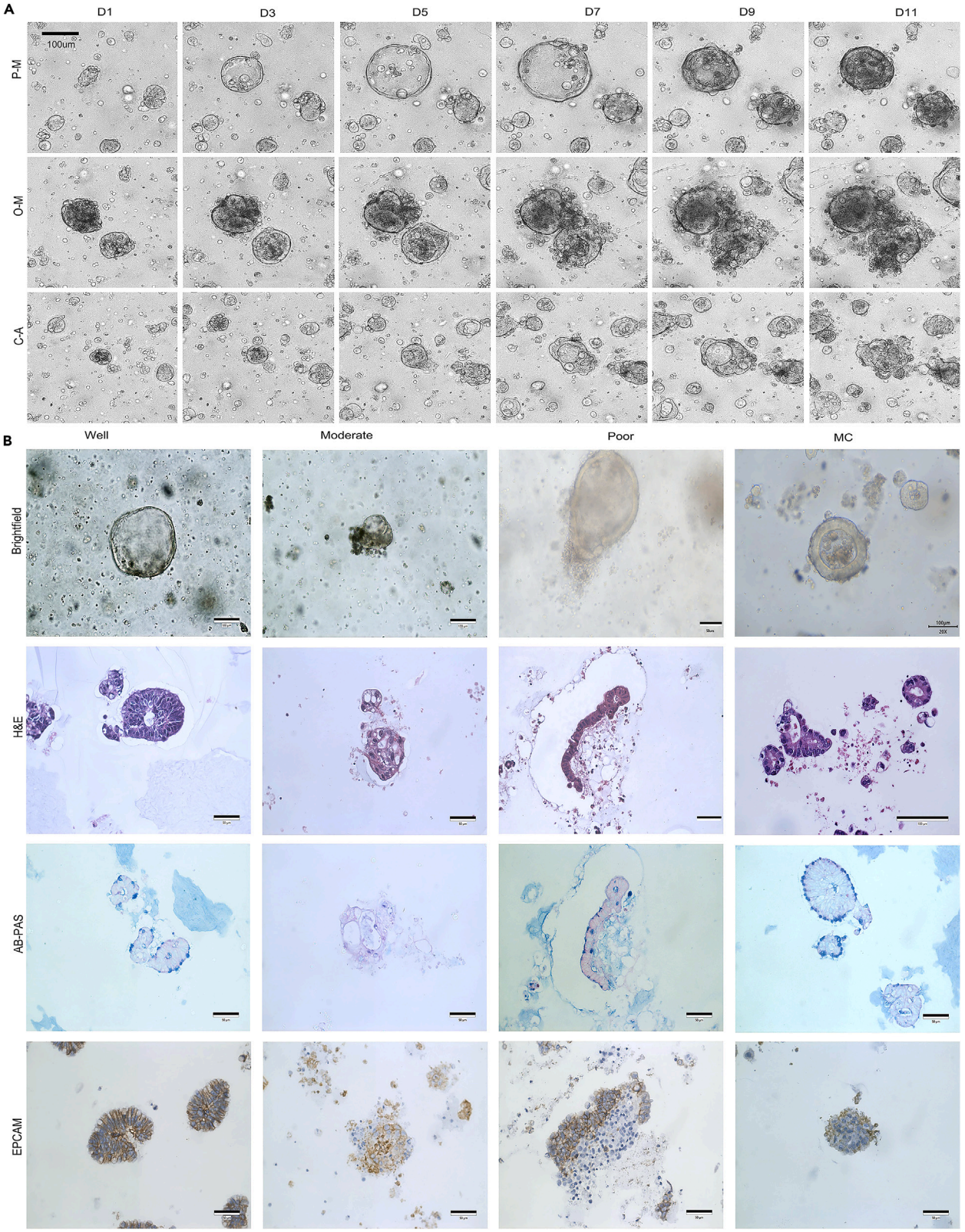
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Figure 1. Establishment and identification of organoids from CRC patients

(A) Dynamic growth changes in organoids from primary tumors and different metastases of one patient. P-M = primary tumor, O-M = omental metastases, C-A = cancerous ascites. Scale bar: 100 μm.

(B) Brightfield images, hematoxylin and eosin (H&E) staining, Alcian blue/periodic acid-Schiff (AB-PAS) staining, and immunohistochemistry staining of epithelial cell adhesion molecule (EPCAM) in organoids derived from different pathological types, including well-differentiated adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma and mucinous adenocarcinoma (MC). Scale bar: 100 μm.

RESULTS

Establishment and identification of organoids from CRC patient samples

We obtained tumor tissues and cultured them within 30 min after sample dissection. CRC samples were cultured as previously described[19] (see also Methods). There may be mycoplasma contamination in organoid culture, which looks similar to organoids in culture medium. Therefore, we tested for mycoplasma using the MycoAlert Mycoplasma Detection Kit, and the results showed that all MycoAlert values were less than 0.9 (Figure S1), which meant that no mycoplasma contamination was found in our medium according to the manual. We performed short-tandem repeat (STR) analysis of primary tumor tissues and matched organoids using a multiple amplification kit (PowerPlex 21 System, Promega). STR profiles showed a mean ± SD of 96.82 ± 4.38% between the primary tumor tissue and the corresponding organoids. Previous studies indicated that organoids established from different patients typically displayed different phenotypes.[20] Notably, there were visible differences in growth rates and morphology in the organoids from multiple lesions in one patient (Figure 1A). As the results showed, primary lesion-derived organoids displayed a completely hollow appearance and good growth, omentum-derived organoids displayed a completely cystic and solid appearance with moderate growth, and ascites-derived organoids presented a mixed, bubbly structure with poor growth. Considering that the cell activity of exfoliated cells in ascites is relatively low, ascites-derived organoids developed relatively slowly.

Currently, the classification of different CRC subtypes is based on histological examination. To verify whether phenotypic features are retained in organoids, we histologically characterized the CRC organoids (Figure 1B). CRC organoids presented with an epithelial architecture and typical tumorous features, including an enlarged nucleus and increased nuclear atypia. Epithelial tumor origin was assessed by immunostaining for the protein EPCAM. Moreover, mucinous adenocarcinoma organoids exhibited an apparent mucus lake dyed blue by AB-PAS staining, which was still clearly visible by H&E staining. The expression levels of Ki67 and P53 were 72% and 59.5%, respectively, in organoids generated from primary tumors. Combined with HE staining, these organoid cultures were from CRC cells without normal colorectal cells (Figure S2A). The PDTOs of liver metastases originating from intestinal epithelium rather than normal liver epithelium were assessed by immunostaining for the proteins CDX2 and HepPar-1. The positive rates of CDX2 and HepPar-1 expression were 94.7% and 0%, respectively, which indicated that these organoid cultures were from intestinal epithelium without normal liver epithelium (Figure S2B). The above results demonstrated that the tumor organoids generally resembled the parental tumor tissue.

Drug sensitivity tests of organoids from CRC patient samples

All PDTOs were evaluated for sensitivities to multiple clinically relevant drugs, and sensitivities were calculated as IC_{50} values, representing the half-maximal inhibitory concentration over the dose−response curve across six different concentrations of each drug. The concentration of chemotherapy drugs ranged from 6.25 μmol/L to 200 μmol/L, whereas that of targeted drugs ranged from 0.01236 μmol/L to 3 μmol/L. For combination regimens, the corresponding chemotherapy drugs were added at a 1:1 ratio, and the concentration ranged from 6.25 μmol/L to 200 μmol/L. Organoids exposed to different drug concentrations showed different growth trends (Figure S3A). A reduced area or even disappearance was observed in organoids exposed to a high drug concentration after 96 h, whereas organoids exposed to a low drug concentration displayed a similar or even enlarged area in contrast to organoids before dosing. In addition, we found that different organoids from different lesions in one patient showed different sensitivities to one drug. As illustrated in Figure S3B, this is a CRC patient with liver metastases. Organoids from primary tumor exposed to the FOLFOX regimen decreased in size and amount. In addition, there was no obvious change in organoids from liver metastases after exposure to the FOLFOX regimen.

