



"Panitumumab as a radiosensitizing agent in KRAS wild-type locally advanced rectal cancer"

Mardjuadi, Feby ; Carrasco, Javier ; Coche, Jean-Charles ; Sempoux, Christine ; Jouret-Mourin, Anne ; Scalliet, Pierre ; Goeminne, Jean-Charles ; Daisne, Jean-François ; Delaunoy, Thierry ; Vuylsteke, Peter ; Humblet, Yves ; Meert, Nicolas ; Van den Eynde, Marc ; Moxhon, Anne ; Haustermans, Karin ; Canon, Jean-Luc ; Machiels, Jean-Pascal

ABSTRACT

Our goal was to optimize the radiosensitizing potential of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies, when given concomitantly with preoperative radiotherapy in KRAS wild-type locally advanced rectal cancer (LARC). Based on pre-clinical studies conducted by our group, we designed a phase II trial in which panitumumab (6 mg/kg/q2 weeks) was combined with preoperative radiotherapy (45 Gy in 25 fractions) to treat cT3-4/N+KRAS wild-type LARC. The primary endpoint was complete pathologic response (pCR) ($H_0=5\%$, $H_1=17\%$, $\alpha=0.05$, $\beta=0.2$). From 19 enrolled patients, 17 (89 %) were evaluable for pathology assessment. Although no pCR was observed, seven patients (41 %) had grade 3 Dworak pathological tumor regression. The regimen was safe and was associated with 95 % of sphincter-preservation rate. No NRAS, BRAF, or PI3KCA mutation was found in this study, but one patient (5 %) showed loss of PTEN expression. The quantification of plasma EGFR ligands during treatment showed significant upregulation of plasma TGF- α and EGF following panitumumab administration ($p<0.05$). At surgery, patients with important pathological regression (grade 3 Dworak) had higher plasma TGF- α ($p=0.03$) but lower plasma EGF ($p=0.003$) compared to those with grade 0-2 Dworak. Our study suggests that concomitant panitumumab and preoperative radiotherapy in KRAS wild-type LARC is feasible and results in some tumor regression. However, pCR rate remained modest. Given that the primary endpoint of our study was not reached, we rema...

CITE THIS VERSION

Mardjuadi, Feby ; Carrasco, Javier ; Coche, Jean-Charles ; Sempoux, Christine ; Jouret-Mourin, Anne ; et. al. *Panitumumab as a radiosensitizing agent in KRAS wild-type locally advanced rectal cancer*. In: *Targeted Oncology : biotherapies for the clinicians in oncology*, Vol. 10, no. 3, p. 375-383 (2015) <http://hdl.handle.net/2078.1/151838> -- DOI : 10.1007/s11523-014-0342-9

Le dépôt institutionnel DIAL est destiné au dépôt et à la diffusion de documents scientifiques émanant des membres de l'UCLouvain. Toute utilisation de ce document à des fins lucratives ou commerciales est strictement interdite. L'utilisateur s'engage à respecter les droits d'auteur liés à ce document, principalement le droit à l'intégrité de l'œuvre et le droit à la paternité. La politique complète de copyright est disponible sur la page [Copyright policy](#)

DIAL is an institutional repository for the deposit and dissemination of scientific documents from UCLouvain members. Usage of this document for profit or commercial purposes is strictly prohibited. User agrees to respect copyright about this document, mainly text integrity and source mention. Full content of copyright policy is available at [Copyright policy](#)

Panitumumab as a radiosensitizing agent in *KRAS* wild-type locally advanced rectal cancer

Febby Ingriani Mardjuadi • Javier Carrasco • Jean-Charles Coche • Christine Sempoux • Anne Jouret-Mourin • Pierre Scalliet • Jean-Charles Goeminne • Jean-François Daisne • Thierry Delaunoit • Peter Vuylsteke • Yves Humblet • Nicolas Meert • Marc van den Eynde • Anne Moxhon • Karin Haustermans • Jean-Luc Canon • Jean-Pascal Machiels

Received: 17 January 2014 / Accepted: 25 September 2014
© Springer International Publishing Switzerland 2014

Abstract Our goal was to optimize the radiosensitizing potential of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies, when given concomitantly with pre-operative radiotherapy in *KRAS* wild-type locally advanced rectal cancer (LARC). Based on pre-clinical studies conducted by our group, we designed a phase II trial in which panitumumab (6 mg/kg/q2 weeks) was combined with pre-operative radiotherapy (45 Gy in 25 fractions) to treat cT3-4/N+ *KRAS* wild-type LARC. The primary endpoint was complete pathologic response (pCR) (H0=5 %, H1=17 %, $\alpha=0.05$, $\beta=0.2$). From 19 enrolled patients, 17 (89 %) were evaluable for

pathology assessment. Although no pCR was observed, seven patients (41 %) had grade 3 Dworak pathological tumor regression. The regimen was safe and was associated with 95 % of sphincter-preservation rate. No *NRAS*, *BRAF*, or *PI3KCA* mutation was found in this study, but one patient (5 %) showed loss of *PTEN* expression. The quantification of plasma EGFR ligands during treatment showed significant upregulation of plasma TGF- α and EGF following panitumumab administration ($p<0.05$). At surgery, patients with important pathological regression (grade 3 Dworak) had higher plasma TGF- α ($p=0.03$) but lower plasma EGF

Electronic supplementary material The online version of this article (doi:10.1007/s11523-014-0342-9) contains supplementary material, which is available to authorized users.

