

# A Phase IIa Randomized, Double-Blind Trial of Erlotinib in Inhibiting Epidermal Growth Factor Receptor Signaling in Aberrant Crypt Foci of the Colorectum

Daniel L. Gillen<sup>1,2</sup>, Frank L. Meyskens<sup>2</sup>, Timothy R. Morgan<sup>2,3</sup>, Jason A. Zell<sup>2,4</sup>, Robert Carroll<sup>5</sup>, Richard Benya<sup>5</sup>, Wen-Pin Chen<sup>2</sup>, Allen Mo<sup>6</sup>, Chris Tucker<sup>7</sup>, Asmita Bhattacharya<sup>8</sup>, Zhiliang Huang<sup>8</sup>, Myra Arcilla<sup>8</sup>, Vanessa Wong<sup>2</sup>, Jinah Chung<sup>2</sup>, Rachel Gonzalez<sup>3</sup>, Luz Maria Rodriguez<sup>9,10</sup>, Eva Szabo<sup>9</sup>, Daniel W. Rosenberg<sup>6</sup>, and Steven M. Lipkin<sup>8</sup>

## Abstract

Colorectal cancer progresses through multiple distinct stages that are potentially amenable to chemopreventative intervention. Epidermal growth factor receptor (EGFR) inhibitors are efficacious in advanced tumors including colorectal cancer. There is significant evidence that EGFR also plays important roles in colorectal cancer initiation, and that EGFR inhibitors block tumorigenesis. We performed a double-blind randomized clinical trial to test whether the EGFR inhibitor erlotinib given for up to 30 days had an acceptable safety and efficacy profile to reduce EGFR signaling biomarkers in colorectal aberrant crypt foci (ACF), a subset of which progress to colorectal cancer, and normal rectal tissue. A total of 45 patients were randomized to one of three

erlotinib doses (25, 50, and 100 mg) with randomization stratified by nonsteroidal anti-inflammatory drug (NSAID) use. There were no unanticipated adverse events with erlotinib therapy. Erlotinib was detected in both normal rectal mucosa and ACFs. Colorectal ACF phosphorylated ERK (pERK), phosphorylated EGFR (pEGFR), and total EGFR signaling changes from baseline were modest and there was no dose response. Overall, this trial did not meet its primary efficacy endpoint. Colorectal EGFR signaling inhibition by erlotinib is therefore likely insufficient to merit further studies without additional prescreening stratification or potentially longer duration of use. *Cancer Prev Res*; 8(3); 222–30. ©2015 AACR.

## Introduction

Colorectal cancer is the second leading cause of cancer-related death in the United States. Tragically, a large proportion of colorectal cancer is preventable because tumors associated with the disease are relatively slow growing and early detection is feasible through screening. Most colorectal cancer cases are preceded by precursor adenomas, and recent decreases in colorectal cancer in the United States are attributable to early detection of

adenomas (1, 2). There has therefore been intensive interest in preventing colorectal cancer by targeting precancerous colorectal cancer precursors in colorectal epithelium. Nonsteroidal anti-inflammatory drugs (NSAID) and COX-2 inhibitors have shown activity in adenoma prevention; however, cardiovascular side effects have created uncertainty as to their suitability for this indication (3, 4). Therefore, new agents are needed.

Epidermal growth factor receptor (EGFR) is expressed at high levels in a variety of epithelial tumors, including colorectal, pancreatic, head and neck, breast, kidney, bladder, and glioblastomas (5, 6), and its inhibition has significant activity to shrink tumors in colorectal cancer, non-small cell lung cancer (NSCLC), and pancreas cancers (7–10). Although most of the focus on EGFR inhibitors has been in the treatment of advanced malignancies, there is significant evidence that EGFR also plays important roles in colorectal cancer initiation, and that EGFR inhibitors block tumor initiation. In *Apc<sup>Mim</sup>* mice, EGFR inactivation essentially abolishes adenoma formation (11). Similarly, treatment of mouse and rat colorectal cancer models with the EGFR small-molecule inhibitor gefitinib also blocks adenoma formation (12, 13). Therefore, there is evidence that EGFR plays a role in initiation of adenomas, in addition to its more intensively studied role in tumor progression.

Erlotinib (Tarceva, OSI-774) is an orally active EGFR tyrosine kinase inhibitor used as an antitumor agent for the treatment of solid tumors, including NSCLC and pancreatic cancer (14). Erlotinib at 150 mg p.o. once daily is used in patients with locally advanced or metastatic NSCLC after failure of at least one prior

<sup>1</sup>Department of Statistics, University of California at Irvine, Irvine, California. <sup>2</sup>Chao Family Comprehensive Cancer Center, University of California at Irvine, Irvine, California. <sup>3</sup>Department of Medicine, VA Long Beach Health Care System, Long Beach, California. <sup>4</sup>Department of Epidemiology, University of California at Irvine, Irvine, California. <sup>5</sup>Department of Medicine, University of Illinois Medical Center at Chicago, Chicago, Illinois. <sup>6</sup>Center for Molecular Medicine, University of Connecticut Health, Farmington, Connecticut. <sup>7</sup>Astellas Pharmaceuticals, Long Island, New York. <sup>8</sup>Division of Gastroenterology and Hepatology, Weill Cornell Medical College, New York, New York. <sup>9</sup>Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland. <sup>10</sup>Department of Surgery, Walter Reed National Military Medical Center, Bethesda, Maryland.

**Note:** Supplementary data for this article are available at Cancer Prevention Research Online (<http://cancerprevres.aacrjournals.org/>).

**Corresponding Author:** Steven M. Lipkin, Weill Cornell Medical College, 541 E 71 Street, Rm 718 New York, NY 10021. Phone: 212-774-7160; Fax: 212-774-7167; E-mail: stl2012@med.cornell.edu

doi: 10.1158/1940-6207.CAPR-14-0148

©2015 American Association for Cancer Research.

chemotherapy regimen, and was approved in the United States in 2004 (15). A supplemental NDA (sNDA) was also approved to add pancreatic cancer (erlotinib 100 mg p.o. once daily in combination with gemcitabine) as an indication in 2005 (16).

Erlotinib was identified via high-throughput drug screening for direct and reversible inhibition of EGFR (HER1 in humans) tyrosine kinase, but has "off-target" inhibition of other kinases with lower affinities, including STK10, and HER2 (17). Erlotinib inhibits human EGFR tyrosine kinase with an  $IC_{50}$  of 2 nmol/L (0.79 ng/mL) in an *in vitro* enzyme assay and reduces EGFR autophosphorylation in intact tumor cells with an  $IC_{50}$  of 20 nmol/L (7.9 ng/mL; ref. 18).

