

40. Chen CN, Hsieh FJ, Cheng YM et al.. The significance of placenta growth factor in angiogenesis and clinical outcome of human gastric cancer. *Cancer Lett* 2004; 213: 73–82.
41. Jain RK, Duda DG, Willett CG et al.. Biomarkers of response and resistance to antiangiogenic therapy. *Nat Rev Clin Oncol* 2009; 6: 327–338.
42. Okugawa Y, Miki C, Toiyama Y et al.. Serum soluble VCAM-1 as a valuable prognostic marker in colorectal carcinoma [abstract]. Presented at: American Society of Clinical Oncology Gastrointestinal Cancers Symposium, San Francisco, CA, 2008.
43. Pasięka Z, Kuzdak K, Czyz W et al.. Soluble intracellular adhesion molecules (sICAM-1, sVCAM-1) in peripheral blood of patients with thyroid cancer. *Neoplasma* 2004; 51: 34–37.
44. Silva HC, Garcao F, Coutinho EC et al.. Soluble VCAM-1 and E-selectin in breast cancer: relationship with staging and with the detection of circulating cancer cells. *Neoplasma* 2006; 53: 538–543.

*Annals of Oncology* 24: 718–725, 2013

doi:10.1093/annonc/mds519

Published online 8 November 2012

## Neoadjuvant chemoradiotherapy with or without panitumumab in patients with wild-type *KRAS*, locally advanced rectal cancer (LARC): a randomized, multicenter, phase II trial SAKK 41/07

D. Helbling<sup>1</sup>, G. Bodoky<sup>2</sup>, O. Gautschi<sup>3</sup>, H. Sun<sup>4</sup>, F. Bosman<sup>5</sup>, B. Gloor<sup>6</sup>, R. Burkhard<sup>7</sup>, R. Winterhalder<sup>8</sup>, A. Madlung<sup>9</sup>, D. Rauch<sup>10</sup>, P. Saletti<sup>11</sup>, L. Widmer<sup>7</sup>, M. Borner<sup>12</sup>, D. Baertschi<sup>4</sup>, P. Yan<sup>5</sup>, J. Benhattar<sup>5</sup>, E. O. Leibundgut<sup>13</sup>, S. Bougel<sup>5</sup> & D. Koeberle<sup>14</sup>

<sup>1</sup>Department of Medical Oncology, Gastrointestinal Tumorcenter Zurich, Zurich, Switzerland; <sup>2</sup>Department of Oncology, St. Laszlo Hospital, Budapest, Hungary; <sup>3</sup>Department of Medical Oncology, Luzerner Kantonsspital, Lucerne; <sup>4</sup>Swiss Group for Clinical Cancer Research, Coordinating Center, Bern; <sup>5</sup>Department of Pathology, Lausanne; <sup>6</sup>Department of Visceral Surgery and Medicine, University Hospital Bern, Bern; <sup>7</sup>Onkozentrum Hirslanden, Hirslanden Hospital Zurich, Zurich; <sup>8</sup>Department of Medicine, Medical Oncology, Kantonsspital, Luzern; <sup>9</sup>Department of Radiation Oncology with Division of Medical Radiation Physics, University Hospital, Bern; <sup>10</sup>Department of Medicine, Medical Oncology, Regionalspital Thun, Thun; <sup>11</sup>Ospedale San Giovanni, Oncology Institute of Southern Switzerland, Bellinzona; <sup>12</sup>Department of Medicine, Medical Oncology, Spitalzentrum, Biel; <sup>13</sup>Central Haematology Laboratory, University Hospital Bern, Bern; <sup>14</sup>Division of Oncology, Kantonsspital St. Gallen, St. Gallen, Switzerland

Received 15 July 2012; revised 19 August 2012; accepted 30 August 2012

**Background:** We conducted a randomized, phase II, multicenter study to evaluate the anti-epidermal growth factor receptor (EGFR) mAb panitumumab (P) in combination with chemoradiotherapy (CRT) with standard-dose capecitabine as neoadjuvant treatment for wild-type *KRAS* locally advanced rectal cancer (LARC).

**Patients and methods:** Patients with wild-type *KRAS*, T3-4 and/or N+ LARC were randomly assigned to receive CRT with or without P (6 mg/kg). The primary end-point was pathological near-complete or complete tumor response (pNC/CR), defined as grade 3 (pNCR) or 4 (pCR) histological regression by Dworak classification (DC).

**Results:** Forty of 68 patients were randomly assigned to P + CRT and 28 to CRT. pNC/CR was achieved in 21 patients (53%) treated with P + CRT [95% confidence interval (CI) 36%–69%] versus 9 patients (32%) treated with CRT alone (95% CI: 16%–52%). pCR was achieved in 4 (10%) and 5 (18%) patients, and pNCR in 17 (43%) and 4 (14%) patients. In immunohistochemical analysis, most DC 3 cells were not apoptotic. The most common grade  $\geq 3$  toxic effects in the P + CRT/CRT arm were diarrhea (10%/6%) and anastomotic leakage (15%/4%).

**Conclusions:** The addition of panitumumab to neoadjuvant CRT in patients with *KRAS* wild-type LARC resulted in a high pNC/CR rate, mostly grade 3 DC. The results of both treatment arms exceeded prespecified thresholds. The addition of panitumumab increased toxicity.

