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

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ABSTRACT

Colorectal cancer (CRC) progresses through multiple distinct stages that are potentially amenable to chemopreventative intervention. Epidermal Growth Factor Receptor (EGFR) inhibitors are efficacious in advanced tumors including CRC. There is significant evidence that EGFR also plays important roles in CRC 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 CRC, and normal rectal tissue. A total of N=45 patients were randomized to one of three erlotinib doses (25 mg, 50 mg, 100 mg) with randomization stratified by non-steroidal 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 phosphoERK, phosphoEGFR and total EGFR signaling changes from baseline were modest and there was no dose response. Overall, this trial did not meet is primary efficacy endpoint.Colorectal EGFR signaling inhibition by erlotinib is therefore likely insufficient to merit further studies without additional pre-screening stratification or potentially longer duration of use.

INTRODUCTION

Colorectal cancer (CRC) is the 2nd leading cause of cancer death in the United States. Tragically, a large proportion of CRC is preventable because tumors associated with the disease are relatively slow growing and early detection is feasible through screening. Most CRC cases are preceded by precursor adenomas, and recent decreases in CRC in the US are attributable to early detection of adenomas(1, 2). There has therefore been intensive interest in preventing CRC by targeting precancerous CRC precursors in colorectal epithelium. Non-steroidal anti-inflammatory drugs (NSAIDs) 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 (CRC), non-small cell lung cancer and pancreas cancers (7-10). While 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 CRC initiation, and that EGFR inhibitors block tumor initiation. In *Apc*^{Min} mice EGFR inactivation essentially abolishes adenoma formation (11). Similarly, treatment of mouse and rat CRC 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 non-small cell lung cancer (NSCLC) and pancreatic cancer(14). Erlotinib at 150mg po qD is used in patients with locally advanced or metastatic NSCLC after failure of at least 1 prior chemotherapy regimen, and was approved in the US in 2004(15). A supplemental NDA (sNDA) was also approved to add pancreatic cancer (erlotinib 100mg po qD 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 (HERl 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 nM (0.79 ng/mL) in an *in vitro* enzyme assay and reduces EGFR autophosphorylation in intact tumor cells with an IC_{50} of 20 nM (7.9 ng/mL) (18).

Aberrant crypt foci (ACF) were first described as collections of colonic crypts with expanded peri-cryptal 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 32-63% and 30-37% 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 thegrowth 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 10cm of rectum in 77% of subjects with no colonic abnormalities, 83% of subjects with an adenoma(s), and 93% of subjects with colorectal cancer (CRC). 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 pre-clinical azoxymethane induced mouse model of colon cancer, oral gefininib qOD treatment significantly decreased colonocyte proliferation 49% and large ACF formation 50%(26). In the normal mouse colon, gefitinib decreased 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 CRC and the use of erlotinib as a EGFR inhibitor, we designed and conducted a multi-site 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 acceptable side effect profile for secondary prevention in subjects at high risk for CRC.

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 prior to 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- vs. post-erlotinib treated ACF at doses of 25mg, 50mg or 100mg.

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, (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 ≥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 aswere 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 4 or more ACFs on initial chromoendoscopy were be randomized 1:1:1 to treatment with 25 mg, 50 mg 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 3 treatment arms throughout accrual. Randomization was stratified by NSAID use (≥10 days per month vs. < 10 days per month). 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. In order 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 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 mg 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. However, the initial ACF samples tested did not demonstrate clear phosphoERK signal on Western blot,

while phosphoEGFR and total EGFR signals were robust (Supplemental Figure 1). 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 were performed by Western blot analysis as key secondary endpoints. Detailed Protocols for tissue handling and biomarker measurement are included in Supplemental Methods.

Safety analyses were based on investigator-reported adverse events, serious adverse events, laboratory measurements, and physical examinations. Adverse events (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- vs. post-erlotinib treated ACF from patients randomized to doses of 25mg, 50mg or 100mg. Key secondary endpoints specified in the trial were change in levels of pEGFR and total EGFR between pre- vs. post-erlotnib treated ACF and normal mucosa. The study was powered (n=15 subjects per group) to provide approximately 83% power to detect a 0.8 standard deviation change in the primary endpoint using a level .05 test assuming a correlation of conservative correlation assumption of 0.5 between pre- and post-measures.

