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Cancer Therapy: Clinical

Clinical Cancer Research

Phase I Dose-Escalation Study of Linsitinib (OSI-906) and Erlotinib in Patients with Advanced Solid Tumors

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Abstract

Purpose: Cross-talk between type I IGF receptor (IGF1R), insulin receptor (INSR), and epidermal growth factor receptor (EGFR) mediates resistance to individual receptor blockade. This study aimed to determine the MTD, safety, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of linsitinib, a potent oral IGF1R/INSR inhibitor, with EGFR inhibitor erlotinib.

Experimental Design: This open-label, dose-escalation study investigated linsitinib schedules S1: once daily intermittent (days 1–3 weekly); S2, once daily continuous; S3, twice-daily continuous; each with erlotinib 100–150 mg once daily; and a non–small cell lung cancer (NSCLC) expansion cohort.

Results: Ninety-five patients were enrolled (S1, 44; S2, 24; S3, 12; expansion cohort, 15) and 91 treated. Seven experienced dose-limiting toxicities: QTc prolongation (3), abnormal liver function (2), hyperglycemia (1), and anorexia (1). Common adverse events included drug eruption (84%), diarrhea (73%), fatigue (68%), nausea (58%), vomiting (40%). MTDs for linsitinib/erlotinib were 450/150 mg (S1), 400/100 mg (S2). On the basis of prior monotherapy data, S3 dosing at 150 mg twice daily/150 mg once daily was the recommended phase II dose for the expansion cohort. There was no evidence of drug–drug interaction. Pharmacodynamic data showed IGF-1 elevation and reduced IGF1R/INSR phosphorylation, suggesting pathway inhibition. Across schedules, 5/75 (7%) evaluable patients experienced partial responses: spinal chordoma (268+ weeks), rectal cancer (36 weeks), three NSCLCs including 2 adenocarcinomas (16, 72 weeks), 1 squamous wild-type EGFR NSCLC (36 weeks). Disease control (CR+PR+SD) occurred in 38 of 75 (51%), and 28 of 91 (31%) patients were on study >12 weeks.

Conclusions: The linsitinib/erlotinib combination was tolerable with preliminary evidence of activity, including durable responses in cases unlikely to respond to erlotinib monotherapy. *Clin Cancer Res; 22(12); 2897–907. ©2016 AACR.*

Background

Erlotinib is a potent first-generation inhibitor of EGFR, and is an established first-line therapy for patients with NSCLC positive for exon 19 deletions or exon 21 mutations (1). Erlotinib blocks EGFR kinase activity, suppressing downstream signaling via multiple intermediates including the MAPK and PI3K–AKT pathways (2). These signaling pathways are activated by additional receptors including IGF1R (3). IGF1R is expressed almost ubiquitously by normal tissues, is activated by ligands IGF-1 and -2, and is required for embryonic development and postnatal growth (4, 5). IGF1R has become a target for cancer therapy, because components of the IGF axis are often aberrantly expressed in cancers, and IGF pathway activation promotes tumorigenesis and metastasis (4, 6). Furthermore, IGF1R overexpression is associated with adverse survival in several tumor types (7–10). Cancers also express a variant form of the insulin receptor (INSR-A) that is activated by IGF-2 and insulin to drive proliferation and cell survival (11). INSR-A signaling can compensate for IGF1R inhibition (12), and coinhibition of IGF1R and INSR may provide enhanced antitumor activity (13, 14).

Linsitinib (OSI-906) is a potent, orally bioavailable dual IGF1R and INSR tyrosine kinase inhibitor (TKI) with antiproliferative effects in a variety of tumor cell lines, and antitumor activity in an IGF1R-driven xenograft model (15, 16). Preliminary antitumor activity has been reported for single-agent linsitinib in patients with solid tumors including partial responses (PR) in melanoma and adrenocortical carcinoma (17–19). Combined IGF1R/INSR and EGFR blockade may enhance inhibition of common downstream signaling pathways, and suppress resistance to single receptor blockade (6, 14, 20). Preclinical data indicate that IGF1R mediates acquired resistance to erlotinib in lung cancers with wild-type EGFR, and combined inhibition of IGF1R/INSR and EGFR results in supra-additive inhibition of tumor growth *in vitro*

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Translational Relevance

This is the first published study to test the efficacy of combined insulin-like growth factor receptor (IGF1R)/EGFR inhibition using two small-molecule inhibitors, linsitinib plus erlotinib. The combination has acceptable tolerability, with no pharmacokinetic evidence of significant drug–drug interaction, and detectable but modest antitumor activity in unselected patients. Measurement of circulating IGF-1 and IGF1R/insulin receptor (INSR) phosphorylation suggests that receptor blockade was induced, but may have been intermittent. Mutation analysis identified *EGFR* mutations in 3 non–small cell lung cancer patients, correlating with response (exon 19 del, 1 case) or resistance (exon 19 del, T790M, 2 cases) to trial therapy. There was also evidence of clinical activity in tumors lacking detectable *EGFR* mutation, unlikely to respond to single-agent erlotinib. These results highlight two issues for successful application of this approach: the importance of assessing IGF axis activity in clinical material and the need for predictive biomarkers.

and *in vivo* in NSCLC, breast, pancreatic, and colorectal cancer (CRC; refs. 21–25). Furthermore, in a recent CRC xenograft study, erlotinib-resistant tumors had marked IGF-2 overexpression, and were sensitized to EGFR inhibition by a small-molecule IGF1R TKI (26).

In the current study, linsitinib was combined with erlotinib in patients with advanced solid tumors. The primary objectives were to determine the maximum tolerated dose (MTD) and define the recommended phase II dose (RP2D) of linsitinib plus erlotinib. Secondary objectives were to evaluate safety, preliminary antitumor activity, pharmacokinetic, and pharmacodynamic profiles.

Patients and Methods

Patient population

Male and female patients ≥18 years were eligible if they had a histologically or cytologically confirmed advanced solid tumor and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2. Patients were required to be nonsmokers for ≥3 months prior to study entry, have a negative urine cotinine test, and have adequate cardiac, hematopoietic, hepatic, and renal function, including corrected QT interval (QTc) ≤450 ms with no concurrent use of drugs that may prolong QTc, fasting glucose ≤125 mg/dL (7.0 mmol/L), and blood ketones equal to or below the upper limit of normal. Patients were excluded for a history of diabetes mellitus or significant heart disease, prior EGFR or IGF1R inhibitor therapy, or use ≤14 days of strong or moderate CYP3A4 or CYP1A2 inhibitors/inducers, proton pump inhibitors, or drugs with an established risk of causing QTc prolongation. Prior anticancer therapy was permissible if chemotherapy was discontinued 3 weeks prior to the study (4 weeks for carboplatin or investigational agents, 6 weeks for nitrosoureas and mitomycin C), hormonal therapy was discontinued prior to trial therapy and patients had recovered from any acute radiation toxicity and recent surgery. For inclusion in the advanced NSCLC expansion cohort, patients required measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 (27), and archival tumor tissue available for analysis. Study approval was obtained from the Independent Ethics Committee or Institutional Review Board at each site. This study was conducted according to the principles of the Declaration of Helsinki, Good Clinical Practice, International Conference on Harmonization guidelines and applicable laws and regulations. Patients signed written informed consent prior to initiation of study-specific procedures.

