Research Article

Impact of CAPOX or FOLFOX4 on Spleen size, Platelet Count and Liver Function when Partnered Cetuximab as First-line Treatment for KRAS Wild-type Metastatic Colorectal Cancer

Victor HF Lee[1*], Wei-Jia Fang[2], Ka-On Lam[1], Cheuk-Wai Choi[3], Sherry CY Ng[1], Garrett CL Ho[4], Thomas KC Cheng[4], Patty PY Ho[1], Rico KY Liu[1], To-Wai Leung[1], Dora LW Kwong[1], Shu-Sen Zheng[2*]

[1]Department of Clinical Oncology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong
[2]The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, People's Republic of China
[3]Department of Community Medicine, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong
[4]Department of Nuclear Medicine & Positron Emission Tomography, Hong Kong Sanatorium & Hospital, Hong Kong

Abstract

Objectives: Oxaliplatin can cause hepatic sinusoidal injury and splenomegaly. It remains unknown if the magnitude of injury would differ when oxaliplatin is combined with capecitabine or 5-FU with/without cetuximab. We investigated the impact of 1st line CAPOX or FOLFOX4 and the additional cetuximab on spleen size, platelet count and liver function in patients with KRAS wild-type metastatic colorectal cancer (mCRC).
Methods: 101 Patients planned to receive either CAPOX or FOLFOX4 with/without cetuximab as first-line treatment were prospectively recruited. Changes in spleen size by volumetric measurement after treatment were determined. Correlation studies were performed for factors associated with changes in spleen size, thrombocytopenia and impaired liver function.
Results: The spleen enlarged (median +17.9%, $P < 0.001$) after treatment. Multivariable analysis revealed that capecitabine, its dose intensity and cumulative dose (per 10000mg increase) correlated with splenomegaly ($P = 0.01$, $P = 0.02$ and $P = 0.006$, respectively). Increase in spleen size ($P = 0.004$) and splenomegaly ($P = 0.002$) correlated with thrombocytopenia. Dose intensity and cumulative dose of capecitabine (per 10000mg increase) and increase in spleen size correlated with grade ≥1 impaired liver function ($P = 0.01$, $P = 0.003$ and $P = 0.04$, respectively). Use of cetuximab correlated with less splenic enlargement (+13.7% vs. +22.7%; $P = 0.04$), especially when coupled with FOLFOX4 rather than CAPOX (+1.1% vs. + 23.0%; $P = 0.003$).
Conclusions: Capecitabine was associated with more splenomegaly which in turn correlated with thrombocytopenia and impaired liver function. Cetuximab offered some protection from further splenic enlargement especially when combined with FOLFOX4.

Keywords: cetuximab, fluoropyrimidine, impaired liver function, splenomegaly, thrombocytopenia

*Corresponding author: Victor HF Lee and Shu-Sen Zheng, Department of Clinical Oncology, 1/F, Professorial Block, Queen Mary Hospital, 102 Pokfulam Road, Pokfulam, Hong Kong. Email: vhflee@hku.hk and shusenzheng@zju.edu.cn
Citation: Lee VH, et al. Impact of CAPOX or FOLFOX4 on Spleen size, Platelet Count and Liver Function when Partnered Cetuximab as First-line Treatment for KRAS Wild-type Metastatic Colorectal Cancer. Cancer Research Frontiers. 2015 Feb; 1(1): 60-74. doi: 10.17980/2015.60

Competing Interests: The authors declare that they have no competing interests.
Received Nov15, 2014; Revised Jan 11, 2015; Accepted Feb 16, 2015; Published Feb 28, 2015.

Introduction

Fluoropyrimidines and oxaliplatin have been used in metastatic colorectal cancer (mCRC) for more than ten years (1). Oxaliplatin when combined with capecitabine (CAPOX) or infusional 5-FU and folinic acid (FOLFOX regimen) was found equally efficacious in first-line setting (2-4). Addition of targeted therapy including anti-epidermal growth factor receptor (EGFR) or anti-vascular endothelial growth factor (anti-VEGF) monoclonal antibody further improves the response rate and overall survival in first and subsequent lines of treatment (5-11). The Medical Research Council (MRC) COIN trial, was the largest phase III randomized controlled trial which investigated the effect of cetuximab, a monoclonal antibody against EGFR, on overall survival when it was added to oxaliplatin- and fluoropyrimidine-based chemotherapy as first-line therapy in mCRC. The choice between oxaliplatin plus infusional 5-FU and oxaliplatin plus capecitabine was not randomized but it was an agreement between patients and treating physicians before treatment commencement. Its first publication revealed that severe grade 3/4 diarrhea was observed in 30% of patients who received oxaliplatin and capecitabine, leading to study protocol amendment in 2007 with dose reduction of capecitabine from 1000mg/m^2 to 850mg/m^2 twice daily in future patients (12). This may be one of the reasons of failure to improve overall survival as published in 2011 (13). At the same time, with growing experience of using oxaliplatin in the past decade, this drug was also noted to have close association with hepatic sinusoidal injury and post-hepatectomy morbidity and mortality when given pre-operatively (14-19). Moreover increase in spleen size was recently proven an effective biomarker for such hepatic adverse event after oxaliplatin (20). This hepatic complication is definitely a particular concern to the surgeons and patients when perioperative chemotherapy is increasingly adopted for potentially resectable liver metastases (21). On the other hand, while bevacizumab was previously shown to carry a protective effect from excessive increase in spleen size, there has been so far no similar report for cetuximab and whether the choice between 5FU and capecitabine would pose any extra effect on the spleen size (22,23). Based on all of the above, we initiated a prospective study in 2010 to assess the change in spleen size, platelet counts and liver function in patients with KRAS wild-type mCRC treated with either CAPOX or FOLFOX4 with or without cetuximab as first-line treatment.