Aberrant crypt foci (ACF) were first described as collections of colonic crypts with expanded pericryptal zones and increased dye staining (19). These lesions are monoclonal and are believed to be the earliest identifiable precursors of colon cancer (20, 21). The prevalence, number, and size of human ACFs also increase with age. Furthermore, many of the molecular derangements described in colon cancers are also found in ACF, including *KRAS*, *APC*, and *CTNNB1* mutations and growth-promoting alterations in cell cycle-controlling genes (22). In the distal colon and rectum, approximately 32% to 63% and 30% to 37% of ACFs, respectively, have *KRAS* and *BRAF* mutations (20–23), which can drive downstream RAS–RAF–ERK pathway activation. Along with less common *EGFR* mutations, *KRAS* and *BRAF* mutations are thought to drive the growth of almost all ACFs, and are typically mutually exclusive in individual ACFs (23).

The proliferative rates were increased in dysplastic ACF, supporting the significance of crypt cell hyper-proliferation as a biomarker of ACF with greater neoplastic potential. In a well-performed study, ACFs were identified in the distal 10 cm of rectum in 77% of subjects with no colonic abnormalities, 83% of subjects with an adenoma(s), and 93% of subjects with colorectal cancer. The mean numbers of ACFs in these groups respectively were: endoscopically normal colon, 5.0; adenomatous polyp 6.9; and colorectal cancer, 9.9. (17).

Normal colon and ACFs express EGFR, and many hyperproliferative ACFs overexpress EGFR (20, 21). Furthermore, ACFs also express higher levels of EGFR ligands, *PCNA* and *CCND1*, which are important downstream targets of EGFR activation (24, 25). Because the EGFR regulates colonocyte growth and differentiation, it has been implicated in premalignancy. In the preclinical azoxymethane-induced mouse model of colon cancer, oral gefitinib qOD treatment significantly decreased colonocyte proliferation 49% and large ACF formation 50% (26). In the normal mouse colon, gefitinib decreased phosphorylated ERK (pERK) 50% (26, 27). This treatment also significantly reduced the number of ACFs and decreased microadenoma cyclin D1. This study concluded that EGFR inhibitors may be useful for colorectal cancer chemoprevention and that human trials should be undertaken (27). Because gefitinib decreased COX-2 expression, the study suggested that EGFR inhibition may be mediated, at least in part, through COX-2 inhibition. Therefore, it is possible that in human chemoprevention trials, NSAIDs may be a confounding factor.

In light of previous findings regarding the role of EGFR in development of colorectal cancer and the use of erlotinib as a EGFR inhibitor, we designed and conducted a multisite randomized phase IIa trial to quantify the ability of erlotinib to decrease EGF signaling and to identify the lowest erlotinib dose at which ACF EGF signaling is inhibited and for which there is an accept-

able side effect profile for secondary prevention in subjects at high risk for colorectal cancer.

## Materials and Methods

### Study design

The study was a three-arm, randomized, double-blind trial to test the ability of erlotinib to reduce EGF pathway signaling and to identify the lowest erlotinib dose at which ACF EGF signaling is inhibited and for which there is an acceptable side effect profile for secondary prevention. The trial was conducted at three clinical sites. The Institutional Review Board (IRB) at each site approved the study protocol and written informed consent was provided by all patients before study enrollment. An independent Data and Safety Monitoring Board (DSMB) established at the UCI Chao Comprehensive Cancer Center reviewed safety data for the trial bi-annually. The primary endpoint of the trial was the difference in pERK levels between pre- versus post-erlotinib-treated ACF at doses of 25, 50, or 100 mg.

### Eligibility and exclusion criteria

Eligibility for enrollment into the study required that participants have one or more of the following: (i) a history of stage I–III colorectal cancer, not treated in the past 6 months with no anticipated treatment in the next 3 months, (ii) current adenoma  $\geq 1$  cm in size, (iii) three or more adenomas (of any size) removed at one colonoscopy within past 6 years, (iv) a sessile serrated adenoma  $\geq 5$  mm in size, and (v) an adenoma (of any size) with villous features (villous, tubulovillous) or with high-grade dysplasia. In addition, participants must have been found to have  $\geq 4$  ACFs at their baseline colonoscopy or flexible sigmoidoscopy, normal baseline laboratory evaluations for hematologic, renal, and hepatic function, and ECOG performance status of 0 or 1.

Patients were deemed ineligible for study participation if they reported a history of inflammatory bowel disease (IBD), a history of interstitial or chronic lung disease, smoking within the past 3 months, increased risk of bleeding from rectal biopsy, currently taking warfarin, or a significant CYP 3A4 inhibitor, uncontrollable diarrhea, prior receipt of radiation to the rectum or pelvis, or active keratoconjunctivitis. Women who were pregnant or breast-feeding were also deemed ineligible for the study as were subjects taking any other investigational pharmaceutical agent or those with a previous history of sensitivity to erlotinib, gefitinib, or cetuximab.

### Randomization and study treatment

Subjects ( $n = 45$ ) meeting inclusion and exclusion criteria with four or more ACFs on initial chromoendoscopy were randomized 1:1:1 to treatment with 25, 50, or 100 mg doses of erlotinib and treated for up to 30 days ( $n = 15$ /arm). A block size of 6 was used to maintain balance across the three treatment arms throughout accrual. Randomization was stratified by NSAID use ( $\geq 10$  d/mo vs.  $< 10$  d/mo). Randomization assignment lists were prepared by the central study statistician and supplied to study coordinators at each site. Randomization assignment was double-blinded for all participants and investigators.

Erlotinib drug product was supplied by Astellas Pharma Global Development, Inc. To protect the blinding of treatment assignment, each patient was given three bottles and asked to take one pill from each bottle daily for up to 30 days, until their scheduled follow-up clinic visit. Patients randomized to the 100-mg dose

Gillen et al.

were given one bottle of 100-mg active pills and two bottles of 25-mg placebo pills, patients randomized to the 50-mg dose were given one bottle of 100-mg placebo pills and two bottles of 25-mg active pills, and patients randomized to the 25-mg dose were given one bottle each of 100- and 25-mg placebo pills along with one bottle of 25-mg active pills.

#### Patient follow-up, assessment of study endpoints, and assessment of adverse events

Patients were contacted via phone by the Study Coordinator between 4 and 14 days following randomization to monitor study safety and compliance. In addition, subjects were asked to keep a diary to document consumption of medication and to bring their diary to their follow-up visit occurring up to 30 days following randomization.

The endpoints for the trial were molecular biomarkers of EGFR signaling activity measured at the follow-up clinic visit occurring up to 30 days after randomization. The original proposed pERK primary analysis method was Western blot analysis. However, the initial ACF samples tested did not demonstrate clear pERK signal on Western blot analysis, while phosphorylated EGFR (pEGFR) and total EGFR signals were robust (Supplementary Fig. S1). Therefore, we used a nanofluidic proteomic immunoassay (NIA) that has greater sensitivity and, to our knowledge, is the only published method successfully reporting quantitative pERK levels in human distal colorectal ACF (28). Quantification of pEGFR and total EGFR was performed by Western blot analysis as key secondary endpoints. Detailed protocols for tissue handling and biomarker measurement are included in Supplementary Methods.

