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ORIGINAL RESEARCH—CLINICAL

Epidermal Growth Factor Receptor Inhibition With Erlotinib in Liver: Dose De-Escalation Pilot Trial as an Initial Step in a Chemoprevention Strategy

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BACKGROUND AND AIMS: Effective approaches for prevention of hepatocellular carcinoma (HCC) will have a significant impact on HCC-related mortality. There are strong preclinical data and rationale to support targeting epidermal growth factor receptor (EGFR) for HCC chemoprevention. Small molecule inhibitors of EGFR have been Food and Drug Administration–approved for cancer therapy, which provides an opportunity to repurpose one of these drugs for chemoprevention of HCC. Unfortunately, the frequency of side effects associated with administration of these drugs at oncology doses renders them ineffective for chemoprevention. This clinical trial assesses whether lower doses of one of these inhibitors, erlotinib, still engages EGFR in the liver to block signaling (eg, EGFR phosphorylation). The objective of this clinical trial was determination of a safe and minimum effective dose of erlorinib for which $\geq$ 50% reduction phospho-EGFR immunohistochemical staining in the liver was observed. **METHODS:** Forty six participants were preregistered and 25 participants were registered in this multicenter trial. By dose de-escalation trial design, cohorts of participants received a 7-day course of erlotinib 75 mg/day, 50 mg/day or 25 mg/day with liver tissue acquisition prior to and after erlotinib. **RESULTS:** A $\geq$50% reduction phospho-EGFR immunohistochemical staining in the liver was observed in a minimum of 40% of participants (predetermined threshhold) at each of the dose levels. Erlotinib was very well tolerated with few side effects observed, particularly at the dose of 25 mg/day. Favorable modulation of the Prognostic Liver Signature was observed in participants who received erlotinib. **CONCLUSION:** These data support the selection of erlotinib doses as low as 25 mg/day of for a longer intervention to assess for evidence of efficacy as an HCC chemoprevention drug (ClinicalTrials.gov NCT02273362).

Keywords: Erlotinib; Hepatocellular Carcinoma; Cirrhosis; EGFR; Prevention

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common solid tumor worldwide, and due to its poor prognosis, it is the third-leading cause of cancer-related death.[1,2] Identification of high-risk populations suitable for screening and chemoprevention has been proposed

Abbreviations used in this paper: **AE, Adverse event; ALT, Alanine aminotransferase; APCI LC/MS/MS, Atmospheric Pressure Chemical Ionization with Liquid-Chromatography and tandem mass spectrometry; AST, Aspartate aminotransferase; CI, Confidence interval; CPN, Cancer Prevention Network/Mayo Clinic Consortium; CTCAE, Common Terminology Criteria for Adverse Events; DAB, diaminobenzidine; EGCG, Epigallocatechin Gallate; EGF, Epidermal growth factor; EGFR, Epidermal growth factor receptor; FDA, Food and Drug Administration; FFPE, Formalin fixed paraffin embedded; FPS, Fibrosis Progression Signature; FXR, Farnesoid X receptor; HBV, Hepatitis B virus; HCC, Hepatocellular carcinoma; HCV, Hepatitis C virus; IHC, Immunohistochemical; IND, Investigational new drug; IRB, Institutional Review Board; JPEG, Joint Photographic Experts Group; MED, Minimum effective dose; NASH, Nonalcoholic steatohepatitis; PLS, Prognostic Liver Signature; QC, Quality Control; TIFF, Tagged information file format.**

Most current article

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as an effective and efficient strategy to impact HCC-related mortality.[3] Identification of key pathways to target for prevention represents an important step in this strategy. While the cause of HCC is multifactorial, the common pathway for the majority of cases is cirrhosis. Molecular pathways have been identified that play key roles in progression of fibrosis and cirrhosis across several etiologies of cirrhosis. These include activation of hepatic stellate cells, reactive oxygen species-induced necrosis and apoptotic cell death, cell-cell signaling via cytokines and chemokines, and activation of the TGF-β pathway.[4–7]

Many agents have been investigated in the context of HCC prevention. Some fall into a category of those that address an altered metabolic milieu in the liver. These include metformin (NCT02319200), pioglitazone,[8] and experimental therapies for nonalcoholic steatohepatitis including farnesoid X receptor agonists,[9] and apoptosis signal-regulating kinase 1 (Ask1) inhibitors such as selonsertib.[10] Natural components found in foods have been investigated such as glycyrrhizin—the chief sweet-tasting constituent of licorice,[11] epigallocatechin Gallate (EGCG)—the major catechin in green tea,[12] and turmeric—a commonly used spice.

Several lines of experimental evidence provide strong rationale for epidermal growth factor receptor (EGFR) as a target for prevention of HCC. Epidermal growth factor (EGF) stimulates hepatocyte growth and plays a central role in liver regeneration. EGF is upregulated in cirrhosis,[13] and a correlation between EGF and risk for HCC in cirrhotic patients has been observed.[14] Of note, experimental overexpression of EGF in animal models gives rise to HCC, and blockade of its receptor EGFR with small molecular inhibitors effectively prevents HCC.[15,16] Furthermore, there are several small-molecule EGFR inhibitors that have regulatory approval for use in other indications, suggesting a potential strategy of repurposing existing, Food and Drug Administration–approved drugs. Erlotinib is a small molecule tyrosine kinase inhibitor that effectively targets EGFR.[17] It is used in cancer treatment (eg, nonsmall cell lung cancer, pancreas cancer) and has also been evaluated for cancer prevention. In the case of liver cancer prevention, additional support for consideration of erlotinib comes from studies demonstrating that EGFR is a co-factor important for Hepatitis C virus (HCV) entry into cells, and EGFR tyrosine kinase inhibitors including erlotinib have substantial antiviral activity.[18]

Tyrosine kinase inhibitors frequently cause side effects in cancer patients, which raise important concerns about their potential as cancer prevention drugs because they may require long-term administration. Erlotinib has common side effects of cutaneous rash and fatigue when administered at 150 mg/day for prolonged periods to cancer patients. Acceptance and compliance with a prescribed medication for cancer prevention would be unachievable if side effects were frequent. Thus, prior to giving any consideration to large-scale, long-term clinical trials of erlotinib, determination of the lowest dose of erlotinib that inhibits EGFR signaling in the target tissue is important. And similarly, understanding of the safety and toxicity profile of erlotinib at this lowest dose is equally important.

The purpose of the present study was to identify a dose schedule of erlotinib lower than the 150 mg/day oncology dose yet effective in blocking EGFR signaling in the liver. We conducted a single-arm, open label clinical trial to (1) determine the minimum dose of erlotinib administered to participants with liver fibrosis or cirrhosis that effectively reduces EGFR signaling in the liver and (2) determine the frequency and severity of side effects. EGFR signaling in the liver was assessed by measurement of phospho-EGFR immunohistochemical (IHC) staining on liver biopsy specimens obtained prior to and after erlotinib administration. The primary objective of the trial was determination of a safe and minimum effective dose (MED) based on predefined dose-limiting toxicities (DLTs) and based on the frequency by which a $\geq$ 50% reduction phospho-EGFR IHC staining in the liver was observed.

Methods

Study Design and Patient Population

This single-arm phase 1 trial with expansion cohort (NCT02273362) was designed to enroll patients with a clinical diagnosis of liver fibrosis or cirrhosis from 5 institutions through the Cancer Prevention Network. The study was approved by the Institutional Review Boards of each institution, monitored twice annualy by the Data and Safety Monitoring Board of the Mayo Clinic Cancer Center and conducted in accordance with recognized ethical guidelines. The trial protocol is available for review in Supplement Methods 1 and eligibility criteria are summarized in Supplemental Methods 2. Written informed consent was obtained from all patients prior to participation.

