Cancer Therapy: Clinical

A Phase I Trial of LY2510924, a CXCR4 Peptide Antagonist, in Patients with Advanced Cancer

Matthew D. Galsky1, Nicholas J. Vogelzang1, Paul Conkling2, Eyaas Raddad3, John Polzer3, Stephanie Roberson3, John R. Stille3, Mansoor Saleh5, and Donald Thornton4

Abstract

Purpose: Overexpression of C-X-C motif receptor 4 (CXCR4) is implicated in tumor progression. LY2510924 is a peptide antagonist, which blocks stromal cell–derived factor-1 (SDF1) from CXCR4 binding.

Experimental Design: This phase I study included two parts: a 3+3 dose escalation (part A) and dose confirmation (part B). LY2510924 was administered as a daily subcutaneous injection on a 28-day cycle. The primary objective was to determine the recommended phase II dose. Secondary objectives included safety, pharmacokinetics, efficacy, and pharmacodynamic response, including mobilization of CD34+ hematopoietic stem cells into the peripheral blood.

Results: Forty-five patients were enrolled, 25 in part A and 20 in part B. Patients were administered increasing doses of LY2510924: 1.0, 2.5, 5.0, 10, 20, and 30 mg/day for part A and 2.5 or 20 mg/day for part B. Two patients (30-mg/day cohort) experienced dose-limiting toxicities of grade 3 increased neutrophil count. The maximum tolerated dose (MTD) was 20 mg/day. The most common drug-related treatment-emergent adverse events were fatigue (9%), injection-site reaction (9%), injection site pruritus (7%), and nausea (7%). The best response was stable disease for nine patients (20%). At the end of cycle 1, mean peak LY2510924 plasma concentration and the 24-hour area under the plasma concentration versus time curve increased slightly more than dose proportionally. LY2510924 dose dependently increased CD34+ cell counts in peripheral blood up to 18-fold.

Conclusions: LY2510924 demonstrated CD34+ cell mobilization at doses ≥2.5 mg/day with a tolerable safety profile up to an MTD of 20 mg/day. Clin Cancer Res; 20(13); 1–8. ©2014 AACR.

Introduction

Metastatic tumor spread is the leading cause of cancer deaths (1). The tumor microenvironment has several biologic processes that can contribute to the metastatic process including chemokine signaling, which regulates cell migration. Cells with chemokine receptors migrate in response to cytokine concentration gradients within the microenvironment. The chemokine C-X-C motif receptor 4 (CXCR4) is often overexpressed on cancer cells (2) and is involved in the metastasis of solid tumors such as breast, ovarian, and thyroid tumors and progression of lymphomas and chronic lymphocytic leukemia (CLL; refs. 3–7). The α-chemokine stromal cell-derived factor 1 (SDF1/CXCL12) signals through CXCR4 and promotes tumor growth by stimulation of cell proliferation and survival processes (8–10). Indirectly, secretion of SDF1 promotes tumor growth by attracting endothelial cells to the tumor microenvironment, which contribute to angiogenesis (11, 12).

LY2510924 is a potent selective peptide antagonist of CXCR4. LY2510924 inhibits SDF1 binding to CXCR4 and blocks downstream signaling. Preclinical data show that CXCR4 antagonists can cause the mobilization of leukocytes and stem cells in vivo (13), a clear indication of target modulation. In addition, peptide antagonists can retard the growth of primary tumors and prevent metastases in mouse cancer models (14–17). Leucocyte mobilization effect of LY2510924 was confirmed in mice, dogs, and monkeys. In addition, antitumor effects of LY2510924 were shown in xenograft mouse models with a multitude of human cancer types including non-Hodgkin lymphoma (NHL), colon cancer, non–small cell lung cancer, renal cell carcinoma (RCC), and breast cancer (unpublished results). Where assessed, the concentration of LY2510924 needed to achieve 50% of maximal leukocyte mobilization (EC50) was approximately 10-fold lower than the concentration to achieve 50% tumor growth inhibition (IC50) in the NHL xenograft mouse model.

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Here, we report the first in-human phase I clinical trial of LY2510924 in patients with advanced cancer. The primary objective of this phase I study was to determine the recommended phase II dose of LY2510924. Secondary objectives included characterization of safety and toxicity profiles, estimation of pharmacokinetic parameters, pharmacodynamic response, which included mobilization of CD34+ hematopoietic stem cells (HSC) into the peripheral blood, and to record any antitumor activity observed.