**Key words:** capecitabine, chemoradiotherapy, panitumumab, radiotherapy, rectal cancer, wild-type *KRAS*

\*Correspondence to: Dr D. Helbling, Department of Medical Oncology, Gastrointestinal Tumorcenter Zurich, Seestrasse 259, Zurich 8038, Switzerland. Tel: +41-43-344-33-33; E-mail: daniel.helbling@gitz.ch

## introduction

In patients with locally advanced rectal cancer (LARC), complete surgical resection is a prerequisite for cure. Neoadjuvant chemoradiotherapy (CRT) is frequently used to downstage LARC tumors and to enable surgery to be more effective and provide locoregional control [1–3]. The standard neoadjuvant regimen is 5-fluorouracil (5-FU) and external radiotherapy (RT). Neoadjuvant CRT with capecitabine has been shown to be tolerated with comparable activity to 5-FU regimens [4, 5].

The epidermal growth factor receptor (EGFR)-targeted monoclonal antibodies (mAbs) cetuximab and panitumumab have shown efficacy as monotherapy in phase III studies in patients with chemotherapy-refractory metastatic colorectal cancer [6–8] and also improved outcomes when added to standard chemotherapy in the first-line and subsequent lines of therapy [7, 9, 10]. Preliminary data suggest that EGFR-targeted agents in combination with RT may be synergistic, as RT increases EGFR expression within tumor cells, while EGFR blockade sensitizes the cells to the effects of RT [11, 12]. In the setting of locally advanced head and neck cancer, addition of cetuximab to RT enhanced locoregional control and survival [13, 14].

Intensive research investigating the response according to molecular tumor markers in order to allow better patient selection, has shown that *KRAS*-activating mutations predict resistance to cetuximab and panitumumab [15, 16]. Mutations in *KRAS* codons 12 or 13 occur in ~35%–45% of colorectal cancers and lead to constitutive signaling via impaired ability of GTPase-activating proteins to hydrolyze *KRAS*-bound guanosine triphosphate (GTP) [17]. Other molecular markers that have been linked to response/nonresponse to EGFR-targeted mAbs in the colorectal cancer setting include mutations of *BRAF* [18, 19] or *PIK3CA* [19], *EGFR* gene copy number [20] and levels of EGFR ligands such as amphiregulin (AREG) and epiregulin (EREG) [21].

We conducted a randomized, phase II, multicenter study to evaluate the efficacy and safety of panitumumab in combination with capecitabine and external beam RT as a neoadjuvant regimen for patients with advanced wild-type *KRAS* LARC.

## patients and methods

Patients with centrally tested wild-type *KRAS* LARC were recruited at centers in Switzerland and from one center in Budapest, Hungary. The study was conducted according to the Declaration of Helsinki. The protocol was approved by the independent ethics committee at participating centers. Patients provided written informed consent before randomization. Separate informed consent was obtained for *KRAS* testing, biobanking and translational research.

## inclusion criteria

The eligibility criteria included: previously untreated histologically confirmed locally advanced adenocarcinoma of the rectum with or without nodal involvement and requiring surgery; stage cT3/4 and/or cN1/2 cM0 and wild-type *KRAS* gene status, as assessed by a central pathology; World Health

Organization (WHO) performance status 0–1; age  $\geq 18$  years and adequate organ function. Patients with distant metastases were excluded from the study.

## pretreatment evaluation

Baseline evaluation included computed tomography of the abdomen and thorax, and magnetic resonance imaging of the pelvis.

*KRAS* mutation status and EGFR gene copy number analyses were conducted at a central laboratory (Institute of Pathology, University of Lausanne, CHUV, Switzerland), on formalin-fixed paraffin-embedded tissues (5  $\mu$ m paraffin sections, microdissected to contain >70% tumor tissues) prepared from diagnostic tumor biopsy samples collected before the trial. The *KRAS* mutation status was analyzed by pyrosequencing of a PCR-amplified exon 2 sequence containing codons 12 and 13. EGFR copy number analysis was carried out by quantitative PCR of tumor DNA. Serum samples were collected for *KRAS* mutational screening and biomarker testing and were assessed at the laboratory of Molecular Diagnostics, University Hospital Bern.

## study conduct

After screening, the eligible patients were randomly assigned to receive either CRT alone or panitumumab + CRT (P + CRT) as neoadjuvant treatment. Randomization was stratified by: T-stage (T3 versus T4); tumor localization (<10 cm versus  $\geq 10$  cm); institution and EGFR gene copy number (<2.9 versus  $\geq 2.9$  copies per nucleus).

## treatment

Panitumumab (6 mg/kg every 2 weeks for 8 weeks) was administered i.v. over ~60 min.

Capecitabine (825 mg/m<sup>2</sup>) was taken twice daily orally throughout RT. RT was applied with a total dose of 45 Gy in 25 fractions of 1.8 Gy over 5 weeks, starting from 7 days after the first panitumumab administration (P + CRT arm) (supplementary Figure S1, available at *Annals of Oncology* online). Patients were irradiated in the prone position using a linear accelerator (3D-conformal or, preferably intensity-modulated, RT).

## surgery and histopathology

Surgery was planned 6 weeks ( $\pm 1$  week) after completion of CRT. Total mesorectal excision with sphincter preservation was to be carried out whenever feasible, according to the standardized techniques, using a laparoscopic or open approach. After fixation and processing, resected specimens were microscopically examined for the presence of tumor cells, fully evaluated and staged according to the American Joint Committee on Cancer TNM system [22]. Tumor regression was graded by the local pathologists according to the regression grading scale proposed by Dworak et al. [grade 0 = none; 1 = minimal; 2 = moderate (predominantly fibrotic changes with few tumor cells); 3 = good (very few tumor cells); 4 = complete (no tumor cells)] [23]. A central pathology review (by F. Bosman, P. Yun) was carried out for samples classified as Dworak grade 2 or 3, as this cut-off was decisive regarding the primary end-point. Radical resection (R0) was defined as microscopic and macroscopic tumor-free resection with clear circumferential resection margins (invasion front  $\geq 2$  mm from the lateral resection margin).

