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ORIGINAL ARTICLE

Cetuximab and circadian chronomodulated chemotherapy as salvage treatment for metastatic colorectal cancer (mCRC): safety, efficacy and improved secondary surgical resectability

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Abstract
Background Circadian rhythm disruption was linked to high serum levels of Transforming Growth Factor Receptor α, an Epidermal Growth Factor Receptor (EGFR) ligand and poor survival in patients with metastatic colorectal cancer (mCRC). We hypothesized that EGFR blockade with cetuximab would enhance the activity of chronotherapy as a result of improved circadian coordination.
Methods All the patients with mCRC referred to our unit for progression on prior chemotherapy over a 30-month-period received weekly cetuximab and fortnightly chronotherapy.
Results Fifty-six patients were treated with a median of six courses of fluoropyrimidine-based chemotherapy and irinotecan (61%), oxaliplatin (25%) or both (14%) after a median of three prior regimens. We found no *EFGR* amplification by FISH in the tumor of 27 consecutive patients. Acneiform rash and diarrhea were the most common toxicities. Objective response rate was 32.1% and positively correlated with rash grade ($p = 0.025$). None of the responders had K-Ras mutation in their tumor. Median progression-free and overall survival were 4.6 and 13.7 months, respectively. Complete macroscopic resections of metastases in liver, lung or other abdominopelvic sites were performed following tumor downstaging by the treatment regimen in 11 patients (21%), 8 of whom being alive at 3 years. These figures are twice as high as those reported for first-line combination of cetuximab with conventional chemotherapy or for third line chronotherapy.
Conclusions The addition of cetuximab to chronotherapy allowed safe and effective therapeutic control of metastases, including their complete resection, despite previous failure of several treatment regimens.

Keywords Cetuximab · Chronotherapy · Liver resection · Metastatic colorectal cancer · Neoadjuvant chemotherapy · Circadian clocks

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Introduction

The downsizing of metastatic colorectal cancer (mCRC) with safe and effective neo-adjuvant chemotherapy allows subsequent radical surgical resection of residual metastases. This medico-surgical strategy offers long-term survival to patients despite initially unresectable disease, as initially proven with circadian-based chronomodulated chemotherapy [1–3]. The incorporation of cetuximab in the neo-adjuvant chemotherapy for unresectable mCRC was recently found to be an important asset for the success of this medico-surgical strategy in previously treated patients.

Thus, addition of cetuximab to conventional chemotherapy allowed hepatic resections in 7% of the patients despite prior failure of cytotoxic chemotherapy alone [4]. Cetuximab is a chimeric monoclonal antibody directed against the extracellular domain of the epidermal growth factor receptor (EGFR). It displays activity against mCRC as a single agent [5–7]. It further enhances the efficacy of cytotoxic chemotherapy, either in first-line [7, 8], or in pretreated patients, partly because it reverts resistance to standard chemotherapy [9, 10].

Patients with mCRC can display increased circulating levels of Transforming Growth Factor α (TGF α), a natural ligand of EGFR [11, 12]. It has been previously shown that serum levels of TGF α significantly correlated with poor survival outcome as well as with circadian disruption in mCRC patients [12]. In rodents, only intracerebral infusion of TGFα or EGF, disrupted the circadian clocks in the brain. Of 40 other cytokines tested, no other produced these effects on the circadian rhythm [13, 14]. These preclinical findings emphasize the key role of TGFα/EGF/EGFR ligand receptor interaction and their downstream pathways in the regulation of the circadian clocks that determine 24-h changes in anticancer drug tolerability and efficacy. Circadian clocks are found in all mammalian cells and are comprised of 15 specific genes that control cell proliferation, DNA repair, apoptosis, angiogenesis, metabolism and drug detoxification [15–17]. The adjustment of chemotherapy delivery to circadian clocks—i.e. chronotherapy—improved tolerability and efficacy compared to constant rate infusion first-line treatment for mCRC [18–20]. In addition, a four- to fivefold increased tolerability was achieved with optimally versus poorly timed chronotherapy in 159 pretreated patients with colorectal or lung cancer [21]. The optimal timing ranged 1:00–4:00 a.m. for 5-fluorouracil-leucovorin (5-FU-LV) and 1:00–4:00 p.m. for oxaliplatin or carboplatin. The combination of EGFR antagonists with chronotherapy could further enhance the resectability of CRC metastases, beyond what has already been achieved with cetuximab and standard chemotherapy, as a result of improved coordination of the molecular circadian clocks that rhythmically control nearly 10% of the human transcriptome [15]. This work examines the effects of the addition of cetuximab to chronotherapy for the safe and effective therapeutic control of metastases, and even their complete resection, despite previous failure of several treatment regimens.