F. I. Mardjuadi • J.-P. Machiels
Institut de Recherche Expérimentale et Clinique, Pôle d'imagerie médicale, radiothérapie et oncologie (IREC/MIRO), Université catholique de Louvain, Brussels, Belgium

J. Carrasco • J.-L. Canon
Service d'oncologie médicale, Grand Hôpital de Charleroi (GhdC), Charleroi, Belgium

J.-C. Coche
Service de gastroentérologie, Clinique St-Pierre, Ottignies, Belgium

C. Sempoux • A. Jouret-Mourin
Service d'anatomie pathologie, Cliniques universitaires St-Luc, Brussels, Belgium

C. Sempoux • A. Jouret-Mourin • P. Scalliet • Y. Humblet • M. van den Eynde • A. Moxhon
Clinique des Pathologies Tumorales du Colon et du Rectum, Centre du Cancer, Cliniques universitaires St-Luc, Brussels, Belgium

J.-C. Goeminne • P. Vuylsteke
Service d'oncologie médicale, Clinique et Maternité St-Elisabeth, Namur, Belgium

J.-F. Daisne
Service de radiothérapie, Clinique et Maternité St-Elisabeth, Namur, Belgium

T. Delaunoit
Service d'oncologie, Hôpital de Jolimont, Haine-St-Paul, Belgium

N. Meert
Service de radiothérapie, Hôpital St-Joseph, Gilly, Belgium

K. Haustermans
Radiation Oncology, University Hospitals Leuven, Katholieke Universiteit Leuven, Leuven, Belgium

F. I. Mardjuadi • Y. Humblet • M. van den Eynde • A. Moxhon • J.-P. Machiels (✉)
Service d'oncologie médicale, Cliniques universitaires St-Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium
e-mail: jean-pascal.machiels@uclouvain.be

($p=0.003$) compared to those with grade 0–2 Dworak. Our study suggests that concomitant panitumumab and preoperative radiotherapy in *KRAS* wild-type LARC is feasible and results in some tumor regression. However, pCR rate remained modest. Given that the primary endpoint of our study was not reached, we remain unable to recommend the use of panitumumab as a radiosensitizer in *KRAS* wild-type LARC outside a research setting.

Keywords Panitumumab · Locally advanced rectal cancer · Tumor regression

Introduction

In locally advanced rectal cancer (LARC), preoperative chemoradiation followed by total mesorectal excision (TME) is the standard treatment [1–3]. While the addition of chemotherapy to preoperative radiotherapy (RT) significantly improves pathologic complete response (pCR) and loco-regional control (LRC) rates, it does not influence disease-free survival (DFS) or overall survival (OS) [4, 5]. Novel approaches are therefore needed to achieve better long-term outcomes.

In LARC to date, no radiosensitizing agent has surpassed a 5-fluorouracil-based regimen in terms of efficacy. The anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (moAbs), cetuximab and panitumumab, are approved by the US Food and Drug Administration for the treatment of *RAS* wild-type metastatic colorectal cancer [6, 7]. These anti-EGFR moAbs have established radiosensitizing properties [8, 9]. Panitumumab is a fully humanized monoclonal antibody with a lower incidence of infusion hypersensitivity reaction than cetuximab. Panitumumab can be administered without premedication using a validated bi-weekly dosing [10].

Several trials, including one by our group, have investigated EGFR-targeting moAbs in combination with a preoperative chemoradiation regimen in LARC. Early efficacy results in terms of pCR rate were modest (5 % to 10 %) [11–14]. However, the patient population in these studies was not selected according to the *RAS* mutational status. In metastatic colorectal cancer, the absence of a *RAS* mutation is a predictive factor of response to anti-EGFR moAbs [15, 16].

It has also been suggested that optimal sequencing of chemotherapy, radiotherapy, and the EGFR inhibitor might unlock the full radiosensitizing potential of anti-EGFR moAbs [17–21].

Previously in a phase I/II study, we safely combined cetuximab and capecitabine with preoperative RT in patients with LARC [11]. However, the study produced a pCR rate of only 5 %. The first loading dose of cetuximab was given before the start of chemoradiotherapy. Since tumor cells undergoing mitosis are described as relatively more radio- and

chemosensitive than quiescent ones, the low rate of pCR was likely caused by a significant decrease in the proliferation of tumor cells following the first dose of cetuximab, as shown by our translational research [20]. The low pCR observed in our earlier trial may have also been caused by a decrease in the radiosensitizing effects of the chemotherapy and/or cetuximab when administered with RT using the investigated sequence [17–21].

To evaluate the impact of radiosensitization using anti-EGFR moAb in LARC, we conducted a phase II trial of concomitant panitumumab with preoperative RT in *KRAS* wild-type tumors. In the current study, the first dose of panitumumab was given after the start of RT. With the objective of identifying predictive biomarkers of response, we also performed quantification of plasma EGFR ligands during the course of the study treatment.

Patients and methods

Study objectives

We conducted an open-label, single-arm, multicenter phase II trial to investigate the activity of panitumumab as a radiosensitizing agent in *KRAS* wild-type LARC. The trial was conducted in accordance with the 1996 Guideline for Good Clinical Practice, the Declaration of Helsinki, and the 2004 European regulations. Approval from ethics committees was obtained and all patients signed a written informed consent.

The primary endpoint was pCR rate in accordance with our statistical hypothesis. Secondary endpoints included tumor regression grade according to Dworak et al. [22], negative circumferential radial margin (CRM) rate, toxicity, LRC rate at 5 years, and DFS rate at 5 years.

Eligibility

Eligible patients had histologically confirmed *KRAS* wild-type rectal adenocarcinoma, localized within 15 cm of the anal margin, cT3–cT4 tumors and/or with nodal involvement (N+) as determined by endoscopic transrectal ultrasound or pelvic magnetic resonance imaging, Eastern Cooperative Oncology Group (ECOG) performance status 0–1, normal bone marrow, chemistry, and hepatic and renal functions. Exclusion criteria included distant metastases, *KRAS* mutation, and prior exposure to EGFR-targeting therapy.

RAS, *BRAF*, *PI3KCA*, and *PTEN* analyses

Prior to inclusion, eligible patients were screened for the presence of *KRAS* codon 12 and 13 mutation. The analysis was centralized in a certified laboratory (Centre de

Technologies Moléculaires Appliquées, Université catholique de Louvain, Belgium). Extracted DNA (EZ1 DNA Tissue kit, Qiagen) from formalin-fixed, paraffin-embedded rectal adenocarcinoma tissues was amplified through polymerase chain reaction (AmpliTaQ® Gold DNA Polymerase, Applied Biosystems).

