Identification of LY2510924, a Novel Cyclic Peptide CXCR4 Antagonist That Exhibits Antitumor Activities in Solid Tumor and Breast Cancer Metastatic Models


Abstract

Emerging evidence demonstrates that stromal cell-derived factor 1 (SDF-1) and CXCR4, a chemokine and chemokine receptor pair, play important roles in tumorigenesis. In this report, we describe a small cyclic peptide, LY2510924, which is a potent and selective CXCR4 antagonist currently in phase II clinical studies for cancer. LY2510924 specifically blocked SDF-1 binding to CXCR4 with IC₅₀ value of 0.079 nmol/L and inhibited SDF-1–induced GTP binding with Kb value of 0.38 nmol/L. In human lymphoma U937 cells expressing endogenous CXCR4, LY2510924 inhibited SDF-1–induced cell migration with IC₅₀ value of 0.26 nmol/L and inhibited SDF-1/CXCR4–mediated intracellular signaling. LY2510924 exhibited a concentration–dependent inhibition of SDF-1–stimulated phospho-ERK and phospho-Akt in tumor cells. Biochemical and cellular analyses revealed that LY2510924 had no apparent agonist activity. Pharmacokinetic analyses suggested that LY2510924 had acceptable in vivo stability and a pharmacokinetic profile similar to a typical small-molecular inhibitor in preclinical species. LY2510924 showed dose-dependent inhibition of tumor growth in human xenograft models developed with non–Hodgkin lymphoma, renal cell carcinoma, lung, and colon cancer cells that express functional CXCR4. In MDA-MB-231, a breast cancer metastatic model, LY2510924 inhibited tumor metastasis by blocking migration/homing process of tumor cells to the lung and by inhibiting cell proliferation after tumor cell homing. Collectively, the preclinical data support further investigation of LY2510924 in clinical studies for cancer.

Introduction

Stromal cell–derived factor 1 (SDF-1, also called CXCL12) and CXCR4, a chemokine and chemokine receptor pair play important roles in many stages of tumorigenesis (1–3). CXCR4 is overexpressed in a variety of human cancers, and this overexpression is correlated with increased risk for recurrence and poor overall survival in multiple cancer patients including breast, lung, kidney, colon, ovarian, and brain cancers, as well as lymphoma and leukemia (1–5). SDF-1/CXCR4 interaction activates multiple signal transduction pathways including PI3K/Akt, Ras/Raf/MAPK cascades (6, 7). The paracrine and endocrine effects mediated through SDF-1/CXCR4 are critical for tumor growth, invasion, angiogenesis, and metastasis (2, 3). Within hypoxic areas of tumors, both SDF-1 secretion by fibroblasts and CXCR4 expression on tumor cells increase, which stimulate tumor cell growth, mobility, and invasion. SDF-1 promotes tumor growth in a paracrine fashion by directly stimulating tumor cell proliferation and survival via CXCR4. SDF-1 also induces recruitment of endothelial progenitors, which allows for tumor angiogenesis (3). In addition, targeted metastasis of tumor cells to sites with high levels of SDF-1 expression, such as bone marrow, lung, liver, and lymph nodes, involves CXCR4 activation on circulating tumor cells (2). Therefore, a CXCR4 antagonist with desirable in vivo pharmacologic and toxicologic properties would have potential for treatment of human cancers. Interruption of CXCR4 and SDF-1–axis has demonstrated antitumor growth activities in a variety of preclinical tumor models (2, 3, 8–12).

The critical roles of CXCR4 in cancer and other diseases have triggered the development of selective CXCR4 inhibitors for clinical use. SDF-1, the only ligand of CXCR4, is a highly basic and cationic chemokine and contains approximately 25% basic residues (13). For development of a small-molecule inhibitor, it generally requires multiple basic centers in a molecule to achieve high enough affinity for blocking the SDF-1 and CXCR4 interaction effectively (14). One such small-molecule inhibitor is the well-characterized bicyclam Plerixafor, also called AMD3100 (15). Because of its basic property, Plerixafor is not orally bioavailable, and causes significant safety concerns in chronic dose schedules due to compound-associated toxicity (16). Plerixafor can only be utilized for short-term treatment in the clinic.
Alternatively, many potent peptide antagonists for CXCR4 have been identified (17). However, these peptide antagonists generally have a short in vivo half-life. Two such examples are BTK140 (18) and CITEC-9908 (19).