Patients and methods

Objective

Preliminary assessment of safety, efficacy and surgical resection rate of cetuximab associated with chronomodulated chemotherapy in patients having failed at least one chemotherapy line.

Selection of subjects

All consecutive patients referred to our unit between March 2004 and November 2006 were given cetuximab combined with chronomodulated chemotherapy in second- or higher line of chemotherapy for metastatic disease provided they had: (1) histologically confirmed mCRC; (2) progressive disease on prior chemotherapy; (3) measurable metastases considered as non resectable with curative intent because of additional bulky disease or ill-placed lesions and/or lesions at multiple sites, (4) no overt brain metastases, (5) WHO performance status (PS) < 3.

Lack of tumor EGFR expression or presence of *K-Ras* mutation did not constitute an exclusion criterion.

Treatment

Cetuximab was given intravenously at a loading dose of 400 mg/m^2 over 2 h, then weekly at a dose of 250 mg/m^2 over 1 h. Chronotherapy consisted of day 1 irinotecan (180 mg/m^2 over 6 h with peak flow rate at 5:00 a.m.), and day 2–4 5-FU-LV (900 and 400 mg/m^2/day, respectively, over 12 h with peak flow rate at 4:00 a.m.) (Fig. 1a). In patients with prior clinical intolerance to irinotecan (repeat grade 3 or 4 gastro-intestinal toxicities or grade 3 asthenia), this chronotherapy regimen consisted of day 1–4 oxaliplatin (20 mg/m^2/day over 12 h with peak flow rate at 4:00 p.m.) and 5-FU-LV (700 and 300 mg/m^2/day, respectively), on the same chronomodulated infusion rate as reported above (Fig. 1b). Chemotherapy courses were repeated every 2 weeks. Patients with grade 3 toxicity had their treatment postponed until at least partial recovery (grade ≤ 2), then resumed with a 25% dose reduction. Treatment was pursued until disease progression, grade 4 intolerable toxicity, or patient refusal.

Assessment procedures

All patients receiving at least one full course of combined cetuximab and chronomodulated chemotherapy course were assessed for toxicity and antitumor efficacy. Tumor response was determined according to RECIST criteria [23], using thoraco-abdomino-pelvic spiral CT-scan and abdomino-pelvic ultrasound (US) at baseline, then repeated every four cycles. All imaging assessments were reviewed by an independent radiologist. Adverse events were graded according to National Cancer Institute of Canada Common Toxicity Criteria (NCIC-CTC) version 3. Decision of metastases resection was made in multidisciplinary staff meeting, based upon imaging and clinical assessment,

