Epiregulin gene expression as a biomarker of benefit from cetuximab in the treatment of advanced colorectal cancer

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Background: Anti-EGFR antibody, cetuximab, improves overall survival (OS) in K-ras wild-type chemotherapy-refractory colorectal cancer. Epidermal growth factor receptor ligand epiregulin (EREG) gene expression may further predict cetuximab benefit.

Methods: Tumour samples from a phase III clinical trial of cetuximab plus best supportive care (BSC) vs BSC alone (CO.17) were analysed for EREG mRNA gene expression. Predictive effects of high vs low EREG on OS and progression-free survival (PFS) were examined for treatment–biomarker interaction.

Results: Both EREG and K-ras status were ascertained in 385 (193 cetuximab, 192 BSC) tumour samples. Within the high EREG and K-ras wild-type status (co-biomarker)-positive group (n = 139, 36%), median PFS was 5.4 vs 1.9 months (hazard ratio (HR) 0.31; P < 0.0001), and median OS was 9.8 vs 5.1 months (HR 0.43; P < 0.001) for cetuximab vs BSC, respectively. In the rest (n = 246, 64%), PFS (HR 0.82; P = 0.12) and OS (HR 0.90; P = 0.45) were not significantly different. Test for treatment interaction showed a larger cetuximab effect on OS (HR 0.52; P = 0.007) and PFS (HR 0.49; P = 0.001) in the co-biomarker-positive group.

Conclusion: In pre-treated K-ras wild-type status colorectal cancer, patients with high EREG gene expression appear to benefit more from cetuximab therapy compared with low expression. Epiregulin as a selective biomarker requires further evaluation.

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Results were previously presented in part as a poster discussion session at the 2009 Annual Meeting of the American Society of Clinical Oncology (J Clin Oncol 2009; 27:15s (suppl; abstr 4016)).
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Received 28 August 2013; revised 4 November 2013; accepted 7 November 2013; published online 12 December 2013
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Cetuximab, an IgG1 monoclonal antibody targeting the epidermal growth factor receptor (EGFR), is approved for the treatment of colorectal cancer. As demonstrated in CO.17, a randomised trial of the NCIC Clinical Trials Group (NCIC CTG) and the Australasian Gastro-Intestinal Trials Group (AGITG), cetuximab + best supportive care (BSC) improves overall survival (OS) and progression-free survival (PFS), and better preserves quality of life for advanced chemotherapy-refractory colorectal cancer compared with BSC alone (Au et al, 2009; Jonker et al, 2007). Benefit was limited to patients whose tumours express the wild-type K-ras gene (Karapetis et al, 2008). However, cetuximab resistance remains common, with 35% of patients progressing at the first disease response assessment (Karapetis et al, 2008). Further predictive biomarkers are needed to improve therapeutic index and cost-effectiveness, and to determine resistance pathways to aid in future drug development.

Epiregulin (EREG), a ligand for EGFR, is a putative biomarker for benefit from cetuximab through gene expression profiling (Kambata-Ford et al, 2007; Baker et al, 2011). In cetuximab-treated patients, high tumour EREG mRNA expression was associated with better disease control and PFS (Kambata-Ford et al, 2007). Epiregulin may stimulate EGFR through an autocrine loop with positive feedback, and elevated EREG may indicate tumour dependence on the EGFR pathway. Whether EREG is merely prognostic or is a true biomarker of benefit from cetuximab requires a randomised trial with a comparator not exposed to cetuximab.

We undertook a correlative analysis of CO.17 trial patients to determine whether tumour EREG expression is predictive of benefit from cetuximab therapy beyond K-ras status. We also assessed the prognostic implications of EREG expression within the patients receiving BSC.

### MATERIALS AND METHODS

This correlative study was designed by a committee including members of the NCIC CTG and AGITG. The relevant institutional review boards approved the study protocol. This included approval for research involving archived tumour tissue, in accordance with patient consent.

