**EGFR Fluorescence In situ Hybridization Pattern of Chromosome 7 Disomy Predicts Resistance to Cetuximab in KRAS Wild-type Metastatic Colorectal Cancer Patients**

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**Abstract**

**Purpose:** Metastatic colorectal cancer patients with low epidermal growth factor receptor (EGFR) gene copy number are unlikely to respond to anti-EGFR monoclonal antibody (mAb) treatment. The objective of this study was to investigate EGFR fluorescence in situ hybridization (FISH) patterns of chromosome 7 disomy with efficacy of cetuximab therapy in metastatic colorectal cancer patients.

**Experimental Design:** We detected the EGFR FISH patterns and KRAS status in 74 tumors from cetuximab-treated metastatic colorectal cancer patients and analyzed with response rate (RR) and progression-free survival (PFS).

**Results:** One of the 16 (6.25%) patients with chromosome 7 homogeneous disomy (defined as FISH negative) had objective response to cetuximab. A total of 53 (76.8%) patients with chromosome 7 pattern of variable ratios of disomy versus polysomy (defined as FISH positive) had a significantly higher RR (37.7% versus 6.25%; P = 0.01), a trend towards longer PFS (4.5 versus 2.9 months; P = 0.07). Among 54 KRAS wild-type patients, EGFR FISH-positive patients had significantly higher RR (51.3% versus 9%; P = 0.01) and longer PFS (5.0 versus 2.3 months; P = 0.02) than EGFR FISH-negative patients. However, among 20 KRAS mutant-type patients, there was no difference in RR (0% versus 0%) and PFS (2.5 versus 3.8 months; P = 0.51) between EGFR FISH-positive and -negative patients.

**Conclusion:** Our results show firstly that patients with EGFR FISH pattern of chromosome 7 disomy have a very low chance to benefit from cetuximab-based therapy. EGFR FISH pattern of chromosome 7 disomy may be as a negative predictive factor for cetuximab response in KRAS wild-type metastatic colorectal cancer patients. *Clin Cancer Res;* 17(2); 382–90. ©2011 AACR.

**Introduction**

It has been shown that the anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody (mAb) cetuximab (Erbitux, developed by Merck KGaA) as monotherapy or for combination with chemotherapy can improve responsiveness and prolong survival in patients with metastatic colorectal cancer (1–4), but only 10% to 20% of patients respond to this agent. Several recent clinical studies have shown that the presence of a KRAS mutation is a significant predictor of resistance to anti-EGFR mAbs (5–7). On the basis of this finding, the European Union drug regulatory body, the European Medicines Agency, has approved the use of anti-EGFR mAbs only for metastatic colorectal cancer patients whose tumors display wild-type (WT) KRAS. However, the occurrence of KRAS mutations only accounts for approximately 30% to 40% of nonresponsive patients. Therefore, the identification of additional genetic determinants of treatment benefit still needs to be defined.

Recently, studies have suggested that an increased EGFR gene copy number (GCN) analyzed by the fluorescence in situ hybridization (FISH) technique could be a promising predictor of anti-EGFR mAb therapy in metastatic colorectal cancer (8–11). Patients with low GCN are indeed unlikely to respond to anti-EGFR mAb treatment and have less progression-free time than patients with increased GCN. However, the EGFR FISH pattern of metastatic colorectal cancer is often not homogeneous, and has variable ratios of disomy versus polysomy or amplification. In these situations, the definition of EGFR patterns and the reproducibility of data lead to difficulties in direct comparison and clinical application. Moroni et al. reported that chromosome 7 homogeneous disomy is the most frequent

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Translational Relevance

Metastatic colorectal cancer patients with low epidermal growth factor receptor (EGFR) gene copy number (GCN) are unlikely to respond to anti-EGFR monoclonal antibody treatment. However, the definition of GCN and the reproducibility of data lead to the difficulties in clinical application. In this article, we describe the EGFR FISH patterns of chromosome 7 homogeneous disomy, which is the most frequent pattern of nonincreased EGFR GCN in colorectal cancer and is easy to detect as a negative predictive factor for cetuximab response in KRAS wild-type metastatic colorectal cancer. Together with KRAS mutation, chromosome 7 disomy may predict more patients who will not respond to cetuximab.