In this report, we have combined a medium throughput screen and a rational design approach that identified LY2510924 as a potent and selective CXCR4 antagonist currently in phase II clinical studies (NCT13911130 and NCT1439568). LY2510924 is a small cyclic peptide containing non-native amino acids with a molecular weight of 1,189.74 dalton. LY2510924 has a dramatically improved in vivo stability with a half-life of 3 to 5 hours in preclinical species and 9.16 hours in humans at the recommended phase II dose (20). It is a potent inhibitor of the SDF-1 and CXCR4 interaction and the downstream signaling and functions. LY2510924 has shown dose-dependent inhibition of tumor growth in many cancer xenograft models utilizing cell lines that express high levels of CXCR4. LY2510924 also inhibits breast cancer metastasis in an experimental metastasis model.

Materials and Methods

Reagents

- Anti–ERK1/2, anti–phospho-ERK1/2, anti-Akt, and anti–phospho-Akt polyclonal antibodies were purchased from Cell Signaling Technology. Anti–β-actin monoclonal antibody was from Sigma. Anti-hCXCR4 monoclonal antibody for immuno-fluorescent staining was from R&D Systems. Recombinant human SDF-1α was purchased from PeproTech EC Ltd.

Cell culture

Human cervical carcinoma Hela Cells, leukemia CCRF-CEM cells, non–Hodgkin lymphoma Namalwa cells, histiocytic lymphoma U937 cells, renal cancer A498 cells, lung cancer A549 cells, colon cancer HCT116 cells, and breast cancer MDA-MB-231 cells were purchased from the ATCC. All these cells were passaged for fewer than 2 months after which time new cultures were initiated from vials of frozen cells. Characterization of the cell lines was done by a third party vendor (RADIL), which included profiling (by PCR) for contamination by various microorganisms of bacterial and viral origin. As a result, no contamination was detected. The samples were also verified to be of human origin without mammalian interspecies contamination. The alleles for nine genetic markers were used to determine that the banked samples detected by measuring the absorbance at 492 nm with a Tecan Spectrafluor Plus Microplate Reader.

Western blot analysis

The cell treatment, cell lysate preparation, and Western blot analyses were performed as described previously (6).

Pharmacokinetic analysis in preclinical species

Pharmacokinetic studies of LY2510924 were conducted in female C57/BL6 mice, male Sprague–Dawley rats, male Beagle dogs, and male Cynomolgus monkeys at fasted state. LY2510924 at different doses was administered by subcutaneous (s.c.) or intravenous (i.v.) injection, and the plasma samples were collected at different time points. The compound concentrations were determined by high-performance liquid chromatography (HPLC) and mass spectrum analysis.

In vivo studies

The Eli Lilly and Company Animal Care and Use Committee approved all the experimental protocols. For the non–Hodgkin lymphoma Namalwa model, 200,000 Namalwa cells mixed with Matrigel (1:1) were implanted subcutaneously into the rear flank of SCID mice (Jackson), and the implanted tumor cells grew as solid tumors. For other solid tumor xenograft models, 3 × 10^6 (A498) or 5 × 10^5 (HCT116 and A549) cells in a 1:1 Matrigel mix

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(0.2 mL volume) were implanted by subcutaneous injection in hind leg of athymic nude female mice (A498 and HCT-116) or SCID female mice (A549).

The tumor volumes were measured every third or fourth day using a caliper. A high-content multiplexed tissue imaging and quantification method was utilized for tumor tissue staining of nuclear intensity, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) area, CD31, and Ki-67 as described (22).

