ERCC overexpression associated with a poor response of cT4b colorectal cancer with FOLFOX-based neoadjuvant concurrent chemoradiation

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Abstract. Colorectal cancer (CRC) of the clinical tumor stage T4b (cT4b) refers to advanced tumors with direct invasion of adjacent structures and the tumors are considered unresectable. Despite advancements in aggressive surgery and combination chemotherapy, the prognosis of cT4b CRC remains poor. Optimizing the therapeutic sequence administered to patients with cT4b CRC to improve clinical outcomes is crucial. In the present study, patients with unresectable cT4b and nodal stage N1-2 CRC were investigated at a single institution. A total of 20 consecutive patients were treated with pre-operative concurrent chemoradiation by using 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX) since February 2015 and were regularly followed up until March 2020. Due to their poor response to concurrent chemoradiation (CCRT) with FOLFOX, the chemotherapy regimen was changed to irinotecan plus 5-fluorouracil/leucovorin (FOLFIRI) as the second-line neoadjuvant treatment. Genetic alterations, such as microsatellite instability (MSI), were documented, and the expression levels of excision repair cross-complementing group 1 (ERCC1) and ERCC2 were examined. Of the 20 patients, the tumors of 14 patients (70%) became resectable after FOLFIRI administration. The median duration between the last date of radiotherapy and surgery was 32.7 weeks (range, 10.1-59.3 weeks). Of note, 4 of the 14 patients with resectable tumors (28.6%) achieved a pathologic complete response. The median overall survival and progression-free survival were 27.5 months (range, 12-39 months) and 27.5 months (range, 8-39 months), respectively. The cancerous specimens of all of the patients (100%) exhibited ERCC2 overexpression and 18 specimens (90%) had ERCC1 overexpression. Only one tumor (5%) exhibited high MSI. The present study indicated that ERCC overexpression associated with the poor response of FOLFOX-based CCRT and FOLFIRI after FOLFOX-based CCRT failure may have a potential role in conversion to resectable tumors by neoadjuvant treatment in cT4b CRC. However, a further prospective study with more patients is required to improve the precision of the conclusions.

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Introduction

The highest incidence of colorectal cancer (CRC) is observed in certain European countries, Australia, New Zealand, Northern America and East Asia (1). The global burden of CRC is expected to persist at least until the year 2035 (2,3). The highest number of cancer-associated deaths are caused by cancers of the lungs, prostate gland, and colon/rectum in males and cancers of the lungs, breasts and colon/rectum in

females (4). CRC is the most commonly diagnosed cancer type in Taiwan (5). Despite significant improvements achieved with multidisciplinary treatment, CRC remains one of the major causes of cancer-associated mortality. Of note, the death rate for CRC decreased by 53% from 1970 to 2016 (4).

A growing body of evidence supports the concept of perioperative treatment for CRC. Concurrent chemoradiation (CCRT) followed by surgery and adjuvant chemotherapy has become the standard treatment for locally advanced rectal cancer (6). An analysis of the National Cancer Database (NCDb) revealed that neoadjuvant radiotherapy (RT) for clinical T4 stage (*c*T4) disease may be associated with superior R0 resection rates and improved overall survival (OS) (7). The clinical T4b classification refers to advanced tumors with direct invasion of adjacent structures and those tumors are unresectable (8).

Excision repair cross-complementing (ERCC) genes encode proteins involved in a complex DNA repair mechanism that is responsible for the removal of DNA lesions and the maintenance of chromosome stability. ERCC1 gene polymorphisms have been investigated as a potential predictive biomarkers of the efficacy of oxaliplatin and platinum treatment in various cancer types (9). Increased ERCC1 expression is associated with clinical resistance to platinum-based chemotherapy. In a previous study by our group, ERCC1 overexpression was indicated to be an independent predictor of poor disease-free survival (DFS) and OS in patients with stage III CRC who received adjuvant 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX) (10). Despite advancements in aggressive surgery and combination chemotherapy, the prognosis of *c*T4b CRC remains poor, with a 5-year survival rate of 15.7-38.5% (11-14). Therefore, identifying potential prognostic biomarkers and optimizing the therapeutic sequence administered to patients with cT4b CRC to improve clinical outcomes are crucial. Whether neoadjuvant treatment for cT4b CRC improves the resectability of tumors and patient survival has remained to be fully determined. A previous study by our group demonstrated that neoadjuvant chemoradiotherapy is feasible and safe with a prominent pathologic complete response in locally advanced colon cancer (15). In the present study, its capacity to convert the tumor into a resectable tumor was comprehensively studied for patients with *c*T4b CRC after neoadjuvant CCRT.