Materials and Methods

Patients and study design

This study was initiated in January 2010 with approval from local institutional review board. Informed consent was obtained from every patient recruited into this study. Patients with histologically proven KRAS wild-type mCRC who planned to receive either CAPOX or FOLFOX4 with or without cetuximab as first-line systemic treatment were prospectively recruited into this study. Determination of KRAS mutations from formalin-fixed paraffin-embedded tumor biopsies was made by QIAmp Deoxyribonucleic acid (DNA) FFPE tissue kit (Qiagen, Hilden, Germany), followed by polymerase chain reaction (PCR) amplification and direct sequencing using enriched tumor genomic DNA before treatment (24). All patients had baseline contrast-enhanced computed tomography (CT) scans of 5mm slice thickness of the thorax, abdomen and pelvis performed for the confirmation of primary tumor if present, regional nodal involvement and distant metastasis by board-certified radiologists within 4 weeks before treatment. After baseline physical examination and blood tests for hematology and biochemistry, they discussed with their treating physicians for choices between CAPOX and FOLFOX4 and whether to add cetuximab in addition to the chemotherapy regimen as a self-financed drug. Baseline liver impairment was defined according to liver biochemistry. Serum total bilirubin, albumin, alkaline phosphatase (ALP), alanine transferase (ALT) and aspartate transferase (AST) were each scored on a 0-4 scale according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 3.0 (25). These values were then summed up to give a baseline liver impairment score, which was further subclassified into 4 groups (baseline liver impairment score 0, 1-4, 5-8 and ≥9) accordingly, slightly modified and adapted from the criteria devised by Twelves et al (26). CAPOX was given as a 3-weekly regimen with oxaliplatin 130mg/m^2 infused over 2 hours on day 1 followed by capecitabine 1000mg/m^2 orally twice a day for 2 weeks followed by a 1 week rest period. FOLFOX4 consisted of oxaliplatin 85mg/m^2 infused over two hours concurrently with folinic acid 200mg/m^2 on day 1, followed by bolus 5-FU 400mg/m^2 and continuous 5-FU infusion over 22 hours on day 1 and 2, given as a 2-weekly regimen. For those who also opted for cetuximab, they received an initial loading dose of cetuximab 400mg/m^2 infused over 2 hours followed by subsequent 250mg/m^2 over 1 hour once weekly. Dose reduction of chemotherapeutic drugs and cetuximab was in accordance with the departmental guidelines of the treating institution and recommendation from drug manufacturers. Blood tests for hematology and

biochemistry were performed before every cycle of treatment and additional blood tests were also arranged if clinically necessary. Thrombocytopenia was defined as platelet count less than 150×10^9/liter (20). All toxicities and adverse events were graded with NCI-CTCAE Version 3.0 (25). Treatment was discontinued at the time of development of disease progression, cumulative toxic events or patient's preference. Patients who discontinued one or more agents within the treatment regimen as a result of toxic events while continuing on the remaining agent(s) or those who switched from CAPOX to FOLFOX4 or vice versa (with/without cetuximab) for whatever reason were excluded from this study. Patients with past history of chronic hepatitis C infection were excluded but those with chronic hepatitis B infection were allowed provided that their e antigen was absent and they had received anti-viral therapy at least 1 week before systemic treatment and continued the therapy until at least 3 months beyond completion of systemic treatment.

Volumetric Evaluation of Spleen Size

After baseline CT evaluation, subsequent contrast-enhanced CT scan of the same regions with the same slice thickness was repeated 9 to 10 weeks later (i.e. after 5-6 cycles for FOLFOX4 and after 3-4 cycles for CAPOX (CT Time Point 1) and then again after the same time interval (CT Time Point 2) and so on until treatment discontinuation. All patients in this study had at least one reassessment CT scan at CT Time Point 1. All CT images were then uploaded into *Eclipse Treatment Planning System* (Palo Alto, CA) version 8.0 for determination of spleen size changes. One designated clinical oncologist (WJ Fang) who was blinded to the patient identity and demographics as well as treatment details, contoured the spleen on the CT images at baseline and all subsequent time points for all patients, with the method described in our previous publication (27). No patients were identified to have splenic metastasis. The whole liver, as well as any liver metastases if present and the resultant net liver were also contoured as well for subsequent statistical analysis. The resulting sum of the areas of the net liver and spleen were calculated by the treatment planning system to generate the respective volumes. Patients who had previous splenectomy or radiological features of cirrhosis were not allowed in this study. Changes in spleen size were then determined at each CT time point and compared to those at baseline. Splenic enlargement meant any increase in spleen size while splenomegaly was defined as an increase by 50% or more, compared with the baseline (20).

Statistical Analysis

Patient demographics were compared using Mann-Whitney U tests for continuous variables and Chi-square tests for categorical variables. Changes in spleen size were calculated by Mann-Whitney U tests for different groups of patients. Univariable and multivariable logistic regression were performed to assess the correlation of the following covariates with splenic enlargement, splenomegaly, thrombocytopenia and impaired liver function: age, sex, baseline body surface area, baseline body weight, Hepatitis B infection, history of diabetes mellitus, smoking history, alcoholic history, baseline liver impairment scores, baseline creatinine clearance, baseline net liver and spleen volume, presence of liver metastasis, number of liver metastasis, volume of liver metastases, use of 5-FU, capecitabine and cetuximab, dose intensity of oxaliplatin, 5-FU, capecitabine and cetuximab and cumulative dose of oxaliplatin, 5-FU, capecitabine and cetuximab. Only covariates considered significant at P value < 0.1 in the univariable analysis were included in the multivariable model. Statistical results were considered significant if the P value was < 0.05. All statistical analyses were performed by Statistical Package for Social Sciences (SPSS Institute, Chicago, IL) version 20.

Results

Overall study population

Altogether 101 patients were recruited and evaluated (Table 1 & 2). 32, 24, 25 and 20 patients received CAPOX, FOLFOX4, CAPOX plus cetuximab and FOLFOX4 plus cetuximab respectively. 23, 17, 19 and 9 patients from CAPOX, FOLFOX4, CAPOX plus cetuximab and FOLFOX4 plus cetuximab respectively had their primary tumor resected before study entry (Table 1). No patients received surgery for any of the metastatic nodules before and after this study. After reassessment CT scan at CT Time Point 1, 43 (42.6%) patients continued the treatment beyond CT Time Point 1 without disease progression or signs of intolerable toxicities and had their second scan at CT Time Point 2. The remaining 58 patients stopped treatment after CT time point 1 because of disease progression in 30 (29.7%) patients as well as intolerable toxicities secondary to prolonged immunosuppression (20 patients, 19.8%), grade 3 oxaliplatin-related hypersensitivity reaction (3 patients, 2.9%) and oxaliplatin-related peripheral neuropathy (5 patients, 5.0%).