The primary end point was a binary outcome of whether a participant achieved a $\geq$ 50% reduction from baseline in liver phospho-EGFR staining, defined as the percentage of positive pixels, immediately after a 7-day intervention period with daily erlotinib. Because of the need for liver tissue for analysis at 2 separate time points (baseline and immediately after the final dose), a window-of-opportunity trial design was used. Eligible patients were required to have a clinical documentation of a diagnosis of liver fibrosis or cirrhosis and scheduled for elective liver resection. Eligible, registered participants underwent a liver biopsy to serve as baseline liver tissue, after which they received daily erlotinib leading up to the day of scheduled liver resection. At time of liver resection, a portion of the resected liver was obtained and analyzed as the posterlotinib tissue for comparison with baseline. An additional pathway of eligibility was subsequently established to increase the rate of accrual yet continue to subject participants to only a single research-related liver biopsy. This second pathway included patients who had undergone a liver biopsy for clinical indications in the preceeding 3 months, and who had residual tissue in the biopsy specimen which could be analyzed for the primary end point. Registered participants in this pathway underwent additional screening by assessment of phospho-EGFR staining in the liver biopsy, which served as the baseline measurement. Those with positive staining ($>$ 100 stained pixels) remained eligible. They received daily erlotinib following which they underwent a liver biopsy after the final dose of erlotinib for comparison against their baseline liver biopsy.

Dose Administration and Dose Levels

Five dose levels were scheduled: 150 mg/day (dose level +2), 100 mg/day (dose level +1), 75 mg/day (dose level 0), 50 mg/day (dose level −1), and 25 mg/day (dose level −2). Dose level 0 served as the starting dose level, with plans to either escalate or de-escalate based on results. For example, de-escalation to dose level −1 would occur if at least 2 of the first 5 evaluable participants (40%) demonstrate a response and the dose level was deemed to be safe. Alternatively, the dose would be escalated to dose level +1 for the next cohort. To be considered evaluable for response in this cohorts-of-5 design, a participant needed to have phospho-EGFR staining performed at both baseline and immediately after the final erlotinib dose. Alternatively, the starting dose level was scheduled to be escalated to dose level +1 if less than a 40% response rate was observed. In the dose level −1 cohort, if at least 2 of the first 5 evaluable participants (40%) demonstrated a response and the dose level was deemed to be safe, participants would be scheduled for enrollment at the next lower dose level (dose level −2). If at least 2 responses were observed at this dose level in the first 5 evaluable (40%) and this dose level was deemed to be safe, then this dose level was defined as the MED for consideration in future clinical trials. And upon designation of the MED, the study design called for a dose-expansion cohort at that dose level to a total of 10 evaluable participants to further evaluate the safety and response rate. The trial design also called for raising the dose level from dose level 0 to dose level +1 and possibly dose level +2 if the targeted response rate was not observed using a lower dose of erlotinib. On the day of liver resection or biopsy, erlotinib was administered to participants by study staff within hours of blood draw to assess erlotinib plasma levels. Study design also called for enrollment to be temporarily suspended after each cohort of 5 evaluable participants to evaluate the safety and the phospho-EGFR response.

To be evaluable for safety analysis, a participant needed to receive at least one dose of erlotinib. Safety was defined as the absence of any DLTs, designated as a rash determined to be grade 4 or higher, or any other adverse event (AE) grade 3 or higher and deemed at least possibly related to study agent based on the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version 4.

Participants were scheduled to take erlotinib once daily for 7 days including the day of scheduled liver resection or biopsy. Because operative schedules sometimes change for clinical reasons, the range of daily erlotinib permitted by protocol was 5–14 days. Participants were instructed to self-administer erlotinib orally each morning on an empty stomach at least 1 hour before or 2 hours after eating. Because cigarette smoking has been shown to reduce erlotinib exposure, smokers were required to discontinue smoking 2 weeks prior to starting the study and to continue not smoking while taking erlotinib. And because co-administration of erlotinib with a proton pump inhibitor decreases erlotinib exposure and maximum concentration, concomitant use of proton pump inhibitors with erlotinib was not permitted.

Erlotinib is manufactured as the hydrochloride salt by Astellas Pharma Global Development, Inc (Northbrook, IL; Astellas). NCI, Division of Cancer Prevention sponsored the IND (64,808) and provided erlotinib as 25 mg tablets for this clinical trial.

Quantification of Phospho-EGFR in Tissues

Biospecimen collection. Details of the methods used for collection, processing, storage, and transport of biospecimens are provided at the end of the clinical trial protocol (Supplemental Methods 1). As described above, some participants in this trial were enrolled on the basis of a recent clinically indicated liver biopsy, in which case the preanalytic handling methodology was not prescribed.

Immunohistochemical (IHC) staining. IHC staining was performed at the Pathology Research Core (Mayo Clinic, Rochester, MN) using the Leica Bond RX stainer (Leica). Formalin-fixed paraffin-embedded tissues were sectioned at 5 microns and IHC staining was performed online. Slides for phospho-EGFR stain were retrieved for 20 minutes using Epitope Retrieval 1 (Citrate; Leica). The phospho-EGFR primary antibody (Clone: 1H12; Cell Signaling) was diluted to 1:200 or 1:400 in Background Reducing Diluent (Dako) and incubated for 15 minutes.

The detection system used was Polymer Refine Detection System (Leica). This system includes the hydrogen peroxidase block, post primary and polymer reagent, diaminobenzidine (DAB), and hematoxylin. Immunostaining visualization was achieved by incubating slides 10 minutes in DAB and DAB buffer (1:19 mixture) from the Bond Polymer Refine Detection System. To this point, slides were rinsed between steps with 1X Bond Wash Buffer (Leica). Slides were counterstained for 5 minutes using Schmidt hematoxylin and molecular biology grade water (1:1 mixture), followed by several rinses in 1X Bond wash buffer and distilled water, this is not the hematoxylin provided with the Refine kit. Once the immunochemistry process was completed, slides were removed from the stainer and rinsed in tap water for 5 minutes. Slides were dehydrated in increasing concentrations of ethyl alcohol and cleared in 3 changes of xylene prior to permanent coverslipping in xylene-based medium.

Digital image analysis. The pEGFR slides were scanned at 40x magnification on the Aperio ScanScope AT Turbo brightfield instrument (Leica Biosystems) at a resolution of 0.25 microns per pixel. The images were 24-bit contiguous standard pyramid tiled TIFFs compressed via JPEG2000 with a quality setting of 70. For digital image analysis, the Aperio ImageScope Software (Leica Biosystems) was used. A minimum 75% normal liver tissue present was annotated. Care was taken to avoid and/or eliminate staining artifacts, tissue folds, portal tracts, or large pools of inflammatory cells. A Positive Pixel Count (Leica) algorithm was used to analyze the annotations. Results were exported and a ratio was calculated using the Number of Strong Positive pixels divided by the Total Number (Positive + Negative) of pixels. This ratio was converted to a percent. For Quality Control, 2 experienced, board-certified anatomic pathologists independently reviewed the annotations and verified the overall results. During this Quality Control, the pathologists evaluated the presence of portal tracts and entered appropriate information on the data worksheet provided for each case. The pathologists agreed with the analysis of the tissues.

