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A Phase 1 Dose-Escalation, Pharmacokinetic, and Pharmacodynamic Evaluation of eIF-4E Antisense Oligonucleotide LY2275796 in Patients with Advanced Cancer

David S. Hong¹, Razelle Kurzrock¹, Yun Oh², Jennifer Wheler¹, Aung Naing¹, Les Brail³, Sophie Callies⁴, Valérie André⁵, Sunil K Kadam³, Aejaz Nasir³, Timothy R. Holzer³, Funda Meric-Bernstam¹, Mayer Fishman⁶, and George Simon^{6,*}

¹MD Anderson Cancer Center, Houston, TX

²Cancer Care Center of Frederick, Frederick, MD

³Eli Lilly and Company, Indianapolis, IN

⁴Eli Lilly France

⁵Eli Lilly UK

⁶H. Lee Moffitt Cancer Center, Tampa, FL

Abstract

Purpose—The antisense oligonucleotide, LY2275796, blocks expression of eIF-4E, an mRNA translation regulator upregulated in tumors. This Phase I study sought an appropriate LY2275796 dose in patients with advanced tumors.

Experimental Design—A 3-day loading dose, then weekly maintenance doses, were given to 1–3 patient cohorts, beginning with 100 mg and escalating. Plasma samples were collected to determine LY2275796 concentrations; tumor biopsies, to quantify eIF-4E mRNA/protein.

Results—Thirty patients with Stage 4 disease received 1 LY2275796 dose. A dose-limiting toxicity was observed at 1200 mg, with 1000 mg the maximum-tolerated dose. Across all dose levels, most patients (87%) had only grade 1–2 toxicities. LY2275796 pharmacokinetics supported the dosing regimen. Comparison of pre- and post-dose biopsies showed eIF-4E decreased in most patients. Fifteen patients had progressive disease, and seven patients achieved stable disease (minimum of 6 weeks) as best response, with two patients on therapy >3 months (one with melanoma, one with cystadenocarcinoma of the head/neck).

Conclusions—LY2275796 was well tolerated up to 1000 mg. Since tumor eIF-4E expression was decreased, but no tumor response observed, LY2275796 should be studied combined with other treatment modalities.

Corresponding author: David S. Hong, MD, Medical Director, Clinical Center for Targeted Therapy, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, Room Number: FC8.3050, Phone: 713.563.5844, Fax: 713.563.0533. dshong@mdanderson.org.

*Current address: Medical University of South Carolina, Charleston, SC

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INTRODUCTION

Genetic and epigenetic alterations can act together to release a cell from its normal growth constraints, enabling formation of a primary tumor (1). Expression of growth-critical proteins is regulated at multiple levels by disparate stimuli. Protein expression is controlled at the level of translation primarily through the activity of messenger RNA (mRNA) cap-binding protein eukaryotic initiation factor 4E (eIF-4E), which is frequently upregulated in tumors (2–4). The second generation 20-mer antisense oligonucleotide (ASO), LY2275796, was designed to bind to human eIF-4E mRNA. While first generation ASOs contained a phosphorothioate (a sulphur substitution of a nonbridging O) backbone, second generation ASOs, contain the phosphorothioate backbone plus the addition of 2' *O*-methoxyethyl modification of riboses at the 5' and 3' ends. These modifications enhance affinity for target RNA, thus improving stability and potency, improving antitumor potential and decreasing toxicity (5,6). As with other ASOs, inhibition of gene expression is mainly accomplished by recruitment of endogenous RNase H (7–9).

In preclinical studies, administration of LY2275796 to tumor-bearing mice resulted in a dose-related reduction in eIF-4E, protein expression, and suppression of tumor growth (8). The high dose of 25 mg/kg LY2275796 resulting in a 56% reduction in eIF4E protein expression relative to the mismatch control ASO and tumor growth suppression as assessed by no increase in tumor volume. Treatment with 5 mg/kg or 12.5 mg/kg dose group, showed statistically non-significant reduction in eIF4E expression, with no and intermediate reduction in tumor growth, respectively (8). Measures of generalized toxicity, body weight, organ weight, and liver transaminase levels were not affected despite an 80% reduction of eIF-4E levels in the liver, an organ where ASOs preferentially accumulate.