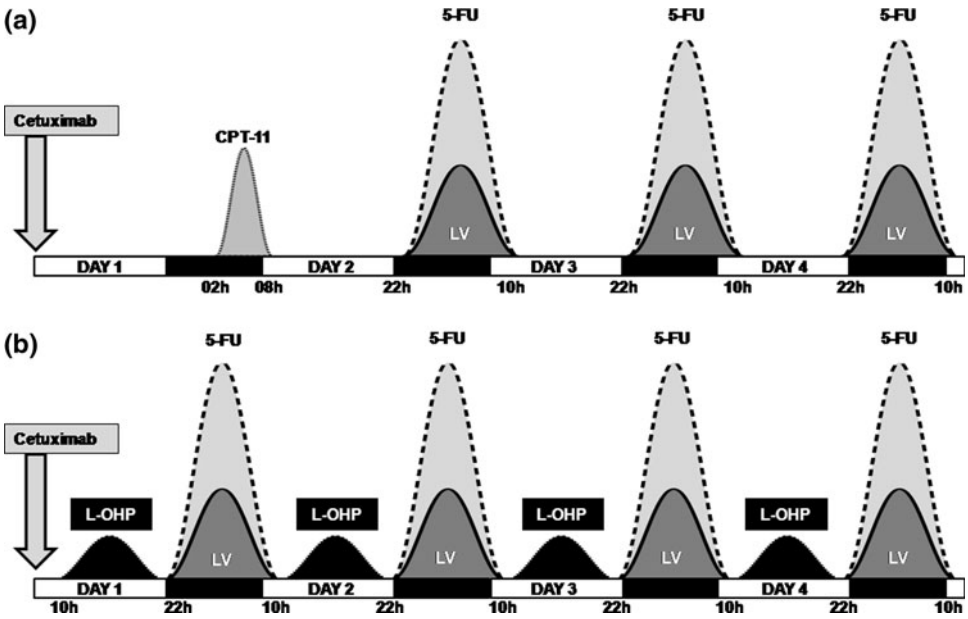

Fig. 1 Chronotherapy schedules automatically infused with a programmable multichannel pump in outpatients every other week, following the administration of cetuximab (250 mg/m^2/week) on day 1. **a** Chronomodulated irinotecan-5FU-LV: Day 1, irinotecan (CPT11, 180 mg/m^2); Days 2–4, 5-fluorouracil (5-FU, 900 mg/m^2/day) and leucovorin (LV, 400 mg/m^2/day). **b** Chronomodulated oxaliplatin-5-FU-LV: Days 1–4, 5-FU (700 mg/m^2/day); LV (300 mg/m^{2}day) and oxaliplatin (l-OHP, 20 mg/m^2/day)

consistently with prior experience [1, 2]. Progression-free survival (PFS) was calculated from inclusion to the first documentation of disease progression. Overall survival (OS) was computed from inclusion in the study until death (from any cause) or December 2009 for patients alive at the conclusion of the study.

Expression of epidermal growth factor receptor

Epidermal Growth Factor Receptor analyses were performed on 4 μm-thick paraffin-embedded sections from primary colorectal cancer and/or resected metastases. EGFR protein expression was assessed with immunohistochemistry (IHC) using EGFR Pharm Dx kit (Dako, Trappes, France) for all the patients. Only cell membrane staining with anti-EGFR antibody (clone 2-18C9) was considered to be specific. EGFR status was considered positive when $\geq$1% of the tumor cells had a complete or incomplete membrane staining. Percentage of tumor cells expressing EGFR was determined and the intensity of staining was semi-quantitatively assessed as follows: 0, no staining; 1+, weak; 2+, moderate; 3+, strong. When the staining intensity was heterogeneous, the highest intensity was utilized. Hepatocytes and peripheral nerves served as positive internal controls and positive (HT-29 cell line) and negative (CAM-1 cell line) external controls were also used.

EGFR gene copy number was assessed by fluorescent in situ hybridization (FISH) using the EGFR/CEN-7 FISH Probe Mix (Dako, Trappes, France), the Texas Red-labeled DNA probe (EGFR) bound to a 196 kb segment containing the *EGFR* gene on chromosome 7q11.2 and the fluorescein-labeled DNA probe (CEN-7) bound to the centromeric region of chromosome 7. *EGFR* amplification was assessed in the tumors from the initial consecutive 27 patients.

Determination of KRAS mutations

The KRAS mutational status was performed on available and technically adequate primary tumor and/or subsequently resected metastases from 14 responders and 2 patients with stable disease (Table 4). Genomic DNA purified from paraffin-embedded tissues was used after histological quantification of tumor tissue in each tumor sample by HES coloration. KRAS mutations located within codons 12 and 13 were screened using the allelic discrimination assay. The detection threshold of our technique was tested using dilution of DNA bearing the various KRAS mutation into normal DNA. All mutations were detectable up to dilution of 1%, except G12V up to 5% and G12S up to 10%. Each sample analysis was performed in duplicate, and wild type and mutated KRAS controls were used in each experiment.