Materials and methods

Patients. The present study assessed patients with unresectable *c*T4b and clinical nodal stage N1-2 (cN1-2) CRC between February 2015 and March 2019 at Kaohsiung medical university hospital. A total of 20 consecutive patients were treated with preoperative CCRT by using the FOLFOX regimen (15). The resectability of their tumors was assessed through imaging techniques such as CT, after 6 to 12 cycles of FOLFOX with duration of 12 to 24 weeks. Only patients who did not respond to this first-line CCRT regimen were included in the present study. This poor response was not expected. However, all patients exhibited a poor response to CCRT and all tumors were still unresectable after CCRT with FOLFOX. Therefore, the chemotherapy regimen was changed to irinotecan plus fluorouracil/leucovorin (FOLFIRI) after FOLFOX-based CCRT (16). The clinical course of the patients was followed up from the time of cancer diagnosis through to their last available clinical record. Genetic alterations, such as microsatellite instability (MSI) and ERCC1 and ERCC2 expression, were examined. Clinicopathological variables, treatment outcomes and adverse events were also analyzed. Patients were included in this study if they had pathologically proven colorectal adenocarcinoma and a clinical diagnosis of cT4b with cN1 or cN2. Patients were excluded if they had a history of prior pelvic irradiation or malignancies other than CRC. A total of two patients were excluded because one underwent prior pelvic irradiation for cervical cancer and the other one underwent the same for prostate cancer. Furthermore, patients with distant metastasis were also not included in this study. Patient follow-ups were performed by visits to the clinic until the end of March 2020.

All of the patients underwent pretreatment workups comprising a physical examination, a history review, chest radiography, bronchoscopy with tumor biopsy, contrast-enhanced CT or MRI and routine laboratory tests. The tumor stage was classified according to the seventh edition of the Cancer Staging Manual and Handbook of the American Joint Committee on Cancer (8). The Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) guidelines, which are also routinely used in clinical practice, were used to evaluate the CRC tumor response (17,18). Side effects were graded according to the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE) (http://ctep.cancer.gov/reporting/ctc.html). The following variables (patient characteristics) were recorded: Age, sex, tumor location, initial clinical tumor and nodal classification, tumor size, ERCC1 and ERCC2 overexpression levels and MSI status in the cancerous specimen by diagnostic biopsy, best objective response according to the RECIST criteria and adverse events.

Ethics approval statement. The present study was approved by the ethical and research committee of Kaohsiung Medical University Hospital [approval no. KMUHIRB-E(I)-20190182]. This study was conducted in compliance with institutional review board regulations in accordance with the Helsinki Declaration of 1975 as revised in 1983. All patients provided written informed consent for the addition of their sample to the collection, use for scientific research and added to a specimen bank; patient information was anonymized prior to the analysis.

Immunohistochemical (IHC) staining of ERCC1 and ERCC2. All incubations were performed at room temperature unless otherwise specified. Formalin-fixed and paraffin-embedded tissue blocks of samples from each patient were used to obtain 4-μm-thick sections, and the sections were deparaffinized in xylene (10,19). They were then rehydrated in a graded alcohol series (100, 95 and 75%). Each rehydration step was performed for 1 min at room temperature. Next, the sections were washed with tap water for 5 min at room temperature. Antigen retrieval was performed using target retrieval solution (pH 9.0; DAKO) in an autoclave (121˚C, 1.2 kg/cm^2) for 10 min, and endogenous peroxidase was blocked in the sections by incubating them in 3% hydrogen peroxide for 5 min. Finally, for antigen retrieval, the sections were immersed in citrate buffer (ERCC1: pH 9.0, ERCC2: pH 9.0) prior to immunostaining at room temperature. The sections were incubated for

15 min at room temperature with antibodies against ERCC1 (dilution, 1/25; cat. no. #ab2356; Abcam) and ERCC2 (dilution, 1/250; #ab111596; Abcam). Next, the samples were treated with the DAKO REAL EnVision Detection System-HRP (DAKO) for 30 min. Finally, the sections were incubated in 3',3-diaminobenzidine for 5 min, followed by Mayer's hematoxylin counterstaining, and dehydration was then performed through 2 changes of 95% ethanol and 2 changes of 100% ethanol. Subsequently, the samples were cleared in 3 changes of xylene and then mounted on slides for observation by using a microscope. Negative controls were prepared by replacing the primary antibody with distilled water.

To improve the accuracy and reduce interobserver differences, the immunostaining of ERCC1 and ERCC2 was scored by two independent pathologists (CYC and YTC). They evaluated slides and scored the extent of immunostaining through light microscopy. They analyzed gene expression based on the intensity of IHC staining and the percentage of positive cancerous cells. Samples with nuclear ERCC1 and cytoplasmic ERCC2 immunostaining were considered positive. ERCC1 and ERCC2 overexpression was defined as a score of 2 (positive staining in >50% of cells), whereas absence of overexpression was defined as a score of 0 or 1.