**Patients and trial design.** The CO.17 trial design and eligibility criteria were reported previously (Jonker et al, 2007). The primary end point of the phase III study was to determine the effect of cetuximab on OS in patients with anti-EGFR therapy-naive advanced colorectal cancer who had failed all chemotherapy and for whom no standard anticancer therapy was available. Patients were randomised to receive cetuximab + BSC or BSC alone. Cetuximab was administered as an intravenous loading dose of 400 mg/m² over 120 min on day 1, followed by a maintenance schedule of 250 mg/m² intravenously over 60 min once a week until disease progression or intolerable toxicity. Eligible patients were enrolled between December 2003 and August 2005. Patients in both arms were evaluated for tumour response or progression every 8 weeks.

**Tumour collection and processing.** Formalin-fixed, paraffin-embedded tumour samples from archival (e.g. diagnostic or prior colectomy) specimens were banked at Queen’s University in Kingston, Ontario, Canada. Tissue samples were assayed for EREG mRNA expression in a blinded manner by the Department of Clinical Biomarkers-Oncology at Bristol-Myers Squibb, Hopewell, NJ, USA.

**Epiregulin expression analysis.** Blinded to clinical outcome, EREG expression analysis was performed using quantitative real-time PCR followed by extracting total RNA from formalin-fixed, paraffin-embedded tissue slides or sections (RNeasy FFPE kit; Qiagen, Venlo, The Netherlands). Epiregulin gene expression levels were detected by quantitative real-time PCR. Total RNA was isolated from whole FFPE tissue sections using the RNeasy FFPE kit (Qiagen). Quality and quantity of RNA were measured using the NanoDrop spectrophotometer (Thermo Scientific, Waldham, MA, USA), and 1 mg of RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Polymerase chain reactions were performed using 140 ng cDNA and Assay on Demand primer/probe reagents from Applied Biosystems (GAPDH: Hs00266705_g1; EREG: Hs00154995_m1). Using the delta cycle threshold (ΔCt) method, which measures the difference between the cycle time of the biomarker and that of a reference gene, a normalised ΔCt value for EREG expression relative to GAPDH expression was determined for each sample. A smaller normalised ΔCt for the biomarker corresponds to higher gene expression.

**Statistical analysis.** Statistical analyses were performed at the NCIC CTG in accordance with a prespecified statistical analysis protocol. The co-biomarker analysis was undertaken after a preliminary analysis of EREG alone. All randomised subjects who had both K-ras mutation and EREG expression data available were included in the analyses, representing the EREG evaluable data set. The primary end point, OS, was defined as the time from randomisation until death from any cause. The secondary end points were PFS, defined as the time from randomisation until the first objective observation of disease progression or death from any cause, and response rates, defined by the RECIST criteria. Epiregulin expression, as a continuous variable, was assessed using both a prespecified threshold (ΔCt = 6.27) derived from a prior study (Kambata-Ford et al, 2007), as well as a more exploratory analysis using a threshold that had a minimum P-value, among all potential thresholds, for the interaction term in a Cox model for OS, which included treatment, EREG expression dichotomised at the threshold and their interaction as covariates, without adjustment for multiple comparisons. The survival of subjects by K-ras mutation status, EREG expression and/or treatment group was summarised using Kaplan–Meier curves and differences compared by log-rank test with the hazard ratio (HR) and its 95% confidence interval (CI) calculated based on the Cox regression model with a single covariate. A ‘co-biomarker’-positive group predicted to have greatest benefit from cetuximab therapy was defined as those patients with both K-ras wild-type status and high EREG expression, using each threshold. A Cox regression model with ‘co-biomarker’ status, treatment and ‘co-biomarker’ status by treatment interaction as covariates was studied. To assess the independent prognostic effects of EREG expression, a multivariate Cox regression model was fitted to only BSC patients, including the following protocol-specific covariates: ECOG performance status (0–1 vs 2), gender (male vs female), age (≥65 vs <65 years), baseline lactate dehydrogenase level (>UNL vs ≤UNL), baseline alkaline phosphatase (>UNL vs ≤UNL), baseline haemoglobin (<LLN vs ≥LLN), number of disease sites (>2 vs ≤2), number of previous chemotherapy drug classes (>2 vs ≤2), primary tumour site (rectum only vs colon) and presence of liver metastases (yes vs no). We used the Cox model with treatment, K-ras mutation status, EREG expression and their interaction as covariates to assess the interaction between treatment and biomarker status, with the primary analysis being among patients with K-ras wild-type status. This interaction test assesses whether the treatment effect was significantly different for patients with different biomarker status. All reported P-values were two-sided and not adjusted for multiple testing.