Study design

This was a multicenter, phase I, open-label study of linsitinib plus erlotinib in patients with advanced solid tumors (NCT00739453). The primary objectives were to determine the MTD and establish the RP2D of linsitinib plus erlotinib. Secondary study objectives included safety, pharmacokinetic and pharmacodynamic profiles, antitumor activity, and potential correlation between exploratory biomarkers and clinical outcomes in an expansion cohort of patients with advanced NSCLC.

A standard "3 + 3" dose-escalation design was used in 3 oral linsitinib dose schedules of 21-day treatment cycles. In the S1 intermittent schedule, linsitinib was administered on days 1–3 every 7 days, starting at 50 mg once daily, escalating to 600 mg once daily. Schedule 2 (S2) continuous dosing of linsitinib started at 50 mg once daily, escalating to 400 mg, and in schedule 3 (S3) twice daily continuous schedule, linsitinib was administered at 100 or 150 mg twice daily. Oral erlotinib was administered at 100 or 150 mg QD on days 2–21 of the first cycle and days 1–21 for the remaining cycles. Initiation of schedules S1–S3 occurred consecutively with the next schedule starting following clinically significant related grade ≥2 toxicity in the previous schedule, or achievement of ≥2 dose levels in the previous schedule without dose-limiting toxicity (DLT). Following completion of S1–S3, a NSCLC expansion cohort was initiated, administering linsitinib 150 mg twice daily, the established monotherapy RP2D (18), starting at day 1, and erlotinib at 150 mg QD starting at day 8 of cycle 1.

Toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (AEs) v3.0 and DLTs were defined as clinically significant toxicity considered to be related to study drug and occurring during the initial cycle. DLTs included standard hematologic (grade 4 neutropenia or thrombocytopenia, grade ≥3 febrile neutropenia, documented grade ≥3 infection with grade ≥3 neutropenia) and nonhematologic criteria (any grade ≥3 toxicity excluding fatigue, γ-glutamyl transferase elevation, nausea, or rash). Additional DLTs included glucose intolerance, defined as grade 3 symptoms of hyperglycemia accompanied by grade 2 hyperglycemia (fasting glucose >160 mg/dL or 8.9 mmol/L), grade 3 fasting glucose >250 mg/dL (13.9 mmol/L), grade 3 electrolyte abnormalities due to glucose intolerance, positive ketones (above the upper limit of normal), or grade 4 hyperglycemia (glucose >500 mg/dL or 27.8 mmol/L). Also included as DLTs were drug-related toxicity of any severity causing inability to begin a second cycle by day 36 in any schedule, or to complete the first cycle in S1, or requiring interruption of dosing ≥5 continuous days in S2 and S3. DLTs were assessed during the initial 21-day cycle with final assessment on day 22, along with the need for subsequent dose interruptions, delays, or occurrence of cumulative toxicity. DLTs were assessed in ≥3 patients per cohort and patients had to complete a full cycle before the next dose level could be opened. Following a DLT, 3 additional patients were treated at that dose level, up to 6 per

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cohort. The DLT population comprised patients in S1 who did not miss >3 days of linsitinib and >5 days of erlotinib during the initial cycle, and patients in S2 and S3 who did not miss >5 days dosing of either drug during the initial cycle. The MTD was defined as the highest dose at which no more than 1 of 6 patients experienced a DLT (i.e., the dose level below that which induced a DLT in $\geq$33% of patients). The NSCLC expansion cohort was enrolled using dosing based on the S3 schedule, and clinical safety and pharmacokinetic data from the twice daily monotherapy study (18).

Safety and efficacy

AEs were recorded from the signing of informed consent to 30 days after the final dose of study drug. Laboratory data included hematology (full blood count including hemoglobin, hematocrit, platelets, and reticulocyte counts) and biochemistry data: blood glucose, insulin, lactate, blood ketones, blood urea nitrogen, creatinine, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, total protein, electrolytes, γ-glutamyl transferase, lactate dehydrogenase, lipase, total cholesterol, and triglycerides. During the initial cycle, blood glucose was monitored twice daily by patients using a home glucometer. Safety was also monitored by physical examination and electrocardiogram predose on day 1, postdose on days 1, 2, 8, and 15 of the first cycle, pre- and postdose on day 22, predose on day 1 of every subsequent cycle, and at last study visit. Measurable disease was required only for enrollment of the expansion cohort, but disease status, evaluated by RECIST, was assessed in all patients every 6 to 8 weeks by physical and radiologic examination. Patients were evaluable for efficacy if they had measurable disease according to RECIST, received at least 21 days of therapy, and underwent disease re-evaluation.

Pharmacokinetics

For patients on schedules S1–S3, plasma samples were collected on day 1 predosing of linsitinib, at intervals between 1 and 24 hours postdose, and at the same timepoints on day 2 pre- and postdosing of linsitinib with the first dose of erlotinib. Further samples were collected on days 3 and 15 immediately prior to linsitinib and erlotinib dosing, and on day 22 (day 1 of cycle 2) predose and at intervals 1–24 hours postdose. Patients in the NSCLC expansion cohort were sampled on day 7 pre-linsitinib dosing and 1 to 10 or 12 hours postdose (the latter immediately prior to the second linsitinib dose of the day), on day 8 predosing with linsitinib, and 1, 2 (immediately before the first erlotinib dose), 3, 4, 6, 8, 10, or 12 hours postdose, also on days 9 and 15 predose, and days 22–23 (i.e., cycle 2 days 1–2) as days 8–9. Plasma concentration versus time profiles of linsitinib, erlotinib, and OSI-420 (metabolite of erlotinib) were obtained from the analysis of plasma samples, using a validated liquid chromatography-tandem mass spectrometry method. The WinNonlin program (Pharsight Corporation) with standard noncompartmental methods was used to determine the following pharmacokinetic variables for linsitinib, erlotinib, and OSI-420: maximum observed plasma concentration (C_{max}), time to maximum observed plasma concentration (T_{max}), trough plasma concentration (C_{trough}), and area under the plasma concentration–time curve (AUC) over a dosing interval. Oral clearance (CL/F) of linsitinib was calculated by dividing the dose by AUC. Descriptive statistics were used to summarize the pharmacokinetic parameters for each dose cohort.

