Reducing Skin Toxicities from EGFR Inhibitors with Topical BRAF Inhibitor Therapy

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ABSTRACT

Treatment of cancer with EGFR inhibitors is limited by on-target skin toxicities induced by inhibition of the MAPK pathway. BRAF inhibitors are known to paradoxically activate the MAPK downstream of EGFR, which we confirmed using human skin keratinocytes. We then conducted a phase I clinical trial testing the hypothesis that topical therapy with the BRAF inhibitor LUT014 could improve skin toxicities induced by EGFR inhibitors. Ten patients with metastatic colorectal cancer who had developed acneiform rash while being treated with cetuximab or panitumumab were enrolled in three cohorts. LUT014 was well tolerated, and there were no dose-limiting toxicities. The acneiform rash improved in the 6 patients who started with grade 2 rash in the low and intermediate cohorts. We conclude that topical LUT014 is safe and efficacious in improving rash from EGFR inhibitors, consistent with the mechanism of action inducting paradoxical MAPK activation.

SIGNIFICANCE: BRAF inhibitor topical therapy could avoid dose reductions of EGFR inhibitors, locally treating the main dose-limiting skin toxicity of this class of agents.

INTRODUCTION

Studies have reported that 75% to 90% of patients treated with EGFR inhibitor therapy experience some form of papulopustular, acneiform rash, which frequently leads to impaired quality of life and suboptimal anticancer treatment due to treatment interruptions, dose reductions, or permanent discontinuation of EGFR inhibitor therapy (1–7). Inhibition of the activated EGFR in normal epithelial tissues results in inhibition of ERK 1/2 phosphorylation (pERK) and decreased keratinocyte proliferation and migration, and premature differentiation (8), with increases in chemokines attracting proinflammatory cells that trigger the resulting acneiform skin rash (Fig. 1A; ref. 9). However,
Figure 1. Paradoxical MAPK activation, reversion of EGFR inhibition, and cellular proliferation with LUT014. A and B, Hypothesis of the mechanism of action of topical LUT014 to reverse the pathogenesis of acneiform rash induced by EGFR inhibitor (EGFRi) therapy. In cancer cells (A), oncogenic EGFR signaling leads to increased MAPK signaling and cancer cell proliferation. With EGFR inhibitor treatment, the MAPK signaling pathway is inhibited resulting in tumor shrinkage. In skin cells (B), administration of EGFR inhibitor treatment leads to decreased MAPK signaling and acneiform rash. Topical therapy with LUT014 would override the MAPK pathway inhibition, resulting in an increase in phosphorylated ERK1/2 (pERK) signaling in skin cells.

C, Chemical structure of LUT014.

D, Effects of LUT014 on MAPK signaling in primary adult HEKa cells. Western blot analysis of HEKa lysates for pERK and total ERK2 following 2-hour exposure to DMSO vehicle control, human keratinocyte growth supplement (HKGS) to stimulate the MAPK pathway, the positive control BRAF inhibitor vemurafenib and LUT014. The bar graph represents data from two replicate experiments with error bars representing SEM.

E, Reversal of EGFR inhibitor–mediated pERK inhibition with LUT014. HEKa cells were cultured with HKGS to increase pERK, and exposed to the presence of the EGFR inhibitors erlotinib or cetuximab, without or with the addition of LUT014. Cell lysates were analyzed by Western blot for pERK and total ERK2. Results are representative of two replicate experiments.

F, Bell-shaped curve of paradoxical proliferation with increasing concentrations of LUT014. Proliferation assay of MIA-PaCa-2 cells treated with various concentrations of LUT014 for 72 hours.
EGFR inhibitor–induced acneiform rash is markedly reduced or does not develop in patients who receive a combination of an EGFR inhibitor with a systemic BRAF inhibitor for the treatment of BRAF V600-mutated colorectal carcinomas (10). The decrease in toxicities with the combination is attributed to the paradoxical activation of the MAPK pathway induced by the BRAF inhibitor offsetting the decrease in pERK induced by the EGFR inhibitor (11–16). Paradoxical MAPK activation with a BRAF inhibitor refers to the increased MAPK pathway output, measured by increase in pERK and cell proliferation, when exposing cells that are wild-type for BRAF to a BRAF inhibitor, in particular if there is strong upstream receptor tyrosine kinase or RAS activation (17, 18). On the basis of this mechanistic understanding, we hypothesized that a topical therapy with a BRAF inhibitor could reduce the severity of dose-limiting acneiform lesions associated with EGFR inhibitor treatment (Fig. 1B). The topical BRAF inhibitor would reverse the pERK inhibition through the induction of paradoxical MAPK activation in skin cells with wild-type BRAF, leading to reactivation of the MAPK pathway (19), and it would avoid interference with the anticancer treatment and other toxicities seen with systemic BRAF inhibitors.