Statistical analyses

Fisher's exact test was used to calculate the univariate correlation between EGFR expression, acneiform rash, and chronotherapy regimen with response to cetuximab. Survival curves were calculated using the Kaplan–Meier method and compared using the log rank test. Univariate analysis was performed on the following factors: gender, age, performance status, site of primary tumor, number of metastatic sites, percent liver involvement, presence of lung metastases, presence of peritoneal metastases, CEA

level, CA19.9 level, EGFR IHC status, number of prior chemotherapy lines, prior failure to fluoropyrimidines, oxaliplatin or irinotecan, prior surgery for metastases and acneiform rash. Those factors with $p < 0.20$ were tested in multivariate analyses based on the Cox model for PFS and OS. Analyses were carried out using the SPSS software (SPSS Inc, USA, version 16.0). The level of significance was set at $p = 0.05$.

Results

Patient characteristics

The baseline characteristics of the 56 consecutive patients meeting the selection criteria are presented in Table 1. The median age was 61 years (range 35–80 years), and 61% were male patients. Patients were heavily pretreated as 86% of them had received two or more previous chemotherapy regimens and 66% had undergone prior resection of metastases. Of note, 75% of the patients had already been given chronotherapy and 84% had been exposed to all three major cytotoxic drugs used for treatment of mCRC, including 5-FU, irinotecan and oxaliplatin. A total of 367 fortnightly treatment courses were administered [median 6 (range 1–22)].

Table 1 Patient characteristics ($N = 56$)

	No. of patients	%
Gender (M/F)	34/22	61/39
PS (WHO)		
0	37	66
1	14	25.0
2	5	9
Primary tumor		
Colon	32	57
Rectum	24	43
Number of metastatic sites		
1	15	27
2	23	41
>2	18	32
Organs involved		
Liver	43	77
Lung	38	68
Peritoneum	6	11
Other	18	32
Baseline serum CEA		
≤Normal (N)	11	20
N to 10 × N	23	41
>10 × N	22	39
Baseline serum CA19-9		
Not determined	3	5
≤Normal (N)	20	36
N to 10 × N	20	36
>10 × N	13	23
No. of prior chemotherapy lines		
1	8	14
2	12	21
≥3	33	65
Prior exposure to cytotoxic drugs		
5-Fluorouracil	56	100
Oxaliplatin	54	96
Irinotecan	49	88
All 3	47	84
Other	25	45
Modality of prior chemotherapy		
Conventional regimen only	14	25
Chronomodulated only	11	20
Both	31	55
Prior surgery		
For primary tumor	54	96
For metastases	37	66

Toxicity and dose intensities

Three patients displayed grade 4 allergic reactions during the first cetuximab infusion prompting discontinuation of the drug. Thus, 53 patients received at least one full course of cetuximab and chemotherapy and were fully assessable. No toxic death was encountered.

Skin toxicity, diarrhea and peripheral neuropathy were the most frequent grade 3–4 adverse events (Table 2). Pre-existing Grade 2–3 sensory neuropathy was reported for 16 patients (28%, including 21% of the patients with Grade 2 and 7% of the patients with Grade 3).

Skin reactions of any kind and any grade were observed in 94% of the patients. Acneiform rash occurred in 83% of patients and reached grade 3 in 34% of the patients. Paronychial cracking on the fingers and/or toes was observed in 19% of the patients. Forty percent of the patients displayed skin cracks, 28% dry skin (xerosis) and 24% facial erythema.

Hypomagnesaemia was grade 1 in 40% of patients, grade 2 in 7% and grade 3 in 2%. Hematological toxicity was mild with grade 4 neutropenia recorded in less than 10% of the patients, without febrile neutropenia or infection, or influence on cetuximab dosing (Table 2). More patients given irinotecan-based regimens experienced grade 3–4 neutropenia (13/39 vs. 0/14; $p = 0.007$) and skin rash (17/39 vs. 1/14; $p = 0.039$) as compared to those receiving oxaliplatin-based regimen.