Pattern in metastatic colorectal cancer with decreased EGFR GCN (12). They suggested that chromosome 7 disomy is easier to detect than an increase in EGFR copy number and therefore, might enable a more reproducible FISH result. However, no clinical data have supported his hypothesis yet. In addition, EGFR is a transmembrane tyrosine kinase receptor that, on ligand binding, mainly triggers the RAS-RAF-MAPK and PI3K-PTEN-AKT signaling pathways. The resistance to anti-EGFR mAbs may be due to constitutive activation of the downstream genes of the EGFR signaling pathway such as KRAS, BRAF, or PIK3C2A, or to the loss of a tumor suppressor gene such as PTEN. This implies that pathways rather than single genes should be the focus of studies aimed at analyzing anti-EGFR mAb therapy.

The aim of the present study was therefore to examine EGFR FISH patterns combined with KRAS mutation status in metastatic colorectal cancer patients, and investigate their associations with response to cetuximab therapy. To this end, we first evaluated whether previously generated cutoff points could be validated in our independent series. Second, we assessed whether chromosome 7 disomy could be used as EGFR FISH result criteria on this data set. Furthermore, we explored the combination of EGFR GCN with KRAS status which currently is the best-established marker for outcome prediction after cetuximab is administered for colorectal cancer.

Patients and Methods

Patients

This retrospective study enrolled 74 consecutive metastatic colorectal cancer patients treated with cetuximab-containing regimens between May 2005 and March 2010 from three institutions in China, including Sun Yat-sen University Cancer Center (Guangzhou), Beijing Cancer Hospital (Beijing), and The First People’s Hospital of Foshan (Guangdong Province). Patients were selected based on the following criteria: histologically proven metastatic colorectal adenocarcinoma; presence of at least one measurable lesion; cetuximab-containing regimens were received after failure of irinotecan- and/or oxaliplatin-based regimens; sufficient specimens of formalin-fixed paraffin-embedded tissue were available from primary colorectal and/or metastatic tumors; never previously received EGFR-targeted therapy; having signed informed consent form. The study was approved by the Research Ethics Committee of the Sun Yat-sen University Cancer Center (reference YP-2009177).

Cetuximab was administered as a loading dose of 400 mg/m² i.v., followed by a dose of 250 mg/m² once a week. All patients received cetuximab in combination with cytotoxic drugs; 63 (85.1%) patients received cetuximab plus irinotecan or irinotecan-based chemotherapy, 10 (13.5%) received cetuximab plus oxaliplatin-based chemotherapy, and 1 (1.4%) received cetuximab plus capecitabine chemotherapy.

Clinical response was assessed every 6 to 8 weeks by radiologic examination (computed tomography or magnetic resonance imaging). The Response Evaluation Criteria in Solid Tumors guidelines (13) were adopted for evaluation, and objective tumor response was classified as complete response, partial response, stable disease, or progressive disease. Patients with complete response or partial response were defined as responders, whereas patients with stable disease or progressive disease were defined as non-responders. Progression-free survival (PFS) was calculated from the time of first cetuximab infusion to the time of disease progression or death from any cause. Overall survival (OS) was calculated from the time of first cetuximab infusion to patient death or last contact.

DNA extraction and KRAS mutation analysis

DNA was extracted from paraffin-embedded colorectal cancer samples using the QiAmp DNA Mini Kit (Qiagen) according to the manufacturer’s recommendations after a histologic control for the presence of tumor cells (>70%) in each tumor sample. A real-time PCR genotyping method was done for the detection of KRAS codon 12 and codon 13 mutations. The presence of KRAS mutations (6 at codon 12 and 1 at codon 13) was determined by allelic discrimination assay on an ABI 7900HT Sequence Detection System (Applied Biosystems). Specific probes for each allele (mutated and wild alleles) were labeled with the fluorescence reporter dyes FAM or VIC at their 5’-end. Briefly, reactions were done in a 25 μl mixture comprising 50 ng of DNA, 0.2 μL (20 μmol/L) of specific primers and probes, and 12.5 μL 1 × TaqMan Universal PCR Master Mix (Applied Biosystems). The PCR amplification was done under the following cycle conditions: 95 °C for 10 minutes; 40 cycles, 95 °C for 30 seconds; and 60 °C for 1 minute. Data were analyzed with SDS2.0 software (Applied Biosystems). Each mutation detected by allelic discrimination was validated by direct sequencing analysis.

