Invest New Drugs (2015) 33:710–719
DOI 10.1007/s10637-015-0241-7

PHASE I STUDIES

A first-in-human phase I trial of LY2780301, a dual p70 S6 kinase and Akt Inhibitor, in patients with advanced or metastatic cancer

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Received: 11 December 2014 / Accepted: 10 April 2015 / Published online: 24 April 2015

Summary The primary objective of this phase I study of LY2780301, a dual p70 S6 kinase and Akt inhibitor, was to determine the recommended phase II dose as a single agent in patients with advanced cancer. Secondary objectives included safety, pharmacokinetic, and pharmacodynamic analyses, and co-clinical analyses in Avatar models. Eligible patients received total daily doses of LY2780301 100–500 mg, given orally as a single dose or divided into 2 doses for 28-day cycles. Dose escalation followed 3+3 design. The primary pharmacodynamic endpoint was inhibition of S6 assessed by skin and tumor biopsy. Thirty-two patients were treated. Common toxicities possibly related to treatment included constipation (19 %), fatigue (13 %), nausea (9 %), and diarrhea (9 %). Grade 3/4 toxicities potentially related to treatment were anemia (n=2), increased alanine aminotransferase/aspartate aminotransferase (ALT) (n=1), and increased gamma-glutamyl transpeptidase (GGT) (n=1). One patient experienced best overall response of prolonged stable disease for 6 cycles. Plasma exposures of LY2780301 exceeded predicted efficacious exposures, but were not dose proportional. Among patients receiving 500 mg daily >50 % exhibited reduced S6 in skin biopsies at Day 8 of treatment, but the effect was not maintained. Plasma concentrations of LY2780301 and/or its metabolites were not correlated with S6 expression in the epidermis. There was minimal antitumor activity against the model, CRC 019. Avatar models showed minimal pharmacodynamic effects consistent with the observed antitumor effects. This study suggests a dose of LY2780301 500 mg QD for future studies.

Keywords p70 S6 kinase (p70S6K) · Akt · mTOR · PI3K/Akt/mTOR signaling pathway · Cancer · LY2780301

At the time of this study, Dr. Miller and Mr. Bumgardner were employed by Eli Lilly and Company. Dr. Miller is currently employed by Everest Clinical Research, Little Falls, NJ, USA. Mr. Bumgardner is currently employed by Onyx Pharmaceuticals, a subsidiary of Amgen, South San Francisco, CA, USA.

Electronic supplementary material The online version of this article (doi:10.1007/s10637-015-0241-7) contains supplementary material, which is available to authorized users.

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Introduction

The phosphatidlyinositol-3-kinase (PI3K)/Protein Kinase B (PKB, Akt/mammalian target of the rapamycin (mTOR) signaling pathway is a key regulator of cell proliferation and survival [1], and has been described as a "master switch" for cell growth and proliferation [2]. The PI3K/Akt/mTOR signaling pathway is frequently mutated [3–5] and is constitutively activated in human tumors [6]. The Akt family of serine-threonine protein kinases includes three isoforms: Akt1, Akt2, and Akt3 [7–12]. Activation of the PI3K/Akt/mTOR pathway results in the activation of the mTOR complex 1 (mTORC1), and p70 S6 kinase (p70S6K) is a key effector of mTOR. Activation of p70S6K and subsequent phosphorylation of the S6 ribosomal protein (S6) up-

regulates mRNA translation, which promotes sustained cell growth and proliferation. Blockage of PI3K/Akt/mTOR pathway activation has been shown to result in apoptosis or cell cycle arrest in several different models [13]. Therapeutic targeting of the PI3K/Akt/mTOR signaling pathway may provide a method of inhibiting protein synthesis similar to that of rapamycin and its analogues.

LY2780301 is a highly selective adenosine triphosphate (ATP)-competitive dual inhibitor of p70S6K and Akt. Preclinically, this small molecule exhibited antiproliferative activity in a broad range of cell lines using monolayer and colony formation assays (data on file, Eli Lilly and Company). LY2780301 effectively inhibited the growth of A2780 (ovarian), H460 (lung), PC3 (prostate), and HCT116 (colon) xenograft models. Pharmacodynamic relationships of LY2780301 with phospho-S6 (pS6) and other markers were dose-, exposure-, and time-dependent (data on file, Eli Lilly and Company).

This first-in-human phase I study of LY2780301 had a primary objective to determine the recommended phase II dose and schedule as an orally administered single agent in patients with advanced solid tumors. Secondary objectives included evaluation of safety, pharmacokinetics (PK), and pharmacodynamics (PD) of the compound and its metabolites. Parallel clinical and nonclinical investigations (co-clinical trials), which included Avatar mouse models of cancer, were conducted and included models with tumor tissues obtained from clinical study patients. Co-clinical studies provided relevant preclinical models to test the study agent for further mechanistic and preclinical studies. This approach has been used in clinical studies with novel anticancer agents and has resulted in a better understanding of mechanisms of action [14].

Patients and methods

Ethics statement

This study followed the guiding principles of the Declaration of Helsinki [15] and the Good Clinical Practice Guidelines of the International Conference on Harmonisation [16]. All patients provided written informed consent prior to study enrollment.