To further explore heterogeneous drug responses, we assembled a 39-compound library for drug screening. In total, 127 organoids from 113 metastatic CRC (mCRC) patients were successfully generated and subjected to customized drug screening. The demographics of the patients are summarized in

Table 1. Patient demographics and tumor characteristics in mCRC patients

Characteristic	N = 113 patients
Age, median (range), years	56 (25–75)
Sex	
Male	66 (58.4)
Female	47 (41.6)
BMI, median (range), kg/m^2	23.0 (15.2–30.9)
Primary tumor location, No (%)	
Right side	27 (23.9)
Left side	86 (76.1)
Pathological type, No (%)	
Adenocarcinoma	91 (80.5)
Mucinous adenocarcinoma	22 (19.5)
Differentiation grade, No (%)	
Well	5 (4.4)
Moderately	84 (74.3)
Poor	24 (21.2)
Pathologic T category, No (%)	
T2	17 (15)
T3	52 (46)
T4	44 (38.9)
Pathologic N category, No (%)	
N0	26 (23)
N1	60 (53.1)
N2	27 (23.9)
Metastatic site, No (%)	
Liver	56 (49.6)
Lung	23 (20.4)
Peritoneal/Omental	19 (16.8)
Ovary	3 (2.7)
Multiple metastases	12 (10.6)

Table 1. A heatmap of sensitivities to the 39 evaluated drugs confirmed a range of responses across the different organoids (Figure 2B). Previous studies have shown that the molecular landscape of tumors is associated with sensitivity to chemotherapy and targeted therapy.[21,22] We divided the tumors according to their mutation background among our 127 samples and determined whether there was statistically significant variance. Resistance to EGFR inhibition was confirmed in PDTOs with KRAS mutations (Figure 2A). PDTOs with wild-type KRAS were observed to have significantly higher sensitivity to four EGFR inhibitors (cetuximab, lapatinib, neratinib and erlotinib). Similarly, the difference in sensitivity to vemurafenib and GDC0941 was defined by BRAF-V600E and PIK3CA activity sensitivity (Figure 2A). In addition, no significant drug sensitivity difference was observed for APC and TP53 mutations regarding all detected drugs in our samples (Figures S4A–S4B). Then, we attempted to explore whether the clinicopathological characteristics are related to drug responses. Previous studies have indicated that the sidedness of CRC is associated with the response to certain chemotherapies.[23] We separated the left- and right-sided tumors among our 127 samples and compared whether there was a statistically significant variance according to sidedness. The results revealed that dovitinib exhibited a better response in our right-sided CRC samples. No correlation between sidedness and cetuximab was found in our samples (Figures S4C–S4D). Next, the samples were grouped into adenocarcinoma (AC) and mucinous adenocarcinoma (MC), and we further explored the correlation between pathology and drug responses. As shown in Figures S4E–S4F, lapatinib resulted in a better response in the MC samples, and no significant difference was observed for the other drugs. We continued to investigate whether there was any difference in sensitivity

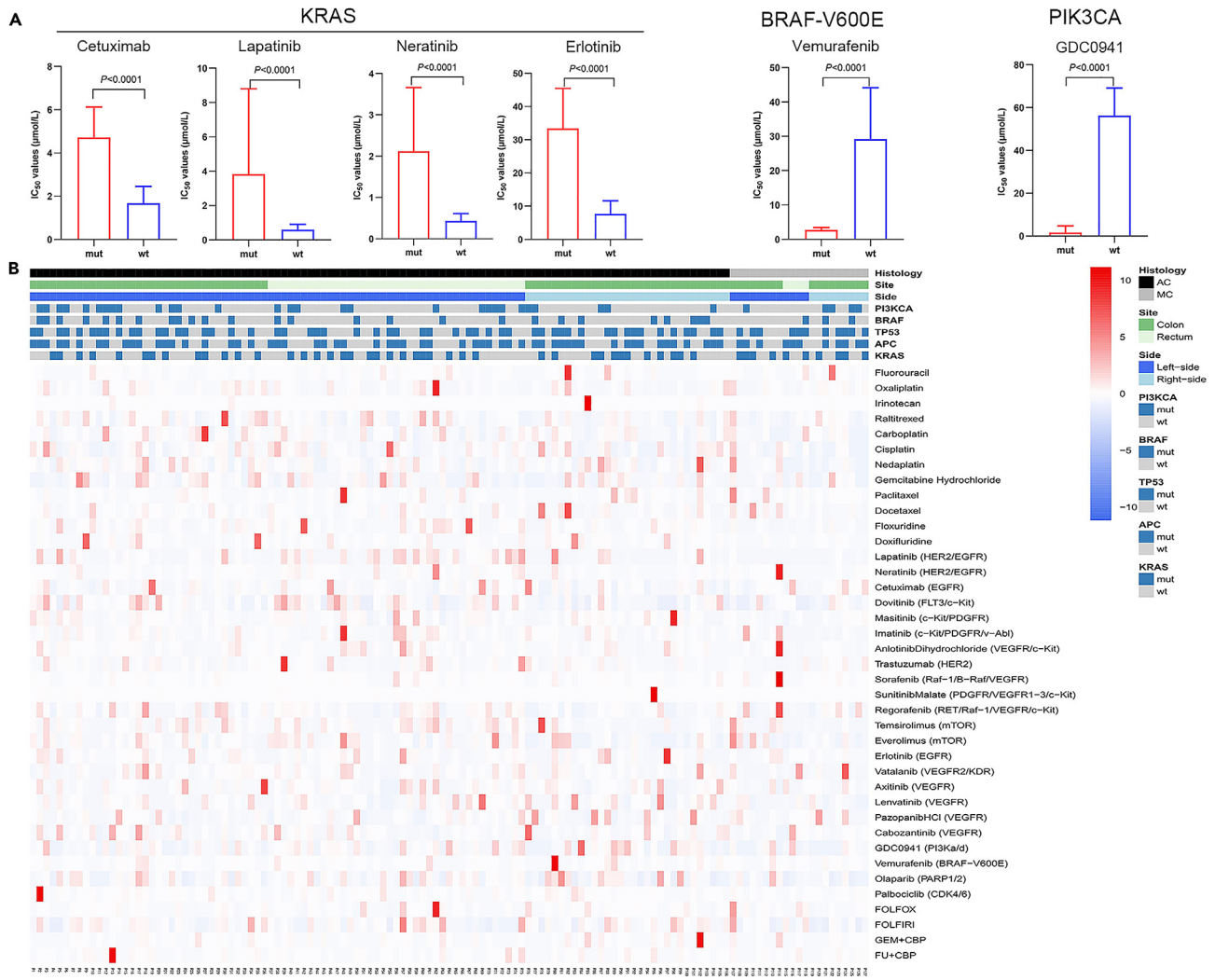

Figure 2. Drug sensitivity tests of organoids from CRC patients

(A) Pharmacogenomic relationship between KRAS mutation status and response to EGFR inhibition, BRAF-V600E mutation status and response to vemurafenib, and PIK3CA mutation status and response to GDC0941. Data are represented as median with 95% CI.

(B) A heatmap of IC_{50} values of different organoids to different drugs. Organoids showed heterogeneous responses to various drugs. AC = adenocarcinoma, MC = mucinous adenocarcinoma.

to various drugs in colonic and rectal tumors. The results indicated that the rectal tumor samples exhibited a better response to nedaplatin, whereas the colonic tumor samples exhibited a better response to lapatinib (Figures S4G–S4H).

Association of organoid drug response with patient clinical response

Next, we attempted to evaluate the potential of using organoids to predict patient response to chemotherapy. A total of 189 samples from 136 mCRC patients were collected from November 2018 to July 2021. Among them, 148 samples from 113 mCRC patients were successfully used to establish PDTOs, and 41 samples from 23 mCRC patients could not be cultured. The success rate of organoid establishment was 78.3% (148/189). All organoids were exposed to fluorouracil combined with oxaliplatin at concentrations ranging from 200 μmol/L to 6.25 μmol/L at a 1:1 ratio. At 96 h after drug exposure, we assayed the cell viability of each condition and fitted a dose–response curve (DRC) to calculate IC_{50} values and the area under the DRC (AUC_{DRC}) (Figure 3F). The clinical characteristics of 113 mCRC patients are presented in Table 1. We defined response in the clinic as CR/PR, AJCC/CAP regression grade 0 to 1, or decreased CEA levels, whereas nonresponse was defined as SD/PD, AJCC/CAP regression grade 2 to 3, or elevated