## dose modifications

Chemotherapy and RT were interrupted if grade  $\geq 3$  toxicity developed (capecitabine and panitumumab were also interrupted in case of diarrhea and mucositis grade 2). Study treatment was restarted when toxicity had resolved to grade  $\leq 1$ . Dose reductions were required after grade  $\geq 3$

toxicity. If the treatment was delayed for  $\geq 2$  weeks, the patient was withdrawn from the study treatment.

### adjuvant chemotherapy and follow-up evaluations

It was recommended that patients received adjuvant therapy for 4–6 months according to local practice. All patients with R0 resection were followed (every 3 months during the first year and every 6 months thereafter) until disease progression or for 3 years after surgery.

### concomitant therapy

All patients randomly assigned to panitumumab received a pre-emptive supportive treatment regimen, consisting of a skin moisturizer, sunscreen and doxycycline 100 mg orally twice daily.

### study end-points

The primary end-point of the study was pathological near-complete or complete tumor response (pNC/CR), prospectively defined as grade 3 (near-complete) or 4 (complete) histological regression using the Dworak classification (DC) [23].

The early secondary end-points were R0 or R1 resection, sphincter preservation, downstaging of primary tumor and/or lymph nodes (comparison between cT/N and ypT/N) and postoperative complications (within 8 weeks after surgery) defined as insufficiency of anastomosis; fistula; severe local infection requiring antibiotics; bladder or erectile dysfunction or need for additional interventions/operation. The late secondary end-points (in the R0 population) were time (from randomization) to local relapse, time to distant relapse and disease-free survival.

### translational studies

The following markers were evaluated in baseline serum (for full methodological details, see supplementary information, available at Annals of Oncology online): circulating tumor *KRAS* mutations by real-time quantitative PCR and concentrations of EGFR and the related growth factors AREG, betacellulin, epidermal growth factor (EGF), EGFR, EREG, fibroblast growth factor-basic, heparin-binding EGF-like growth factor (HB-EGF), platelet-derived growth factor  $\beta$  polypeptide, placental growth factor, tenascin C, transforming growth factor  $\alpha$  and vascular endothelial growth factor by using antibody suspension bead arrays and suspension array system.

Pretreatment tumor specimens were examined for mutations in *KRAS* exon 3 (codons 59 and 61), *KRAS* exon 4 (codons 117 and 146), *BRAF* exon 15 (codon 600), *NRAS* exon 2 (codons 12 and 13), *PIK3CA* exon 9 (codons 542, 545 and 546) and *PIK3CA* exon 20 (codons 1043 and 1047) (by pyrosequencing after PCR amplification).

To assess the viability of the remaining tumor cells following neoadjuvant treatment, the available post-surgery tumor specimens were tested for the Ki-67 proliferation marker MIB-1 and the early apoptosis marker M30 (by immunohistochemistry). Quantitative methylation analysis of *RASSF2A* and *EGFR* promoter regions was also carried out (by pyrosequencing after bisulfite modification of genomic DNA and PCR amplification).

### statistical considerations

The required sample size was calculated separately for each treatment arm using the Fleming's single-stage design [24] with 80% power and 5% significance level. For the P + CRT arm, 40 patients were required to test the null hypothesis of the pNC/CR rate  $\leq 10\%$  versus the alternative of  $\geq 25\%$ . If there were  $\leq 7$  patients with pNC/CR, the treatment would not be

recommended for further investigation. Twenty-seven patients would be needed in the CRT arm to test the null hypothesis of the pNC/CR rate  $\leq 5\%$  versus the alternative of  $\geq 20\%$ . Analyses of the primary end-point, as well as pathological tumor response by the Dworak regression grade, were based on the intention-to-treat analysis set (all randomly assigned patients). The remaining early secondary end-points were based on the resection population (all patients who underwent surgical resection). Statistical comparisons between the treatment arms were not planned.

## results

Between March 2009 and May 2010, a total of 68 patients (53 men and 15 women, aged 31–80 years) at 19 centers were randomly assigned, 40 to P + CRT and 28 to CRT (Figure 1). Most patients ( $n = 65$ ) had WHO performance status 0. The most common T-stage was cT3. The majority of patients had tumor localization  $< 10$  cm from the anal margin, lymph node involvement and  $< 2.9$  EGFR gene copies (Table 1).