Table 1. Distribution of Baseline Demographics in the Study Population

	CAPOX (n = 32)	**FOLFOX4 (n = 24)**	**CAPOX + cetuximab (n = 25)**	**FOLFOX4 + cetuximab (n = 20)**	***P***
Median age (range)	59.5 (43–80)	59.0 (44–76)	60.0 (42-78)	57.0 (40–80)	0.88
Sex					
Male	20 (62.5%)	12 (50.0%)	16 (64.0%)	7 (35.0%)	0.18
Female	12 (37.5%)	12 (50.0%)	9 (36.0%)	13 (65.0%)	
ECOG performance status					
0	3 (9.4%)	2 (8.3%)	3 (12.0%)	4 (20.0%)	0.42
1	29 (90.6%)	22 (31.7%)	22 (88.0%)	15 (75.0%)	
2	0 (0%)	0 (0%)	0 (0%)	1 (5.0%)	
Median baseline body surface area (range)	1.56 (1.35–1.85)	1.62 (1.35–1.83)	1.63 (1.37–1.78)	1.54 (1.34–1.99)	0.50
Median baseline body weight (kg) (range)	57.1 (42.7–78.2)	61.5 (43.2–73.5)	58.3 (40.5–69.8)	57.1 (40.5–95.0)	0.79
Median baseline creatinine clearance by Cockroft and Gault formula (ml/minute) (range)	69.4 (33.9–111.0)	75.3 (36.4–98.7)	66.8 (38.4–149.2)	77.9 (36.9–156.2)	0.84
Previous alcoholic consumption					
Never	27 (84.4%)	21 (87.5%)	17 (68.0%)	19 (95.0%)	0.09
Current or previous drinker	5 (15.6%)	3 (12.5%)	8 (32.0%)	1 (5.0%)	
Smoking status					
Never smoker	24 (75.0%)	21 (87.5%)	17 (68.0%)	17 (85.0%)	0.32
Current or ex-smoker	8 (25.0%)	3 (12.5%)	8 (32.0%)	3 (15.0%)	
Hepatitis B surface antigen (HBsAg) positivity	2 (6.3%)	4 (16.7%)	1 (4.0%)	1 (5.0%)	0.33

Table 1. (continued)

Liver impairment score (median), range)	0 (0–6)	0 (0–3)	0 (0–3)	1 (0–10)	0.23
0	23 (71.9%)	16 (66.7)	14 (56.0%)	9 (45.0%)	0.20
1 – 4	7 (21.9%)	8 (33.3)	11 (44.0%)	8 (40.0%)	
5 – 8	2 (6.2%)	0 (0%)	0 (0%)	2 (10.0%)	
≥9	0 (0%)	0 (0%)	0 (0%)	1 (5.00%)	
History of diabetes mellitus	2 (6.3%)	3 (12.5%)	2 (8.0%)	1 (5.0%)	0.79
Site of primary					
Colon	14 (43.8%)	16 (66.7%)	18 (72.0%)	16 (80.0%)	0.10
Rectum	15 (46.9%)	7 (29.2%)	7 (28.0%)	4 (20.0%)	
Synchronous colon and rectal cancer	3 (9.3%)	1 (4.1%)	0 (0%)	0 (0%)	
Primary resected	23 (71.9%)	17 (70.8%)	19 (76.0%)	9 (45.0%)	0.12
Median number of sites of metastasis (range)	1 (1–5)	1 (1–4)	1 (1–4)	2 (1–4)	0.08
Presence of liver metastasis	11 (34.4%)	5 (20.8%)	16 (64.0%)	16 (80.0%)	< 0.001
Types of metastasis					
Liver limited disease only	4 (12.5%)	2 (8.3%)	8 (32.0%)	7 (35.0%)	0.05
Liver and others	7 (21.9%)	3 (12.5%)	8 (32.0%)	10 (50.0%)	0.04
Non-liver	21 (65.6%)	19 (79.2%)	9 (36.0%)	3 (15.0%)	< 0.001
Median number of liver metastasis (range)	0 (0–17)	0 (0–8)	2 (0–17)	3 (0–16)	< 0.001
0 lesion	22 (68.8%)	18 (75.0%)	9 (36.0%)	6 (30.0%)	0.004
1-5 lesions	4 (12.5%)	4 (16.7%)	8 (32.0%)	8 (40.0%)	
>5 lesions	6 (18.9%)	2 (8.3%)	8 (32.0%)	6 (30.0%)	

Table 1. (continued)

Median volume of liver metastases (cm^3) (range)	0 (0–840.80)	0 (0–201.33)	2.1 (0–1621.15)	24.4 (0–4091.78)	0.001
Lung metastasis	12 (37.5%)	10 (41.7%)	2 (8.0%)	5 (25.0%)	0.04
Bone metastasis	3 (9.4%)	1 (4.2%)	3 (12.0%)	1 (5.0%)	0.70
Brain metastasis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA
Distant lymph node metastasis	15 (46.9%)	11 (45.8%)	10 (40.0%)	10 (50.0%)	0.92
Median baseline net liver volume (cm^3) (range)	1077.45 (759.06–1907.82)	1048.49 (724.72–1766.63)	1091.13 (851.38–1475.85)	1115.84 (438.35–2140.68)	0.55
Median baseline spleen volume (cm^3) (range)	112.61 (46.74–258.76)	136.39 (23.37–339.66)	125.52 (38.92–261.38)	132.40 (77.52–305.63)	0.31

Serial volumetric evaluation of the spleen revealed that 85 patients (84.2%) had their spleen enlarged while 30 patients (29.7%) had splenomegaly at CT Time Point 1 after treatment. Median increase in spleen size was 17.9% (range, –27.7% to +296.6%, $P < 0.001$) at CT Time Point 1 in whole study population. Patients who received cetuximab had less splenic enlargement than those who did not (median +13.7% vs. +22.7%, $P = 0.04$) (Fig. 1a). Also fewer patients (68.9%) who received cetuximab had their spleen enlarged as compared with those who did not receive cetuximab (85.7%, $P = 0.04$). This was especially seen in those when FOLFOX4 was added to cetuximab than those who had FOLFOX4 alone (median +1.1% vs. +18.0%, $P = 0.009$), and to a lesser and non-significant extent, in those who had CAPOX plus cetuximab as compared with CAPOX alone (median +23.0% vs. +32.5%, $P = 0.46$) (Table 2).

Thrombocytopenia was noted in 56 (55.5%) patients with 5.0% being grade ≥3 events. Grade ≥1 and grade ≥3 impaired liver function was noted in 51.5% and 1.0% respectively.

CAPOX vs. FOLFOX4

Use of CAPOX appeared to cause greater enlargement of spleen (median +32.5%) when compared with FOLFOX4 (median +18.0%, $P = 0.12$) (Fig. 1b). Splenomegaly was also more commonly detected in those who received CAPOX (34.4%) compared with FOLFOX4 (12.5%, $P = 0.06$). Splenomegaly in turn correlated with thrombocytopenia ($P = 0.03$).