**RESULTS**

**Characteristics of the patients.** Five hundred and seventy-two patients were randomly assigned to receive cetuximab (287) or...
BSC (285). A total of 385 tumour specimens (193 cetuximab arm; 192 BSC arm) were evaluable for both K-ras mutation status and EREG expression, representing 67% of the total study population. Tumour specimens from the remaining patients were not retrievable for this analysis for reasons including lack of consent for tissue research, insufficient tissue and refusal or inability of the laboratory of origin to release tissue for research. Of these 385 EREG evaluable tumours, 42% had detectable K-ras mutations. The distribution of low vs high EREG varied depending on the threshold selected as summarised in Table 1 and Supplementary Figure A. The prespecified normalised cycle time value was 6.27. The minimum P-value threshold identified a cycle time of 7.21, defining fewer K-ras wild-type patients as low EREG expression and then the prespecified threshold (56 vs 86 patients). Co-biomarker positivity (K-ras wild-type/high EREG) was found in 139 (36.1%) and 169 (43.9%) of EREG evaluable patients using the prespecified and minimum P-value thresholds, respectively.

The baseline demographic and disease characteristics of patients in the total study population and the EREG evaluable data set are summarised in Table 2. The EREG low and high groups defined by the minimum P-value threshold were similar with respect to these baseline characteristics including ECOG performance status and other variables found to be associated with survival in the multivariate analysis. The distribution of each of these characteristics in the EREG evaluated data set was also similar to that observed in the total study population. Non-protocol crossover to cetuximab treatment occurred in 13 BSC patients (four before and nine after progression).

**EREG as a continuous variable.** The median EREG normalised ΔCt was 5.56 (range 0.79–11.89). As a continuous variable and adjusted for prognostic factors, lower EREG expression (higher normalised ΔCt) was associated with worse OS in both BSC (HR 1.17; 95% CI: 1.04–1.32; \( P = 0.01 \)) and cetuximab-treated patients (HR 1.13; 95% CI: 1.01–1.27; \( P = 0.04 \)) in the K-ras wild-type subset. Lower EREG expression was also associated with worse PFS in the cetuximab-treated patients (HR 1.13; 95% CI: 1.01–1.26; \( P = 0.03 \)), but not in the BSC arm \( (P = 0.48) \). As a biomarker for benefit from cetuximab therapy in K-ras wild-type patients, EREG as a continuous variable did not achieve statistical significance for OS \((P = 0.30)\) or PFS \((P = 0.08)\) with interaction testing.