Table 1 continued

	No. of patients	%
EGFR protein expression (IHC)		
Negative	15	27
Primary tumor only	8	14
Metastasis only	3	5
Primary tumor and metastasis	4	7
1–10%	22	39
Primary tumor only	8	14
Metastasis only	3	5
Primary tumor and metastasis	11	20
>10%	19	34
Primary tumor only	9	16
Metastasis only	3	5
Primary tumor and metastasis	7	13

Table 2 Main toxicities per patient

	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Neutropenia	10 (19.2)[a]	10 (19.2)	8 (15.4)	5 (9.6)
Thrombocytopenia	8 (14.4)	2 (3.8)	0 (0)	1 (1.9)
Anemia	11 (21.2)	8 (15.4)	3 (5.8)	0 (0)
Febrile neutropenia	NA	NA	0	0
Gastrointestinal				
Vomiting	14 (26.4)	23 (43.4)	4 (7.5)	0 (0)
Diarrhea	17 (32.1)	14 (26.4)	11 (20.8)	4 (7.5)
Mucositis	14 (26.4)	21 (39.6)	5 (9.4)	2 (3.8)
Anorexia	11 (20.8)	19 (35.8)	6 (11.3)	0 (0)
Skin and nails				
Acneiform rash	8 (15.1)	18 (34.0)	18 (34.0)	0 (0)
Crack	9 (17.0)	10 (18.9)	2 (3.8)	0 (0)
Perionyxis	6 (11.3)	3 (5.7)	1 (1.9)	0 (0)
Other				
Fatigue	18 (34.0)	26 (49.1)	5 (9.4)	1 (1.9)
Peripheral neuropathy	4 (7.5)	21 (39.6)[b]	12 (22.6)[c]	0 (0)
Alopecia	12 (22.6)	24 (45.3)[c]	–	–

NA not applicable

[a] Number of patients (%)

[b] Including 12 patients with baseline grade 2 sensory neuropathy

[c] Including 4 patients with baseline grade 3 sensory neuropathy

[d] Including 8 patients with baseline grade 2 alopecia

Median dose intensities (mg/m^2/week) over the four initial courses were 250 for cetuximab (range 104–325), 69 for irinotecan (24–105), 32 for oxaliplatin (16–51) and 1,080 for 5-FU (372–1,600). Relative dose intensities were 93.0% for cetuximab, 76.7% for irinotecan, 79.4% for oxaliplatin, and 80.0% for 5-FU.

Efficacy

Among the 53 evaluable patients, 17 patients (32% [95% CI 19.4–44.6]) had an objective response to treatment (26.4% partial and 5.6% complete). Disease remained stable in 18 patients (34%) and progressed in another 18 patients (34%). With a median follow up of 56 months (range 37–69), median PFS was 4.6 months [3.3–5.9] and median overall survival (OS) was 13.7 months [8.1–19.2] (Fig. 2). The median duration of response was 11.7 months [95% CI 9.8–13.6]. Efficacy parameters were similar across the chemotherapy regimens used. There was a significant correlation between tumor response or disease control and rash occurrence and severity (Table 3). Univariate analyses showed that median PFS and OS were prolonged in patients with severe acneiform rash, without lung metastases, or with normal CA19.9 (Table 3). Multivariate analyses confirmed that acneiform rash, lung involvement, and serum level of CA19.9 were independent prognostic factors for PFS. These three factors and age were independent and statistically significant predictors for overall survival. These analyses did not identify any other prognostic factor including PS, gender, number of prior chemotherapy lines, serum CEA level or EGFR protein expression.

Surgical resection of metastases

Eleven patients underwent surgical resection of metastases. Nine of them had received 2 or more prior chemotherapy lines before neoadjuvant cetuximab-chronotherapy. Nine patients also had prior surgery for metastatic disease. Eight patients with an objective response and three patients with stable disease or minor response underwent metastases resection after a median of seven courses of cetuximab-chronotherapy (3–15). Nine patients had R0 resection in liver (4 patients), lung (3 patients), lymph nodes (1 patient) or pelvic recurrence (1 patient), while 2 patients had R1 resection in liver. Overall, the rate of patients whose metastases were macroscopically resected was 20.7% [95% CI 10–32], including liver for 11.3% of the patients. With a postsurgical median follow up of 40.8 months (range 38–55 months), the median relapse-free survival (RFS) for the 11 operated patients was 10.0 months [95% CI 4.0–15.9] with a 80 and 43% survival estimate at 3 and 5 years, respectively.