The forward and reverse *KRAS* amplification primers were as follows: KRAS-F, forward, 5'-NNNGCCTGCTGAAAA TGACTGAA-3'; KRAS-R, reversed biotinylated primer, 5'-TTAGCTGTATCGTCAAGGCACTCT-3'; KRAS-2F, forward biotinylated primer, 5'-TGACTGAATATAAACTTG TGGTAGTTG-3'; and KRAS-2R, reversed primer, 5'-TCGT CCACAAAATGATTCTGAA-3'.

Samples were run together with a positive control, a negative control, and a *KRAS* wild-type control using the following cycling conditions: 95 °C for 5 min; 40 cycles of 95 °C for 40 s, 58 °C for 40 s, and 72 °C for 80 s; and 72 °C for 7 min.

The next step consisted of immobilizing the PCR products on streptavidin-coated beads, separating the DNA double strands, and hybridization of the samples with pyrosequencing primers to enable the detection of *KRAS* codon 12 and codon 13 mutations. To do this, 40 µL of streptavidin-sepharose beads (Streptavidin Sepharose™ High Performance, Qiagen) and binding buffer (PyroMark™, Qiagen) mixture were added to the plate containing the PCR products for 5 min at room temperature with constant agitation. Afterwards, the plate was put into a Vacuum Prep Tool (Qiagen) to prepare the single strands by denaturation. The beads were then suspended into a PSQ™ 96 Plate containing 40 µL of pyrosequencing primers and annealing buffer mixture (PyroMark™, Qiagen).

The forward sequencing primers KRAS-PF1 (5'-TGTGGT AGTTGGAGCTG-3'), KRAS-PF2 (5'-TGTGGTAGTTGG AGCT-3'), and KRAS-PF3 (5'-TGGTAGTTGGAGCTGGT-3') were used to detect codon 12 (GTT and GAT), codon 12 (TGT), and codon 13 (GAC) mutations, respectively.

The reverse sequencing primer, KRAS-2PF (5'-GCACTC TTGCCTACG-3'), was used to confirm and validate the reading. Primers hybridization was done by heating the PSQ™ 96 Plate to 80 °C for 2 min. Pyrosequencing analyses were performed in a PSQ96MA pyrosequencer (Qiagen) using the software provided by the manufacturer (PSQ96MA2.1.1 software, Qiagen).

NRAS, *BRAF*, and *PI3KCA* mutations as well as PTEN expression analyses were also centralized and conducted at another certified laboratory (Institut de Pathologie et de Génétique, Gosselies, Belgium). Briefly, after standard DNA extractions followed by genes amplification using the appropriate primers by PCR, the *NRAS*, *BRAF*, and *PI3KCA* genes were sequenced using SNaPshot assays [23]. PTEN protein expression was studied using standard immunohistochemistry with rabbit moAb of PTEN XP D4.3 as primary antibody (Cell Signaling Technology, Inc). Details on *NRAS*, *BRAF*, *PI3KCA*,

and PTEN analyses are included in the [Supplementary Materials](#).

Study design

RT (45 Gy in 25 fractions) was delivered on weekdays (days 1 to 33). To optimize its radiosensitizing activity, panitumumab (6 mg/kg/q2 weeks, intravenously) was initiated after the start of RT (day 8) and was administered concomitantly with RT on days 8, 22, and 36. After the end of RT, a maintenance dose of panitumumab alone was given on day 50. The conception of the study design was heavily based upon prior pre-clinical investigations conducted in our laboratories using *KRAS* wild-type human colorectal cancer xenograft models (unpublished data). Our pre-clinical data suggest that radiosensitization by anti-EGFR moAb is enhanced when the moAb is introduced during the course of RT and continued after the completion of RT. Further details on our laboratory experiments and results are included in the [Supplementary Materials](#).

Radiotherapy

A dummy run was performed in all participating centers to standardize the delineation and treatment planning. Megavoltage equipment was used with 6 MV as minimal energy. A dose of 45 Gy in 25 fractions (1.8 Gy daily on weekdays, days 1–33) was delivered. Three-dimensional conformal radiotherapy was used for all patients based on a contrast CT scan of the pelvis. The planning CT was carried out in the treatment position, with 3- to 5-mm-thick slices. Clinical target volume (CTV) included the entire mesorectum. Internal iliac nodes were included up to the venous bifurcation, together with the presacral nodes (limit S1/S2) [24]. Planning target volume (PTV) was an isotropic expansion of the CTV (10 mm). Maximum, mean, and median dose to the PTV were calculated.

Surgery

TME was performed 6–8 weeks after the end of RT. Postoperative chemotherapy was recommended but was left at the discretion of each investigator. Follow-up was scheduled every 3 months for two consecutive years then every 6 months for a total of five years.

Pathology assessment

Surgical specimens were assessed according to the current standardized procedure in Belgium [25]. Mesorectum quality was scored as complete (smooth), nearly complete (mildly irregular), or incomplete (severely irregular). The surgical specimen was fixed for 72 h in formalin and sectioned in parallel cuts of 3 mm to evaluate CRM. CRM was considered negative if no tumor cells were visible microscopically at

<2 mm from the radial margins. Classification of tumors was performed using the WHO guidelines. Tumors' histological differentiation was graded as well, moderately, or poorly differentiated. Tumor regression was evaluated according to Dworak's rectal cancer regression grading [22].

In case of residual macroscopic tumor, standard examination was performed with three to five sections to investigate the deepest invasion area. If no macroscopic tumor was observed, the scar area plus a 2-cm margin were sampled to search for residual cancer structures. If no tumor cells were visible, three additional levels from each block of the total scar area were made before a pCR could be confirmed. All lymph nodes included in the specimen were examined.

Pathology staging was established according to the 5th edition of the American Joint Committee on Cancer Staging Manual. Pathology central review was blindly and independently performed by two specialized pathologists.

Enzyme-linked immunoabsorbent assay (ELISA)

Plasma was taken at baseline (day 1), after 1 week of treatment with RT alone (day 8, 1 h before panitumumab administration), during the combined RT and panitumumab treatment (day 22, 1 h after panitumumab administration), during panitumumab maintenance (day 50, 1 h after panitumumab administration), and at the time of surgery.