For an experimental lung metastasis model, SCID female mice from Taconic were injected intravenously via the tail vein with 5 × 10^6 MDA-MB-231 cells, and were treated subcutaneously with vehicle (1× PBS) or 3 mg/kg of LY2510924 formulated in 1× PBS. Group 1 and 2 animals received vehicle or 3 mg/kg of LY2510924 twice daily for 14 days with treatment beginning on one day before tumor cell injection. Group 3 animals received 3 mg/kg of LY2510924 twice daily for 13 days with treatment beginning one day after tumor cell injection. After treatment, lung tissues were fixed in 10% neutral-buffered formalin for at least 24 hours and embedded and processed whole so that all lung lobes were present in histologic sections. Three-micrometer sections were stained with hematoxylin and eosin (H&E) and were immunostained for human cytokeratin 18 (Dako) to highlight the metastatic cells and differentiate them from the murine lung tissue. The H&E-stained sections of the entire lung field were examined per animal for the presence of micro- and/or macrometastases. For quantitative evaluation, images of the cytokeratin 18-stained lungs were collected with an Aperio ScanScope. Images were imported into the eCognition image analysis software written by Definiens and an algorithm was written to determine the area of lung containing metastatic cells, the number of metastases, and the size of metastatic foci. LY2510924 is highly soluble in 1× PBS, and for all in vivo studies, it was formulated with 1× PBS.

Results

Identification of LY2510924

We combined a medium throughput screen and a rational design approach and identified LY2510924 as a CXCR4 antagonist. As shown in Fig. 1A, LY2510924 is a small and cyclic peptide composed of Cyclo[Pe-Tyr-Lys(iPr)-D-Arg-2-Nal-Gly-D-Glu-Lys(iPr)-NH2]. It has a molecular weight of 1189.74 Dalton. On the basis of the published X-ray crystal structures of CXCR4 (23), we conducted a structural modeling analysis. The tentative binding pose of LY2510924 suggested that it occupied a binding pocket (Fig. 1B) and possessed ligand–receptor interactions with CXCR4 residues such as Asp187, Arg188, Glu202, His113, and Tyr190 (Fig. 1C). LY2510924 appeared to have a complementary pocket (Fig. 1B) and possessed ligand ing pose of LY2510924 suggested that it occupied a binding

LY2510924 is a potent and selective CXCR4 antagonist

As shown in Fig. 1D, LY2510924 inhibited SDF-1 binding to human CXCR4 in a concentration-dependent fashion with IC50 of 0.0797 nmol/L, or Kd of 0.0495 nmol/L. The results suggest that LY2510924 has a high affinity in interacting with CXCR4 and blocking SDF-1 binding. To further confirm the inhibitory activity of LY2510924, an SDF-1–induced GTP-binding assay was developed with radioactively labeled GTPyS35 and purified CEM cell membrane. As demonstrated in Fig. 1E, LY2510924 completely inhibited SDF-1–mediated GTPyS35 binding in a concentration-dependent manner with Kd of 0.38 nmol/L. The result further suggests that LY2510924 functions as an antagonist. One of the important functions of CXCR4/SDF-1 interaction is to regulate cell migration. To determine the cellular activity of LY2510924, we developed a cell migration (or chemotaxis) assay with U937 cells that express endogenous CXCR4. As demonstrated in Fig. 1F, LY2510924 inhibited SDF-1–induced migration of U937 cells in a concentration-dependent manner with IC50 of 0.26 nmol/L, further confirming that LY2510924 acted as an antagonist. To assess the selectivity, LY2510924 was tested in ligand binding assays of a panel of selected chemokine and other G protein-coupled receptors. As listed in Supplementary Table S1, LY2510924 had no inhibitory activities against other chemokine receptors including CCR1, CCR2, CXCR2, and CXCR3 at the concentrations tested. Similarly, there was no activity observed among serotonin, dopamine, and opioid receptors.