Tumor molecular predictors of response to cetuximab-chronotherapy

EGFR immunostaining and gene amplification, and KRAS status in responders

The tumor of 15 patients (27%) was EGFR negative with IHC. On the opposite, high positivity was observed in 34%

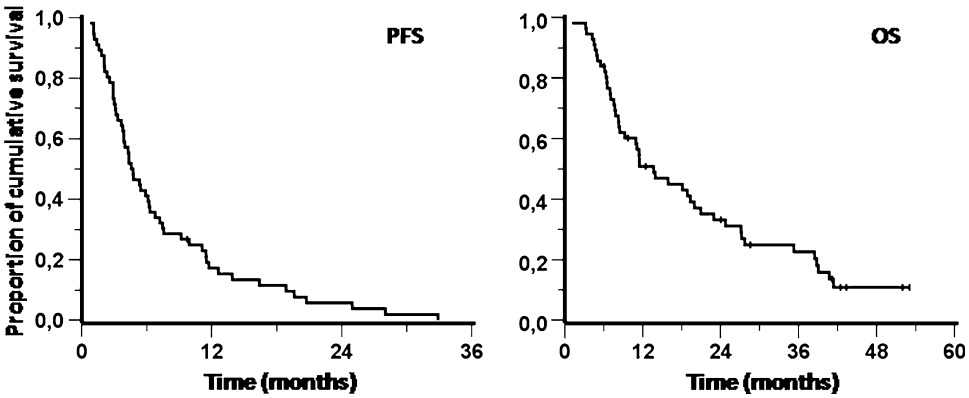

Fig. 2 Progression-free survival (PFS, *left panel*) and overall survival (OS, *right panel*) curves for the 56 patients included in the study (intent to treat, PFS, 55 events; OS, 46 events). Median PFS [95% CI], 4.6 months [3.3–5.9]; median overall survival, 13.7 months [8.1–19.2]

Table 3 Correlations between acneiform rash and response, progression-free survival (PFS) and overall survival (OS) in 53 patients evaluated for efficacy

Rash grade	0	1	2	3	*p* Value
Number of patients	9	8	18	18	
Objective response	0 (0)[a]	1 (12)	9 (50)	7 (39)	0.018
Disease control	3 (33)	4 (50)	12 (67)	16 (89)	0.002
PFS[b]	2.3 [1.7–3.0]	3.3 [1.4–5.3]	6.2 [5.9–6.6]	5.9 [2.9–8.9]	0.003
OS[b]	8.4 [0.0–17.0]	7.8 [4.6–11.1]	13.9 [0.0–28.9]	20.9 [11.5–30.3]	0.002

[a] Number of patients (percent)

[b] Median time, months, with [95% CI]

of the patients (Table 1). Tumor EGFR positive staining did not seem to significantly influence either objective response rate (EGFR+, 26%; EGFR−, 48%, $p = 0.09$), or median PFS (4.7 vs. 7.6 months, $p = 0.10$), or median overall survival (11.5 vs. 22.9 months, $p = 0.15$), even though all these efficacy endpoints appeared to be best in the patients whose tumor did not express EGFR.

No *EGFR* gene amplification was documented by FISH in the tumor specimens from any of the 27 consecutive patients tested, which led us to discontinue this assay. In these patients, objective response rate was 41%; median duration of response was 11.5 months (11 patients); median PFS was 5.9 months, and median survival was 13.7 months; the secondary resection rate was 18.5%. These figures were similar to those obtained in the 53 evaluable patients.

Of note, EGFR expression was undetectable in the tumor of 7 of the 17 responders (41%). No EGFR expression either was found in the tumor of 6 of the 11 patients with complete macroscopic surgical resections (55%).

Tumor *K-Ras* mutation was found in neither the 13 patients who achieved an objective response, nor in the 10 patients undergoing surgery, whose tumor specimens were adequate for such determinations (Table 4).