### treatment exposure

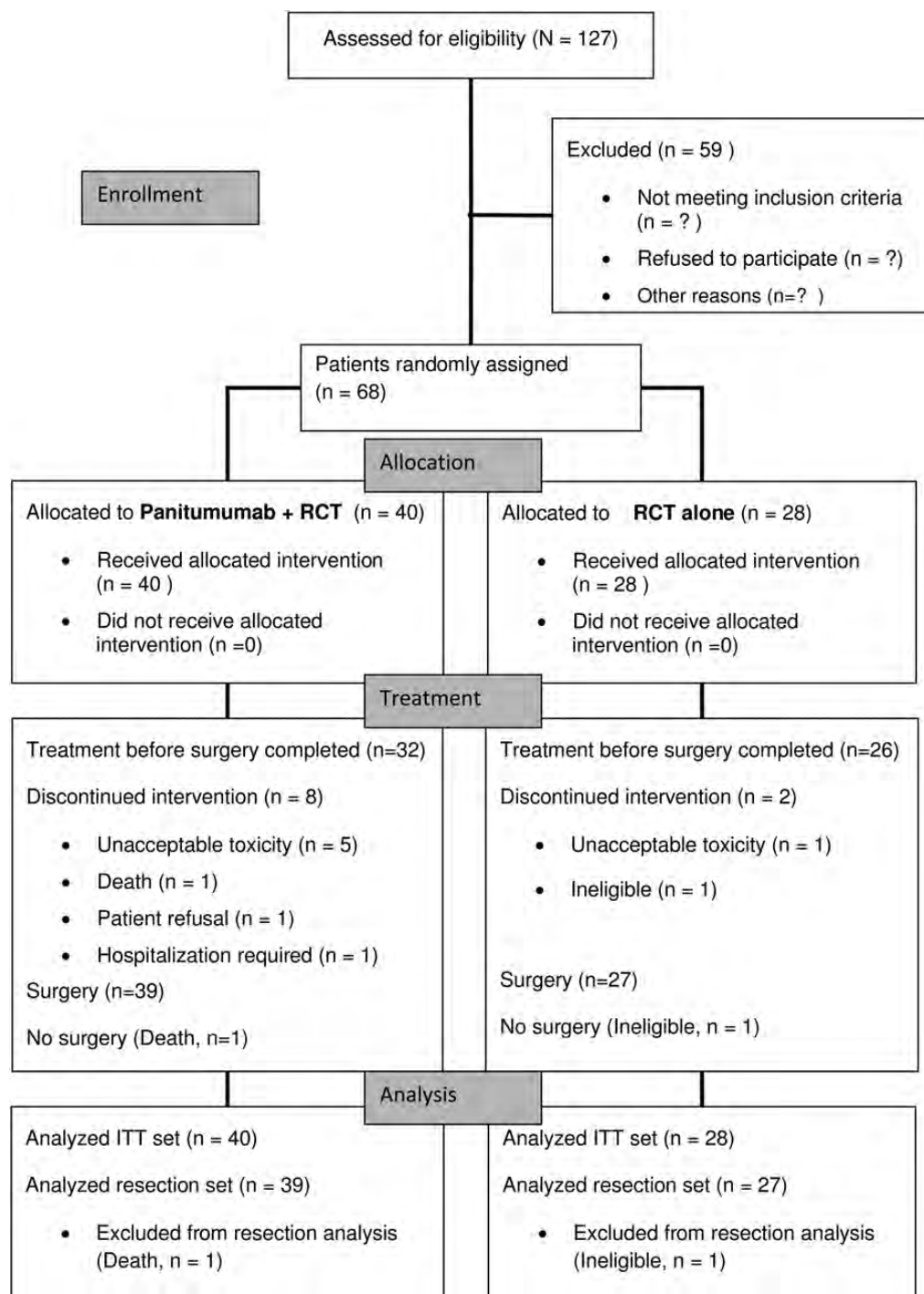
The median total CRT doses were similar in the P + CRT and the CRT arms: RT 45 and 45 Gy; capecitabine 98% and 97% of target dose; and panitumumab 88% of target dose. There were four panitumumab dose delays (for diarrhea, and/or hand-foot syndrome,  $n = 3$ ; skin toxicity,  $n = 1$ ) and 12 reduced/omitted doses (mainly due to diarrhea or proctitis/ulceration,  $n = 6$  or skin toxicity/dry skin/erythema multiforme,  $n = 4$ ).

### surgery and outcomes

One patient (P + CRT) died of unknown causes and another patient (CRT) was withdrawn from the study because of incorrect diagnosis. A further eight patients stopped treatment because of adverse events (P + CRT,  $n = 5$ ; CRT,  $n = 1$ ) or other reasons, including patient's wish or renal failure requiring hospitalization (P + CRT, one patient each). A total of 66 patients underwent surgery (P + CRT,  $n = 39$ ; CRT,  $n = 27$ ). A median of 15 lymph nodes per patient was harvested in both the treatment arms. Tumor regression grade is shown in Table 2 and achievement of the primary and early secondary end-points is summarized in Table 3.

Complete regression by the Dworak criteria (grade 4) was achieved in four patients (10%) and near-complete regression (grade 3) in 17 patients (43%) in the P + CRT arm. In the CRT arm, five patients (18%) and four patients (14%), respectively, achieved complete and near-complete regression (Table 2). A central pathology review for grade 2 or 3 regression was completed for all but two patients (both in the P + CRT arm). The primary end-point, pNC/CR, was achieved in 21 patients (53%; 95% CI 36%–69%) in the P + CRT arm and 9 patients (32%; 95% CI 16%–52%) in the CRT arm.

R0 resection was achieved in 33 patients (85%) in the P + CRT arm and in 25 patients (93%) in the CRT arm. Downstaging of the primary tumor and/or lymph nodes was achieved in 34 patients (87%) and 23 patients (85%), with sphincter preservation being achieved in 27 (69%) and 19 (70%) patients.



**Figure 1.** Consort diagram.

### postoperative complications

Postoperative complications are listed in Table 4. The most common complications were anastomosis leakage, local infection and requirement for further intervention/operation [ $n = 7$  patients each (18%) in the P + CRT arm versus 2, 4 and 4 patients (7%, 15% and 15%) in the CRT arm].

### follow-up and deaths

The median follow-up time for 68 assessable patients was 9.1 months. So far only four patients died (two in each arm). Both

the patients in the P + CRT arm died perioperatively, whereas the patients in the CRT arm died after tumor recurrences during the follow-up phase. Both of the latter patients had R0 resection, but the circumferential resection margin was <2 mm in one patient.