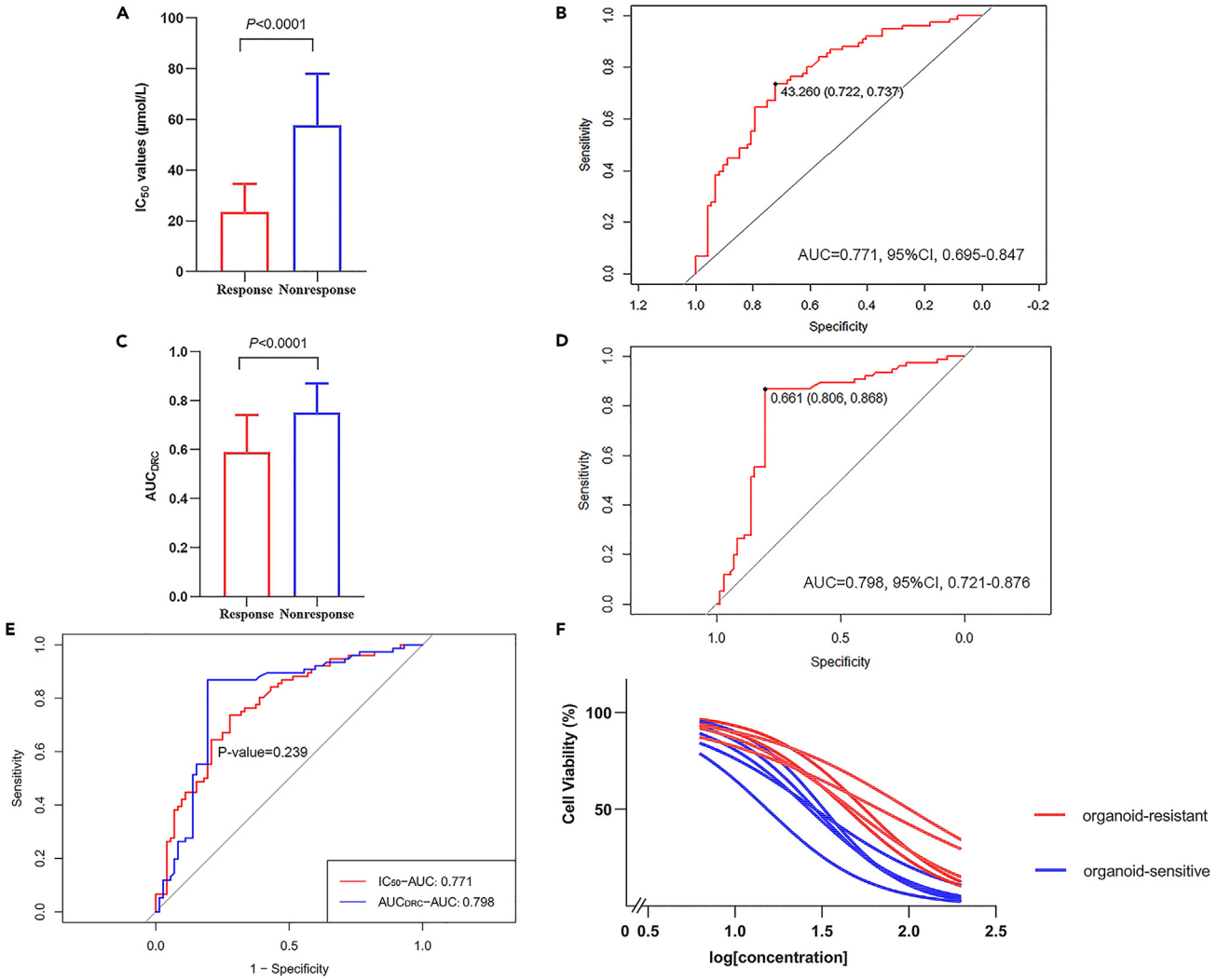

Figure 3. Association of organoid drug responses with patient clinical responses

(A) Column chart depicting the difference in IC_{50} values for the FOLFOX regimen between the clinical response and clinical nonresponse groups. The IC_{50} value of the clinical response group was significantly lower than that of the clinical nonresponse group ($p < 0.0001$). Data are represented as median with 95% CI.

(B) Receiver operating characteristic (ROC) curve of IC_{50} values for the FOLFOX regimen tested in organoids. The IC_{50} value for the organoid drug sensitivity test was associated with the patients' clinical responses, with an area under the ROC curve of 0.771 (95% CI, 0.695–0.847), and the optimal IC_{50} cutoff value for the FOLFOX regimen was 43.26 μmol/L, as determined from the ROC curve. An IC_{50} value higher than 43.26 μmol/L indicated organoid resistance, and an IC_{50} value lower than 43.26 μmol/L indicated organoid sensitivity.

(C) Column chart depicting the difference in the AUC_{DRC} for the FOLFOX regimen between the clinical response and clinical nonresponse groups. The AUC_{DRC} of the clinical response group was significantly lower than that of the clinical nonresponse group ($p < 0.0001$). Data are represented as median with 95% CI. AUC_{DRC}: area under the dose–response curve.

(D) Receiver operating characteristic (ROC) curve of the AUC_{DRC} for the FOLFOX regimen tested in organoids.

(E) Comparison of IC_{50} and AUC_{DRC} values in evaluating the clinical response.

(F) Fitted dose–response curves (DRCs) of 10 PDTOs exposed to the FOLFOX regimen *in vitro*. Blue lines represent PDTOs derived from the organoid-sensitive group, and red lines represent PDTOs derived from the organoid-resistant group.

CEA levels. After the clinical follow-up (median time: 12 months) in the mCRC patients, the treatment responses were revealed. In total, 69 organoids were derived from lesions that were classified as responsive, and 79 organoids were derived from lesions that were classified as nonresponsive. We quantified responses to FOLFOX by calculating the IC_{50} and AUC_{DRC}, and the results showed that the IC_{50} values of organoids generated from the responsive group were significantly lower than those of organoids from the nonresponsive group (Figure 3A, $p < 0.0001$). Similarly, the AUC_{DRC} of organoids generated from the

Table 2. Predictive ability of the defined IC_{50} cutoff value in the PDTO drug test for predicting chemotherapy responses

		Response in the clinic		
N = 148		Response N = 69	Nonresponse N = 79	
Drug response in PDTO	Sensitive N=72	52	20	PPV=72.22% (52/72)
	Resistant N=76	17	59	NPV=77.63% (59/76)
		Sens=75.36% (52/69)	Spec=74.68% (59/79)	Accuracy=75% (111/148)

PDTO: patient-derived tumor organoid; PPV: positive predictive value; NPV: negative predictive value; Sens: sensitivity; Spec: specificity.

responsive group was significantly lower than that of organoids from the nonresponsive group (Figure 3C, $p < 0.0001$). We next constructed a receiver operating characteristic (ROC) curve to explore the association of organoid drug response with patient clinical response. The dependent variable of the ROC curve was categorized by clinical responses assessed using the above definitions in 148 samples. The area under the ROC curve were 0.771 (95% CI, 0.695–0.847) and 0.798 (95% CI, 0.721–0.876) based on the IC_{50} and AUC_{DRC} respectively (Figures 3B and 3D). Further comparison showed that there was no significant difference between IC_{50} and AUC_{DRC} in evaluating the clinical response (Figure 3E, $p = 0.239$), which suggests that the IC_{50} and AUC_{DRC} values for the organoid drug-sensitivity test were both associated with the corresponding patients' clinical responses. In addition, the optimal IC_{50} cutoff value for the FOLFOX regimen was 43.26 μmol/L, as determined from the ROC curve. An IC_{50} value higher than 43.26 μmol/L indicated organoid resistance, and an IC_{50} value lower than 43.26 μmol/L indicated organoid sensitivity. In total, 72 organoids were classified as sensitive, and 76 organoids were classified as resistant. Next, we compared the clinical responses and organoid responses to evaluate the ability of this defined cutoff value to predict chemotherapy responses. As Table 2 shows, the sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the defined cutoff value for predicting chemotherapy responses in CRC were 75.36% (95% CI: 63.16%–82.89%), 74.68% (95% CI: 61.11%–81.94%), 75% (95% CI: 65.54%–80.41%), 72.22% (95% CI: 66.25%–81.82%) and 77.63% (95% CI: 64.56%–80.56%), respectively. Subsequently, we performed further stratifications considering several clinical features, and the results are shown in Table 3 (see Tables S1–S17 for details). For patients with multiple different lesions, we attempted to assess the predictive ability of spatially distinct individual cultures from the same patient. A total of 25 patients had multiple lesions, and 60 lesions (25 primary lesions and 35 metastatic lesions) were included in the analysis. As Table 4 and Table 5 show, the predictive accuracy values were 84% and 77.14% for primary and metastatic lesions, respectively. Overall, all of these results suggested that our defined cutoff value of the IC_{50} value can be used to effectively distinguish between chemosensitive and nonsensitive patients receiving the FOLFOX regimen.