The objective was to study the influence of the study regimen on EGFR ligands: EGF, transforming growth factor (TGF)- α , heparin-binding EGF (HB-EGF), betacellulin, amphiregulin, and epiregulin. Plasma concentrations of these ligands were quantified using human ELISA Kits (R & D Systems, UK; Abcam, UK; Gentaur, Belgium) according to the manufacturer's instructions.

Statistics

The trial's sample size was calculated using Simon's two-step sequential design with 80 % power for a significance level of 0.05 to reject the null hypothesis of a 5 % pCR rate (based on the pCR rate obtained with RT alone in the EORTC 22921 trial) and to accept the alternative hypothesis of a 17 % pCR rate which is averagely produced by concomitant chemoradiotherapy [3]. An interim analysis was planned after 12 patients had undergone surgery and all the pathology data had been collected. In accordance with the study protocol, accrual was not stopped during the interim analysis. If at least one pCR was observed among the first 12 patients, 30 additional patients were to be recruited.

Kaplan–Meier curves were used to obtain LRC, DFS, and OS probabilities. The probability of LRC at 5 years was calculated from the date of inclusion to the date of confirmed loco-regional disease relapse. DFS probability was calculated from the date of inclusion to the date of confirmed disease

progression or death. OS at 5 years was calculated from the date of inclusion to the date of death. Statistical analysis was performed using SPSS 16.0.1. Paired and unpaired *t* tests were used to compare the means of EGFR ligand concentrations within and between patient groups, respectively. A two-sided *p* value ≤ 0.05 was considered significant.

Results

Baseline characteristics

From October 2009 to June 2010, 26 eligible patients with rectal adenocarcinoma were screened for *KRAS* mutation. *KRAS* mutation was detected in five patients (19 %) and they were considered as screening failures. Nineteen *KRAS* wild-type patients were enrolled in the phase II trial. The patients' characteristics are summarized in Table 1.

Toxicity

Toxicities were graded using the National Cancer Institute's Common Toxicity Criteria (NCI CTC) version 3.0 (Table 2). The most frequent grade 1–2 toxicities were asthenia (89 %), diarrhea (89 %), and acneiform rash (84 %). The most common

Table 1 Baseline characteristics

Study population (N=19)	Number of patients (%)
Median age, years	63 (range 52–78)
Gender	
Male	13 (68 %)
Female	6 (32 %)
ECOG performance status	
0	14 (74 %)
1	5 (26 %)
<i>KRAS</i> mutation (exon 2)	0
Baseline TNM staging ^a	
cT2N+	3 (16 %)
cT3N0	6 (32 %)
cT3N+	9 (47 %)
cT4N0	0
cT4N+	1 (5 %)
Distance from anal margin	
<5 cm	4 (21 %)
5–10 cm	13 (68 %)
>10 cm	2 (11 %)

ECOG Eastern Cooperative Oncology Group

^a As determined by endoscopic transrectal ultrasound or pelvic magnetic resonance imaging (taking into account the poorest TNM stage when both investigations were performed)

Table 2 Toxicity

Adverse events	NCI CTC version 3.0 grade (N=19)			
	1	2	3	4
N (%)				
Hematologic				
Anemia	8 (42)	1 (5)	1 (5)	0
Leucopenia	0	4 (21)	5 (26)	0
Thrombopenia	3 (16)	0	0	0
Gastrointestinal				
Anorexia	3 (16)	1 (5)	1 (5)	0
Constipation	2 (10)	5 (26)	0	0
Diarrhea	15 (79)	2 (10)	0	0
Low gastrointestinal bleeding	2 (10)	4 (21)	0	0
Stomatitis	3 (16)	1 (5)	0	0
Proctitis	2 (10)	5 (26)	0	0
Nausea	3 (16)	0	0	0
Vomiting	1 (5)	0	0	0
Other (tenesma)	1 (5)	4 (21)	0	0
Nail toxicity	0	1 (5)	1 (5)	0
Skin toxicity				
Acneiform rash	9 (47)	7 (37)	1 (5)	0
Dermatitis	0	0	1 (5)	0
Dry skin	12 (63)	4 (21)	0	0
Erythema	7 (37)	4 (21)	0	0
Pruritis	5 (26)	3 (16)	0	0
Other (fissures, local skin infection)	3 (16)	1 (5)	0	0
Other				
Asthenia	14 (74)	3 (16)	1 (5)	0
Cardiac arrhythmia	1 (5)	0	1 (5)	0
Creatinine increased	1 (5)	2 (10)	0	0
Elevated ASAT or ALAT	4 (21)	0	0	0
Elevated GGT	1 (5)	0	3 (16)	0
Hypocalcemia	6 (31)	1 (5)	0	0
Hypokalemia	1 (5)	0	1 (5)	0
Hypomagnesemia	3 (16)	3 (16)	0	1 (5)
Hypophosphatemia	5 (26)	2 (10)	3 (16)	1 (5)
Hypotension	1 (5)	1 (5)	0	0
Infection	0	0	3 (16)	1 (5)
Local-regional pain (anal, perineal)	4 (21)	8 (42)	0	0
Thromboembolic event	0	0	0	1 (5)
Weight loss	4 (21)	3 (16)	0	0

NCI CTC National Cancer Institute's Common Toxicity Criteria, AST aspartate aminotransferase, ALAT alanine aminotransferase, GGT gamma-glutamyl transpeptidase

grade 3 toxicity was leucopenia (26 %). Four grade 4 adverse events were reported: sepsis ($n=1$), pulmonary embolism ($n=1$), hypomagnesemia ($n=1$), and hypophosphatemia ($n=1$). The median relative dose intensity of panitumumab and

radiotherapy was 99.4 % (95 % confidence interval=91.3–100) and 94.3 % (95 % confidence interval=92.8–99.2 %), respectively.

