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


Abstract

Purpose: The antisense oligonucleotide LY2275796 blocks expression of cap-binding protein eukaryotic initiation factor 4E (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 to 3 patient cohorts, beginning with 100 mg and escalating. Plasma samples were collected to determine LY2275796 concentrations and tumor biopsies to quantify eIF-4E mRNA/protein.

Results: Thirty patients with stage 4 disease received 1 or more LY2275796 dose. A dose-limiting toxicity was observed at 1,200 mg, with 1,000 mg the maximum-tolerated dose. Across all dose levels, most patients (87%) had only grade 1 to 2 toxicities. LY2275796 pharmacokinetics supported the dosing regimen. Comparison of pre- and postdose biopsies showed eIF-4E decreased in most patients. Fifteen patients had progressive disease, and 7 patients achieved stable disease (minimum of 6 weeks) as best response, with 2 patients on therapy for more than 3 months (one with melanoma, one with cystadenocarcinoma of the head/neck).

Conclusions: LY2275796 was well tolerated up to 1,000 mg. Because tumor eIF-4E expression was decreased, but no tumor response observed, LY2275796 should be studied combined with other treatment modalities.

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 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. Whereas first generation ASOs contained a phosphorothioate (a sulfur 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 results in a 56% reduction in eIF-4E 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 showed statistically nonsignificant reduction in eIF-4E 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.
**Translational Relevance**

Protein expression is controlled at the level of translation primarily through 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 1,000-mg dose level with only grade 1 to 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. Because tumor eIF-4E expression was inhibited, but no tumor response observed, results suggest that future trials should examine LY2275796 in combination with other treatment modalities.

**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 or more weeks, performance status (PS) 0/1 on the Eastern Cooperative Oncology Group (ECOG) scale (10), age 18 years or older, and written informed consent. Other criteria were adequate bone marrow, liver, and renal function. In parts B and C of the study, patients were to maintain the patency of central venous catheters.

Key exclusion criteria included previous treatment with antisense therapies, serious preexisting medical conditions, and symptomatic central nervous system metastasis, hematologic 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 297,300 ng/h/mL, 65,910 ng/mL (C_{\text{AUC}0–\text{t}}), and 96 ng/mL (C_{\text{AUC}0–\text{tmin}}) would be needed to achieve the 50% to 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 3 days (loading doses on days 1, 2, and 3) and weekly thereafter (maintenance doses starting at 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 PK 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 1,285–1,304), administered intravenously as a loading dose over 3 days, and thereafter as a weekly maintenance dose (Supplementary Fig. S1). In part A of the 3-part study, the drug dose was escalated by increments of 100% or less, using single patient cohorts, until a significant toxicity was observed, or ongoing PK modeling suggested the need for PD data. In part B, the study drug dose was escalated by increments of 50% or less, using 3-patient minimum cohorts, until a dose-limiting toxicity (DLT) was observed, or until the biological effective dose (BED) was established after considering the MTD, toxicity, and efficacy indications, and the PK-PD profile. A DLT was defined as any grade 4 hematologic toxicity lasting more than 5 days, grade 3 aPTT lasting more than 48 hours post infusion, or any grade 3/4 nonhematologic 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 to 3 as a 3-hour...
infusion. At concentrations of 1,000 mg or higher, if toxicities associated with peak plasma concentrations were observed at previous doses, 3 consecutive 24-hour infusions were permitted. Cycle 2 (days 8–28) consisted of a 3-hour infusion maintenance dose administered weekly (days 8, 15, and 22). Cycle 3 started on day 29; this and subsequent cycles were 28-day cycles with 4 weekly maintenance doses. Patients remained on the study until progressive disease, a DLT, requested discontinuation, or noncompliance.

