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

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Running header: LY2510924 in patients with advanced cancer

Keywords: CXCR4, LY2510924, cancer, CD34+

Word Count Abstract- 245 (Clinical Cancer Research limit = 250)
Word Count Text- 4030 (CCR limit = 5000)
Tables/Figures-6 (CCR limit = 6)

Research support: The study was sponsored by Eli Lilly and Company.
Statement of clinical relevance (required for submission to journal):

This manuscript 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 (SDF-1) 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.
Conflict of Interest

Dr. Galsky is supported by a Prostate Cancer Foundation Young Investigator Award, has received honoraria for participating on advisory boards for Dendreon, Astellas, Janssen, and Eli Lilly and Company, and has received research funding from BioMotiv, Novartis, Dendreon, Janssen, and Celgene.

Abstract

Purpose: Over-expression 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 (SDF-1) 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 maximally 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 9 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.
Introduction

Metastatic tumor spread is the leading cause of cancer deaths (1). The tumor microenvironment has several biological 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) (3-7). The $\alpha$-chemokine stromal-cell derived factor 1 (SDF-1/CXCL12) signals through CXCR4 and promotes tumor growth by stimulation of cell proliferation and survival processes (8-10). Indirectly, secretion of SDF-1 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 SDF-1 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. Additionally, peptide antagonists can retard the growth of primary tumors and prevent metastases in mouse cancer models (14-17). Leukocyte mobilization effect of LY2510924 was confirmed in mice, dogs and monkeys. Additionally, anti-tumor effects of LY2510924 were shown in xenograft mouse models with a multitude of human cancer types including non-Hodgkins Lymphoma, colon cancer, non-small cell lung cancer, renal cell carcinoma, 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 non-Hodgkin’s lymphoma xenograft mouse model.

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 (PK) parameters, pharmacodynamic (PD) 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) (18) and had discontinued all previous chemotherapy and immunotherapy at least 3 weeks (2 weeks for radiotherapy and 6 weeks for mitomycin-C or nitrosoureas) prior to enrollment and had a performance status of ≤2 on the Eastern Cooperative Oncology Group (ECOG) scale. Required laboratory tests included adequate hematopoietic and hepatic function defined as: absolute neutrophil count (ANC) ≥1.5 × 10^9/L; platelets ≥100 × 10^9/L; hemoglobin ≥8 g/dL; serum creatinine clearance ≥60 mL/min; bilirubin ≤1.5 × upper limits of normal (ULN); alanine transaminase and aspartate transaminase ≤2.5 × ULN (≤5 × ULN for patients with liver tumor); proteinuria > +1. Patients were excluded from participation for any of the following reasons: treatment with investigational drug within 28 days of first dose of LY2510924; symptomatic...
central nervous system malignancy or metastasis; history of major organ transplant; current acute leukemia; human immunodeficiency virus, hepatitis B or hepatitis C infection; QTc >470 msec (female), >450 msec (male), or history of long QT syndrome; previously treated with CXCR antagonist; uncontrolled hypertension; pregnancy; lactation. Patients with treated CNS metastases were eligible provided their disease was radiographically stable, asymptomatic, and they were not currently receiving corticosteroids and/or anticonvulsants.

Study Design

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 prior to enrollment. Eligible patients received LY2510924 as a daily subcutaneous (s.c.) injection on 28-day cycles. Doses were not administered on days 29 and 30 during cycle 1 in order to permit PK 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 one using the standard scoring system, Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0). Any adverse events (AEs) possibly related to LY2510924 were considered toxicities using available PK/PD data. Patients received two cycles of treatment unless one or more criteria for discontinuation were met. If a single patient of the 3 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 MTD. A DLT was defined as any
of the following adverse events occurring during cycle 1 as per the Common Terminology Criteria for Adverse Events Version 4.0 (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 2 doses of LY2510924 in cohorts of 10 patients each: 1) the MTD defined in Part A, and 2) a biologically effective dose (BED), determined by PD 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 (ASCT), 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+, Cytokeratins 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 employed to quantitate CTCs at the following time points during cycle 1: baseline (within 7 days of cycle 1 day 1), day 1 pre-dose, day 1 8 hours, day 2
post-dose, day 8 post-dose, and day 28 post-dose. 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 & Company (Indianapolis, IN) 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 pre-dose, 0.5, 2, 4, 6, and 8 hours; day 2 pre-dose; day 7, 8, or 9 post-dose; day 28 0.5, 2, 4, 6, and 8 hours; day 29 (24-hour post-dose); day 30 (48-hour post-dose) for the determination of plasma LY2510924 concentration. Doses were not administered on days 29 and 30 specifically to allow for extended PK 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 0.2-100 ng/mL, with up to 200-fold dilution. Intra- and inter-assay precision were better than 7% (coefficient of variance %), and accuracy was within 10% of nominal value. PK parameters were derived from the concentration-time profiles using standard noncompartmental analysis method. The primary PK parameters were peak concentrations ($C_{\text{max}}$) and the area under the concentration-time curve during the dosing period ($\text{AUC}_\tau$). The power model (20) was fitted to the relationship of LY2510924 dose with each of $C_{\text{max}}$ and $\text{AUC}_\tau$ (20).
Pharmacodynamic Assessments