Pharmacodynamics

Exploratory analyses of potential pharmacodynamic and molecular markers for response to linsitinib and erlotinib were performed where possible on plasma and tumor samples. Blood samples were collected predose on days 1, 2, 3, 15, 22, and 23, and at 2 and 4 hours postdose on days 1, 2, and 22. Samples from patients on the NSCLC expansion cohort were collected prior to linsitinib dosing on days 1, 8, 9, 15, and 22 (only on odd-numbered cycles) to assess levels of IGF-1 using the Total IGF-1 ELISA Kit (DSL/Beckman Coulter) or human insulin-like growth factor-1 E20 Total IGF-1 ELISA (Mediagnost GmbH). Peripheral blood mononuclear cells (PBMC) were isolated from blood samples obtained predose on days 1, 2, 3, 15, 22, and 23 and postdose 2 and 4 hours on days 1, 2, and 22. NSCLC expansion cohort samples were collected prior to linsitinib dosing on days 1, 8, 9, 15, and 22. PBMCs were lysed and used to assess phosphorylation of IGF1R and INSR using the Proteome Profiler Human Phospho-RTK Array Kit (R&D Systems) according to the manufacturer's protocol. Mutation status of EGFR, K-RAS, PIK3CA, and BRAF was determined on available archival tumor samples (10 patients) and plasma (30 patients) from a total of 34 patients in the dose-escalation or NSCLC expansion phases using standard PCR, ICE-COLD-PCR, and SURVEYOR and WAVE HS System analysis followed by Sanger dideoxy sequencing (Transgenomic) as described previously (18).

Results

Ninety-five patients were enrolled at four sites in the United States and United Kingdom, including 44 in S1, 24 in S2, 12 in S3, and 15 in the NSCLC expansion cohort. Patient demographics and baseline characteristics are shown in Table 1. Patients were ages 20–85 years and 98% had an ECOG PS of 0 or 1. The largest proportion of patients presented with NSCLC (34%) followed by prostate cancer (5%), pancreatic cancer (4%), and CRC (4%). Approximately 50% of patients had received at least 3 previous lines of treatment (Table 1). Of the 95 patients, 91 were treated and included in the safety and pharmacokinetic populations, 67 in the DLT population, and 75 in the efficacy population. Ninety patients discontinued the study, 69 (77%) due to disease progression, 13 (14%) due to an AE, and 8 (9%) by patient request.

Dose escalation and MTD

In the dose-escalation phase, 7 patients experienced 10 DLTs (4 in S1, 2 in S2, and 1 in S3), including QTc prolongation, hyperglycemia, elevated liver function tests, and anorexia (Table 2A). All DLTs were considered related to linsitinib alone or the linsitinib/erlotinib combination. MTDs were established for the intermittent S1 schedule as 450 mg linsitinib once daily days 1–3 every 7 days and 150 mg erlotinib once daily, and for the continuous S2 schedule as 400 mg linsitinib once daily and 100 mg erlotinib once daily. The S3 schedule did not achieve $\geq$33% of patients with DLTs; dose escalation was not pursued further because the RP2D for linsitinib as monotherapy was established at 150 mg linsitinib twice daily in a separate clinical study (18), and erlotinib was already at the approved dose. This dose combination was chosen as the RP2D for the expansion cohort due to the acceptable safety profile in the S3 schedule (no AEs of grade >3), and because pharmacokinetic and pharmacodynamic data from the monotherapy study indicated that continuous dosing of 150 mg linsitinib twice daily reached adequate exposure predicted for IGF1R inhibition (18).

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Table 1. Baseline demographic and patient characteristics

	S1 ($n = 44$)	S2 ($n = 24$)	S3 ($n = 12$)	NSCLC expansion ($n = 15$)	Total ($n = 95$)
Age, median (range)	63 (34–83)	60 (20–72)	49 (28–85)	62 (45–71)	62 (20–85)
Age group, *n* (%)					
18–39	4 (9)	2 (8)	2 (17)	0 (0)	8 (8)
40–64	23 (52)	13 (54)	7 (58)	10 (67)	53 (56)
≥65	17 (39)	9 (38)	3 (25)	5 (33)	34 (36)
Sex, *n* (%)					
Female	21 (48)	12 (50)	6 (50)	9 (60)	48 (51)
Male	23 (52)	12 (50)	6 (50)	6 (40)	47 (49)
Race, *n* (%)					
White	38 (86)	20 (83)	12 (100)	14 (93)	84 (88)
Hispanic	1 (2)	0 (0)	0 (0)	0 (0)	1 (1)
Black	5 (11)	4 (17)	0 (0)	1 (7)	10 (11)
ECOG PS, *n* (%)					
0	13 (30)	12 (50)	8 (67)	3 (20)	36 (38)
1	30 (68)	12 (50)	3 (25)	12 (80)	57 (60)
2	1 (2)	0	1 (8)	0	2 (2)
Cotinine test, *n* (%)					
Negative	43 (98)	24 (100)	11 (92)	14 (93)	92 (97)
Positive	0	0	0	1 (7)	1 (1)
Not done	1 (2)	0	1 (8)	0	2 (2)
Tumor type, *n* (%)					
NSCLC	8 (18)	6 (25)	3 (25)	15 (100)	32 (34)
Pancreatic	4 (9)	0	0	0	4 (4)
Colorectal	4 (9)	0	0	0	4 (4)
Prostate	3 (7)	2 (8)	0	0	5 (5)
Other	25 (57)	16 (67)	9 (75)	0	50 (53)
Prior chemotherapy regimens, *n* (%)					
0–2	21 (48)	11 (46)	6 (50)	8 (53)	46 (48)
3–5	18 (41)	11 (46)	6 (50)	7 (47)	42 (44)
6–8	5 (11)	2 (8)	0	0	7 (7)
Prior radiotherapy, *n* (%)	29 (66)	13 (54)	7 (58)	12 (80)	61 (64)
Prior disease-related surgery, *n* (%)	33 (75)	16 (67)	11 (92)	6 (40)	66 (69)
Prior hormonal therapy and immunotherapy, *n* (%)	10 (23)	3 (12)	0	0	13 (14)

NOTE: Patient characteristics are shown for each cohort: S1, intermittent linsitinib once daily (days 1–3 every 7 days); S2, continuous linsitinib once daily; S3, continuous linsitinib twice daily, each with erlotinib 100 or 150 mg once daily, and NSCLC expansion cohort treated at 150 mg twice daily linsitinib, 150 mg once daily erlotinib.