Determination of EGFR gene copy number by FISH

EGFR GCN per cell was investigated by FISH using the LSI EGFR Spectrum Orange/CEP 7 Spectrum Green probe.
(Vysis, Abbott Laboratories) according to the manufacturer’s protocol. Briefly, 2-μm-thick tissue sections were cut and incubated at 56 °C overnight; after being deparaffinized and dehydrated, the sections were incubated in 2 × saline sodium citrate buffer (2 × SSC; pH 7.0) at 75 °C for 20 minutes. Then the sections were digested with proteinase K (0.2 mg/mL in 2 × SSC, pH 7.0) at 37 °C for 20 minutes, rinsed in 2 × SSC (pH 7.0) at room temperature for 5 minutes, fixed in 10% neutral buffered formalin, and dehydrated using ethanol in a series of increasing concentrations (70%, 85%, 100%). The probe sets were applied onto the tissue areas on each slide, and the hybridization area was covered with a glass coverslip and sealed with rubber cement. The slides were incubated in a humidified atmosphere at 85 °C for 5 minutes for codenaturation of probe and target DNA, and subsequently at 37 °C for 16 hours for hybridization. Posthybridization washes were done in 1.5 mol/L Urea and 0.1 × SSC (pH 7.0–7.5) at 45 °C for 30 minutes and in 2 × SSC for 2 minutes at room temperature. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). FISH signals for each locus-specific FISH probe were assessed under an Olympus BX51 TRF microscope (Olympus, Japan) equipped with a triple-pass filter (DAPI/green/orange; Vysis).

Without knowledge of the patients’ clinical molecular characteristics, two independent observers (WF and SQ) scored at least 100 nonoverlapping interphase nuclei for the number of copies of EGFR and CEP7 by use of predefined scoring guidelines. The negative controls consisted of a healthy colorectal mucosa adjacent to malignant disease; the control for amplified EGFR was an amplified colonic adenocarcinoma. FISH patterns were defined as described in (14, 15): Briefly, the samples were grouped as follows: normal disomy, ≤2 gene copies in >90% of cells; trisomy, 3 gene copies in >10% of cells and ratio gene/chromosomes ≤2; low polysomy, ≥4 gene copies in >10% but <40% of cells and ratio gene/ chromosomes ≤2; high polysomy, ≥4 gene copies in >40% cells and ratio gene/chromosomes ≤2; and gene amplification, ratio gene/chromosome >2 or 15 gene copies in ≥10% of cells. Trisomy, low polysomy, high polysomy, and/or gene amplification were considered EGFR-FISH positive. Normal disomy was considered EGFR-FISH negative.

Statistics

Differences in response rate (RR) were tested by the Fisher’s exact test; PFS, OS, and the 95% confidence intervals (95% CI) were evaluated by Kaplan-Meier survival analysis. Comparisons of PFS and OS between different groups were done by the log-rank test. EGFR sensitivity and specificity were expressed in terms of percentage, and the value for which sensitivity and specificity were the highest was chosen as the best cutoff point. All statistical analyses were carried out on SPSS 13.0 software and P < 0.05 was considered statistically significant.

Table 1. Characteristics of 74 patients of metastatic colorectal cancer patients

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total evaluated</td>
<td>74 (100)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>43 (58.1)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (41.9)</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53</td>
</tr>
<tr>
<td>Range</td>
<td>23–82</td>
</tr>
<tr>
<td>Primary tumor site</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>43 (58.1)</td>
</tr>
<tr>
<td>Rectum</td>
<td>31 (41.9)</td>
</tr>
<tr>
<td>Previous chemotherapy regimens</td>
<td></td>
</tr>
<tr>
<td>Irinotecan containing</td>
<td>59 (79.7)</td>
</tr>
<tr>
<td>Oxaliplatin containing</td>
<td>69 (93.2)</td>
</tr>
<tr>
<td>Cetuximab treatment line</td>
<td></td>
</tr>
<tr>
<td>First line</td>
<td>0</td>
</tr>
<tr>
<td>Second line</td>
<td>15 (20.3)</td>
</tr>
<tr>
<td>Third line and more</td>
<td>59 (79.7)</td>
</tr>
<tr>
<td>Treatment regimens</td>
<td></td>
</tr>
<tr>
<td>Cetuximab monotherapy</td>
<td>0</td>
</tr>
<tr>
<td>Cetuximab plus chemotherapy</td>
<td>74 (100)</td>
</tr>
<tr>
<td>Treatment duration (weeks)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11</td>
</tr>
<tr>
<td>Range</td>
<td>2–38</td>
</tr>
<tr>
<td>Kras mutation status</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>54 (73)</td>
</tr>
<tr>
<td>Mutation</td>
<td>20 (27)</td>
</tr>
</tbody>
</table>