**Overall survival.** In the co-biomarker-positive group (K-ras wild-type/EREG high), the median OS was 9.8 months for cetuximab vs 5.1 months for BSC (HR 0.43; 95% CI: 0.29–0.64; \( P < 0.001 \)) when defined by the prespecified EREG threshold. Using the minimum P-value threshold, the median OS was 9.9 months for cetuximab vs 5.0 months for BSC (HR 0.46; 95% CI: 0.32–0.65; \( P < 0.001 \)) (Figure 1A). In the co-biomarker-negative group (K-ras mutant or EREG low), OS was not significantly improved with the addition of cetuximab therapy, whether the EREG expression was dichotomised by the prespecified threshold (HR 0.90; 95% CI: 0.68–1.19; \( P = 0.45 \)) or the minimum P-value threshold (HR 0.97; 95% CI: 0.72–1.30; \( P = 0.82 \)). In the subset with both K-ras wild-type and low EREG expression (Figure 1B), cetuximab therapy was not associated with an improvement in OS (HR 0.93; 95% CI: 0.51–1.71; \( P = 0.81 \)), with a median of 6.51 vs 4.80 months and a 1-year OS of 20.7% vs 24.5% in the cetuximab vs BSC arms, respectively. Among patients with K-ras wild-type tumours, interaction testing was nonsignificant for treatment effect by EREG status on OS (HR 0.62; 95% CI: 0.33–1.15; \( P = 0.13 \)) using the prespecified threshold or using the minimum P-value threshold (HR 0.54; 95% CI: 0.27–1.08; \( P = 0.08 \)). Among all patients, co-biomarker positivity was a significant predictor for OS benefit from cetuximab therapy using either the prespecified threshold (HR 0.53; 95% CI: 0.32–0.87; \( P = 0.01 \)) or the minimum P-value threshold (HR 0.45; 95% CI: 0.28–0.73; \( P = 0.001 \)). Figure 2 illustrates a Forest plot for OS by treatment arm and subgroup using the minimum P-value method (adjusted analysis). Epiregulin expression was found to be correlated with OS benefit from cetuximab in K-ras wild-type but not mutant status patients (adjusted HR 0.45; 95% CI: 0.21–0.97 vs 1.03, 95% CI: 0.48–2.22).

**Progression-free survival.** In the co-biomarker-positive group (K-ras wild-type and EREG high), the median PFS was 5.4 months for cetuximab vs 1.9 months for BSC (HR 0.31; 95% CI: 0.29–0.64; \( P < 0.0001 \)) when defined by the prespecified EREG threshold. Using the minimum P-value threshold, the median PFS was 5.1 months for cetuximab vs 1.9 months for BSC (HR 0.33; 0.04). As a biomarker for benefit from cetuximab therapy in K-ras wild-type patients, EREG as a continuous variable did not achieve statistical significance for OS \((P = 0.30)\) or PFS \((P = 0.08)\) with interaction testing.

**Table 1. Distribution of K-ras mutations and EREG expression by treatment arm**

| Cetuximab (N = 287) | Best supportive care (N = 285) |
|---------------------|-------------------------------|
| K-ras evaluable (N=394) |                                |
|                      | K-ras status                  | K-ras status                  |
|                      | Wild-type                     | Mutant                        |
|                      | Wild-type                     | Mutant                        |
|                      | EREG evaluable (N=385)        |                                |
|                      | High\(^a\)                    | Low                           | High\(^a\)                    | Low                           |
|                      | 117                           | 81                            | 113                           | 83                            |
|                      | 79                            |                                | 83                            |                                |
| EREG expression      |                                |                                |                                |                                |
| Pre-specified threshold | 66                            | 48                            | 33                            | 46                            |
| Minimum P-value threshold | 84                            | 30                            | 47                            | 32                            |

Abbreviation: EREG = epiregulin.

\(^a\)Those with both wild-type K-ras status and high EREG were defined as the ‘co-biomarker’-positive group.
In the K-ras wild-type subset with low EREG expression, cetuximab was associated with an improvement in PFS using the prespecified threshold (HR 0.61; 95% CI: 0.38–0.98; P = 0.035) with a median of 1.9 months in both arms, but not when the minimum P-value threshold was used (HR 0.70; 95% CI: 0.39–1.27; P = 0.24) threshold nor the minimum P-value threshold (HR 0.54; 95% CI: 0.28–1.06; P = 0.074). Figure 2 illustrates a Forest plot for PFS by treatment arm and subgroup.

**Response to treatment.** There were no objective tumour responses in patients treated with BSC. Among K-ras mutant patients, there was one response documented among 33 patients.