RESULTS

In Vitro Testing of Paradoxical Activation with a BRAF Inhibitor to Overcome EGFR Inhibitor–Induced MAPK Pathway Inhibition

LUT014 is a small-molecule inhibitor of the serine/threonine–protein kinase BRAF (compound structure in Fig. 1C). We characterized the kinase inhibitory activity of LUT014 compared with the reference BRAF inhibitor vemurafenib (formerly PLX4032; ref. 20). In these studies, LUT014 potency relative to vemurafenib was approximately 3-fold higher for mutated BRAF (0.013 μmol/L for LUT014 and 0.04 μmol/L for vemurafenib), and approximately 4-fold lower for wild-type BRAF (Supplementary Table S1). We then tested LUT014 at two concentrations in an in vitro kinase inhibition profiling study against 59 human recombinant kinases. At the concentration of 0.01 μmol/L, which significantly blocks mutated BRAF, LUT014 had no significant inhibitory effect on any of the other kinases tested (Supplementary Table S2). At 100-fold higher concentration of the 50% inhibition concentration (IC50), 1 μmol/L, LUT014 loses its specificity for BRAF as it also has significant inhibitory effect (30% inhibition) against ABL, CRAF, EPHA5, EPHB4, LYN, and SAPK2a. These data demonstrate that LUT014 is a potent and specific BRAF kinase inhibitor with higher selectivity for the mutated kinase than vemurafenib. We then set up in vitro models to test the ability of LUT014 to induce paradoxical MAPK activation and reverse the effects of EGFR inhibition. Culturing primary adult human epidermal keratinocyte (HEKa) cells in the presence of human keratinocyte growth supplement (HKGS) increased pERK, representing activation of the MAPK pathway. The increase in pERK was similar with the addition of the BRAF inhibitor vemurafenib, which is known to induce paradoxical MAPK activation, and it was higher with LUT014, which had been selected to maximize the paradoxical increase in pERK (Fig. 1D). Addition of the EGFR kinase inhibitor erlotinib, or the antibody blocking EGFR cetuximab, partially inhibited the HKGS stimulation of the MAPK pathway in HEKa cells, demonstrated by decreased pERK. The addition of LUT014 abrogated the inhibitory effect of both EGFR inhibitors, increasing pERK to levels comparable with that seen with HKGS alone (Fig. 1E). As it has been reported that at higher concentrations of a BRAF inhibitor the paradoxical activation effects decrease (17, 18), we tested the effects of LUT014 on the proliferation of the KrasG12C-mutated human pancreatic cancer cell line Mia-PaCa-2 (21). Increasing concentrations of LUT014 induced a concentration-dependent increased proliferation of Mia-PaCa-2 cells, with a peak proliferation at 0.041 μmol/L. At higher concentrations, LUT014 induced a concentration-dependent decreased proliferation, resulting in Mia-PaCa-2 growth arrest at the highest concentrations (Fig. 1F). Therefore, LUT014 tested in vitro reverses the MAPK pathway inhibition induced by EGFR inhibitors, through a bell-shaped paradoxical MAPK activation and downstream cell proliferation.