LY2510924 inhibits SDF-1– and CXCR4-mediated cell signaling in tumor cells

We have previously demonstrated that SDF-1 stimulates Akt and ERK phosphorylation in Hela cells (6). As shown in Fig. 2A, SDF-1 treatment of Hela cells stimulated the phosphorylation of ERK and Akt, and LY2510924 inhibited SDF-1–stimulated ERK and Akt phosphorylation in a concentration-dependent manner (Fig. 2A and B). Further quantitation from Western blot analyses revealed that the IC50 values of LY2510924 inhibition of p-ERK and p-Akt in Hela cells were 3.3 and 0.33 nmol/L, respectively. Similarly, in Namalwa cells, which also express high level of CXCR4, treatment of SDF-1 enhanced the phosphorylation of ERK and Akt, and LY2510924 again inhibited SDF-1–stimulated Akt phosphorylation with IC50 values of 1.4 and 1.2 nmol/L, respectively (Fig. 2C and D). These results suggest that LY2510924 blocks CXCR4/SDF-1–regulated cell signaling in tumor cells.

LY2510924 has no apparent agonist activity

Functional studies from cell migration, GTP binding, and cell signaling analyses demonstrated that LY2910924 inhibited SDF-1/CXCR4–mediated cellular functions. This suggests that LY2510924 acted as an antagonist of CXCR4. To evaluate whether LY2510924 had agonist activity, we tested it in GTPyS35–binding and cell migration assays in agonist mode. As illustrated in Fig. 2E and F, SDF-1x stimulated GTPyS35 binding and cell migration in a concentration-dependent manner with ED50 of 1.32 nmol/L and 1.2 nmol/L, respectively. However, in a wide range of concentrations of LY2510924 tested, there was no observed stimulation of GTPyS35 binding (Fig. 2E) or cell migration (Fig. 2F) in the same assay conditions.

LY2510924 has good in vivo stability and pharmacokinetic profile

Pharmacokinetic (PK) studies were conducted in different preclinical species using subcutaneous and intravenous dosing to assess exposure and determine relative bioavailability. As demonstrated in Fig. 3A, rat and dog show a similar trend of exposure and relative bioavailability. As illustrated in Fig. 3B, C, and D, LY2510924 exhibited good in vivo stability under in vivo conditions.

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PK parameters are summarized in Table 1. The intravenous clearance of LY2510924 was less than hepatic plasma flow in all preclinical species tested. Volume of distribution was relatively small (0.5–0.7 L/kg). Subcutaneous relative bioavailability was 100%, 68%, and 100% in rat, dog, and monkey, respectively. Terminal elimination half-life was estimated to be 2.5 to 5.5, 1.7, 3.2, and 3.1 hours in mouse, rat, dog, and monkey, respectively. The in vivo half-life and other PK properties support further investigation of LY2510924 in the clinic. Indeed, in a completed phase I clinic study, LY2510924 has a half-life of 9.16 hours in the human lymphoma xenograft model.

LY2510924 inhibits tumor growth in a human non–Hodgkin lymphoma xenograft model

To evaluate in vivo efficacy of LY2510924 in a cancer disease model, we established a tumor xenograft model with human non–Hodgkin lymphoma Namalwa cells as described (9). The Namalwa cells were carefully characterized for CXCR4 expression and function by multiple approaches including SDF-1 binding (data not shown) and CXCR4-dependent ERK and Akt activation (Fig. 2C and D). In addition, LY2510924 is active against mouse CXCR4, and human CXCR4 can be activated by mouse SDF-1. To test in vivo efficacy of LY2510924 in this model, we treated the animals subcutaneously with 0.1 mg/kg twice daily, 0.3 mg/kg twice daily, 1 mg/kg twice daily, 3 mg/kg twice daily, or 0.6 mg/kg once daily. As demonstrated in Fig. 4A, a dose-dependent tumor growth inhibition was observed among compound-treated groups, and no significant body weight changes were observed in any dose group throughout the study. Statistical analysis using ANOVA with Dunnett comparisons to baseline revealed that all the treated groups, with the exception of the 0.3 mg/kg twice-daily group, showed significant tumor growth reduction compared with the vehicle group.