In the Phase I clinical trial summarized here, a dose escalation design was used to determine a Phase 2 LY2275796 dose that could be safely administered to patients with advanced solid tumors. Secondary objectives of this study were to characterize LY2275796 toxicities, determine its effect on eIF-4E expression, estimate its pharmacokinetic parameters, explore pharmacokinetic-pharmacodynamic relationships, and document antitumor activity.

MATERIALS AND METHODS

Patient Selection

Eligibility criteria included histologic or cytologic documentation of a malignancy for which no proven therapy exists; discontinuation of previous cancer therapies for 4 weeks; performance status (PS) 0/1 on the Eastern Cooperative Oncology Group (ECOG) scale (10); age ≥ 18 years; and written informed consent. Other criteria were adequate bone marrow, liver, and renal (11) function and adequate coagulation (activated partial

thromboplastin time [aPTT] and prothrombin time [PT] upper limit of normal). In parts B and C of the study, patients were to have disease amenable to biopsy.

Key exclusion criteria included previous treatment with antisense therapies; serious pre-existing medical conditions; and symptomatic central nervous system metastasis, hematological malignancies, or bleeding diathesis. Anticoagulant therapy (with the exception of the use of heparinized saline to maintain the patency of central venous catheters) was prohibited.

Dose Selection

An indirect PK-PD model was fitted to the tumor-bearing mouse model preclinical data (8) to describe the relationship between concentration and target inhibition. The model indicated that LY2275796 plasma exposure/concentrations of 297300 ng/h/mL, 65910 ng/mL (C_{max}) and 96 ng/mL (C_{trough} or C_{min} concentration) would be needed to achieve the 50–60% inhibition of target expression shown in the preclinical model to be necessary for tumor growth suppression. The preclinical model indicated that LY2275796 dose level of 800 mg (or greater) given daily for three days (loading doses on day 1, day 2, and day 3) and weekly thereafter (maintenance doses starting day 8) would lead to the targeted exposure.

A maximum tolerated dose (MTD) was not defined in the preclinical safety studies and a lowest no observed-adverse-effect level (NOAEL) for LY2275796 was defined at 5 mg/kg in mice and 2 mg/kg in monkeys. The pharmacokinetics of LY2275796 were characterized in monkeys following single and multiple doses in the toxicology study, with the monkey pharmacokinetic parameters then scaled to predict the human LY2275796 pharmacokinetics. Plasma concentrations following different doses were simulated in humans using the predicted human PK parameters. Data supported fixed-dose administration rather than dosing normalized by body weight or surface area. The proposed starting dose was 100 mg, which is approximately 1.3 mg/kg assuming an average weight of 75 kg. This dose had been shown to be tolerated in clinical studies with other ASOs and PK modeling predicted peak plasma concentrations following a 3-hour infusion, as had been found with a similar dose with other ASOs.

Study Design and Treatment Scheme

This was a nonrandomized, dose-escalating, Phase 1 investigation of LY2275796 (Accession # M15353, nucleotide 1285-1304), administered intravenously as a loading dose over 3 days, and thereafter as a weekly maintenance dose (Figure A; Supplementary data, online-only). In part A of the three-part study, the drug dose was escalated by increments of 100%, using single patient cohorts, until a significant toxicity was observed, or ongoing pharmacokinetic modeling suggested the need for pharmacodynamic data. In part B, the study drug dose was escalated by increments of 50%, using three-patient minimum cohorts, until a dose-limiting toxicity (DLT) was observed, or until the biological effective dose (BED) was established after considering the maximum tolerated dose (MTD), toxicity and efficacy indications, and the pharmacokinetic-pharmacodynamic profile. A DLT was defined as any grade 4 hematological toxicity lasting >5 days, grade 3 aPTT lasting >48 hours past infusion, or any grade 3/4 non-hematological toxicity within the first cycle. The

MTD was defined as the dose level below the DLT level. In part C, no further dose escalation occurred; rather, qualified patients were administered the MTD/BED to confirm pharmacology, further characterize toxicities, and to document any antitumor activity