Safety analyses were based on investigator-reported adverse events (AE), serious AEs (SAE), laboratory measurements, and physical examinations. AEs were identified using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. AEs were assessed according to the CTCAE grade associated with the AE term.

#### Statistical analysis

The protocol-specified primary endpoint for the trial was change in pERK levels between pre- versus post-erlotinib-treated ACF from patients randomized to doses of 25, 50, or 100 mg. Key secondary endpoints specified in the trial were change in levels of pEGFR and total EGFR between pre- versus post-erlotinib-treated ACF and normal mucosa. The study was powered ( $n = 15$  subjects/group) to provide approximately 83% power to detect a 0.8 standard deviation change in the primary endpoint using a level 0.05 test assuming a correlation of conservative correlation assumption of 0.5 between pre- and postmeasures.

The distribution of baseline characteristics was summarized using the mean, standard deviation, median, and range for continuous covariates, and frequency and percentage for discrete covariates. AEs were summarized by grade, type, and frequency of occurrence. For the primary analysis, pERK was standardized by total ERK for each participant by NIA. The mean change in percentage of pERK (post-pre) for each dose group was estimated and then tested via a paired  $t$  test as defined in the protocol. Corresponding 95% confidence intervals (CI) and  $P$  values for the mean change were computed using the  $t$  distribution with the relevant number of degrees of freedom for each treatment group. A pooled analysis combining all treatment groups was also

performed, as specified in the trial protocol. For the analysis of key secondary endpoints, a log-transformation of pEGFR and total EGFR was used in primary analyses because of the highly skewed distribution observed in these outcomes. After the log-transformation, the distribution of pEGFR and total EGFR was roughly symmetric. The general linear model was used to estimate and compare the relative change in median pEGFR and total EGFR, separately, with adjustment for actin as a normalizing factor. The estimated relative change (post:pre) in the median, corresponding 95% CI, and  $P$  value for testing the null hypothesis of equal medians comparing pre- and postmeasurements were reported. The Bonferroni-Holm adjusted method for multiple comparisons was applied to maintain an experiment-wise significance level of 0.05.

Exploratory analyses considered additional adjustment and stratification by plasma erlotinib concentration (ng/mL), plasma OSI-420 concentration (ng/mL), normal mucosa erlotinib concentration (ng/mg), normal mucosa OSI-420 concentration (ng/mg), total duration of use (days), and NSAID use ( $< 10$  d/mo vs.  $\geq 10$  d/mo). All analyses were performed in SAS 9.2 (SAS Institute).

## Results

### Baseline characteristics and patient follow-up

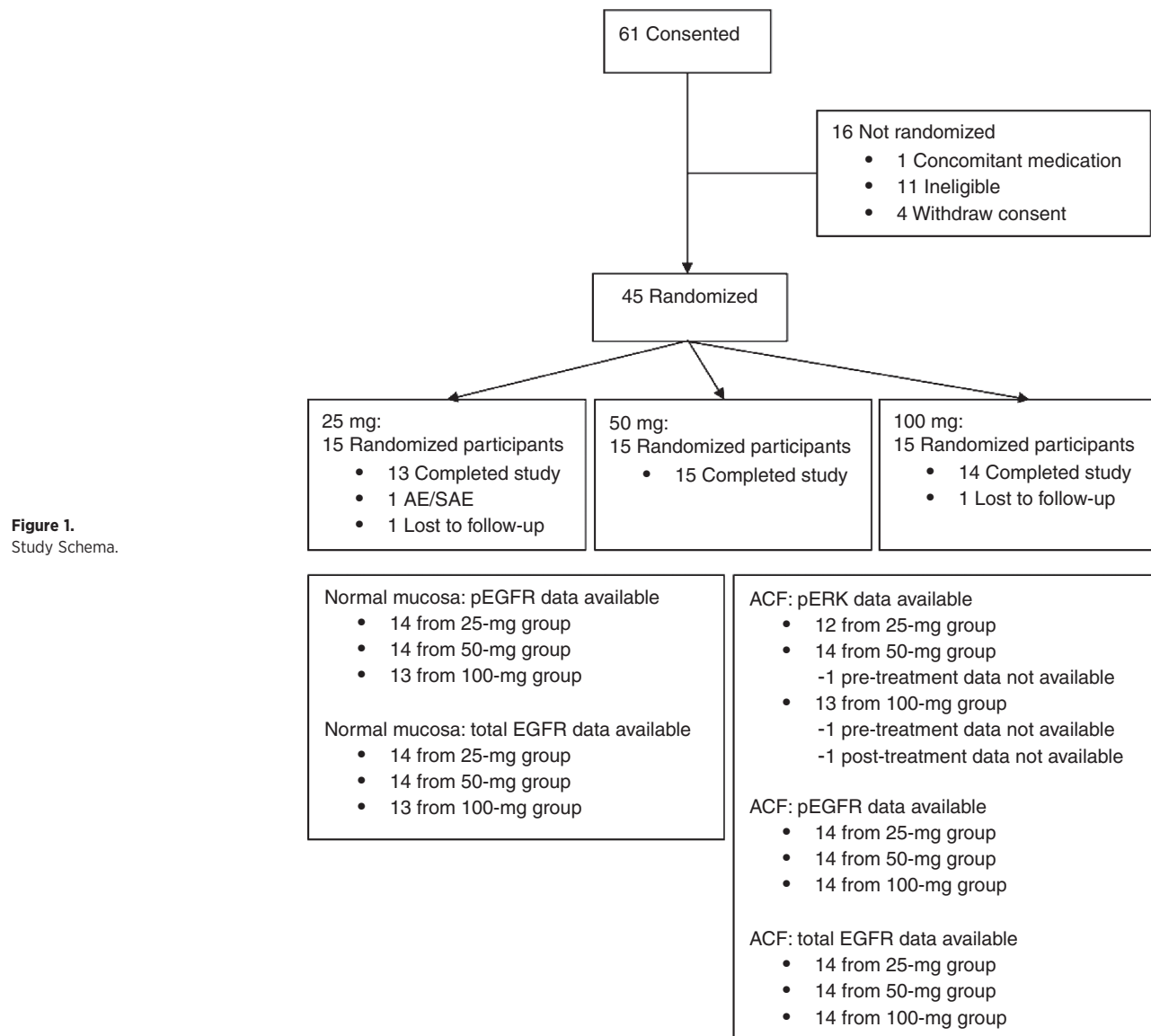
Figure 1 displays the study schema. In total 61 patients were consented for the study with 45 patients ultimately randomized. Of the 16 patients not randomized, 11 did not meet the study eligibility criteria, 1 was ineligible due to the use of concomitant medications, and 4 withdrew consent before randomization. Fifteen patients were randomized to each dose group.