### Table 2. Characteristics of the patients included in the analysis for EREG expression, divided according to minimum P-value threshold

| Characteristic                        | All randomised patients (N = 572) | EREG evaluable* (N = 394) | High EREG (n = 270) | Low EREG (n = 124) | P-valueb |
|---------------------------------------|-----------------------------------|---------------------------|---------------------|-------------------|----------|
| Age, median (range) (in years)        | 63.2 (28.6–88.1)                 | 63.3 (28.6–88.1)          | 63.4 (28.6–88.1)    | 62.8 (30.8–85.9)  | 0.68     |
| < 65 years, no. (%)                   | 335 (58.6)                       | 31 (58.6)                 | 152 (56.3)          | 79 (63.7)         |          |
| ≥ 65 years, no. (%)                   | 237 (41.4)                       | 163 (41.4)                | 118 (43.7)          | 45 (36.3)         |          |
| Sex, no. (%)                          | 204 (35.7)                       | 133 (33.8)                | 86 (31.9)           | 47 (37.9)         | 0.24     |
| Male                                  | 368 (64.3)                       | 261 (66.2)                | 184 (68.1)          | 77 (62.1)         |          |
| ECOG performance status, no. (%)     |                                   |                           |                     |                   | 0.48     |
| 0                                     | 136 (23.8)                       | 90 (22.8)                 | 64 (23.7)           | 26 (21.0)         |          |
| 1                                     | 302 (52.6)                       | 224 (56.9)                | 148 (54.8)          | 76 (61.3)         |          |
| 2                                     | 134 (23.4)                       | 80 (20.3)                 | 58 (21.5)           | 22 (17.7)         |          |
| Site of primary cancer, no. (%)       |                                   |                           |                     |                   | 0.87     |
| Colon only                            | 332 (58.0)                       | 245 (62.2)                | 170 (63.0)          | 75 (60.5)         |          |
| Rectum only                           | 133 (23.3)                       | 84 (21.3)                 | 57 (21.1)           | 27 (21.8)         |          |
| Colon and rectum                      | 107 (18.7)                       | 65 (16.5)                 | 43 (15.9)           | 22 (17.7)         |          |
| Any prior radiotherapy, no. (%)       | 202 (35.3)                       | 127 (32.2)                | 82 (30.4)           | 45 (36.3)         | 0.24     |
| Prior chemotherapy, no. (%)           |                                   |                           |                     |                   |          |
| Adjuvant therapy                      | 211 (36.9)                       | 142 (36.0)                | 99 (36.7)           | 43 (34.7)         | 0.70     |
| No. of regimens                       |                                   |                           |                     |                   | 0.79     |
| 1–2                                   | 104 (18.2)                       | 74 (18.8)                 | 49 (18.1)           | 25 (20.2)         |          |
| 4                                     | 159 (27.8)                       | 109 (27.7)                | 75 (27.8)           | 32 (24.7)         |          |
| > 5                                   | 92 (16.1)                        | 54 (13.7)                 | 40 (14.8)           | 16 (11.3)         |          |
| Thymidylate synthase inhibitor        | 572 (100.0)                      | 394 (100)                 | 270 (100)           | 124 (100)         | NA       |
| Irinotecan                            | 550 (96.2)                       | 379 (96.2)                | 259 (95.9)          | 120 (96.8)        | 0.68     |
| Oxaliplatin                           | 559 (97.7)                       | 385 (97.7)                | 263 (97.4)          | 122 (98.4)        | 0.55     |
| Sites of disease, no. (%)             |                                   |                           |                     |                   |          |
| Liver                                 | 463 (80.9)                       | 313 (79.4)                | 220 (81.5)          | 93 (75.0)         | 0.14     |
| Lung                                  | 368 (64.3)                       | 243 (61.7)                | 164 (60.7)          | 79 (63.7)         | 0.57     |
| Nodes                                 | 247 (43.2)                       | 167 (42.4)                | 115 (42.6)          | 52 (41.9)         | 0.90     |
| Ascites                               | 86 (15.0)                        | 61 (15.5)                 | 41 (15.2)           | 20 (16.1)         | 0.81     |
| Number of sites of disease, no. (%)   |                                   |                           |                     |                   | 0.18     |
| 1                                     | 93 (16.3)                        | 68 (13.7)                 | 44 (16.3)           | 24 (19.4)         |          |
| 2                                     | 153 (26.7)                       | 107 (22.7)                | 79 (29.3)           | 28 (22.6)         |          |
| 3                                     | 173 (30.2)                       | 118 (29.9)                | 85 (31.5)           | 33 (26.6)         |          |
| > 4                                   | 153 (26.7)                       | 101 (25.6)                | 62 (23.0)           | 39 (31.5)         |          |
| K-ras status, no. (% of evaluable)    |                                   |                           |                     |                   | 0.004    |
| Wild-type                             | 230 (58.4)                       | 225 (58.4)                | 169 (63.5)          | 56 (47.1)         |          |
| Mutant                                | 164 (41.6)                       | 160 (41.6)                | 97 (36.5)           | 63 (52.9)         |          |
| Treatment, no. (%)                    |                                   |                           |                     |                   | 0.62     |
| Cetuximab + BSC                       | 287 (50.2)                       | 196 (49.7)                | 132 (48.9)          | 64 (51.6)         |          |
| BSC only                              | 285 (49.8)                       | 198 (50.3)                | 138 (51.1)          | 60 (48.4)         |          |