To understand the molecular mechanism of tumor growth inhibition in this model, we collected fresh tumor tissues at the end of the study and conducted a high content multiplexed imaging analysis with Hoechst, CD31, caspase-3, and Ki-67 antibodies. As shown in Fig. 4B, a dose-dependent and significant
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A decrease of nuclear intensity was observed, suggesting a potential cell-cycle effect (likely G1 to G2 decrease of nuclear intensity was observed, suggesting a potential agent inhibited tumor growth significantly. As demonstrated in Fig. 5A, LY2510924 at 2 mg/kg in single xenograft models, LY2510924 inhibits tumor growth in multiple solid tumor xenograft models. A number of tumor xenograft models were utilized to further evaluate the antitumor growth activities of LY2510924 as a single agent. As demonstrated in Fig. 5A, LY2510924 at 2 mg/kg in single agent inhibited tumor growth significantly in a renal cell carcinoma (RCC) xenograft model of A-498, a cell line with von Hippel–Lindau (VHL) mutation and high level CXCR4 expression. Similarly, LY2510924 also inhibited tumor growth of a tumor xenograft model of A549, a lung cancer cell line with a KRas mutation and CXCR4 expression (Fig. 5B). Significant tumor growth inhibition was also observed in a colon tumor xenograft model of HCT-116 cells expressing functional CXCR4 (Fig. 5C). Overall, the study results suggest that LY2510924 has single-agent antitumor growth activities in a variety of preclinical tumor models. The molecular mechanisms of tumor growth inhibition in these solid tumors are under investigation.

LY2510924 inhibits tumor cell metastasis in an experimental breast cancer metastatic model

SDF-1 and CXCR4 were demonstrated to play a critical role in breast cancer metastasis (2, 24, 25). To evaluate the activity of LY2510924 inhibition of breast cancer metastasis, an experimental lung metastasis model was developed with breast cancer MDA-MB-231 cells. The mice were treated subcutaneously with vehicle (1× PBS) or LY2510924 either 24 hours before or 24 hours posttumor cell injection. Fourteen days after tumor cell implant, the lungs were collected for H&E stain and IHC analyses with human cytokeratin 18 antibody. The lung metastases were identified and quantitated in histologic sections using cytokeratin 18 staining. As demonstrated in Fig. 6A, in both twice daily ×15 (compound treatment 24 hours before tumor cell implant) and
twice daily ×13 (compound treatment 24 hours posttumor cell implant) treatment groups, total percentage area of lung metastasis was significantly decreased. A more detailed analysis revealed that the inhibition of lung metastasis in the 15 × twice-daily group was due to a decrease in both the number of metastases and the size of individual colony. However, in the twice daily ×13 group, the inhibition of lung metastasis was primarily due to decreased size of lung metastases, while the difference in number of colonies was not statistically significant (Fig. 6B–D). Figure 6E showed representative images of the lungs staining with human cytokeratin 18 from three groups. These results suggest that LY2510924 is able to inhibit tumor cell metastasis by blocking migration/homing process of tumor cells to reduce the colony numbers of lung metastasis, and also by inhibiting cell proliferation to reduce the size of the colonies after tumor cell homing to the lung.

Discussion

SDF-1 and CXCR4 mediate multiple signal transduction pathways and a variety of cellular functions such as cell migration, proliferation, invasion, and survival. In spite of significant efforts to develop a CXCR4 antagonist for clinic utilization, an ideal compound with desirable PK profile and potency suitable for chronic dosing for cancer indications is not clinically available. In this report, we identified and characterized LY2510924, which functions as a selective CXCR4 antagonist and is suitable for chronic dosing in the clinical setting. LY2510924 has acceptable in vivo stability with half-life of 3 to 5 hours. It is a potent inhibitor of the SDF-1 and CXCR4 interaction, downstream signaling, and cellular function without agonist activity. LY2510924 demonstrated dose-dependent inhibition of tumor growth in human cancer xenograft models. In an experimental metastasis model, LY2510924 inhibited breast cancer cell metastasis by blocking migration/homing process of tumor cells to the lung and by inhibiting cell proliferation after tumor cell homing in the lung. Overall, the preclinical data of LY2510924 supported its further investigation in the clinic.