Results

Patient characteristics and KRAS status

The baseline and treatment characteristics of the 74 patients are listed in Table 1. Among the patients, 25 T1 (33.8%) achieved an objective tumor response (all partial responses and no complete response), 24 (32.4%) had stable disease, and 25 (33.8%) had progressive disease. Median PFS time was 4.4 months (95% CI, 3.3–5.6), and median OS time was 18.6 months (95% CI, 13.9–23.2). Of the 74 primary tumors analyzed, 20 had KRAS mutations (27%). None of the 20 KRAS mutation patients had an objective response to cetuximab, whereas 21 of the 54 KRAS WT patients were responders (0% versus 38.9%, respectively; P < 0.001). Patients with KRAS WT had significantly longer OS (25.3 versus 16.0 months; P = 0.05) and a significantly longer PFS (5.0 versus 2.5 months; P = 0.004) compared with KRAS mutation patients.

EGFR FISH analyses

EGFR FISH analysis was successfully detected in 69 of the tumor samples. In the remaining five cases adequate
samples for analysis were unavailable due to lack of thin tumor sections of 2 μm provided by a local hospital (n = 4) and tissue calcification (n = 1). Primary tumor tissues were obtained in 66 cases. There was metastasis in only three cases (tissue samples were obtained from liver, left cervical lymph node, and retroperitoneal lymph node, respectively). Representative patterns of EGFR gene signals evaluated by FISH are shown in Fig. 1. Among the 69 patients, 2 (2.9%) had EGFR gene amplification in focal areas of the tumor cells, 51 (73.9%) had an EGFR FISH pattern of variable ratios of disomy versus polysomy, and 16 (23.2%) had chromosome 7 homogeneous disomy.

We initially analyzed our patients’ EGFR FISH data according to other scoring systems previously reported in colorectal carcinomas (score B, scores C and D; refs. 9, 11), and in lung carcinoma (score E; refs. 14, 17), as presented in Table 2. When score B was used, 41 patients (59.4%) were EGFR FISH positive. EGFR FISH-positive patients had a significantly higher RR (39% versus 17.9%; P = 0.05) and a trend towards longer OS (18.6 versus 16 months; P = 0.09); there was no significant difference in PFS (4.2 versus 3.8 months; P = 0.69). Score B showed a 47.9% sensitivity (95% CI, 36.1–59.7) and 76.2% specificity (95% CI, 66.1–86.2). When score C was used, 28 patients (40.6%) were EGFR FISH positive. No significant differences in RR (39.3% versus 24.4%; P = 0.15), PFS (4.6 versus 3.5 months; P = 0.97), and OS (18.9 versus 17.5 months; P = 0.27) were observed between EGFR FISH-negative and EGFR FISH-positive patients. For this model, sensitivity was 64.6% (95% CI, 53.2–76.0) and specificity was 52.4% (95% CI, 40.6–64.2). When score D was used, 16 patients (23.2%) were classified as EGFR FISH positive and no association was detected between EGFR FISH positive and clinical outcomes such as RR (P = 0.34), PFS (P = 0.75), and OS (P = 0.80).

We further defined chromosome 7 homogeneous disomy as EGFR FISH negative; and gene amplification and pattern of variable ratios of disomy versus polysomy as EGFR FISH positive. According to these criteria, 16 (23.2%) cases were classified as EGFR FISH negative and 53 (76.8%) as EGFR FISH positive. EGFR FISH-positive patients had a significantly higher RR (37.7% versus 6.25%; P = 0.01) and a trend towards longer PFS (4.5 versus 2.9 months; P = 0.07); there was no significant difference in OS (18.6 versus 11.3 months; P = 0.11; Table 2 and Fig. 2). This model showed a specificity of 95.2% (95% CI, 90.1–100.3) and a sensitivity of 31.2% (95% CI, 20.3–42.1; Table 2).