Phase I Clinical Trial Testing the Use of Topical BRAF Inhibitor Therapy for EGFR Inhibitor–Induced Acneiform Rash

To test whether topical administration of LUT014 would benefit patients with acneiform rash induced by anti-EGFR therapies, we enrolled 10 patients with metastatic colorectal carcinoma who developed grade 1 or 2 skin rash while on therapy with cetuximab or panitumumab (Table 1). Three patients were assigned to the 0.3 mg/g dose cohort 1, four to the 1.0 mg/g dose cohort 2, and three to the 2.5 mg/g dose cohort 3 (Supplementary Fig. S1). Supplementary Table S3 provides the baseline assessment of anti-EGFR–induced skin toxicity; Supplementary Table S4 provides the anti-EGFR agent and dosing regimen while receiving LUT014; and Supplementary Table S5 provides the LUT014 treatment adherence. In general, the study drug was well tolerated and no dose-limiting toxicity (DLT) or MTD was defined. A total of 38 adverse events of any attribution were reported, with comparable numbers for each dose cohort (Table 2). Seven patients experienced an adverse event considered to be related to the study drug, including pruritus, dry skin, and stinging sensation (all were grades 1–2 in severity). There were no local or systemic toxicities that have been associated with the clinical use of BRAF inhibitors administered as oral systemic therapies, such as photosensitivity, maculopapular rash, or hyperkeratosis (22). Pharmacokinetic (PK) analysis using blood samples of patients treated with topical LUT014 showed negligible absorption and minimal systemic exposure, with plasma concentrations in the pg/mL range (Supplementary Fig. S2A and S2B). These are concentrations that are 3 to 4 orders of magnitude lower than the effective systemic concentrations of other BRAF inhibitors, which are measured in μg/mL (23–25).

We used three scales to evaluate the severity of acneiform lesions during the clinical trial period. The primary readout was based on the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 for grading acneiform rash. The clinical trial eligibility criteria were based on having an acneiform skin rash of grade 1 or 2 while on anti-EGFR therapy, and therefore we measured the grading change from baseline with

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the topical application of LUT014. The Multinational Association of Supportive Care in Cancer (MASCC) Study Group EGFR Inhibitor–dermatologic adverse event grading scale is a central reviewer assessment based on the review of pictures provided to one remote reviewer (26). The Functional Assessment of Cancer Therapy (FACT) is a health-related quality-of-life questionnaire to assess symptoms associated with EGFR inhibitors (27). We report the score based on the 13 skin-specific questions (FACT-EGFRI-13), and which was calculated by the change in total score relative to baseline, with a decrease in value representing worsening and an increase in value representing improvement of skin-related symptoms.

The three patients in cohort 1 entered the study with grade 2 acneiform rash, and all improved to grade 1 during the 28-day treatment period, with two of the patients showing improvement within the first week. All three patients continued to have a sustained benefit by day 55, a month after administration had terminated (Fig. 2A and B). Using the central reviewer MASCC grading, two patients improved and one patient did not change in the central assessment during the treatment period (Fig. 2C). With the FACT-EGFRI-13 skin-related symptom questionnaire, the three patients had symptomatic improvement during the 28-day treatment period (Fig. 2D). Cohort 2 accrued four patients, two with grade 2 at baseline that improved to grade 1 during the 28-day treatment period (one improved at one week and the other at the second week), and two with grade 1 at baseline that did not change in grading during the treatment period.

### Table 1. Patient characteristics

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<th>2.5 mg/g LUT014 (N = 3)</th>
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EGFR inhibitor treatment of metastatic colorectal carcinoma—number of days prior to screening visit that treatment initiated

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### Table 2. Treatment-related adverse events

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<th>2.5 mg/g (N = 3)</th>
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<td>Grade 3 or 4</td>
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<td>Grade 3 or 4</td>
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<td>Local pain</td>
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<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dry skin</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>3</td>
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<td>1</td>
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Figure 2. Efficacy of topical LUT014 for EGFR inhibitor-induced acneiform rash. A, Pictures of baseline and on-therapy areas of rash in patient 104001 from cohort 1, after 1 week of treatment with LUT014. B–D, Evolution of rash following the CTCAE scale (B), MASCC (C), and FACT-13 (D).
Topical BRAF Inhibitor for Anti-EGFR Toxicities