Patients

This multicenter Phase 1 study had the following patient eligibility criteria for enrollment: age ≥18 years; histologically confirmed solid tumors or Non-Hodgkin's lymphoma (NHL) refractory to standard therapy; measurable or nonmeasurable disease defined by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) [17]; discontinued previous treatments for cancer and recovered from the acute effects of therapy for at least 28 days for myelosuppressive agents or 14 days for nonmyelosuppressive agents; a performance status of ≤1 on the Eastern Cooperative Oncology Group (ECOG) scale; and baseline laboratory tests to determine adequate hematopoietic, renal, and hepatic function [defined as absolute neutrophil count $\geq 1.5\times10^9$/L; platelets $\geq 100\times10^9$/L; hemoglobin ≥8 g/dL; serum creatinine ≤1.5×upper limits of normal (ULN) or calculated clearance >45 mL/min; bilirubin ≤1.5× ULN; and alanine transaminase (ALT) ≤2.5 x ULN (≤5× ULN was acceptable for patients with liver tumors)].

Patients were excluded for any of the following reasons: received treatment within 28 days of the initial dose of study drug with a drug that had not received regulatory approval for any indication; symptomatic central nervous system malignancy or metastasis (except for patients no longer receiving corticosteroids and/or anticonvulsants with asymptomatic and stable disease for at least 60 days); current acute or chronic leukemia; positive test results for human immunodeficiency virus, hepatitis A, B, or C; corrected QT interval (QTc) >470 msec on an electrocardiogram; treatment with a strong cytochrome P450 3A4 (CYP3A4) substrate with a narrow therapeutic range, or classification as a strong inhibitor or inducer; history of pituitary adenoma; or pregnancy or lactation.

Study design

This nonrandomized, open-label dose escalation phase I study of LY2780301 in patients with advanced solid tumors or NHL incorporated once-daily (QD) (Part A) and twice-daily (BID) (Part B) dose regimens as part of a 28-day dosing cycle. Dose escalation was to follow a 3+3 design until the criteria for maximum tolerated dose (MTD) were met. Part B initiation was based on patient toxicity and PK/PD data from Part A. Part A dosing began at 100 mg in a flat dosing scheme and escalated by 100 mg in each subsequent cohort, up to 500 mg. In Part B, dosing began at 150 mg BID and escalated by 50 mg for the second cohort (200 mg BID).

In Parts A and B, each cohort initially included 3 patients. At the end of the first treatment cycle, each patient was clinically evaluated for safety by the investigator before being allowed to receive the next treatment cycle. Eligible patients received 2 cycles of LY2780301, unless one or more criteria for discontinuation were met. Discontinuation criteria included: progressive disease; unacceptable toxicity; noncompliance of the patient; a dosing delay of more than 2 weeks due to an AE; a dose-limiting toxicity (DLT) leading to dose reduction with another DLT-equivalent toxicity occurring at the reduced dose in Cycle 2 or greater; and withdrawal by the patient, attending physician, or sponsor for any reason. Patients who, in the opinion of the investigator, demonstrated clinical benefit may have received treatment beyond 2 cycles.

Dose escalations were considered following an assessment of toxicity using the Common Terminology Criteria for Adverse Events Version 4(CTCAE) Version 4.02) [18]. Dose escalation decisions primarily considered any adverse events (AEs) possibly related to LY2780301, along with PK/PD data, when available, as a secondary consideration. Patients received at least two cycles of treatment unless one or more criteria for discontinuation were met. DLT was defined as an AE possibly related to LY2780301 occurring during cycle 1 following the CTCAE v4.02 criteria: Grade 4 thrombocytopenia or neutropenia >5 days' duration; febrile neutropenia; ≥Grade 3 non-hematological toxicity except nausea/vomiting/diarrhea, skin rash that was responsive to medical treatment; transient (≤5 days) Grade 3 elevations of ALT/aspartate aminotransferase (AST) without evidence of other hepatic injury; transient Grade 3 hyperglycemia; and Grade 3 hypertriglyceridemia or hyperlipidemia without optimal treatment. If a single patient experienced DLT during Cycle 1 of LY2780301, three additional patients were enrolled at that dose level. If a DLT was observed in two or more patients at any dose level, escalation ceased and the previous dose was declared the MTD. An expansion cohort of up to 30 patients was planned once the recommended dose was reached.

Response analyses were based on RECIST Version 1.1 [17] for patients with solid tumors, or the Revised Response Criteria for Malignant Lymphoma or patients with non-Hodgkin's lymphoma [19].

Drug supply

LY2780301 was provided by Eli Lilly & Company (Indianapolis, IN, USA) as capsules for oral administration containing 25 or 100 mg of active drug.

