The Role of HER-3 Expression in the Prediction of Clinical Outcome for Advanced Colorectal Cancer Patients Receiving Irinotecan and Cetuximab

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LEARNING OBJECTIVES:

After completing this course, the reader will be able to:

1. Describe the relationship between HER-3 status and response to treatment with cetuximab and irinotecan in patients with advanced colorectal cancer and EGFR+ wild-type *K-RAS* mutation in this study.
2. Evaluate data that support using HER-3 status as a predictive factor for anti-EGFR treatment response in patients with colorectal cancer.

ABSTRACT

Preclinical data suggested that, in the presence of human epidermal growth factor receptor (HER)-3–altered activation, colorectal cancer cells may escape anti–epidermal growth factor receptor (EGFR)–mediated cell death. HER-3 overexpression may then represent a key factor for resistance to anti-EGFR antibodies in colorectal cancer. The aim of our analysis was to investigate a possible correlation between HER-3 expression and clinical outcome in wild-type *K-RAS* advanced colorectal cancer patients receiving cetuximab and irinotecan.

We retrospectively analyzed immunoreactivity for HER-3 in wild-type *K-RAS* advanced colorectal cancer patients receiving irinotecan and cetuximab.

Eighty-four advanced wild-type *K-RAS* colorectal cancer patients were available for HER-3 analysis. Forty patients (48%) had a HER-3[−] colorectal tumor, whereas the remaining 44 cases (52%) were deemed

HER-3⁺. In patients with HER-3[−] and HER-3⁺ tumors, we observed a partial response in 17 (42%) and eight (18%) patients respectively; progressive disease occurred in 11 (35%) and 26 (53%) patients with HER-3[−] and HER-3⁺ tumors, respectively ($p = .003$). The median progression-free survival time was 6.3 months in patients with HER-3[−] tumors and 2.8 months for those who had HER-3–overexpressing tumors ($p < .0001$). The median overall survival time was 13.6 months in patients showing HER-3[−] tumors and 10.5 months for those who had HER-3–expressing tumors ($p = .01$).

HER-3 proved to be a predictive factor for clinical outcome in wild-type *K-RAS* colorectal cancer patients treated with cetuximab. Combined HER-3 and *K-RAS* analysis may represent an effective strategy for better selection of responding colorectal cancer patients. *The Oncologist* 2011;16:53–60

INTRODUCTION

Clinical reports with the use of monoclonal antibodies directed against the ligand-binding site of the epidermal growth factor receptor (EGFR) have shown practice-changing results in the treatment of colorectal cancer patients and will hopefully further improve the available therapeutic options for patients diagnosed with this highly deadly disease in the near future [1–5].

After several years of intense translational research and the clinical absence of predictive factors, the introduction of *K-RAS* mutational status seemed to possess the necessary potential for full translation of the concept of targeted therapy in this setting into clinical practice [5–7].

However, if on the one hand we are now able to exclude from anti-EGFR treatment patients with putative refractory colorectal tumors (i.e., those harboring a *K-RAS*–mutant status), on the other hand we are still incapable of accurately selecting responding patients among those without *K-RAS* mutations. In fact, clinical observations have suggested that a non-negligible proportion of patients, in the range of 70%–40%, do not seem to benefit from the use of anti-EGFR targeted antibodies, even in the absence of mutation in the *K-RAS* gene (i.e., wild-type *K-RAS*) [2, 4, 5–7].

These considerations have thus dampened enthusiasm about the possibility of reliable selection of appropriate antineoplastic therapy based on molecular characteristics of the tumor, which seemed initially possible with the introduction of anti-EGFR treatment modalities.

The human epidermal growth factor receptors (EGFR, HER-2, HER-3, and HER-4) are transmembrane receptors

able to activate downstream signaling pathways in response to extracellular signals. Early biological analyses regarding these receptors demonstrated a marked interdependence on each other and complementarity in their functions with lateral signaling constituting a biologically significant aspect of their activity. Among this family of receptors, HER-3 is the only one lacking tyrosine kinase function, thus demonstrating biological activity only as a consequence of heterodimerization [8–10]. In particular, the HER-3 heterodimer is a highly functional signaling unit with a central role in mediating the phosphoinositide 3-kinase (PI3K)–Akt downstream signaling pathway. Preclinical data have suggested that this HER-3 biological activity as an EGFR lateral signaling partner may have a crucial function in mediating de novo or acquired resistance to anti-EGFR treatment [11–15]. A comparative analysis of cetuximab-resistant versus parental lines showed that EGFR, along with HER-3, was highly activated in resistant clones [11]. Further analyses also indicated that biological resistance to cetuximab might reflect dysregulation of EGFR internalization/degradation and subsequent EGFR-dependent activation of HER-3. HER-3 activity may then represent a critical step for tumor cells to escape cetuximab inhibition [8, 9, 11].