Pre-operative CCRT and post-operative chemotherapy or RT regimen. All of the patients received CCRT once the diagnosis of stage IIIC CRC was confirmed. For pelvic RT, each patient was placed in a customized thermoplastic immobilization cast. The primary and boost beams were combined in a single integrated treatment plan. A total of 19 patients received intensity-modulated RT. The fractionation scheme was 45 Gy in 25 fractions to the pelvic lymphatic drainage area with a simultaneous boost of 50 Gy delivered to primary tumors and also to metastatic lymph nodes for 18 patients. Furthermore, one patient received 49.5 Gy in 27 fractions to the pelvic lymphatic drainage area with a simultaneous boost of 54 Gy delivered to primary tumors and metastatic lymph nodes. Three-dimensional conventional RT was delivered to one patient with a 44 Gy/22 fraction to the pelvis with a boost of 6 Gy/3 fractions to the tumor. RT was delivered without interruption.

The FOLFOX regimen followed a biweekly schedule concurrent with RT. Each cycle of FOLFOX consisted of oxaliplatin (85 mg/m^2) through a 2-h infusion concurrently with folinic acid (400 mg/m^2) through a 2-h infusion on day 1 and 5-FU (2,800 mg/m^2) through a 46-h infusion repeated every 2 weeks. The FOLFIRI regimen after CCRT consisted of leucovorin calcium (calcium folinate), 5-fluorouracil and irinotecan. Each cycle of FOLFIRI consisted of irinotecan (180 mg/m^2) through a 2-h infusion concurrently with folinic acid (400 mg/m^2) through a 2-h infusion on day 1 and 5-FU (2,800 mg/m^2) through a 46-h infusion repeated every 2 weeks. Furthermore, seven patients received bevacizumab (Avastin; Roche) 5 mg/kg repeated every 2 weeks combined with FOLFIRI.

After a median post-RT follow-up of 24.3 months (range, 7.8-37 months), radical protectomy or colectomy was performed. A total of five patients with negative pathological margins were treated using capecitabine (Xeloda; Roche) at a dose of 850 mg/m^2 every 12 h between days 1 and 14.

Table I. Demographic and clinicopathological characteristics of 20 patients with cT4b colorectal cancer.

Characteristic	Value
Age, years	60.5 (34-75)
Sex	
Male	14 (70)
Female	6 (30)
Tumor location	
Cecum	1 (5)
Ascending colon	4 (20)
Descending colon	2 (10)
Sigmoid colon	4 (20)
Rectosigmoid colon	3 (15)
Rectum	6 (30)
Clinical T classification	
cT4b	20 (100)
Clinical N classification	
cN1	7 (35)
cN2	13 (65)
Tumor size (cm)	
≤5	2 (10)
>5	18 (90)
ERCC1 overexpression	
Yes	18 (90)
No	2 (10)
ERCC2 overexpression	
Yes	20 (100)
No	0 (0)
MSI	
High	1 (5)
Low	2 (10)
MSS	17 (85)
Best objective response (RECIST)	
Complete response	4 (20)
Partial response	12 (60)
Stable disease	2 (10)
Progressive disease	2 (10)
Grade 1/2 adverse events	
Nausea/vomiting	20 (100)
Diarrhea	8 (40)
Skin itchy rash	1 (5)
Leukopenia	16 (80)
Anemia	17 (85)
Thrombocytopenia	6 (30)
Liver toxicities	7 (35)
Oral mucositis	2 (10)
Peripheral neuropathy	5 (25)
Grade 3 adverse events	
Skin itchy rash	1 (5)
Leukopenia	3 (15)
Liver toxicity	1 (5)

Values are expressed as n (%) or the median (range). ERCC1, excision repair cross-complementing 1; MSI, microsatellite instability; MSS, microsatellite stable; RECIST, Response Evaluation Criteria in Solid Tumors.