Pharmacokinetic studies

Pharmacokinetic analyses were performed on all patients who received at least one dose of study drug and contributed post-dose blood samples for bioanalysis according to the study protocol. Whole blood samples were collected pre-dose and at 0.5, 1, 2, 3, 5, and 8 h post-dose on days 1 and 8 of Cycle 1. Concentrations of LY2780301 parent drug and its two principal metabolites (desmethyl and didesmethyl) were measured by a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method [data on file, Eli Lilly and Company]. Pharmacokinetic parameters following single and multiple doses of LY2780301 included partial area under the plasma concentration-time curve (AUC), peak observed concentration (C_{max}), time to C_{max} (t_{max}), half-life ($t_{½}$), apparent clearance (CL/F), and apparent steady-state volume of distribution (V_{ss}/F).

Pharmacodynamic studies

Pharmacodynamic analyses were performed in skin biopsies collected on days 1, 8, and 22 of Cycle 1. The pS6 expression was investigated in two scoring schemes: 1) levels of pS6 expression in the entire epidermis, and 2) levels of pS6 expression in the epidermis minus the stratum granulosum (epidermis-SG).

Antitumor activity

Patients' tumor measurements were assessed by one or more of the following radiologic tests: computerized tomography (CT) scan, magnetic resonance imaging (MRI), and/or chest x-ray. The extent of each patient's disease was assessed using the following procedures: tumor measurement of palpable or visible lesions for patients with solid tumors (RECIST v1.1 guidelines), [17] or the Revised Response Criteria for Malignant Lymphoma [19] for patients with NHL.

Animal studies

The study protocol for the parallel co-clinical study in Avatar mouse models of cancer was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The care and use of animals in this study complied with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Six-week old female athymic nude-*Foxn1^{nu}* mice (Harlan) were housed in individual high-efficiency particulate air (HEPA) ventilated cages (Sealsafe® Plus, Techniplast). Tumor fragments were subcutaneously implanted bilaterally on the right and left flank, and included colon cancer (three models: CRC 005, 019, 012), pancreatic cancer (three models: Panc 031, 198, 215), lung cancer (two models: Pulm 021, 024), and one melanoma model (Mel001) obtained from one of the study subjects. Animals were randomized into treatment and control groups when tumors reached ~200 mm^3, at which point dosing was initiated (Day 1). LY2780301 was administered at a dose of 12.5 or 50 mg/kg daily Monday-Friday by oral gavage. Animals were checked daily for mobility, body weight, morbidity and other abnormal effects, and mortality. Tumor sizes were measured (in triplicate) twice weekly in two dimensions using an electronic caliper, and the tumor volume was expressed in mm^3 using the formula $TV=width^2\ x\ length\ x\ 0.5$. Percent tumor growth inhibition (%TGI) values were calculated for each treatment group (T) versus control (C) using initial (i) and final (f) tumor measurements by the equation $\%TGI=1-[(Tf-Ti)/(Cf-Ci)]$. TGI values were compared between treatment and control groups.

Western blot analysis of LY2780301

Total protein from xenograft samples was extracted with RIPA buffer (R0278, Sigma Aldrich, St. Louis, Missouri, USA) plus protease inhibitor cocktail tablet (11836170001, Roche Diagnostics, Indianapolis, Indiana, USA) and phosphatase inhibitor cocktail 2 and 3 (P5726, P0044 Sigma Aldrich, St. Louis, Missouri, USA). Analyses were conducted after SDS-PAGE electrophoresis and transferred to polyvinylidene difluoride Immobilon®-P membranes (Millipore®). Immunoblotting was performed according to the antibody manufacturers' recommendations. The following antibodies were used: anti-S6 (2217), anti-pS6 (4857), anti-Akt (4691), anti-phospho-Akt (pAkt) (4060), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (5174) (Cell Signaling Technology®, Danvers, Massachusetts, USA). Secondary antibodies included goat anti-rabbit-horseradish peroxidase (HRP) (P0448, Dako, Carpinteria, California, USA).

Results

Phase I study patient demographics and treatment

Thirty-two patients completed at least one cycle of LY2780301. The mean treatment durations were 2.9 cycles for both Parts A and B (range Part A: 1–9, Part B: 1–5). Most patients had numerous prior therapies; 72 % of patients had ≥3 previous lines of systemic treatment. Patient characteristics and the dose-escalation scheme are summarized in Tables 1 and 2, respectively.

Table 1 Baseline Patient Characteristics

Characteristic	*N*=32 n, (%)
Sex	
Male	17 (53)
Female	15 (47)
Age, years	
Mean	55.6
Range	22–79
Race	
Caucasian	32 (100)
ECOG Performance status	
0	13 (41)
1	19 (59)
Tumor Type	
Colorectal	9 (28)
Mesothelioma	7 (22)
Breast	5 (16)
Sarcoma	4 (13)
Other	7 (22)
Prior Therapy	
Chemotherapy	32 (100)
1 Line	2
2 Lines	7
≥3 Lines	23
Surgery	28 (88)
Radiotherapy	13 (41)

Abbreviations: *ECOG*, Eastern Cooperative Oncology Group

Safety and tolerability

Twenty-five patients in Part A and seven patients in Part B received at least one dose of LY2780301. There were no DLTs reported in either Part A or Part B. Eight patients (25 %) experienced a serious adverse event (SAE) during treatment: 500 mg QD cohort (*n*=3), 300 mg QD cohort (*n*=2), 400 mg QD cohorts (*n*=2), and 100 mg QD cohort (*n*=1). The most common SAEs were dyspnea and respiratory failure, experienced by two patients each. One patient each experienced the following SAEs: gastrointestinal disorder, small intestine obstruction, enterocolitis infection, upper respiratory infection, acute kidney injury, renal colic, and surgical procedure. None of these SAEs were considered related to study drug.