Abbreviations: BSC = best supportive care; ECOG = Eastern Cooperative Oncology Group; EREG = epiregulin.

*Although 394 patients were EREG evaluable, there were 9 for whom K-ras status was not available, leaving 385 for the combined analysis.

bBetween high and low EREG using χ² for categorical variables and t-test for continuous variables.

95% CI: 0.22–0.46; P < 0.0001). In the K-ras wild-type subset with low EREG expression, cetuximab was associated with an improvement in PFS using the prespecified threshold (HR 0.61; 95% CI: 0.38–0.98; P = 0.035) with a median of 1.9 months in both arms, but not when the minimum P-value threshold was used (HR 0.70; 95% CI: 0.39–1.24; P = 0.21), with a median of 1.8 months both with and without cetuximab. Interaction testing after adjustment for covariates demonstrated that in K-ras wild-type patients, high EREG expression does not significantly predict for PFS benefit from cetuximab therapy using neither the prespecified (HR 0.70; 95% CI: 0.38–1.27; P = 0.24) threshold nor the minimum P-value threshold (HR 0.54; 95% CI: 0.28–1.06; P = 0.074). Figure 2 illustrates a Forest plot for PFS by treatment arm and subgroup. In K-ras mutant patients, EREG expression was not found to be correlated with PFS benefit from cetuximab (adjusted HR 0.91; 95% CI: 0.45–1.83; P = 0.79).

**Response to treatment.** There were no objective tumour responses in patients treated with BSC. Among K-ras mutant patients, there was one response documented among 33 patients.
with high EREG by the prespecified threshold, but none in the low EREG group. This patient did not have a G13D mutation (De Roock et al, 2010). In the K-ras wild-type subset, the response rate was 16.7% vs 6.3% in patients with high vs low EREG status by the prespecified threshold, respectively. Using the minimum P-value threshold, the response rate was 15.5% vs 3.3% in patients with high vs low EREG status, respectively. When comparing the response by study arm (cetuximab vs BSC), the rate was significantly higher with the addition of cetuximab in those with high EREG (16.7% vs 0%, P < 0.0001), but not for those with low EREG expression (P = 0.25).

**Effect of EREG in the best supportive care group.** In the BSC arm, EREG expression was not associated with a significant difference in OS. The OS HR of overexpressed to normal EREG was 1.14 (95% CI: 0.84–1.55; P = 0.41) (Figure 3). The PFS HR of overexpressed to normal EREG was 0.85 (95% CI: 0.64–1.14; P = 0.28). The difference remains nonsignificant after adjusting other protocol-specified factors (for OS: adjusted HR 0.82; 95% CI: 0.58–1.15; P = 0.24; for PFS: adjusted HR 0.80; 95% CI: 0.59–1.09; P = 0.16).