Baseline characteristics were similar across the three erlotinib dosage groups (Table 1). The study sample had a mean age range between 60 and 63 years across treatment arms, was predominantly male (ranging from 67% male in the 100 mg group to 93% male in the 50 mg group), and non-Hispanic white. Baseline hematology and blood chemistry levels were similar across the three dose groups with the largest differences observed in basophils and serum albumin. Data on baseline pERK levels were successfully captured and analyzed for 12, 13, and 12 subjects in the 25-, 50-, and 100-mg dose groups, respectively. Data on baseline pEGFR and total EGFR data were successfully captured and analyzed for 14 subjects in each group (Fig. 1). Baseline pERK, percentage of pERK, pEGFR, and total EGFR levels were overall similar across treatment arms at baseline (Table 2).

Table 3 presents summaries of the distribution of erlotinib concentrations at the follow-up visit by dose group. Among subjects in the 100-mg dose group mean erlotinib concentrations in plasma were estimated to be 1,048.55 ng/mL (95% CI, 1,414.59–682.50) and 794.28 ng/mL (95% CI, 1,160.33–428.24) higher when compared with those in the 25- and 50-mg arms, respectively. Similarly, among subjects in the 100-mg dose group, mean OSI-420 concentrations in plasma were estimated to be 100.21 ng/mL (95% CI, 139.42–61.00) and 84.12 ng/mL (95% CI, 122.63–45.61) higher when compared with those in the 25- and 50-mg arms, respectively.

### pERK signaling in ACF

A total of 12, 13, and 11 patients had complete data on pre- and post-pERK levels in the 25-, 50-, and 100-mg dose groups, respectively (Fig. 1). Follow-up visits ranged from 7 to 28 days following randomization and were roughly uniformly distributed



across the study sample. The primary reason for failure to complete the trial was patient loss-to-follow-up. One patient in the 25-mg dose group was discontinued from the study due to a SAE. No viable outcome data on pERK changes was obtained for two patients in the 25-mg group, 1 patient in the 50-mg group, and 2 patients in the 100-mg group due to sample technical failure.

Figure 2 displays the estimated within subject change in percentage pERK in ACFs (post-pre) by dose group along with corresponding 95% CIs and *P* values for a test of the null hypothesis that the true mean change is equal to zero. The percentage of pERK decreased in each treatment arms after erlotinib treatment, although this difference was not statistically significant from zero in any treatment group or in the total sample when data from all treatment groups were pooled together. Absolute decreases in the percentage of pERK ranged from 0.13% (95% CI, -3.9% to 3.7%) in the 100-mg dose group to 2.52% (95% CI, -7.9% to 2.8%) in the 50-mg dose group. No dose response trend was observed. The absolute decrease in the

percentage of pERK in the pooled trial sample was -1.23% (95% CI, -5.0% to 2.5%).

#### ACF biomarkers of EGF receptor activation

Secondary endpoints comparing pre- and post-erlotinib-treated median pEGFR and total EGFR levels on Western blot analyses were used to analyze each trial participant's ACF and normal colon tissue biospecimens. A total of 13, 15, and 14 patients completed the trial in the 25-, 50-, and 100-mg dose groups, respectively (Fig. 1).

Overall, the median pEGFR and total EGFR at posttreatment were higher relative to pretreatment levels across all the three dose levels in both normal mucosa and ACF (Fig. 3). Among subjects randomized to the 50-mg dose group, median posttreatment pEGFR and total EGFR in normal mucosa was estimated to be 65% (unadjusted 95% CI, 0.87-3.10) and 91% (unadjusted 95% CI, 0.91-4.00) higher relative to baseline levels. Similar results

Gillen et al.

**Table 1.** Baseline characteristics of patients by randomization group

Characteristic	Erlotinib dose		
	25 mg (N = 15)	50 mg (N = 15)	100 mg (N = 15)
<b>Demographics</b>			
Age (y), mean (SD)	63.67 (4.43)	62.47 (6.03)	60.67 (7.42)
Male, n (%)	13 (87%)	14 (93%)	10 (67%)
<b>Ethnicity</b>			
Hispanic or Latino, n (%)	4 (27%)	2 (13%)	1 (7%)
Non-Hispanic or Latino, n (%)	8 (53%)	11 (73%)	10 (67%)
Unknown, n (%)	3 (20%)	2 (13%)	4 (27%)
Race-White, n (%)	13 (87%)	13 (87%)	12 (80%)
Weight (kg), mean (SD)	83.66 (16.83)	96.42 (19.01)	91.14 (29.52)
NSAID use < 10 d/month, n (%)	9 (60.0)	9 (60.0)	9 (60.0)
<b>Hematology</b>			
Hemoglobin, mean (SD)	14.05 (1.06)	14.05 (1.66)	14.24 (1.19)
Hematocrit, mean (SD)	40.75 (3.19)	41.15 (4.27)	41.99 (3.56)
WBC, mean (SD)	6.67 (2.27)	6.19 (1.36)	6.91 (1.97)
Neutrophils, % mean (SD)	61.53 (9.86)	61.87 (8.85)	60.69 (8.04)
Lymphocytes, % mean (SD)	28.47 (8.21)	27.16 (8.14)	27.65 (8.75)
Monocytes, % mean (SD)	7.51 (2.7)	8.26 (2.83)	8.11 (2.31)
Eosinophils, % mean (SD)	1.98 (1.61)	2.16 (1.26)	2.76 (2.54)
Basophils, % mean (SD)	0.44 (0.37)	0.55 (0.4)	0.79 (0.32)
Platelet, mean (SD)	226.07 (67.99)	224.73 (52.87)	230.64 (43.84)
<b>Blood chemistry</b>			
Total protein, mean (SD)	6.86 (0.26)	6.71 (0.46)	6.66 (0.59)
Albumin, mean (SD)	4.16 (0.21)	4.01 (0.21)	3.91 (0.31)
BUN, mean (SD)	15.67 (2.61)	17.2 (9.89)	15.07 (4.39)
Creatinine, mean (SD)	0.99 (0.2)	1.08 (0.17)	1.03 (0.24)
Bilirubin, direct, mean (SD)	0.12 (0.04)	0.17 (0.12)	0.11 (0.03)
Bilirubin, total, mean (SD)	0.99 (0.4)	0.75 (0.3)	0.71 (0.24)
Alkaline phosphatase, mean (SD)	69.47 (16.26)	60.27 (16.82)	64.86 (27.27)
Sodium, mean (SD)	138.67 (2.53)	138 (1.77)	139.71 (2.92)
Potassium, mean (SD)	4.29 (0.4)	4.36 (0.57)	4.17 (0.45)
Chloride, mean (SD)	103.67 (3.72)	103.67 (3.04)	104 (2.83)
Bicarbonate, mean (SD)	26.64 (2.11)	27.33 (3.11)	28.46 (1.33)
SGOT/AST, mean (SD)	24.07 (4.48)	26.8 (9.07)	27.14 (9.05)
SGPT/ALT, mean (SD)	25.33 (9.63)	25.87 (14.35)	27.14 (11.09)

were observed when considering pEGFR and total EGFR values in ACF mucosa.