For Parts A and B, 21 patients (66 %) reported at least one treatment-emergent adverse event (TEAE) possibly related to study drug (Table 2). There were four Grade 3/4 TEAEs possibly related to study drug: Grade 3 anemia (*n*=1), increased ALT /AST (*n*=1), increased GGT (*n*=1), and Grade 4 anemia (*n*=1). The most common TEAEs possibly related to study drug were constipation (19 %), fatigue (13 %), nausea (9 %), and diarrhea (9 %). Laboratory abnormalities possibly related to study drug included increased ALT/AST (3 %), hyperglycemia (6 %), anemia (6 %), and thrombocytopenia (3 %). No patients discontinued treatment due to an adverse event.

Pharmacokinetics

In Part A, LY2780301 exposures, as assessed by AUC from time zero to infinity following the first dose on day 1($AUC_{[0-\infty]}$), increased with doses from 100 to 500 mg QD. A similar trend in AUC was observed during one dosing interval at steady state ($AUC_{[\tau,ss]}$) on Day 8, with the exception of the 200-mg QD cohort, in which the average AUC was slightly lower than the 100-mg QD cohort. Exposures across the QD dose range were not dose-proportional. Corresponding median t_{max} across treatment groups ranged from 3 to 6 h. PK results are summarized in Table 3.

Twice-daily dosing was evaluated for the 150-mg and 200-mg doses and compared with QD dosing at steady state (Day 8). Mean exposures in the former groups ranged from 36,700 to 39,000 ng·hr/mL (73,400-78,000 ng·hr/mL extrapolated over a 24-hour period). Although sample sizes were small,

Table 2 Treatment Emergent Adverse Events Possibly Related to Study Drug (all grades[a] that occurred in ≥5 % of patients)

Clinical	100 mg QD		200 mg QD		300 mg QD		400 mg QD		500 mg QD		150 mg BID		200 mg BID		Total	
	n=3		*n*=3		*n*=4		*n*=3		*n*=12		*n*=3		*n*=4		*N*=32	
Grade	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	N	%
Constipation	1	0							3	0			2	0	6	19
Fatigue			1	0			1	0	2	0					4	13
Diarrhea							1	0	1	0	1	0			3	9
Nausea									1	0	1	0	1	0	3	9
Anorexia			1	0							1	0			2	6
Heartburn											2	0			2	6
Vomiting					1	0			1	0					2	6
Lab																
Grade	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	1/2	≥3	N	%
Anemia			0	1			0	1							2	6
Hyperglycemia											1	0	1	0	2	6

Abbreviations: BID = twice daily; QD = once daily

[a] Grades are according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.02

these results demonstrated BID dosing resulted in lower overall daily exposures compared to QD dosing. Therefore, additional BID dosing -regimens were not evaluated.

Using pooled data from Part A and Part B, a PK model determined the mean CL/F and V_{ss}/F estimates ranged from 2.23 to 5.48 L/hr and from 43.4 to 207 L, respectively. Mean $t_{1/2}$ ranged from 8.58 to 29.3 h, with the $t_{1/2}$ generally increasing with increasing dose. Exposures at steady state were within the anticipated efficacious range of exposures based on preclinical data (data on file, Eli Lilly and Company).

Plasma concentrations of the desmethyl (LSN2804018) and didesmethyl (LSN2804027) metabolites of LY2780301, both of which are known to exhibit measurable, sub-potent activities relative to the parent molecule, were analyzed (data on file, Eli Lilly and Company). Median plasma concentrations of these metabolites varied among treatment groups, ranging from 5 to 29 % for the desmethyl metabolite, and from 0.07 to 0.89 % for the didesmethyl metabolite on a molar basis (Table 3).

Pharmacodynamics

The level of ribosomal pS6 inhibition was evaluated in pre-treatment and on-treatment skin biopsies *via* quantitative immunohistochemistry (IHC). Skin samples were taken from 15 patients at the highest administered doses. Eleven patients had samples available at baseline and also at Cycle 1 day 8, pre-dose and 5 h post-dose. Ten of the 11 patients were treated at the 500 mg QD dose, and one patient was treated at the 200 mg BID dose. In the 500 mg QD cohort, six of the ten patients achieved pS6 inhibition, which was in alignment with a pre-established relevant threshold. Seven of the ten patients had a decrease in pS6 expression levels post-LY2780301 when compared with baseline epidermis-SG measures. Six of these ten patients exhibited a decrease in pS6 levels using the entire epidermis measures (Fig. 1). However, the decrease in pS6 expression was transient and was not maintained in the 5-hour post-dose samples collected on Day 8 of cycle 1 (data on file, Eli Lilly and Company). Only two patients had pre- and post-dose pS6 levels measured on Day 22, and both of these patients exhibited a decrease in pS6 expression. (data on file, Eli Lilly and Company).