It is challenging to develop a drug-like agent suitable for clinical use due to the nature of SDF-1 and CXCR4 interaction. Plerixafor, a small-molecule inhibitor of SDF-1 and CXCR4, was approved by FDA in 2008 for the mobilization of hematopoietic stem cells in multiple myeloma and non–Hodgkin lymphoma patients (26, 27). Plerixafor is not orally bioavailable and is utilized for relatively short courses of therapy. Another small-molecular inhibitor is AMD-11070, which is in early-stage clinic development for anti-HIV infection (28). AMD11070 has a reduced basic property compared with Plerixafor and is orally bioavailable. However, it did not achieve maximal pharmacodynamics effect in human based on the elevation of white blood cells (28).

Alternatively, many peptide antagonists have been identified for CXCR4, and include T22, T140 and its analogues, a cyclic pentapeptide FC131 and its analogues, CTCE-9908 and BKT140 (17–19). Some of them, such as CTCE-9908 and BKT140, were moved into clinic studies. Although these peptides were generally potent in antagonizing CXCR4, their in vivo stability and PK profile were generally poor. For example, BKT140 has a half-life of 0.29 to 0.72 hour in a clinical study (18), and the half-life of CTCE-9908 was even shorter. LY2510924 is a small cyclic peptide containing a
number of non-natural amino acids with improved in vivo stability. In a completed phase I study of patients with advanced cancer, the in vivo half-life of LY2510924 at 20 mg, the recommended phase II dose was 9.16 hours, and the pharmacodynamic effect based on stem cell mobilization was robust (20). Because of its high potency and in vivo stability, LY2510924 was suitable for once daily injection for chronic treatment in a clinical setting.
Antitumor growth activities of LY2510924 in solid cancer xenograft models.

Figure 5.


CXCR4 is a G protein-coupled receptor, and studies have demonstrated that SDF-1 and CXCR4 interaction causes mobilization of calcium, decrease of cAMP within the cells, and activation of multiple signaling pathways, including PI3 kinase/AKT, PLC-γ/protein kinase c, and MAPKs ERK1/2 (6, 7, 29). Functionally, CXCR4 plays a role in many stages of tumor biology, including tumor growth, survival, invasion, metastasis, and angiogenesis. The molecular mechanism of antitumor activities by SDF-1 and CXCR4 inhibition could be complex in different models. In this report, we showed that LY25109204 caused a significant and concentration-dependent decrease of nuclear intensity in the Namalwa xenograft model, suggesting that it may affect the G1 to G2–M progression of the cell cycle. The cell cycle and proliferation effects observed in this model were consistent with other reports (3, 12, 30). Surprisingly, in the lymphoma Namalwa xenograft model, we have not observed significant impact on the tumor vasculature based on CD31 staining. It is likely due to the low base level of vasculature in this specific model. The molecular mechanisms of LY2510924 in other solid tumor models tested in this study were not characterized, and additional investigations to define the molecular mechanisms including antiangiogenic effect in these models are ongoing.