CAPOX plus cetuximab vs. FOLFOX4 plus cetuximab

Again, spleen size was significantly increased with CAPOX plus cetuximab when compared with FOLFOX4 plus cetuximab (median +23.0% vs. +1.1%, $P = 0.003$) (Fig. 1c). In addition, more patients who received CAPOX plus cetuximab developed splenomegaly than those who received FOLFOX4 plus cetuximab (40.0% vs. 10.0% respectively, $P = 0.02$). An example from one patient whose spleen enlarged after CAPOX plus cetuximab was illustrated in Fig. 2. Splenomegaly was also significantly associated with thrombocytopenia ($P = 0.02$) and marginally associated with grade ≥1 impaired liver function ($P = 0.05$).

Univariable and multivariable analysis

Univariable and multivariable analysis were performed for factors associated with splenic enlargement, splenomegaly, thrombocytopenia and grade ≥1 impaired liver function. Use of capecitabine correlated significantly in univariable analysis ($P = 0.05$) and marginally in multivariable analysis with splenic enlargement (Odds ratio

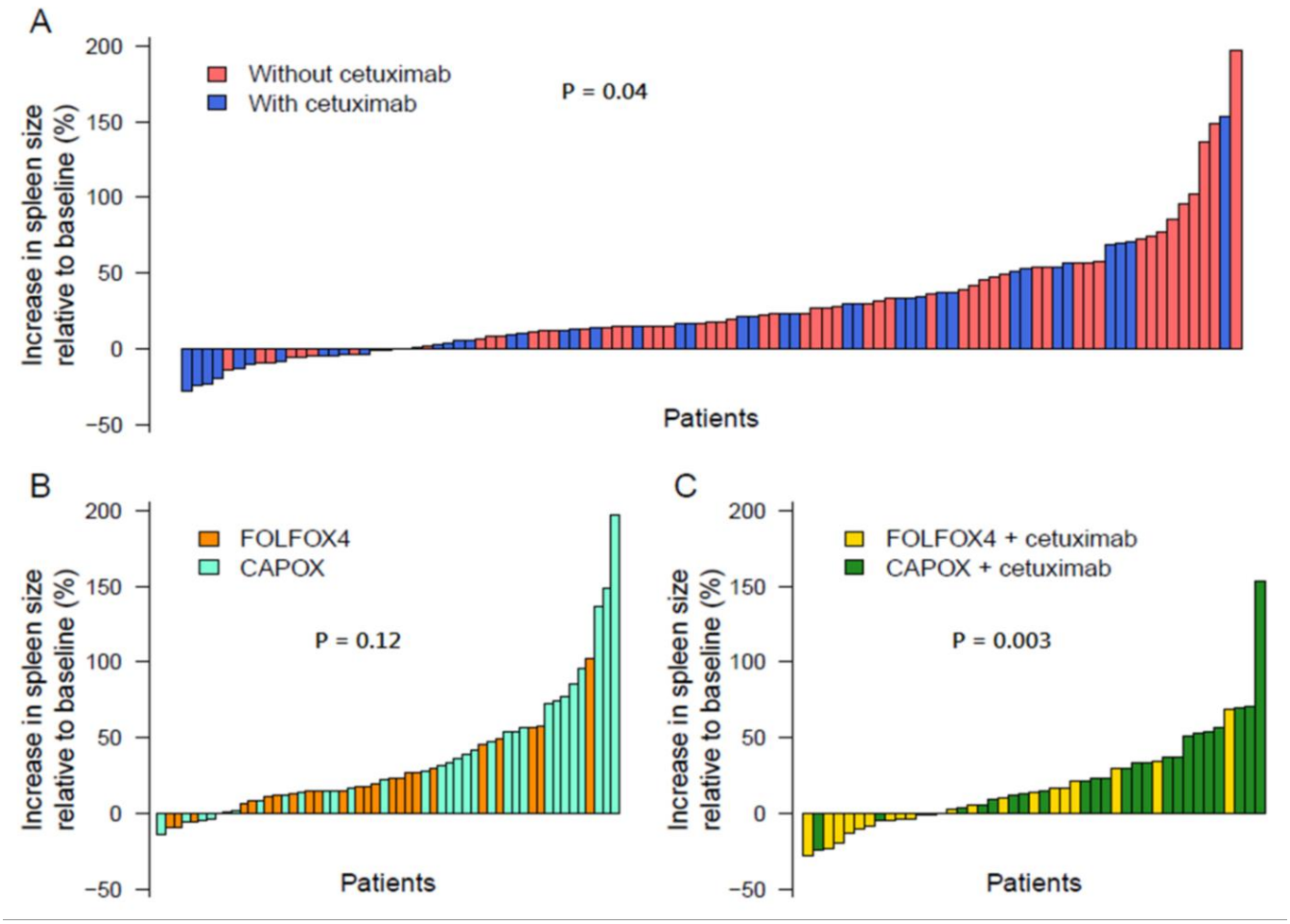

FIGURE 1. Change in spleen size after systemic treatment in the study population who received chemotherapy with and without cetuximab (A), CAPOX and FOLFOX4 (B) and CAPOX plus cetuximab and FOLFOX4 plus cetuximab (C).