Antitumor activity

There were 28 evaluable patients for disease response. Of these, 8 (29 %) patients exhibited stable disease. Five of the eight patients had a progression free survival (PFS) >120 days (mesothelioma *n*=2, both 500 mg QD; breast cancer *n*=2, 400 mg QD, 150 mg BID; colon cancer *n*=1, 200 mg BID). There were no responders in this study. A molecular tumor profile is available for 10 of 32 patients, and is summarized in the Supplemental Table. One patient with colon cancer and PIK3CA E545K mutation had stable disease for 125 days.

Co-clinical analyses of LY2780301

Figure 2 shows the tumor growth inhibition curves of Avatar models treated with LY2780301 at the indicated doses. Overall tumor growth inhibition (TGI) and the genetic characteristics of the tumors tested are shown in Fig. 3. Overall, there was minimal antitumor activity against the model CRC 019, with a TGI of 68 and 64 % for the 12.5 and 50-mg/kg doses, respectively, on day 28 that was not maintained on day 50.

Table 3 LY2780301 PK Parameters, Geometric Mean (CV%)

	Day 1							Day 8						
	Part A: QD Dosing					Part B: BID Dosing		Part A: QD Dosing					Part B: BID Dosing	
Dose	100 mg	200 mg	300 mg	400 mg	500 mg	150 mg	200 mg	100 mg	200 mg	300 mg	400 mg	500 mg	150 mg	200 mg
N	3	3	3	3	12	3	4	3	3	3	3	12	3	4
t_{max}[a]	3.00	3.00	3.00	5.00	6.00	3.00	2.50	5.00	4.85	3.00	5.50	3.04	3.00	3.00
(h)	(2.00–5.00)	(2.00–8.00)	(3.00–8.00)	(2.00–8.00)	(2.98–22.67)	(3.00–8.00)	(2.00–3.00)	(3.00–5.17)	(2.00–5.00)	(2.08–3.00)	(3.00–8.00)	(0.00–8.00)	(3.00–5.00)	(3.00–3.00)
C_{max} (ng/mL)	1960	1530	5970	3010	4710	2230	2910	3270	3030	7050	6400	6390	3850	3950
	(67)	(130)	(139)	(71)	(55)	(102)	(19)	(86)	(80)	(48)	(40)	(41)	(45)	(21)
Day 1 AUC(0-∞) or Day 8 $AUC_{(\tau,ss)}$ (ng·h /mL)	37,100	37,900	66,200	80,100	173,000	52,300	38,400	44,800	36,500	106,000	125,000	125,000	39,000	36,700
	(63)	(386)[c]	(53)[c]	(30)[c]	(121)[b]	(24)[b]	(51)[c]	(93)	(2)[c]	(96)	(39)	(47)	(38)	(31)
R_a[a,f]								1.61 (1.49–2.04)	3.32[c] (2.05–4.59)	1.37 (1.15–3.79)	2.37[c] (1.98–2.76)	1.91 (1.13–11.9)	1.71 (1.51–5.31)	1.55 (1.29–1.85)
CL/F	2.69	5.28	4.53	4.99	2.89			2.23	5.48	2.83	3.20	4.00		
(L/h)	(63)	(386)[c]	(53)[c]	(30)[c]	(121)[b]			(93)	(2)[c]	(96)	(39)	(47)		
V_{ss}/F	55.2	153	65.6	176	115	43.4	72.1	55.2	162	75.6	102	207	113	117
(L)	(58)	(300)[c]	(7)[c]	(57)[c]	(65)[b]	(24)[b]	(22)[c]	(61)	NA[d]	(27)	NA[d]	(50)[e]	Na[d]	(24)[c]
$t_{1/2}$	12.9	19.3	9.5	22.9	26.0	9.43	8.58	15.7	18.4	17.8	27.4	29.3	18.1	12.8
(h)	(29)	(11)[c]	(62)[c]	(31)[c]	(74)[b]	(2)[b]	(33)[c]	(29)	NA[d]	(108)	NA[d]	(43)[e]	Na[d]	(53)[c]
% desmethyl 2LSN2804018[a,g,h]	8 (4–9)	5 (3–10)	7 (4–7)	7 (5–12)	5 (2–19)	10 (5–16)	15 (6–17)	15 (8–18)	16[c] (10–22)	14 (10–38)	24[c] (20–28)	16 (8–28)	23 (22–47)	29 (23–37)
% didesmethyl LSN2804027[a,g,i]	0.18[c]	0.25[d]	0.16	0.16	0.07	0.21	0.41	0.3	0.32[c]	0.31[c]	0.72[c]	0.31	0.58	0.89
	(0.16–0.19)		(0.06–1.22)	(0.09–0.30)	(0.00–0.50)	(0.14–0.58)	(0.12–0.55)	(0.09–0.45)	(0.18–0.46)	(0.14–2.00)	(0.54–0.89)	(0.11–0.83)	(0.55–2.39)	(0.64–1.26)