**DISCUSSION**

Our findings demonstrate, for the first time, the potential predictive value (as opposed to simply prognostic value) of tumour EREG mRNA expression as a biomarker for OS benefit from cetuximab in patients with pre-treated advanced colorectal cancer. Cetuximab treatment was associated with a doubling of both the median OS and PFS in patients with wild-type K-ras tumours that also had high EREG expression. There was no significant OS benefit observed from cetuximab treatment in patients with K-ras wild-type tumours that displayed low EREG expression. The differences in treatment effect by EREG expression are suggestive of a different treatment effect but require validation in independent studies.

Epidermal growth factor receptor pathway ligands EREG and amphiregulin (AREG) may stimulate EGFR through an autocrine loop with positive feedback, and elevated EREG may indicate tumour dependence on the EGFR pathway. The role of EREG and AREG as predictive biomarkers of benefit from cetuximab therapy has been evaluated previously. Khambata-Ford et al (2007) evaluated tumour EREG and AREG mRNA expression from 110 patients with advanced colorectal cancer treated on a single-arm cetuximab monotherapy. Subsequently, tumour EREG and AREG mRNA expression were evaluated in 220 patients with chemotherapy–refractory colorectal cancer treated with cetuximab plus irinotecan (Jacobs et al, 2009). Another group analysed 226 cetuximab-treated patients in the first- to third-line setting (Pentheroudakis et al, 2013). A smaller study of 26 K-ras wild-type status patients suggested high EREG in combination with other EGFR ligand expression predicted for improved disease control and PFS (Yoshida et al, 2013). Like our study, these studies demonstrated that high EREG expression was associated with higher tumour response rates and better OS and PFS. However, the absence of a non-treatment control group in both of these studies did not allow for separation of predictive (treatment-dependent) vs prognostic (treatment-independent) effects through interaction testing. In CO.17 only 7% of patients in the BSC control arm ultimately received anti-EGFR therapy (postprotocol), making it ideal to differentiate these effects. The consistency of the findings from the above single-arm studies with our controlled study strengthens our findings. Clarifying inconsistencies between studies, such as the finding of an OS predictive effect of EREG in K-ras mutant status patients (Pentheroudakis et al, 2013), which was not observed in our study, may require additional studies. Possible explanations may include the combination with chemotherapy in many of these patients, thus possibly indicating that EREG expression may be predictive of chemotherapy benefit.

Amphiregulin expression was also assessed in our study, but no significant association with survival according to K-ras status was observed. Epiregulin and AREG are ligands that are coregulated, bind the same receptor and have genes found on same chromosomes. As such, similar prognostic or predictive effects would be expected. Most data show similar results for AREG and EREG, but usually favouring EREG as being the better predictor. For example, one study found a prognostic association for EREG but not for AREG (Kuramochi et al, 2012). Further research exploring the biological impact of AREG is needed. Other investigators have focused on the prognostic implications of high tumour expression of the ligands. One study evaluated tumour EREG and AREG expression by IHC and in the serum by ELISA in
73 patients with colorectal cancer (Li et al., 2010). They reported 90% coexpression of the ligands and concluded that high ligand expression is a poor prognostic factor, associated with T stage and distant metastases. Our trial, with its uniform advanced colorectal cancer population and a BSC-only control arm refutes this finding. Although a weak prognostic effect was suggested assessing EREG as a continuous variable (HR 1.17; \( P = 0.01 \)), neither the minimum \( P \)-value nor the prespecified thresholds identified a prognostic effect, whether by univariate analysis or after controlling for other prognostic factors. Thus, there appears little prognostic effect, at least using archival tissue for patients now in a chemotherapy–refractory setting.

A high degree of concordance between primary and metastatic paired tumours has been established for K-ras mutation status. Modest but significant concordance for EREG mRNA was reported in 120 patients with paired liver metastases and primary tumours (Rs = 0.58, \( P < 0.0001 \)) (Kuramochi et al., 2012). Although EREG levels in metastasis appear to correlate with outcome (Kambata-Ford et al., 2007), as do levels in older archival primary samples (Baker et al., 2011), it is unknown which is superior.

Unlike the dichotomous K-ras status (wild vs mutant), EREG expression is a continuous variable with arbitrary categorising thresholds requiring derivation and validation. Several studies have derived thresholds that could be utilised in prospective validation studies. Our own efforts to validate a predetermined threshold suggested a more discriminatory level.

