**In Vitro and In Vivo Activity of Lucitanib in FGFR1/2 Amplified or Mutated Cancer Models**

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**Abstract**

The fibroblast growth factor receptor (FGFR) pathway has been implicated both as an escape mechanism from anti-angiogenic therapy and as a driver oncogene in different tumor types. Lucitanib is a small molecule inhibitor of vascular endothelial growth factor (VEGF) receptors 1 to 3 (VEGFR1 to 3), platelet derived growth factor α/β (PDGFRα/β) and FGFR1–3 tyrosine kinases and has demonstrated activity in a phase I/II clinical study, with objective RECIST responses in breast cancer patients with FGFR1 or FGF3/4/19 gene amplification, as well as in patients anticipated to benefit from anti-angiogenic agents. We report here the in vitro and in vivo antitumor activity of lucitanib in experimental models with or without FGFR1/2 amplification or mutations. In cell assays, lucitanib potently inhibited the growth of tumor cell lines with amplified FGFR1 or mutated/amplified FGFR2. In all xenograft models studied, lucitanib demonstrated marked tumor growth inhibition due to potent inhibition of angiogenesis. Notably, in two lung cancer models with FGFR1 amplification, the antitumor efficacy was higher, suggesting that the simultaneous inhibition of VEGF and FGF receptors in FGFR1 dependent tumors can be therapeutically advantageous. Similar antitumor activity was observed in FGFR2 wild-type and amplified or mutated xenograft models. Pharmacokinetic studies showed lucitanib plasma concentrations in the micro/sub-micromolar range demonstrated drug accumulation following repeated lucitanib administration.

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**Introduction**

Fibroblast growth factor receptors (FGFRs) play several roles in the control of cell proliferation, cell differentiation, angiogenesis, development and survival [1]. While the FGFR pathway was initially studied as a promoter of tumor angiogenesis with synergistic effects on the vascular endothelial growth pathway [2], recent studies have suggested that the pathway is involved both as an escape mechanism from anti-angiogenic therapy [3] and as a driving oncogene in certain tumors [4,5]. De-regulation of the FGFR pathway has been shown to induce tumor cell growth and to maintain the malignant properties of cancer cells. Alterations of the FGFR pathway in cancer include: gene amplification with receptor over-expression, FGFR mutations with...
constitutive active receptor or reduced dependency on ligand binding, translocation to produce FGFR fusion proteins with constitutive kinase activity, alternative FGFR splicing leading to a different but broader ligand specificity and up-regulation of the FGF expression with subsequent paracrine/autocrine activation of the pathway [6–8]. The dependency on FGFR pathway has been reported in different human malignancies [9,10]. Amplification of FGFR1 has been reported in breast cancers (~10%) [11,12], ovarian cancers (5%), lung squamous cell carcinomas (~20%) and lung adenocarcinomas (~3.5%) [13–15]. Approximately 5% of gastric cancers have been reported to bear FGFR2 amplification [16]. FGFR2 mutations occur in about 12% of endometrial carcinoma. Up to 80% of low-grade and 20% of high grade bladder cancer harbor activating mutations in FGFR3 [17,18].

The inhibition of the aberrant FGFR pathway in cancer models by anti-FGFR antibodies and/or small molecules has been shown to have robust antitumor effect [4]. Several multitarget tyrosine kinase inhibitors in clinical development, originally identified as ATP competitors of the vascular endothelial growth factor receptor (VEGFR) family, have been shown to have activity against one or more FGFRs in preclinical models (for an updated review see (4)): nintedanib [19] brivanib [20], dovitinib [21,22] and ponatinib [23]. Recently, more specific FGFR inhibitors lacking significant activity against VEGFR have been synthesized and are under clinical evaluation [19], specifically designed for copy number analysis (Applied Biosystems). Thermal cycle conditions were 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Data were analyzed using the CopyCaller 2.0 Software (Applied Biosystems).

Real Time (RT)-PCR was used for relative quantification of FGFR1, FGFR2 and FGFR2 mRNA. Total RNA from human umbilical vein/endothelium HUVEC and lung cancer cell lines was purified with the SV40 Total RNA Isolation System (Promega) and reverse transcribed with the cDNA Archive Kit (Applied Biosystems). FGFR1, FGFR2, FGF2, and ACTB expression was detected using TaqMan assays (Applied Biosystems). Reactions were run in a total volume of 20 μL containing 8 ng of cDNA with TaqMan PCR Master Mix, following the manufacturer’s instructions (Applied Biosystems). All the data were normalized to ACTB, and data are expressed as fold increase over the normalized value of HUVEC cells.

