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

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This manuscript was previously presented in part in poster session in ESMO 14th World Congress on Gastrointestinal Cancer, June 27-30, 2012, Barcelona, Spain and presented in part in abstract published only in 49th Annual Meeting of the American Society of Clinical Oncology, May 29-June 2, 2013, Chicago, IL, USA.
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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
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 (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, initiated in 2005, 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 reporting its toxicities profile 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.\textsuperscript{14–19} Moreover, increase in spleen size was recently proven an effective biomarker for such hepatic adverse event after oxaliplatin.\textsuperscript{20} This adverse hepatic complication is definitely a particular concern to the surgeons and patients when perioperative chemotherapy is increasingly adopted for potentially resectable liver metastases.\textsuperscript{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.\textsuperscript{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 DNA FFPE tissue kit (Qiagen, Hilden, Germany), followed by polymerase chain reaction (PCR) amplification and direct sequencing using enriched tumour genomic DNA before treatment. 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 tumour 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. 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. CAPOX was given as a 3-weekly regimen with oxaliplatin 130mg/m² infused over 2 hours on day 1 followed by capecitabine 1000mg/m² orally twice a day for 2 weeks followed by a
1 week rest period. FOLFOX4 consisted of oxaliplatin 85mg/m² infused over two hours concurrently with folinic acid 200mg/m² on day 1, followed by bolus 5-FU 400mg/m² 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/m² infused over 2 hours followed by subsequent 250mg/m² 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 $150 \times 10^9$/liter. All toxicities and adverse events were graded with NCI-CTCAE Version 3.0. 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. 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. 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%).

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 $\geq 1$ 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 $\geq 1$ impaired liver function. Use of capecitabine correlated significantly in univariable analysis ($P = 0.05$) and marginally in multivariable analysis with splenic enlargement (Odd ratio [OR] 2.36, 95% CI, 0.90%–617%, $P = 0.07$) (Table 3). 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. These three factors also correlated significantly with splenomegaly in multivariable analysis ($P = 0.01$, $P = 0.02$ and $P = 0.006$ respectively) (Table 3). 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 \( \geq 1 \) 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
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. 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.

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. VEGF has been known to regulate activation of matrix metalloproteinase (MMP)-9 by inducing its expression, which in turn, together with MMP-2, triggers the early steps of hepatic sinusoidal injury. 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/m² nearly fully saturates its clearance and dose reduction is not necessary in patients with renal and hepatic failure.\textsuperscript{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. 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. 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.

Conflict of interest

All authors declare that they have no competing interests.

References


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Figure legends:

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).