Safety

Of the 91 treated patients, 89 of 91 (98%) experienced treatment-related AEs, of maximum severity grade 1 in 15 patients (16%), grade 2 in 47 (52%), grade 3 in 24 (26%), and grade 4 in 3 (3%). The most common treatment-emergent AEs across all cohorts were drug eruption (84%), diarrhea (73%), fatigue (68%), nausea (58%), and vomiting (40%), with frequencies similar across the dosing schedules except that vomiting occurred more often in S3 (67% compared with 32%–40% in other cohorts; Table 2B). Fatigue was more common in S1 (72%) and S2 (75%) compared with S3 (58%) and the expansion cohort (53%), and was the only treatment-emergent AE to occur at grade ≥3 in ≥5% of patients, observed only in S1 and S2.

AE causality was determined by investigator judgment based on known AEs of erlotinib and linsitinib. AEs related specifically to linsitinib occurred in 43% of patients, including 48% in S1, 33% in S2, 25% in S3, and 60% in the expansion cohort. The most common were fatigue (12%), nausea (11%), vomiting (8%), and prolonged QTc interval (8%); all the linsitinib-related AEs in S1 and S2 occurred in patients receiving ≥300 mg linsitinib. Erlotinib-related AEs were reported in 84% of patients and the most common were drug eruption (74%), dry skin (26%), pruritus (26%), and diarrhea (26%). AEs attributed to both linsitinib and erlotinib were reported in 82% of patients; the most common were fatigue (45%), diarrhea (43%), nausea (37%), and anorexia (20%).

Serious AEs (SAEs) were reported in 42% including 52% of patients in S1, 21% in S2, 42% in S3, and 47% in the expansion cohort, with nausea (7%) and vomiting (7%) the most frequent drug-related SAEs. Treatment-related SAEs occurred in 13 patients (14%) including 10 of 40 (25%) patients in S1 and 3 of 15 (20%) in the expansion cohort. These included SAEs related to both linsitinib and erlotinib: fatigue (1 patient), malaise (1), nausea (1), vomiting (3), diarrhea (2), anemia (1), increased ALT (2), increased AST (1), acute renal failure (1), QTc prolongation (1), and gastrointestinal perforation (1). SAEs attributed to erlotinib alone were rash (1), pneumonitis (1), gastrointestinal perforation (1), gastrointestinal hemorrhage (1). One SAE was considered to be related to linsitinib alone: grade 3 hyperglycemia occurred in 2 patients in S1 and 1 patient in the expansion cohort. AEs led to study discontinuation for 13 patients, including 6 (15%) in S1, 2 (8%) in S2, 3 (25%) in S3, and 2 (13%) in the expansion cohort. Of those, 3 patients had an event related to erlotinib only, 1 to linsitinib only (prolonged QTc interval), and 5 to the combination. Nine patients died within 30 days of the last linsitinib dose, due to underlying disease (7 patients), pneumonia (1), or dehydration following a suicide attempt (1).

AEs of special interest to linsitinib include hyperglycemia, hypoglycemia, and prolonged QTc interval. Linsitinib-related hyperglycemia occurred in 5 patients (5%) including 3 in S1 and 2 in the expansion cohort, at grade 3 (3 cases) and grade 2 (2). All instances of hyperglycemia responded to temporary (3–5 days)

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Table 2. Toxicities of linsitinib/erlotinib combination

A. DLTs

Linsitinib/erlotinib dose (mg)	Grade of AE[a]	Relation to treatment
S1		
600/100 QD	G3 hyperglycemia[b]	Linsitinib
600/100 QD	G3 increased AST	Linsitinib and erlotinib
	G3 increased ALP	Linsitinib and erlotinib
	G2 increased ALT	Linsitinib and erlotinib
450/100 QD	G4 increased ALT[c]	Linsitinib and erlotinib
	G4 increased AST[c]	Linsitinib and erlotinib
450/150 QD	G3 prolonged QTc interval	Linsitinib
S2		
400/150 QD	G2 prolonged QTc interval[d]	Linsitinib
400/150 QD	G3 prolonged QTc interval	Linsitinib
S3		
150 BID /150 QD	G3 anorexia	Linsitinib and erlotinib

B. Treatment-emergent AEs occurring in at least 5% of patients

AE	S1 ($n = 40$)	S2 ($n = 24$)	S3 ($n = 12$)	NSCLC expansion ($n = 15$)	Total ($n = 91$)
Fatigue	29 (72)	18 (75)	7 (58)	8 (53)	62 (68)
Malaise	2 (5)	2 (8)	1 (8)	1 (7)	6 (7)
Nausea	25 (62)	11 (46)	9 (75)	8 (53)	53 (58)
Vomiting	13 (32)	9 (38)	8 (67)	6 (40)	36 (40)
Diarrhea	28 (70)	16 (67)	9 (75)	13 (87)	66 (73)
Rash	32 (80)	20 (83)	11 (92)	13 (87)	76 (84)
Pruritis	11 (28)	8 (33)	6 (50)	5 (33)	30 (33)
Anorexia	12 (30)	11 (46)	5 (42)	4 (27)	32 (35)
Dehydration	9 (22)	2 (8)	4 (33)	1 (7)	16 (18)
Hyperglycemia	3 (8)	0	0	2 (13)	5 (5)
QTc prolongation	5 (12)	3 (12)	0	1 (7)	9 (10)
ALT elevation	3 (8)	1 (4)	2 (17)	0	6 (7)
AST elevation	3 (8)	1 (4)	2 (17)	0	6 (7)

NOTE: Table shows number (%) of patients experiencing each AE.

Abbreviations: ALP, alkaline phosphatase; BID, twice daily; G, grade; QD, once daily.

[a]Unless otherwise noted, DLT defined by ≥grade 3 toxicity.

[b]Glucose intolerance: Fasting glucose >250 mg/dL (13.9 mmol/L).

[c]Inability to complete designated schedule in first treatment period due to drug-related toxicity.

[d]Interruption of dosing for ≥5 continuous days within first 21 days due to drug-related toxicity.

interruption of linsitinib, followed by reduction of linsitinib to the next lowest dose. Glucometer readings indicated grade 3–4 hypoglycemia in 8 patients, including 2 patients treated on schedule S3 at the RP2D. Only 1 of these low readings, in an S1 patient, was accompanied by simultaneous serum glucose measurement showing low glucose (grade 2). Prolonged QTc occurred in 9 patients (10%; see Table 2B): grade 1 in 2 patients (S1: 450/100 mg and S2: 300/150 mg), grade 2 in 5 patients (S1: 400/150 mg, 1 case, and 450/150 mg, 2 cases; S2: 1 case at 400/150 mg; NSCLC expansion cohort: 1 case at 150/150 mg), and grade 3 in 2 patients (S1: 450/150 mg and S2: 400/150 mg); 7 of these were attributed to linsitinib. It was unclear whether there was a relationship between occurrence of QTc prolongation and peak circulating drug concentrations; however, in another study QTc prolongation was associated with peak concentrations of linsitinib at the 600 mg dose (unpublished data).