Cycle 1 (days 1–7) consisted of a loading dose of LY2275796 administered daily on days 1–3 as a 3-hour infusion. At concentrations of 1000 mg or higher, if toxicities associated with peak plasma concentrations were observed at previous doses, three consecutive 24-hour infusions were permitted. Cycle 2 (days 8–28) consisted of a 3-hour infusion maintenance dose administered weekly (days 8, 15, 22). Cycle 3 started on day 29; this and subsequent cycles were 28-day cycles with four weekly maintenance doses. Patients remained on the study until progressive disease, a DLT, requested discontinuation, or non-compliance.

Treatment could be delayed up to 2 weeks to allow hematological toxicities to resolve to grade 2, and non-hematological toxicities to grade 1. Patients experiencing a DLT could be dose-reduced to the previous dose level, if they recovered sufficiently from the toxicity. Patients requiring a second dose reduction or experiencing a DLT in the first week of treatment were discontinued from the study.

Baseline and Treatment Assessments

Baseline assessments included medical history, vital signs, PS, radiologic tumor measurement, and measurement of palpable or visible lesions. Hematology, coagulation, blood chemistry, complement split products, and platelet activation markers were assessed at baseline, following cycle 1 and 2 infusions, and as needed following later cycle infusions. Hematologic and nonhematologic data were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) rating scale, Version 3.0 (12). At the discretion of the investigator, tumor response was assessed at intervals using Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 guidelines (13).

Pharmacokinetic and Pharmacodynamic Analyses

Plasma samples were collected at prespecified intervals for determination of LY2275796 concentrations using a validated enzyme-linked immunosorbent assay at a central laboratory. Patients participating in parts B and C of the study had pre-treatment tumor biopsies collected within 4 weeks before day 1 of the loading dose. Post-treatment biopsies were collected between days 21 through 28, and for some patients, an additional biopsy between days 8 through 11, times chosen to correspond to the expected timing of protein and mRNA reduction, respectively. To determine the pharmacodynamic effect of LY2275796, a validated branched DNA (bDNA) assay was used to measure eIF-4E mRNA, and immunohistochemistry was used to measure eIF-4E protein content using a mouse monoclonal antibody (BD Biosciences, clone 87). Tumor tissue samples were also analyzed by immunohistochemistry for related downstream markers of pharmacology and tumor growth using mouse monoclonal antibodies raised against Cyclin D1 (Novocastra, clone P2D11F11) and vascular endothelial growth factor (VEGF; Novus Biologicals, clone VG1).

Statistical Evaluation Methods

Pharmacokinetic, safety, and response data were analyzed for all patients who received at least one dose of LY2275796.

A non-linear mixed effect modeling technique (NONMEM, version VI) was used to analyze plasma LY2275796 data (log-transformed), allowing estimation of mean pharmacokinetic parameter values and inter-individual and intra-individual variability. Model parameters were estimated using the first order conditional estimation method with interaction (14). The inter-individual and intra-individual variability were coded as an exponential and a proportional relationship, respectively. Three and four compartmental pharmacokinetic models with elimination from the peripheral compartment and a similar elimination rate constant were fitted to the pharmacokinetic data. This approach was justified since second generation ASOs distribute extensively into tissues with different rates of uptake depending on tissue type (15). Additionally, ASOs are cleared through tissue nuclease cleavage; hence, the elimination rate in the peripheral compartment is dependent on nuclease activity rather than the usual elimination processes (glomerular filtration), which are generally reflected in the central plasma compartment.

LY2275796 plasma concentration data were also analyzed using a standard non-compartmental method (WinNonlin Enterprise, Version 5.2) to derive pharmacokinetic parameters for LY2275796: maximum plasma concentration (C_{max}), area under the plasma concentration versus time curve (AUC) and clearance (CL).

To quantify eIF-4E mRNA expression using the bDNA assay, the ratio of geometric means for post-dose to pre-dose eIF-4E expression was computed using a mixed model on log-transformed data with patient as a random factor and time as a fixed factor (pre- and post-dose). The log-transformed mean of the three available housekeeping genes (Actin B, HPRT1, RPS20) was to be used to normalize the ratio of geometric means for post-dose to pre-dose eIF-4E mRNA expression.