Measurement of Plasma Drug Concentration

Plasma erlotinib levels were measured by Q2 Solutions (Ithaca, NY)) using a solid-phase extraction procedure and

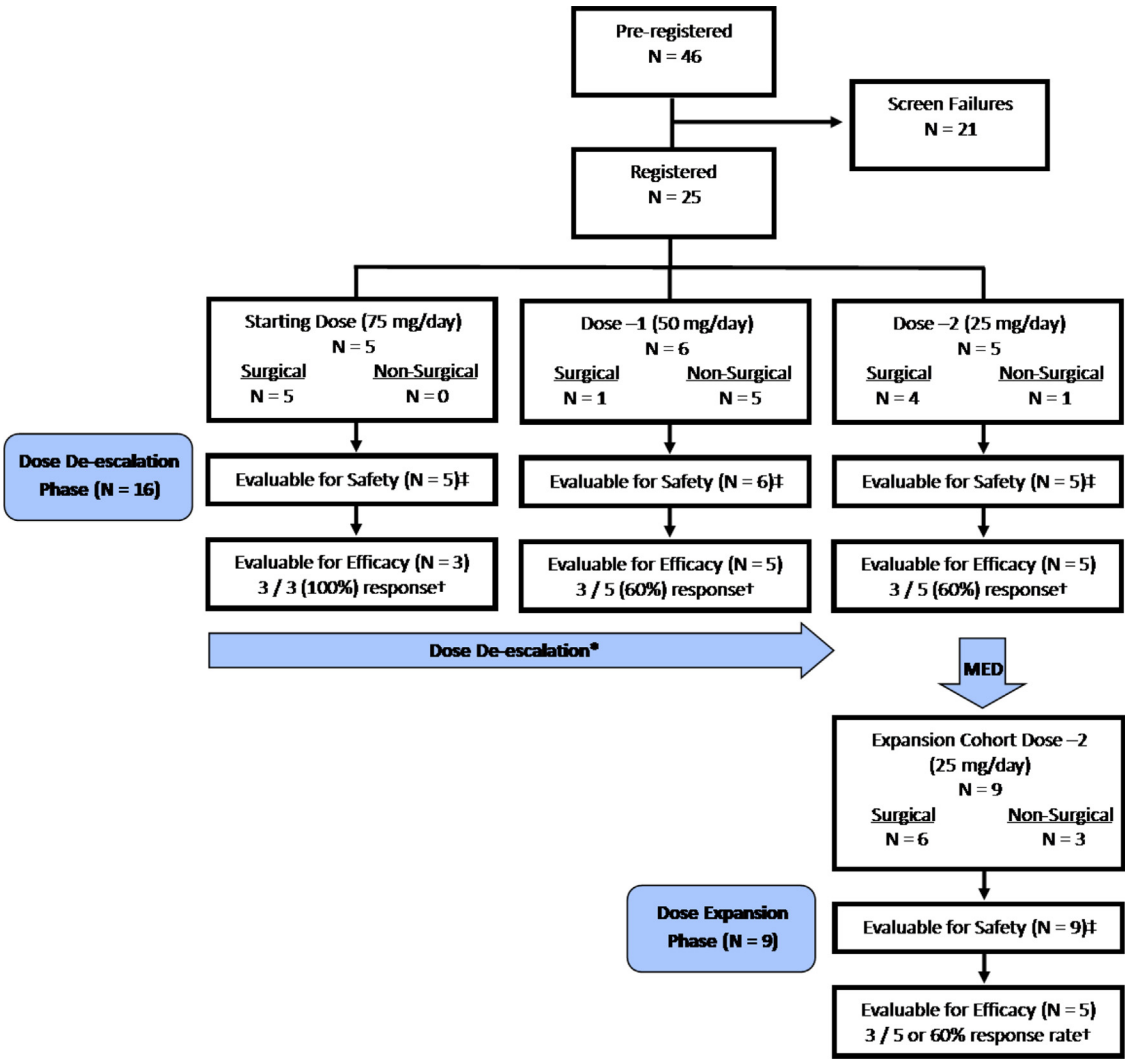

Figure 1. CONSORT Diagram. "Surgical" reference participants in whom scheduled liver resection was the method obtaining liver tissue after erlotinib. "Nonsurgical" references participants for whom liver biopsy was the method for obtaining liver tissue after erlotinib. MED, Minimum Effective Dose.

atmospheric pressure chemical ionization with liquid-chromatography and tandem mass spectrometry instrumentation.

Statistics

Statistical considerations regarding sample size with the cohorts-of-5 design were based on the response rate of phospho-EGFR staining from baseline to postintervention. Assuming a binomial distribution for the number of responses in 5 evaluable participants at a given dose level and a true response rate of 60%, the probability of observing at least 2 responses in 5 participants ($\geq$ 40%) is 91%, which was considered adequate evidence of clinical activity in a small pilot study, recognizing that a true response rate of 60% could not be ruled out even if only a 40% response rate was observed. In the dose expansion phase, 10 participants evaluable for response were targeted as such a sample size would result in a maximum width of a 2-sided 90% confidence interval (CI) for the true but unknown response rate to be $\pm$25%.

The constellation of AEs was summarized by reporting the number and percentage of participants based on the maximum grade for each type of AE experienced by a participant. Furthermore, the maximum grade for any AE was calculated for each participant and the number and percentage for grade 1+ AEs were described in detail and summarized separately as well as by attribution. For the primary end point response, we summarized the frequency and percentage of participants who achieved a response, along with the 90% CI, by dose level, including within all participants treated at the MED across the dose-finding and dose-expansion phases. In a similar manner, we descriptively summarized the percent change in ratio (%) from baseline to postintervention.

Data Availability

All authors had access to the study data and had reviewed and approved the final manuscript. Data and materials are available in the NCI's Cancer Data Acquisition System and may be requested using the standardized process.

Table 1. All Registered Participants

Characteristic	Dose level 0 (N = 5)	Dose level −1 (N = 6)	Dose level −2 (N = 14)	Total (N = 25)
Age				
Mean (SD)	63.6 (7.96)	61.2 (10.89)	63.1 (10.17)	62.8 (9.60)
Median	61.0	58.5	66.0	64.0
Range	55, 76	48, 80	38, 75	38, 80
Sex, n (%)				
Female	2 (40.0%)	2 (33.3%)	4 (28.6%)	8 (32.0%)
Male	3 (60.0%)	4 (66.7%)	10 (71.4%)	17 (68.0%)
Race, n (%)				
Asian	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (4.0%)
Black or African American	1 (20.0%)	0 (0.0%)	1 (7.1%)	2 (8.0%)
Not reported	2 (40.0%)	0 (0.0%)	0 (0.0%)	2 (8.0%)
White	2 (40.0%)	6 (100.0%)	12 (85.7%)	20 (80.0%)
Ethnicity, n (%)				
Hispanic or Latino	1 (20.0%)	0 (0.0%)	1 (7.1%)	2 (8.0%)
Not Hispanic or Latino	3 (60.0%)	4 (66.7%)	11 (78.6%)	18 (72.0%)
Not reported	1 (20.0%)	2 (33.3%)	1 (7.1%)	4 (16.0%)
Unknown	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (4.0%)
Dose cohort, n (%)				
Dose de-escalation	5 (100.0%)	6 (100.0%)	5 (35.7%)	16 (64.0%)
Dose expansion	0 (0.0%)	0 (0.0%)	9 (64.3%)	9 (36.0%)
Surgical cohort, n (%)				
Surgical	5 (100.0%)	1 (16.7%)	10 (71.4%)	16 (64.0%)
Nonsurgical	0 (0.0%)	5 (83.3%)	4 (28.6%)	9 (36.0%)

Results

Participant Characteristics

Between November 24, 2014 and October 22, 2019, 46 participants (dose level 0: n = 5, dose level −1: n = 10, dose level −2: n = 31) were preregistered and 25 participants (dose level 0: n = 5, dose level −1: n = 6, dose level −2: n = 14) were registered at 6 participating institutions (Figure 1). The median age was 64 years (range, 38–80 years), 8 (32%) participants were female, 2 (8%) participants were Black or African American, 1 (4%) participant was Asian, and 2 (8%) participants were Hispanic or Latino (Table 1). Sixteen participants (64%) were in the surgical cohort and 9 (36%) were in the nonsurgical cohort. Including all dose levels, 25 (100%) of registered participants started intervention and 18 (72%) participants were evaluable for the primary end point.