Among subjects randomized to the 50-mg dose group, median posttreatment pEGFR and total EGFR in ACF was estimated to be 93% (unadjusted 95% CI, 1.07–3.48) and 2.22-fold (unadjusted 95% CI, 1.08–4.54) higher relative to baseline levels. Although posttreatment levels tended to be higher across all dose groups, after adjustment for multiple comparisons, no statistically signif-

icant within-subject changes in pEGFR or EGFR were observed. Secondary exploratory analyses considering adjustment and effect modification by erlotinib plasma concentration, duration of use, and NSAID use did not result in qualitatively differential results.

#### Safety

Table 4 depicts the frequency of AE reports by grade and dose group. Only two grade 3 AEs were reported in the trial, one in the

**Table 2.** Distributional summaries of baseline pEGFR and total EGFR values by randomization group

Characteristic	Erlotinib dose		
	25 mg (N = 14)	50 mg (N = 14)	100 mg (N = 14)
<b>pEGFR in normal tissue</b>			
Median	8,433.9	6,938.7	12,098.2
Mean (SD)	14,004.2 (10,935.0)	8,802.4 (6,935.6)	16,673.3 (14,344.9)
Range	1,491.0–33,325.3	112.5–28,728.2	4,892.9–39,215.4
<b>pEGFR in ACF tissue</b>			
Median	7,959.0	6,612.5	7,450.7
Mean (SD)	12,729.1 (9,942.8)	5,868.0 (3,090.9)	10,528 (10,150.4)
Range	2,707.0–39,617.1	168.3–10,955.3	517.2–40,051.7
<b>Total EGFR in normal tissue</b>			
Median	7,359.3	11,927.6	12,415.2
Mean (SD)	8,840.7 (6,824.7)	13,285.2 (8,311.4)	14,003.4 (7,587.1)
Range	26.0–26,333.3	122.0–27,957.6	4,892.9–36,742.6
<b>Total EGFR in ACF tissue</b>			
Median	9,342.2	8,560.4	6,623.2
Mean (SD)	10,300.7 (5,758.0)	10,212.4 (7,473.4)	9,507.5 (9,780.9)
Range	23.5–20,579.0	29.3–28,622.7	280.2–36,132.3

**Table 3.** Summary measures of the distribution of erlotinib concentration in plasma and normal mucosa at the follow-up visit by dosage level

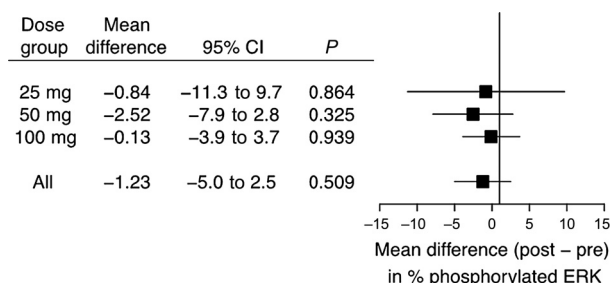
Characteristic	Erlotinib dose		
	25 mg	50 mg	100 mg
Plasma erlotinib concentration, ng/mL			
N	14	14	13
Median	222.58	480.95	1,118.34
Mean (SD)	232.29 (160.6)	486.56 (211.8)	1,280.84 (788.3)
Plasma OSI-420 concentration, ng/mL			
N	13	14	13
Median	15.92	36.94	98.01
Mean (SD)	17.77 (12.3)	33.87 (14.1)	117.98 (84.5)
Normal mucosa erlotinib concentration, ng/mg			
N	12	12	12
Median	0.32	1.10	1.68
Mean (SD)	0.36 (0.18)	1.38 (1.23)	3.25 (4.62)
Normal mucosa OSI-420 concentration, ng/mg			
N	4	10	11
Median	0.04	0.14	0.23
Mean (SD)	0.04 (0.01)	0.17 (0.15)	0.29 (0.24)

25-mg dose group and one in the 100-mg dose group. The grade 3 AE occurring in the 25-mg dose group was incarcerated hernia and subsequently led to withdrawal of the patient from the study by the investigator. A single SAE of chest pain was observed in the 25-mg dose group and was considered unrelated to study drug.

Table 5 presents the most frequently reported AEs ( $\geq 5\%$ ) by dose group. The most commonly reported AE was rash, observed in 33%, 40%, and 80% of patients in the 25-, 50-, and 100-mg dose groups, respectively. This was followed by dryness or itchiness of the skin, eyes, or mouth, which was reported in 47% of patients in each of the dose groups. Finally, diarrhea was reported in nearly one third of study participants overall.

## Discussion

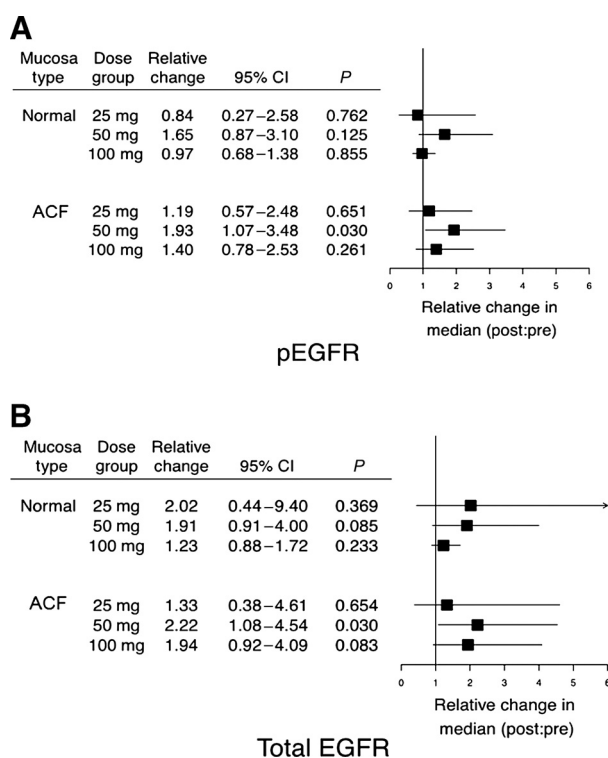
Previous randomized trials have established the role of erlotinib as established therapies for refractory NSCLC and pancreas adenocarcinoma (29, 30). Because EGFR inhibition through monoclonal antibodies cetuximab and panitumumab has established activity to induce tumor responses in *KRAS* wild-type colorectal cancer (31), this trial sought to determine (i) the ability of erlotinib to decrease EGF signaling for up to 30 days in rectal ACFs and normal colon and (ii) to identify the lowest efficacious erlotinib dose for which there is an acceptable side effect profile for potential follow up in the setting of secondary chemoprevention of colorectal cancers.