Chromosome 7 disomy for prediction with different KRAS status

Among the 54 KRAS WT patients, 11 (20.4%) had an EGFR FISH pattern of chromosome 7 disomy (EGFR FISH negative), whereas 37 (68.5%) had variable ratios of disomy versus polysomy, and 2 (3.7%) had gene amplification (EGFR FISH positive); the other 4 (7.4%) patients did not have EGFR FISH results (Table 3). One of the EGFR FISH-negative patients had an objective response

Fig. 1. Representative FISH detection of EGFR copy and chromosome 7 disomy. A, EGFR gene focal amplification in tumor cells. B, increase in EGFR gene copy number and polysomy of chromosome 7. C, homogenous of EGFR gene copy number with a homogeneous chromosome 7 Trisomy. D, no EGFR gene copy number and chromosome 7 disomy alteration pattern.
to cetuximab, whereas 20 of the EGFR FISH-positive patients were responders (9% versus 51.3%, respectively; \( P < 0.01 \)). Patients who were EGFR FISH positive had a significantly longer PFS (5.0 versus 2.3 months; \( P = 0.02 \)) than EGFR FISH-negative patients, but there was no significant difference in OS (18.6 versus 10.0 months; \( P = 0.16 \); Fig. 5A and B). Among the 20 KRAS mutation patients, 5 (25%) had an EGFR FISH pattern of chromosome 7 disomy (EGFR FISH negative), 14 (70.0%) had variable ratios of disomy versus polysomy, and none had gene amplification (EGFR FISH positive); 1 (5.0%) patient did not have an EGFR FISH result (Table 3). KRAS mutation patients, whether EGFR FISH positive or negative, had no objective response to cetuximab. There were no significant differences in RR (0% versus 0%), PFS (2.5 versus 3.8 months; \( P = 0.51 \)), or OS (15.9 versus 11.3 months; \( P = 0.43 \)) between EGFR FISH-positive and EGFR FISH-negative patients (Fig. 3C and D).

### Table 2. Clinical outcomes of the patients according to EGFR copy numbers detected by FISH

<table>
<thead>
<tr>
<th>Patients, n (%)</th>
<th>Response, n (%)</th>
<th>PFS (months)</th>
<th>OS (months)</th>
<th>Specificity (95% CI)</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR FISH+ (cut point A) 53 (76.8)</td>
<td>20 (37.7)</td>
<td>4.5</td>
<td>18.6</td>
<td>95.2 (90.1–100.3)</td>
<td>31.2 (20.3–42.1)</td>
</tr>
<tr>
<td>EGFR FISH- (cut point A) 16 (23.2)</td>
<td>1 (6.25)</td>
<td>2.9</td>
<td>11.3</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>EGFR FISH+ (cut point B) 41 (59.4)</td>
<td>16 (39.0)</td>
<td>4.2</td>
<td>18.6</td>
<td>76.2 (66.1–86.2)</td>
<td>47.9 (36.1–59.7)</td>
</tr>
<tr>
<td>EGFR FISH- (cut point B) 28 (40.6)</td>
<td>5 (17.9)</td>
<td>3.8</td>
<td>16</td>
<td>0.05</td>
<td>0.69</td>
</tr>
<tr>
<td>EGFR FISH+ (cut point C) 28 (40.6)</td>
<td>11 (39.3)</td>
<td>4.6</td>
<td>18.9</td>
<td>52.4 (40.6–64.2)</td>
<td>64.6 (53.2–76.0)</td>
</tr>
<tr>
<td>EGFR FISH- (cut point C) 41 (59.4)</td>
<td>10 (24.4)</td>
<td>3.5</td>
<td>17.5</td>
<td>0.15</td>
<td>0.97</td>
</tr>
<tr>
<td>EGFR FISH+ (cut point D) 16 (23.2)</td>
<td>6 (37.5)</td>
<td>5.1</td>
<td>18.9</td>
<td>28.6 (18.0–39.2)</td>
<td>79.2 (69.6–88.8)</td>
</tr>
<tr>
<td>EGFR FISH- (cut point D) 53 (76.8)</td>
<td>15 (28.3)</td>
<td>3.5</td>
<td>17.9</td>
<td>0.34</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Cut point A, a mean of \( \geq 2.0 \) EGFR gene copy number qualifies the tumor as FISH positive.

Cut point B, a mean of \( \geq 2.47 \) EGFR gene copy number qualifies the tumor as FISH positive.