The distribution of baseline characteristics were summarized using the mean, standard deviation, median, and range for continuous covariates, and frequency and percent for discrete covariates. Adverse events 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 percent of phosphorylated ERK (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 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 utilized 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% confidence interval, and p-value for testing the null hypothesis of equal medians comparing pre- and post-measurements 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 days/month vs. ≥ 10 days/month). All analyses were performed in SAS 9.2 (SAS Institute, Cary NC).

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 prior to 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 25mg, 50mg, and 100mg dose groups, respectively. Data on baseline pEGFR and total EGFR data were successfully captured and analyzed for 14 subjects in each group. (Figure 1). Baseline pERK, percent of

phosphorylated ERK, 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 1048.55 ng/mL (95% CI: 1414.59, 682.50) and 794.28 ng/mL (95% CI: 1160.33, 428.24) higher when compared to those in the 25 mg 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 to those in the 25 mg 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 mg, 50 mg, and 100 mg dose groups, respectively (Figure 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 serious adverse event (SAE). No viable outcome data on pERK changes was obtained for two patients in the 25 mg group, one patient in the 50 mg group, and 2 patients in the 100mg group due to sample technical failure.

Figure 2 displays the estimated within subject change in percent phosphorylated ERK in ACFs (post – pre) by dose group along with corresponding 95% confidence intervals and p-values for a test of the null hypothesis that the true mean change is equal to zero. The percent of phosphorylated ERK 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 percent of phosphorylated ERK ranged from 0.13% (95% CI: -3.9%, 3.7%) in the 100 mg dose group to 2.52% (95% CI: -7.9%, 2.8%) in the 50 mg dose group. No dose response trend was observed. The absolute decrease in the percent of phosphorylated ERK in the pooled trial sample was -1.23% (95% CI: -5.0%, 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 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 mg, 50 mg, and 100 mg dose groups respectively (Figure 1).

Overall, the median pEGFR and total EGFR at post-treatment were higher relative to pre-treatment levels across all the three dose levels in both normal mucosa and ACF (Figure 3). Among subjects randomized to the 50 mg dose group median post-treatment 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 were observed when considering pEGFR and total EGFR values in ACF mucosa.

Among subjects randomized to the 50 mg dose group median post-treatment 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. While post-treatment levels tended to be higher across all dose groups, after adjustment for multiple comparisons, no statistically significant 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 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 serious adverse event 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 adverse events (≥5%) by dose group. The most commonly reported AE was rash, observed in 33%, 40%, and 80% of patients in the 25 mg, 50 mg, 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 have established activity to induce tumor responses in *KRAS* wild-type CRC(31), this trial sought to determine (a) the ability of erlotinib to decrease EGF signaling for up to 30 days in rectal ACFs and normal colon and (b) 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 CRCs.

The primary endpoint of the trial was the difference in pERK levels comparing paired pre-post ACF at 25mg, 50mg and 100mg 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 non-significant trends towards reduced pERK levels with erlotinib treatment. There was no dose dependence. Combining all three arms

similarly showed a statistically non-significant trend towards reduced pERK levels (Figure 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 pre-treatment trial participant biospecimens. Rather, median pEGFR and total EGFR levels were consistently upregulated at all three doses (up to 2-fold higher) when post-treatment levels compared to pre-treatment paired measurements. Though not statistically significant after adjustment for multiple comparisons, this finding was consistent in both normal mucosa and ACFs and across all dose groups (Figure 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 (50mg, 75mg and 100mg) concordantly reduced pEGFR, total EGFR and pERK levels in normal oral mucosa and pre-malignant 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, since 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 to 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.