Samples for PD analysis were taken during cycle 1: day 1 pre-dose, 2, or 8 hours; day 2 pre-dose, day 7, 8, or 9 post-dose; day 28 pre-dose; day 29 (24-hour post-dose); day 30 (48-hour post-dose). Samples were also collected at cycle 3 and subsequent cycles on day 1 and at study termination. PD 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 5 concentrations (0, 0.16, 1.6, 16, 160 nM) 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 RO assay did not become available until Part A was already in progress, samples were taken from only 2 cohorts in Part A, 20 and 30 mg, and both cohorts in Part B, 2.5 and 20 mg. Samples for RO analyses were collected on day 1 pre-dose, 0.5 hour and 4 hour and day 2 pre-dose from 3 cohorts: 2.5, 20 and 30 mg/day.

Immunogenicity

Analysis of immunogenicity used the cut point titration method which utilized an enzyme-linked immuno sorbent assay (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 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 (OD) 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 (SD), 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 1 cycle, 19 (42%) patients completed 2 cycles, 2 patients each completed 3 and 4 cycles, and 4 patients completed ≥5 cycles (Table 3). Progressive disease (35 subjects, 78%) was the most common reason for discontinuation. Other reasons were AE (3, 7%), physician decision (2, 4%), and subject withdrew consent, subject required anti-cancer 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/μL for >5 days. Therefore, the MTD was determined to be 20 mg/day. Nineteen patients (42%) experienced treatment-emergent adverse events (TEAEs) 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 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 due to AE 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 AE (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 appear to be a pattern in CTC count change; change from pre-dose 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 pre-dose 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 7–5 per 7.5 mL blood (11 CTCs at cycle 3 day 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 colorectal cancer patients had levels which
remained relatively constant from baseline to discontinuation from study (10 to 17, 2.5 mg/day; 49 to 37, 2.5 mg/day; 31 to 37, 10 mg/day; 34 to 40, 20 mg/day). The remaining 3 patients had elevated CTCs post-baseline compared to 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 s.c. 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 the 24-hour AUC increased more than dose proportionally (Fig 1A, B). Mean $C_{\text{max}}$ and AUC ranged from 18.8 to 1250 ng/mL, and 61.5 to 5720 ng·h/mL, respectively, in the 1- to 30-mg dose range. PK parameters are summarized in Table 4.

**Receptor Occupancy and Pharmacodynamics**

Receptor occupancy (RO) 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 (Figure 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 1-10 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 RO 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 \( \mu \)g/mL for all subjects except for 1 patient in the 30-mg/day cohort whose antibody titer was 10 \( \mu \)g/mL.

Antitumor activity

There were no patients with partial or complete response. Nine patients (20%) had a best response of SD at the first restaging assessment. Seven patients (16%) completed at least 2 cycles of treatment. Four patients had SD for \( \geq 4 \) cycles, and one patient had SD for \( \geq 12 \) 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 SDF-1 binding to CXCR4 in several species and demonstrated the ability to block SDF-1-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). Based
on the nonclinical safety and efficacy data and the potential clinical utility of disrupting the SDF-1/CXCR4 pathway in cancer, LY2510924 was evaluated in patients with advanced cancer.

This first in-human phase I study in patients with advanced or metastatic cancer explored the safety and tolerability of LY2510924. As a daily s.c. injection on a 28-day cycle, LY2510924 was tolerated up to and including 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/SDF-1 axis with CXCR4 antagonists is known to mobilize CD34+ HSC in healthy volunteers (21). When administered to multiple myeloma and non-Hodgkin’s lymphoma (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 G-CSF for HSC transplantation (HSCT) in multiple myeloma (MM) 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 PD response as observed by mobilization of CD34+ cells (Figure 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 appeared 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 (Figure 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 PD response led to the only DLTs observed during this study. Both DLTs were pre-defined ANC increases that exceeded >25,000 cells/µL that persisted for >5 days 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 post-baseline 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 rather 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 PD responses for CD34+ and ANC increases paralleled the RO data obtained at the 2.5-, 20-, and 30-mg/day dose levels. After a single 2.5-mg/day dose, RO was essentially
complete with median values of 96.9% to 100% from 0.5 hours through 24 hours. Single doses of 20 and 30 mg/day resulted in comparable measurements.