Cut point C, a mean of \( \geq 2.92 \) EGFR gene copy number qualifies the tumor as FISH positive.

Cut point D, according to the score system proposed in non–small cell lung cancer, a tumor is defined as FISH positive when \( \geq 40\% \) of cells have \( \geq 4 \) copies of EGFR or in presence of gene amplification.
Combination of KRAS status and chromosome 7 disomy for prediction

Sixty-nine patients who were successfully tested for EGFR by FISH were also tested for KRAS mutational status. The combination of KRAS status and EGFR FISH patterns were analyzed for response and survival prediction in 41 (55.4%) patients with KRAS WT and EGFR FISH-positive status and 28 (37.8%) patients with KRAS mutation and/or EGFR FISH-negative status. One of the 28 KRAS mutation and/or EGFR FISH-negative patients had an...

![Graphs showing Kaplan-Meier estimates of progression-free survival and overall survival in KRAS WT and KRAS mutation metastatic colorectal cancer patients treated with cetuximab according to EGFR GCN patterns evaluated by FISH.](image)

**Table 3.** Relationship between tumor response and KRAS status combined with EGFR GCN

<table>
<thead>
<tr>
<th></th>
<th>KRAS wild-type (n = 54)*</th>
<th>KRAS mutation (n = 20)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR GCN</td>
<td>No. = 2.0</td>
<td>No. &gt; 2.0</td>
</tr>
<tr>
<td>Response (CR+PR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponse (SD+PD)</td>
<td>10</td>
<td>19</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

*Fifty metastatic colorectal cancer patients got the result of EGFR GCN by FISH.

†Nineteen metastatic colorectal cancer patients got the result of EGFR GCN by FISH.
objective response to cetuximab, whereas 20 of 41 KRAS WT and EGFR FISH-positive patients were responders (3.6% versus 48.8%, respectively; \( P < 0.001 \)). Patients with KRAS WT and EGFR FISH-positive status had a significantly longer PFS (5.0 versus 2.6 months; \( P = 0.005 \)) than KRAS mutation and or EGFR FISH-negative patients, and a trend towards longer OS (18.6 versus 16.0 months; \( P = 0.27 \)) (Fig. 4). This model showed a sensitivity of 65.2% (95% CI, 51.7–78.7) and a specificity of 100% (95% CI, 100–100).

Discussion and Conclusion

Although recent studies have confirmed that EGFR GCN assessed by FISH can influence the response to anti-EGFR mAb therapy in metastatic colorectal cancer, methods of tissue processing and EGFR scoring systems were not standardized among these studies (8–11). In our present study, we assessed the value of an EGFR GCN cutoff point according to what has been previously reported in colorectal carcinomas and in lung carcinomas (9–11, 14–17). Our results agree with other studies that the cutoff point defined in lung carcinomas is not suitable for predicting the response to cetuximab in metastatic colorectal cancer. For metastatic colorectal cancer patients, the nonincreased EGFR GCN status rather than the increased is the most accurate predictive factor for clinical outcome, so effort should be made to better define the low copy number pattern. In our study, using the cutoff of \( \geq 2.92 \) EGFR GCN as FISH positive as proposed by Cappuzzo (11), there was no difference in RR PFS, or OS between the negative and positive groups. Using the cutoff of \( \geq 2.47 \) EGFR GCN as positive as proposed by Sartore-Bianchi (9), positive patients had a significantly higher RR (39% versus 17.9%; \( P = 0.05 \)) and a trend towards longer OS (18.6 versus 16 months; \( P = 0.09 \)), suggesting that the cutoff point of 2.47 EGFR GCN seems more suitable for our study.

The thickness of tumor sections may influence the judgment and definition of EGFR GCN. Our study evaluated a large number of cells in thin sections of 2 \( \mu \)m to avoid overlapping of nuclei, which was the same procedure as in Sartore-Bianchi’s study (9), whereas Cappuzzo’s (11) analysis was conducted using 4-\( \mu \)m tissue sections. The thinner tumor sections could be responsible for the lower cutoff point associated with clinical outcome reported in different studies.