While 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 analyzedpERK levels in human distal colorectal ACFemployed a nanofluidic proteomic immunoassay (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, arguing against confounding by non-specific 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 pre-existing mutations and induction of mutations from EGFR-inhibitor exposure. Vogelstein and colleagues have recently shown that ~50% of somatic mutations in CRC occur in normal tissue and early stage pre-malignant lesions (such as ACF) before tumorigenesis and cell transformation in an age dependent process (38).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 towards reduced downstream pERK signaling levels. Future pre-clinical 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 adverse events, 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 adverse events 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%-93% of all patients experienced Grade 1 rash, and nearly half of patients receiving the 100mg 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 (25mg to 100mg) 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.

While 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 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 histopathological or endoscopic endpointswere 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 is primary efficacy endpoint. Colorectal EGFR signaling inhibition by erlotinib is therefore likely insufficient to merit further studies without additional pre-screening 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.

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Table 1. Baseline characteristics of patients by randomization group.

Characteristic	*Erlotinib Dose*		
	25 mg (N = 15)	*50 mg (N = 15)*	*100 mg (N = 15)*
Demographics			
- Age (yrs), Mean (SD)	63.67 (4.43)	62.47 (6.03)	60.67 (7.42)
- Male, n (%)	13 (87%)	14 (93%)	10 (67%)
- Ethnicity			
Hispanic or Latino, n (%)	4 (27%)	2 (13%)	1 (7%)
Not 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 days/mth, n (%)	9 (60.0)	9 (60.0)	9 (60.0)
Hematology			
- 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)
Blood Chemistry			
- 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)

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

Characteristic	*Erlotinib Dose*		
	25mg (N=14)	*50mg (N=14)*	*100mg (N=14)*
pEGFR in normal tissue			
Median	8433.9	6938.7	12098.2
Mean (SD)	14004.2 (10935.0)	8802.4 (6935.6)	16673.3 (14344.9)
Range	1491.0 - 33325.3	112.5 - 28728.2	4892.9 - 39215.4
pEGFR in ACF tissue			
Median	7959.0	6612.5	7450.7
Mean (SD)	12729.1 (9942.8)	5868.0 (3090.9)	10528 (10150.4)
Range	2707.0 - 39617.1	168.3 - 10955.3	517.2 - 40051.7
Total EGFR in normal tissue			
Median	7359.3	11927.6	12415.2
Mean (SD)	8840.7 (6824.7)	13285.2 (8311.4)	14003.4 (7587.1)
Range	26.0 - 26333.3	122.0 - 27957.6	4892.9 - 36742.6
Total EGFR in ACF tissue			
Median	9342.2	8560.4	6623.2
Mean (SD)	10300.7 (5758.0)	10212.4 (7473.4)	9507.5 (9780.9)
Range	23.5 - 20579.0	29.3 - 28622.7	280.2 - 36132.3

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

	Erlotinib Dose		
Characteristic	*25mg*	*50mg*	*100mg*
Plasma Erlotinib Concentration (ng/mL)			
- N	14	14	13
- Median	222.58	480.95	1118.34
- Mean (SD)	232.29 (160.6)	486.56 (211.8)	1280.84 (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)

Table 4. Frequency of the adverse event grades by dosage arm.

Adverse Event Grade	*Erlotinib Dose*					
	25 mg		*50 mg*		*100 mg*	
	No. of events	*No. Participants (N=15)*	*No. of events*	*No. Participants (N=15)*	*No. of events*	*No. Participants (N=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%)

Table 5. Frequency of most commonly observed adverse events (≥5%) by dosage arm.

Adverse event	*Erlotinib Dose*					
	25 mg		*50 mg*		*100 mg*	
	No. of events	*No. Participants (N=15)*	*No. of events*	*No. Participants (N=15)*	*No. of events*	*No. Participants (N=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%)