Association of organoid response with prognosis in mCRC patients

We continued to explore the relationship of organoid drug response with patient survival. All samples were divided into an organoid-sensitive group and an organoid-resistant group according to the defined cutoff value, and the clinical features of the two groups were similar (Table 6). The progression-free survival (PFS) rate in the organoid-resistant group was significantly worse than that in the organoid-sensitive group, as expected, with an HR of 4.315 (95% CI: 2.585–7.202; $p < 0.001$) (Figure 4). The median PFS of the mCRC patients was 11 months in the organoid-sensitive group and 8 months in the organoid-resistant group. For patients with multiple different lesions, we attempted to assess the prognostic value of spatially distinct individual cultures from the same patient. A total of 25 patients had multiple lesions, and 60 lesions (25 primary lesions and 35 metastatic lesions) were included in the analysis. Similarly, the PFS rate in the organoid-resistant group was significantly worse than that in the organoid-sensitive group in both samples from primary lesions (HR: 4.437; 95% CI: 1.279–15.39; $p = 0.007$) (Figure 5A) and samples from metastatic lesions (HR: 4.003; 95% CI: 1.629–9.834; $p = 0.001$) (Figure 5B). In the multivariate Cox regression analysis, the organoid response remained an independent prognostic predictor of PFS (HR: 3.865; 95% CI: 2.299–6.496; $p < 0.001$) (Table 7).

Table 3. Predictive ability of the defined IC_{50} cutoff value in the PDTO drug test for predicting chemotherapy responses in mCRC patients with different characteristics

Characteristic	$IC50_{Sensitivity}$	$IC50_{Specificity}$	$IC50_{Accuracy}$	$IC50_{PPV}$	$IC50_{NPV}$
Gender					
Male	77.5%	79.25%	78.49%	73.81%	82.35%
Female	72.41%	65.38%	69.09%	70%	68%
Primary tumor					
Left side	74%	76.92%	75.49%	75.51%	75.47%
Right side	78.95%	70.37%	73.91%	65.22%	82.62%
Histological type					
AC	72.88%	70%	71.56%	74.14%	68.63%
MC	90%	82.76%	84.62%	64.29%	96%
Differentiation grade					
Well	83.33%	100%	87.5%	100%	66.7%
Moderate	72.55%	74.55%	73.58%	72.55%	74.55%
Poor	83.33%	72.73%	76.47%	62.5%	88.89%
T stage					
2	72.73%	50%	63.16%	66.67%	57.14%
3	75%	65.71%	70.15%	66.67%	74.19%
4	76.92%	88.89%	83.87%	83.33%	84.21%
N stage					
0	86.96%	87.5%	92.31%	90.91%	82.35%
1	76.47%	76.46%	76.62%	72.22%	80.49%
2	50%	60%	56.25%	42.86%	66.67%
Location of sample					
Primary lesion	74.07%	75%	74.49%	78.43%	70.21%
Metastatic lesion	80%	74.29%	76%	57.14%	98.66%

Prognostic value of organoid response in stage II to III CRC samples

To evaluate whether organoid responses determined by this cutoff value could be applied to predict the survival of patients with stage II/III CRC, surgical samples were collected after excision, and organoid culture and drug tests were performed. A total of 173 samples from 173 CRC patients were collected from November 2018 to December 2019. Among them, 142 samples from 142 CRC patients were successfully used to establish PDTOs, and 31 samples from 31 CRC patients could not be cultured. The success rate of organoid establishment was 82.1% (142/173). In the subsequent follow-up (median time: 35 months), 10 patients refused chemotherapy, 1 patient had other tumors, and 2 patients were lost to follow-up. Therefore, 129 samples from 129 patients with stage II/III CRC were finally included in the analysis, of which 45

Table 4. Predictive ability of the defined IC_{50} cutoff value in the PDTO drug test for predicting the chemotherapy responses of mCRC patients with multiple lesions

Location of sample = primary lesion N = 25		Response in the clinic		
		Response N = 6	Nonresponse N = 19	
Drug response in PDTO	Sensitive N=8	5	3	PPV=65.5% (5/8)
	Resistant N=17	1	16	NPV=94.12% (16/17)
		Sens=83.33% (5/6)	Spec=84.21% (16/19)	Accuracy=84% (21/25)

Table 5. Predictive ability of the defined IC_{50} cutoff value in the PDTO drug test for predicting the chemotherapy responses of mCRC patients with multiple lesions

Location of sample = metastatic lesion N = 35		Response in the clinic		
		Response N = 12	Nonresponse N = 23	
Drug response in PDTO	Sensitive N=18	11	7	PPV=61.11% (11/18)
	Resistant N=17	1	16	NPV=94.12% (16/17)
		Sens=91.67% (11/12)	Spec=69.57% (16/23)	Accuracy=77.14% (27/35)

(34.9%) were stage II and 84 (65.1%) were stage III. The clinicopathological characteristics of these patients are presented in Table 8. According to the defined cutoff value, 48 samples were classified as organoid resistant (16 with stage II and 32 with stage III), whereas 81 samples were classified as organoid sensitive (29 with stage II and 52 with stage III). Low recurrence risk was observed in the organoid-sensitive patients, with a 2-year disease-free survival (DFS) rate of 86.4% (95% CI: 79.0%–93.8%). In contrast, the organoid-resistant patients had a significantly higher recurrence risk than the organoid-sensitive patients (HR: 3.009; 95% CI: 1.409–6.427; p = 0.004), with a 2-year DFS rate of 64.6% (95% CI: 51.1%–78.1%) (Figure 6A). Subsequently, we attempted to assess the prognostic value of the organoid response in stage II and stage III CRC patients. As expected, the organoid-resistant patients had a significantly poorer DFS rate than the organoid-sensitive patients in both patients with stage II disease (HR: 5.318; 95% CI: 1.030–27.450; p = 0.046) (Figure 6B) and those with stage III disease (HR: 2.444; 95% CI: 1.029–5.803; p = 0.043) (Figure 6C).

DISCUSSION

The current treatment for CRC is mainly based on traditional tumor node metastasis (TNM) staging.[24] However, increasing evidence has demonstrated that patients with the same stage who receive the same treatment have different outcomes, which highlights the need for individualized treatment.[25] In addition, it is currently not possible to test the drug response in individual patients before treatment in the clinic. An accurate prediction of drug sensitivity and survival benefits in patients with CRC is of great importance in chemotherapy decision-making and improvement of prognosis. In this study, we defined the IC_{50} cutoff value of the FOLFOX regimen for the PDTO drug sensitivity test based on clinical responses and evaluated the association of PDTO drug responses determined from the defined cutoff value with prognosis in CRC patients. We found that our defined cutoff value could be used to predict the chemotherapy efficacy and prognosis of CRC patients who received the FOLFOX/XELOX regimen.

Despite the development of novel effective strategies for the treatment of tumors, including the targeted therapy and immunotherapy, chemotherapy still plays crucial roles in the fight against cancer alone or in combination with other regimens.[26] Fanget et al.[27] revealed that triplet-drug combination (FOLFIRINOX) was associated with better long-term outcomes for CRC peritoneal metastases patients after complete cytoreductive surgery compared with doublet drug (FOLFOX/FOLFIRI) plus targeted therapy. Napolitano et al.[28] showed that chemotherapy combined with targeted therapy has a stronger anti-tumor activity than targeted therapy alone in BRAFV600E mutant CRC. Torregrosa et al.[29] demonstrated that FOLFIRI-bevacizumab combination was associated with better survival after failure of first line FOLFOX-bevacizumab for mCRC. In short, standard chemotherapy in CRC plays the pivotal role despite the availability of different targeted approaches or immunotherapy. As a first-line chemotherapy regimen, the FOLFOX/XELOX regimen only works in approximately one-third of CRC patients.[30] Therefore, approximately two-thirds of patients receive ineffective chemotherapy because of the lack of a drug test for predicting response in the clinic. Organoids are an emerging technology that has been used for drug screening, drug repositioning and dose adjustment. Combining high-throughput sequencing and drug testing, organoids can be used to reverse drug resistance or improve treatment efficiency.[31–33] The matched healthy and tumor organoids are applicable to adjust drugs with the best appropriate dose, considering both tumor efficacy and normal tissue toxicity.[32] For some off-label drugs, the use of organoids can cut the cost of development and validation.[34] In addition, organoids have displayed potential in response prediction in multiple tumors, including CRC.[35–37] However, the specific cutoff value for the