Discussion

The current study demonstrates that a combined medical–surgical strategy with curative intent can still provide meaningful clinical benefits and prolonged survival in heavily pretreated patients with mCRC. This strategy was based on both safety and antitumor efficacy of a combination of chronomodulated chemotherapy with cetuximab. Single agent cetuximab achieved an objective response in 8–12% of the patients with characteristics similar to our cohort [5, 9]. In these multicenter studies, median PFS was <2 months and median overall survival times were less than 7 months [5, 9]. The combination of cetuximab with single agent irinotecan or oxaliplatin-capecitabine produced response rates of less than 25% as second or third line chemotherapy for metastatic disease [22–24]. This antitumor efficacy further translated into median PFS of 3–5.4 months and median survival of 8.9–10.7 months [25–27]. Importantly, none of these studies reported any subsequent secondary resection of metastatic disease in such heavily pretreated patients. In our study, the combination of cetuximab with chronotherapy achieved an objective response rate of 32.1%, a median PFS of 4.6 months and a median OS of 13.7 months, in patients whose clinical and/or tumor characteristics were by far

Table 4 Survival outcomes and molecular determinants in patients with objective response and/or macroscopically complete surgical resection of metastases from colorectal cancer following salvage therapy with neo-adjuvant chronotherapy and cetuximab

Objective response	Patient ID #	Tumor site(s)	K-Ras status	Max EFGR + cells (%)	R0–R1 resection	DFS (months)	PFS (months)	Survival (months)
CR	18	T	NAv	0	Yes	6.0	11.1	11.1
		M	wt	NAv				
	26	T	NAv	10	Yes	8.3	13.9	38.8
		M	wt	NAv				
	38	T	NAv	1	Yes	10.6	20.7	53.1+
		M	wt	1				
PR	28	T	wt	0	Yes	6.0	11.8	52.0+
		M[a]	wt	NAv				
	39	T	NAv	1	Yes	1.4	6.2	39.0
		M	wt	1				
	41	M	wt	10	Yes	2.6+	9.7+	9.7+
	47	T	wt	NAv	Yes	27.3	32.9	43.3+
		M	wt	0				
	50	M	wt	50	Yes	21.9	24.9	42.5+
	9	T	wt	30	No	NAp	7.5	13.7
		M	wt	NAv				
	11	T	NAv	10	No	NAp	6.3	18.9
		M	NAv	10				
	15	T	NAv	0	No	NAp	12.6	27.7
		M	wt	0				
	19	T	wt	20	No	NAp	5.9	28.6+
		M	NAv	20				
	23	M	NAv	0	No	NAp	19.6	22.9
	25	T	wt	1	No	NAp	11.5	38.4
	34	T[b]	NAv	20	No	NAp	28.0	40.7
	40	T	NAv	0	No	NAp	4.6	18.1
	44	T	wt	0	No	NAp	4.3	24.7
SD	43	M	wt	0	Yes	10.0	16.4	41.4
	45	T	NAv	0	Yes	12.4	18.9	27.2
		M	wt	NAv				
	51	T	NAv	0	Yes	0.5	3.8	41.2+

NAp not applicable, *NAv* not available, *T* primary tumor, *wt* wild type

[a] A mutation in K-Ras codon12 was found in a liver metastasis resected after cetuximab treatment

[b] A mutation in K-Ras codon13 was found in a local relapse resected after cetuximab treatment

worse than those in the above reports [5–9, 25–27]. The efficacy of cetuximab combination with chronotherapy also compares favorably with that obtained with the chronomodulated administration of irinotecan, 5-FU-LV and oxaliplatin in 77 patients with characteristics as pejorative as in the current study [28]. Thus, a macroscopically complete surgical resection of metastases was performed in only ~1/3 of the patients that were successfully resected in the current study [28].

The incidence of adverse events in our heavily pretreated patients cohort was acceptable, based on a comparison with the toxicities reported for first-line cetuximab-FOLFIRI against mCRC [8]. Thus, the incidence of severe neutropenia was 30% in our study, as compared to 28.2% in the Crystal trial. However, the incidence of non haematological toxicities was higher in our series as compared to the Crystal one for diarrhea (28.3 vs. 15.7%) and for acneiform rash (34 vs. 16.2%). We believe that the main reason for this apparent difference in non haematological toxicities relates to the fact that 86% of the patients in our study had received two prior chemotherapy lines, 84% had already been exposed to 5-FU, irinotecan and oxaliplatin, and 66% had previously undergone prior metastases resections, while no prior therapy had been offered to the patients in the Crystal

trial. In addition, the chronomodulated administration of irinotecan with peak delivery at 5 a.m. resulted in increased biotransformation into SN-38 as compared to conventional 30-min infusion at 10 a.m. [29]. The enhanced SN-38 exposure brought about by chronomodulated irinotecan could account both for increased diarrhea and increased antitumor efficacy in our series. This might also plays a role in the severity of the acneiform rash although mechanisms currently remain speculative.