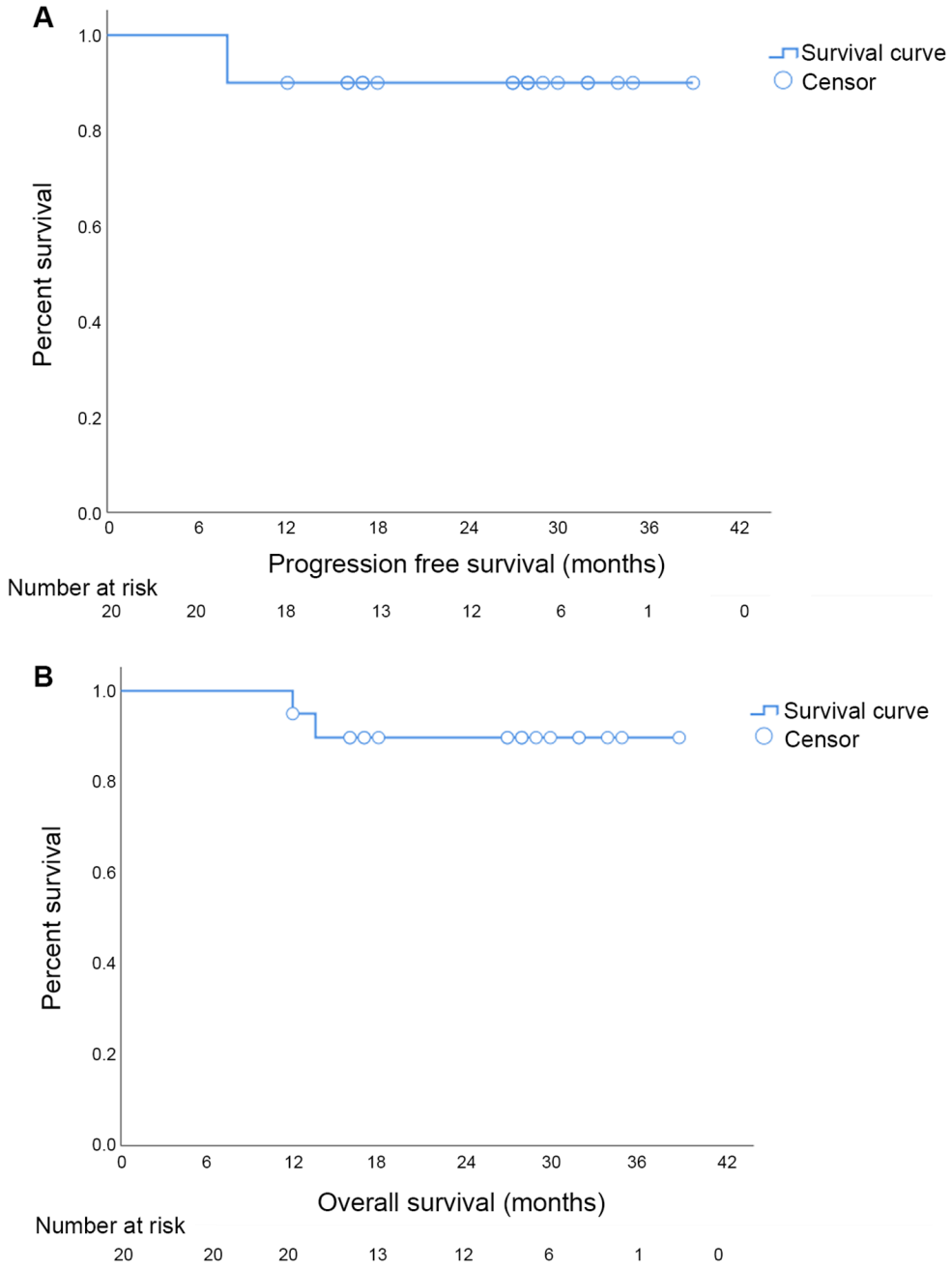

Figure 1. Kaplan-Meier survival analysis for (A) progression-free survival of all 20 patients (range, 8-39 months) and (B) overall survival of all 20 cases (range, 12-39 months).

This protocol was applied every 21 days in 6 to 8 cycles and capecitabine was prescribed for up to 6 months. The other five patients with negative pathological margins and were treated using oral tegafur 100 mg-uracil 224 mg (UFUR; TTY Biopharm Co., Ltd.) at a dose of one capsule three times a day for up to 6 months. Adjuvant FOLFIRI was administered to four patients (three with positive circumferential radial margin involvement and one with 0.5 cm circumferential radial margin).

Statistical analysis. Primary endpoints were OS and progression-free survival (PFS). OS was defined as the period from the date of the start of treatment to the date of death from any cause or until the date of the last follow-up. PFS was measured from the start date of treatment to the date of any type of progression or the final follow-up. OS and PFS rates were assessed using the Kaplan-Meier method. Statistical analyses were performed using the SPSS software package, version 19.0 for Windows (IBM Corp.).