More findings from these studies also showed that cetuximab had no effect on the autophosphorylation of EGFR or the activity of HER-3 and Akt in resistant tumor cell lines. In contrast, cetuximab was able to interfere with mitogen-activated protein kinase (MAPK) activity. This implies that MAPK probably plays only a minor role in anti-

EGFR treatment resistance [11]. Globally, HER-3–driven activation of the PI3K–Akt signaling pathway emerged as a relevant critical factor for resistance to anti-EGFR treatment.

The aim of our analysis was to evaluate the role of HER-3 expression in terms of the response rate, time to progression, and overall survival time of wild-type *K-RAS* advanced colorectal cancer patients treated with irinotecan and cetuximab. The interaction between HER-3 expression and wild-type *K-RAS* mutational status was tested in order to verify the ability of HER-3 to identify a subgroup of patients more likely to benefit from EGFR-targeted antibody treatment.

MATERIALS AND METHODS

Patient Selection

Patients with histologically proven EGFR⁺ wild-type *K-RAS* metastatic colorectal cancer receiving a combination of cetuximab and irinotecan after at least one line of previous chemotherapy were eligible for our analysis. This study was approved by the institutional ethics committee. All patients received cetuximab at an initial dose of 400 mg/m² followed by weekly infusions of 250 mg/m². Irinotecan was administered at a dose of 180 mg/m² every 2 weeks either alone or in combination with 5-fluorouracil and leucovorin. Tumor response was evaluated every 8 weeks by clinician assessment and according to the Response Evaluation Criteria in Solid Tumors.

HER-3 Immunohistochemistry

HER-3 was evaluated using an immunohistochemical technique on 3- to 5- μ m thick tissue sections obtained from paraffin-embedded specimens fixed in 10% (volume/volume) neutral buffered formalin. The sections were deparaffinized in xylene, rehydrated in graded ethanol, washed in phosphate-buffered saline, and heated in a microwave at 98°C, with buffer (pH 9) for 40 minutes.

Peroxide blocking was performed with 3% H₂O₂ at room temperature for 10 minutes. The monoclonal mouse antibody anti-human HER-3 (DAK-H3-IC) was used (1:50 dilution; Dako, Glostrup, Denmark) and samples were incubated overnight at 4°C.

Incubation with the secondary antibody (EnVision System®, Dako HRP; Dako) was performed for 30 minutes, followed by application of diaminobenzidine chromogen for 5 minutes. Subsequently, the slides were counterstained with Meyer's hematoxylin for 1 minute, dehydrated in a graded series of alcohol, treated with xylene, and cover-slipped.

The slides were evaluated using light microscopy independently by two pathologists (I.B. and A.M.).

HER-3 expression was detected as homogeneous finely granular cytoplasmatic staining of neoplastic cells and, rarely, membrane staining [16]. Stained slices were evaluated using the Rajkumar score [17] resulting from the product of the score for the fraction of positively stained tumor cells (0–4, scored as the percentage of positively stained tumor cells as follows: 0, <10%; 1, 10%–25%; 2, 26%–50%; 3, 51%–75%; 4, >75%) and the score for staining intensity (0–3).

Adult stomach sections were used as positive controls in each immunostaining experiment, because parietal cells of the gastric glands are distinctly immunoreactive for HER-3 [16].

Negative controls were used during optimization of the method and did not show any staining.

K-RAS Mutational Analysis

Tumor samples, formalin-fixed and paraffin-embedded, were analyzed for *KRAS* exon 2 mutations, located within codon 12 and codon 13 (Gly12Asp, Gly12Ala, Gly12Val, Gly12Ser, Gly12Arg, Gly12Cys, and Gly13 Asp).