Using pre-treatment and post-treatment tumor biopsies, change in immunohistochemical expression of cytoplasmic and nuclear eIF-4E protein was assessed using H-scores (range 0–300), a system based on the proportions of viable tumor cells showing various levels of staining intensity (0, 1+, 2+, 3+), where 1+ represents mild, 2+ moderate, and 3+ the most intense staining (16).

RESULTS

Patients

Beginning January 2006 at two study centers, 30 patients enrolled and received at least one dose of study drug, with the last patient completing the study in March 2009. Table 1 summarizes patient demographics and disease characteristics. The allocation of patients among the three study parts (A–C), cohorts, and doses is summarized in Figure B (Supplementary data, online-only).

Treatment

Patients received a median of two completed cycles, with a range of one-to-five, and a total of 81 cycles during which 94% of the doses were given as planned). The most common reasons for study discontinuation were progressive disease (17 patients, 56.7%), followed by patient decision and physician decision (five patients each, 16.7%), and patient death (two patients, 6.7%). Additionally, one patient discontinued because of congestive heart failure, an event considered possibly study drug and protocol procedure-related, with the intravenous fluids necessary to administer the study drug as a possible contributing factor.

Among the 267 infusions of LY2275796 planned, 251 were administered (treatment compliance rate 94.0%), two (0.7%) were withdrawn, and 14 (5.2%) were omitted. Both dose withdrawals and most dose omissions (nine of 14, 64.3%) were attributed to adverse events (AEs). There were no dose reductions.

Biologically Effective Dose and Toxicity

The study proceeded through part A and B of the dose escalation scheme (Figure A, online) without any DLTs arising until the 1200 mg dose level, at which time grade 3 fatigue was observed in one of six patients dosed at this level. In addition, three of the six patients at 1200 mg had doses interrupted or withdrawn because of grade 1/2 AEs (pruritus, chills, hypersensitivity, pyrexia). The decision to designate 1000 mg as the MTD and the BED was based on an integrated analysis of the clinical, pharmacokinetic, and pharmacodynamic observations. Clinically, the 1200 mg dose was associated with dose delays and toxicities, whereas the 1000 mg dose had been well tolerated. Taken together, the pharmacokinetic differences (Table 3) did not suggest a substantial change between 1000 mg and 1200 mg. Finally, the assayed biomarkers (immunohistochemistry staining from baseline of eIF-4E nuclear, cytoplasmic; bDNA of eIF-4E gene, mRNA expression) showed little change between the 1000 mg dose and the 1200 mg dose.

Table 2 summarizes the toxicities at all dose levels possibly related to study drug according to investigator assessment. Across all dose levels, most (87%) patients had no or only low grade (1/2) toxicities; no grade 4 toxicities were reported. Four patients reported grade 3 toxicities: two patients with fatigue; one patient each with thrombocytopenia and lymphopenia. Fatigue (47%), nausea (33%), fever (27%), and vomiting (20%) were among the most frequently reported low-grade non-laboratory AEs. Prolongation of aPTT (37%) and PT (30%) and thrombocytopenia (17%) were the most frequently reported laboratory toxicities. Generally, the aPTT returned to baseline values within 24 hours, and only one patient experienced epistaxis (grade 1). Three patients required transfusions of packed red blood cells on study: two patients (both 1000 mg dose) received one transfusion each after a drop in hemoglobin level, and one patient (800 mg LY2275796 dose) received 3 transfusions, with the first transfusion coincident with non-study drug-related grade 1 hemolysis.

Four patients died while on study, three from progressive disease and one secondary to multi-organ failure, attributed to disease progression or non-study drug-related sepsis.

Efficacy

Of the 30 patients who received at least 1 dose of LY227596, 22 had at least one post-baseline reassessment by RECIST. Among these patients, 15 patients had progressive disease, and seven patients achieved stable disease (minimum of 6 weeks) as best response, with two patients on therapy >3 months (one with melanoma, one with cystadenocarcinoma of the head/neck). Among the patients with no post-baseline response assessment, four discontinued from the study, two died (one due to disease progression, the other from non-drug-related multi-organ failure), and one patient each discontinued for clinical progression and congestive heart failure.