Table 6. Patient demographics and tumor characteristics of the organoid-sensitive and organoid-resistant groups of mCRC patients

Characteristic	Organoid-sensitive group (N = 72	Organoid-resistant group (N = 76)	p value
Age, median (IQR)	58.0 (50.0–63.0)	56.0 (45.0–61.2)	0.087
Sex			0.351
Male	42 (58.3%)	51 (67.1%)	
Female	30 (41.7%)	25 (32.9%)	
BMI, median (IQR)	23.0 (20.7–24.3)	22.8 (21.1–24.2)	0.621
Primary tumor			0.966
Left-side	49 (68.1%)	53 (69.7%)	
Right-side	23 (31.9%)	23 (30.3%)	
Histological type			0.095
Adenocarcinoma	58 (80.6%)	51(67.1%)	
Mucinous adenocarcinoma	14 (19.4%)	25 (32.9%)	
Tumor differentiation, no (%)			0.769
Well	5 (7.0%)	3 (3.95%)	
Moderate	51 (70.8%)	55 (72.4%)	
Poor	16 (22.2%)	18 (23.7%)	
T stage			0.093
2	12 (16.7%)	7 (9.2%)	
3	36 (60.0%)	31 (40.8%)	
4	24 (33.3%)	38 (50.0%)	
N stage			0.507
0	22 (30.6%)	17 (22.4%)	
1	36 (50.0%)	41 (53.9%)	
2	14 (19.4%)	18 (23.7%)	
Resection			1.000
R0 resection	43(59.7%)	45 (59.2%)	
R1 resection	29 (40.3%)	31 (40.8%)	
Location of distant disease			0.431
Liver	35 (48.6%)	39 (51.3%)	
Lung	13 (18.1%)	11 (14.5%)	
Peritoneal	8 (11.1%)	15 (19.7%)	
Multiple metastases	11 (15.3%)	6 (7.9%)	
Other	5 (6.94%)	5 (6.6%)	
Neoadjuvant chemotherapy			0.342
No	51 (70.8%)	60 (78.9%)	
Yes	21 (29.2%)	16 (21.1%)	
Lymphovascular invasion			0.183
No	42 (58.3%)	35 (46.1%)	
Yes	30 (41.7%)	41 (53.9%)	
Preoperative CEA			0.493
≤5	53 (73.6%)	51 (67.1%)	
>5	19 (26.4%)	25 (32.9%)	

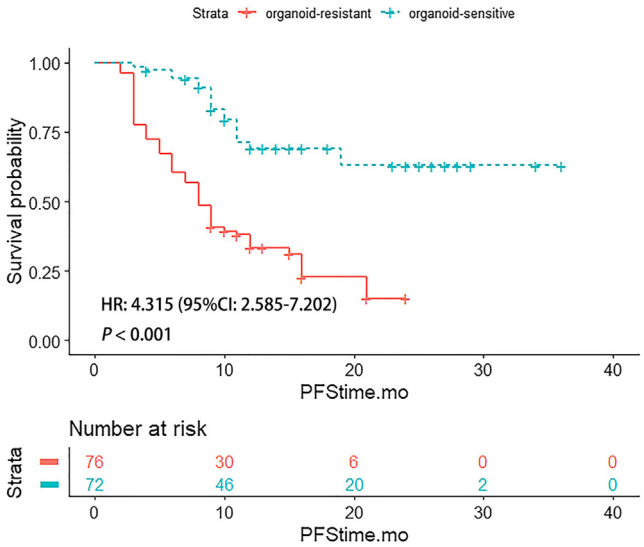

Figure 4. Association of organoid response with prognosis in mCRC patients

The Kaplan–Meier survival curve indicated that the organoid-resistant group showed a significantly lower PFS rate than the organoid-sensitive group (HR: 4.315; 95% CI: 2.585–7.202; p < 0.001). PFS = progression-free time, HR = hazard ratio.

FOLFOX regimen to determine whether the IC_{50} of the PDTO drug-sensitivity test indicates sensitivity or resistance remains unknown because of a lack of clinical cohort data. In this research, we performed a cohort study to define the IC_{50} cutoff value for the FOLFOX regimen to explore the association of the PDTO drug response with the patient clinical response. Our results indicated that the IC_{50} cutoff value for the FOLFOX regimen was 43.26 μmol/L, with an area under the ROC curve of 0.771 (95% CI, 0.695–0.847). An IC_{50} higher than 43.26 μmol/L indicated organoid resistance, and an IC_{50} lower than 43.26 μmol/L indicated organoid sensitivity. The sensitivity, specificity, accuracy, PPV and NPV of the organoid response determined by our defined cutoff value for predicting the chemotherapy response in CRC patients were 75.36%, 74.68%, 75%, 72.22% and 77.63%, respectively. The above results suggested that the defined IC_{50} cutoff value can be used to effectively distinguish CRC patients with chemosensitivity or nonsensitivity to the FOLFOX regimen.

As a heterogeneous disease, CRC shows differences in prognosis among patients.[23] Previous studies have shown that the TNM staging system and some histopathological factors, including tumor grade, histological type and lymphovascular invasion, could be used to predict prognosis. However, their predictive values were limited.[38–40] In this study, mCRC patients with organoid sensitivity showed a significantly higher PFS rate than patients with organoid resistance (p < 0.001). The PDTO drug response determined by the IC_{50} cutoff value was identified as an independent prognostic predictor. Furthermore, despite prognosis prediction reaching statistical significance, the incremental value of the PDTO drug response played the most important role among all prognostic factors, and the HR of the PDTO drug test was 3.856, which was higher than those of other factors. From a clinical perspective, the PDTO drug response provides vital prognostic information. Therefore, the PDTO drug test is a promising clinical application.

Postoperative adjuvant chemotherapy is the standard treatment for CRC patients with high-risk stage II and stage III disease to improve survival outcomes.[23,41] In this study, we attempted to explore whether this cutoff value could predict the survival benefits of CRC patients with stage II to III disease. We found that the PDTO drug response determined by the cutoff value could be used to predict the DFS of stage II and III CRC patients, similar to stage IV CRC patients. Both stage II and stage III patients with organoid sensitivity had a significantly lower recurrence risk than those with organoid resistance.

The potential paradigm-changing clinical applications of this PDTO-guided strategy can be summarized as follows: CRC patients with organoid sensitivity are predicted to respond to the FOLFOX/XELOX regimen and have a good prognosis. Therefore, these patients are recommended to receive the FOLFOX/XELOX regimen. Organoid-resistant CRC patients are predicted to be nonresponsive to the FOLFOX/XELOX

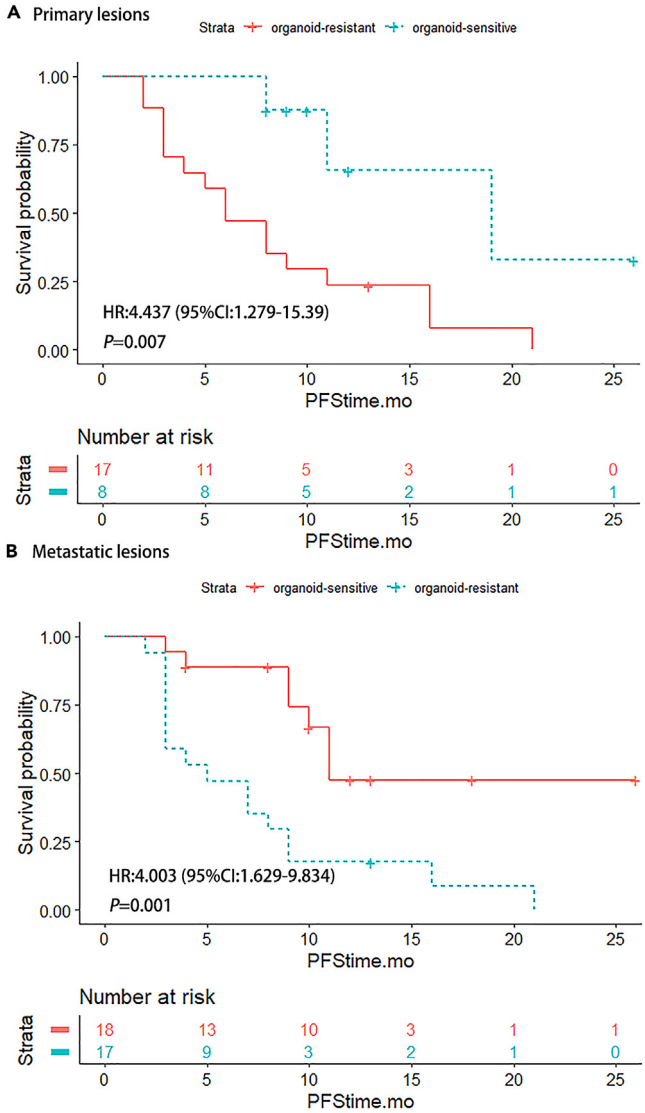

Figure 5. Association of the organoid response with prognosis in mCRC patients with multiple lesions

(A) The Kaplan–Meier survival curve indicated that the organoid-resistant group had a significantly lower PFS rate than the organoid-sensitive group (HR: 4.437; 95% CI: 1.279–15.39; p = 0.007) in organoids derived from primary lesions.