At lower concentrations there is no activation of pERK, then there is a range of concentrations with increased pERK in a dose-dependent manner, and at the highest concentrations pERK is eventually inhibited (17, 18). We confirmed this concentration-dependent modulation of pERK, with inhibition of paradoxical cellular proliferation at the highest concentrations of LUT014 used in vitro. Results of the clinical trial also seem to suggest a similar phenomenon, as the clinical benefit was greater at the lowest concentration of LUT014 in cohorts 1 and 2, whereas there was no consistent evidence of improvement in the highest dose cohort. Of note, there were more cases with baseline grade 2 rash in the first two cohorts, which may result in a greater ability to detect a change in the rash score, as it is harder to demonstrate improvement when starting with less evident lesions. These clinical data are reminiscent of the bell-shaped curve of paradoxical MAPK activation with BRAF inhibitors, with increase in pERK at certain concentrations of the BRAF inhibitor and decreased pERK at higher concentrations (17, 18).

Acneiform rash is a frequent reason for dose reductions, drug holidays, or discontinuation of therapy with EGFR inhibitors, which may limit the clinical benefit of this mode of therapy to treat cancer (30). The acneiform lesions appear relatively early, with initial onset typically from one to three weeks after initiation of EGFR inhibitor therapy. The severity has been reported to generally increase during the days after onset, peaking at two to four weeks after initiation of therapy and decreasing thereafter, but it frequently remains a problem even months after initiation of therapy (2, 4, 6). The rash erythematous papules can frequently be painful and tender to the touch (2, 6). There is currently no approved treatment for this side effect, and patients receiving this mode of therapy have limited options other than EGFR inhibitor dose decreases or discontinuations. On the basis of our preclinical modeling and early clinical trial testing, we conclude that improving a topmost adverse event of EGFR inhibitor therapy with topical LUT014 could allow maintaining quality of life and dose intensity, thereby maximizing the antitumor effects while locally inhibiting dose-limiting skin toxicities.

METHODS

LUT014 Drug Characterization and BRAF Kinase Assay

LUT014 has the chemical name of 5-N(3-(9H-purin-6-yl)pyridin-2-yl)-6-methyl-1-N(3-(trifluoromethoxy)phenyl)isoquinoline-1,5-diamine, with a molecular weight of 528.49 daltons (Da) and a molecular formula of C27H19F3N2O. Three strengths of LUT014 formulated as an aqueous gel for topical administration were used in the clinical trial: 0.3 mg/g (0.03% weight/weight; w/w), 1.0 mg/g (0.10% w/w), and 2.5 mg/g (0.25% w/w). Different concentrations of LUT014 were tested to assess its ability to reverse serine/threonine-protein wild-type kinase BRAF and mutated BRAFV600E activity, and compare the respective half maximal inhibitory concentrations (IC50) against the positive control BRAF inhibitor vemurafenib (Reaction Biology Corporation). The assay used 75.88 μmol/L MEK1 (K07R) as substrate solution to which each kinase (4.5 μL of 5.56 μmol/L BRAF or 1.87 μL of 4.02 μmol/L BRAFV600E) was added to the diluted substrate in reagent reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive control reservoirs (Fisherbrand, Thermo Fisher Scientific). Five nanoliters of each test/reference item working solution or 10 nL of each positive
control working solution was added to 5 mL of the appropriate kinase/substrate reaction mixture utilizing acoustic sound waves (Echo 520, Labcyte), and were incubated for 20 minutes. 33P-ATP (specific activity 0.01 μCi/μL final) was added to the reaction mixtures to initiate the reactions and incubated for 120 minutes at room temperature with no motion. Reactions were spotted onto P81 ion exchange paper (Whatman #3698-915, Sigma-Aldrich) to terminate the reactions. Filters were washed 4× in 0.75% phosphoric acid. The radioactive phosphorylated substrate remaining on the filter paper after washing was measured using miniaturized radiotracers-based filter binding assay. Kinase activity data were expressed as the percent remaining kinase activity in test samples compared with vehicle (DMSO) reactions. IC50 values and curve fits were obtained using Prism4 Software (GraphPad). An in vitro kinase inhibition profiling study was designed to investigate the specificity and selectivity of LUT014 on a group of 59 human recombinant kinases selected based on their relationship with BRAF (Eurolin Scientific). Two concentrations of LUT014 (0.01 μmol/L and 1 μmol/L) were tested, with the protein kinases assayed in a radiometric format, whereas the selected lipid kinases were assayed using homogeneous time-resolved fluorescence, a combination of fluorescence resonance energy transfer and time-resolved fluorescence (Eurolin Scientific). On the day of the assay, the LUT014 10 mmol/L stock solution was thawed at room temperature and diluted serially in 100% DMSO to prepare a 50 μmol/L working stock (50× final assay concentration). The working stock was prepared in a 96-well deep well plate at room temperature and was used for assay within 8 days of preparation. 0.2 μL of the 10 μmol/L LUT014 working stock (for final dose in assay of 1 μmol/L) or 0.2 μL of the 0.1 μmol/L LUT014 diluted working stock (for final dose in assay of 0.01 μmol/L) was added to the assay well, before a reaction mix containing the enzyme and substrate was added. The reaction was initiated by the addition of ATP. There was no preincubation of the compound with the enzyme/substrate mix prior to ATP addition.