**Patients and Methods**

**Patients**

Eligible patients were male or female, age ≥18 years, with histologically confirmed solid tumors refractory to standard therapy. Patients had measurable disease as defined by the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1; ref. 18) and had discontinued all previous chemotherapy and immunotherapy at least 3 weeks (2 weeks for radiotherapy and 6 weeks for mitomycin-C or cytotoxic chemotherapy and immunotherapy at least 3 weeks [29 and 30 during cycle 1 to permit pharmacokinetic analyses]. A 3+3 design was used for dose escalation (part A), and dosing began at 1 mg/day. Dose escalation was guided by safety assessments during cycle 1 using the standard scoring system, Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0). Any adverse events possibly related to LY2510924 were considered toxicities using available pharmacokinetic/pharmacodynamic data. Patients received two cycles of treatment unless one or more criteria for discontinuation were met. If a single patient of the three enrolled in the cohort experienced a dose-limiting toxicity (DLT) within the first cycle of LY2510924 treatment, 3 additional patients were enrolled at that dose level. If a DLT was observed in 2 or more patients at any dose level, escalation ceased and the previous dose level was declared the maximum tolerated dose (MTD). A DLT was defined as any of the following adverse events occurring during cycle 1 as per the CTCAE v4.0: ≥ grade 3 nonhematological toxicity except nausea/vomiting/diarrhea responsive to medical treatment; grade 4 neutropenia for >5 days duration; febrile neutropenia of any duration; ≥ grade 2 seizure; grade 3 injection-site reaction; ≥ grade 3 cytokine release syndrome/acute infusion-style reaction; ANC < 25,000 cells/μL for >5 days duration. If a patient did not complete cycle 1 for a reason other than DLT, the patient was replaced.

Dose confirmation (part B) examined two doses of LY2510924 in cohorts of 10 patients each: (i) the MTD determined in part A and (ii) a biologically effective dose (BED), determined by pharmacodynamic response. The minimal BED was defined as the first dose level where there is a statistically significant increase in the number of CD34+ cells in the peripheral blood. The BED dose level would be conducted only if the BED was determined to be a dose ≥30% lower than MTD.

The ability of CXCR4 antagonists to induce mobilization of cells in autologous stem cell transplantation, such as the use of CXCR4 antagonist plerixafor (AMD3100), raised questions whether the same treatment might mobilize cells from solid tumors that express CXCR4 (19). To monitor patients for mobilization of cells in this study, circulating tumor cell (CTC) counts were quantified. The CELLSEARCH CXC test was used for the immunomagnetic selection and enumeration of rare circulating epithelial cells from whole blood using the CELLSEARCH System (Janssen Diagnostics LLC). These rare circulating cells of epithelial origin are defined as cells being EpCAM+, cytokeratin 8, 18, and/or 19+, and CD45−. To correlate the number of CTCs at baseline and at various times during study treatment with LY2510924, this validated assay was used to quantitate CTCs at the following time points during cycle 1:

**Translational Relevance**

This article reports the results of a phase I study designed to evaluate the safety and tolerability of the C-X-C motif receptor 4 (CXCR4) inhibitor LY2510924 in patients with advanced cancer. LY2510924 is a peptide antagonist, which blocks stromal cell–derived factor-1 (SDF1) from CXCR4 binding. CXCR4 is often overexpressed in many cancers and involved in the metastasis of solid tumors. LY2510924 was tolerated with mostly grade 1/2 adverse events, revealed favorable pharmacokinetics, and demonstrated evidence of target engagement as indicated by dose-dependent increases in CD34+ cells.