Pharmacokinetic analysis

Figure 1A shows median plasma linsitinib levels in patients treated at S1, S2, or S3 MTDs. Levels peaked at 4 hours, declined over 24 hours in the S1 and S2 cohorts to near or below the 400 ng/mL (~1μmol/L) level associated with preclinical activity (15, 16), and were maintained above this threshold in the twice daily S3 cohort. Profiles were similar following day 1 (linsitinib alone) and day 2 (combination) dosing. Linsitinib C_{max} and AUC from time zero to infinity increased in a dose-proportional manner in a comparison of dose-normalized parameters in S1 and S2. In the NSCLC expansion cohort, where linsitinib was dosed from day 1 and erlotinib from day 8, linsitinib pharmacokinetic parameters were similar after dosing 150 mg linsitinib twice daily as a single agent (day 7) and in combination with 150 mg erlotinib once daily (days 8 and 22; Table 3, Fig. 1B). These data suggest that there was no significant drug–drug interaction effect on linsitinib pharmacokinetic parameters. Furthermore, analysis of erlotinib and OSI-420 pharmacokinetics after dosing the linsitinib/erlotinib combination (Table 3, Fig. 1) showed parameters similar to previously published data after erlotinib monotherapy (28, 29), suggesting that linsitinib did not alter the pharmacokinetics of erlotinib.

Efficacy

Of 75 patients evaluable for efficacy, none achieved complete response, 5 (7%) achieved PR, and 33 (44%) had stable disease (SD) for a minimum of 6 weeks (Table 4A). The 5 patients achieving PR included 2 on the intermittent S1 schedule (NSCLC, rectal cancer), 1 on S2 (spinal chordoma), and 2 with NSCLC treated at the RP2D in the expansion cohort. Disease control rate (CR + PR + SD) occurred in 10 patients in S1 (32%), 13 patients in S2 (68%), 6 patients in S3 (55%), and 9 patients in the expansion cohort (64%). Duration on study by treatment schedule is shown in Fig. 2A. The median (mean ± SE) duration of treatment was 6 (12 ± 3) weeks in S1, 10 (23 ± 9) weeks in S2,

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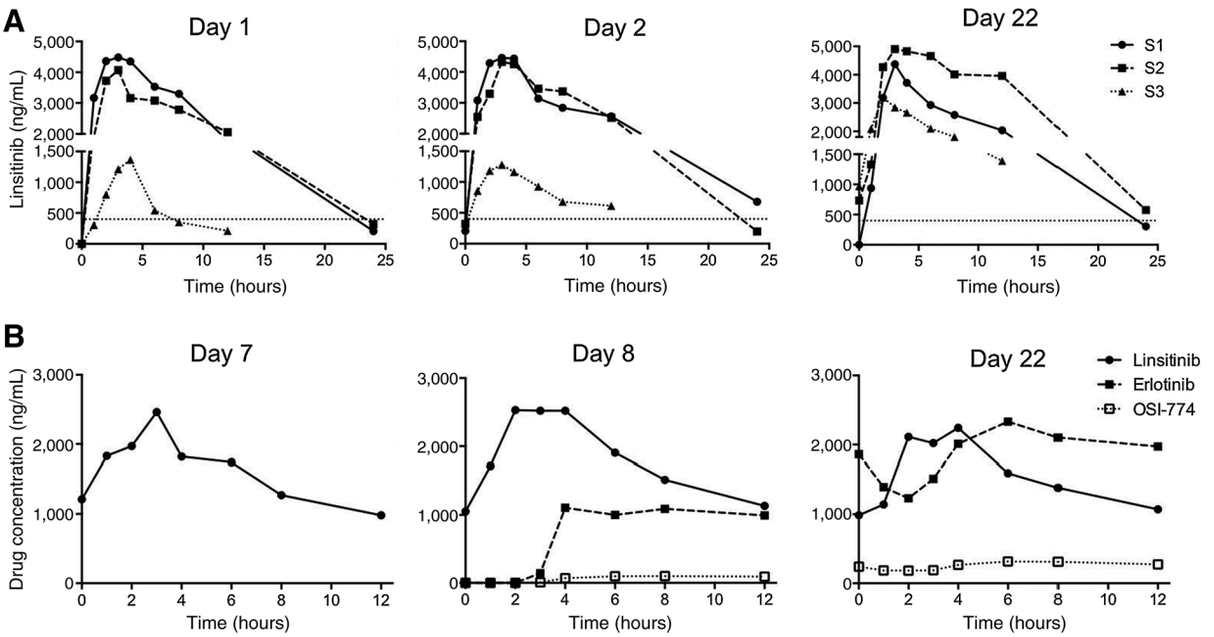

Figure 1.
Pharmacokinetic analysis of linsitinib, erlotinib and OSI-420. A, median plasma levels of linsitinib during day 1 (linsitinib alone), day 2 (first day of erlotinib dosing), and day 22 (day 1 cycle 2) in patients treated at the MTDs in the S1 (450/150 mg), S2 (400/100 mg), and S3 (150/150 mg) dose-escalation cohorts. Dotted line indicates 400 ng/mL concentration predicted from (15, 16) to be required for efficacy. B, median plasma levels of linsitinib, erlotinib, and OSI-420 during day 7 (linsitinib alone), day 8 (first day of erlotinib dosing), and day 22 (day 1, cycle 2) in NSCLC patients treated on the expansion cohort.

7 (17 ± 7) weeks in S3 and 6 (11 ± 2) weeks in the expansion cohort. Overall 28 of 91 (31%) patients were on study for >12 weeks, including 9 of 40 (22.5%) patients in S1, 11 of 24 (46%) in S2, 3 of 12 (25%) in S3, and 5 of 15 (33%) in the NSCLC expansion cohort. The 5 patients achieving PR remained on study for 16, 36, 36, 72, and 268+ weeks.