Abbreviations: $AUC_{(0-\infty)}$, area under the concentration time curve from zero to infinity; $AUC_{(\tau,ss)}$, area under the concentration time curve over the dosing interval at steady state; BID, twice daily; CL/F, apparent total body clearance; C_{max}, maximum observed concentration; h, hours; LY2780301, parent molecule; LSN2804018, desmethyl metabolite; LSN2804027, didesmethyl metabolite; N, number of patients; QD, once daily; RA, intra- or inter cycle accumulation ratio; $t_{1/2}$, elimination half life; t_{max}, time of C_{max}; V_{ss}/F, apparent volume of distribution at steady state

[a] Median (range)

[b] n=6

[c] n=2

[d] n=1

[e] n=5

[f] Ra=AUC(0–8) day 8/AUC(0–8) day 1

[g] Calculated based on partial area through 8 h post-dose ($AUC_{[0-8]}$): ($AUC_{[0-8]}$metabolite, day x / MWmetabolite) / ($AUC_{[0-8]}$parent, day x / MWparent), where MW represents molecular weight

[h] LSN2804018/ LY2780301=% desmethyl metabolite (LSN2804018) relative to parent

LSN2804027/ LY2780301=% didesmethyl metabolite (LSN2804027) relative to parent

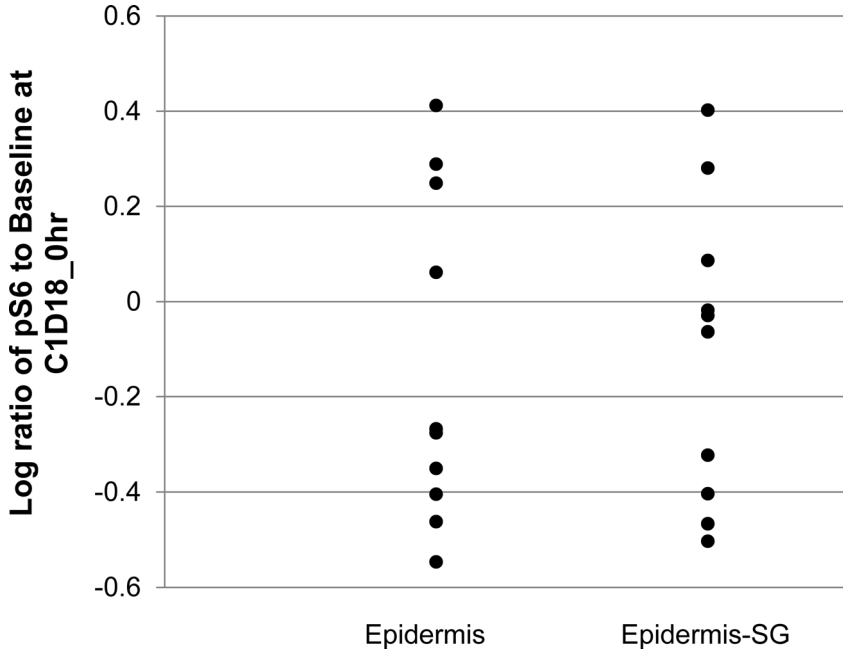


Fig. 1 pS6 levels in skin biopsies. The graph represents change in pS6 levels in skin biopsies from the epidermis and epidermis minus the stratum granulosum (epidermis-SG) for patients in the 500-mg QD cohort at Cycle 1 day 8 postdose (C1D8_ 0hr) compared to baseline *Each dot represents a single patient Epidermis SG = epidermis minus the stratum granulosum*