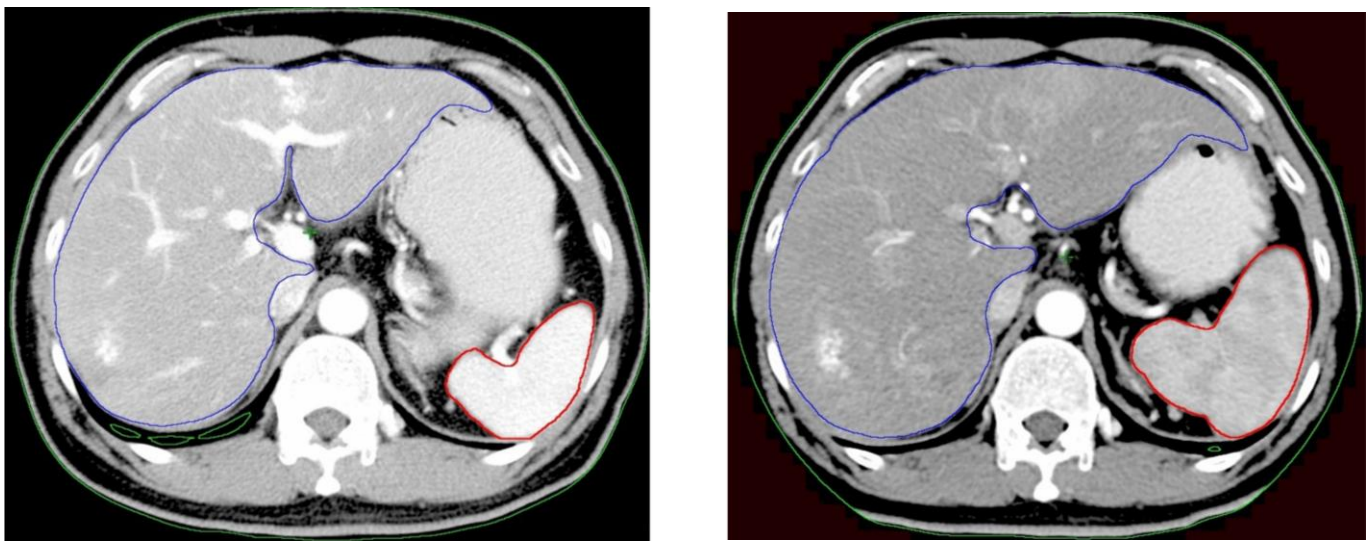

FIGURE 2. Computed tomography images of a patient with his spleen increased from baseline (A) to after 4 cycles of CAPOX plus cetuximab (B).

[OR] 2.36, 95% CI, 0.90%–617%, $P = 0.07$) (Table 3 & 4). Univariable analysis revealed that use of capecitabine (OR 4.61, 95% CI, 143%–1493%, $P = 0.007$), dose intensity of capecitabine (OR 3.81, 95% CI, 126%–1591%, $P = 0.02$) and cumulative dose of capecitabine per 10000mg increase (OR 1.08, 95% CI, 108%–128%, $P = 0.02$) correlated with splenomegaly (Table 3). These three factors also correlated significantly with splenomegaly in multivariable analysis ($P = 0.01$, $P = 0.02$ and $P = 0.006$ respectively) (Table 4).

In addition, increase in spleen size and splenomegaly were the only factors which correlated with thrombocytopenia in both univariable (OR 4.50, 95% CI, 166%–1210%, $P = 0.01$ and OR 4.60, 95% CI, 201%–1289%, $P = 0.04$ respectively) and multivariable analysis ($P = 0.004$ and $P = 0.002$ respectively).
Furthermore, dose intensity of capecitabine, cumulative dose of capecitabine (per 10000mg increase) and splenic enlargement correlated with grade ≥1 impaired liver function in both univariable ($P = 0.05$, $P = 0.03$ and $P = 0.02$) and multivariable analyses ($P = 0.01$, $P = 0.003$ and $P = 0.04$) respectively. On the contrary, use of cetuximab offered protection from splenic enlargement in both univariable ($P = 0.04$) and multivariable analysis ($P = 0.05$) and marginal protection from splenomegaly ($P = 0.09$ and $P = 0.11$ respectively).

Discussion

To the best of our knowledge, this is the first prospective study demonstrating the unfavorable coupling effect of CAPOX on the spleen size and its related complications when compared with FOLFOX4, and the difference was more distinguished when cetuximab was added. Production of reactive oxygen radicals and depletion of glutathione in hepatic endothelial cells by oxaliplatin was previously found to cause hepatic sinusoidal injury in both in-vitro and in-vivo studies (28,29). Overman et al further established the etiological relation between oxaliplatin and hepatic sinusoidal injury and noted that 86% of his patients had their spleen enlarged after adjuvant FOLFOX, which was similar to ours (84.2%) (20). He also confirmed the dose-dependent effect of oxaliplatin on increasing the spleen size in these patients. In his another cohort of patients with liver metastasis in the same study, splenomegaly (with the same definition stated in our study) correlated with moderate to severe hepatic sinusoidal injury in 55% of patients at the time when their liver metastases were resected. The correlation between splenomegaly and thrombocytopenia was also found significant. We are the first demonstrating that use of capecitabine correlated with splenomegaly. More importantly, we also proved that, instead of creatinine clearance, the overall drug intensity and the cumulative dose of capecitabine, which had already incorporated creatinine clearance into consideration, was a more important and reliable factor correlating with splenomegaly.

Patients who received CAPOX showed a trend of greater splenic enlargement ($P = 0.12$) and splenomegaly ($P = 0.06$) as compared with those who received FOLFOX4. Therefore it was reasonable to speculate that capecitabine predisposed to these splenic and hepatic sequelae and subsequent thrombocytopenia secondary to splenic enlargement. In fact, this oral prodrug has to be transformed by carboxylesterase, cytidine deaminase and thymidine phosphorylase before activated to 5-FU, with the first two of the whole three step-wise enzymatic conversions involving substantial hepatic functional workload. We postulated that these frequent and overwhelming enzymatic processes may pose a detriment to liver injury and subsequent splenic enlargement, although future confirmation studies are necessary. A meta-analysis of randomized-controlled clinical trials including CRYSTAL, OPUS, COIN, NORDIC VII, AIO KRK-0104 and CECOG also echoed that cetuximab should only be used with infusional 5-FU rather than capecitabine or bolus 5-FU in KRAS wild-type mCRC for a better reduction in risk of progression and death (30). This is not just a concern to oncologists but also surgeons who operate on patients after perioperative chemotherapy in patients who have potentially resectable liver metastases, as illustrated in the recent EORTC 40983 study which demonstrated an improved progression-free survival after perioperative chemotherapy with FOLFOX4 (21).

Cetuximab was first shown in our current study providing a protective effect on further enlargement of spleen size as compared with chemotherapy alone without cetuximab. Of note, cetuximab protected against further splenic enlargement and splenomegaly when partnered FOLFOX4 rather than CAPOX. The underlying reason why cetuximab offered better protection from splenic enlargement when coupled with 5-fluorouracil rather than capecitabine is unknown. Similar protective effect on spleen and hepatic sinusoidal injury was also noted in bevacizumab before (20,22,23,31). VEGF has been known to regulate activation of matrix metalloproteinase (MMP)-9 by inducing its expression, which in turn, together with

Table 2. Characteristics of Individual Treatment Regimens and Their Respective Outcomes