(B) The Kaplan–Meier survival curve indicated that the organoid-resistant group had a significantly lower PFS rate than the organoid-sensitive group (HR: 4.003; 95% CI: 1.629–9.834; p = 0.001) in organoids derived from metastatic lesions. PFS = progression-free survival, HR = hazard ratio.

regimen and have a poor prognosis. Therefore, these patients may be classified as high risk, and this classification might be helpful in decision-making regarding whether the FOLFIRI regimen should be recommended directly for these patients, especially stage IV CRC patients.

Although the IC_{50} value is an effective response metric and can be used to predict the chemotherapy efficacy and prognosis of CRC patients, it still has some limitations in evaluating the drug response. When two or more drugs are involved in the comparison of efficacy, the IC_{50} value is not accurate enough as an evaluation metric. In this case, the AUC_{DRC} may be a more accurate and objective evaluation method. In addition, as an *in vitro* drug response metric, the value of IC_{50} in predicting drug sensitivity or drug resistance still needs to be validated by clinical data. Moreover, PDTOs may not completely mimic the *in vivo* tumor because of the lack of some components of the tumor microenvironment, and by using PDTOs, it is not possible to evaluate the antitumor effects of the immune system. In addition, the purpose of this study

Table 7. Univariate and multivariate Cox regression analyses of the association of the PDTO-based drug test and clinicopathological characteristics with progression-free survival (PFS) in stage IV CRC patients

	Univariate		Multivariate	
Variable	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years)	0.989 (0.969–1.009)	0.288		
BMI	1.004 (0.931–1.082)	0.921		
Sex				
Male	1			
Female	0.766 (0.478–1.224)	0.265		
Location				
Left	1			
Right	1.239 (0.778–1.973)	0.366		
Histological type				
Adenocarcinoma	1		1	
Mucinous adenocarcinoma	3.356 (2.075–5.426)	<0.001	2.321 (1.378–3.909)	0.002
Differentiation				
Well	1		1	
Moderate	1.650 (1.072–2.541)	0.023	1.424 (0.333–6.085)	0.633
Poorly	1.890 (1.241–3.278)	0.020	1.741 (0.390–7.772)	0.467
T stage				
2	1			
3	1.416 (0.656–1.682)	0.249		
4	1.765 (0.777–2.896)	0.150		
N stage				
0	1			
1	1.344 (0.756–2.387)	0.314		
2	1.500 (0.734–3.399)	0.227		
R0 resection				
Yes	1		1	
No	2.187 (1.389–3.442)	<0.001	1.723 (1.070–2.777)	0.025
Neoadjuvant chemotherapy				
No	1			
Yes	1.132 (0.681–1.882)	0.633		
Lymphovascular invasion				
No	1		1	
Yes	1.797 (1.150–2.806)	0.01	1.662 (1.053–2.624)	0.029
CEA (ng/mL)				
≤5	1		1	
>5	1.881 (1.192–2.970)	0.007	1.531 (0.952–2.462)	0.079
Organoid response				
Sensitive	1		1	
Resistant	4.315 (2.585–7.202)	<0.001	3.865 (2.299–6.496)	<0.001
Location of distant disease				
Liver	1			
Lung	0.221 (0.088–0.557)	0.001		
Peritoneal	1.073 (0.609–1.891)	0.807		
Multiple metastases	0.483 (0.217–1.075)	0.075		
Other	0.391 (0.122–1.256)	0.115		

Table 8. Patient demographics and tumor characteristics in stage II to III CRC patients

Characteristic	Stage II-III (N = 129)	Stage II (N = 45)	Stage III (N = 84)
Age, median (range)	58 (20–75)	61 (25–75)	56.5 (20–75)
Sex			
Male	70 (54.3%)	30 (66.7%)	40 (47.6%)
Female	59 (45.7%)	15 (33.3%)	44 (52.4%)
BMI, median (range)	22.1 (15.7–39.6)	21.8 (16.9–39.6)	22.8 (15.7–30.9)
Primary Tumor			
Right-side	37 (28.7%)	19 (42.2%)	18 (21.4%)
Left-side	92 (71.3%)	26 (57.8%)	66 (78.6)
Histological Type			
AC	107 (82.9%)	38(84.4%)	69 (82.1%)
MC	22 (17.1%)	7 (15.6%)	15 (17.9%)
Tumor Differentiation			
Well	5 (3.9%)	3 (6.7%)	2 (2.4%)
Moderate	96 (74.4%)	34 (75.6%)	62 (73.8%)
Poor	28 (21.7%)	8 (17.8%)	20 (23.8%)
T stage			
2	9 (7.0%)	/	9 (10.7)
3	60 (46.5%)	21 (46.7%)	39 (46.4%)
4	60 (46.5%)	24 (53.3%)	36 (42.9%)
N stage			
0	45 (34.9%)	45 (100%)	/
1	57 (44.2%)	/	57 (67.9%)
2	27 (20.9%)	/	27 (32.1%)
Lymphovascular Invasion			
Yes	86 (66.7%)	45 (100%)	41 (48.8%)
No	43 (33.3%)	/	43 (51.3%)
Drug Test			
Resistant	48 (37.2%)	16 (35.6%)	32 (38.1%)
Sensitive	81 (62.8%)	29 (64.4%)	52 (61.9)

AC = adenocarcinoma, MC = mucinous adenocarcinoma.

was to define the IC_{50} cutoff value for the PDTO drug test to effectively distinguish CRC patients who underwent first-line chemotherapy such as FOLFOX or XELOX. We did not test the IC_{50} values of second-line chemotherapy such as FOLFOXIRI. Of course, the synergy between/among drugs may influence the therapeutic response of patients. Some monoclonal antibodies, such as cetuximab or trastuzumab, may have at least two mechanisms of action: (1) inhibition of EGFR and HER2 and (2) immune system-mediated cytotoxicity. Regarding the second mechanism of action, by using tumor organoids, it is not possible to establish immune system-mediated cytotoxicity. In this study, the purpose was to define the IC_{50} cutoff value of first-line chemotherapy regimens such as FOLFOX or XELOX, and we also tested some monoclonal antibodies such as cetuximab or trastuzumab. We admitted that we did not establish immune system-mediated cytotoxicity. This is also a limitation in this study. Our next step will consider the use of more complex models of PDTOs cocultured with lymphocytes.

To the best of our knowledge, this is the first study to define the IC_{50} cutoff value for PDTO drug sensitivity based on clinical responses in CRC patients, which provides a broad reference for clinical translation and subsequent research. Organoids have been recognized as a novel model for disease research.[12,42] Although some studies have shown that organoids have the potential to be used to predict treatment