For each tumor sample, the neoplastic area was selected and DNA was extracted using a DNA extraction kit from Qiagen (QIAamp® DNA Mini and Blood Mini; Qiagen, Hilden, Germany). The extracted DNA was polymerase chain reaction (PCR) amplified using the following sense and antisense primers: 5'-AAGGCCT-GCTGAAAATGACTG-3' and 5'-CAAAGAATGGTC-CTGCACCAG-3'.

After purification using the QIAquick® PCR Purification kit (Qiagen), PCR products were direct sequenced using the Big Dye V1.1 Terminator Kit (Applied Biosystems, Foster City, CA) and an ABI Prism 3100 DNA sequencer (Applied Biosystems).

Statistical Analysis

Statistical analyses were performed using MedCalc package (MedCalc® v9.4.2.0; MedCalc Software bvba, Mariakerke, Belgium).

A receiver operating characteristic (ROC) curve analysis was performed to determine a cutoff value for the HER-3 expression results.

Association between categorical variables was analyzed using the χ^2 test. The Kruskal–Wallis test was used to analyze the possible association between HER-3 expression and response results.

Survival distribution was estimated by the Kaplan–Meier method. Significant differences in the probability of

Table 1. Patient characteristics and results for HER-3 immunohistochemistry expression

	Whole group (n = 84)	HER-3 ⁻ (n = 40; 48%)	HER-3 ⁺ (n = 44; 52%)	p-value
Age (range), yrs	64 (36–80)	65 (38–78)	64 (36–80)	
Sex				
Male	56 (67%)	27 (67%)	29 (66%)	
Female	28 (33%)	13 (33%)	15 (34%)	
Prior oxaliplatin	84 (100%)	40 (100%)	44 (100%)	
Previous lines of treatment				
1	9 (11%)	4 (10%)	5 (11%)	
2–3	75 (89%)	36 (90%)	39 (89%)	
Treatment				
mFOLFIRI ^a + cetuximab	30 (36%)	13 (32%)	17 (39%)	
Irinotecan + cetuximab	54 (64%)	27 (68%)	27 (61%)	
Response				
PR	25 (30%)	17 (42%)	8 (18%)	
SD	22 (26%)	12 (30%)	10 (23%)	
PD	37 (44%)	11 (28%)	26 (59%)	.003
Median PFS (mos)	3.2	6.3	2.8	<.0001
Median OS (mos)	11.3	13.6	10.5	.01

Only statistically significant *p*-values are shown.

^amFOLFIRI consisted of irinotecan, 180 mg/m² on day 1, bolus 5-FU, 400 mg/m² on day 1, and continuous infusion 5-FU, 2,400 mg/m² over 46 hours.

Abbreviations: 5-FU, 5-fluorouracil; HER-3, human epidermal growth factor receptor 3; mFOLFIRI, modified 5-fluorouracil, leucovorin, and irinotecan; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial remission; SD, stable disease.

relapsing between strata were evaluated using the log-rank test.

A significance level of 0.05 was chosen to assess statistical significance.

For statistical analyses, overall survival (OS) and progression-free survival (PFS) were defined, respectively, as the interval between the start of cetuximab–irinotecan therapy and death or last follow-up visit and as the interval between the start of cetuximab–irinotecan therapy and clinical progression or death or last follow-up visit if there was no progression.

RESULTS

Eighty-four advanced wild-type *K-RAS* colorectal cancer patients were available for HER-3 immunohistochemistry analysis. Patient characteristics are shown in Table 1. All patients were previously treated with oxaliplatin-based chemotherapy, and most of them (89%) were treated with the cetuximab–irinotecan combination after failure of at least two previous lines of chemotherapy. Overall, we observed a partial response in 25 patients (30%) and progressive disease in 37 cases (44%). Twenty-two patients (26%) showed tumor stabilization, whereas no complete remissions were obtained (Table 1).