Indeed, an important finding in the current study is the high neoadjuvant potential activity of cetuximab's addition to chronotherapy, since 21% of the evaluable patients underwent subsequent macroscopic resection of metastases previously considered as unresectable. In this population, with very advanced disease, surgery involved multiple sites of extrahepatic metastases, consistently with the late dissemination of the disease beyond the liver itself. Despite this, the overall benefit of an aggressive medico-surgical strategy in the resected patients of our study is supported by an estimated survival rate of 43% at 5 years. This latter figure compares favorably with a 5-year survivorship of 33% in a series of 184 patients undergoing neoadjuvant chemotherapy and partial hepatectomy for liver metastases from colorectal cancer. Only 29% of the patients in this series had received prior chemotherapy [30].

The neoadjuvant potential of combining cetuximab with chemotherapy was recently shown by our team in previously treated patients [4]. An updated assessment of this latter series reveals that partial hepatectomies were performed in 5 of 45 patients on cetuximab-chronotherapy (11.1%) as compared to 5 of 98 patients on conventional delivery (5.1%), a difference that supports the apparently increased rate of complete macroscopic resections with cetuximab and chronotherapy in the current study, and merits further investigation.

Patient outcome in our cohort was correlated with the occurrence and severity of skin toxicity, as reported in most studies with cetuximab [9, 10, 23–27, 31].

A wild type *K-Ras* status has been demonstrated to predict positive response to cetuximab therapy [30, 32] and was confirmed in this cohort of patients receiving cetuximab combined with chronotherapy. Our study also confirmed that the IHC detection of EGFR protein does not predict for cetuximab efficacy [33–35]. No *EGFR* gene amplification was found in any of the tumor specimens studied. The lack of *EGFR* gene amplification was reported to predict a lack of activity of cetuximab or panitumumab, eventually combined with irinotecan-based chemotherapy [36–39]. In our study, the lack of *EGFR* amplification was consistent with its recently reported low incidence [40]. Moreover, it did not impair any of the efficacy endpoints in the patients receiving chronotherapy and cetuximab.

The improved efficacy of circadian chronomodulated chemotherapy with addition of cetuximab as compared to conventional delivery of infusion chemotherapy could stem from specific effects of cetuximab on the circadian timing system that governs chronopharmacological processes [15, 29]. Cetuximab could not only revert resistance to chemotherapy, but also prevent EGFR binding of TGF-α and restore circadian function in host cells, further improving the circadian control of malignant growth and the sensitivity to chronotherapy itself through host-mediated mechanisms [12]. In such case, the antitumor activity of cetuximab addition to chronotherapy would remain unaffected by expression of EGFR protein or *EGFR* gene amplification in tumor. Indeed, this was the case in our study, where all efficacy endpoints were highest in patients whose tumor had undetectable EGFR. Experimental and clinical data support the role of the circadian timing system as a control point in tumor progression [41, 42]. Circadian disruption has been independently associated with poor tumor response to therapy and poor survival in patients with mCRC [43], with a prospective confirmation in an international study [44]. Clinical data further support the ability of gefitinib, an inhibitor of the tyrosine-kinase domain of the EGFR, to improve circadian coordination [45]. This small molecule that impairs intracellular signaling pathways downstream of the EGFR receptor, concurrently restored a near normal rest-activity circadian rhythm and improved fatigue in advanced non-small cell lung cancer patients, despite an absence of demonstrable tumor response to therapy [45].

In conclusion, the combination of cetuximab and chronotherapy offers an effective option for salvage therapy in heavily pretreated patients. Importantly, this strategy is now demonstrated to render initially unresectable distant metastases resectable with the expectation of improved survival as part of an aggressive combined medical-surgical treatment plan.

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