Treatment could be delayed up to 2 weeks to allow hematologic toxicities to resolve to grade 2 or less, and nonhematologic toxicities to grade 1 or less. 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 ELISA at a central laboratory. Patients participating in parts B and C of the study had pretreatment tumor biopsies collected within 4 weeks before day 1 of the loading dose. Posttreatment 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 PD 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 VEGF (Novus Biologicals; clone VG1).

Statistical evaluation methods
PK, safety, and response data were analyzed for all patients who received at least a single dose of LY2275796.

A nonlinear mixed effect modeling technique (NONMEM, version VI) was used to analyze plasma LY2275796 data (log-transformed), allowing estimation of mean PK parameter values and interindividual and intraindividual variability. Model parameters were estimated using the first order conditional estimation method with interaction (14). The interindividual and intraindividual variability were coded as an exponential and a proportional relationship, respectively. Three and 4 compartmental PK models with elimination from the peripheral compartment and a similar elimination rate constant were fitted to the PK data. This approach was justified because second generation ASOs distribute extensively into tissues with different rates of uptake depending on tissue type (15). In addition, 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 noncompartmental method

**Table 1.** Patient demographics and disease characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range), y</td>
<td>58.5 (25–79)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (46.7)</td>
</tr>
<tr>
<td>Origin</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>26 (86.7)</td>
</tr>
<tr>
<td>African</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>ECOG performance scale, n (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12 (40)</td>
</tr>
<tr>
<td>1</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Disease stage at entry, n (%)</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Pathologic diagnosis</td>
<td></td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Head and neck carcinoma</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Colon, adenocarcinoma</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Lung, non-small cell carcinoma</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Lung, small cell carcinoma</td>
<td>2 (6.7)</td>
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<tr>
<td>Uterine leiomyosarcoma</td>
<td>2 (6.7)</td>
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<tr>
<td>Sarcoma</td>
<td>2 (6.7)</td>
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<tr>
<td>Other</td>
<td>7 (23.3)</td>
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<td>Prior treatment</td>
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<td>Systemic therapy</td>
<td>28 (93)</td>
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<tr>
<td>Surgery</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>19 (63)</td>
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</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NOS, not otherwise specified.
(WinNonlin Enterprise, Version 5.2) to derive PK parameters for LY2275796: maximum plasma concentration ($C_{\text{max}}$), 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 postdose to predose 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 postdose). The log-transformed mean of the 3 available housekeeping genes (Actin B, HPRT1, and RPS20) was to be used to normalize the ratio of geometric means for postdose to predose eIF-4E mRNA expression.

Using pretreatment and posttreatment 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+, and 3+), in which 1+ represents mild, 2+ moderate, and 3+ the most intense staining (16).

### Results

#### Patients

Beginning January 2006 at 2 study centers, 30 patients enrolled and received at least a single 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 3 study parts (A–C), cohorts, and doses is summarized in Supplementary Figure S2.

#### Treatment

Patients received a median of 2 completed cycles, with a range of 1 to 5, 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 (5 patients each, 16.7%), and patient death (2 patients, 6.7%). In addition, one patient discontinued because of congestive heart failure.

### Table 2. Treated patients ($n = 30$) with CTCAE 3.0 possibly drug-related toxicities

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Total no. pts (%)</th>
<th>Gr 1/2 n (%)</th>
<th>Gr 3 n (%)</th>
<th>Dose 100/200/400 mg (n = 4)</th>
<th>Dose 600/800 mg (n = 6)</th>
<th>Dose 1,000 mg (n = 14)</th>
<th>Dose 1,200 mg (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>3 (10)</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>2 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>2 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>1 (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aPTT prolonged</td>
<td>11 (37)</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT prolonged</td>
<td>9 (30)</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR increased</td>
<td>4 (13)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5 (17)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Allergic hypersensitivity</td>
<td>2 (7)</td>
<td></td>
<td></td>
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<tr>
<td>Anorexia</td>
<td>4 (13)</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (7)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>14 (47)</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>8 (27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>10 (33)</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropathy: sensory</td>
<td>3 (10)</td>
<td></td>
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<tr>
<td>Pruritus/itching</td>
<td>2 (7)</td>
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<tr>
<td>Rigors/chills</td>
<td>4 (13)</td>
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<tr>
<td>Vomiting</td>
<td>6 (20)</td>
<td></td>
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<tr>
<td>Weight loss</td>
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<td></td>
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</tr>
</tbody>
</table>