The immunohistochemistry analysis for HER-3 expression was performed in primary tumors in all cases. HER-3 results according to the Rajkumar score were as follows: score 0, 15 cases (18%); score 1, seven cases (8%); score 2, two cases (3%); score 3, four cases (5%); score 4, five cases (6%); score 6, seven cases (8%); score 8, 26 cases (31%); score 9, 12 cases (14%); and score 12, six cases (7%). The cutoff point with the highest sensitivity and specificity for estimating HER-3 expression was set at ≤8 after the ROC curve analysis (Fig. 1). Consequently, tumors showing a HER-3 score ≥8 were classified as highly expressing HER-3 (or HER-3⁺) tumors, in contrast to tumors with a HER-3 score <8 (low-expressing HER-3 or HER-3⁻ tumors).

Forty (48%) patients had HER-3⁻ colorectal tumors, whereas the remaining 44 patients (52%) were deemed to be HER-3⁺. All major clinical characteristics were comparable between the HER-3⁻ and HER-3⁺ groups of patients. In particular, no differences were noticed for sex, age at diagnosis, and previous lines of chemotherapy (Table 1). In contrast, in patients with HER-3⁻ and HER-3⁺ tumors, respectively, we observed a partial response in 17 (42%) and eight (18%) patients; progressive disease was obtained in

Figure 1. Receiver operating characteristic analysis based on human epidermal growth factor receptor 3 expression results with response to cetuximab therapy as the endpoint. In this model, the sensitivity was 92% (95% confidence interval [CI], 74%–99%) and specificity was 96.7% (95% CI, 88.5%–99.6%). The area under the curve was 0.95 ($p = .0001$).

11 (35%) and 26 (53%) patients, respectively, with HER-3[−] and HER-3⁺ tumors ($p = .003$) (Table 1). The median PFS interval was 6.3 months in patients with HER-3[−]

tumors and 2.8 months for those who had HER-3-expressing tumors ($p < .0001$) (Fig. 2). The median OS time was 13.6 months in patients with HER-3[−] tumors and 10.5 months for those who had HER-3-expressing tumors ($p = .01$) (Fig. 3).

DISCUSSION

Since the introduction of *K-RAS* mutational status analysis as a predictive factor for anti-EGFR antibody treatment, further biological determinants have been investigated in order to better identify responding colorectal cancer patients. It has been, in fact, evident since the very beginning that although a mutant *K-RAS* status was able to exclude many resistant patients from treatment, a considerable proportion of patients with wild-type *K-RAS* tumors were still refractory to this therapeutic approach. Unfortunately, to date it is not possible to further characterize this latter group of wild-type *K-RAS* patients who, in the end, receive ineffective therapy at the cost of unwanted side effects.

Most biological factors analyzed in the attempt to improve patient selection in this setting focused either on the EGFR downstream signaling pathway or on the receptor itself. Published research data suggested that EGFR polymorphisms [18], gene copy number [19, 20], *PI3K* and *b-RAF* mutational status [21, 22, 23], and phosphatase and tensin homologue deleted on chromosome ten or nuclear factor κB expression [24, 25] may all represent predictive

Figure 2. Kaplan-Meier curves for the median progression-free survival intervals of colorectal cancer patients treated with irinotecan and cetuximab showing human epidermal growth factor receptor (HER)-3 tumor overexpression (dotted line) and without HER-3 tumor overexpression (solid line)—2.8 versus 6.3 months, respectively ($p < .0001$).

Figure 3. Kaplan–Meier curves for the median overall survival duration of colorectal cancer patients treated with irinotecan and cetuximab showing tumor human epidermal growth factor receptor (HER)-3 overexpression (dotted line) and without tumor HER-3 overexpression (solid line)—10.5 months versus 13.6 months, respectively ($p = .01$).

determinants for anti-EGFR therapy. However, initial observations were often conflicting and limited to a small proportion of patients. For example, preliminary data on the role of *b-RAF* mutational status seemed initially promising for a straightforward application in clinical practice, but a large subsequent analysis from the Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer trial demonstrated that *b-RAF*, although possessing a possible prognostic role, had no predictive value [26].

A global analysis of all biological markers that have been suggested as possible predictive factors for anti-EGFR treatment would be extremely relevant at this point. However, several technical and biological limitations prevented us from performing such in-depth analysis. First of all, the tumor samples at our disposal were insufficient for all these analyses (insufficient materials). Furthermore, some biological factors cited, such as *b-RAF* and PI3K, are too rarely altered (about 10% of all cases) to significantly affect the results of our case series, and last but not least, the sample size (84 patients) allowed us to speculate about one new, unexplored factor (HER-3), but it would have been clearly statistically inadequate for multiple comparisons of multiple factors.