Materials and Methods

Cell Lines and Drug Treatment

Ten non-small cell lung cancer (NSCLC) cell lines (H1299, H1975, H2342, H1650, H358, A549, H2228, H23, H1581, H520), one small cell lung cancer (SCLC) cell line (DMS114), three gastric (KATOIII, SNU16, MNK45) and three endometrial (HEC1A, MFE296, AN3CA) cancer cell lines were maintained in RPMI supplemented with 1% glutamine and 10% fetal bovine serum (FBS). Human umbilical vein cells (HUVEC) were maintained as monolayer in MCDB131 medium supplemented with 20% (v/v) FBS, 1% (v/v) L-glutamine, 5 Units/ml Heparin and 50 μg/ml endothelial cell growth factor (ECGF) using culture flasks or plates pre-coated with 1% (v/v) gelatine. All cell lines were obtained from the American Type Culture Collection and their authentication was carried out by the authors within the last 6 months.

Lucitaniib was dissolved in DMSO in a stock solution of 10 mM and stored at −20 °C. All cell lines were treated with a range (0.01 to 50 μM) of lucitaniib concentrations in 96-well plates 48 hours after seeding. A cell viability assay (CellTiter MTS, Promega, Italy) was performed 72 hours after treatment. Quantitation of fluorescence was measured at 490 nm in a microplate reader (Infinite M200, TECAN) after 2 hours of incubation with MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H–tetrazolium) according to manufacturer’s instruction. The efficacy of the treatment (half maximal inhibitory concentration, IC50) was calculated by CalcuSyn Software (Biosoft, Cambridge, UK).

Molecular Analyses

DNA from lung cancer cell lines was extracted using the Maxwell 16 Cell DNA Purification Kit (Promega, Milan, Italy). The FGFR1 and FGFR2 gene copy numbers were assessed using a TaqMan Copy Number assay (no Hs00241111 and Hs01552926 respectively, Applied Biosystems, Monza, Milan). RNaseP copy number was used as reference gene. The PCR was carried out in 96-well plates with a reaction volume of 15 μL containing 10 ng of DNA, TaqMan Genotyping Master Mix (Applied Biosystems), primers and probes specifically designed for copy number analysis (Applied Biosystems). Thermal cycle conditions were 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Data were analyzed using the Copy Caller 2.0 Software (Applied Biosystems).

Two-Parameter Flow Cytometry Analysis: DNA Content and FITC-Conjugated dUTP

DNA fragmentation during apoptosis was detected by the TdT-mediated dUTP nick-end labeling technique (TUNEL), which uses terminal deoxynucleotidyl transferase (TdT) to catalyze the binding of FITC-conjugated dUTP to DNA strand breaks. DMS114 and H1299 cells treated either with DMSO or with the corresponding lucitaniib IC50 doses, were fixed at different time points after treatment, washed in PBS and permeabilized for 2 minutes on ice in 0.1% Triton X-100, 0.1% sodium citrate. The cells were washed, re-suspended in 50 μL of TUNEL reaction mixture (Roche, Mannheim, Germany) containing dUTP–FITC and TdT, and incubated for 90 minutes at 37 °C in the dark. After that, the samples were washed and re-suspended in 1 μg/ml propidium iodide plus 25 μL of 1 mg/ml RNase in water and incubated overnight at 4 °C.

Western Blotting

Protein extraction and Western Blot were performed as previously described [31]. Anti-Phospho(Y653/654)-panFGFR, anti-FGFR1, anti-FGFR2, anti-Phospho(Y436)FRS2, anti-Phospho(T202/Y204)ERK1/2 and anti-ERK1/2 antibodies were purchased from Cell Signaling (Danvers, MA), while anti-FRS2 antibody was purchased from R&D systems (Minneapolis, MN). Secondary anti-rabbit and anti-sheep

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**Xenograft Models**

Female NCr-nu/nu mice (6 weeks old) were obtained from ENVIGO RMS srl (Correzzana, Italy). They were maintained under specific pathogen free conditions, housed in isolated vented cages, and handled using aseptic procedures. The IRCCS-Istituto di Ricerche Farmacologiche Mario Negri adheres to the principles set out in the following laws, regulations, and policies governing the care and use of laboratory animals: Italian Governing Law (D. lg 26/2014; Authorization n.19/2008-A issued March 6, 2008 by Ministry of Health); Mario Negri Institutional Regulations and Policies providing internal authorization for persons conducting animal experiments (Quality Management System Certificate – UNI EN ISO 9001:2008 – Reg, No.6121); the NIH Guide for the Care and Use of Laboratory Animals (2011 edition) and EU directives and guidelines (EEC Council Directive 2010/63/UE). The Statement of Compliance (Assurance) with the Public Health Service (PHS) Policy on Human Care and Use of Laboratory Animals was recently reviewed (9/9/2014) and will expire on September 30, 2019 (Animal Welfare Assurance #A5023–01). H1581, DMS114, H1299, MFE296, SNU16, MNK45 and HEC1A tumors were subcutaneously transplanted into the flanks of nude mice as fragments. Mice were randomized when the average tumor size was 200–250 mm³ (n = 8–10 per group), to receive lucitanib given orally at the dose of 2.5, 5, 10 or 20 mg/kg daily or vehicle (Methocel 0.5%) for 30 days. Tumor growth was measured twice weekly with a Vernier caliper, and the tumor volumes (mm³) were calculated as follows: (length [mm] × width [mm]²)/2. Tumor growth data were expressed as the mean ± SEM. Treatment efficacy was expressed as best tumor growth inhibition [%T/C = (median volume of treated tumors/median volume of control tumors) × 100]. Animals were euthanized when primary tumor volume exceeded 10% of body weight, evaluated the same day.