**Abbreviations:** AST, aspartate transaminase; INR, international normalized ratio; Gr, grade; pts, patients.

*Adverse 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 or more 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.*
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%), 2 (0.7%) were withdrawn, and 14 (5.2%) were omitted. Both dose withdrawals and most dose omissions (9 of 14, 64.3%) were attributed to adverse events (AE). There were no dose reductions.

**Biologically effective dose and toxicity**

The study proceeded through part A and B of the dose escalation scheme (Supplementary Fig. S1) without any DLTs arising until the 1,200 mg dose level, at which time grade 3 fatigue was observed in 1 of 6 patients dosed at this level. In addition, 3 of the 6 patients at 1,200 mg had doses interrupted or withdrawn because of grade 1/2 AEs (pruritus, chills, hypersensitivity, and pyrexia). The decision to designate 1,000 mg as the MTD and the BED was based on an integrated analysis of the clinical, PK, and PD observations. Clinically, the 1,200 mg dose was associated with dose delays and toxicities, whereas the 1,000 mg dose had been well tolerated. Taken together, the PK differences (Table 3) did not suggest a substantial change between 1,000 mg and 1,200 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 1,000-mg dose and the 1,200-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: 2 patients with fatigue; 1 patient each with thrombocytopenia and lymphopenia. Fatigue (47%), nausea (33%), fever (27%), and vomiting (20%) were among the most frequently reported low-grade nonlaboratory 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 1 patient experienced epistaxis (grade 1). Three patients required transfusions of packed red blood cells on study: 2 patients (both 1,000 mg dose) received 1 transfusion each after a drop in hemoglobin level, and 1 patient (800 mg LY2275796 dose) received 3 transfusions, with the first transfusion coincident with nonstudy drug-related grade 1 hemolysis.

Four patients died while on study, 3 from progressive disease and 1 secondary to multiorgan 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 1 postbaseline reassessment by RECIST. Among these patients, 15 patients had progressive disease, and 7 patients achieved stable disease (minimum of 6 weeks) as best response, 1 with melanoma, 1 with cystadenocarcinoma of the head/neck. The patients with no postbaseline response assessment, 4 discontinued from the study, 2 died (one due to disease progression, the other from non–drug-related multiorgan failure), and 1 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 LY2275796 into tissue and its elimination from plasma were found to occur at multiple rates or half-lives, leading to different
concentrations in different tissues (Fig. 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 9–25 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 LY2275796 pharmacokinetics. Supplementary Table S1 details the compartmental PK model parameters.

Table 3 summarizes the LY2275796 plasma PK parameters illustrating that the increase in LY2275796 exposure is less than dose proportional. No significant accumulation in plasma exposure (\(C_{\text{max}}\) 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 pretreatment biopsy; 18 patients also had a biopsy 21 to 28 days...
posttreatment, and 3 patients had a second posttreatment biopsy 8 to 11 days posttreatment. Due to the limited quantity of tumor tissue in some biopsies, not all immunohistochemistry and bDNA assays were done on each biopsy. Compared with pretreatment tumor biopsies, posttreatment tumor biopsies in patients receiving the BED (1,000 mg) or more, showed a reduction in cytoplasmic expression of eIF-4E protein in 9 of 12 patients, whereas the nuclear eIF-4E protein level was reduced in 3 of 6 patients (Figs. 2, 3). Likewise, at doses ≥1,000 mg, cytoplasmic VEGF was reduced in 8 of 12 patients, nuclear VEGF in 5 of 11 patients, cytoplasmic cyclin D1 in 2 of 4 patients, and nuclear cyclin D1 in 5 of 11 patients.