Pharmacokinetics

Fitting the data to a multicompartment model, with the rate of input into circulation determined by the rate of intravenous infusion, the distribution of LY227596 into tissue and its elimination from plasma were found to occur at multiple rates or half-lives, leading to different concentrations in different tissues (Figure 1). The half-lives of these disposition phases were approximately 9 minutes, 2 hours, 5 hours, and 15 days, accounting for approximately 11%, 74%, 11%, and 4% of the overall plasma exposure, respectively, and indicating that the majority of the plasma exposure is distributed into the tissues within 24 hours of administration. The terminal elimination phase half-life of 15 days (range 9 to 25 days) corresponds to a small percentage of the plasma exposure (approximately 4%). This long terminal half-life is due to the moderate clearance and high volume of distribution that characterizes LY227596 pharmacokinetics. Table A (Supplementary data, online only) details the compartmental pharmacokinetic model parameters.

Table 3 summarizes the LY227596 plasma pharmacokinetic parameters illustrating that the increase in LY227596 exposure is less than dose proportional. No significant accumulation in plasma exposure (C_{max} and AUC from 0–24 hours) was observed over the first month of treatment (loading and maintenance doses).

Pharmacodynamics

Of the 25 patients in parts B and C, each had a pre-treatment biopsy; 18 patients also had a biopsy 21–28 days post-treatment, and three patients had a second post-treatment biopsy 8–11 days post-treatment. Due to the limited quantity of tumor tissue in some biopsies, not all immunohistochemistry and bDNA assays were performed on each biopsy. Compared to pre-treatment tumor biopsies, post-treatment tumor biopsies in patients receiving the BED (1000 mg) or more, showed a reduction in cytoplasmic expression of eIF-4E protein in nine of twelve patients, while the nuclear eIF-4E protein level was reduced in three of six patients (Figure 2,3). Likewise, at doses 1000 mg, cytoplasmic VEGF was reduced in eight of twelve patients, nuclear VEGF in five of eleven patients, cytoplasmic Cyclin D1 in two of four patients, and nuclear Cyclin D1 in five of eleven patients.

Using the bDNA assay to quantify eIF-4E gene expression, eIF-4E mRNA was found to be reduced in six of seven evaluable pairs. The estimate of the ratio of the least squares geometric means for post-treatment to pre-treatment bDNA eIF-4E expression was 0.19 (90% CI: 0.06 to 0.58), corresponding to an 80% reduction in eIF-4E mRNA expression

post-treatment compared to pre-treatment (90% CI: 42% to 96%). The eIF-4E expression results could not be normalized via the housekeeping genes, since the housekeeping genes appear to have been affected by the eIF-4E ASO, with an estimated reduction in expression of 64% (90% CI: 7% to 86%).

DISCUSSION

Using a loading and maintenance dose regimen, the MTD and BED of the eIF-4E ASO, LY2275796, was identified as 1000 mg. LY2275796 was well tolerated with a low rate of grade 3/4 toxicity. The only DLT noted was grade 3 fatigue (at 1200 mg). As observed with other ASOs, by some unknown mechanism, a portion of the patients (37%) exhibited aPTT prolongation; however, it was grade 1/2 and recovered to baseline levels within 24 hours. Low grade fatigue and fever were also observed, similar to the febrile events noted with other ASOs, and thought to be non-sequence specific and related to the polyanionic nature of these compounds and their effects on cytokine release.

Prior pre-clinical pharmacology, toxicology, and biomarker studies with LY2275796 permitted the construction of a pharmacokinetic/pharmacodynamic model to approximate the dose range required to produce an anti-tumor response (17). A long terminal half-life (>10 days) was predicted based on the greater resistance of second generation ASOs to tissue nuclease-mediated degradation. Further, a loading dose followed by a weekly maintenance dose was proposed to rapidly achieve an effective LY2275796 concentration in the tumor and to maintain this effect over time. The pharmacokinetic profile observed in this study matched that anticipated by the model. The predicted LY2275796 pharmacokinetic properties of extensive volume of distribution and moderate clearance leading to a long terminal half-life were confirmed and found to support the dosing strategy.