**Table 1. Molecular Characteristics of the Cancer Cell Lines**

| Cell Line | Type   | Gene Copy Number | mRNA Mutational Status |
|-----------|--------|------------------|------------------------|
| H1299     | NSCLC  | FGFR1: 1, FGFR2: 2 | mRNA Mutational Status |
| H1975     | NSCLC  | FGFR1: 1, FGFR2: 1 | mRNA Mutational Status |
| H2342     | NSCLC  | FGFR1: 1, FGFR2: 1 | mRNA Mutational Status |
| H1650     | NSCLC  | FGFR1: 2, FGFR2: 4 | mRNA Mutational Status |
| H538      | NSCLC  | FGFR1: 1, FGFR2: 1 | mRNA Mutational Status |
| A549      | NSCLC  | FGFR1: 2, FGFR2: 2 | mRNA Mutational Status |
| H2228     | NSCLC  | FGFR1: 2, FGFR2: 1 | mRNA Mutational Status |
| H23       | NSCLC  | FGFR1: 1, FGFR2: 1 | mRNA Mutational Status |
| H1581     | NSCLC  | FGFR1: 4, FGFR2: 2 | mRNA Mutational Status |
| H520      | NSCLC  | FGFR1: 4, FGFR2: 1 | mRNA Mutational Status |
| DMS114    | NSCLC  | FGFR1: 5, FGFR2: 3 | mRNA Mutational Status |
| HEC1A     | Endometrial | FGFR1: 2, FGFR2: 4 | mRNA Mutational Status |
| MFE296    | Endometrial | FGFR1: 2, FGFR2: 2 | mRNA Mutational Status |
| AN3CA     | Endometrial | FGFR1: 2, FGFR2: 2 | mRNA Mutational Status |
| MNK45     | Gastric | FGFR1: 1, FGFR2: 1 | mRNA Mutational Status |
| SNU16     | Gastric | FGFR1: 2, FGFR2: 1 | mRNA Mutational Status |
| KATO III  | Gastric | FGFR1: 2, FGFR2: 1 | mRNA Mutational Status |

Gene copy number and mRNA expression levels were determined as described in Material and Methods. Mutational status derived from Cosmic database (http://cancer.sanger.ac.uk/cosmic). wt, wild type; mut, mutated; na: not available.

**Figure 1.** Lucitanib in vitro activity (IC₅₀) in lung, endometrial and gastric cancer cell lines. wt FGFR1/2: (○); amplified FGFR1: (■); amplified FGFR2: (□); mutated FGFR2: (□). IC₅₀ values have been calculated as described in Materials and Methods and are the arithmetic mean IC₅₀ (µM) ± SEM.
Lucitanib Pharmacokinetics

Pharmacokinetic sampling was performed after 12 daily repeated lucitanib oral administrations (qdx12) in different xenograft models at several time intervals after dosing. Blood samples were collected at 4 and 24 hours post-dose for H1299 and DMS114 tumor bearing mice receiving daily doses of 2.5, 5, 10 and 20 mg/kg. Blood samples were collected at 4 hours and 24 hours post-dose for H1581 tumor bearing mice treated at 2.5, 5, 10 and 20 mg/kg daily doses and specifically for the 5 and 20 mg/kg doses, additional samplings were performed at 1, 2, and 8 hours post-treatment. Three to four mice were used per time-point; animals were anesthetized with isoflurane, blood was drawn into heparinized tubes from the retro-orbital plexus, centrifuged 10 min at 4000 rpm at 4 °C and plasma was separated and stored frozen at −20 °C. Mice were sacrificed by cervical dislocation and tumor samples were removed, snap-frozen and stored at −20 °C. Plasma and tumor concentrations of lucitanib after repeated oral doses were measured by LC–MS/MS analysis, as previously reported[32,33]. The lower limit of quantification of the method was 2 and 5 ng/ml lucitanib in plasma and tumors, respectively. The tumor-to-plasma lucitanib concentration ratio was calculated in mice sacrificed at 4 and 24 hours post-dosing. Data are expressed as μM concentration (mean ± standard deviation).