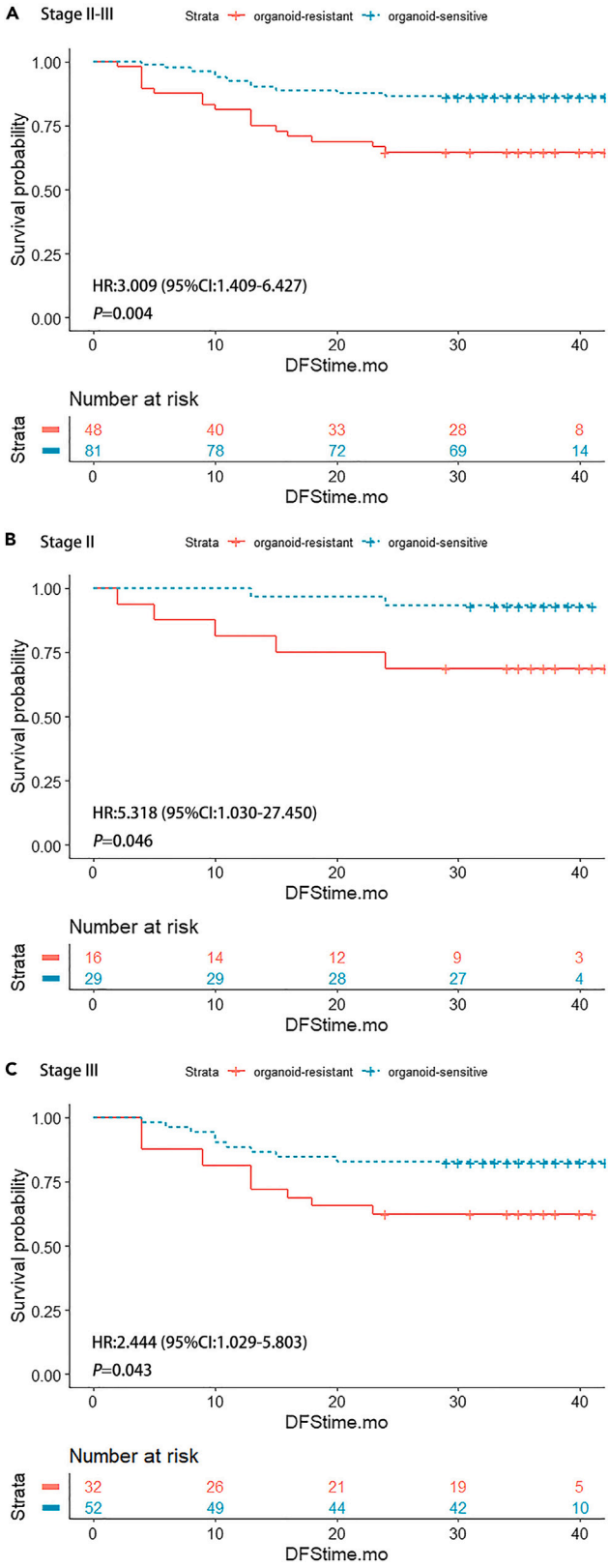
A
Stage II-III
Strata organoid-resistant organoid-sensitive
Survival probability
1.00
0.75
0.50
0.25
0.00
HR:3.009 (95%CI:1.409-6.427)
P=0.004
0 10 20 30 40
DFStime.mo
Number at risk
Strata
48 40 33 28 8
81 78 72 69 14
0 10 20 30 40
DFStime.mo
B
Stage II
Strata organoid-resistant organoid-sensitive
Survival probability
1.00
0.75
0.50
0.25
0.00
HR:5.318 (95%CI:1.030-27.450)
P=0.046
0 10 20 30 40
DFStime.mo
Number at risk
Strata
16 14 12 9 3
29 29 28 27 4
0 10 20 30 40
DFStime.mo
C
Stage III
Strata organoid-resistant organoid-sensitive
Survival probability
1.00
0.75
0.50
0.25
0.00
HR:2.444 (95%CI:1.029-5.803)
P=0.043
0 10 20 30 40
DFStime.mo
Number at risk
Strata
32 26 21 19 5
52 49 44 42 10
0 10 20 30 40
DFStime.mo

Figure 6. Prognostic value of organoid response in stage II to III CRC patients

(A) Association of organoid response with survival in CRC patients with stage II to III disease. The organoid-resistant group showed a significantly poorer DFS rate than the organoid-sensitive group (HR: 3.009; 95% CI: 1.409–6.427; p = 0.004).

(B) Association of organoid response with survival in CRC patients with stage II disease. Organoid-resistant patients had a significantly poorer DFS rate than organoid-sensitive patients (HR: 5.318; 95% CI: 1.030–27.450; p = 0.046).

(C) Association of organoid response with survival in CRC patients with stage III disease. Organoid-resistant patients had a significantly poorer DFS rate than organoid-sensitive patients (HR: 2.444; 95% CI: 1.029–5.803; p = 0.043). DFS = disease-free time, HR = hazard ratio.

response, the number of patients analyzed has been insufficient, and the specific cutoff value indicating whether the IC_{50} indicates drug sensitivity or resistance is lacking.[43,44] Therefore, our study showing the defined cutoff value is promising for clinical application. We believe that clinicians could obtain organoid drug sensitivity results in the near future, and the IC_{50} cutoff value could be used to guide individualized treatment.

In conclusion, our study is the first to define the IC_{50} cutoff value for the FOLFOX regimen in the PDTO drug test, which could be used to effectively distinguish CRC patients with chemosensitivity or nonsensitivity and predict survival benefits.

Limitations of the study

This study was retrospective and may have selection bias.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107116.

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AUTHOR CONTRIBUTIONS

Y.T., C.D., X.W., and J.Y. conceived and designed the study. Y.T., T.W., Y.H., H.J., and B.Y. performed organoid culture and drug test. X.H., Y.Z., Y.H., and W.X. collected the clinicopathological data. Y.W. and H.D. contributed to patient recruitment. Z.C. and J.L. performed immunohistochemistry. Data analysis and result interpretation were performed by Y.T. Y.T. and J.Y. drafted the manuscript. C.D., X.W., Y.W.,

and H.D. critically revised the manuscript. All authors approved the final draft submitted. Y.T., T.W., and Y.H. contributed equally as first authors. C.D., X.W., and J.Y. contributed equally as corresponding authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-EPCAM	Affinity	# BF0159; RRID: AB_2833813
Mouse monoclonal anti-HepPar-1	Abnova	#MAB14639; RRID: AB_2909615
Rabbit monoclonal anti-CDX2	Affinity	#DF4679; RRID: AB_2837030
Rabbit polyclonal anti-Ki67	Affinity	#AF0198; RRID: AB_2834152
Rabbit polyclonal anti-P53	Affinity	#AF0879; RRID: AB_2827700
Chemicals, peptides, and recombinant proteins		
5-Fluorouracil	Selleck	S1209; CAS: 51-21-8
Irinotecan	Selleck	S2217; CAS: 136572-09-3
Oxaliplatin	Selleck	S1224; CAS: 61825-94-3
Raltitrexed	GLPBIO	GC17750; CAS: 112887-68-0
Carboplatin	GLPBIO	GC11207; CAS: 41575-94-4
Cisplatin	GLPBIO	GC11908; CAS: 15663-27-1
Nedaplatin	GLPBIO	GC15786; CAS: 95734-82-0
Gemcitabine	GLPBIO	GC16805; CAS: 95058-81-4
Paclitaxel	GLPBIO	GC12511; CAS: 33069-62-4
Docetaxel	GLPBIO	GC16684; CAS: 114977-28-5
Floxuridine	GLPBIO	GC18014; CAS: 50-91-9
Doxifluridine	GLPBIO	GC11099; CAS: 3094-09-5
Cetuximab	Macklin	C873860; CAS: 205923-56-4
Lapatinib	GLPBIO	GC13608; CAS: 231277-92-2
Sunitinib Malate	GLPBIO	GC14683; CAS: 341031-54-7
Imatinib	Macklin	I823217; CAS: 152459-95-5
Masitinib	GLPBIO	GC13410; CAS: 790299-79-5
Dovitinib	GLPBIO	GC11372; CAS : 852433-84-2
Sorafenib	GLPBIO	GC17369; CAS: 284461-73-0
Anlotinib Dihydrochloride	GLPBIO	GC25073; CAS: 1360460-82-7
Neratinib	GLPBIO	GC10362; CAS: 89785-84-2
Erlotinib	GLPBIO	GC10627; CAS: 183321-74-6
Trastuzumab	GLPBIO	GC61473; CAS: 1826843-81-5
Pazopanib HCl	Selleck	S1035; CAS: 444731-52-6
Vatalanib	GLPBIO	GC14464; CAS: 212141-54-3
Cabozantinib	GLPBIO	GC12531; CAS: 1140909-48-3
Lenvatinib	GLPBIO	GC36438; CAS: 857890-39-2
Axitinib	GLPBIO	GC12216; CAS: 319460-85-0
Regorafenib	GLPBIO	GC10111; CAS: 755037-03-7
Temsirolimus	GLPBIO	GC12573; CAS: 162635-04-3
Everolimus	GLPBIO	GC13601; CAS: 159351-69-6
Palbociclib	GLPBIO	GC15173; CAS: 571190-30-2
Vemurafenib	GLPBIO	GC13412; CAS: 918504-65-1
GDC0941	Biorbyt	orb154703; CAS: 957054-30-7
Olaparib	GLPBIO	GC17580; CAS: 763113-22-0

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
R version 4.0.3	Open source	https://cran.rproject.org/bin/windows/base/old/4.0.3/
R Studio	Open source	https://www.rstudio.com/
survival package in R	R CRAN	https://cran.rproject.org/web/packages/survival/index.html
survminer package in R	R CRAN	https://cran.rproject.org/web/packages/survminer/index.html
pROC package in R	R CRAN	https://cran.rproject.org/web/packages/pROC/index.html
rms package in R	R CRAN	https://cran.rproject.org/web/packages/rms/index.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Jun Yan (yanjunfudan@163.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The clinicopathological, survival and drug test data of all participants after deidentification are available from the corresponding author; proposals or written requests for access should be directed to Dr. Jun Yan (yanjunfudan@163.com). The data sharing process should be approved by the Ethics Committee of Nanfang Hospital, and a data access agreement should be signed.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request, Dr. Jun Yan (yanjunfudan@163.com).