Figure 1

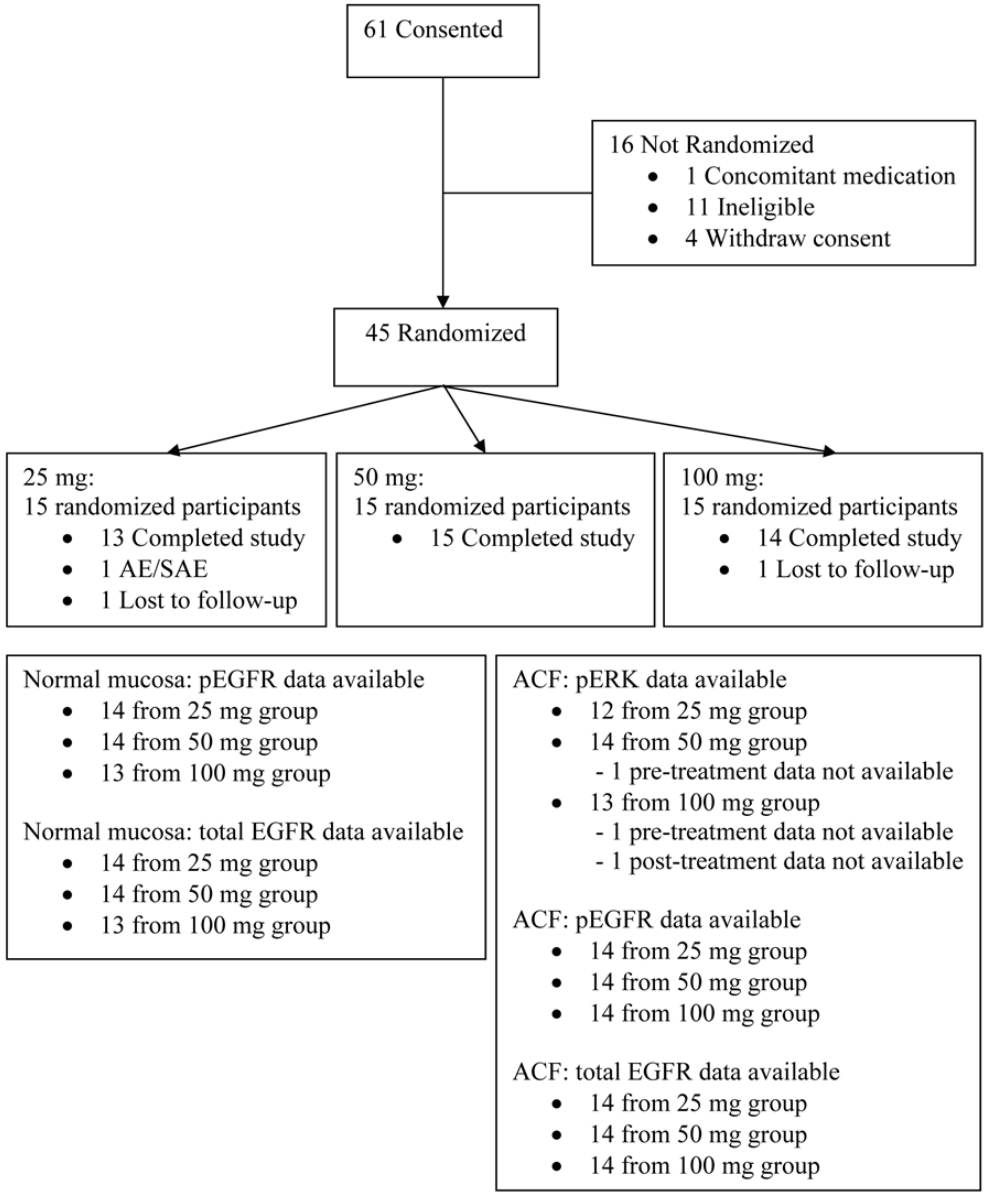


Figure 1. Study Schema

Figure 2

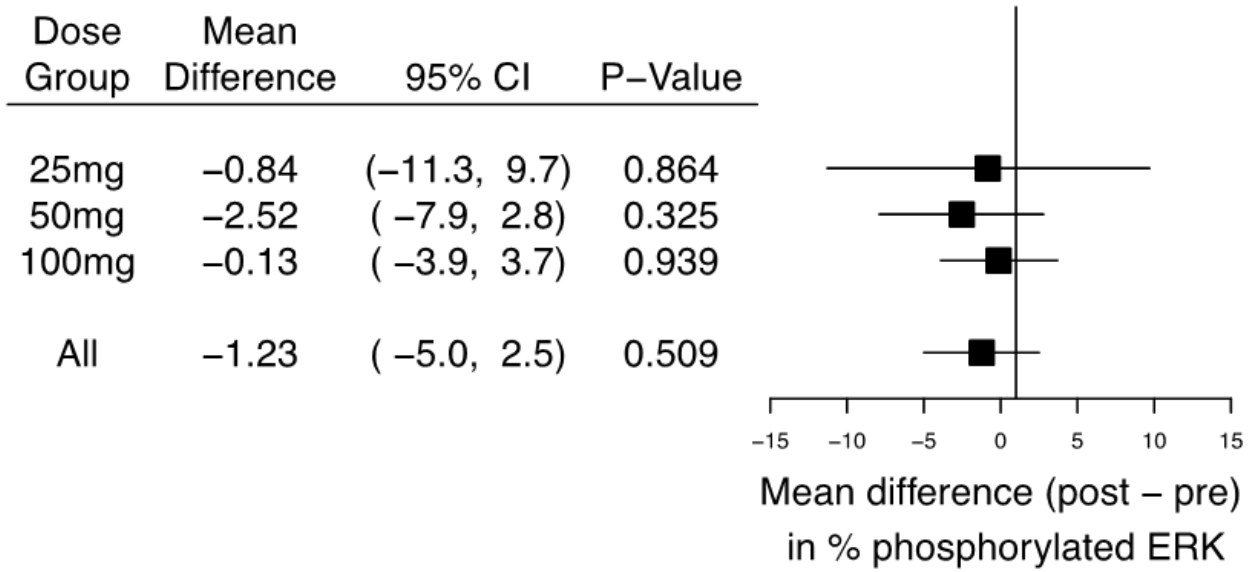
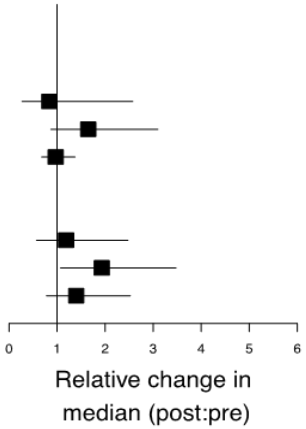


Figure 2. Primary analysis of mean within-subject change (post - pre) in the percent of phosphorylated ERK in ECFs by dose group. "All" represents pooled data from all dose groups.

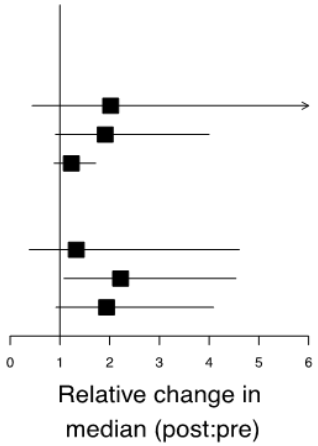
Figure 3

Mucosa Type	Dose Group	Relative Change	95% CI	P–Value
Normal	25mg	0.84	(0.27, 2.58)	0.762
	50mg	1.65	(0.87, 3.10)	0.125
	100mg	0.97	(0.68, 1.38)	0.855
ACF	25mg	1.19	(0.57, 2.48)	0.651
	50mg	1.93	(1.07, 3.48)	0.030
	100mg	1.40	(0.78, 2.53)	0.261



(a) pEGFR

Mucosa Type	Dose Group	Relative Change	95% CI	P–Value
Normal	25mg	2.02	(0.44, 9.40)	0.369
	50mg	1.91	(0.91, 4.00)	0.085
	100mg	1.23	(0.88, 1.72)	0.233
ACF	25mg	1.33	(0.38, 4.61)	0.654
	50mg	2.22	(1.08, 4.54)	0.030
	100mg	1.94	(0.92, 4.09)	0.083



(b) Total EGFR

Figure 3. Relative change (post : pre) in median phosphorylated and total EGFR by dose group and mucosa strata. Figure 3(a) depicts estimates of the relative change in the median of phosphorylated EGFR. Figure 3(b) depicts estimates of the relative change in the median of total EGFR.

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