This study was a multicenter, nonrandomized, open-label phase I trial of LY2510924 in patients with advanced cancer. The study was approved by the institutions’ respective Institutional Review Boards and each patient provided written informed consent before enrollment. Eligible patients received LY2510924 as a daily subcutaneous injection on 28-day cycles. Doses were not administered on days 29 and 30 to permit pharmacokinetic analyses. A 3+3 design was used for dose escalation (part A), and dosing began at 1 mg/day. Dose escalation was guided by safety assessments during cycle 1 using the standard scoring system, Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0). Any adverse events possibly related to LY2510924 were considered toxicities using available pharmacokinetic/pharmacodynamic data. Patients received two cycles of treatment unless one or more criteria for discontinuation were met. If a single patient of the three enrolled in the cohort experienced a dose-limiting toxicity (DLT) within the first cycle of LY2510924 treatment, 3 additional patients were enrolled at that dose level. If a DLT was observed in 2 or more patients at any dose level, escalation ceased and the previous dose level was declared the maximum tolerated dose (MTD). A DLT was defined as any of the following adverse events occurring during cycle 1 as per the CTCAE v4.0: ≥ grade 3 nonhematological toxicity except nausea/vomiting/diarrhea responsive to medical treatment; grade 4 neutropenia for >5 days duration; febrile neutropenia of any duration; ≥ grade 2 seizure; grade 3 injection-site reaction; ≥ grade 3 cytokine release syndrome/acute infusion-style reaction; ANC < 25,000 cells/μL for >5 days duration. If a patient did not complete cycle 1 for a reason other than DLT, the patient was replaced.

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baseline (within 7 days of cycle 1 day 1), day 1 predose, day 1 8 hours, day 2 postdose, day 8 postdose, and day 28 postdose. Additional samples were collected at cycle 2 day 1 (if >3 days after cycle 1 day 28), cycle 3 and every other subsequent cycle on day 1, and study termination.

Drug supply
LY2510924 was provided by Eli Lilly and Company as 20 mg of lyophilized active drug, which was reconstituted for injection per USP in 0.9% sodium chloride.

Pharmacokinetic assessments
Whole blood samples were collected on the following days of cycle 1: day 1 predose, 0.5, 2, 4, 6, and 8 hours; day 2 predose; day 7, 8, or 9 postdose; day 28 0.5, 2, 4, 6, and 8 hours; day 29 (24-hour postdose); day 30 (48-hour postdose) for the determination of plasma LY2510924 concentration. Doses were not administered on days 29 and 30 specifically to allow for extended pharmacokinetic analyses. LY2510924 plasma concentrations were determined using a validated liquid chromatography/mass spectrometry/mass spectrometry method, on samples prepared using solid phase extraction. The analytical method was validated over 0.2 to 100 ng/mL, with up to 200-fold dilution. Intra- and interassay precision were better than 7% (coefficient of variance%), and accuracy was within 10% of nominal value. Pharmacokinetic parameters were derived from the concentration–time profiles using standard noncompartmental analysis method. The primary pharmacokinetic parameters were peak concentrations (C\text{max}) and the area under the concentration–time curve during the dosing period (AUC\text{t}). The power model (20) was fit to the relationship of LY2510924 dose with each of C\text{max} and AUC\text{t} (20).

Pharmacodynamic assessments
Samples for pharmacodynamic analysis were taken during cycle 1: day 1 predose, 2, or 8 hours; day 2 predose, day 7, 8, or 9 postdose; day 28 predose; day 29 (24-hour postdose); and day 30 (48-hour postdose). Samples were also collected at cycle 3 and subsequent cycles on day 1 and at study termination. Pharmacodynamic analyses included receptor occupancy (RO), ANC, absolute lymphocyte count, and peripheral blood CD34+ counts.

An assay utilizing phycoerythrin (PE) to measure CXCR4 RO was developed and validated in conjunction with Covance Central Laboratories. Briefly, the fluorescence of the PE-labeled monoclonal antibody to the CXCR4 receptor was measured at five concentrations (0, 0.16, 1.6, 16, 160 nmol/L) per sample, and a mean molecules of equivalent soluble fluorophore (MESF) was derived at each concentration. A percent inhibition was calculated from the mean MESF at each concentration for each sample. Because the receptor occupancy assay did not become available until part A was already in progress, samples were taken from only two cohorts in part A, 20 and 30 mg, and both cohorts in part B, 2.5 and 20 mg. Samples for receptor occupancy analyses were collected on day 1 predose, 0.5 hour and 4 hour and day 2 predose from three cohorts: 2.5, 20 and 30 mg/day.