**Figure 2.** Primary analysis of mean within-subject change (post-pre) in the percentage of pERK in ECFs by dose group. "All" represents pooled data from all dose groups.

The primary endpoint of the trial was the difference in pERK levels comparing paired pre-post ACF at 25, 50, and 100 mg doses of erlotinib. Key secondary endpoints included differences in pEGFR and total EGFR in biospecimens for each dose tested. For all three arms, there were statistically nonsignificant trends toward reduced pERK levels with erlotinib treatment. There was no dose dependence. Combining all three arms similarly showed a statistically nonsignificant trend toward reduced pERK levels (Fig. 2). At the same time, contrary to our original hypothesis, pEGFR and total EGFR levels were not observed to decrease in rectal ACFs and normal tissue when compared with paired pretreatment trial participant biospecimens. Rather, median pEGFR and total EGFR levels were consistently upregulated at all three doses (up to 2-fold higher) when posttreatment levels compared with pretreatment paired measurements. Although not statistically significant after adjustment for multiple comparisons, this finding was consistent in both normal mucosa and ACFs and across all dose groups (Fig. 3).

In a recent early-phase chemoprevention trial of head and neck squamous cell cancer with erlotinib and celecoxib, treatment with similar doses of erlotinib (50, 75, and 100 mg) concordantly reduced pEGFR, total EGFR, and pERK levels in normal oral mucosa and premalignant oral leukoplakia (32). In this trial, the precise mechanism as to why pERK levels trend lower while EGFR signaling biomarkers, including total EGFR levels, are paradoxically increased with erlotinib in rectal ACF is not understood. However, because erlotinib is not effective as therapy for advanced colorectal cancer while it does demonstrate efficacy in other cancer types (lung and pancreas cancers most notably), this may reflect EGFR signaling differences specific to colorectum compared with



**Figure 3.** Relative change (post:pre) in median phosphorylated and total EGFR by dose group and mucosa strata. A, estimates of the relative change in the median of pEGFR. B, estimates of the relative change in the median of total EGFR.

Gillen et al.

**Table 4.** Frequency of the AE grades by dosage arm

AE grade	Erlotinib dose					
	25 mg		50 mg		100 mg	
	Events, <i>n</i>	Participants ( <i>N</i> = 15)	Events, <i>n</i>	Participants ( <i>N</i> = 15)	Events, <i>n</i>	Participants ( <i>N</i> = 15)
No AE reported	—	3 (20%)	—	2 (13%)	—	1 (7%)
Grade 1 (or higher)	37	12 (80%)	38	13 (87%)	51	14 (93%)
Grade 2 (or higher)	4	3 (20%)	5	5 (33%)	12	7 (47%)
Grade 3 (or higher)	1	1 (7%)	0	0	1	1 (7%)

other tissues. Future experiments will be required to determine precisely why the same EGFR inhibitor can affect this pathway signaling in different cell types with opposite effects.

Although there are many studies in the literature that have successfully measured pERK in cell lines and various human tissues using different techniques, to our knowledge, the only study that has successfully analyzed pERK levels in human distal colorectal ACF used a NIA (28). Precisely why distal colorectal ACF pERK levels are difficult to measure is presently unclear. However, at the same time, pEGFR and total EGFR levels were readily measurable from the same biospecimens by Western blot analysis, arguing against confounding by nonspecific tissue degradation, general loss of phosphoproteins from colonoscopy bowel preparation regimens, or other artifacts of tissue handling. However, as with any trial using rectal tissue that did not achieve concordant primary and secondary endpoints, we cannot completely exclude that bowel preparation regimens or sampling may have influenced analyses of rectal ACFs and mucosa in an unanticipated manner.

Previously, a significant percentage of distal colon and rectum ACF were shown to have significant rates of *KRAS* and *BRAF* mutations (20–23) that can drive ACF growth. In colorectal and non-small cell lung adenocarcinomas, *KRAS* (33, 34), *BRAF* (35, 36), and *EGFR* kinase domain mutations (37) have been previously associated with anti-EGFR targeted therapy chemoresistance, which is thought to arise from both preexisting mutations and induction of mutations from EGFR-inhibitor exposure. Tomasetti and colleagues (38) have recently shown that approximately 50% of somatic mutations in colorectal cancer occur in normal tissue and early-stage premalignant lesions (such as ACF) before tumorigenesis and cell transformation in an age-dependent process. It is tempting to speculate that in our trial, post-therapy ACF might represent expansion of an EGFR inhibitor-resistant population existing before erlotinib exposure, or ACFs with activated *KRAS*, *BRAF*, *EGFR*, or other mutations under evolutionary selection from EGFR inhibition. If correct, paradoxical feedback could then cause a trend toward reduced downstream pERK signaling levels. Future preclinical experiments and correlative studies in ACF and normal colon mucosa from this and other trials will be required to understand the precise molecular

mechanisms of resistance, genetic, or otherwise, in patients taking erlotinib as targeted therapies for other malignancies.

Analysis of plasma and tissue levels confirmed that sufficient doses of erlotinib were received in each study group for detection of erlotinib and its major metabolite OSI-420. This is consistent with excluding patient compliance as a factor in the observed findings, which is further supported by the observation of expected AEs, such as diarrhea and rash, in this trial's participants. In addition, secondary exploratory analyses considering adjustment and effect modification by plasma erlotinib levels and duration of use did not reveal qualitatively different results in EGFR signaling biomarkers in subgroups of patients with higher erlotinib levels or those with longer duration of use.

Reported AEs were largely expected given past experience with erlotinib treatment. The most commonly reported AE in the trial was rash. Consistent with previous studies, the incidence of rash was observed to increase with increasing erlotinib dose. Depending on erlotinib dose, 80% to 93% of all patients experienced grade 1 rash, and nearly half of patients receiving the 100-mg dose experienced grade 2 rash. Dryness or itchiness of the skin, eyes, or mouth together with diarrhea were the next most commonly observed AEs in the trial. As such, given the lack of efficacy, the investigators believe that erlotinib toxicity at the higher doses investigated in this trial makes its use problematic for chemoprevention clinical trials. The occurrence of AEs at these doses (25–100 mg) in healthy outpatient trial participants may mean that robust efficacy may be required for individuals to consider the benefit:risk ratio to be acceptable in the chemoprevention setting. Only one SAE (chest pain) was reported over the course of the trial, though this event was deemed unrelated to study drug by the local investigator.