Pre- and post-treatment biopsies were obtained during the study at time points where reductions in eIF-4E mRNA and protein were predicted. A decrease in eIF-4E expression was observed in the majority of patients by both immunohistochemistry and/or bDNA at doses 1000 mg (Figures 2,3). Two targets of eIF-4E, VEGF and cyclin D1, were also found by immunohistochemistry to be reduced (~35–60%) in some patients, but not all. Notably, the bDNA assay is a more sensitive and quantitative assay while immunohistochemistry allows assessment of biomarker expression in viable tumor cells. Limitations include inherent sampling error associated with tumor biopsies, the relatively small number of subjects, and the inability to normalize the mRNA results due to apparent downward change of housekeeping gene expression which may also be related directly to eIF-4E inhibition..

Our determination of BED at 1000mg was based on both the pharmacokinetic and pharmacodynamic findings in the study. Pharmacokinetic and pharmacodynamics parameters did not change substantially from the 1000 mg to the 1200 mg dose. Additionally, there was an increase in toxicity and decreased tolerability at the 1200 mg dose. The preclinical models had shown a 50–60% knockdown in eIF4E protein was sufficient for tumor growth suppression in a tumor-bearing mouse model (8). In our study we observed an 80% reduction in eIF-4E mRNA in post-treatment biopsies as compared to pretreatment biopsies. In addition, we observed a reduction in cytoplasmic expression of

eIF-4E protein in nine of twelve patients and nuclear eIF-4E protein in three of six patients. Although, eIF-4E mRNA and nuclear protein expression were reduced in most tumor biopsies, no tumor responses were observed. Possibly, the eIF-4E downregulation achieved clinically is not as robust as that achieved in preclinical models where anti-tumor effects of eIF-4E downregulation were observed (8,18). Alternately, blocking a component of one of the protein translational complexes may not immediately result in cytotoxic/apoptotic events and tumor size reduction, as seen with cytotoxic agents. Instead, the effect might be cytostasis, resulting in stable disease. However, the relatively low (32%) stable disease rate in this study and short duration (only 2 patients >3 months) would suggest that anti-tumor effects of LY2275796 might best be achieved in combination with chemotherapy or radiation.

The pharmacokinetic profile of LY2275796 in this first-in-human study matched the anticipated profile based on the model and supports the present dosing schedule of a 3-day consecutive loading dose followed by weekly maintenance dose. The 1000 mg dose level was identified as being well tolerated and effective at inhibiting eIF-4E mRNA and protein expression, with some evidence of the expression of two relevant downstream targets also being impacted. As with all ASO therapy, the main limitation of LY2275796 may be that it impacts a single target in a disease state maintained by genetically independent, functionally redundant alterations. Certainly, using LY2275796 ASO therapy in combination with other treatment modalities might achieve improved anti-tumor effects, especially if combined with drugs that have additive or synergistic preclinical effects when combined with eIF-4E suppression. Recent studies pairing an ASO with chemotherapy have included OGX-011 in combination with docetaxel (19) and an ASO against the R2 subunit of ribonucleotide reductase also in combination with docetaxel (20). An additional approach to further potentiate the therapeutic efficacy of eIF-4E ASO therapy may be the selection of patients with tumors that overexpress eIF-4E. Currently, two phase I/II trials with combination chemotherapy are underway, in which eIF-4E ASO is being examined for the treatment of patients with castrate-resistant prostate cancer and for the treatment of patients with stage IV non-small cell lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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STATEMENT OF TRANSLATIONAL RELEVANCE

Protein expression is controlled at the level of translation primarily through messenger RNA (mRNA) cap-binding protein eukaryotic initiation factor 4E (eIF-4E), which is frequently upregulated in tumors. LY2275796, an antisense oligonucleotide to eIF-4E, was developed to decrease eIF-4E expression in tumors, thereby inhibiting their growth. Following successful studies in mice, a Phase I dose escalation design was used to determine a dose level that could be safely administered to patients with advanced solid tumors. LY2275796 was well tolerated at the 1000 mg dose level with only grade 1–2 toxicities. Plasma samples and tumor biopsies taken before and during the study characterized LY2275796 pharmacokinetics and quantified changes in eIF-4E mRNA and protein, thus fulfilling an important prerequisite for continuing study of LY2275796 in a Phase II trial. Since tumor eIF-4E expression was inhibited, but no tumor response observed, results suggest future trials should examine LY2275796 in combination with other treatment modalities.