However, it is difficult to determine exactly whether the EGFR GCN is more or less than 2.47/nucleus in clinical practice. From a morphologic point of view, chromosome 7 disomy is easier to identify and assess than an increase in EGFR GCN. Sartore-Bianchi et al. have reported that most metastatic colorectal cancer patients with nonincreased EGFR GCN displayed homogeneous disomy (9). There are no data, however, to support chromosome 7 disomy being used in clinical practice at this time. Our present study revealed that only 1 of the 16 (6.2%) disomy patients responded to treatment with cetuximab. The specificity of prediction for nonresponsive patients was 95.2%, but the sensitivity of prediction for responsive patients was quite low (31.2%). A biomarker that definitively predicts a negative response is as useful as one that predicts a positive response. Using chromosome 7 disomy as criteria, our study indicated that 23% of metastatic colorectal cancer patients could be excluded from unnecessary treatment with cetuximab. Sartore-Bianchi et al. have reported that none of the 38 patients with a mean EGFR GCN of \(<2.47\) nucleus responded to panitumumab whereas 6 of 20 patients with a mean EGFR GCN of \(\geq 2.47\) nucleus achieved a response (9). If their data are analyzed using chromosome 7 disomy as criteria, the specificity of predic-
tion for nonresponsive patients is still 100%, but the sensitivity of prediction for responsive patients is lower than they reported. We agree with Mauro Moroni’s opinion that from a clinical point of view, we can risk treating a nonresponsive patient, but we cannot risk not treating a potentially responsive patient (12). Furthermore, the most important point is that chromosome 7 disomy is easier to detect than an increase in EGFR copy number, and therefore, might enable a more reproducible FISH assay in clinical practice.

Using the cutoff value of chromosome 7 disomy, our data showed a trend towards longer PFS (4.5 versus 2.9 months), but the difference did not achieve statistical significance. However, a subgroup of KRAS WT patients who were EGFR FISH positive had a significantly longer PFS (5.0 versus 2.3 months; P = 0.02) than EGFR FISH-negative patients, whereas in KRAS mutant patients, no matter whether they were EGFR FISH positive or negative, there were no significant differences in RR (0% versus 0%), PFS (2.5 versus 3.8 months; P = 0.51), or OS (15.9 versus 11.3 months; P = 0.43). Our study supported the hypothesis that in the presence of the KRAS gene wild-type, tumor growth is probably mainly driven by the EGFR pathway and this biological characteristic is evoked by an increase in EGFR copy number. In the presence of KRAS mutation, however, EGFR signaling transduction gets rid of the control of the upstream receptor and resistance to anti-EGFR treatments.

Furthermore, our study showed that the combined detection of EGFR GCN with KRAS mutations provided better predictive values for selecting metastatic colorectal cancer patients who would respond to cetuximab, and especially with regard to identifying those patients with tumors which are either EGFR FISH negative or KRAS mutant-type status and are unlikely to benefit from anti-EGFR mAb therapy. To the best of our knowledge, there has been only one previous report, that from Personeni et al. (10), that described that the relationship between mean EGFR GCN and survival differs between wild-type and mutant patients, but there was no assessment of the value of combination detection of the two markers for selection of patients for anti-EGFR therapies.

Despite the high negative predictive value of both EGFR FISH and KRAS mutation status in our cohort, the positive predictive value was still low, there was still a significant percentage of patients with an increased gene copy number and KRAS WT status who were nonresponsive. Therefore, further identification and combination evaluation of other predictive biomarkers, e.g., EGFR downstream genes such as BRAF, PIK3CA, and PTEN, is imperative to improve the selection of candidates for mAb treatment. Due to the retrospective nature and limited number of cases of this study, a prospective, large sample and multicenter study on KRAS mutation status and EGFR GCN and their predictive value for selecting individual metastatic colorectal cancer patients who would respond to cetuximab is further needed to validate our findings.

In conclusion, the results of the current study show firstly that it may be feasible to consider EGFR FISH pattern of chromosome 7 disomy as a negative predictive factor for cetuximab response in KRAS WT metastatic colorectal cancer. Together with KRAS mutation, chromosome 7 disomy predicts metastatic colorectal cancer patients will not respond to cetuximab.

Disclosure of Potential Conflicts of Interest

Y-H. Li, commercial research grant, Beijing Merck Pharmaceutical Consulting Let. The other authors disclosed no potential conflicts of interest.

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EGFR Fluorescence In situ Hybridization Pattern of Chromosome 7 Disomy Predicts Resistance to Cetuximab in KRAS Wild-type Metastatic Colorectal Cancer Patients

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