Preclinical In Vitro Testing of pERK Induction

Primary HEKa cells, isolated from adult skin of a single donor and cryopreserved at the end of primary culture (Gibco catalog number C0055C, Thermo Fisher Scientific), were cultured in T25 flasks at 37°C, 5% CO2 in growth medium (Epilife CF Kit, Gibco) supplemented with 60 μmol/L calcium (Gibco) and HKGS (Gibco). After at least three passages, cells were harvested with trypsin when flask reached 70%–80% confluence, and seeded in 10-cm culture dishes (250,000 cells/dish) in 9 mL of growth medium overnight. To analyze the effect of HKGS or a BRAF inhibitor on pERK, cells were starved for 2.5 hours in 5 mL of starvation medium without HKGS, and then individual plates were incubated for two hours with DMSO 0.1%, HKGS, vemurafenib 0.3 μmol/L (0.146 μg/mL), or LUT014 0.3 μmol/L (0.158 μg/mL). To analyze the effects of LUT014 to reactivate pERK in HKGS-activated HEKa cells cultured in an EGFR inhibitor, cells were seeded in 60-mm dishes (120,000 cells/dish) in 4 mL growth medium. After overnight incubation, cells were starved for 2.5 hours in 3 mL of starvation medium, and then individual plates were incubated for two hours with DMSO 0.1%, HKGS, HKGS plus erlotinib stock solution (10 μmol/L) plus DMSO (0.1%), HKGS plus erlotinib stock solution (10 μmol/L) plus LUT014 (0.3 μmol/L; 0.158 μg/mL), HKGS plus cetuximab stock solution (50 μg/mL) plus DMSO (0.1%), or HKGS plus cetuximab stock solution (50 μg/mL) plus LUT014 (0.3 μmol/L; 0.158 μg/mL).

Cells were harvested with 1 mL cold PBS containing EDTA-free, phosphate absurfacel cocktail (Millipore Sigma) using a cell scraper. The cell suspensions were then used for Western blot as previously described (31). We used the monoclonal anti-diphosphorylated ERK-1&2 (clone MAPK-YP Mouse Ascenty Fluide, Millipore Sigma), and the anti-ERK 2 antibody (C-14, rabbit polyclonal IgG, Santa Cruz Biotechnology). The membranes were developed with WesternBright Sirius chemiluminescent substrate (Advansa) and photographed with an Omega LumG imager (Aplegen, Gel Company). The signals of phosphorylated ERK1/2 and total ERK2 were quantified using ImageJ software and the phosphorylated ERK1/2/total ERK2 ratio was calculated. For the quantification, equal-sized rectangles were drawn around the bands of the phosphorylated ERK1/2 and the total ERK2, and the pixels in each rectangle were measured.

Preclinical In Vitro Testing of Cellular Proliferation Induction

MIA-PaCa-2 cells (ATCC catalog number CRM-CRL-1420) were cultured at 37°C in 775 flask in growth medium DMEM (ATCC) supplemented with 10% FBS and 2.5% horse serum. After 4 days, when the flask reached 50% confluence, cells were harvested with trypsin and seeded in 96-well plates at a concentration of 5,000 cells/well, in a total volume of 0.1 mL/well starvation medium, and incubated 24 hours at 37°C, 5% CO2. The next morning, the medium was replaced with 180 μL growth medium (containing 10% FBS and 2.5% horse serum) and 20 μL of each concentration of LUT014, DMSO, or PBS. Each concentration of each compound was added to triplicate wells. The plates were incubated for 72 hours at 37°C, 5% CO2. Cellular proliferation was measured using the ATP-lite proliferation assay (PerkinElmer) following the manufacturer’s instructions, with the luminescence measured using a CLARIOStar reader (BMG Labtech), and analyzed with the ATP-lite TOP program (PerkinElmer).