Despite the relevant number of preclinical observations, few clinical studies have been conducted to explore the putative function of growth factor receptor interdependence

and complementarity in influencing clinical outcome with anti-EGFR treatment [27].

In our analysis, HER-3 overexpression was able to identify a subgroup of colorectal cancer patients who, despite a wild-type *K-RAS* status, were unlikely to respond to cetuximab. In the present series, HER-3 status seemed to influence not only the response rate and PFS interval but also the OS duration, thus confirming a strong rationale for its use as a predictive factor.

In colorectal cancer, the association between HER-3 expression and clinical outcome was investigated in the past mainly as a predictive variable for tumor prognosis in resected patients. Nevertheless, results in this area often have been contradictory and not easily transferable to clinical practice [28, 29]. One study suggested a trend toward a worse prognosis for patients with abnormal expression of HER-3, but unfortunately without repeatable significance across different studies [29]. Besides, the omission in the analysis of other known factors with established prognostic roles, the low number of patients examined, and the heterogeneity of the population studied prevented investigators from reaching definitive conclusions. Apparently no data investigating HER-3 are available for metastatic colorectal cancer.

In contrast, in agreement with our findings, HER-3–altered expression has been suggested to be crucial in pre-

dicting resistance to anti-EGFR treatment in breast and lung tumors [9, 13, 30].

Taken together, these observations seem to confirm that HER-3 expression is not likely to have a marked prognostic role but should be considered relevant as a predictive factor.

In our series, we decided to analyze HER-3 expression and not phosphorylated HER-3 status. Analysis of phosphorylated HER-3 in paraffin-embedded tissues is not standardized, and results are mostly unreliable and extremely difficult to replicate in different institutions. Only the availability of fresh-frozen samples would allow a reliable analysis of phosphorylated HER-3, but unfortunately this is not possible for a retrospective series. In view of a hopefully larger application/replication of our results, HER-3 expression seems much more reliable and widely applicable. Besides, because HER-3 lacks tyrosine kinase activity, we can postulate that HER-3 expression may be a key factor for the formation of heterodimers and thus for its biological activity. The available clinical data from different tumor types using HER-3 expression seem to confirm this suggestion.

Interestingly, HER-3 has also been demonstrated to be a possible molecular target as part of a treatment protocol focused on control of either HER receptors or the PI3K–Akt pathway. Although HER-3 itself is not an easily druggable target because of the lack of kinase activity, several treat-

ment approaches aimed at this receptor are under study [8, 9]. The possibility of using HER-3 inhibitors in biologically selected anti-EGFR-resistant tumors promises to be a crucial challenge for the future development of targeted therapy in colorectal cancer patients.

CONCLUSIONS

We believe that our data, along with those already available on the biological mechanism putatively connected to tumor resistance to anti-EGFR monoclonal antibody therapy, may help in composing the molecular mosaic of the various aspects related to the use of EGFR-targeted antineoplastic agents. Furthermore, besides identifying colorectal cancer patients refractory to EGFR-directed treatment, HER-3 overexpression may also represent a potential biological indicator for the development of a new class of antineoplastic agents in this setting.