Efficacy

Pathology results

The pathology results are summarized in Table 3. Two patients (10 %) were not evaluable for rectal cancer pathology assessment because their tumors were confirmed to be sigmoid adenocarcinomas (>15 cm from the anal margin). Six patients (35 %) showed tumor downstaging compared to baseline cTNM. The mesorectum excision was considered incomplete in two patients (12 %). Negative CRM was found

Table 3 Pathology central review

Pathology outcomes	(N=17) ^a
Histology differentiation	
Well differentiated	1 (6 %)
Moderately differentiated	15 (88 %)
Poorly differentiated	1 (6 %)
Circumferential radial margin	
>10 mm	9 (53 %)
2–10 mm	4 (23 %)
<2 mm	3 (18 %)
<1 mm	0
Not evaluable	1 (6 %)
ypTN stage	
ypT1N0	3 (18 %)
ypT1N1	0
ypT1N2	0
ypT2N0	3 (18 %)
ypT2N1	0
ypT2N2	0
ypT3N0	7 (41 %)
ypT3N1	3 (18 %)
ypT3N2	1 (6 %)
Dworak tumor regression grade	
4	0
3	7 (41 %)
2	7 (41 %)
1	3 (18 %)
0	0
Oncogene mutation status	
NRAS (exon 2)	0
BRAF (V600E)	0
PI3KCA (exons 9 and 20)	0
PTEN expression	16 (94 %)

^a Two patients were excluded from the pathology efficacy evaluation because their tumors were sigmoid adenocarcinomas

in 13 patients (76 %). Dworak grade 3, 2, and 1 was found in seven (41 %), seven (41 %), and three (18 %) patients, respectively. No pCR (grade 4 Dworak) was observed.

Follow-up

The median time interval from the completion of RT to surgery was 6 weeks (range 5–8 weeks). TME with colo-anal anastomosis was performed in 18 patients (95 %). One patient (5 %) underwent an abdomino-perineal resection. Postoperative serious adverse events included pulmonary embolism ($n=1$), systemic infection ($n=1$), and delayed resumption of bowel motility ($n=1$). Nine patients (47 %) received postoperative 5-fluorouracil-based chemotherapy. With a median follow-up of 53 months (range 14–60 months), two patients (10 %) had developed distant metastases, one patient (5 %) had confirmed loco-regional relapse, and one patient (5 %) had simultaneous loco-regional and distant relapses. In addition, three patients (16 %) died due to disease progression and another (5 %) from a non-cancerous cause. Local-control, disease-free, and overall survival probabilities according to Kaplan–Meier are shown in Fig. 1.

EGFR ligands expression

ELISA results are summarized in Fig. 2. Among the quantified EGFR ligands, only plasma EGF and TGF- α showed significant variations from baseline.

For plasma EGF, no significant change from baseline was observed after treatment with RT alone (day 8) or during concomitant RT and panitumumab (day 22). However, panitumumab treatment after the end of RT (day 50) triggered a significant rise in plasma EGF concentration ($p<0.05$) (Fig. 2a). Similarly, a significant increase in mean plasma TGF- α in relation to panitumumab administration ($p<0.001$) was also observed (Fig. 2b), suggesting that the changes in the expression of EGFR ligands during the study treatment were induced rather by panitumumab than by RT or concomitant RT and panitumumab.

Interestingly, patients with Dworak grade 3 histological regression tended to have a lower plasma EGF concentration during treatment compared to patients with poor histological response (Dworak grade 0–2). However, this difference only reached statistical significance ($p=0.003$) at the time of surgery (Fig. 2c). Conversely, patients with Dworak grade 3 had a higher plasma TGF- α at the time of surgery compared to those with Dworak grade 0–2 ($p=0.03$) (Fig. 2d).

No significant change from baseline was found during treatment for the other EGFR ligands (data not shown).

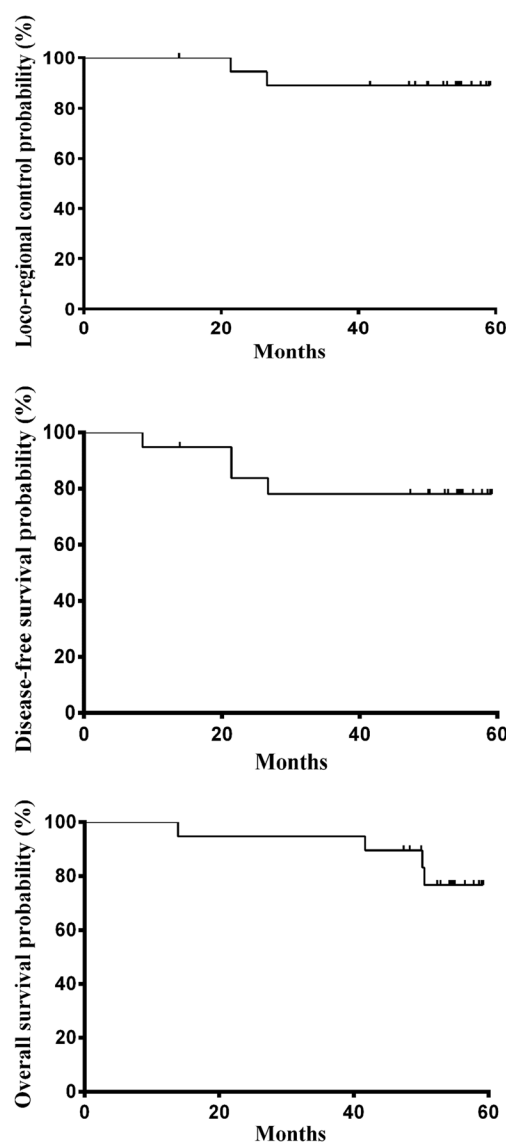


Fig. 1 Local-control, disease-free, and overall survival probabilities at 5 years

Discussion

EGFR-targeting moAbs have proven their efficacy in patients with metastatic colorectal cancer [6, 7, 15, 16]. However, their potential to improve outcome in the preoperative setting of LARC remains uncertain [11–14]. Our work set out to challenge key hypotheses that may explain the lack of improvement in the pCR rate obtained when anti-EGFR moAbs are added to preoperative chemoradiation in rectal cancer. We and others have previously highlighted the importance of optimal treatment sequencing when combining an EGFR inhibitor with chemoradiation [17–21]. In our study, the bimodality approach of panitumumab and RT was chosen for two reasons. First, it enabled us to evaluate the full capacity of panitumumab as a radiosensitizer in *KRAS* wild-type LARC,