Immunogenicity
Analysis of immunogenicity used the cut point titration method, which utilized an ELISA format to detect antibodies to LY2510924 in human serum. The samples were incubated with LY2510924, which had been immobilized on an ELISA plate. After incubation and washing, the bound antibodies were detected with Goat anti-Human immunoglobulin G (IgG), IgA, IgM horseradish peroxidase, and then visualized with a tetramethylbenzidine substrate solution. The color development was stopped by the addition of an acidic solution and the optical density was measured at 450 nm with wavelength correction set to 650 nm.

Antitumor activity
As a secondary objective, any antitumor activity observed was documented. Objective responses, stable disease, and disease progression were defined as per RECIST v1.1 (18) and were assessed at the end of cycle 2 and every cycle thereafter.

Results
Forty-five patients were enrolled onto the study, 25 in part A and 20 in part B. Patient characteristics and dose escalation are summarized in Tables 1 and 2. The median duration of treatment was 1.9 months (range 0.46–11.0). Eighteen (41%) patients completed a maximum of one cycle, 19 (42%) patients completed two cycles, 2 patients each completed three and four cycles, and 4 patients completed ≥5 cycles (Table 3). Progressive disease (35 subjects, 78%) was the most common reason for discontinuation. Other reasons were adverse events (3, 7%), physician decision (2, 4%), and subject withdrew consent, subject required anticancer treatment, and other subject request (each 1 subject, 2%).

Safety and tolerability
Two patients experienced DLTs at the 30-mg/day dose: both had a grade 3 increase in ANC (Table 2) >25,000 cells/\muL for >5 days. Therefore, the MTD was determined to be 20 mg/day. Nineteen patients (42%) experienced treatment-emergent adverse events (TEAE) at least possibly related to study drug. The most common TEAEs were fatigue (9%), injection-site reaction (9%), injection-site pruritus (7%), and nausea (7%). Seventeen patients (38%) experienced grade ≥3 TEAEs, and the most common are summarized in Table 2. Seven patients experienced a serious adverse event (SAE): grade 2 abdominal abscess (n = 1, 2.5 mg/day), grade 4 cerebellar tumor (n = 1, 2.5 mg/day), grade 5 ovarian cancer metastases (n = 1, 20 mg/day), grade 5 colorectal cancer (CRC) metastases (n = 2, 1.0 and 2.5 mg/day), grade 3 dyspnea and chest pain (n = 1, 2.5 mg/day), grade 2 pulmonary embolism, and grade 5 respiratory arrest (n = 1, 10 mg/day). None of the SAEs were deemed related to study drug. Three patients discontinued study drug due to drug-
related TEAEs: grade 3 increase in ANC (n = 2) and grade 3 jugular vein thrombosis (n = 1).

Six total patients died because of adverse event or progressive study disease. Four patients died within 28 days of their last dose of study drug, and three of the deaths were due to progression of study disease (1 patient in each the 1.0, 2.5 and the 20 mg/day dose cohort). One death, (10 mg/day) was due to an adverse event (respiratory arrest) secondary to progressive disease. Two patients (1 each the 2.5 and the 20 mg/day dose cohort) died 29 days following the last dose of study drug due to progression of study disease.

There did not seem to be a pattern in CTC count change; change from predose CTC count was variable across dose cohorts at the time points assessed. Of the 42 patients who received ≥2.5 mg/day of LY2510924, 35 patients had predose CTC counts of <5 per 7.5 mL of blood, the minimum value acknowledged as meaningful for this methodology. After treatment with LY2510924 at doses up to and including 30 mg/day, 3 of the 35 patients reached minimal CTC counts above 5 per 7.5 mL blood (11 CTCs at cycle 3 days 1, 2.5 mg/day, colorectal; 8 CTCs at cycle 5 day 1, 5.0 mg/day, prostate; 9 CTCs at cycle 1 day 8, 20 mg/day, ovarian). Of the 7 patients with elevated baseline CTC counts, 4 patients with CRC had levels that remained relatively constant from baseline to discontinuation from study (10–17, 2.5 mg/day; 49–37, 2.5 mg/day; 31–37, 10 mg/day; 34–40, 20 mg/day). The remaining 3 patients had elevated CTCs postbaseline compared with baseline. One patient in the 30 mg/day cohort (pancreas) had an increase of 73 to 124 (cycle 1 day 28). The second patient in the 2.5 mg/day cohort (prostate) had an increase of 53 to 48 (cycle 1 day 8), to 253 (cycle 2 day 1), and to 235 (cycle 3 day 1). The third patient in the 20 mg/day cohort (prostate) had an increase of 24 to 30 (cycle 2 day 1) and to 400 (cycle 3 day 1).