AUTHOR CONTRIBUTIONS

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REFERENCES

- Goldberg RM, Rothenberg ML, Van Cutsem E et al. The continuum of care: A paradigm for the management of metastatic colorectal cancer. *The Oncologist* 2007;12:38–50.
- Jonker DJ, O'Callaghan CJ, Karapetis CS et al. Cetuximab for the treatment of colorectal cancer. *J Clin Oncol* 2007;357:2040–2048.
- 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.
- Van Cutsem E, Peeters M, Siena S et al. 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* 2007;25:1658–1664.
- Van Cutsem E, Köhne CH, Hitt E et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408–1417.
- Lièvre A, Bachet JB, Boige V et al. Kras mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008;26:374–379.
- Di Fiore F, Blanchard F, Charbonnier F et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. *Br J Cancer* 2007;96:1166–1169.
- Sithanandam G, Anderson LM. The ERBB3 receptor in cancer and cancer gene therapy. *Cancer Gene Ther* 2008;15:413–448.
- Hsieh AC, Moasser MM. Targeting HER proteins in cancer therapy and the role of the non-target HER-3. *Br J Cancer* 2007;97:453–457.
- Campbell MR, Amin D, Moasser MM. HER3 comes of age: New insights into its functions and role in signaling, tumor biology, and cancer therapy. *Clin Cancer Res* 2010;16:1373–1383.
- Wheeler DL, Huang S, Kruser TJ et al. Mechanisms of acquired resistance to cetuximab: Role of HER (ErbB) family members. *Oncogene* 2008;27:3944–3956.
- Buck E, Eyzaguirre A, Haley JD et al. Inactivation of Akt by the epidermal growth factor receptor inhibitor erlotinib is mediated by HER-3 in pancreatic and colorectal tumor cell lines and contributes to erlotinib sensitivity. *Mol Cancer Ther* 2006;5:2051–2059.
- Xu JM, Han Y, Duan HQ et al. EGFR mutations and HER2/3 protein expression and clinical outcome in Chinese advanced non-small cell lung cancer patients treated with gefitinib. *J Cancer Res Clin Oncol* 2009;135:771–782.
- Frolov A, Schuller K, Tzeng CW et al. ErbB3 expression and dimerization with EGFR influence pancreatic cancer cell sensitivity to erlotinib. *Cancer Biol Ther* 2007;6:548–554.
- Jain A, Penuel E, Mink S et al. HER kinase axis receptor dimer partner switching occurs in response to EGFR tyrosine kinase inhibition despite failure to block cellular proliferation. *Cancer Res* 2010;70:1989–1999.
- Prigent SA, Lemoine NR, Hughes CM et al. Expression of the c-erbB-3 protein in normal human adult and fetal tissues. *Oncogene* 1992;7:1273–1278.
- Tanner B, Hasenclever D, Stern K et al. ErbB-3 predicts survival in ovarian cancer. *J Clin Oncol* 2006;24:4317–4323.
- Lurje G, Nagashima F, Zhang W et al. Polymorphisms in cyclooxygenase-2 and epidermal growth factor receptor are associated with progression-free survival independent of K-ras in metastatic colorectal cancer patients treated with single-agent cetuximab. *Clin Cancer Res* 2008;14:7884–7895.

- 19 Moroni M, Veronese S, Benvenuti S et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: A cohort study. *Lancet Oncol* 2005;6:279–286.
- 20 Scartozzi M, Bearzi I, Mandolesi A et al. Epidermal growth factor receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: A fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis. *BMC Cancer* 2009;9:303–310.
- 21 Perrone F, Lampis A, Orsenigo M et al. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. *Ann Oncol* 2009;20:84–90.
- 22 Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F et al. Oncogenetic activation of RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res* 2007;67:2643–2648.
- 23 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.
- 24 Loupakis F, Pollina L, Stasi I et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. *J Clin Oncol* 2009;27:2622–2629.
- 25 Scartozzi M, Bearzi I, Pierantoni C et al. Nuclear factor- κ B tumor expression predicts response and survival in irinotecan-refractory metastatic colorectal cancer treated with cetuximab-irinotecan therapy. *J Clin Oncol* 2007;25:3930–3935.
- 26 Köhne C, Stroiakovski D, Chang-Chien C et al. Predictive biomarkers to improve treatment of metastatic colorectal cancer (mCRC): Outcomes with cetuximab plus FOLFIRI in the CRYSTAL trial [abstract 4068]. *J Clin Oncol* 2009;27(15 suppl):15.
- 27 Scartozzi M, Mandolesi A, Giampieri R et al. Insulin-like growth factor 1 expression correlates with clinical outcome in K-RAS wild type colorectal cancer patients treated with cetuximab and irinotecan. *Int J Cancer* 2010;127:1941–1947.
- 28 Leung SP, Griffith OL, Masoudi H et al. Clinical utility of type 1 growth factor receptor expression in colon cancer. *Am J Surg* 2008;195:604–610.
- 29 Kountourakis P, Pavlakis K, Psyrri A et al. Prognostic significance of HER3 and HER4 protein expression in colorectal adenocarcinomas. *BMC Cancer* 2006;6:46–54.
- 30 Sergina NV, Rausch M, Wang D et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature* 2007;445:437–441.