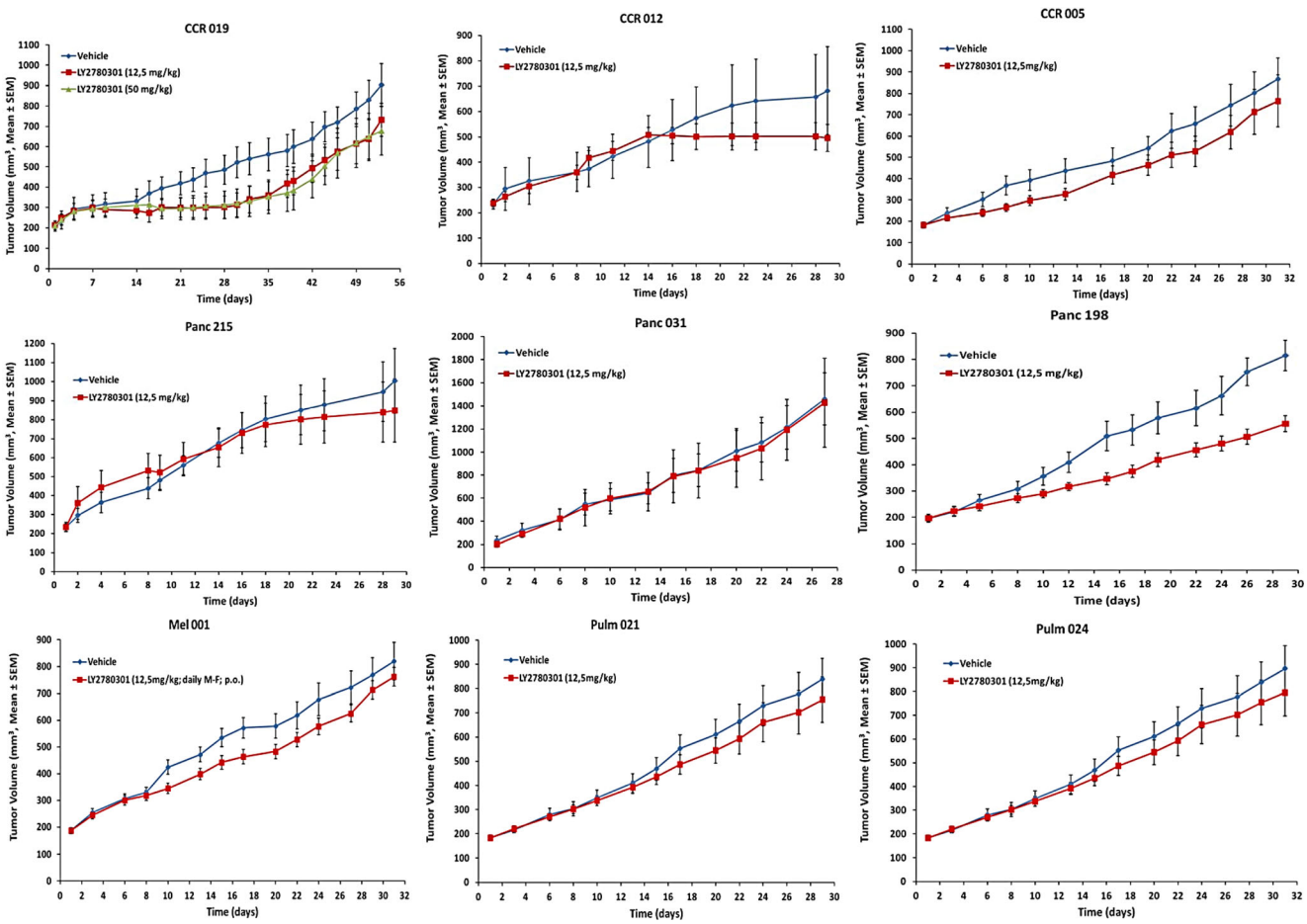
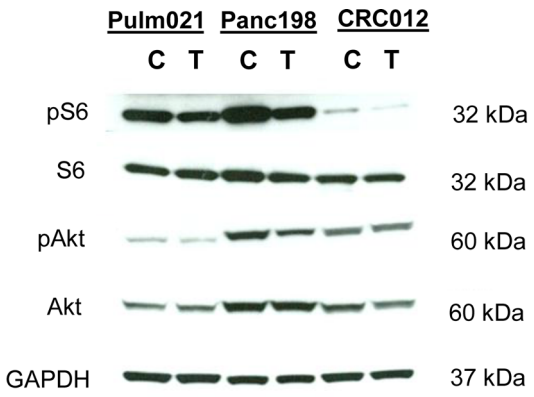


Fig. 2 Tumor growth inhibition curves of LY2780301 against a series of Avatar mouse models of cancer Groups of xenograft tumors (with indicated tumor model) were treated at the dose and schedule indicated. Tumor sizes were monitored and tumor volumes determined as described in the Methods section CRC, colorectal cancer; *Panc*, pancreatic cancer; *Pulm*, pulmonary cancer; *Mel*, melanoma



Preclinical Results

Model	Relevant Genetic Abnormalities	TGI
CRC005	*KRAS* (mut)	14
CRC012		38
CRC019		68[1]
Panc198	*KRAS* (mut); *TP53* (mut)	42
Panc215	*KRAS* (mut)	15
Panc031	*PI3K* (mut)	0
Pulm021	*KRAS* (mut)	13
Pulm024	*KRAS* (mut)	13
Mel001		18

[1] 12.5 mg/Kg dose level

Fig. 3 Tumor xenograft characteristics and expression of pS6 and pAkt. TOP Expression of (p)S6 and (p)Akt in xenograft samples from control (C) mice and mice treated for 29 days with LY2780301 (T), analyzed by western blot BOTTOM Genetic characteristics of the patient-derived tumor xenografts from (Pulm021) (Panc198) and (CRC012) Pulm, pulmonary cancer; *Panc*, pancreatic cancer; *CRC*, colorectal cancer

Assessment of pathway inhibition in treated models showed minimal PD effects consistent with the observed antitumor effects (Fig. 3).

Discussion

The primary objective of this phase I study was to recommend a phase II dose and schedule for LY2780301. However, because there were no DLTs, the MTD was not identified. Based on PK, PD, and clinical results, the dose of 500 mg QD was recommended for future studies . Signs of clinical benefit were observed in a few patients at different dose levels with different pretreated and progressing tumor types, including mesothelioma and breast cancer.