Clinical Study Design

This was an open-label, dose-escalation study of LUT014 gel topically administered for four weeks. A 2-week screening period (day 1 to day 14) was followed by a 4-week treatment period (day 0 to day 1). Patients applied the study drug once daily to their face, neck, chest, and upper back. The posttreatment safety follow-up period ran from day 28 to day 55. In addition, two long-term safety follow-up visits were done 3 and 6 months after completion of treatment, including a dermatological examination to detect any potential BRAF inhibitor skin toxicities within the treatment area.

Three dose levels of LUT014 gel were tested: 0.3, 1.0, and 2.5 mg/g. The dose of LUT014 was escalated in sequential dose cohorts of three to six patients, in a conventional 3 + 3 escalating dose design. Escalation to the next dose level was permitted if no DLT was reported by the three patients in the cohort within 4 weeks of receiving the first dose of LUT014. If one out of three patients developed a DLT, an additional three patients were planned to be treated with study drug at the same dose level. At any given dose level, if more than one out of the three or six patients experienced a DLT within 4 weeks of receiving the first dose of LUT014, the dose level was determined to have exceeded the MTD, and the MTD was set to be the next lower dose.

A DLT was defined as any treatment-emergent grade 2 or higher clinically significant adverse event, with the exception of a transient grade 2 adverse event (i.e., one that returns to grade 1 or baseline within 7 days) or grade 2 adverse event that was manageable with standard of care (i.e., one that returns to grade 1 or baseline within 14 days) that occurred within 4 weeks of the first dose of study drug for which there was a reasonable possibility that LUT014 had caused the event. Upon completion of the 4-week treatment period of the third patient in each dosing cohort, a Safety Review Committee meeting was set to review the safety data and to provide recommendation/decision on dose escalation/deescalation, or expansion of dosing cohort.

Study Patients

Eligible patients were 18 years or older, with a diagnosis of a metastatic cutaneous carcinoma treated with cetuximab (Erbitux) or panitumumab (Vectibix) and who had developed a grade 1 or 2 acneiform skin toxicity, based on CTCAE version 5.0–skin and subcutaneous tissue disorders grading scale. The anti-EGFR treatment must have...
Topical BRAF Inhibitor for Anti-EGFR Toxicities

been initiated within 12 weeks prior to the screening visit and continued through the day 55 visit at the same dose, except if required per the dose-modification section of the approved product labeling. Unless required to treat an adverse event, patients were not allowed to receive any systemic antibiotic treatment within 7 days prior to screening until 1 month after the completion of the treatment with the study drug (day 55). In addition, topical corticosteroids to the targeted treatment areas, as well as systemic corticosteroids therapy, except for low-dose systemic corticosteroids given as part of standard of care for the prevention or treatment of chemotherapy-induced nausea and vomiting, were prohibited 14 days prior to study entry through day 55 in the study. Eleven patients were screened, and one did not proceed due to not meeting the eligibility criteria. Two patients in cohort 2 had started anti-EGFR therapy greater than 12 weeks prior to the screening visit, and one patient in cohort 3 who was treated with systemic antibiotic within 7 days prior to screening but 9 days prior to baseline; these three patients were given waivers by the study sponsor and the local institutional review boards (IRB) to participate in the trial, and thus all 10 patients were enrolled.

Study Objectives

The primary objective of the study was to evaluate the safety and tolerability of LUT014 topicalically applied daily for 4 weeks in patients with metastatic colorectal carcinoma with anti-EGFR therapy-induced acneiform lesions. The secondary objectives were to assess the PK of the LUT014 in plasma after a single administration and after 8 days of daily administrations and to evaluate the preliminary efficacy of 4-week treatment of the LUT014 on anti-EGFR therapy-induced acneiform lesions. Patients underwent oncologic treatment efficacy assessments of their colorectal cancer as per the institutional standard of care.