The study group characteristics were also assessed separately for the 2 cohorts: (1) dose de-escalation cohort and (2) dose expansion cohort. In the dose de-escalation cohort, 29 participants (dose level 0: n = 5, dose level −1: n = 10, dose level −2: n = 14) were preregistered and 16 participants (dose level 0: n = 5, dose level −1: n = 6, dose level −2: n = 5) were registered (Figure 1). The median age was 60.5 years (range, 48–80 years), 5 (31.3%) participants were female, 1 (6.3%) participant was Black or African American, 1 (6.3%) participant was Asian, and 1 (6.3%) participant was Hispanic or Latino. Ten (62.5%) participants were in the surgical cohort and 6 (37.5%) participants were in the nonsurgical cohort.

In the dose expansion cohort at the lowest erlotinib dose of 25 mg/day, 17 participants were preregistered and 9 participants were registered. The median age was 69 years (range, 38–75 years). Three (33.3%) participants were female, 1 (11.1%) participant was Black or African American, and 1 (11.1%) participant was Hispanic or Latino. Six (66.7%) participants were in the surgical cohort and 3 (33.3%) participants were in the nonsurgical cohort.

Of the 14 study participants in either the dose de-escalation or dose expansion cohort who received erlotinib at 25 mg per day, 4 (28.6%) participants were female, 1 (7.1%) participant was Black or African American, 1 (7.1%) participant was Asian, and 1 (7.1%) participant was Hispanic or Latino. There are no reported differences of EGFR expression and signaling in normal cells based on race or ethnicity.

Relationship Between Erlotinib Dose and Reduction in Liver Phopho-EGFR Staining

Eighteen participants (dose level 0: n = 3, dose level −1: n = 5, dose level −2: n = 10) were evaluable for the primary end point and 7 participants were not evaluable for the primary end point. One participant in dose level 0 terminated intervention early due to physician decision before receiving erlotinib, 1 participant in dose level 0 had lost preintervention tissue specimens, 1 participant in dose level −1 terminated intervention early due to AE before receiving erlotinib, 3 participants in dose level −2 did not have additional preintervention tissue specimens available beyond eligibility, and 1 participant in dose level −2 was not evaluable for phospho-EGFR staining at post-intervention due to the absence of any nontumor in the submitted specimen.

Table 2. Phospho-EGFR Staining Results

Characteristic	Dose level 0 (75 mg/day)	Dose level −1 (50 mg/day)	Dose level −2 (25 mg/day)
Dose de-escalation participants			
Evaluable for primary end point (n = 13)			
Response, n/total (%)			
No	0/3 (0.0%)	2/5 (40.0%)	2/5 (40.0%)
Yes	3/3 (100.0%)	3/5 (60.0%)	3/5 (60.0%)
Percent change in ratio (%)			
Mean (SD)	−69.6 (25.92)	1315.9 (2770.12)	18.7 (140.23)
Median	−55.5	−66.6	−56.6
Range	−99.6, −53.9	−83.1, 6246.4	−94.1, 223.2
Dose expansion participants			
Evaluable for primary end point (n = 5)			
Response, n/total (%)			
No			2/5 (40.0%)
Yes			3/5 (60.0%)
Percent change in ratio (%)			
Mean (SD)			−20.5 (79.11)
Median			−72.4
Range			−84.8, 76.8
All participants evaluable for primary end point (n = 18)			
Response, n/total (%)			
No	0/3 (0.0%)	2/5 (40.0%)	4/10 (40.0%)
Yes	3/3 (100.0%)	3/5 (60.0%)	6/10 (60.0%)
Percent change in ratio (%)			
Mean (SD)	−69.6 (25.92)	1315.9 (2770.12)	−0.9 (109.30)
Median	−55.5	−66.6	−64.5
Range	−99.6, −53.9	−83.1, 6246.4	−94.1, 223.2

At dose level 0 (75 mg/day), 3 of 3 participants evaluable for the primary end point (100% [90% CI, 36.8%–100%]) achieved at least 50% reduction in the percentage of positive pixels in the phospho-EGFR staining in the liver from baseline after at least a 6-day intervention period with daily erlotinib (Table 2).

Selected examples of phopho-EGFR staining at baseline and after erlotinib are provided in Figures 2A and B. One participant had 2 separate preintervention liver samples and 2 separate postintervention liver samples, thereby permitting 4 possible pairings for comparision of pre-intervention vs postintervention phospho-EGFR staining for 1 participant (Table 3). The possibility of multiple potential comparisons had not been considered during study design. The study investigators and medical monitor at the Division of Cancer Prevention agreed on a decision algorithm by which the assessment would be scored as meeting the threshold of at least 50% reduction in percentage of positive pixels if at least half of the possible comparisons yielded this result. One participant had 4 of 4 comparisons reveal a more than 50% reduction, and 2 participants had 1 of 2 comparisons with a more than 50% reduction. The heterogeneity of phospho-EGFR staining among different samples for a single participant at baseline was modest. The heterogeneity of phospho-EGFR staining among different samples from a single participant after erlotinib was more pronounced, thereby suggesting greater heterogeneity within a liver in response to EGFR inhibition. As shown in Table 2, at this dose level (75 mg/day), the median change in liver phospho-EGFR staining from baseline to at least 6 days of intervention was −55.5 percentage points (range, −99.6 to −53.9). When the reductions in phosphos-EGFR staining are combined with the AE profile (see below), the results met the requirements to proceed with dose de-escalation to dose level −1.

At dose level −1 (50 mg/day), 3 of 5 participants evaluable for the primary end point (60% [90% CI, 18.9%–92.4%]) achieved at least 50% reduction in the percentage of positive pixels in the phospho-EGFR staining in the liver from baseline after daily erlotinib. The 3 participants scored as having more than 50% reduction in phospho-EGFR staining each had multiple liver tissue specimens that generated more than a single comparison of pre-erlotinib vs posterlotinib tissue. More than 50% reduction in phospho-EGFR staining was observed in 4 of 6 comparisons, 1 of 2 comparisons, and 2 of 2 comparisons in these 3 participants, respectively. The median change in liver phospho-EGFR staining was −66.6 percentage points (range, −83.1 to 6246.4). When combined with the AE profile at this dose level (see below), the results met the requirements to dose de-escalate to dose level −2 (25 mg/day).

At dose level −2 (25 mg/day), 5 participants were evaluable for the primary end point, and 3 of these participants (60% [90% CI, 18.9%–92.4%]) achieved at least 50% reduction in phospho-EGFR staining compared to baseline after daily erlotinib. The median change in liver