Although this was a carefully controlled, double-blind, phase IIa clinical trial, it also has limitations. The lack of concordant changes in pERK, pEGFR, and total EGFR endpoints may be due to the relatively short duration of treatment exposure. Patients received erlotinib from 7 to 28 days. Previously reported trials establishing the efficacy of erlotinib as a treatment have considered median durations of 2 to 4 months. It is possible that given the short duration of exposure, there was not sufficient time for EGF signaling inhibition to be observed. In addition, this trial data

**Table 5.** Frequency of most commonly observed AEs ( $\geq 5\%$ ) by dosage arm

AE	Erlotinib dose					
	25 mg		50 mg		100 mg	
	Events, <i>n</i>	Participants ( <i>N</i> = 15)	Events, <i>n</i>	Participants ( <i>N</i> = 15)	Events, <i>n</i>	Participants ( <i>N</i> = 15)
Diarrhea	4	4 (27%)	5	4 (27%)	6	5 (33%)
Dryness/itchiness (includes skin, eyes, and mouth)	9	7 (47%)	8	7 (47%)	10	7 (47%)
Fatigue	1	1 (7%)	0	0	3	3 (20%)
Flatulence	0	0	2	1 (7%)	0	0
Nausea	2	2 (13%)	2	2 (13%)	0	0
Oral sores	0	0	2	2 (13%)	1	1 (7%)
Rash	5	5 (33%)	7	6 (40%)	14	12 (80%)

included only three biomarkers of EGF signaling, pERK, pEGFR, and total EGFR. It is possible that EGF signaling inhibition by erlotinib may give different results if histopathologic or endoscopic endpoints were used, but the trial was not statistically powered for these endpoints. Finally, large heterogeneity in pERK, pEGFR, and total EGFR levels was observed across patients in all dose groups. It is possible that more homogeneous subpopulations may exhibit EGF signaling inhibition with erlotinib use, but again the current study was not designed or powered to investigate the existence of these groups.

Overall, this trial did not meet its primary efficacy endpoint. Colorectal EGFR signaling inhibition by erlotinib is therefore likely insufficient to merit further studies without additional prescreening stratification or potentially longer duration of use. Future studies will be necessary to evaluate erlotinib in the setting of chemoprevention for other types of solid tumors.

### Disclosure of Potential Conflicts of Interest

F.L. Meyskens has ownership interest (including patents) in Cancer Prevention Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

### Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

### Authors' Contributions

**Conception and design:** D.L. Gillen, F.L. Meyskens, L.M. Rodriguez, S.M. Lipkin  
**Development of methodology:** F.L. Meyskens, A. Mo, D.W. Rosenberg, S.M. Lipkin

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** F.L. Meyskens, T.R. Morgan, J. Zell, R. Carroll, A. Mo, C. Tucker, A. Bhattacharya, V. Wong, J. Chung, R. Gonzalez, D.W. Rosenberg, S.M. Lipkin

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** D.L. Gillen, F.L. Meyskens, T.R. Morgan, J. Zell, W.-P. Chen, A. Bhattacharya

**Writing, review, and/or revision of the manuscript:** D.L. Gillen, F.L. Meyskens, T.R. Morgan, J. Zell, R. Benya, C. Tucker, A. Bhattacharya, L.M. Rodriguez, E. Szabo, D.W. Rosenberg, S.M. Lipkin

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** D.L. Gillen, R. Benya, W.-P. Chen, Z. Huang, M. Arcilla, V. Wong, J. Chung, R. Gonzalez, D.W. Rosenberg, S.M. Lipkin

**Study supervision:** F.L. Meyskens, T.R. Morgan, R. Gonzalez, E. Szabo, S.M. Lipkin

**Other (provided guidance, trial design, intellectual, and financial support):** L.M. Rodriguez

### Acknowledgments

The authors thank Matt Bell for his donation to support this project.

### Grant Support

The project described was supported by NCI N01-CN-35160 (to F.L. Meyskens), NCI P30CA062203, and the National Center for Research Resources and the National Center for Advancing Translational Sciences, NIH, through grant U11 TR000153.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 6, 2014; revised January 8, 2015; accepted January 15, 2015; published OnlineFirst January 20, 2015.

### References

- Zauber AG, Winawer SJ, O'Brien MJ, Lansdorf-Vogelaar I, van Ballegoijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med* 2012;366:687-96.
- Winawer SJ, Zauber AG. Incidence reduction following colonoscopic polypectomy. *Am J Gastroenterol* 2011;106:370.
- Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med* 2006;355:885-95.
- Bertagnoli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, et al. Celecoxib for the prevention of sporadic colorectal adenomas. *N Engl J Med* 2006;355:873-84.
- Bronte G, Terrasi M, Rizzo S, Sivestris N, Ficorella C, Cajozzo M, et al. EGFR genomic alterations in cancer: prognostic and predictive values. *Front Biosci* 2011;3:879-87.
- Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Mol Diagn* 2013;15:415-53.
- Hackel PO, Zwick E, Prenzel N, Ullrich A. Epidermal growth factor receptors: critical mediators of multiple receptor pathways. *Curr Opin Cell Biol* 1999;11:184-9.
- Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys* 2004;59:21-6.
- Lyseng-Williamson KA. Erlotinib: a guide to its use in first-line treatment of non-small-cell lung cancer with epidermal growth factor-activating mutations. *Mol Diagn Ther* 2013;17:57-62.
- Tsien CI, Nyati MK, Ahsan A, Ramanand SG, Chepeha DB, Worden FP, et al. Effect of erlotinib on epidermal growth factor receptor and downstream signaling in oral cavity squamous cell carcinoma. *Head Neck* 2013;35:1323-30.
- Roberts RBML, Washington MK. Importance of epidermal growth factor receptor signaling in establishment of adenomas and maintenance of carcinomas during intestinal tumorigenesis. *Proc Natl Acad Sci U S A* 2002;99:1521-6.
- Little NMR, Cerda S. EGFR antagonist Iressa inhibits ERK activation, colonic crypt cell proliferation and ACF growth in mouse azoxymethane model of colon cancer. *Gastroenterology* 2005;28A-175.
- Jagadeeswaran SDU, Little. EGFR antagonist gefitinib inhibits ErbB signaling and tumor development in the rat azoxymethane model of colon cancer. *Gastroenterology* 2006;130A-180.
- Blais N, Kassouf E. Maintenance therapies for non-small cell lung cancer. *Front Oncol* 2014;4:213.
- Herbst RS, Prager D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* 2005;23:5892-9.
- Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007;25:1960-6.
- Fabian MA, Biggs WH III, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, et al. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol* 2005;23:329-36.
- Lu JF, Eppler SM, Wolf J, Hamilton M, Rakhit A, Bruno R, et al. Clinical pharmacokinetics of erlotinib in patients with solid tumors and exposure-safety relationship in patients with non-small cell lung cancer. *Clin Pharmacol Ther* 2006;80:136-45.
- Yokota T, Sugano K, Kondo H, Saito D, Sugihara K, Fukayama N, et al. Detection of aberrant crypt foci by magnifying colonoscopy. *Gastrointest Endosc* 1997;46:61-5.
- Pretlow TP, Pretlow TG. Mutant KRAS in aberrant crypt foci (ACF): initiation of colorectal cancer? *Biochim Biophys Acta* 2005;1756:83-96.
- Rosenberg DW, Yang S, Pleau DC, Greenspan EJ, Stevens RG, Rajan TV, et al. Mutations in BRAF and KRAS differentially distinguish serrated versus