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

A total of 242 CRC patients who received neoadjuvant chemotherapy or adjuvant chemotherapy with the FOLFOX/XELOX regimen were enrolled in this cohort study from November 2018 to July 2021 at NanFang Hospital (Tables 1 and 8 and Figure S5). Among these patients, 113 patients (median [range] age: 56[25–75] years, including 66 men [58.4%]) were in stage IV, and 129 patients (median [range] age: 58[20–75] years, including 70 men [54.3%]) were in stage III and high-risk stage II. The inclusion criteria were as follows: aged between 18 and 75 years; pathologically diagnosed with CRC; American Society of Anesthesiologists (ASA) score ≤ 3; and underwent surgery or biopsy to obtain samples. The exclusion criteria were as follows: other malignancies; chemotherapy contraindications; lost to follow-up; pregnant or breastfeeding women; and patients diagnosed with middle and lower rectal cancer who planned to receive chemoradiotherapy. For patients who needed neoadjuvant chemotherapy or translational therapy, a biopsy was performed to obtain tumor specimens. For patients who needed adjuvant chemotherapy, surgery was performed to obtain tumor specimens.

The clinicopathological characteristics, including age, sex, body mass index (BMI), tumor location, histological type, tumor differentiation, T stage, N stage, location of distant disease, receipt of neoadjuvant chemotherapy, lymphovascular invasion, carcinoembryonic antigen (CEA) level, and follow-up data, were documented.

Tumor specimens were sampled within 30 min after excision and then immediately sent to the laboratory and stored in RPMI 1690 (L220KJ, Basalmedia) supplemented with 5% penicillin-1-streptomycin (BL505A, Biosharp) at 4°C.

All tissues collected were verified as CRC by pathological examination. This study was approved by the Institutional Review Board of the Nanfang Hospital of Southern Medical University (NFEC-2021-447). All

patients signed an informed consent to participate in the study. All procedures performed in the study involving human participants were in accordance with the Declaration of Helsinki.

METHOD DETAILS

Clinical treatment and follow-up

For a CRC patient with stage IV disease, one multidisciplinary treatment (MDT) team consultation was carried out. If the MDT team decided that simultaneous resection of the primary tumor and metastases could be achieved, the patient received radical surgery and postoperative adjuvant chemotherapy. If the MDT team decided that the primary tumor and metastases could not be resected simultaneously or needed to be resected in stages, the patient received neoadjuvant chemotherapy or translational therapy, and the MDT consultation was performed again to evaluate the timing of surgery after 4 cycles of chemotherapy. For a CRC patient with stage III and high-risk stage II disease, curative surgery was performed, followed by adjuvant chemotherapy.

Patients were regularly tested for blood CEA levels, chest and abdominal enhanced CT, liver MRI or PET-CT if necessary. For patients who received neoadjuvant chemotherapy or translational therapy and then underwent surgery, the blood CEA level was measured every cycle, and CT (MRI or PET-CT if necessary) was performed after 4 cycles of chemotherapy. The pathologic response was assessed using the American Joint Committee on Cancer/College of American Pathologists (AJCC/CAP) regression grade. For patients who received postoperative adjuvant chemotherapy, blood CEA levels were measured every cycle, and CT (MRI or PET-CT) was performed every 4 cycles until the end of 8 cycles of FOLFOX/XELOX chemotherapy. The imaging response was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (RECIST 1.1), which includes complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Stage II or III CRC patients who completed half year chemotherapy were followed up. CEA levels were measured every 3 months for the first 3 years and every 6 months to 5 years. Enhanced CT of the chest and abdomen was checked every 6–12 months.

PDTO culture

Organoid culture was performed as previously described with minor modifications.[19] In brief, tumor samples were washed with 10 mL of Hank's balanced salt solution (HBSS) containing 5% antibiotics 8–10 times and minced with scissors to 1x1x1 mm in size. The minced tissue was digested in 5 mL of 5 mg/mL collagenase type II (Invitrogen) in DMEM/F12 for approximately 3 h at 37°C on a shaker. After filtering, the suspension was centrifuged at 1200 × g for 2 minutes, and red blood cells (RBCs) were removed by adding RBC lysis buffer (00443357, Invitrogen eBioscience) for 5 minutes. Tumor cells were washed, counted and resuspended in a mixture of Matrigel basement membrane Matrix (Corning, 356235) and organoid culture medium-Advanced DMEM/F12 (Gibco, 12634010). Then, 30 μL drops of the Matrigel cell suspension were incubated in a 37°C and 5% CO2 cell culture incubator for 10 min. Once complete gelation occurred, the corresponding culture medium was added to each plate, and the cells were incubated in a 37°C and 5% CO2 cell culture incubator. The medium was refreshed every 2–3 days. All cultures were periodically examined for mycoplasma contamination using the MycoAlert Mycoplasma Detection Assay (Lonza #LT07-218).

Drug library and response assay

A customized 39-drug library was designed for drug sensitivity profiling of the organoids, including 12 chemotherapy drugs, 23 targeted drugs and 4 combination agents. For subsequent organoid drug sensitivity analyses, organoids were harvested from the Matrigel using 1x TrypLE (Gibco) and then dissociated into small clusters. Next, organoids were resuspended in 2% Matrigel/organoid culture medium (200–1000 clusters/ml) and dispensed into 384-well plates (Corning) in triplicate. To maximize cell viability, plates were coated with 0.1% collagen (Thermo Fisher) prior to plating. At 48 h after plating, the drug and vehicle (DMSO, ABT-263/navitoclax) were preprinted onto 384-well tissue culture plates. Chemotherapy drug concentrations typically ranged from 200 μmol/L to 6.25 μmol/L at a 6-point 2-fold dilution. Targeted drug concentrations typically ranged from 3 μmol/L to 0.01236 μmol/L at a 6-point 3-fold dilution. The combination agent combined the corresponding drugs at a 1:1 ratio, and the concentrations typically ranged from 200 μmol/L to 6.25 μmol/L at a 6-point 2-fold dilution. Cell viability was assayed after 96 h of drug exposure using a CellTiter-Glo 3D Cell Viability Assay (Promega) according to the manufacturer's instructions, and the results were normalized to vehicle controls.

Data were analyzed using GraphPad Prism 7.0 software, and the half-maximal inhibitory concentration (IC_{50}) values were calculated by the dose–response curve visualized using nonlinear regression (curve fitting). Drug sensitivity was evaluated by the dose–response curve of cell viability and the IC_{50} value.

H&E staining, AB-PAS staining and immunostaining

All organoids were processed for paraffin embedding and stained with hematoxylin & eosin. Alcian blue/periodic acid-Schiff (PAS) staining and epithelial cell adhesion molecule (EPCAM), Ki67, P53, CDX2 and HepPar-1 immunohistochemical staining were performed as previously described.

QUANTIFICATION AND STATISTICAL ANALYSIS

A receiver operating characteristic (ROC) curve was used to explore the association of organoid drug response with patients' clinical response, by which the optimal cutoff value of IC_{50} was generated. Kaplan–Meier survival curves were used to compare the survival of different groups. Univariate and multivariate Cox proportional hazard regression analyses were performed to estimate the hazard ratio (HR) with a 95% CI and identify the independent predictors for PFS. Student's *t* test was carried out to analyze the IC_{50} values. All statistical analyses were performed in R (version 3.6.0) and GraphPad Prism 7.0. A 2-sided p value < 0.05 was considered to represent a significant difference.

ADDITIONAL RESOURCES

We have no relevant resources.