In total, the study recruited 31 NSCLC patients including 22 with adenocarcinoma and 9 with nonadenocarcinoma histology (4 squamous, 2 papillary, 1 mixed adenosquamous, 2 unclassified). Of the patients with adenocarcinoma, 2 (9%) achieved PR, 1 in S1 and 1 in the expansion cohort, 7 (32%) SD, and 13 (59%) PD. Equivalent figures for the nonadenocarcinoma cases were 1 (11%) PR in a patient treated on the expansion cohort, 5 (56%) SD, 3 (33%) PD. The 15 NSCLC patients treated in the expansion cohort at the RP2D included 11 with adenocarcinoma (1 with PR, 5 with SD, 5 with PD) and 4 with nonadenocarcinoma histology (1 PR, 2 SD, 1 PD). Figure 2B shows chest CT images from a patient with squamous NSCLC who achieved PR during treatment at the RP2D.

Pharmacodynamic analysis

PBMCs from 9 patients were analyzed for IGF1R and INSR phosphorylation, including 3 patients in S1 (2 at 150 mg, 1 at 450 mg), 5 in S2 (2 at 300 mg, 3 at 400 mg), and 1 in S3 (150 mg twice daily). Two cases showed no detectable signal; data for the remaining 7 cases are shown in Fig. 2C. IGF1R/INSR

Table 3. Pharmacokinetic parameters for NSCLC expansion cohort

	Linsitinib (mg twice daily)/erlotinib (mg once daily)		
	150/0 (Day 7)	**150/150 (Day 8)**	**150/150 (Day 22)**
Evaluable, *n*	15	15	11
Linsitinib			
C_{max} (ng/mL)	2,580 (1,070–6,420)	2,890 (1,200–8,100)	2,340 (942–5,750)
T_{max} (h)	3.0 (0.0–6.1)	2.0 (1.0–10.5)	3.0 (1.0–8.0)
AUC_{0-last} (h.ng/mL)	17,906 (6,979–52,597)	18,668 (843–64,587)	16,220 (6,483–47,145)
Erlotinib			
C_{max} (ng/mL)	–	1,285 (628–1,790)	2,390 (1,050–5,530)
T_{max} (h)	–	4.1 (1.9–8.6)	4.1 (1.9–8.4)
AUC_{0-last} (h.ng/mL)	–	21,061 (10,363–29,919)	42,484 (16,318–105,521)
OSI-420			
C_{max} (ng/mL)	–	125 (62.4–331)	342 (163–877)
T_{max} (h)	–	5.2 (1.9–21.9)	6.0 (1.9–8.1)
AUC_{0-last} (h.ng/mL)	–	2,039 (1,061–5,455)	5,727 (2,706–17,052)

NOTE: Table shows pharmacokinetic parameters expressed as median (range).
Abbreviations: AUC_{0-last}, area under the concentration–time curve; C_{max}, maximum observed concentration; max, maximum; min, minimum; T_{max}, time to maximum concentration.

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Table 4. Efficacy and mutation detection

A: Best response by cohort	S1 ($n = 31$)	S2 ($n = 19$)	S3 ($n = 11$)	NSCLC expansion ($n = 14$)	Total ($n = 75$)
Overall best response, *n* (%)					
Complete response (CR)	0	0	0	0	0
Partial response (PR)	2 (6)	1 (5)	0	2 (14)	5 (7)
Stable disease (SD)	8 (26)	12 (63)	6 (55)	7 (50)	33 (44)
Progressive disease	21 (68)	6 (32)	5 (45)	5 (36)	37 (49)
Overall response rate (CR + PR)					
n (%)	2 (7)	1 (5)	0	2 (14)	NA
95% CI	0.8–21.4	0.1–26.0	0.0–28.5	1.8–42.8	NA
Disease control rate (CR + PR + SD)					
n (%)	10 (32)	13 (68)	6 (55)	9 (64)	NA
95% CI	16.7–51.4	43.4–87.4	23.4–83.3	35.1–87.2	NA

B: Mutation detection[a]

Diagnosis	Linsitinib/erlotinib dose (mg)	Response (weeks)	ctDNA	Tumor DNA
S1				
CRC	400/150	SD (6)	KRAS G12V, PIK3CA E542K	NA
NSCLC (adenocarcinoma)	450/150	PR (72)	EGFR exon 19 del	NA
Rectal (squamous)	450/150	PR (36)	NVD EGFR, KRAS, PIK3CA	NVD in EGFR, KRAS, BRAF, PIK3CA
S2				
Chordoma	50/100	PR (>268)	PIK3CA exon 9 E542K	NVD EGFR exons 18–21[b], PIK3CA exon 9, BRAF exon 15
Expansion NSCLC				
Adenocarcinoma	150 BID/150	SD (6)	EGFR exon 19 del and T790M	NA
Poorly diff adenocarcinoma	150 BID/150	PR (16)	NVD EGFR, KRAS	NA
Adenocarcinoma	150 BID/150	SD (6)	KRAS G12D	KRAS G12D
Adenocarcinoma	150 BID/150	PD	EGFR exon 19 del, T790M	NA
Squamous	150 BID/150	PR (36)	NVD EGFR exons 18–21	NVD
Adenocarcinoma	150 BID/150	SD (30)	KRAS G12D	KRAS G12D

Abbreviations: adeno, adenocarcinoma; BID, twice daily; CI, confidence interval; CRC, colorectal cancer; diff, differentiated; NA, not available; NSCLC, non–small cell lung cancer; NVD, no variant detected; poorly diff, poorly differentiated; sq, squamous.

[a]Mutation detection in ctDNA or archival tumor tissue, by cohort and dose (mg) of linsitinib/erlotinib.

[b]Assay failure for exon 20 EGFR.

phosphorylation was substantially inhibited 2 to 24 hours after the first dose. Later time points showed fluctuating receptor inhibition in patients on the intermittent S1 schedule and persisting receptor inhibition in patients treated with ≥300 mg linsitinib daily in S2 and S3. However, there was considerable variation between patients, some showing recovery in predose samples taken on days 3, 15, and 23. Circulating IGF-1 was measured in 72 cases to assess endocrine response to pathway blockade. Compared with baseline (predose) levels, IGF-1 increased by ≥130% during cycle 1 in 15 of 31 (48%) patients with informative data in S1, 13 of 19 (68%) in S2, 11 of 12 (92%) in S3, and 10 of 10 expansion cohort patients (Fig. 2D). Although not statistically significant, these results suggest a trend to greater fold increase in IGF-1 in patients receiving linsitinib twice daily. Of 10 evaluable patients in the NSCLC expansion cohort, there was a consistent increase in circulating IGF-1 >130% at all sampled time points in 5 patients, and some values <130% in 5 cases, suggesting variable IGF1R inhibition.