Pharmacokinetics
LY2510924 was quickly absorbed after subcutaneous dosing, with a median peak concentration at 0.5 hour, the first sampling time. Half-life increased with dose up to geometric mean of 11.3 hours. At the end of cycle 1, mean peak LY2510924 plasma concentration (C\text{max}) and

### Table 2. Dose escalation, DLTs, and grade 3/4 TEAEs possibly related to LY2510924: parts A and B combined

<table>
<thead>
<tr>
<th>Dose</th>
<th>1 mg/day</th>
<th>2.5 mg/day</th>
<th>5 mg/day</th>
<th>10 mg/day</th>
<th>20 mg/day</th>
<th>30 mg/day</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3</td>
<td>15(^a)</td>
<td>3</td>
<td>3</td>
<td>14(^b)</td>
<td>7</td>
<td>45</td>
</tr>
<tr>
<td>DLT Event</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a^\)Five patients in part A and 10 patients in part B.
\(^b^\)Four patients in part A and 10 patients in part B.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Anemia</th>
<th>Fatigue</th>
<th>Dyspnea</th>
<th>Neutrophil count increased</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>0</td>
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<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a^\)Five patients in part A and 10 patients in part B.
\(^b^\)Four patients in part A and 10 patients in part B.
the 24-hour AUC increased more than dose proportionally (Fig 1A and B). Mean $C_{\text{max}}$ and AUC ranged from 18.8 to 1,250 ng/mL, and 61.5 to 5,720 ng·h/mL, respectively, in the 1 to 30 mg dose range. Pharmacokinetic parameters are summarized in Table 4.

### Table 3. Treatment exposure and best response for parts A and B combined ($N = 45$)

<table>
<thead>
<tr>
<th>Treatment exposure</th>
<th>Median duration of exposure, mo (range)</th>
<th>1.9 (0.46–11.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of cycles completed:</td>
<td>N (%)</td>
<td>2</td>
</tr>
<tr>
<td>Cycles completed</td>
<td>&lt;1 cycle</td>
<td>7 (16)</td>
</tr>
<tr>
<td>1 cycle</td>
<td>11 (24)</td>
<td></td>
</tr>
<tr>
<td>2 cycles</td>
<td>19 (42)</td>
<td></td>
</tr>
<tr>
<td>3 cycles</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>4 cycles</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>≥ 5 cycles</td>
<td>4 (9)</td>
<td></td>
</tr>
<tr>
<td>Best response</td>
<td>N (%)</td>
<td>29 (64)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>9 (20)</td>
<td></td>
</tr>
<tr>
<td>≥2 cycles</td>
<td>7 (16)</td>
<td></td>
</tr>
<tr>
<td>≥4 cycles</td>
<td>4 (9)</td>
<td></td>
</tr>
<tr>
<td>≥12 cycles</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Progressive disease</td>
<td>7 (16)</td>
<td></td>
</tr>
</tbody>
</table>

### Receptor occupancy and pharmacodynamics

Receptor occupancy was consistently high with median values of 96.9% to 100% for 2.5, 20, and 30 mg between 0.5 hours through 24 hours after 1 dose. ANC increased after a single dose and remained elevated at 28 days of dosing (Fig. 2A). LY2510924 dose dependently increased CD34$^+$ cell counts in peripheral blood up to 18-fold after a single dose, with an apparent dose–response relationship between 1 mg and 10 mg, with little additional response with 20 mg or 30 mg. The increase persisted to the end of cycle 1, but was somewhat blunted relative to day 1 (Fig. 2B). A dose of 2.5 mg/day was deemed biologically effective, as it resulted in high levels of receptor occupancy and a meaningful increase in CD34$^+$ cell counts. Thus, this dose was further explored in part B. Absolute lymphocyte count increased in all dose cohorts beginning at cycle 1 day 2 and remained increased over the course of the study with the exception of cycle 2 day 1 for dose cohorts 2.5, 5.0, and 10 mg/day.

### Immunogenicity

Production of anti-LY2510924 antibodies was examined for all patients. Anti-LY2510926 antibody titer was $<10$ µg/mL for all subjects except for 1 patient in the 30-mg/day cohort whose antibody titer was 10 µg/mL.