### translational studies

The median EGFR gene copy number was 1.0. The EGFR copy number did not associate with pNC/CR (data not shown). Mutational analysis in tissue sections revealed a total of 23

**Table 1.** Baseline characteristics of patients

	Panitumumab + chemoradiotherapy (CRT) (n = 40)	CRT (n = 28)	All patients (n = 68)
Age (median/range)	62/31–80	60/35–77	61/31–80
Sex			
Male	33 (82%)	20 (71%)	53 (78%)
Female	7 (18%)	8 (29%)	15 (22%)
World Health Organization (WHO) performance status			
0	38 (95%)	27 (96%)	65 (96%)
1	2 (5%)	1 (4%)	3 (4%)
N stage			
cN0	8 (20%)	7 (25%)	15 (22%)
cN1	24 (60%)	13 (46%)	37 (54%)
cN2	8 (20%)	8 (29%)	16 (24%)
T stage <sup>b</sup>			
cT2	4 (10%)	2 (7%)	6 (9%)
cT3	34 (85%)	26 (93%)	60 (88%)
cT4	2 (5%)	0 (0%)	2 (3%)
Histological grade			
G1	0 (0%)	2 (7%)	2 (3%)
G2	35 (88%)	23 (82%)	58 (85%)
G3	3 (8%)	3 (11%)	6 (9%)
Gx	2 (5%)	0 (0%)	2 (3%)
Tumor localization <sup>a,b</sup> (cm)			
<10	32 (80%)	22 (79%)	54 (79%)
≥10	8 (20%)	6 (21%)	14 (21%)
EGFR gene copy number <sup>b</sup>			
<2.9	38 (95%)	26 (93%)	64 (94%)
≥2.9	2 (5%)	2 (7%)	4 (6%)

Categorical parameters shown as frequency and percentage.

<sup>a</sup>In centimeters from the anal verge.

<sup>b</sup>Stratification factor.

**Table 2.** Number and percentage of patients achieving each Dworak regression grade (intent-to-treat analysis)

Dworak regression grade	Panitumumab + chemoradiotherapy (CRT, n = 40)	CRT (n = 28)	All patients (n = 68)
None (grade 0)	0 (0%)	1 (4%)	4 (6%)
Minimal (grade 1)	9 (23%)	11 (40%)	20 (29%)
Moderate (grade 2)	7 (18%)	6 (21%)	13 (19%)
Good (grade 3)	17 (43%)	4 (14%)	21 (31%)
Total (grade 4)	4 (10%)	5 (18%)	9 (13%)

mutations in 18 tumors (26%), including 12 mutations in *KRAS* exons 3 and 4, 7 mutations in *PIK3CA*, 2 mutations in *NRAS* and 1 mutation in *BRAF*. Thirteen tumors harbored a single mutation and five tumors had two concomitant mutations in *PIK3CA* and *KRAS* (exons 3 and 4) (n = 4) and *NRAS* (n = 1). There was no significant association between additional mutations and with pNC/CR (data not shown).

The *RASSF2A* promoter was hypermethylated in 75% of the rectal tumors, but no significant association was found with

**Table 3.** Number and proportion of patients achieving primary and key secondary end-points

	No. of patients	Percent <sup>a</sup>	95% CI (exact)
Pathological near-complete or complete tumor response (pNC/CR)			
Panitumumab + chemoradiotherapy (CRT)	21/40	53	(36, 69)%
CRT	09/28	32	(16, 52)%
Total	30/68	44	(32, 57)%
R0 resection <sup>b</sup>			
Panitumumab + CRT	33/39	85	(70, 94)%
CRT	25/27	93	(76, 99)%
Total	58/66	89	(78, 95)%
Sphincter preservation			
Panitumumab + CRT	27/39	69	(52, 83)%
CRT	19/27	70	(50, 86)%
Total	46/66	70	(57, 80)%
Downstaging of primary tumor or lymph nodes			
Panitumumab + CRT	34/39	87	(73, 96)%
CRT	23/27	85	(66, 96)%
Total	57/66	86	(76, 94)%
Downstaging of primary tumor and lymph nodes			
Panitumumab + CRT	16/39	41	(26, 58)%
CRT	08/27	30	(14, 50)%
Total	24/66	36	(25, 49)%

CI, confidence intervals.

<sup>a</sup>Based on the intent-to-treat population for pCR and the operated population for the remaining end-points.

<sup>b</sup>Only one patient (from arm A) had R1 resection.

**Table 4.** Number and percentage of patients with postoperative complications within 8 weeks after surgery

Complication	Panitumumab + chemoradiotherapy (CRT; n = 39)	CRT (n = 27)	Total (n = 66)
Anastomotic leakage	7 (18%)	2 (7%)	9 (14%)
Fistula	2 (5%)	2 (7%)	4 (6%)
Local infection	7 (18%)	4 (15%)	11 (17%)
Bladder dysfunction	3 (8%)	2 (7%)	5 (8%)
Erectile dysfunction	3 (8%)	3 (11%)	6 (9%)
Interventions/operation	7 (18%)	4 (15%)	11 (17%)
Other	6 (15%)	8 (30%)	14 (21%)

pNC/CR (data not shown). In contrast, the *EFGR* promoter was hypomethylated in all tumors.

The remaining tumor cells of the available specimens with regression Dworak grade 3 showed no difference in MIB-1 and M30 expression compared with those with regression Dworak grades 0–2 for both the treatment arms (supplementary Table S1, available at *Annals of Oncology* online).

No mutation of *KRAS* exon 2 (codon 12 and 13) was detected in serum. None of the 12 serum biomarkers analyzed were significantly associated with pNC/CR (supplementary Tables S2 and S3, available at *Annals of Oncology* online).