Mutation analysis was performed on DNA extracted from 10 tumors (8 NSCLC, 1 rectal cancer, 1 chordoma), circulating tumor DNA (ctDNA) of corresponding plasma samples, and an additional 24 ctDNAs without matched tumor. Table 4B shows data from informative cases. Of the 5 PR patients, analysis of ctDNA detected an *EGFR* exon 19 deletion in 1 patient with NSCLC, and *PIK3CA* exon 9 mutation in the spinal chordoma, although the latter was not detected in tumor DNA. Mutations in *EGFR, KRAS, PIK3CA*, and *BRAF* were not detected in plasma or tumor DNA of the other 3 patients achieving PR: 2 with NSCLC, including the patient shown in Fig. 1B, and 1 with rectal cancer. *EGFR* exon 19 mutations were detected in ctDNA of 2 further NSCLC patients, both treated at the RP2D in the NSCLC expansion cohort. Neither responded to trial therapy; both cases had received prior erlotinib and also harbored the exon 20 T790M mutation associated with erlotinib resistance (30, 31). A CRC patient with brief SD as best response had ctDNA mutations in both *PIK3CA* (E542K) and *KRAS* (G12V). *KRAS* codon 12 mutations were also detected in the plasma and tumor DNA of 2 NSCLC patients, both with SD as best response (duration 6 weeks, 30 weeks).

Discussion

This study demonstrates that combination treatment with linsitinib and erlotinib has acceptable tolerability, with no pharmacokinetic evidence of significant drug–drug interaction, and detectable but modest antitumor activity in unselected patients. MTDs were defined as 450 mg linsitinib once daily days 1–3 every 7 days with 150 mg erlotinib once daily in the S1 schedule, and

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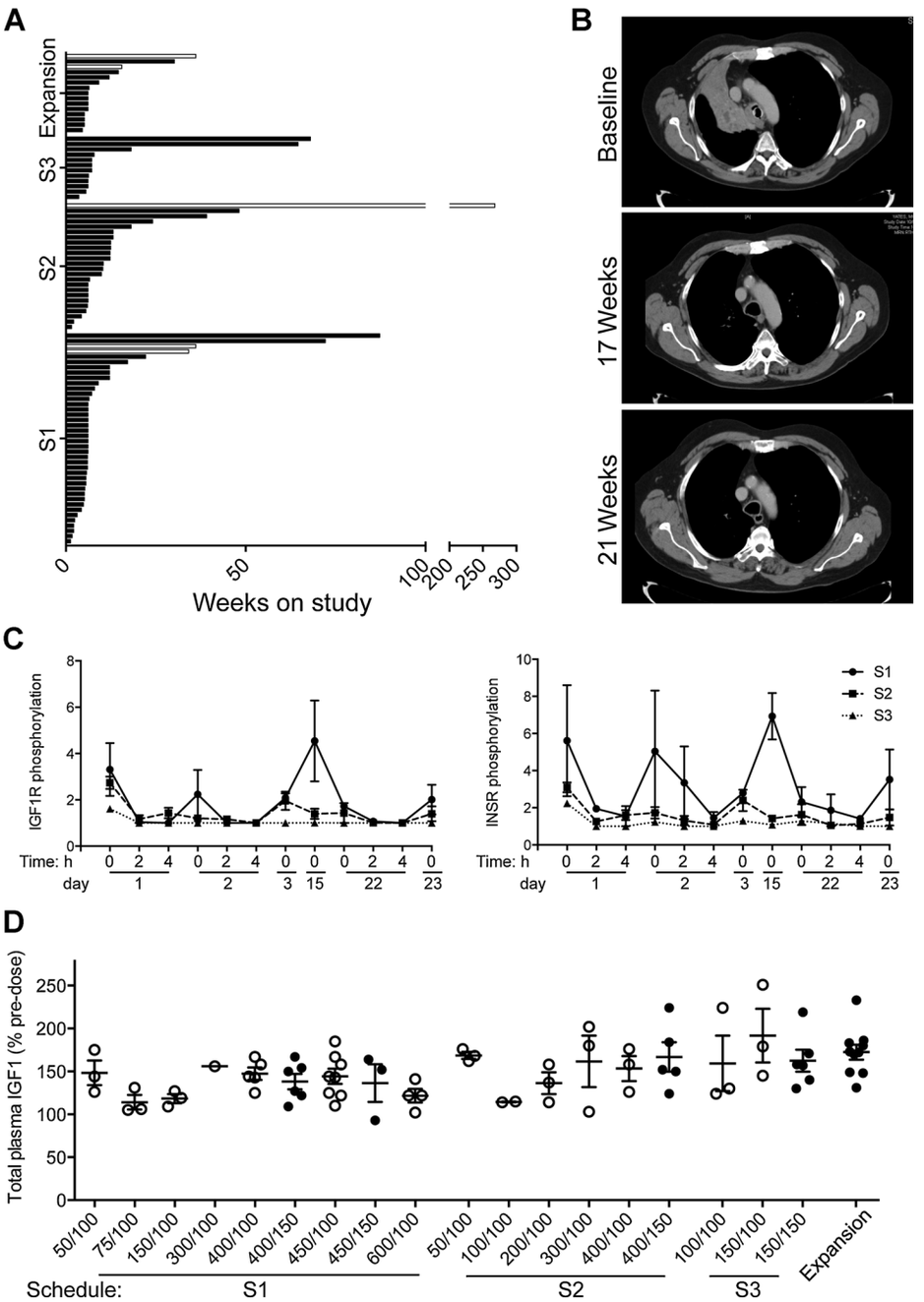

Figure 2.
Efficacy and pharmacodynamic data in patients treated with linsitinib and erlotinib. A, duration on study (weeks) in 90 evaluable patients. White bars, confirmed partial responses. B, CT scan of chest from male patient with squamous NSCLC without detectable EGFR mutation, treated in NSCLC expansion cohort with linsitinib 150 mg twice daily and erlotinib 150 mg once daily. Top, baseline scan showing central mass and right hilar lymphadenopathy causing right upper lobe collapse. Middle and bottom, response scan at 17 weeks, confirmatory scan at 21 weeks. C, effects of linsitinib on phosphorylation of left: IGF1R, right: INSR in PBMCs of patients treated on dose-escalation schedules S1–S3. D, plasma IGF-1 levels expressed as % greatest fold increase over predose value, by dose of linsitinib/erlotinib. Open circles, patients treated with 100 mg erlotinib once daily; closed circles, 150 mg erlotinib once daily.

400 mg linsitinib once daily with 100 mg erlotinib once daily for the S2 once daily continuous schedule. This compares with linsitinib MTDs defined in monotherapy studies of 600 mg linsitinib for intermittent dosing and 400 mg once daily for continuous once daily dosing (17, 18). On the basis of the data from this trial and the monotherapy study, and the finding that linsitinib has a short half-life (5 hours; 18), twice daily dosing is required to achieve continuous inhibition of IGF1R and INSR, with RP2D for the combination of 150 mg linsitinib twice daily and 150 mg erlotinib once daily. In all, 64 patients in the S1 and S2 cohorts received total linsitinib doses above the RP2D (300 mg/day), reflecting the fact that recruitment was conducted in parallel with the monotherapy studies (17, 18), and information on monotherapy RP2D was available only towards the end of the current trial.