Safety and Tolerability Assessments

Safety and tolerability were assessed primarily based on the adverse events from day 0 to day 55. In addition to the adverse events assessment, the safety measurements included physical examination, vital signs and body weight, 12-lead ECG, ECOG performance status, safety labs including complete blood count, comprehensive metabolic panel, and urinalysis. At 3 and 6 months from study start, a dermatologist performed a skin assessment of the areas of the skin where LUT014 gel was applied to screen for potential cutaneous toxicities known to be associated with approved BRAF inhibitors, including erythema nodosum-type rash, photosensitivity, squamous papillomas or warts, keratoacanthomas, cutaneous squamous cell carcinoma, dry skin, and folliculitis or cysts (32).

PK Analyses

Blood samples for PK analysis were taken from the initial three patients enrolled to each dosing cohort (total nine patients). PK samples were collected predose on day 0 and day 7 and at 1, 2, 4, 8, and 24 hours postdose. A qualified LC/MS-MS analytical assay with an LLOQ of 10 pg/mL was used to determine concentrations of LUT014 in plasma. Plasma concentrations below the limit of quantitation (<10 pg/mL) were treated as zero for the calculations of mean (SD).

Efficacy Assessment

Evaluation of the preliminary efficacy of LUT014 gel for the treatment of anti-EGFR therapy-induced acneiform lesions was based on the following: (i) Acneiform lesions grading performed locally by the Investigator using the NCI CTCAE version 5.0 skin and subcutaneous tissue disorders grading scale. (ii) Acneiform lesions grading performed by a central reader, using the MASCC Study Group EGFR Inhibitor–dermatologic adverse event grading scale (26). Grading was based on masked photographs of patients’ face, neck, and upper portion of the anterior and posterior chest. (iii) Patient self-reported quality-of-life FACT-EGFRI-13 questionnaire (27), assessing the physical and psychologic effects of the dermatologic symptoms associated with anti-EGFR treatment on study patients. Only the first 13 questions of the FACT-EGFRI that are relevant for the skin were evaluated. Data from all patients who received at least one dose of study drug were included in the safety and efficacy analyses.

Standard Protocol Approvals, Registration, and Patient Consents

Written informed consent was obtained from all study patients prior to any study-related procedure. The clinical trial was approved by the IRBs of each clinical trial site. The study was conducted in accordance with the applicable regulatory and International Council for Harmonisation–Good Clinical Practice requirements, the ethical principles that have their origin in the principles of the Declaration of Helsinki, and the local laws and regulations of the study sites regarding the conduct of clinical trials and the protection of human patients.

Statistical Analyses

Tabular summaries of the secondary safety assessments included descriptive statistics (i.e., mean, SD, median, and range for continuous data, and frequency for categorical data) for each visit assessed, as well as for the calculated changes from baseline. For the PK analyses, plasma LUT014 concentration–time profiles were analyzed by noncompartmental methods, and PK parameters were calculated by standard equations, when plasma levels are achieved. PK analyses produced using Phoenix WinNonLin (Certara). The efficacy assessments NCI CTCAS skin and subcutaneous tissue disorders grading scale, MASCC Study Group EGFR Inhibitor–dermatologic adverse event grading scale, FACT-EGFRI-13 questionnaire, were summarized by treatment group using descriptive statistics as appropriate (i.e., mean, SD, median, range for continuous data, and frequency for categorical data). All analyses were performed using SAS Software (version 9.2, SAS Institute).

Authors’ Disclosures

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Authors’ Contributions

M.E. Lacouture: Conceptualization, resources, data curation, formal analysis, supervision, investigation, writing–review and editing. Z.A. Wainberg: Data curation, investigation, writing–review and editing. A.B. Patel: Data curation, investigation, writing–review and editing. M.J. Anadkat: Data curation, investigation, writing–review and editing. S.M. Stemmer: Data curation, investigation, writing–review and editing. E. Shacham-Shmueli: Resources, data curation, investigation, writing–review and editing. E. Medina: Data curation, formal analysis, visualization, writing–review and editing. A. Ribas: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing.

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REFERENCES


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