CXCR4 is overexpressed in a variety of cancers including breast (2), lung (31), RCC (5, 32), colon (33), and brain cancer (7), as well as acute myeloid leukemia (34) and non–Hodgkin lymphoma (9). High CXCR4 expression is generally correlated with more invasiveness and high-risk disease and with poor prognosis. LY2510924 was demonstrated to have single-agent activities in xenograft models of RCC, lung, colon, breast cancer, and non-Hodgkin lymphoma. In RCC, particularly clear cell RCC with inactivation of VHL tumor suppressor gene, CXCR4 is significantly upregulated and is an independent prognostic factor associated with poor survival (5, 32). Therefore, LY2510924 in combination with standard of care could be investigated as a strategy for treatment of clear cell RCC with VHL mutation. In lung cancer, particularly small cell lung carcinoma (SCLC), CXCR4 is often overexpressed and correlated with more invasive disease (31, 35). LY2510924 could be investigated as a treatment option of SCLC with high-level CXCR4 expression. In non–Hodgkin lymphoma and other hematologic cancer types, CXCR4 expression is generally high in tumor cells, and SDF-1 is enriched in tumor microenvironment such as bone marrow. Interaction of SDF-1 and CXCR4 retains the tumor cells in the tumor microenvironment and, and refers resistance to the standard chemotherapy. Combination of LY2510924 and chemotherapy could be an important strategy to improve the efficacy of standard of care for acute myelogenous leukemia (36). In breast cancer, CXCR4 is well characterized for its important role in metastasis and survival (2, 24, 25). In an experimental metastasis model, we demonstrated that LY2510924 inhibited breast cancer cell metastasis by blocking migration/homing process of tumor cells to the lung and inhibiting cell proliferation after tumor cell homing. These results are consistent with those obtained previously with RNAi of CXCR4 in a 4T1 breast cancer model (37).

CXCR4 through its interaction with SDF-1 also plays a critical role in tumor microenvironment through local activation of endothelial and myeloid progenitor cells, infiltration of immune cells and progenitor cells, immunosuppression, and T-cell activation (3, 11, 38, 39). Blockade of SDF-1 and CXCR4 axis inhibited tumor angiogenesis in breast (3) and other cancer models (10). In an orthotopic glioblastoma model, CXCR4 inhibition dramatically improved the efficacy of irradiation therapy by blocking infiltration of myeloid progenitor cells and inhibiting myeloid cell-mediated vasculogenesis (11). In an immunocompetent mouse model of ovarian cancer, CXCR4 inhibition selectively reduced intratumoral FoxP3+ T cells and increased T-cell–mediated anti-tumor immune responses (38). Recently, CXCR4 inhibition promoted T-cell accumulation in a pancreatic tumor model and synergized with the checkpoint antagonist, anti–PD-L1 antibody to cause cancer regression (39). On the basis of these data, LY2510924 may be a therapeutic agent with the potential to improve the clinic benefits of some current therapies, such as irradiation, antiangiogenic agent, or immunotherapeutic checkpoint antagonist through a rational combination strategy.
Disclosure of Potential Conflicts of Interest

M. Zamek-Gliszczynski is S.M. Director at GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: S.-B. Peng, Y.-H. Hui, M. Zamek-Gliszczynski, L.Z. Yan

Figure 6.
LY2510924 inhibits breast cancer metastasis in the MDA-MB-231 model. SCID female mice were injected intravenously via the tail vein with 5 × 10^6 MDA-MB-231 cells, and were treated subcutaneously with vehicle or 3 mg/kg of LY3009120. Group 1 and 2 animals received vehicle or 3 mg/kg of LY251092 twice daily (BID) for 15 days with treatment beginning one day before tumor cell injection, and Group 3 animals received 3 mg/kg of LY2510924 twice daily for 13 days with treatment beginning one day after tumor cell injection. After treatment, fresh lung tissues were collected for H&E staining and human cytokeratin 18 IHC as described in Materials and Methods. The H&E-stained sections of the entire lung field were examined per animal for the presence of micro- and/or macrometastases. For quantitative evaluation, images of the cytokeratin 18-stained lungs were collected with an Aperio ScanScope. Images were imported into the eCognition image analysis software system provided by Definiens. A, total lung metastatic area among groups. B, total numbers of lung metastasis among groups. C, maximum size of lung metastasis among groups. D, mean size of lung metastasis among groups. *P < 0.01. E, representative images of cytokeratin 18 IHC among three groups. Red arrows, positive cytokeratin 18 staining of lung metastatic colonies.
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