When considering doses for Part B, the 2.5-mg/day dose of LY2510924 showed a high RO that was sustained over the 24-hour dosing interval. This dose also produced a significant PD response as analyzed by increased ANC and CD34+ cell counts. ANC and CD34+ mobilization appears to be saturated at the 20 mg/day dose. Since the preclinical potency for leukocyte mobilization was approximately 10-fold higher than that of antitumor effect, seeking to saturate leukocyte mobilization is advisable to assure efficacy. Furthermore, mean peak LY2510924 serum levels achieved at the 20 mg/day are approximately 4-fold higher than the IC$_{50}$ for tumor growth suppression in the non-Hodgkin’s xenograft tumor mouse model. Therefore, the MTD of 20 mg/day was also explored in Part B to ensure efficacious exposures are reached.

Based on the well-characterized roles of SDF-1 and CXCR4 in chemotaxis and the similarities between chemotactic cell migration and cancer cell movement to distant sites, this receptor-ligand pair is hypothesized to play a role in cancer pathogenesis and metastasis. Significant CXCR4 expression is associated with advanced differentiated renal cell carcinoma (RCC) reported by in vitro and murine in vivo models (24). In approximately 70% to 90% of RCC cases, CXCR4 and SDF-1 are overexpressed in tumor and vascular cells of RCC patients (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 colorectal cancer (CRC) patients is characterized by up-regulation of SDF-1 (3 to 90-fold) and CXCR4 (3 to 1,000 fold) (26) while sunitinib (Sutent) treatment resulted in higher
levels of inflammatory molecules SDF-1, IL-6, and soluble c-KIT as well as circulating progenitor cells in hepatocellular carcinoma (HCC) patients (27); these increase levels were associated with a poor outcome. Additionally, 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 SDF-1 with an increase in cell proliferation, adhesion, and motility which may be attributed, in part, to increased PI3K 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 SD for at least 1 cycle, and 26 subjects (57.8%) had a best response of progressive disease. A neuroendocrine lung cancer patient in the 20-mg/day cohort had SD 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 RO, and produced a strong PD 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.
Acknowledgments

The authors would like to thank Dr. Muhammad Khan and TOP Translational Oncology Program of US Oncology lead by Dr. Daniel Von Hoff for their insight and expertise in the cancer research field. They would also like to express their appreciation to Sheng-Bin Peng (Lilly Research Laboratories, Indianapolis, IN) for his insight on the biology of this molecule and the CXCR4 target. Authors also express their gratitude for the expertise of Lisa Green (Covance Laboratories, Greenfield, IN) for the development of the CXCR4 receptor occupancy assay for clinical use.
References


Table 1. Patient demographics (N=45)

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Abbreviations: ECOG=Eastern Cooperative Oncology Group.

*Other cancers include: bladder, clear cell renal carcinoma, endometrium, gastrointestinal stromal tumor, lymphoma, oropharyngeal, sarcoma, thyroid.
Table 2. Dose-escalation, dose limiting toxicities (DLT) and Grade 3/4 treatment emergent adverse events (TEAE) possibly related to LY2510924: Parts A and B combined

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<td>3</td>
<td>3</td>
<td>14</td>
<td>7</td>
<td>45</td>
</tr>
<tr>
<td>DLT</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>

<table>
<thead>
<tr>
<th>Event</th>
<th>Grade 3 neutrophil increased for both patients</th>
</tr>
</thead>
</table>

| Toxicity | | | | | | | |
|----------| | | | | | | |
| Anemia   | 0 | 1 | 0 | 0 | 1 | 0 | 2 (4) |
| Fatigue  | 0 | 1 | 0 | 0 | 1 | 0 | 2 (4) |
| Dyspnea  | 0 | 1 | 0 | 0 | 0 | 1 | 2 (4) |
| Neutrophil count increased | 0 | 0 | 0 | 0 | 0 | 2 | 2 (4) |

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

<table>
<thead>
<tr>
<th>Treatment Exposure</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Median duration of exposure, months (range)</td>
<td>1.9 (0.46-11.0)</td>
</tr>
<tr>
<td>Mean number of cycles completed:</td>
<td>2</td>
</tr>
<tr>
<td>Cycles completed</td>
<td></td>
</tr>
<tr>
<td>&lt; 1 cycle</td>
<td>7 (16)</td>
</tr>
<tr>
<td>1 cycle</td>
<td>11 (24)</td>
</tr>
<tr>
<td>2 cycles</td>
<td>19 (42)</td>
</tr>
<tr>
<td>3 cycles</td>
<td>2 (4)</td>
</tr>
<tr>
<td>4 cycles</td>
<td>2 (4)</td>
</tr>
<tr>
<td>≥ 5 cycles</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Best Response</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>9 (20)</td>
</tr>
<tr>
<td>≥ 2 cycles</td>
<td>7 (16)</td>
</tr>
<tr>
<td>≥ 4 cycles</td>
<td>4 (9)</td>
</tr>
<tr>
<td>≥ 12 cycles</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>29 (64)</td>
</tr>
<tr>
<td>Not assessed(^a)</td>
<td>7 (16)</td>
</tr>
</tbody>
</table>