	CAPOX (n = 32)	**FOLFOX4 (n = 24)**	***P***	**CAPOX + cetuximab (n = 25)**	**FOLFOX4 + cetuximab (n = 20)**	***P***
Median number of weeks of treatment (range)	12 (9–12)	10 (10–12)	0.55	12 (9–12)	10 (10–12)	0.41
Median dose intensity (range)						
Oxaliplatin	0.84 (0.50–1.00)	0.81 (0.53–1.00)	0.80	0.77 (0.27–1.00)	0.76 (0.53–1.00)	0.52
5-FU	NA	0.73 (0.47–1.00)	NA	NA	0.78 (0.55–1.00)	NA
Capecitabine	0.80 (0.50–1.00)	NA	NA	0.73 (0.29–1.00)	NA	NA
Cetuximab	NA	NA	NA	0.81 (0.55–1.00)	0.83 (0.56–1.00)	0.27
Median cumulative dose (mg/m^2) (range)						
Oxaliplatin	520 (128–978)	409 (252–939)	0.03	500 (264–905)	337 (169–923)	0.12
5-FU	NA	9000 (5963–22483)	NA	NA	8000 (3961–21679)	NA
Capecitabine	112783 (29167–210000)	NA	NA	104407 (63553–197126)	NA	NA
Cetuximab	NA	NA	NA	2744 (1780–5190)	2218 (1000–5420)	0.40
Median change in spleen size (range)	+32.5% (–13.9% – +196.6%)	+18.0% (–9.6% – +102.2%)	0.12	+23.0% (–24.4% – +153.2%)	+1.1% (–27.7% – +69.2%)	0.003
Number of patients with increase in net spleen size (%)	27 (84.4%)	21 (87.5%)	0.74	21 (84.0%)	10 (50.0%)	0.01
Splenomegaly (%)	11 (34.4%)	3 (12.5%)	0.06	10 (40.0%)	2 (10.0%)	0.02
Thrombocytopenia (%)	18 (56.3%)	14 (58.3%)	0.88	14 (56.0%)	10 (50.0%)	0.69
Impaired liver function ≥grade 1 (%)	17 (53.1%)	12 (50.0%)	0.82	15 (60.0%)	8 (40.0%)	0.18

Abbreviations: NA = not applicable.

Table 3. Univariable analysis on the effect of various covariates on the development of splenic enlargement, splenomegaly, thrombocytopenia and grade ≥1 impaired liver function

	Splenic enlargement			**Splenomegaly**			**Thrombocytopenia**			**Grade ≥1 Impaired liver function**		
Covariates	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*
Age	0.96	0.88–1.04	0.34	0.98	0.92–1.04	0.44	0.99	0.95–1.04	0.74	0.94	0.89–1.08	0.19
Sex (male)	0.17	0.03–1.03	0.12	0.70	0.17–2.87	0.62	0.63	0.19–2.05	0.44	1.34	0.21–2.60	0.65
Baseline body surface area	0.52	0.26–1.35	0.41	0.23	0.23–2.18	0.80	0.62	0.35–2.70	0.93	0.33	0.29–3.02	0.84
Baseline body weight	1.14	0.94–1.39	0.19	1.01	0.87–1.18	0.86	0.98	0.86–1.12	0.77	0.97	0.84–1.11	0.62
Hepatitis B infection	2.79	0.39–19.87	0.31	2.19	0.22–21.73	0.50	1.97	0.40–9.80	0.41	8.27	0.85–8.82	0.17
History of diabetes mellitus	1.94	0.30–12.78	0.49	1.61	1.01–3.62	0.60	0.72	0.15–3.37	0.67	1.49	0.29–7.79	0.64
Smoking history	2.66	0.41–17.26	0.30	1.63	0.26–10.10	0.87	0.98	0.23–4.13	0.59	3.44	0.68–17.46	0.19
Alcoholic history	2.22	0.09–53.33	0.62	4.00	0.01–51.13	0.35	2.93	0.18–48.68	0.60	2.22	0.10–8.68	0.40
Baseline liver impairment score	0.85	0.36–2.00	0.70	1.01	0.71–1.43	0.96	0.78	0.60 – 1.03	0.11	0.83	0.64–1.07	0.15
Baseline creatinine clearance	0.98	0.95–1.02	0.31	1.00	0.98–1.02	0.80	1.00	0.98–1.02	0.68	1.00	0.98–1.01	0.34
Baseline net liver volume	1.00	0.99–1.01	0.60	1.00	0.99–1.01	0.85	1.00	0.99–1.01	0.31	1.00	0.99–1.01	0.32
Baseline net spleen volume	0.99	0.98–1.01	0.20	1.00	0.98–1.01	0.14	1.00	0.99–1.01	0.40	1.00	0.99–1.01	0.83
Presence of liver metastasis	3.01	0.91–11.69	0.11	1.36	0.34–4.69	0.66	1.01	0.38–3.15	0.87	0.93	0.31–2.90	0.89
Number of liver metastasis	0.94	0.85–1.05	0.26	1.13	0.98–1.30	0.12	0.99	0.88–1.12	0.90	0.94	0.82–1.07	0.35
Volume of liver metastasis	1.00	0.99–1.01	0.15	1.00	0.99–1.01	0.61	1.00	0.98–1.01	0.21	1.00	0.99–1.01	0.56
Use of capecitabine	2.09	0.56–1.03	0.08	4.97	0.60–1.02	0.05	1.21	0.28–1.66	0.12	0.65	0.30–1.44	0.29
Dose intensity of oxaliplatin	2.77	0.12–1435.96	0.66	2.34	0.09–1240.54	0.23	1.26	0.25–1.08	0.18	2.75	0.64–81.70	0.11

Table 3. (continued)

Dose intensity of 5-FU	0.21	0.18–81.89	0.36	0.12	0.21–78.87	0.76	0.53	0.46–25.40	0.92	0.49	0.18–1.33	0.16
Dose intensity of capecitabine	0.66	0.11–21.97	0.92	4.71	1.24–17.02	0.02	1.00	0.94–1.07	0.95	2.68	1.02–7.39	0.03
Cumulative dose of oxaliplatin (mg)	1.01	0.99–1.02	0.46	1.01	0.99–1.01	0.67	1.01	0.99 – 1.03	0.15	1.00	0.99–1.01	0.30
Cumulative dose of 5-FU (mg)	0.92	0.32–1.56	0.67	1.00	0.99–1.01	0.49	1.00	0.99–1.01	0.47	1.00	0.99 – 1.01	0.12
Cumulative dose of capecitabine (per 10000mg increase)	0.95	0.52–1.73	0.86	1.09	1.05–1.28	0.006	1.00	0.99–1.01	0.53	1.19	1.05–1.53	0.04
Use of cetuximab	0.27	0.01–0.92	0.04	0.12	0.08–1.02	0.09	0.29	0.15–1.54	0.59	1.03	0.47–2.25	0.95
Dose intensity of cetuximab	0.48	0.04–116.26	0.72	0.44	0.06–160.54	0.79	0.51	0.12–79.98	0.38	1.11	0.43–2.90	0.83
Cumulative dose of cetuximab (mg)	0.96	0.99–1.01	0.07	0.99	0.99–1.02	0.28	1.00	0.99–1.01	0.53	0.99	0.99–1.02	1.00
Splenic enlargement	-	-	-	-	-	-	4.49	1.58–12.12	0.008	2.80	1.05–7.31	0.04
Splenomegaly	-	-	-	-	-	-	3.60	1.32–7.35	0.05	1.08	0.42–2.78	0.88

MMP-2, triggers the early steps of hepatic sinusoidal injury (31). Bevacizumab, a monoclonal antibody against VEGF, may alleviate this hepatic injury by down-regulation of MMP-9 production. Cetuximab, a monoclonal antibody against EGFR, is not likely to protect the spleen by the same mechanism. Previous studies have demonstrated that weekly dosing of cetuximab with 250mg/m^2 nearly fully saturates its clearance and dose reduction is not necessary in patients with renal and hepatic failure (32-36). Perhaps the antibody-dependent and complement-mediated immune responses elicited by cetuximab may modulate the inflammatory response to the hepatic sinusoids, and the exact underlying pathophysiology remains to be deciphered.