Results

Patient and treatment characteristics. A total of 20 patients were retrospectively enrolled after applying the inclusion and exclusion criteria. The median age was 60.5 years (range, 34-75 years). Table I summarizes the clinical characteristics of the 20 patients. All of the patients had overexpression of ERCC2, which was detected using IHC staining. Furthermore,

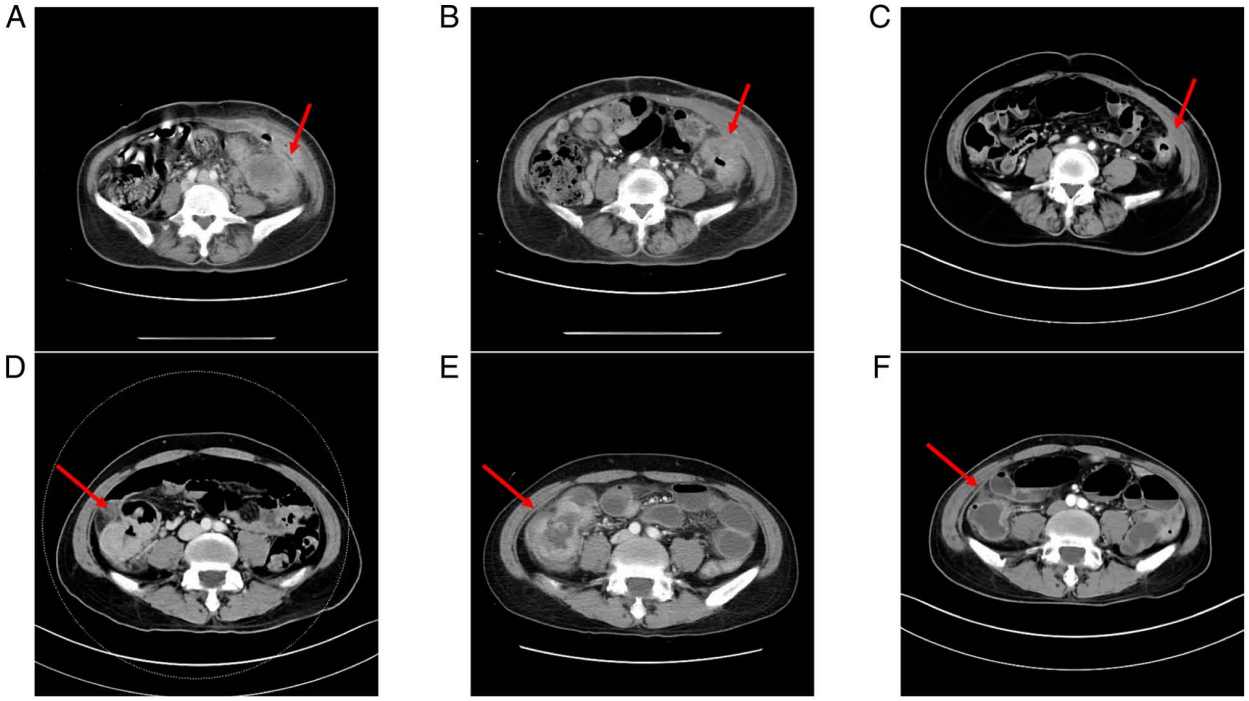

Figure 2. Representative CT images. (A-C) Case no. 5 (A) prior to neoadjuvant CCRT, (B) prior to FOLFIRI treatment and (C) after FOLFIRI treatment. (D-F) Case no. 10 (D) prior to neoadjuvant CCRT, (E) prior to FOLFIRI treatment and (F) after FOLFIRI treatment (red arrows denote the location of the tumor). CCRT, concurrent chemoradiation; FOLFIRI, irinotecan plus fluorouracil/leucovorin.

18 patients exhibited ERCC1 overexpression, whereas 2 patients did not exhibit ERCC1 overexpression. Only one patient exhibited high MSI, two patients exhibited low MSI and 17 patients (85%) exhibited microsatellite stability. According to the RECIST criteria (16,17), 12 patients (60%) were categorized as exhibiting partial response (PR), 2 patients (10%) achieved stable disease and 4 patients had a complete response (CR) (20%). Only two patients exhibited progressive disease (PD). Adverse events noted during treatment were mostly CTCAE grade 1-2 toxicity. None of the patients exhibited any grade 4 toxicity. When the patients experienced CTCAE grade 3 neutropenia, dermatitis, diarrhea, stomatitis, liver toxicity or painful paresthesia for up to 7 days, the oxaliplatin dose was reduced by 25%. The median duration between the last date of radiotherapy and surgery was 32.7 weeks (range, 10.1-59.3 weeks). The median follow-up time in all patients was 24.3 months (range, 7.8-37 months).