Another limitation of this analysis is that only 67% of the CO.17 intention-to-treat (ITT) population had the tissue available. Although a greater proportion would have increased statistical power and further reduced the chance of bias, the comparison of EREG-assessed and ITT populations suggest that EREG-assessed patients were representative of the larger population.
Overall survival was not significantly correlated with low vs high EREG expression status. Epiregulin expression was dichotomised using the prespecified threshold. Overall survival was not significantly correlated with low vs high EREG status (adjusted HR 0.82; 95% CI: 0.58 vs 1.15; P = 0.24), suggesting that EREG expression is not a significant prognostic factor.

Whenever a biomarker clearly identifies subgroups that benefit more from a treatment, the biomarker will have limited clinical utility unless it identifies a population that either should or should not receive treatment. In the context of an established therapy such as cetuximab when used in a disease with finite effective treatment options such as colorectal cancer, a decision to forego therapy requires a high level of assurance that the patient could not have benefited from treatment. This study evaluated two thresholds for EREG mRNA expression. We tried to validate a threshold derived from a previous study and found it has a good discriminatory level. We also tried to derive a new threshold based on the minimum P-value approach. The minimum P-value threshold slightly more successfully demonstrated a K-ras wild-type population who currently routinely receive cetuximab, but owing to low tumour EREG expression have no clinically important effect from this therapy. Although it provided a slightly better discriminatory level, this needs validation utilising an independent data set.

The low EREG population represented 56 out of 225 (24.9%) of K-ras wild-type patients in the minimum P-value analysis. Interaction testing provides evidence that patients with low EREG benefit less from cetuximab. Specifically, there was no statistically significant difference in OS (HR 0.93; P = 0.81), PFS (HR 0.70; P = 0.21) or response rate (P = 0.25) with vs without cetuximab. Beyond these statistical tests, in the population with K-ras wild-type but low EREG expression, there appears to be no clinically relevant absolute difference in median OS (increase of 0.7 months), 1-year OS (increase of 3.8%) or median PFS (increase of 0 months; Supplementary Figure B), and 97% of this subgroup did not have an objective response. Furthermore, given the same cost for much less gain, the cost per life year saved is likely to be very high (Mittmann et al., 2009). No apparent benefit is therefore observed in patients with tumours that are either K-ras mutant status or have low EREG expression (Supplementary Figure C).

Beyond a means of better selecting patients who will derive greatest benefit from therapy, identification of markers of resistance provides insight into biology. Exploration of low EREG tumours may identify means to overcome resistance or identify signalling pathways that are relevant for this population. Further evaluation of the interaction between the ligands and other signalling pathways (e.g. PI3K/PTEN/AKT/mTOR) is warranted. An analysis including BRAF and these markers is being conducted.

The era of personalised medicine has dawned for colorectal cancer. Although this is met with excitement, there is also sober recognition that the days of having a newly discovered therapy demonstrating broad benefits for a large population with a particular site of cancer may be behind us. Future improvements in cancer outcomes while minimising patient exposure to ineffective therapy will require a shift in oncology culture toward tumour profiling at diagnosis, with treatments based on molecular signature.

ACKNOWLEDGEMENTS

This work was supported by the Canadian Cancer Society through the NCIC Clinical Trials Group, ImClone Systems and Bristol-Myers Squibb.

CONFLICT OF INTEREST

CJO, DT are employees of the NCIC Clinical Trials Group, which has received grant support from Bristol-Myers Squibb. LLS, RJS and JRZ report receiving research grants from Bristol-Myers Squibb, and JRZ received consulting fees from Amgen. SK-F, CL, CH and DPM own equity in and were or are employees of Bristol-Myers Squibb. No other potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

DJ, CK were responsible for the initial writing of the manuscript. SK, CH and DM conducted the laboratory analyses. DT conducted the statistical analysis. All of the authors contributed to the study design and conduct, as well as review of the revision and approval of the manuscript.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)