The most common linsitinib-related AEs were fatigue, nausea, vomiting, and QTc prolongation, similar to those reported in monotherapy studies (17–19). The three dosing schedules had similar toxicities, except that linsitinib treatment-related SAEs occurred in 10 of 40 (25%) patients in S1 but not in S2 or S3,

suggesting that continuous linsitinib dosing may be better tolerated, perhaps reflecting metabolic changes associated with intermittent IGF1R/INSR blockade. Specific AEs in this study reflected toxicities observed in monotherapy studies of linsitinib and IGF1R antibodies, including hyperglycemia and abnormal liver function (17–19, 32–36), suggesting that these are class effects of IGF1R inhibition. In contrast thrombocytopenia and neutropenia were reported in studies of IGF-1R antibodies (37) but were not observed with linsitinib. QTc prolongation was dose-limiting in this trial, as with other small-molecule drugs, is not considered to be mechanism based (38), and was not observed in IGF1R antibody trials (33–37).

Recent reviews have discussed the limited clinical activity of single-agent IGF1R inhibition, and the degree of IGF1R/INSR blockade achieved by IGF axis inhibitors of different classes (5, 6, 14). IGF1R antibodies downregulate IGF1Rs, whereas IGF1R TKIs suppress receptor activity without influencing IGF1R expression (6). IGF1R antibodies demonstrated single-agent activity in soft tissue sarcomas, Ewing and thymic tumors, but not in common cancers (6, 32, 33, 36,39). Linsitinib is the first IGF1R/INSR TKI to report clinically, and as monotherapy induced partial responses in adrenocortical carcinoma and melanoma (17, 18). In a randomized phase III trial in adrenocortical carcinoma, 3 of 90 patients on the linsitinib arm experienced durable PR, with no difference in overall survival between linsitinib and placebo arms (19). Linsitinib has also been combined with everolimus in metastatic CRC, with no evidence of clinical activity (40).

In light of these concerns about efficacy, pharmacokinetic and pharmacodynamic analyses tested whether IGF1R and INSR were blocked by linsitinib. Dosing at the RP2D maintained median plasma linsitinib levels above the predicted concentration required for antitumor activity (16). IGF1R inhibition induces an increase in circulating IGF-1, attributed to hypothalamic–pituitary axis activation in response to pituitary IGF1R blockade (4). We observed a trend to greater IGF-1 increase in patients dosed with linsitinib twice daily, although differences between cohorts were not significant, and the relative increase in circulating IGF-1 did not appear to correlate with clinical response. There was evidence of durable inhibition of PBMC IGF1Rs/INSRs in patients treated with linsitinib twice daily, but some indications of incomplete receptor blockade even at the RP2D, as judged by variable elevation of circulating IGF-1, and hypoglycemia, likely due to restoration of INSR responsiveness in the context of hyperinsulinemia. These data suggest that receptor blockade may have been intermittent in some patients, highlighting the importance of assessing IGF1R/INSR axis activity in clinical material (6). Key issues for IGF1R/INSR TKIs include the extent to which complete IGF1R/INSR-A blockade is achievable clinically, the consequences of inhibiting the metabolic INSR, and the identification of kinase-independent functions for IGF1R (41, 42), which may be suppressed by IGF1R antibodies but unaffected by kinase inhibition.

This is the first study to assess combined IGF1R/EGFR inhibition using two small-molecule inhibitors. Previous trials evaluated erlotinib with IGF1R antibodies cixutumumab or R1507. In advanced NSCLC, the combination of cixutumumab plus erlotinib was not tolerable and was ineffective, with SD as best response (43). Also in NSCLC, R1507 was tolerable with erlotinib; there was no progression-free survival or overall survival advantage over erlotinib alone in unselected patients, but the 12-week progression-free survival rate in patients with KRAS-mutant tumors was 36% for R1507 versus 0% on the placebo arm (44). In the current study, combination treatment with linsitinib and erlotinib showed preliminary evidence of antitumor activity, and durable objective responses occurred in patients on all three linsitinib schedules, with 28 of 91 (31%) patients on study for >12 weeks.

We did not attempt to quantify IGF1R or EGFR in the tumors of trial subjects, as these parameters have not been shown to be useful predictive biomarkers (6). In a subset of patients, we were able to analyze ctDNA and archival tumor DNA, to investigate correlations between tumor-derived mutations and response. Functionally significant *KRAS* mutations were detected in 2 NSCLC patients, both of whom had SD, one durable. There are conflicting reports of the association of *RAS* mutation and/or RAS–MEK–ERK pathway activation with response to IGF1R inhibition, some (including the clinical R1507 study highlighted above) supporting association with sensitivity to IGF1R inhibition (44–46), and others with resistance (47, 48). *EGFR* mutations were detected in ctDNA of 3 of 30 NSCLC cases, consistent with the published incidence (10%–13%) of NSCLC *EGFR* mutations (49). The clinical response in a patient with *EGFR* exon 19-deleted NSCLC (Table 4B) is consistent with the association between this mutation and erlotinib sensitivity, and the additional "gatekeeper" *EGFR* T790M mutation, detected in 2 nonresponders here, with erlotinib resistance (30, 31, 50). The presence of only 2 informative cases harboring *EGFR* T790M is insufficient to assess activity of the linsitinib/erlotinib combination in this setting. However, there was evidence of clinical activity in tumors unlikely to respond to single-agent erlotinib, including durable partial responses in *EGFR* wild-type squamous lung cancer and spinal chordoma. Further analysis of the latter will be reported separately.

In summary, treatment with linsitinib and erlotinib was found to be tolerable in patients with advanced solid tumors, and induced durable objective responses in a minority of patients. Effective development of this approach will require clarification of the extent of pathway blockade and identification of predictive biomarkers for this combination.

Disclosure of Potential Conflicts of Interest

V.M. Macaulay is a consultant/advisory board member for Boehringer Ingelheim. C.M. Rudin is a consultant/advisory board member for Boehringer Ingelheim, Celgene and GlaxoSmithKline. R.A. Juergens is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim and Roche, and reports receiving commercial research support from Astellas and AstraZeneca. A. Stephens has ownership interest (including patents) in Piramal Imaging GmbH. S.M. Gadgeel is a consultant/advisory board member for Boehringer Ingelheim and Genentech/Roche. No potential conflicts of interest were disclosed by the other authors.

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