Treatment outcomes and failure patterns. Of the 20 inoperable patients, the tumors of 14 patients became resectable after the administration of FOLFIRI. Of the seven patients who received bevacizumab combined with FOLFIRI, three displayed neoplasms that were converted to resectable tumors, while the remaining four patients who received bevacizumab combined with FOLFIRI were still unresectable. A total of six patients underwent radical proctectomy, 5 patients were treated using laparoscopic hemicolectomy and 3 patients received laparoscopy anterior resection. The median OS and PFS were 27.5 months (95% CI: 20.58-28.48) and 27.5 months (95% CI: 19.73-28.37), respectively (Fig. 1). A total of two patients developed distant metastases, but no local recurrence was noted. Furthermore, 18 patients (90%) survived until the end of the present study. The treatment sequence, OS and PFS are listed in Table II. All patients received upfront CCRT with FOLFOX. As a poor response was noted on imaging (Fig. 2), FOLFIRI was added after CCRT.

Among the 14 patients who underwent surgery, 12 patients (85.7%) achieved a pathologic N0 status and 4 patients achieved a pT0 status. Overall, 4 (28.6%) of the 14 cases that became resectable after neoadjuvant treatment achieved pathologic CR (pCR). A total of 12 patients (60%) exhibited an initial CEA level of >5 ng/ml prior to treatment, but only 5 patients (25%) exhibited a CEA level of >5 ng/ml at the last follow-up. Among the 20 patients, after FOLFOX-based CCRT and the FOLFIRI regimen, only one PD patient exhibited an increase of 21.2% in tumor size (longest diameter x widest length) (Table III). The remaining 19 patients had 2.3-73.9% reduction in tumor size compared with the size prior to FOLFIRI administration (Table III).

Discussion

T4b CRC is associated with a poor prognosis due to the direct local extension or infiltration into surrounding structures or organs, thus causing unresectability or a high incidence of nodal and distant metastases (12,20,21). As it involves adhesion to the adjacent organs, complete resection of cT4b CRC is difficult, as the organs may be damaged during resection. A growing body of clinical evidence suggests that the combination of neoadjuvant chemotherapy, specifically pre-operative CCRT, curative surgery and adjuvant chemotherapy, has a favorable prognostic effect on patients with locally advanced CRC (22). In the present study, unresectable tumors in 14 of the 20 patients with CRC (70%), who exhibited a poor response toward CCRT with FOLFOX, were converted to

Table II. Pathological and treatment evaluation of 20 patients with cT4b colorectal cancer.

Case no.	Age, years/sex	Tumor location	Primary tumor resection, yes vs. no	Time intervals between RT completion date to surgery date, weeks	PFS, months	OS, months	Gene overexpression	Survival, yes/no
1	48/M	Sigmoid	No	ND	8	12	ERCC1 (+)/ERCC2 (+)	No (died of cancer)
2	67/M	Rectum	Yes	47	39	39	ERCC1 (+)/ERCC2 (+)	Yes
3	63/M	Rectum	Yes	27.1	35	35	ERCC1 (+)/ERCC2 (+)	Yes
4	65/M	Rectum	No	ND	34	34	ERCC1 (+)/ERCC2 (+)	Yes
5	61/M	Cecum	Yes	13.1	32	32	ERCC1 (+)/ERCC2 (+)	Yes
6	41/M	Ascending	Yes	59.3	32	32	ERCC1 (+)/ERCC2 (+)	Yes
7	60/M	Rectum	Yes	52.9	30	30	ERCC1 (-)/ERCC2 (+)	Yes
8	61/M	Descending	No	ND	8	14	ERCC1 (+)/ERCC2 (+)	No (died of cancer)
9	56/M	Sigmoid	No	ND	29	29	ERCC1 (+)/ERCC2 (+)	Yes
10	61/F	Descending	Yes	35.1	28	28	ERCC1 (+)/ERCC2 (+)	Yes
11	56/F	Ascending	Yes	13.4	28	28	ERCC1 (-)/ERCC2 (+)	Yes
12	75/M	Rectum	Yes	39	27	27	ERCC1 (+)/ERCC2 (+)	Yes
13	39/M	Rectosigmoid	No	ND	28	28	ERCC1 (+)/ERCC2 (+)	Yes
14	53/M	Sigmoid	Yes	25.3	27	27	ERCC1 (+)/ERCC2 (+)	Yes
15	70/F	Ascending	Yes	10.1	18	18	ERCC1 (+)/ERCC2 (+)	Yes
16	64/F	Sigmoid	Yes	34.4	17	17	ERCC1 (+)/ERCC2 (+)	Yes
17	60/F	Rectosigmoid	No	ND	17	17	ERCC1 (+)/ERCC2 (+)	Yes
18	34/F	Rectum	Yes	30.9	16	16	ERCC1 (+)/ERCC2 (+)	Yes
19	68/M	Rectosigmoid	Yes	35.9	16	16	ERCC1 (+)/ERCC2 (+)	Yes
20	34/M	Ascending	Yes	29.3	12	12	ERCC1 (+)/ERCC2 (+)	Yes

ERCC1, excision repair cross-complementing 1; RT, radiotherapy; PFS, progression-free survival; OS, overall survival; ND, not determined; M, male; F, female.