Mean PK exposures of LY2780301 at all dose levels exceeded the *a priori* established threshold of efficacious exposure based on preclinical data (>25,000 ng·hr/mL) and PK modeling. This might explain the high variability of exposure to LY2780301 and the lack of apparent dose proportionality. It was hypothesized that variability in exposures was related to variability of dissolved LY2780301 in the gastric compartment, a phenomenon that is pH-dependent. The terminal slopes for the plasma concentration-time curves across dose ranges were approximately parallel. The absence of dose proportionality was likely due to limited absorption of the higher dose because of low solubility. The sizable inter-individual variability within cohorts and sparse sampling (8 h or- 24-hours post-dose) precluded formal assessment of dose proportionality and limited characterization of the terminal elimination phase. The apparent trend for $t_{1/2}$ likely was caused by the dose-dependent increase in V_{ss}/F. Median accumulation, as calculated by the ratio of AUC from time zero to 8 h [$AUC_{(0–8)}$] on day 8 relative to day 1, ranged from 1.37 to 3.32.

There was evidence to suggest that LY2780301, at the recommended dose of 500 mg QD, affected IHC expression of pS6 in post-LY2780301 skin biopsies. Unfortunately, pS6 inhibition data were not obtained throughout the different dose regimens, limiting the possibility of describing an exposure-response relationship. At the highest dose levels in both schemes, PK/PD plots showed no visible correlation between inhibition of pS6 levels in the epidermis and exposure to LY2780301 or its desmethyl and didesmethyl metabolites.

Conducting parallel clinical and co-clinical investigations has recently been proposed as a strategy to optimize drug development [20–22]. In previous studies, co-clinical models enabled recapitulation of the heterogeneity of human cancer and were predictive of clinical outcome [14, 23, 24]. In this study, at the dose and schedules tested, the LY2780301 was not effective against any selected models (Fig. 2). This suggests that additional doses, schedules, or combinations would be needed for therapeutic efficacy. Of particular interest was model Mel001 developed from a patient with BRAF wild-type malignant melanoma (Fig. 3). The patient developed disease progression after one cycle of treatment, which was consistent with the lack of activity of LY2780301 in the patient's corresponding Avatar model. Patients in this trial were not selected based on their tumor molecular profile; no valid hypothesis was available at the time of study initiation to select patients based on tumor molecular profiling. The analysis of limited tumor profile data did not show a clear link between PI3K mutations and duration of stable disease.

There are several known therapeutic inhibitors of the PI3K/Akt/mTOR signaling pathway. Rapamycin and its analogues temsirolimus/CCI-779 (Torisel®, Wyeth Pharmaceuticals, Madison, NJ, USA) [25–27], everolimus/RAD 001 (Afinitor®, Novartis Pharma, Basel, Switzerland) [28], and ridaforolimus/A23573 (Ariad Pharma, Cambridge, MA, USA) [29] allosterically inhibit mTORC1. Following the approval of everolimus and temsirolimus for treating breast and

renal cancer, other similar compounds were developed, including: ATP-competitive, dual inhibitors of class I PI3K and mTORC1/2; 'pan-PI3K' inhibitors that inhibit all four isoforms of class I PI3K (α, β, δ, γ); isoform-specific inhibitors of the various PI3K isoforms; allosteric and catalytic inhibitors of Akt; and ATP-competitive inhibitors of mTOR only. While these compounds block the same signaling pathway, they have different levels of antitumor activity and varying levels of toxicity depending on the tumor's genetic context.

Similarly, ATP-competitive dual inhibitors of class I PI3K and mTORC1/2 may have a broader activity profile, but toxicity may preclude these compounds from attaining sufficient therapeutic doses. On the opposite extreme of selectivity, isoform-specific inhibitors may be only effective in specific contexts. INK-1402, a selective p110 α inhibitor, was more effective in PI3K catalytic subunit α (PI3KCA) mutated cell lines compared with mutated or absent PTEN. BYL719 (Novartis) and GDC-0032 (Genentech) reported partial responses exclusively in patients with PI3K3CA mutant tumors [30, 31]. Preclinical work showed that Akt inhibitors provide an interesting strategy for tumors with either PIK3CA or PTEN alterations [32–37]. Combined treatment with a PI3K-alpha inhibitor and an mTOR inhibitor was found to be synergistic in PIK3CA mutant tumors [38]. This provides a rationale for future trials in combination with other anticancer agents and molecular selection of patients.

Acknowledgments The study was sponsored by Eli Lilly and Company. The authors acknowledge Suzanne R.L. Young, Ph.D., and Jennifer M. Harris, Pharm.D., employees of Eli Lilly and Company, for writing assistance. Antonio Calles is a "Rio Hortega" fellowship grant recipient from the Instituto de Salud Carlos III (CM09/00283).

Conflicts of interest A.A., J.R., A.C., I.B., P.P.L.C., and J.M. have no conflicts of interest to report. W.B. is an employee and shareholder of Onyx Pharmaceuticals, a subsidiary of Amgen, and is a shareholder of Eli Lilly and Company. M.H. received research support and an honorarium from Eli Lilly and Company. E.C. received clinical research support and an honorarium (Advisory Board) from Eli Lilly and Company. P.W., B.A.M., U.O., and K.A.B... are employees and shareholders of Eli Lilly and Company.

Previous disclosure Data from this study, NCT01115751, were communicated as an oral presentation at The American Society of Clinical Oncology (ASCO) 2012 [J Clin Oncol 30, 2012 (suppl; abstr 3005).

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