Limitations of the study included non-randomized nature, relative small sample size despite being the largest series ever reported and non-specific timing of splenic volume evaluation due to the variation of cycle duration of different chemotherapeutic regimens. There were also few unbalanced distributions of some of the baseline parameters including lung metastasis, liver metastasis, number and volume of liver metastases, cumulative dose of oxaliplatin, as well as uneven distribution of cumulative dose of capecitabine between CAPOX group and CAPOX plus cetuximab group. However no stratification according to presence and number liver metastasis were performed between FOLFOX and CAPOX with or without cetuximab even in COIN study, as the choice between these two chemotherapeutic regimens were made according to the treating physician and patient's own preferences. Despite the difference in the presence, number and volume of liver metastasis, there was no difference in liver impairment scores across each treatment group in our study. Moreover, a previous study demonstrated that mild to moderate liver dysfunction had no clinically significant influence on the pharmacokinetic parameters of capecitabine and its metabolites and there was no need for, a priori, dose reduction of capecitabine in patients with mildly to moderately impaired liver function (26). It is

not practical to conduct a randomized-controlled trial between these two chemotherapeutic regimens in our study again as this has been proven equally efficacious as 1st line treatment for mCRC previously published in phase III randomized-controlled trials before the initiation of our study (2-4). Moreover the role of cetuximab in addition to chemotherapy in KRAS wild-type mCRC had been well established in CRYSTAL and OPUS study. In our study, patients' decision on either FOLFOX4 or CAPOX was mainly based on their concern about financial affordability, hospitalization and their own preference. The decision of adding cetuximab or not was purely their financial consideration as they had to pay at their own cost for cetuximab. In fact, no uneven distribution of the presence, number and volume of liver metastases was found in our patients who received FOLFOX plus cetuximab and CAPOX plus cetuximab (P = 0.24, P = 0.91 and P = 0.17 respectively). Most importantly, unvariable and multivariable analyses did not reveal these factors as predictors of our four treatment outcomes.

In conclusion, our study demonstrated that CAPOX should not be the preferred chemotherapy backbone especially when coupled with cetuximab as first-line treatment for KRAS wild-type mCRC as it gives rise to worse splenic, platelet and hepatic function complications compared to FOLFOX4.

Abbreviations:

ALT – alanine transferase
ALP – alkaline phosphatase
AST – aspartate transferase
CAPOX – capecitabine and oxaliplatin
CI – confidence interval
CT – computed tomography
DNA – deoxyribonucleic acid
EGFR – epidermal growth factor receptor
FOLFOX4 – 5-FU, folinic acid and oxaliplatin
NCI-CTCAE - National Cancer Institute Common Terminology Criteria for Adverse Events
mCRC – metastatic colorectal cancer
MMP - matrix metalloproteinase
OR – odds ratio
PCR – polymerase chain reaction
VEGF – vascular endothelial growth factor

Table 4. Multivariable analysis on the effect of various covariates on the development of splenic enlargement, splenomegaly, thrombocytopenia and grade ≥1 impaired liver function.

	Splenic enlargement			**Splenomegaly**			**Thrombocytopenia**			**Grade ≥1 Impaired liver function**		
Covariates	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*
Use of capecitabine	2.36	0.90–6.17	0.07	4.61	1.43–14.93	0.01	-	-	-	-	-	-
Dose intensity of capecitabine	-	-	-	4.62	1.30– 16.46	0.02	-	-	-	2.71	1.01–7.30	0.05
Cumulative dose of capecitabine (per 10000mg increase)	-	-	-	1.13	1.04–1.23	0.01	-	-	-	1.29	1.06–1.57	0.01
Use of cetuximab	0.37	0.14–0.98	0.05	0.46	0.18–1.18	0.11	-	-	-	-	-	-
Splenic enlargement	-	-	-	-	-	-	4.59	1.62–12.99	0.004	2.83	1.04–7.69	0.04
Splenomegaly	-	-	-	-	-	-	4.88	1.78–13.33	0.002	-	-	-

Only covariates found significant in univariable analysis ($P < 0.1$) were considered in multivariable analysis.

References

1. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol. 2000 Aug;18(16):2938–47.
2. Porschen R, Arkenau HT, Kubicka S, Greil R, Seufferlein T, Freier W, et al. Phase III study of capecitabine plus oxaliplatin compared with fluorouracil and leucovorin plus oxaliplatin in metastatic colorectal cancer: a final report of the AIO Colorectal Study Group. J Clin Oncol. 2007 Sep 20;25(27):4217-23.
3. Diaz-Rubio E, Tabernero J, Gómez-Espana A, Massutí B, Sastre J, Chaves M, et al. Phase III study of capecitabine plus oxaliplatin compared with continuous-infusion fluorouracil plus oxaliplatin as first line therapy in metastatic colorectal cancer: final report of the Spanish Cooperative Group for the Treatment of Digestive Tumors Trial. J Clin Oncol. 2007 Sep 20;25(27):4224-30.
4. Cassidy J, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Randomized phase III study of capecitabine plus oxaliplatin compared with fluorouracil/folinic acid plus oxaliplatin as first-line therapy for metastatic colorectal cancer. J Clin Oncol. 2008 Apr 20;26(12):2006-12. doi: 10.1200/JCO.2007.14.9898.
5. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in first-line treatment of metastatic colorectal cancer. J Clin Oncol. 2009 Feb 10;27(5):663-71. doi: 10.1200/JCO.2008.20.8397.
6. Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. 2009 Apr 2;360(14):1408-17. doi: 10.1056/NEJMoa0805019.
7. Van Cutsem E, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol. 2011 May 20;29(15):2011-9. doi: 10.1200/JCO.2010.33.5091.
8. Bokemeyer C, Van Cutsem E, Rougier P, Ciardiello F, Heeger S, Schlichting M, et al. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomized clinical trials. Eur J Cancer. Eur J Cancer. 2012 Jul;48(10):1466-75. doi: 10.1016/j.ejca.2012.02.057.
9. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004 Jun 3;350(23):2335-42.
10. Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E, Sarkar S. Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. J Clin Oncol. 2005 Jun 1;23(16):3706-12.
11. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol. 2008 Apr 20;26(12):2013–9. doi: 10.1200/JCO.2007.14.9930.
12. Adams RA, Meade AM, Madi A, Fisher D, Kay E, Kenny S, et al. Toxicity associated with combination oxaliplatin plus fluoropyrimidine with or without cetuximab in the MRC COIN trial experience. Br J Cancer. 2009 Jan 27;100(2):251-8. doi: 10.1038/sj.bjc.6604877.
13. Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomized phase 3 MRC COIN trial. Lancet. 2011 Jun 18;377(9783):2103-14. doi: 10.1016/S0140-6736(11)60613-2.
14. Rubbia-Brandt L, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, et al. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. Ann Oncol. 2004 Mar;15(3):460-6.
15. Aloia T, Sebagh M, Plasse M, Karam V, Lévi F, Giacchetti S, et al. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. J Clin Oncol. 2006 Nov 1;24(31):4983-90.
16. Karoui M, Penna C, Amin-Hashem M, Mitry E, Benoist S, Franc B, et al. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. Ann Surg. 2006 Jan;243(1):1–7.