Table III. Treatment outcomes in 20 patients with cT4b colorectal cancer.

Case no.	Sex	Age, years	Pre-CCRT tumor size, cm	Pre-FOLFIRI therapy tumor size, cm	Post-FOLFIRI therapy tumor size, cm	Tumor size reduction, % (post-FOLFIRI minus pre-FOLFIRI)	Post-resection tumor risk	Best overall response (RECIST)
1	Male	48	7.3x5.2	8.0x5.3	9.7x7.5	+21.2	Unresectable	PD
2	Male	67	6.0x3.7	6.5x3.6	2.5x1.5	-61.5	Tumor margin negative	PR
3	Male	63	6.7x3.4	4.6x2.8	1.2x1.0	-73.9	Pathological CR	CR
4	Male	65	7.6x5.8	6.1x4.0	4.6x2.4	-24.6	Unresectable	SD
5	Male	61	7.2x5.3	6.3x5.1	4.6x2.7	-27.0	Tumor margin negative	PR
6	Male	41	6.3x3.2	4.4x2.5	4.3x2.3	-2.3	Tumor margin positive (CRM involved)	PR
7	Male	60	10.9x9.6	6.8x5.5	3.7x3.2	-45.6	Tumor margin negative	PR
8	Male	61	6.0x5.2	5.3x3.5	3.7x3.1	-30.2	Unresectable (due to peritoneal carcinomatosis)	PD
9	Male	56	15.4x10.3	9.8x6.8	6.8x3.1	-30.6	Unresectable (due to tumor invasion to urinary bladder and cecum persisting)	PR
10	Female	61	8.9x4.8	7.5x2.7	3.0x1.3	-60.0	Tumor margin positive (CRM involved)	PR
11	Female	56	5.1x3.2	5.6x3.6	3.6x2.5	-35.7	Tumor margin negative	PR
12	Male	75	5.6x2.7	3.5x2.5	1.0x1.0	-71.4	Pathological CR	CR
13	Male	39	11.6x5.4	10.9x3.7	9.6x3.6	-11.9	Unresectable	SD
14	Male	53	8.6x4.7	6.7x3.4	2.7x1.2	-59.7	Pathological CR	CR
15	Female	70	7.9x3.0	6.0x4.0	4.5x2.4	-25.0	Tumor margin negative	PR
16	Female	64	5.5x5.5	4.9x2.1	1.5x1.0	-69.4	Tumor margin positive (CRM involved)	PR
17	Female	60	7.9x2.8	6.5x2.7	5.1x2.3	-21.5	Unresectable	PR
18	Female	34	5.5x3.5	4.8x1.9	3.2x1.2	-33.0	Tumor margin negative (CRM 0.5 cm)	PR
19	Male	68	4.0x3.5	5.4x3.0	3.0x0.9	-44.0	Pathological CR	CR
20	Male	34	3.9x1.2	4.9x3.0	2.5x1.2	-49.0	Tumor margin negative	PR

CCRT, concurrent chemoradiation; FOLFIRI, irinotecan plus fluorouracil/leucovorin; RECIST, Response Evaluation Criteria in Solid Tumors, the best overall response is evaluated using version 1.1 of the criteria; PD, progressive disease; PR, partial response; CR, complete response; SD, stable disease; CRM, circumferential radial margin. Tumor size, longest length x widest length.

resectable tumors after undergoing FOLFIRI. Gao *et al* (13) analyzed the Surveillance, Epidemiology and End Results (SEER) dataset between 1973 and 2008 in their study. They determined an incidence of 4.4% for pathological T4b CRC. Rectal cancers (21.3%) were reported to have a lower risk of developing a real pathological invasion (pathological T4) than colon cancers (48.8%) (23). The principle of 'en bloc' resection is to resect all invaded organs along with the colon tumor, which may be performed in 65-91.1% of cases (23,24). For unresectable locally advanced disease, pre-operative chemotherapy is delivered to reduce the size of primary tumors and convert them to resectable tumors. The randomized phase III FOxTROT trial of locally advanced, operable colon cancer demonstrated that 3 cycles of pre-operative FOLFOX resulted in significant downstaging compared with post-operative chemotherapy (25). The patients with *c*T4b colon cancer treated with neoadjuvant chemotherapy had a 23% lower risk of death at 3 years than the patients who received post-operative chemotherapy (26). As up to 45% of patients with T4 CRC develop distant metastases, chemotherapy not only results in size regression but also eradicates micrometastatic disease (27). Furthermore, up to 65% of patients with T4 CRC develop nodal dissemination (28), and pre-operative CCRT enhances local RT sensitization and eradication of micrometastases. For locally advanced rectal cancer, pre-operative CCRT also has the potential to increase the rate of sphincter preservation. In the present study, 14 (70%) and 4 patients (20%) with both ERCC1 and ERCC2 overexpression reached PR and pCR after receiving FOLFIRI as a second-line therapy, respectively. In the present study, the conversion to resectability rate (70%) was higher than that observed in previous studies. Population-based data from the SEER dataset from January 1992 to December 2004 on 109,953 patients with colon cancer were compared with NCDb data of 134,206 patients; the 5-year OS rate in the T4bN1-2 patients was 15.8-27.9% (14). Other studies have reported that the 5-year relative survival rate in T4b with nodal involvement was 15.7-38.5% (11-13). A negative resection margin (R0) resulted in a 5-year local control rate for primary locally advanced CRC of up to 89% and 5-year OS of up to 66% (29). In the present study, 11 out of 14 patients had R0 after conversion to resectability and only 3 had positive resection margins.