Figure 1. Simulated Plasma PK Profile Showing Median, 5th and 95th Percentile LY2275796 PK Profile Based on the PK Model and Collected Data after 1000 mg LY2275796

The upper panel illustrates the change in plasma concentration over a 25-day period with loading doses at day 1, 2, and 3 and maintenance doses on day 8 and day 15. The arrows in the figure designate LY2275796 dose administration. The lower panel expands a portion of the upper figure.

Figure 2. Protein Expression (Immunohistochemistry) and Gene Expression Data (branched DNA assay) for Doses of 1000 and 1200 mg

Immunohistochemistry (upper panel) and branched DNA (lower panel) data of the expression of cyclin D1, eIF-4E, and VEGF protein and eIF-4E mRNA, respectively, from patients receiving either a 1000 mg or 1200 mg LY2275796 regimen. For the immunohistochemistry data, open circles correspond to percent post-LY2275796 change in the biomarker expression levels compared to respective baseline expression of various IHC biomarkers for individual patients; closed circles represent medians with inter-quartile range. For the branched DNA data, data are presented with a least square mean and 90%

confidence limits from the model. The asterisks represent data points (outliers) beyond the range of the graph, but still included in summary statistics.

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Figure 3. Decrease in immunohistochemical expression of eIF-4E at 1000 or 1200 mg dose

Left and right column micrographs are paired images derived from tumor tissue from patients with melanoma (3A–D) and epithelial mesothelioma (3E–H), stained with H &E and eIF-4E immunoperoxidase stains, respectively. [Trestle SL4 Slide Scanner, Software Version: 1.0.0.73 (400 dpi). Original magnification: X200].

A. Cycle 1, pre-treatment biopsy of malignant melanoma.

B. Majority of the tumor cells show strong (3+) cytoplasmic staining (cytoplasmic H-score 270). Most tumor cell nuclei show weak (1+) or no nuclear staining (nuclear H-score 30).

C. Cycle 1, post-treatment (Day 25) biopsy of malignant melanoma.

D. Majority of the tumor cells show moderate (2+) or weak (1+) cytoplasmic staining (cytoplasmic H-score 180). The tumor cell nuclei are negative for eIF-4E stain (nuclear H-score 0). Some tumor cell nuclei in the pre-treatment biopsy (3B) show weak staining that is not evident in the post-treatment biopsy (3D).

E. Cycle 1, pre-treatment biopsy of right axillary epithelial mesothelioma featuring nests of tumor cells.

F. Majority of the tumor cells (60%) show 1+ staining, 25% are 2+ and 5% are 3+ (cytoplasmic H-score 125). About one third of the tumor cell nuclei show 1+ staining (nuclear H-score 35).

G. Cycle 1, post-treatment (day 23) biopsy of right axillary epithelial mesothelioma.

H. Only about one third of the tumor cells show weak cytoplasmic staining (cytoplasmic H-score 30). All tumor cell nuclei are negative (nuclear H-score 0). Some tumor cell nuclei in the pre-treatment biopsy (3F) show 1+ staining that is not evident in the post-treatment biopsy (3H)