### Antitumor activity

There were no patients with partial or complete response. Nine patients (20%) had a best response of stable disease at the first restaging assessment. Seven patients (16%) completed at least two cycles of treatment. Four patients had stable disease for ≥ 4 cycles, and one patient had stable disease for ≥ 12 cycles. Patient response and duration of treatment are summarized in Table 3.

### Discussion

LY2510924 is a potent and selective peptide antagonist of CXCR4. In vitro and in vivo studies show that LY2510924 inhibited SDF1 binding to CXCR4 in several species and demonstrated the ability to block SDF1-mediated signaling without possessing agonist activity itself. LY2510924 caused leukocyte and stem cell mobilization in vivo, inhibited the growth of primary tumors, and prevented metastases in relevant mouse cancer models (data on file). On the basis of the nonclinical safety and efficacy data and the potential clinical utility of disrupting the SDF1/CXCR4 pathway in cancer, LY2510924 was evaluated in patients with advanced cancer.

This first in-human phase 1 study in patients with advanced or metastatic cancer explored the safety and
Table 4. LY2510924 pharmacokinetic parameter estimates

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>LY2510924 dose (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 (N = 3)</td>
</tr>
<tr>
<td>Day 1 (n)</td>
<td>3</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>34.6 (13.6)</td>
</tr>
<tr>
<td>Day 28 (n)</td>
<td>3</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>18.8 (12.6)</td>
</tr>
<tr>
<td>AUC (ng·h/mL)</td>
<td>61.5 (22.3)</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>18.1 (7.93)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>4.88 (0.404)</td>
</tr>
<tr>
<td>Vss/F (L)</td>
<td>50.0 (8.28)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC0–∞, area under the plasma concentration time curve from time zero to infinity; CL/F, apparent clearance; Cmax, maximum plasma concentration; N, number of patients who received dose; n, number of patients for whom pharmacokinetic parameters were calculable; t1/2, elimination half-life; Vss/F, apparent volume of distribution at steady state.

| n | 3 | 10 | 9 | 3 |

Tolerability of LY2510924. As a daily subcutaneous injection on a 28-day cycle, LY2510924 was well tolerated and included 20 mg/day in this study. Two DLTs (neutrophil count increased) in the 30-mg/day cohort were identified, and the recommended phase II dose was 20 mg/day. The majority of TEAEs were grade 1 or grade 2 with treatment-related safety profile consisting primarily of mild fatigue, injection-site reaction, and nausea.

Interruption of the CXCR4/SDF1 axis with CXCR4 antagonists is known to mobilize CD34+ HSC in healthy volunteers (21). When administered to multiple myeloma and NHL patients, the CXCR4 antagonist plerixafor (AMD3100) produced an approximate 6- to 7-fold increase in absolute CD34+ cell count from baseline 4 to 6 hours after injection (19). Only grade 1 toxicities were reported for single doses of drug. When used in combination with granulocyte colony-stimulating factor (G-CSF) for HSC transplantation in multiple myeloma and NHL patients, plerixafor in combination with G-CSF (Neupogen) mobilized 3.5- to 4.4-fold more HSCs than G-CSF alone (22). Although plerixafor is tolerated as an acute treatment, extended use in HIV-infected individuals halted the clinical study when it led to premature ventricular contractions in 2 of 40 patients (23).

LY2510924 resulted in a rapid and sustained pharmacodynamic response as observed by mobilization of CD34+ cells (Fig. 2B). There was a dose relationship between CD34+ counts in peripheral blood at 24 hours as the doses increased from 1.0 to 10 mg/day. However, this response seemed to diminish at doses >10 mg/day. After 28 days of treatment, the response was blunted. The 20-mg/day cohort produced the best CD34+ mobilization response during cycle 1 with a minimum median fold increase of 5.5. The diminished response at day 28 may be a result of the increased exposure of CD34+ cells to the clearance mechanisms in the peripheral blood and the inability to regenerate the CD34+ pool as quickly as the cells are mobilized.

ANC increased in subjects in all dose cohorts beginning at cycle 1 day 2 and remained increased through cycle 1 day 28, and in general, was increased over the course of the study (Fig. 2A). Mean ANC increase from baseline across time points was generally greater in the dose cohorts ≥10 mg/day than in the lower dose cohorts. This anticipated pharmacodynamic response led to the only DLTs observed during this study. Both DLTs were predefined ANC increases that exceeded >25,000 cells/μL that persisted for >5-day duration. In both cases, the patients were not symptomatic.