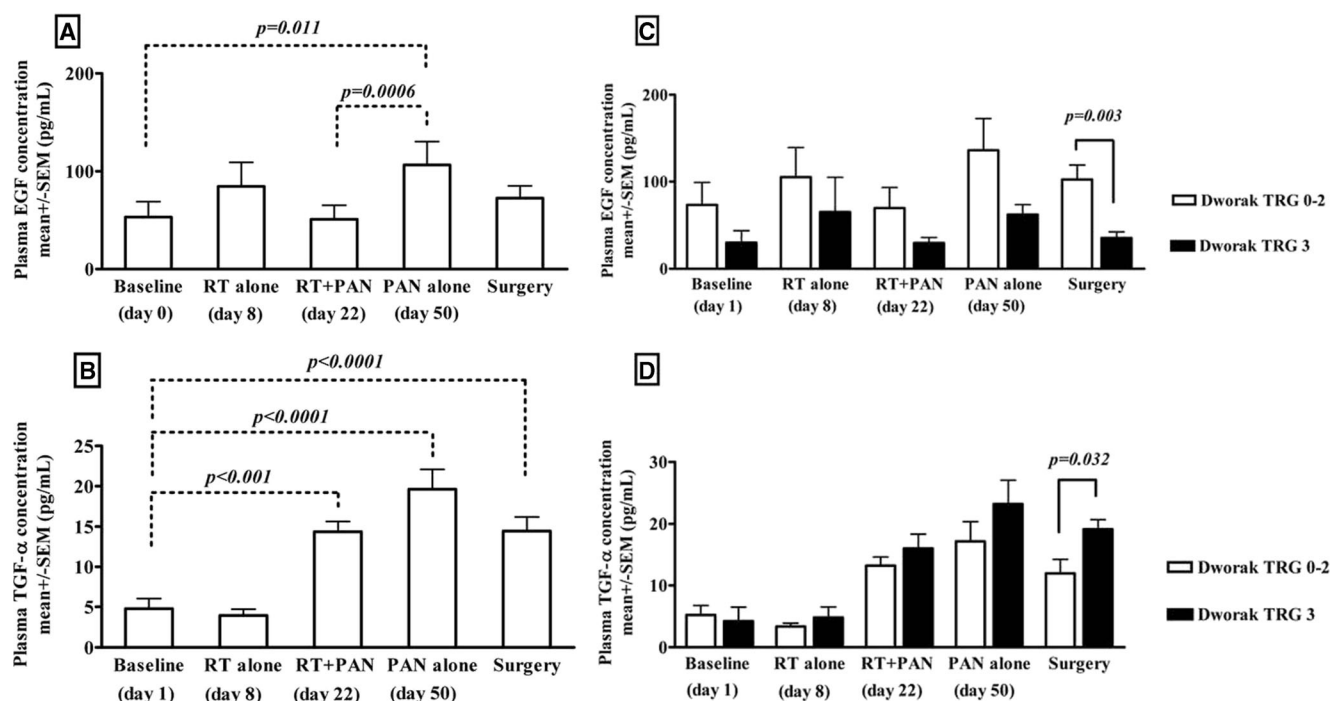


Fig. 2 Plasma EGF and TGF- α . RT radiotherapy, PAN panitumumab, SEM standard error mean, TRG Dworak's tumor regression grade. **a, b** The impact of the study treatment on plasma EGF and TGF- α concentrations ($N=17$). **c, d** Plasma EGF and TGF- α concentrations according

to pathological tumor regression. Dworak grade 0 to 2 ($N=10$) was considered as minimal regression. Dworak grade 3 ($N=7$) was considered as good regression

without the interference of chemotherapy. We intended to investigate whether the addition of anti-EGFR moAb to RT would allow us to achieve a pCR rate of 17 %, which is in the range of pCR obtained with standard chemoradiotherapy [3]. Second, we aimed to test the hypothesis, as described by others [26] and confirmed by our pre-clinical results (see [Supplementary Materials](#)), that radiosensitization with anti-EGFR moAbs could be improved when anti-EGFR moAb is initiated during RT and continued after the end of RT. However, even with an optimized study design, our trial closed prematurely as it failed to achieve its primary endpoint.

Currently, there is no validated predictive biomarker for anti-EGFR-based chemoradiation regimen in LARC. The predictive value of *RAS* mutation, an established molecular marker in metastatic colorectal cancer, remains conflicting in LARC [27–29]. Some promising markers in the metastatic setting, *NRAS*, *BRAF*, and *PIK3CA* mutations, have yet to prove their significance in the neoadjuvant setting [16, 30, 31]. In this study, none of these mutations were found but one patient (5 %) with a poor clinical outcome showed a loss of PTEN expression in the tumor tissue.

Another potential biomarker in LARC is EGFR ligand expression. In our previous trial, we found that cetuximab increased plasma TGF- α concentration and that the increase was significantly correlated with tumor downstaging [20]. Similarly, our present data showed that plasma TGF- α was

significantly increased with panitumumab. Plasma EGF during panitumumab monotherapy was also upregulated; however, no significant change from baseline was observed during treatment with RT alone or during concomitant RT and panitumumab.

At the time of surgery, we found higher plasma TGF- α but lower plasma EGF concentrations in responsive patients with grade 3 Dworak compared to those with grade 0–2 Dworak. TGF- α overexpression has been associated with biological resistance to cetuximab and panitumumab in colorectal cancer cells. Some investigators have suggested that paracrine secretion of TGF- α plays a protective role against EGFR blocking in the surviving cancer cells. This paracrine-derived defense mechanism was not found with other EGFR ligands except for amphiregulin [32]. Other pre-clinical evidence showed that increased TGF- α concentration might trigger an EGFR–MET interaction. TGF- α could promote resistance to anti-EGFR monoclonal antibody by subsequently inducing MET phosphorylation and activating its downstream signaling pathways [33]. In patients with significant response to panitumumab, an increase in plasma TGF- α and consequently an active biological defense mechanism may simply be an indication of a highly effective EGFR blockage by the monoclonal antibody. These findings and hypotheses should be interpreted with caution as it is based on a limited number of patients.