Table 1**Patient Demographics and Disease Characteristics**

Parameter	N=30
Median age (range), years	58.5 (25, 79)
Gender, n (%)	
Male	16 (53.3)
Female	14 (46.7)
Origin	
Caucasian	26 (86.7)
African	2 (6.7)
Hispanic	2 (6.7)
ECOG performance scale, n (%)	
0	12 (40)
1	18 (60)
Disease stage at entry, n (%)	
Stage IV	30 (100)
Pathological Diagnosis:	
Mesothelioma	4 (13.3)
Head & Neck Carcinoma	4 (13.3)
Colon, adenocarcinoma	3 (10)
Malignant Melanoma	3 (10)
Lung, Non-small Cell Carcinoma	3 (10)
Lung, Small Cell Carcinoma	2 (6.7)
Uterine Leiomyosarcoma	2 (6.7)
Sarcoma	2 (6.7)
Other	7 (23.3)
Prior Treatment	
Systemic therapy	28 (93)
Surgery	18 (60)
Radiotherapy	19 (63)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; NOS = not otherwise specified

Table 2

Treated Patients (N=30) with CTCAE 3.0 Possibly Drug-Related Toxicities *

Adverse Event	Total no. pts (%)	Dose 100/200/400 mg (n=4)		Doses 600/800 mg (n=6)		Doses 1000 mg (n=14)		Doses 1200 mg (n=6)	
		Gr 1/2 n (%)	Gr 3 n (%)	Gr 1/2 n (%)	Gr 3 n (%)	Gr 1/2 n (%)	Gr 3 n (%)	Gr 1/2 n (%)	Gr 3 n (%)
Laboratory									
Anemia	3 (10)	1		1		1			
AST	2 (7)			1				1	
Creatinine	2 (7)					1		1	
Lymphopenia	1 (3)								1
aPTT prolonged	11 (37)			2		4		5	
PT prolonged	9 (30)			2		3		4	
INR increased	4 (13)			1		2		1	
Thrombocytopenia	5 (17)			3		1		1	
Non-Laboratory									
Allergic hypersensitivity	2 (7)					1		1	
Anorexia	4 (13)			2		1		1	
Diarrhea	2 (7)			1		1			
Fatigue	14 (47)	1		3	1	5		3	1
Fever	8 (27)					6		2	
Nausea	10 (33)	1		4		2		3	
Neuropathy: Sensory	3 (10)					2		1	
Pruritus/Itching	2 (7)							2	
Rigors/Chills	4 (13)			1		1		2	
Vomiting	6 (20)			2		1		3	
Weight Loss	3 (10)			1		1		1	

Abbreviations: aPTT=activated partial thromboplastin time; AST=aspartate transaminase; INR=International normalized ratio; Gr=grade; PT= prothrombin time; pts=patients

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Averse events thought to be possibly study drug-related by investigators were collected from the first dose of study drug until 30 days after the patient discontinued from the study. Only toxicities reported in 2 patients over all dose levels are reported, except lymphopenia which was only experienced by one patient, but was grade 3. No grade 4 toxicities were reported. Patients are counted only once per event, with the highest grade experienced noted.

*

Table 3

LY2275796 Plasma PK Parameters (Geomean [CV%] or Individual Value) following Day 1, Day 2, and Day 3 Dosing (3 Hour Infusion)

Dose (mg)	Day 1			Day 3		
	No. of patients	C _{max} (ng/mL)	No. of patients	C _{max} (ng/mL)	Plasma CL _{ss} (L/h)	AUC _τ (ng•h/mL)
100	1	15714	1	27645	1.11	90190
200	1	23031	1	20596	2.58	77448
400	2	31843, 42020	2	31840, 37052	2.91, 2.24	136965, 174750
600	1	51773	1	33161	3.39	165764
800	4	38686 (46.8)	4	36709 (36.0)	4.31 (25.5)	178160 (24.7)
1000	11 ^b	85298 (45.7) 38792 – 135262 ^a	14	73738 (39.7) 34423 – 125823 ^a	3.05 (53.5) 1.61 – 8.64 ^a	314284 (52.5) 109980 – 569840 ^a
1200	5 ^c	64536 (11.2)	6	30904 (17.7)	7.75 (15.9)	150219 (16.2)

Abbreviations: AUC=area under the plasma concentration time curve; CL_{ss}=steady state clearance rate; C_{max}= maximum plasma concentration; CV=coefficient of variation (represents the inter-individual variability)

^aRange

^{b,c} Some patients did not have PK samples drawn on Day 1; hence, the number of patients is less on Day 1 than Day 3.