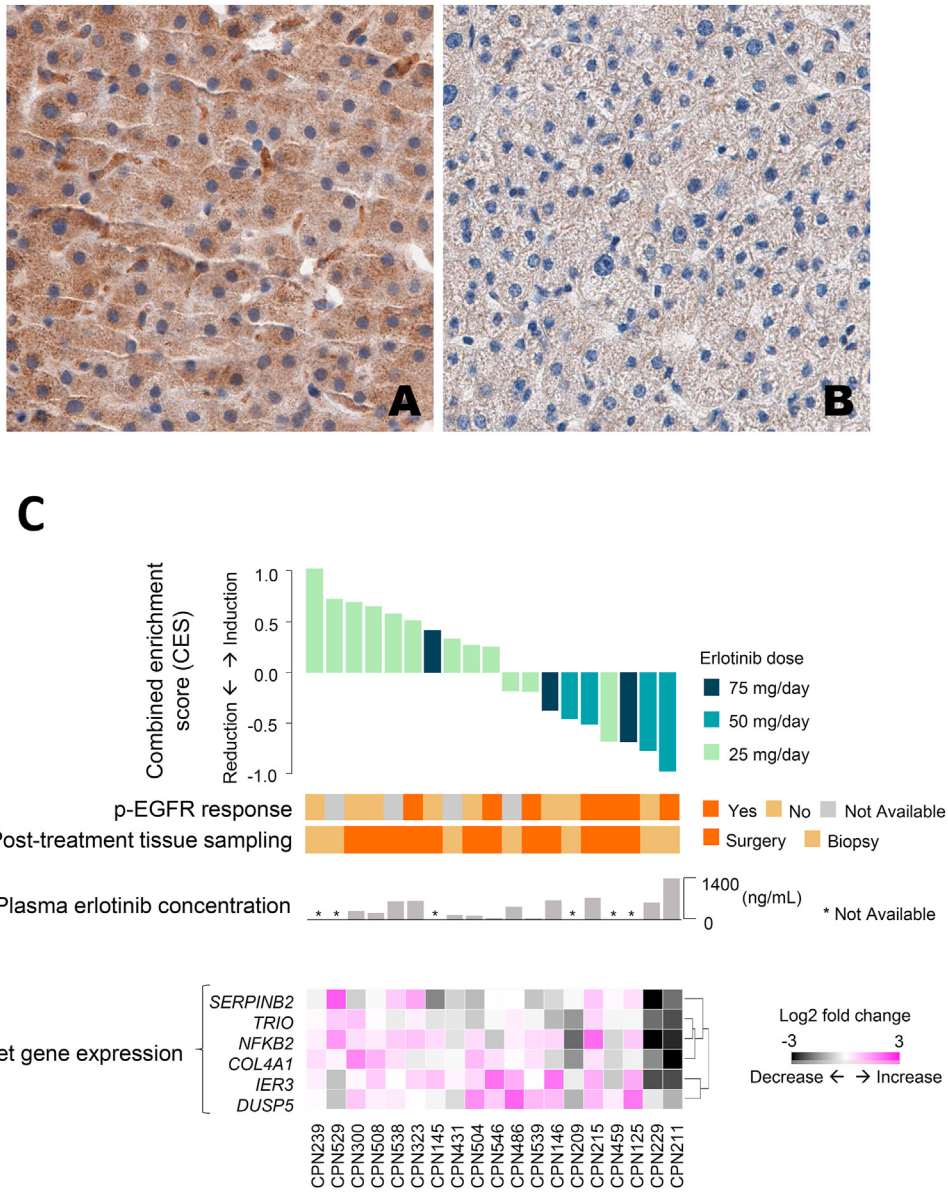

Figure 2. Liver section IHC stains for phospho-EGFR before (A) and after (B) erlotinib. The brown color of the EGFR staining is granular and located in the cytoplasm. In this example, an 85% reduction in positive pixel counts was observed following erlotinib (both photomicrographs were taken at 400×). (C) Correlation of prognostic liver signature before and after erlotinib with phospho-EGFR response, plasma erlotinib concentration, and EGF target gene expression. Modulations of a validated 186-gene hepatic transcriptome signature, prognostic liver signature (PLS), were assessed for individual participants using a combined enrichment score (CES). The CES was plotted from high to low, with each dose-level assignment denoted by color code. For each participant, whether they met primary end point of phospho-EGFR response, and whether their post-treatment tissue was acquired by liver biopsy of surgical resection is displayed by color code. Plasma erlotinib concentation is shown for each participant included in this analysis. The magnitude of modulation of 6 genes that are downstream from EGFR and that are included in the PLS is shown for each participant in this analysis.

phospho-EGFR staining from baseline after at least 6 days of erlotinib was −56.6 percentage points (range, −94.1 to 223.2). When combined with the AE profile at this dose level (see below), the results met the requirements to declare dose level −2 as the MED, and expand this cohort.

In the dose expansion cohort (25 mg/day), 5 participants were evaluable for the primary end point and 4 participants were not evaluable for the primary end point for the reasons described above. Three of the 5 participants (60% [90% CI, 18.9%–92.4%]) achieved at least 50% reduction in the percentage of positive pixels in the phospho-EGFR staining in the liver from baseline after at least a 6-day intervention period with daily erlotinib. The median change in liver phospho-EGFR staining from baseline to at least 6 days of intervention was −72.4 percentage points (range, −84.8 to 76.8).

Table 3. Positive Pixels, Total Pixels, Ratio (%), Percent Change in Ratio (%), and Response

Dose level	Participant	Pixel analysis	Preintervention		Postintervention			Preintervention sample 1 to postintervention sample 1		Preintervention sample 1 to postintervention sample 2		Preintervention sample 1 to postintervention sample 3		Preintervention sample 2 to postintervention sample 1		Preintervention sample 2 to postintervention sample 2		Preintervention sample 2 to postintervention sample 3	
			Sample 1	Sample 2	Sample 1	Sample 2	Sample 3	% change in ratio	Response	% change in ratio	Response	% change in ratio	Response	% change in ratio	Response	% change in ratio	Response	% change in ratio	Response
0	CPN00125[ad]	Positive pixels	2,195,279	1,207,809	3,704,203	579,784	-	−96.8	**Yes**	−99.5	**Yes**	-	-	−96.9	**Yes**	−99.6	**Yes**	-	-
		Total pixels	29,552,852	15,598,288	1,544,913,177	1,695,632,916	-												
		Ratio (%)	7.428315	7.743215	0.239768	0.034193	-												
	CPN00145[ad]	Positive pixels	703,761	463,574	6,290,827	-	-	−53.9	**Yes**	-	-	-	-	−19.9	No	-	-	-	-
		Total pixels	54,386,866	62,204,354	1,054,332,454	-	-												
		Ratio (%)	1.293991	0.745244	0.596664	-	-												
	CPN00146[ad]	Positive pixels	520,766	1,383,858	14,463,042	-	-	−36.4	No	-	-	-	-	−55.5	**Yes**	-	-	-	-
		Total pixels	29,684,196	55,183,970	1,295,664,434	-	-												
		Ratio (%)	1.754354	2.507717	1.116264	-	-												
−1	CPN00209[ac]	Positive pixels	29,031	-	116,137	612,254	-	+563.5	No	+2156.5	No	-	-	-	-	-	-	-	-
		Total pixels	69,240,150	-	41,744,353	64,714,216	-												
		Ratio (%)	0.041928	-	0.278210	0.946089	-												
	CPN00211[ac]	Positive pixels	9757	-	6283	4254	-	−44.1	No	−66.6	**Yes**	-	-	-	-	-	-	-	-
		Total pixels	26,941,822	-	31,046,070	35,138,931	-												
		Ratio (%)	0.036215	-	0.020238	0.012106	-												
	CPN00215[ad]	Positive pixels	96,323	116,015	1,166,156	617,268	379,403	−46.7	No	−77.7	**Yes**	−83.1	**Yes**	+9.0	No	−54.4	**Yes**	−65.5	**Yes**
		Total pixels	34,321,161	84,471,878	779,114,873	986,517,406	799,498,391												
		Ratio (%)	0.280652	0.137342	0.149677	0.062570	0.047455												
	CPN00229[ac]	Positive pixels	13,953	-	1,726,476	-	-	+6246.4	No	-	-	-	-	-	-	-	-	-	-
		Total pixels	67,096,901	-	130,819,140	-	-												
		Ratio (%)	0.020795	-	1.319743	-	-												
	CPN00235[ac]	Positive pixels	320,636	-	83,720	101,395	-	−80.7	**Yes**	−76.1	**Yes**	-	-	-	-	-	-	-	-
		Total pixels	10,689,122	-	14,439,596	14,140,606	-												
		Ratio (%)	2.999648	-	0.579795	0.717048	-												
−2	CPN00239[ac]	Positive pixels	208,488	-	441,414	-	-	+223.2	No	-	-	-	-	-	-	-	-	-	-
		Total pixels	61,873,628	-	40,536,094	-	-												
		Ratio (%)	0.336958	-	1.088941	-	-												
	CPN00240[ad]	Positive pixels	4,085,980	-	4,807,556	-	-	−85.2	**Yes**	-	-	-	-	-	-	-	-	-	-
		Total pixels	105,000,000	-	837,000,000	-	-												
		Ratio (%)	3.891410	-	0.574379	-	-												
	CPN00300[ad]	Positive pixels	153,039	-	1,296,836	-	-	+106.1	No	-	-	-	-	-	-	-	-	-	-
		Total pixels	80,512,552	-	331,000,000	-	-												
		Ratio (%)	0.190081	-	0.391793	-	-												
	CPN00323[ad]	Positive pixels	1,399,998	-	253,718	-	-	−94.1	**Yes**	-	-	-	-	-	-	-	-	-	-
		Total pixels	35,273,445	-	109,000,000	-	-												
		Ratio (%)	3.968986	-	0.232769	-	-												
	CPN00337[ad]	Positive pixels	620,745	-	16,195,878	-	-	−56.6	**Yes**	-	-	-	-	-	-	-	-	-	-
		Total pixels	33,445,921	-	2,010,000,000	-	-												
		Ratio (%)	1.855966	-	0.805765	-	-												
	CPN00459[bd]	Positive pixels	21,240	-	150,443	-	-	−84.8	**Yes**	-	-	-	-	-	-	-	-	-	-
		Total pixels	56,866,628	-	2,641,826,351	-	-												
		Ratio (%)	0.037351	-	0.005695	-	-												
	CPN00504[bd]	Positive pixels	10,247	-	365,891	-	-	+54.4	No	-	-	-	-	-	-	-	-	-	-
		Total pixels	120,153,642	-	2,779,493,941	-	-												
		Ratio (%)	0.008528	-	0.013164	-	-												