**Table 5.** Number and percentage of patients with most common grade 3–4 or 1–2 toxicities by common terminology criteria for adverse events

	Grade 3–4		Grade 1–2	
	Panitumumab + chemoradiotherapy (CRT; n = 40)	CRT (n = 28)	Panitumumab + CRT (n = 40)	CRT (n = 28)
Diarrhea	4 (10%)	1 (4%)	29 (73%)	15 (54%)
Hand-foot syndrome	1 (2%)	0 (0%)	6 (15%)	4 (14%)
Fatigue	1 (2%)	0 (0%)	18 (45%)	9 (32%)
Acneiform skin rash	1 (2%)	0 (0%)	14 (35%)	0 (0%)
Nausea	0 (0%)	0 (0%)	10 (25%)	6 (21%)
Anastomotic leakage	6 (15%)	1 (4%)	1 (3%)	1 (4%)

### tolerability

All 68 patients were assessable for safety. The most common CTC grade  $\geq 3$  toxic effects in the P + CRT and the CRT arm were: diarrhea (10% and 4% of patients), hand-foot syndrome (2% and 0%), fatigue (2% and 0%), acneiform skin rash (2% and 0%) and leakage (15% and 4%) (Table 5; grade 1–2 toxic effects also shown).

Adverse events led to treatment discontinuation in five patients in the P + CRT arm (diarrhea, enterocolitis, ulceration/proctitis, dry skin, erythema multiforme/pruritus/itching) and one patient in the CRT arm (cardiac arrest). Seventeen patients had 18 serious adverse events [12 patients (30%) in the P + CRT arm, five patients (18%) in the CRT arm]. Of these events, three in the P + CRT arm and two in the CRT arm were classified as clearly related to treatment. There were two deaths during the safety monitoring period, both in the P + CRT arm: from acute cardiac failure and sepsis resulting from anastomotic leakage, respectively.

### discussion

In patients with LARC, the rates of pCR have generally been low (<10%) with neoadjuvant regimens consisting of CRT and cetuximab [25–30]. It is possible that this might reflect issues such as the type of chemotherapy used, dosage and scheduling. Moreover, these studies did not preselect patients with wild-type *KRAS* tumors. However, two studies using combination chemotherapies with EGFR-targeted Mab achieved pCR rates of >20% [31, 32].

Our study investigated the addition of panitumumab to neoadjuvant capecitabine and external RT. Using a prospective approach we excluded patients with tumors harboring *KRAS* mutations. Although *KRAS* testing is now integrated into clinical practice before the initiation of cetuximab or panitumumab treatment in patients with metastatic colorectal cancer [33, 34], the role of *KRAS* mutations in determining the response to EGFR-targeted mAbs in the neoadjuvant setting is less clear. In a number of phase II studies in LARC, which did not select patients by *KRAS* status, including the StarPan/STAR-02 study, *KRAS* and/or *BRAF* mutations did not correlate with pCR after neoadjuvant treatment containing cetuximab or panitumumab, probably because of low numbers of patients in these studies [31, 32, 35–38].

The primary end-point of our study was pNC/CR, defined as Dworak grade 3, as well as grade 4 tumor regression. This is

based on the previous data showing that Dworak grade 3 in this setting may confer a similar clinical prognosis to complete response [39, 40]. In the P + CRT arm, there were 4 grade 4 (10%) and 17 grade 3 regressions (43%), compared with only 5 grade 4 (18%) and 4 grade 3 (14%) regressions in the CRT arm, for pNC/CR rates of 53% and 32%. Central pathology review was mandatory for all tumors with Dworak grade 2 or 3 regression. Our M30/Ki-67 results indicate that most of the remaining tumor cells are not apoptotic and show proliferation rates in the range of untreated tumors. This fits with the cancer stem cell hypothesis, in that eradication of responsive cancer cells leaves behind a cancer cell population with clonogenic potential. Although this was true for both the treatment arms, it was more pronounced in the P + CRT arm in which the high pNC/CR rate was mainly achieved by Dworak grade 3 (Table 2). In the light of the ongoing debate regarding the validity of pCR as a surrogate end-point in rectal cancer trials, our results point out the fact that pCR (defined as Dworak grade 4) only reflects a part of the possible destruction following neoadjuvant treatment schedules. This might at least partly explain why in the EXPERT-C trial the pCR was not affected by the addition of cetuximab, whereas the response rate and overall survival were changed significantly [38].

As this was a phase II trial with two parallel treatment arms, formal statistical comparisons between the two treatment arms were not conducted. We included a standard arm in this trial for calibration purposes and to avoid biases due to patient selection and heterogeneous medical care.

The StarPan/STAR-02 study achieved a 21% pCR rate (12/57 patients; 95% CI 10%–32%) based on complete tumor regression (ypT0N0) with a neoadjuvant regimen of panitumumab, 5-FU and oxaliplatin-RT [32]. Grade 3–4 diarrhea occurred in 39% of the patients with one toxic death, and grade 3/4 skin toxicity in 19% of the patients [32]. In our study, all the patients randomly assigned to P + CRT received a pre-emptive supportive treatment including oral doxycycline. Such measures have been shown to markedly reduce the incidence of skin toxicity and diarrhea associated with panitumumab [41]. Grade 3–4 diarrhea occurred in 10% of our patients, with grade 3–4 acneiform rash in only 2%.

Postoperative complications were relatively common in the P + CRT arm. The rate of postoperative anastomotic leakage that we encountered (18% of patients) with one patient developing fatal sepsis is in the upper range [42–44] of the reported literature. In our study, panitumumab was continued until 2 to 3 weeks before surgery and this may have delayed the

healing process, thus contributing to additional perioperative toxicity.