EGFR-targeting moAbs appear to have limited efficacy as radiosensitizers in rectal cancer, but the reason for this remains unclear. Glynne-Jones et al. [19] pointed out that the total radiation dose used in LARC (45–50.4 Gy) is below the curative threshold. In this setting, and unlike cytotoxic chemotherapy, anti-EGFR moAbs may not induce sufficient cell kill, particularly in the presence of subpopulations of radioresistant tumor cells, thus decreasing the probability of achieving pCR. Intrinsic cellular radiosensitivity, accelerated repopulation, hypoxia, and the activation of other growth factor receptors may also have contributed to our study regimen's lack of activity [34–36].

There are limitations but also encouraging facts in our study. First, this was a small and non-randomized phase II trial. Second, chemotherapy was not given concomitantly with RT. The EORTC 22921 rectal cancer trial showed that local recurrences were reduced to the same extent with either postoperative chemotherapy or preoperative chemoradiotherapy [3]. Postoperative chemotherapy was given to 47 % of patients in this study (data not shown). Even so, LRC, DFS, and OS rates fell within the expected ranges for this population. Furthermore, the addition of concurrent chemotherapy to RT has never been shown to improve OS in rectal cancer [4]. While several studies have questioned the relevancy of pCR as an endpoint for neoadjuvant LARC trials, other groups found that patients with pCR have a favorable prognosis [37, 38]. pCR has been correlated with low loco-regional recurrence rates, lower risk of lymph-node metastases, and 5-year survival rates greater than 95 % in several studies [39]. Moreover, the emergence of the “wait and watch” non-operative approach in LARC is based on the hypothesis that patients with radio(chemo)sensitive tumors who will achieve pCR could be spared from surgery [39]. A recent report also showed that patients with “near-pCR” type of response had poor prognosis with DFS and OS rates comparable to the non-responsive patients [40]. Together, these facts support the use of pCR as an endpoint for LARC trials.

Concomitant panitumumab and radiotherapy in this study was feasible, as reflected by the median dose intensity of both regimen, and was associated with acceptable rate of perioperative complications. In addition, we obtained a high rate of colorectal anastomosis in our study (95 %) despite having 21 % of patients with tumors located at less than 5 cm from the anal margin.

A randomized phase II trial, SAKK 41/07, reported that a high proportion of *KRAS* wild-type patients achieved pCR and near-pCR when treated with panitumumab and capecitabine-based chemoradiation [28]. Despite their interesting findings, the pCR rate alone, without taking into account near-pCR response rate, fell within the range of what is expected with a 5-fluorouracil- or capecitabine-based chemoradiation regimen. Due to the overlapping confidence intervals between the

investigational and control arms, the contribution of panitumumab to pCR or pathologic downstaging requires further investigation.

In summary, our work suggests that preoperative radiosensitization using panitumumab in *KRAS* wild-type LARC is associated with pathological tumor regression but without any improvement in the overall pCR rate. Given that pCR was selected as the primary endpoint of our study, we remain unable to recommend the use of panitumumab as a radiosensitizer in *KRAS* wild-type LARC outside a research setting.

Acknowledgments We deeply acknowledge Jean-Luc Gala, Brigitte Honhon, Yannick Neybuch, Anne-France Dekarelle, Annelies Debucquoy, Aline Gillain, Fatima Hammouch, Janique Dewelle, Pierre Lefesvre, and Marie-Lise Vanderhaeghen for their contributions to this study. We also thank Amgen, Belgium. Finally, we thank Aileen Eiszele, BA (Hons), DipEd, GradDipBus, for editing this manuscript.

Funding This study was supported by the Fonds de la Recherche Scientifique (FNRS, grant no. 7.4609.09), the Belgian “Plan National Cancer” (Action 29), and an unrestricted grant from Amgen, Belgium. Feby Mardjuadi is supported by the FNRS (Aspirant F.C. 81552). Karin Haustermans is a fundamental clinical researcher of the Research Foundation—Flanders, Belgium. The preliminary results of this study have been published as an abstract at the American Society of Clinical Oncology 2012 meeting.

Conflicts of interest Jean-Pascal Machiels received an unrestricted grant from Amgen, Belgium to support the phase II clinical trial. All remaining authors have declared no conflict of interest.

References

1. Sauer R, Becker H, Hohenberger W et al (2004) Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* 35: 1731–1740
2. Gerard JP, Conroy T, Bonnetain F et al (2006) Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3–4 rectal cancers: results of FFCD 9203. *J Clin Oncol* 24:4620–4625
3. Bosset JF, Collette L, Calais G et al (2006) Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 355:1114–1123
4. Ceelen WP, Van Nieuwenhove Y, Fierens K (2009) Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer. *Cochrane Database Syst Rev*. doi:10.1002/14651858.CD006041.pub2
5. Bosset J, Calais G, Mineur L et al (2014) Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy in rectal cancer: long-term results of the EORTC 22921 randomized study. *Lancet Oncol* 15(2):184–190. doi:10.1016/S1470-2045(13)70599-0
6. Cunningham D, Humblet Y, Siena S et al (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 351:337–345
7. Van Cutsem E, Peeters M, Siena S et al (2007) Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol* 25(13):1658–1664