Table 3. Continued

Dose level	Participant	Pixel analysis	Preintervention Sample 1	Preintervention Sample 2	Postintervention Sample 1	Postintervention Sample 2	Postintervention Sample 3	Preintervention sample 1 to postintervention sample 1 % change in ratio	Preintervention sample 1 to postintervention sample 1 Response	Preintervention sample 1 to postintervention sample 2 % change in ratio	Preintervention sample 1 to postintervention sample 2 Response	Preintervention sample 1 to postintervention sample 3 % change in ratio	Preintervention sample 1 to postintervention sample 3 Response	Preintervention sample 2 to postintervention sample 1 % change in ratio	Preintervention sample 2 to postintervention sample 1 Response	Preintervention sample 2 to postintervention sample 2 % change in ratio	Preintervention sample 2 to postintervention sample 2 Response	Preintervention sample 2 to postintervention sample 3 % change in ratio	Preintervention sample 2 to postintervention sample 3 Response
	CPN00508[b,d]	Positive pixels	22,318	-	492,728	-	-	+76.8	No	-	-	-	-	-	-	-	-	-	-
		Total pixels	132,400,200	-	1,653,257,062	-	-												
		Ratio (%)	0.016856	-	0.029803	-	-												
	CPN00539[b,d]	Positive pixels	103,365	-	1,691,952	-	-	−72.4	**Yes**	-	-	-	-	-	-	-	-	-	-
		Total pixels	32,589,623	-	1,932,363,860	-	-												
		Ratio (%)	0.317172	-	0.087559	-	-												
	CPN00546[c,d]	Positive pixels	120,871	-	502,154	-	-	−76.5	**Yes**	-	-	-	-	-	-	-	-	-	-
		Total pixels	228,937,246	-	4,054,180,996	-	-												
		Ratio (%)	0.052797	-	0.012386	-	-												

[a]Dose de-escalation cohort.
[b]Dose expansion cohort.
[c]Nonsurgical cohort.
[d]Surgical cohort.

In addition to measurement of phosphoEGFR staining by pixel counts, the slides were reviewed by 2 pathologists for qualitative changes in response to erlotinib. As can be seen in Figure 2A, staining for phospho-EGFR, the majority of the total stain is in the hepatocytes. There is granular staining limited to the cytoplasm. The distribution in hepatocytes was fairly uniform within individual livers but the intensity varied between patients. The degree of reduction in the positive pixel counts correlated with the estimated reduction by visual examination.

Toxicity of Erlotinib Administration

Of 25 registered participants (dose level 0: n = 5, dose level −1: n = 6, dose level −2: n = 14) who started intervention and were evaluable for AEs, 12 (dose level 0: n = 2, dose level −1: n = 3, dose level −2: n = 7) participants reported at least one AE (Table A2, Table 4). One participant (dose level −2: n = 1) reported a grade 3 AE anemia, unrelated to intervention. No grade 4+ AEs or DLTs were reported. Of the 14 participants who received erlotinib 25 mg/day, side effects were rare and only 1 patient reported a rash (grade 1, possibly related) and 1 patient reported epistaxis (grade 1, possibly related). All other side effects were graded as unrelated (Table A2).

Plasma Concentration of Erlotinib

Plasma concentrations of erlotinib were measured prior to any administration of erlotinib and on the final day of erlotinib administration. As expected, plasma erlotinib concentration was below the lower limit of detection on all samples obtained prior to erlotinib intervention. But wide variation was observed in plasma erlotinib concentrations obtained after the final erlotinib dose (Table A2). Of significance, the time interval between the final dose and the blood draw varied from less than an hour to several hours. At dose level 0 (75 mg/day), the plasma concentration ranged from 237 ng/mL to 761 ng/mL (average = 430 mg/mL). At dose level −1 (50 m/day), the plasma erlotinib concentration ranged from 558 to 1380 ng/mL (average = 870 ng/mL). And at dose level −2 (25 mg/day), the erlotinib concentration ranged from 53 to 603 ng/mL (average = 261 ng/mL). No correlation was observed between the plasma erlotinib concentration and either likelihood of > 50% reduction in phospho-EGFR staining, or magnitude of reduction of phospho-EGFR staining.

Modulation of Prognostic Liver Signature by Erlotinib Treatment

Previously, a 186-gene hepatic transcriptome signature, Prognostic Liver Signature (PLS), was defined and validated for its association with long-term risk of developing HCC in multiple independent cohorts of patients with cirrhosis from viral and metabolic etiologies.[19–22] PLS can be therapeutically modulated by various agents, including erlotinib, in animal models of cirrhosis in association with reduced

Table 4. Frequency of Adverse Events

Characteristic	Dose level 0 (75 mg/day)	Dose level −1 (50 mg/day)	Dose level −2 (25 mg/day)
Dose de-escalation participants			
Evaluable for adverse events (n = 16)			
Grade 3+ AE, n/total (%)			
No	5/5 (100.0%)	6/6 (100.0%)	4/5 (80.0%)
Yes	0/5 (0.0%)	0/6 (0.0%)	1/5 (20.0%)
Dose expansion participants			
Evaluable for adverse events (n = 9)			
Response, n/total (%)			
No			9/9 (100.0%)
Yes			0/9 (0.0%)
All participants evaluable for adverse events (n = 25)			
Response, n/total (%)			
No	5/5 (100.0%)	6/6 (100.0%)	13/14 (92.9%)
Yes	0/5 (0.0%)	0/6 (0.0%)	1/14 (7.1%)

HCC nodules and in liver tissues from chronic liver disease patients.[15,23,24] As an exploratory end point in the current clinical trial, we evaluated modulation of the PLS with the 7-day erlotinib treatment, which was assessed by using combined enrichment score (CES) as previously reported[15,23] (Figure 2C). Higher erlotinib doses (50 and 75 mg/day) were associated with more favorable PLS modulation compared to the lowest dose of 25 mg/day (Fisher's exact test, $P = .02$, odds ratio = 14.87) along with a trend of association with the primary study end point (ie, > 50% reduction in phospho-EGFR staining) (Fisher's exact test, $P = .31$, odds ratio = 3.76). No correlation was observed between the PLS modulation and plasma erlotinib concentration, mirroring the observation of an absence of correlation between a reduction in phospho-EGFR staining and plasma erlotinib concentration. Among the PLS member genes, 6 genes (*SERPINB2, TRIO, NFKB2, COL4A1, IER3, and DUSP5*) were overlapped with experimentally defined transcriptional EGF target genes,[25] which were most broadly suppressed in association with the beneficial PLS modulation with the highest erlotinib dose (75 mg/day). We recently defined a subset of PLS genes specifically associated with long-term liver fibrosis progression, Fibrosis Progression Signature (FPS).[26] The FPS showed similar favorable modulation with the higher doses of erlotinib as observed for the PLS (Figure A1).