Gillen et al.

- non-serrated hyperplastic aberrant crypt foci in humans. *Cancer Res* 2007;67:3551-4.
22. Takayama T, Ohi M, Hayashi T, Miyanishi K, Nobuoka A, Nakajima T, et al. Analysis of K-ras, APC, and beta-catenin in aberrant crypt foci in sporadic adenoma, cancer, and familial adenomatous polyposis. *Gastroenterology* 2001;121:599-611.
  23. Drew DA, Devers TJ, O'Brien MJ, Horelik NA, Levine J, Rosenberg DW. HD chromoendoscopy coupled with DNA mass spectrometry profiling identifies somatic mutations in microdissected human proximal aberrant crypt foci. *Mol Cancer Res* 2014;12:823-9.
  24. Khare S, Chaudhary K, Bissonnette M, Carroll R. Aberrant crypt foci in colon cancer epidemiology. *Methods Mol Biol* 2009;472:373-86.
  25. Cohen G, Mustafa R, Chumsangri A, Little N, Nathanson J, Cerda S, et al. Epidermal growth factor receptor signaling is up-regulated in human colonic aberrant crypt foci. *Cancer Res* 2006;66:5656-64.
  26. Dougherty U, Sehdev A, Cerda S, Mustafa R, Little N, Yuan W, et al. Epidermal growth factor receptor controls flat dysplastic aberrant crypt foci development and colon cancer progression in the rat azoxymethane model. *Clin Cancer Res* 2008;14:2253-62.
  27. Fichera A, Little N, Jagadeeswaran S, Dougherty U, Sehdev A, Mustafa R, et al. Epidermal growth factor receptor signaling is required for microadenoma formation in the mouse azoxymethane model of colonic carcinogenesis. *Cancer Res* 2007;67:827-35.
  28. Drew DA, Devers T, Horelik N, Yang S, O'Brien M, Wu R, et al. Nanoproteomic analysis of extracellular receptor kinase-1/2 post-translational activation in microdissected human hyperplastic colon lesions. *Proteomics* 2013;13:1428-36.
  29. Ullivi P, Zoli W, Capelli L, Chiadini E, Calistri D, Amadori D. Target therapy in NSCLC patients: Relevant clinical agents and tumour molecular characterisation. *Mol Clin Oncol* 2013;1:575-81.
  30. Yang ZY, Yuan JQ, Di MY, Zheng DY, Chen JZ, Ding H, et al. Gemcitabine plus erlotinib for advanced pancreatic cancer: a systematic review with meta-analysis. *PLoS One* 2013;8:e57528.
  31. Bekaii-Saab T, Wu C. Seeing the forest through the trees: a systematic review of the safety and efficacy of combination chemotherapies used in the treatment of metastatic colorectal cancer. *Crit Rev Oncol Hematol* 2014;91:9-34.
  32. Saba NF, Hurwitz SJ, Kono SA, Yang CS, Zhao Y, Chen Z, et al. Chemoprevention of head and neck cancer with celecoxib and erlotinib: results of a phase Ib and pharmacokinetic study. *Cancer Prev Res* 2014;7:283-91.
  33. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012;486:532-6.
  34. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014;6:224ra24.
  35. Dahabreh IJ, Terasawa T, Castaldi PJ, Trikalinos TA. Systematic review: Anti-epidermal growth factor receptor treatment effect modification by KRAS mutations in advanced colorectal cancer. *Ann Intern Med* 2011;154:37-49.
  36. Di Nicolantonio F, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008;26:5705-12.
  37. Garm Spindler KL, Pallisgaard N, Rasmussen AA, Lindebjerg J, Andersen RF, Cruger D, et al. The importance of KRAS mutations and EGF61A>G polymorphism to the effect of cetuximab and irinotecan in metastatic colorectal cancer. *Ann Oncol* 2009;20:879-84.
  38. Tomasetti C, Vogelstein B, Parmigiani G. Half or more of the somatic mutations in cancers of self-renewing tissues originate prior to tumor initiation. *Proc Natl Acad Sci U S A* 2013;110:1999-2004.

# Cancer Prevention Research

## A Phase IIa Randomized, Double-Blind Trial of Erlotinib in Inhibiting Epidermal Growth Factor Receptor Signaling in Aberrant Crypt Foci of the Colorectum

Daniel L. Gillen, Frank L. Meyskens, Timothy R. Morgan, et al.

*Cancer Prev Res* 2015;8:222-230. Published OnlineFirst January 20, 2015.

<b>Updated version</b>	Access the most recent version of this article at: doi: <a href="https://doi.org/10.1158/1940-6207.CAPR-14-0148">10.1158/1940-6207.CAPR-14-0148</a>
<b>Supplementary Material</b>	Access the most recent supplemental material at: <a href="http://cancerpreventionresearch.aacrjournals.org/content/suppl/2015/01/21/1940-6207.CAPR-14-0148.DC1">http://cancerpreventionresearch.aacrjournals.org/content/suppl/2015/01/21/1940-6207.CAPR-14-0148.DC1</a>

<b>Cited articles</b>	This article cites 37 articles, 12 of which you can access for free at: <a href="http://cancerpreventionresearch.aacrjournals.org/content/8/3/222.full#ref-list-1">http://cancerpreventionresearch.aacrjournals.org/content/8/3/222.full#ref-list-1</a>
-----------------------	--

<b>E-mail alerts</b>	<a href="#">Sign up to receive free email-alerts</a> related to this article or journal.
<b>Reprints and Subscriptions</b>	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a> .
<b>Permissions</b>	To request permission to re-use all or part of this article, use this link <a href="http://cancerpreventionresearch.aacrjournals.org/content/8/3/222">http://cancerpreventionresearch.aacrjournals.org/content/8/3/222</a> . Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.