\(^a\)Seven patients were not assessed due to early termination for the following reasons: Adverse event =3; physician decision=2; start new chemo=1; withdrew consent=1.
Table 4. LY2510924 pharmacokinetic parameter estimates

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>LY2510924 Dose (mg/day)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 (N=3)</td>
<td>2.5 (N=15)</td>
<td>5.0 (N=3)</td>
<td>10 (N=3)</td>
<td>20 (N=14)</td>
<td>30 (N=7)</td>
</tr>
<tr>
<td>Day 1 (n)</td>
<td>3</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>C_max (ng/mL)</td>
<td>34.6 (13.6)</td>
<td>64.1 (26.5)</td>
<td>138 (58.9)</td>
<td>294 (105)</td>
<td>605 (186)</td>
<td>881 (316)</td>
</tr>
<tr>
<td>Day 28 (n)</td>
<td>3</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>C_max (ng/mL)</td>
<td>18.8 (12.6)</td>
<td>68.3^a (22.5)</td>
<td>145 (55.3)</td>
<td>314 (111)</td>
<td>716^b (232)</td>
<td>1250^c (416)</td>
</tr>
<tr>
<td>AUC_t (ng·hr/mL)</td>
<td>61.5 (22.3)</td>
<td>214 (50.2)</td>
<td>580 (184)</td>
<td>1150 (344)</td>
<td>3800 (886)</td>
<td>5720 (3470)</td>
</tr>
<tr>
<td>CL/F (L/hr)</td>
<td>18.1 (7.93)</td>
<td>12.3 (2.83)</td>
<td>9.39 (3.65)</td>
<td>9.17 (2.77)</td>
<td>5.50 (1.18)</td>
<td>6.38 (2.42)</td>
</tr>
<tr>
<td>t_{1/2} (hr)</td>
<td>4.68 (0.404)</td>
<td>6.45 (1.32)</td>
<td>6.70 (1.10)</td>
<td>6.29 (1.18)</td>
<td>9.16 (1.30)</td>
<td>11.3 (8.04)</td>
</tr>
<tr>
<td>V_{ss/F} (L)</td>
<td>50.0 (8.28)</td>
<td>61.5 (16.6)</td>
<td>57.2 (8.47)</td>
<td>54.1 (12.1)</td>
<td>54.3 (10.7)</td>
<td>61.3 (12.4)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC_{(0-∞)} = area under the plasma concentration time curve from time zero to infinity; CL/F: apparent clearance; C_max = maximum plasma concentration; N: number of patients who received dose; n: number of patients for whom PK parameters were calculable; SD = standard deviation; t_{1/2} : elimination half-life; V_{ss/F} = apparent volume of distribution at steady state.

^a n=10; ^b n=9; ^c n=3.
Figure Legends

Figure 1. LY2510924 Dose relationship with peak concentration ($C_{\text{max}}$) and the area under the concentration-time curve during the dosing interval ($\text{AUC}_\tau$) on Day 28. Graphs depict (A) $C_{\text{max}}$ and (B) $\text{AUC}_\tau$ versus dose for each patient (blue dots) on day 28 of cycle 1. Black and red lines represent power model fit and with the 80% confidence interval, respectively.

Figure 2. Absolute neutrophil counts and CD34+ cell counts. (A) Bars represent mean absolute neutrophil counts (ANC) taken at baseline, 24 hours after first dose of cycle 1 (C1D2 pre-dose), and 24 hours after last dose of cycle 1 (C1D29). Error bars represent standard error. $^a$n=2; $^b$n=12; $^c$n=5; $^d$n=0; $^e$n=11; $^f$n=1; $^g$n=9; $^h$n=3. (B) Bars represent mean CD34+ cell counts at baseline, 24 hours after first dose of cycle 1 (C1D2 pre-dose) and 24 hours after last dose of cycle 1 (C1D29). Error bars represent standard error. $^a$n=14; $^b$n=11; $^c$n=1; $^d$n=9; $^e$n=3.
Figure 1
Figure 2
Clinical Cancer Research

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

Matthew D Galsky, Nicholas J Vogelzang, Paul Conkling, et al.

Clin Cancer Res Published OnlineFirst April 11, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-2686

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