Discussion

The incidence of HCC is rising faster than nearly all other cancers. Disappointingly, there have been few breakthroughs in HCC treatment that have reduced its overall mortality.[2] Potentially curative therapies of ablation, resection, and transplant remain applicable to only a selected minority of patients. Molecularly targeted therapies including immuno-oncology have achieved some successes in HCC.[27] While continued research into therapies is appropriate and much needed, prevention is a tactic that is well suited to HCC. The target population to which prevention efforts should be directed is well defined, and in particular, it is those with underlying chronic liver conditions including cirrhosis, Hepatitis B virus (HBV), HCV, and nonalcoholic steatohepatitis. Seventy percent to 80% of patients afflicted with HCC have an underlying chronic liver disease. And, this well-defined target population is easily identified based on common clinical assessments; it is not necessary to assess for otherwise relatively silent germline mutations.

The efficacy of a strategy of primary prevention of HCC is well known based on results observed after widespread HBV immunization in Taiwan that began in 1984. Since then, the prevalence of HBV carriers has been significantly reduced, particularly in the younger population, and this has been associated with a 75% reduction of HCC incidence in the young.[28] Other examples of primary prevention of HCC include a worldwide effort to reduce exposure to carcinogens such as aflatoxin B1 and identification of patients with chronic HCV to initiate treatment with direct-acting antiviral medications.

Chemoprevention involves administration of natural, synthetic, or biologic agents to prevent or reverse development of cancer.[29] A variety of agents have been evaluated, and some have been approved for prevention of breast, prostate, or colon cancer. Common pathways targeted by these agents are differentiation of cell growth, induction of cell apoptosis, and inhibition of cell growth.[30] To date, no effective chemopreventive agents have been approved for HCC.

The ideal agent for prevention of cancer is defined by several criteria: (1) effective, (2) inexpensive, (3) easily administered (eg, oral, long acting transdermal), (4) few if any side effects, and (5) existence of an accurate biomarker for efficacy. Erlotinib possesses some of these important characteristics, although others remain to be demonstrated. At the lowest daily dose of 25 mg/day, excellent tolerability was observed, and this dose was effective in shutting down

phospho-EGFR signaling in the target organ. Additional studies are in progress to evaluate secondary, explorative end points of the study in hopes of identification of a biomarker of efficacy.

EGFR signaling plays an essential role in cell proliferation, survival, and migration. After binding one of several potential ligands at the extracellular domain, EGFR forms homodimers or heterodimers. Dimerization induces activation of the tyrosine kinase domain, leading to autophosphorylation of tyrosine residues. Adapter proteins associate with the activated EGFR signal intracellularly in pathways that include RAS-RAF-MEK-ERK, AKT, and JAK/STAT pathways.[31] Small molecule inhibitors of EGFR such as erlotinib and gefitinib interact with the cytoplasmic domain of EGFR and compete with adenosine triphosphate to bind and block the catalytic domain of the kinase.[32] This in turn inhibits EGFR autophosphorylation and downstream signaling.

Several scientific observations provide strong rationale for evaluation of inhibition of the EGF-EGFR axis for chemoprevention of HCC. EGF expression is associated with progression of cirrhosis,[13] and in animal models, overexpression of EGF in liver tissue leads to formation of HCC.[33,34] In humans, a functional single nucleotide polymorphism in the EGF gene increases EGF levels in liver tissue, and this single nucleotide polymorphism is also associated with increased risk for HCC.[14] Among cirrhotic patients, those with high EGF expression in their liver tissue have the poorest overall survival. These data further implicate an important role of the EGF-EGFR axis in formation of HCC. In preclinical models, inhibition of the EGFR with erlotinib inhibits and reverses hepatic fibrosis and reduces the rate of transformation to HCC.[15] Accordingly, blockade of EGFR with inhibitors that have been previously approved by Food and Drug Administration for other indications such as cancer presents a promising therapeutic target to manage liver fibrosis and cirrhosis, as well as prevent HCC.

In this clinical trial, reduction of EGFR phosphorylation was observed with doses as low as 25 mg/day. And the impact on signaling downstream of EGFR was also observed with a reduction in phospho-ERK staining and a suppression of EGF target genes in the liver after 1 week exposure to erlotinib. The primary objective of this study was to determine the minimum erlotinib dose that would impact EGFR signaling in the liver, and only short exposure is necessary to assess this end point. Changes in liver histology are not expected after this short of an exposure, and the minimum duration of EGFR inhibition required to change liver histology and function is unknown. The duration of changes in the liver environment after removal of the drug is also unknown.

We are not aware of data—clinical or preclinical—to inform the choice of cutoff threshold for phospho-EGFR reduction. Fifty percent was selected based on the goal of having a clearly defined and reproducible threshold that represents a "low bar" that is appropriate for an early phase pilot trial. Nevertheless, the end point of 50% reduction in EGFR phosphorylation compared to baseline is felt to be indicative of target engagement. And there was a general correlation between frequency of reaching this 50% threshold at the higher doses of erlotinib and a reduction in expression of downstream EGFR target genes (Figure 2C).

It is of interest that some participants who received erlotinib 50 mg/day or 25 mg/day for a minimum of 6 days were observed to have increases of phospho-EGFR staining compared to baseline (Table 3). The mechanisms behind this observation are unclear. Possible explanations include alternative mechanisms of EGF-EGFR axis stimulation such as via response to injury (eg, alcohol, strenous exercise, or trauma), sampling that is not representative of the liver as a whole, and true heterogeneity of phospho-EGFR throughout the liver. And if there is true heterogeneity, the impact on clinical trial design is potentially quite significant. It is unknown whether it is necessary to favorably modulate phopho-EGFR straining in every part of the liver vs a majority of the liver. For purposes of a clinical trial, the ideal assessment of the gold standard of liver histopathology would be noninvasive, hold high predictive value, and not subject to sampling error from heterogeneity. Molecular imaging has appeal but there are not any effective strategies to image tyrosine kinase phosphorylation. The PLS is a potential marker with better demonstrated predictive value than EGFR phosphorylation. And theoretically even better would be a circulating marker, such as serum secretome signature.[35] The ideal predictive marker would be accurate to the extent that it could be incorporated into clinical trial design to enable early discontinuation of the drug intervention or enable individualization of the dose schedule.

The primary end point of this study was to assess the relationship between dose schedule and inhibition of EGFR phosphorylation (eg, signaling) and not to determine the relationship between dose-schedule and efficacy, which would be an unrealistic expectation of exposure to erlotinib for 1 week. It was of interest to observe that even short-term erlotinib exposure in this clinical trial was sufficient to observe a favorable change to the PLS, a transcriptomic signature that has been validated for its association with development of HCC.[19–22] The FPS also showed similar favorable modulation after 1 week of erlotinib, more commonly among those that received 50 mg/day or 75 mg/day compared to 25 mg/day. Given the strong predictive value of these signatures, these observations are very encouraging. And one can envision many strategies that the PLS could be used to prospectively inform clinical trial design. The signature could be assessed early into therapy to discontinue the approach in patients who do not respond favorably to the erlotinib intervention. It may also be used to select the MED individually per patient, or it may be used to monitor patients once they have completed the prescribed duration of erlotinib intervention. There are many challenges to successfully navigate to develop erlotinib as a clinically effective therapy for liver cirrhosis, and use of these signatures may be helpful. It is still an open question whether the CES induction in the 25 mg dose indicates increase of future HCC incidence, and there may be biological

differences between the biopsy and surgical tissues underlying the CES induction. Future studies and trials should address the question. Similarly, cutoff values for PLS/FPS to determine clinically meaningful reduction of HCC risk level should be explored in future studies.