ANC was also increased in all dose cohorts beginning at cycle 1 day 2 and remained increased over the course of the study with the exception of cycle 2 day 1.

Although there were significant mobilization CD34+ cells upon treatment with LY2510924, there was no apparent treatment or time relationships observed for CTC counts. The 3 patients for whom CTC counts increased significantly postbaseline occurred over separate dose levels, 2.5, 20, and 30 mg/day. These patients all started the study with elevated counts, and represented 2 of the 3 patients with prostate cancer, and one with pancreatic cancer. Evaluation of the CTC count data more closely reflects the nature of the tumor type and disease progression than a relationship to LY2510924 dose level or duration of treatment. Given the limitations of the CTC count assay, we cannot rule out mobilization of other potential CTC phenotypes, such as cells that have undergone epithelial–mesenchymal transition.

The pharmacodynamic responses for CD34+ and ANC increases paralleled the receptor occupancy data obtained at the 2.5-, 20-, and 30-mg/day dose levels. After
expression is associated with advanced differentiated RCC reported by in vitro and murine in vivo models (24). In approximately 70% to 90% of RCC cases, CXCR4 and SDF1 are overexpressed in tumor and vascular cells of patients with RCC (25). A positive correlation was demonstrated between strong CXCR4 expression and poor survival in RCC, which is treated with anti-VEGF therapies. As tumors become resistant to treatment during anti-VEGF therapy, circulating cytokines are elevated. Bevacizumab (Avastin) resistance in patients with CRC is characterized by upregulation of SDF1 (3–90-fold) and CXCR4 (3–1,000-fold; ref. 26), whereas sunitinib (Sutent) treatment resulted in higher levels of inflammatory molecules SDF1, interleukin, and soluble c-KIT as well as circulating progenitor cells in patients with hepatocellular carcinoma (27); these increased levels were associated with a poor outcome. In addition, ubiquitous expression of CXCR4 has previously been demonstrated in 10 small cell lung cancer (SCLC) cell lines. Specifically, all 10 SCLC cell lines tested expressed CXCR4 and responded to its ligand SDF1 with an increase in cell proliferation, adhesion, and motility which may be attributed, in part, to increased phosphoinositide 3-kinase signaling (28). SCLC patient samples expressed high levels of CXCR4 (29). In SCLC, CXCR4 activation induced migratory and invasive responses in the extracellular matrix and subsequent adhesion to marrow stromal cells in a CXCR4- and integrin-dependent fashion (30). The protection that resulted from the extracellular matrix response produced drug resistance and residual disease. This stromal cell protection of SCLC cells has been inhibited in vivo by experimental CXCR4 antagonists such as T-140 (29). Therefore, when tumor indications for phase II studies of LY2510924 were selected, there was scientific support for both metastatic clear cell RCC and SCLC. The MTD of 20 mg/day, explored in part B of this phase I first in-human study, was recommended for phase II clinical studies.

There were no objective responses observed in this study. Nine subjects (20%) had a best response of stable disease for at least 1 cycle, and 26 subjects (57.8%) had a best response of progressive disease. A patient with neuroendocrine lung cancer in the 20-mg/day cohort had stable disease for at least 12 cycles. This patient had failed six prior therapies. Tumor shrinkage, as a result of CXCR4 antagonist monotherapy treatment, was not expected in this phase I study.

In summary, LY2510924 interacted with the desired CXCR4 target, as demonstrated by high levels of sustained receptor occupancy, and produced a strong pharmacodynamic response through increased levels of ANC and CD34+ cell counts at several dose levels. The majority of TEAEs were grade 1/2, and there was not an increase in CTC count throughout treatment in patients tested. The recommended phase II dose was 20 mg/day.

Disclosure of Potential Conflicts of Interest

M. Galsky is a consultant/advisory board member for Eli Lilly. N. Vogelzang reports receiving speakers bureau honoraria from Eli Lilly and Medivation/ Astellas, and was an employee of US Oncology. E. Abu-Raddad is an employee of Eli Lilly. No potential conflicts of interest were reported by the other authors.
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References


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