Using the bDNA assay to quantify eIF-4E gene expression, eIF-4E mRNA was found to be reduced in 6 of 7 evaluable pairs. The estimate of the ratio of the least squares geometric means for posttreatment to pretreatment bDNA eIF-4E expression was 0.19 (90% CI: 0.06–0.58), corresponding to an 80% reduction in eIF-4E mRNA expression posttreatment compared with pretreatment (90% CI: 42–96). The eIF-4E expression results could not be normalized via the housekeeping genes because the housekeeping genes seem to have been affected by the eIF-4E ASO, with an estimated reduction in expression of 64% (90% CI: 7–86).

Discussion

Using a loading and maintenance dose regimen, the MTD and BED of the eIF-4E ASO, LY2275796, was identified as 1,000 mg. LY2275796 was well tolerated with a low rate of grade 3/4 toxicity. The only DLT noted was grade 3 fatigue (at 1,200 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 nonsequence specific and related to the polyanionic nature of these compounds and their effects on cytokine release.

Prior preclinical pharmacology, toxicology, and biomarker studies with LY2275796 permitted the construction of a PK-PD model to approximate the dose range required to produce an antitumor 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. Furthermore, 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 PK profile observed in this study matched that anticipated by the model. The predicted LY2275796 PK 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 posttreatment 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 1,000 mg or more (Figs. 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, whereas 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.

Figure 3. Decrease in immunohistochemical expression of eIF-4E at 1,000 or 1,200 mg dose. Left and right column micrographs are paired images derived from tumor tissue from patients with melanoma (A–D) and epithelial mesothelioma (E–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: ×200]. A, cycle 1, pretreatment 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, posttreatment (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 pretreatment biopsy (B) show weak staining that is not evident in the posttreatment biopsy (D). E, cycle 1, pretreatment 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, posttreatment (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 pretreatment biopsy (F) show 1+ staining that is not evident in the posttreatment biopsy (H).
Our determination of BED at 1,000 mg was based on both the PK and PD findings in the study. PK and PD parameters did not change substantially from the 1,000 mg to the 1,200 mg dose. In addition, there was an increase in toxicity and decreased tolerability at the 1,200 mg dose. The preclinical models had shown a 50% to 60% knockdown in eIF-4E protein which 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 posttreatment biopsies as compared with pretreatment biopsies. In addition, we observed a reduction in cytoplasmic expression of eIF-4E protein in 9 of 12 patients and nuclear eIF-4E protein in 3 of 6 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 in which antitumor 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 antitumor effects of LY2275796 might best be achieved in combination with chemotherapy or radiation.

The PK 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 1,000 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 2 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 antitumor 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, 2 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.

Disclosure of Potential Conflicts of Interest

I. Brail, S. Calliers, V. André, S.K. Kadam, A. Nasir, and T.R. Holzer are employees of Eli Lilly and Company and L. Brail has ownership interest (including patents) in the company. The other authors disclosed no potential conflicts of interest.

Acknowledgments

The authors thank all of the patients and institutions involved in this study and also Ugur Aytaç and Linda Ricks, research coordinators at MD Anderson, and Michelle Burton, Jennifer Cooksey, and Anne Dellaportas, research and data coordinators at H. Lee Moffitt Cancer Center. In addition, the authors thank Mary Dugan Wood and Joseph Durrant for writing and editorial assistance, and William Bumgardner and Aimée Bence for assistance with study administration.

Grant Support

This study (H9R-MC-JKAF) was funded by Eli Lilly and Company, Indianapolis, Indiana.

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Received February 16, 2011; revised July 12, 2011; accepted July 27, 2011; published OnlineFirst August 10, 2011.

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Clin Cancer Res 2011;17:6582-6591. Published OnlineFirst August 10, 2011.

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