17. Mehta NN, Ravikumar R, Coldham CA, Buckels JA, Hubscher SG, Bramhall SR, et al. Effect of preoperative chemotherapy on liver resection for colorectal liver metastases. Eur J Surg Oncol. 2008 Jul;34(7):782–6.

18. Nakano H, Oussoultzoglou E, Rosso E, Casnedi S, Chenard-Neu MP, Dufour P, et al. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. Ann Surg. 2008 Jan;247(1):118–24.

19. Vauthey JN, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. J Clin Oncol. 2006 May 1;24(13):2065–72.

20. Overman MJ, Maru DM, Charnsangavej C, Loyer EM, Wang H, Pathak P, et al. Oxaliplatin-mediated increase in spleen size as a biomarker for the development of hepatic sinusoidal injury. J Clin Oncol. 2010 May 20;28(15):2549-55. doi: 10.1200/JCO.2009.27.5701.

21. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomized, controlled, phase 3 trial. Lancet Oncol. 2013 Nov;14(12):1208-15. doi: 10.1016/S1470-2045(13)70447-9.

22. Ribero D, Wang H, Donadon M, Zorzi D, Thomas MB, Eng C, et al. Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. Cancer. 2007 Dec 15;110(12):2761-7.

23. Klinger M, Eipeldauer S, Hacker S, Herberger B, Tamandl D, Dorfmeister M, et al. Bevacizumab protects against sinusoidal obstruction syndrome and does not increase response rate in neoadjuvant XELOX/FOLFOX therapy of colorectal cancer liver metastases. Eur J Surg Oncol. 2009 May;35(5):515-20. doi: 10.1016/j.ejso.2008.12.013.

24. Gonzalez de Castro D, Angulo B, Gomez B, Mair D, Martinez R, Suarez-Cauthier A, et al. A comparison of three methods for detecting KRAS mutations in formalin-fixed colorectal cancer specimens. Br J Cancer. 2012 Jul 10;107(2):345-51. doi: 10.1038/bjc.2012.259.

25. Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, Version. 3.0. Available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf.

26. Twelves C, Glynne-Jones R, Cassidy J, Schüller J, Goggin T, Roos B, et al. Effect of hepatic dysfunction due to liver metastases on the pharmacokinetics of capecitabine and its metabolites. Clin Cancer Res. 1999 Jul;5(7):1696-702.

27. Fang WJ, Lam KO, Ng SC, Choi CW, Kwong DL, Zheng SS, et al. Manual contouring based volumetric evaluation for colorectal cancer with liver limited metastases: a comparison with RECIST. Asian Pac J Cancer Prev. 2013;14(7):4151-5.

28. Laurent A, Nicco C, Chéreau C, Goulvestre C, Alexandre J, Alves A, et al. Controlling tumor growth by modulating endogenous production of reactive oxygen species. Cancer Res. 2005 Feb 1;65(3):948–56.

29. Alexandre J, Nicco C, Chéreau C, Laurent A, Weill B, Goldwasser F, et al. Improvement of the therapeutic index of anticancer drugs by the superoxide dismutase mimic mangafodipir. J Natl Cancer Inst. 2006 Feb 15;98(4):236–44.

30. Ku GY, Haaland BA, de Lima Lopes G Jr. Cetuximab in the first-line treatment of K-ras wild-type metastatic colorectal cancer: the choice and schedule of fluoropyrimidine matters. Cancer Chemother Pharmacol. 2012 Aug;70(2):231-8. doi: 10.1007/s00280-012-1898-7.

31. Deleve LD, Wang X, Tsai J, Kanel G, Strasberg S, Tokes ZA. Sinusoidal obstruction syndrome (veno-occlusive disease) in the rat is prevented by matrix metalloproteinase inhibition. Gastroenterology. 2003 Sep;125(3):882–90.

32. Baselga J, Pfister D, Cooper MR, Cohen R, Burtness B, Bos M, et al. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. J Clin Oncol. 2000 Feb;18(4):904–14.

33. Delbaldo C, Pierga JY, Dieras V, Faivre S, Laurence V, Vedovato JC, et al. Pharmacokinetic profile of cetuximab (Erbitux) alone and in combination with irinotecan in patients with advanced EGFR-positive adenocarcinoma. Eur J Cancer. 2005 Aug;41(12):1739–45.

34. Tan AR, Moore DF, Hidalgo M, Doroshow JH, Poplin EA, Goodin S, et al. Pharmacokinetics of cetuximab after administration of escalating single

dosing and weekly fixed dosing in patients with solid tumors. Clin Cancer Res. 2006 Nov 1;12(21):6517-22.

35. Thariat J, Azzopardi N, Peyrade F, Launay-Vacher V, Santini J, et al. Cetuximab pharmacokinetics in end-stage kidney disease under hemodialysis. J Clin Oncol. 2008 Sep 1;26(25):4223-5. doi: 10.1200/JCO.2008.18.7674.
36. Yalçin S. The increasing role of pharmacogenetics in the treatment of gastrointestinal cancers. Gastrointest Cancer Res. 2009 Sep;3(5):197–203.