None of the mutations in the genes belonging to the EGFR pathway were associated with the response to panitumumab. The rate of *BRAF* mutations in our series of rectal cancers (1.5%) as in the EXPERT-C trial [38] is significantly lower than the rates reported in colon cancers (5%–12%) [19, 45]. This was expected, since *BRAF* mutations are generally associated with tumors with microsatellite instability, which are mainly localized in the proximal colon.

In conclusion, this study showed that the addition of panitumumab to neoadjuvant CRT in patients with *KRAS* wild-type LARC resulted in a high pNC/CR rate, mostly grade 3 DC. The results of both the treatments exceeded prespecified thresholds and are promising for the experimental arm. The addition of panitumumab was well tolerated but increased toxicity. More vigorous validation of these results is required in a phase III trial.

## Acknowledgements

Julia Balfour, a medical writer, Dundee, Scotland, assisted with writing of this manuscript. Julia Balfour was paid by the SAKK with a grant from Amgen, Switzerland. We also thank Barbara Hügli, a laboratory technician, Bern, for excellent technical assistance.

## funding

This work was financially supported by Amgen and Swiss State Secretariat for Education and Research (SER).

## disclosure

The authors have declared no conflicts of interest.

## references

- Bosset JF, Collette L, Calais G et al.. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 2006; 355: 1114–1123.
- Klaassen RA, Nieuwenhuijzen GA, Martijn H et al.. Treatment of locally advanced rectal cancer. *Surg Oncol* 2004; 13: 137–147.
- Sauer R, Becker H, Hohenberger W et al.. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* 2004; 351: 1731–1740.
- Gerard JP, Azria D, Gourgou-Bourgade S et al.. Comparison of two neoadjuvant chemoradiotherapy regimens for locally advanced rectal cancer: results of the phase III trial ACCORD 12/0405-Prodige 2. *J Clin Oncol* 2010; 28: 1638–1644.
- Hofheinz R, Wenz FK, Post S et al.. Capecitabine (Cape) versus 5-fluorouracil (5-FU)-based (neo) adjuvant chemoradiotherapy (CRT) for locally advanced rectal cancer (LARC): long-term results of a randomized, phase III trial. *J Clin Oncol* 2011; 29: (suppl; abstr 3504) 2011.
- Cunningham D, Humblet Y, Siena S et al.. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; 351: 337–345.
- Douillard JY, Siena S, Cassidy J et al.. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010; 28: 4697–4705.
- Jonker DJ, O'Callaghan CJ, Karapetis CS et al.. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007; 357: 2040–2048.
- Peeters M, Price TJ, Cervantes A et al.. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol* 2010; 28: 4706–4713.
- Bokemeyer C, Bondarenko I, Makhson A et al.. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; 27: 663–671.
- Bonner JA, Maihle NJ, Folven BR et al.. The interaction of epidermal growth factor and radiation in human head and neck squamous cell carcinoma cell lines with vastly different radiosensitivities. *Int J Radiat Oncol Biol Phys* 1994; 29: 243–247.
- Liang K, Ang KK, Milas L et al.. The epidermal growth factor receptor mediates radioresistance. *Int J Radiat Oncol Biol Phys* 2003; 57: 246–254.
- Bonner JA, Harari PM, Giralt J et al.. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006; 354: 567–578.
- Bonner JA, Harari PM, Giralt J et al.. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *Lancet Oncol* 2010; 11: 21–28.
- Amado RG, Wolf M, Peeters M et al.. Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008; 26: 1626–1634.
- Karapetis CS, Khambata-Ford S, Jonker DJ et al.. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; 359: 1757–1765.
- Siena S, Sartore-Bianchi A, Di Nicolantonio F et al.. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009; 101: 1308–1324.
- Di Nicolantonio F, Martini M, Molinari F et al.. Wild-type *BRAF* is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008; 26: 5705–5712.
- De Roock W, Claes B, Bernasconi D et al.. Effects of *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; 11: 753–762.
- Sartore-Bianchi A, Moroni M, Veronese S et al.. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. *J Clin Oncol* 2007; 25: 3238–3245.
- Khambata-Ford S, Garrett CR, Meropol NJ et al.. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol* 2007; 25: 3230–3237.
- Greene LP, Balch CM, Fleming ID et al.. *AJCC Cancer Staging Manual*, 6th edition. New York: Springer 2002.
- Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. *Int J Colorectal Dis* 1997; 12: 19–23.
- Fleming TR. One-sample multiple testing procedure for phase II clinical trials. *Biometrics* 1982; 38: 143–151.
- Bertolini F, Chiara S, Bengala C et al.. 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* 2009; 73: 466–472.
- Eisterer W, De Vries A, Oefner D et al.. Neoadjuvant chemoradiation therapy with capecitabine (X) plus cetuximab (C), and external beam radiotherapy (RT) in locally advanced rectal cancer (LARC): ABCSG trial R03. *J Clin Oncol* 2009; 27:15S; Proc ASCO, A4109.
- Horisberger K, Treschl A, Mai S et al.. 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* 2009; 74: 1487–1493.
- Machiels JP, Sempoux C, Scalliet P et al.. Phase I/II study of preoperative cetuximab, capecitabine, and external beam radiotherapy in patients with rectal cancer. *Ann Oncol* 2007; 18: 738–744.
- Rodel C, Arnold D, Hipp M et al.. Phase I-II trial of cetuximab, capecitabine, oxaliplatin, and radiotherapy as preoperative treatment in rectal cancer. *Int J Radiat Oncol Biol Phys* 2008; 70: 1081–1086.