Erlotinib was very well tolerated with few side effects, particularly at the dose of 25 mg/day. It is not surprising that side effects were fewer and less severe compared to those observed with the oncology dose of 150 mg/day, which represents the maximum tolerated dose.[36] It is likely that the high tolerability observed in this study was related to the low dose, and in addition, erlotinib exposure in this clinical trial was typically for only 1 week or less. Subsequent studies of larger cohorts of patients receiving this low dose of erlotinib for significantly longer treatment courses will more accurately define tolerability. In the oncology domain, long-term administration of erlotinib at 150 mg/day is associated with persistent side effects, although the spectrum of side effect in an individual patient does not appear to broaden after prolonged exposure. Rather, the low-grade side effects remain (eg, rash, diarrhea, fatigue) and do not typically resolve over time while still receiving erlotinib.[37] In limited reports of erlotinib 25 mg per day or every other day in elderly patients for ongoing treatment of lung cancer, patients experienced grade 1 rash and/or fatigue combined with reduced kidney function—significant and stable decrease in the glomerular filtration rate—suggesting accumulation of erlotinib despite the low dose.[38] In a prospective study of low-dose erlotinib (25 mg/day) as maintenance treatment for nonsmall cell lung cancer patients, toxicities were generally mild in the 11 patients in this study. Only 1 patient developed a grade 3 toxicity (aspartate aminotransferase/alanine aminotransferase elevation), and 4 patients developed grade 1 skin rash.

Erlotinib at 25 mg/day has been shown to be an effective cancer therapy in some lung cancer patients. However, this provides only limited support for use of this dose in a liver cancer chemoprevention strategy. Lung cancers commonly have activating mutations of EGFR that are known to confer exquisite clinical responsiveness to small molecule EGFR inhibitors.

In this study, we did not observe plasma erlotinib levels that correlated with dose administered or correlated with the primary end point. High interpatient variability of plasma concentrations has been observed in several clinical reports of patients treated with erlotinib[39] and tyrosine kinase inhibitors in general.[40] Clinical responses to erlotinib in cancer patients do not correlate with plasma concentrations, although in a preclinical model of erlotinib inhibition of HCC in the setting of cirrhosis, a dose-response relationship was observed.[15] The absence of a correlation in this study between phospho-EGFR inhibition and erlotinib plasma concentration may be because of variable timing of blood collection for plasma erlotinib level measurement relative to the time the drug was taken by each participant. Participants were generally moved into periprocedural areas (eg, perioperative holding areas) and undergoing clinical assessments by staff for planned procedures (eg, liver resection, liver biopsy) at the time of a planned blood draw for this study. This undoubtedly contributed to the lack of uniformity in timing of collection. An additional important consideration is that many factors are known to influence erlotinib plasma concentration including inhibitors of CYP3A4 (ie, calcium channel blockers, azole antifungals, macrolide antibiotics, fluoroquinolone antibiotics, some HIV antivirals, and grapefruit juice). While medications and foods known to impact erlotinib levels were prospectively controlled, the unknowns of patient compliance and other yet-to-be-described modulators may be factors in this analysis.

There are limitations of this study to be considered when drawing conclusions. Liver fibrosis and cirrhosis in the study population were not scored for severity, and it is possible that there is an innate difference between these 2 patient populations. For example, it is possible that the nonsurgical arm had more advanced fibrosis/cirrhosis from the perspectives that by nature, (1) advanced cirrhosis is a general contraindication to surgery and (2) they had a clinical indication for liver biopsy, which revealed fibrosis/cirrhosis. And it is not known how or if baseline phospho-EGFR varies with severity of liver fibrosis. Consequently, results of this pilot study leave unknown if there is a relationship between MED and severity of fibrosis. Second, the primary end point of reduced EGFR signaling in the liver can be studied with short-term exposure to EGFR inhibitors, and this served as rationale for a 1-week exposure. But this relatively duration likely understates side effects that may be observed with longer exposure. Third, while the validity of phospho-EGFR staining as a marker for EGFR signaling in liver has been demonstrated in rat and mouse liver, this has not been previously examined in human liver. Fourth, while encouraging changes in gene expression profiles were observed with 1 week of erlortinib exposure, results of this pilot study obviously do not address the duration of treatment necessary to observe histologic changes, nor whether the intervention could be withdrawn at that time.

Conclusion

Given the limitations of current treatments for HCC, effective chemoprevention in specific populations is an important objective. There are significant preclinical data that support targeting EGFR. This initial clinical report provides additional support, with results suggesting that an erlotinib dose as low as 25 mg/day is well tolerated and reduces phospho-EGFR signaling in the liver; this dose of erlotinib could be studied for a longer intervention to assess for evidence of efficacy as an HCC chemoprevention drug.

Participating Healthcare Organizations

Mayo Clinic, Jacksonville, Florida.

Massachusetts General Hospital, Boston, Massachusetts.

Mayo Clinic, Rochester, Minnesota.
Mount Sinai Hospital, New York, New York.
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Supplementary Materials

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Authors' Contributions:
Every author provided substantive contributions to each of the following: (1) conception and design of the study; (2) generation, collection, assembly, analysis, and/or interpretation of data; (3) drafting or revision of the manuscript; and (4) approval of the final version of the manuscript.

Conflicts of Interest:
The authors disclose the following: Dr Paul J. Limburg serves as Chief Medical Officer for Screening at Exact Sciences through a contracted services agreement with Mayo Clinic. Dr Paul J. Limburg and Mayo Clinic have contractual rights to receive royalties through this agreement; Exact Sciences. Dr Kenneth K. Tanabe was the recipient of laboratory research funding from NIH, Bridge Biotherapeutics and from Zafgen for research projects unrelated to this clinical trial. Prof Josep M. Llovet is receiving research support from Eisai, Bayer HealthCare Pharmaceuticals Inc, and Ipsen and consulting fees from Eisai Inc, Merck, Bristol-Myers Squibb, Eli Lilly, Roche, Genentech, Glycotest, AstraZeneca, Bayer HealthCare Pharmaceuticals, Omega Therapeutics, Mina Alpha, Boston Scientific, Exelixis, Bluejay and Captor Therapeutics. Prof Josep M. Llovet is receiving research support from Eisai, Bayer HealthCare Pharmaceuticals Inc, and Ipsen and consulting fees from Eisai Inc, Merck, Bristol-Myers Squibb, Eli Lilly, Roche, Genentech, Glycotest, AstraZeneca, Bayer HealthCare Pharmaceuticals, Omega Therapeutics, Mina Alpha, Boston Scientific, Exelixis, Bluejay and Captor Therapeutics. The remaining authors disclose no conflicts.

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Ethical Statement:
The study was approved by the Institutional Review Boards of each institution, monitored twice annually by the Data and Safety Monitoring Board of the Mayo Clinic Cancer Center and conducted in accordance with recognized ethical guidelines. Opinions expressed by the authors are their own and this material should not be interpreted as representing the official viewpoint of the US Department of Health and Human Services, the National Institutes of Health, or the National Cancer Institute.

Data Transparency Statement:
Data and materials are available in the National Cancer Institute's Cancer Data Acquisition System and may be requested using the standardized process.

Reporting Guidelines:
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