The use of peri-operative pelvic RT in the treatment of patients with stage III unresectable CRC continues to develop. Well-established neoadjuvant RT protocols for treating T4 colon tumors are not available (22). At present, several cohorts of patients do not respond to pre-operative therapies, resulting in inoperable status and poor survival. The present study aimed to incorporate the detection of ERCC expression for prescribing precision medicine. Pre-clinical and clinical trials involving ERCC have demonstrated the importance of understanding the pharmacogenetics prior to treatment (30-32).

Protein products of ERCC genes are responsible for nucleotide excision repair (NER) of damaged DNA. Oxaliplatin induces adducts, which are not processed by the mismatch repair mechanism. They are predominantly repaired by components of the NER and base excision repair pathways (33). NER removes a DNA segment with adducts, followed by restoration of that DNA segment (34). ERCC1 is an excision nuclease in the NER pathway that is involved in oxaliplatin metabolism (35). High ERCC1 expression levels are associated with increased platinum drug resistance (36). Shirota *et al* (37) first reported that a high level of intratumoral ERCC1 mRNA was associated with poor prognosis in CRC patients who received oxaliplatin-based chemotherapy. A marked increase in ERCC1 protein expression levels was also noted in patients with C/T or T/T genotypes (70% vs. 20%; P<0.01), which was associated with a significantly lower response to FOLFOX-4 (36.4% vs. 57.5%; P=0.01), shorter PFS (7 vs. 13 months; P<0.01) and shorter OS (16 months vs. 25 months; P<0.01) (38). The ERCC1 rs11615 polymorphism with the T allele was indicated to be associated with a significant increase in the risk of shorter PFS and OS in patients with CRC treated with oxaliplatin-based chemotherapy in a recent meta-analysis (9). However, certain studies have not determined any correlation between ERCC1 mRNA expression and FOLFOX activity (39,40). An increase in ERCC protein expression was noted in the patients of the present study, which may account for a poor response to FOLFOX-based CCRT treatment; this result is compatible with the findings of most previous studies (10,19,36). However, 6 patients (30%) had unresectable tumors that were not conversion to resectable tumors by neoadjuvant treatment. Further validation of the ERCC1 for the FOLFIRI regimen in patients with resistance to CCRT and FOLFOX is mandatory.

The present study has certain limitations. Given its nonrandomized retrospective design, a selection bias that may have affected the present results is likely. In addition, only 20 patients from a single institution were analyzed in the present small-sample study. Despite these drawbacks, the conclusions may provide a path for conversion to resectable tumor by using FOLFIRI in patients with *c*T4b CRC that did not sufficiently respond to FOLFOX-based CCRT. The underlying mechanisms of action require further investigation.

In conclusion, the present retrospective study suggested that overexpression of ERCC is an indicator of poor response to FOLFOX-based CCRT and indicated the potential advantage of FOLFORI as a second-line neoadjuvant treatment in *c*T4b CRC that do not respond to FOLFOX-based CCRT. FOLFIRI may potentiate the antitumor response and thereby improve the efficacy of peri-operative treatment for patients with *c*T4b CRC. The underlying mechanisms of action of FOLFIRI in conversion to resectable tumors by neoadjuvant treatment require additional prospective clinical trials to confirm its validity.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JYW designed the study. MYH performed the statistical analysis, participated in the interpretation of data, and wrote and revised the manuscript. HHL drafted the manuscript. JYW, CWH, CJM, HLT and TCY recruited patients for the study and treated them. MYH, HHL and CMH are radiation oncologists who contributed to the provision of RT. CYC and YTC were the pathologists who scored the IHC staining. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This present study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital [Kaohsiung, Taiwan; no. KMUHIRB-E(I)-20190182].

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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