8. Milas L, Fan Z, Andratschke N, Ang KK (2004) Epidermal growth factor receptor and tumor response to radiation: *in vivo* preclinical studies. *Int J Radiat Oncol Biol Phys* 58(3):966–971
9. Kruser T, Armstrong E, Ghia A et al (2008) Augmentation of radiation response by panitumumab in models of upper aerodigestive tract cancer. *Int J Radiat Oncol Biol Phys* 72(2):534–542
10. Amgen Inc.: Vectibix (panitumumab) package insert. Revised March 2013. Available at http://pi.amgen.com/united_states/vectibix/vectibix_pi.pdf.
11. Machiels JP, Sempoux C, Scalliet P et al (2007) Phase I/II study of preoperative cetuximab, capecitabine, and external-beam radiotherapy in patients with rectal cancer. *Ann Oncol* 18(4):738–744
12. Rödel C, Arnold D, Hipp M et al (2008) Phase I–II trial of cetuximab, capecitabine, oxaliplatin, and radiotherapy as preoperative treatment in rectal cancer. *Int J Radiat Oncol Biol Phys* 70:1081–1086
13. Bertolini F, Chiara S, Bengala C et al (2009) Neoadjuvant treatment with single-agent cetuximab followed by 5-FU, cetuximab, and pelvic radiotherapy: a phase II study in locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 73:466–472
14. Horisberger K, Treschl A, Mai S et al (2009) Cetuximab in combination with capecitabine, irinotecan, and radiotherapy for patients with locally advanced rectal cancer: results of a Phase II MARGIT trial. *Int J Radiat Oncol Biol Phys* 74:1487–1493
15. Peeters M, Douillard JY, Van Cutsem E et al (2013) Mutant KRAS codon 12 and 13 alleles in patients with metastatic colorectal cancer: assessment as prognostic and predictive biomarkers of response to panitumumab. *J Clin Oncol* 31(6):759–765
16. Douillard JY, Oliner KS, Siena S et al (2013) Panitumumab-FOLFOX4 treatment and RAS mutation in colorectal cancer. *N Engl J Med* 369(11):1023–1034
17. Nyati MK, Morgan MA, Feng FY et al (2006) Integration of EGFR inhibitors with radiochemotherapy. *Nat Rev Cancer* 6:876–885
18. Debucquoy A, Machiels JP, McBride WH et al (2010) Integration of epidermal growth factor receptor inhibitors with preoperative chemoradiation. *Clin Cancer Res* 16(10):2709–2714
19. Glynn-Jones R, Mawdsley S, Harrison M (2010) Cetuximab and chemoradiation for rectal cancer—is the water getting muddy? *Acta Oncol* 49(3):278–286
20. Debucquoy A, Haustermans K, Daemen A et al (2009) Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer. *J Clin Oncol* 27(17):2751–2757
21. Ahsan A, Hiniker SM, Davis MA et al (2009) Role of cell cycle in epidermal growth factor receptor inhibitor-mediated radiosensitization. *Cancer Res* 69(12):5108–5114
22. Dworak O, Keilholz L, Hoffmann A (1997) Pathological features of rectal cancer after preoperative radiochemotherapy. *Int J Colorectal Dis* 12:19–23
23. Lurkin I, Stoeck R, Hurst CD et al (2010) Two multiplex assays that simultaneously identify 22 possible mutation sites in the *KRAS*, *BRAF*, *NRAS*, and *PI3KCA* genes. *PLoS One* 5(1):e8802
24. Roels S, Duthoy W, Haustermans K et al (2006) Definition and delineation of the clinical target volume for rectal cancer. *Int J Radiat Oncol Biol Phys* 65(4):1129–1142
25. Demetter P, Vandendael T, Sempoux C et al (2013) Need for objective and reproducible criteria in histopathological assessment of total mesorectal excision specimens: lessons from a national improvement project. *Colorectal Dis*. doi:10.1111/codi.12362
26. Milas L, Fang FM, Mason KA et al (2007) Importance of maintenance therapy in C225-induced enhancement of tumor control by fractionated radiation. *Int J Radiat Oncol Biol Phys* 67(2):568–572
27. Pinto C, Di Fabio F, Maiello E et al (2011) Phase II study of panitumumab, oxaliplatin, 5-fluorouracil, and concurrent radiotherapy as preoperative treatment in high-risk locally advanced rectal cancer patients. *Ann Oncol* 22(11):2424–2430
28. Helbling D, Bodoky G, Gautschi O et al (2013) Neoadjuvant chemoradiotherapy with or without panitumumab in patients with wild-type *KRAS*, locally advanced rectal cancer (LARC): a randomised, multicenter, phase II trial SAKK 41/07. *Ann Oncol* 24:718–725
29. Gaedcke J, Grade M, Jung K et al (2010) KRAS and BRAF mutations in patients with rectal cancer treated with preoperative chemoradiotherapy. *Radiother Oncol* 94(1):76–81
30. Di Nicolantonio F, Martini M, Molinari F et al (2008) Wild-type *BRAF* is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 26(35):5705–5712
31. Mao C, Yang ZY, Hu XF et al (2012) PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in *KRAS* wild-type metastatic colorectal cancer: a systematic review and meta-analysis. *Ann Oncol* 26(6):1518–1525
32. Hobor S, Van Emburgh BO, Crowley E et al (2014) TGF- α and amphiregulin paracrine network promotes resistance to EGFR blockade in colorectal cancer cells. *Clin Cancer Res*, Epub June. doi:10.1158/1078-0432.CCR-14-0774, 10
33. Troiani T, Marinelli E, Napolitano S et al (2013) Increased TGF- α as a mechanism of acquired resistance to the anti-EGFR inhibitor cetuximab through EGFR–MET interaction and activation of MET signaling in colon cancer cells. *Clin Cancer Res* 19(24):6751–6765
34. Pajonk F, Vlashi E, McBride WH (2010) Radiation resistance of cancer stem cells: the 4R's of radiobiology revisited. *Stem Cells* 28(4):639–648
35. Liu B, Fang M, Lu Y et al (2001) Fibroblast growth factor and insulin-like growth factor differentially modulate the apoptosis and G1 arrest induced by anti-epidermal growth factor receptor monoclonal antibody. *Oncogene* 20:1913–1922
36. Liska D, Chen C, Bachleitner-Hofmann T et al (2011) HGF rescues colorectal cancer cells from EGFR inhibition via MET activation. *Clin Cancer Res* 17(3):472–482
37. Maas M, Nelemans PJ, Valentini V et al (2010) Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: a pooled analysis of individual patient data. *Lancet Oncol* 11:835–844. doi:10.1016/S1470-2045(10)70172-8
38. Tural D, Selcukbiricik F, Dztferk MA et al (2013) The relation between pathologic complete response and clinical outcome in patients with rectal cancer. *Hepatogastroenterology* 60. doi:10.5754/hge13138.
39. Vignali A, De Nardi P (2014) Multidisciplinary treatment of rectal cancer in 2014: where are we going? *World J Gastroenterol* 20(32):11249–11261
40. Swellengrebel HA, Bosch S, Cats A et al (2014) Tumour regression grading after chemoradiotherapy for locally advanced rectal cancer: a near pathologic complete response does not translate into good clinical outcome. *Radiother Oncol* 112:44–51