30. Velenik V, Ocvirk J, Oblak I et al. A phase II study of cetuximab, capecitabine and radiotherapy in neoadjuvant treatment of patients with locally advanced resectable rectal cancer. *Eur J Surg Oncol* 2010; 36: 244–250.
31. Kim SY, Hong YS, Kim DY et al. Preoperative chemoradiation with cetuximab, irinotecan, and capecitabine in patients with locally advanced resectable rectal cancer: a multicenter phase II study. *Int J Radiat Oncol Biol Phys* 2010; 81(3): 677–683.
32. Pinto C, Di Fabio F, Maiello E et al. Phase II study of panitumumab, oxaliplatin, 5-fluorouracil, and concurrent radiotherapy as preoperative treatment in high-risk locally advanced rectal cancer patients (StarPan/STAR-02 Study). *Ann Oncol* 2011; 22(11): 2424–2430.
33. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Colon Cancer (version 3. 2011). [www.nccn.org](http://www.nccn.org). Last accessed 29 March 2011.
34. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Rectal Cancer (version 4. 2011). [www.nccn.org](http://www.nccn.org). Last accessed 29 March 2011.
35. Bengala C, Bettelli S, Bertolini F et al. Epidermal growth factor receptor gene copy number, K-ras mutation and pathological response to preoperative cetuximab, 5-FU and radiation therapy in locally advanced rectal cancer. *Ann Oncol* 2009; 20: 469–474.
36. Debucquoy A, Haustermans K, Daemen A et al. Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer. *J Clin Oncol* 2009; 27: 2751–2757.
37. Gaedcke J, Grade M, Jung K et al. KRAS and BRAF mutations in patients with rectal cancer treated with preoperative chemoradiotherapy. *Radiother Oncol* 2010; 94: 76–81.
38. Dewdney A, Cunningham D, Tabernero J et al. Multicenter randomized phase II clinical trial comparing neoadjuvant oxaliplatin, capecitabine, and preoperative radiotherapy with or without cetuximab followed by total mesorectal excision in patients with high-risk rectal cancer (EXPERT-C). *J Clin Oncol* 2012; 30(14): 1620–1627.
39. Beddy D, Hyland JM, Winter DC et al. A simplified tumor regression grade correlates with survival in locally advanced rectal carcinoma treated with neoadjuvant chemoradiotherapy. *Ann Surg Oncol* 2008; 15: 3471–3477.
40. Guillem JG, Chessin DB, Cohen AM et al. Long-term oncologic outcome following preoperative combined modality therapy and total mesorectal excision of locally advanced rectal cancer. *Ann Surg* 2005; 241: 829–836; discussion 836–828.
41. Lacouture ME, Mitchell EP, Piperdi B et al. Skin toxicity evaluation protocol with panitumumab (STEPP), a phase II, open-label, randomized trial evaluating the impact of a pre-emptive skin treatment regimen on skin toxicities and quality of life in patients with metastatic colorectal cancer. *J Clin Oncol* 2010; 28: 1351–1357.
42. Law WJ, Chu KW, Ho JW et al. Risk factors for anastomotic leakage after low anterior resection with total mesorectal excision. *Am J Surg* 2000; 179: 92–96.
43. Nesbakken A, Nygaard K, Lunde OC. Outcome and late functional results after anastomotic leakage following mesorectal excision for rectal cancer. *Br J Surg* 2001; 88: 400–404.
44. Rullier E, Laurent C, Garrelon JL et al. Risk factors for anastomotic leakage after resection of rectal cancer. *Br J Surg* 1998; 85: 355–358.
45. Kalady MF, DeJulius KL, Sanchez JA et al. BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. *Dis Colon Rectum* 2012; 55: 128–133.

*Annals of Oncology* 24: 725–733, 2013  
doi:10.1093/annonc/mds528  
Published online 8 November 2012

## Her2/neu testing in gastric cancer: evaluating the risk of sampling errors

V. S. Warneke<sup>1,†</sup>, H.-M. Behrens<sup>1,2,†</sup>, C. Böger<sup>1</sup>, T. Becker<sup>3</sup>, F. Lordick<sup>4</sup>, M. P. A. Ebert<sup>5</sup> & C. Röcken<sup>1\*</sup>

<sup>1</sup>Department of Pathology, Christian-Albrechts University, Kiel; <sup>2</sup>Department of Pathology, Charité University Hospital, Berlin; <sup>3</sup>Department of General Surgery and Thoracic Surgery, Christian-Albrechts University, Kiel; <sup>4</sup>University Cancer Centre Leipzig, University of Leipzig, Leipzig; <sup>5</sup>Department of Medicine II, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany

Received 11 April 2012; revised 2 September 2012; accepted 4 September 2012

**Background:** We evaluated the risk of sampling errors in specimens of biopsy size, which may be caused by heterogeneous overexpression of Her2/neu in gastric cancer (GC).

**Patients and methods:** The study cohort comprised 454 gastrectomy patients with adenocarcinoma of the stomach or esophago-gastric junction. Tissue micro-arrays (TMAs) served as 'biopsy procedure' and were generated from formalin-fixed and paraffin-embedded tissue: five tissue cylinders were collected randomly from each tumor, rendering 2230 core cylinders. These were compared with 454 whole tissue sections obtained from the same paraffin blocks. Her2/neu expression and gene amplification were analyzed by immunohistochemistry and *in situ* hybridization. The Her2/neu status was determined according to GC scoring system by two independent observers.

\*Correspondence to: Dr C. Röcken, Department of Pathology, Christian-Albrechts University, Arnold-Heller-Str. 3, Haus 14, D-24105 Kiel, Germany.  
Tel: +49-431-597-3401; Fax: +49-431-597-3462; E-mail: christoph.roecken@uk-sh.de

<sup>†</sup>VSW and H-MB contributed equally to this work.