Results

FGFR1 Amplified or FGFR2 Amplified or FGFR2 Mutated Cancer Cell lines are Preferentially Sensitive to Lucitanib In Vitro

The present studies were undertaken to investigate lucitanib cytostatic/cytotoxic activity in FGFR1/2 deregulated cell lines. A panel of 17 cancer cell lines (11 lung, 3 endometrial, and 3 gastric cancer cell lines) was characterized for FGFR1/2 gene copy number, FGFR1/2 and FGF2 mRNA levels and mutational status of FGFR2, TP53 and KRAS (Table 1). Gene amplification was defined positive when the gene copy number was higher or equal to 4. All FGFR1 amplified lung cancer cell lines (DMS114, H1581, H520) overexpressed FGFR1 mRNA (Table 1). On the contrary, FGFR1 non-amplified lung cancer cell lines did not overexpress FGFR1 mRNA. FGFR1 mRNA fold increase correlated with FGFR1 gene copy number (Supplementary Figure 1A).

Figure 2. Cell cycle perturbation and apoptosis induced by lucitanib treatment. A. Flow cytometric analysis of DNA content during treatment with IC50 lucitanib dose in H1299 cells (right panel, 10 μM) and DMS114 cells (left panel, 1 μM). Upper panels show the DNA content of untreated cells (“ctrl”), while lower panels show the DNA content of cells treated with IC50 lucitanib dose for 4, 24, 48 and 72 hours. B. Flow cytometric analysis of apoptotic cells represented by biparametric PI fluorescence (DNA content) and dUTP-FITC fluorescence. Untreated and treated cells are represented as in Panel A at the same times and doses of treatment. Cells with DNA fragmentation induced during apoptosis are dUTP-positive (above the straight line). DNA corresponding to G1 (2 N) and G2 M (4 N) cells is indicated.
FGFR2 amplified or mutated cancer cell lines were markedly more sensitive to lucitanib treatment (IC\textsubscript{50} of 0.045–3.16 μM) than the respective tumor type cell lines with wt FGFR1 and wt FGFR2 (IC\textsubscript{50} of 3–23 μM) (Figure 1). In lung cancer cell lines, lower IC\textsubscript{50} values were associated with higher FGFR1 gene copy number (Supplementary Figure 1B).

Perturbation of the cell cycle was investigated in FGFR1 amplified (DMS114) and non-amplified (H1299) cell lines by treating cells with corresponding lucitanib IC\textsubscript{50} (1 μM and 10 μM respectively) for 4, 24, 48 and 72 hours. A G1 block starting at 24 hours and persisting up to 48 hours of treatment was observed in DMS114 cells, while a clear late S-G2/M blockade of the cell cycle in H1299 cells was observed (Figure 2, upper panel A). Both effects were more evident at twice the IC\textsubscript{50} dose (data not shown). In the same experimental conditions, no apoptosis was detected by TUNEL assay (Figure 2B, lower panel), suggesting a cytostatic, rather than cytotoxic, effect of drug treatment in both the FGFR1 amplified and non-amplified cells used here. Modulation of the FGFR1 pathway signaling was investigated in vitro. As shown in Supplementary Figure 2, a decrease in FGFR, FRS2 and ERK phosphorylation was observed in both H1581 and SNU16 cells after 2 hours of lucitanib treatment ≥100 nM.

Lucitanib Inhibits In Vivo Tumor Growth in Lung, Gastric and Endometrial Carcinoma Xenograft Models

The antitumor activity of lucitanib was evaluated in mice subcutaneously implanted with FGFR1/2 gene amplified (n = 3) and non-amplified cell lines (n = 2). Mice with established tumors were treated with 2.5, 5, 10 or 20 mg/kg QD lucitanib PO. Dose-dependent antitumor activity was observed in all the xenograft models (Figure 3), with a greater antitumor activity at higher doses (Figure 3 and Table 2). At the lowest dose tested (2.5 mg/kg) partial inhibition of tumor growth was observed in all models. In lung xenograft models, lucitanib was preferentially active in the two FGFR1 amplified models than in the non-amplified model (Figure 3A and Table 2). At the 5 mg/kg dose, in H1299 non-amplified FGFR1 xenografts, lucitanib was less active than in the H1581 and DMS114 amplified FGFR1 xenografts, with T/C values of 41% (H1299) compared to 24% (H1581) and 20% (DMS114). The antitumor activities at 5 mg/kg for FGFR1-amplified models were comparable with those observed at the higher doses 10 and 20 mg/kg in the FGFR1 non-amplified model (Figure 3A and Table 2). No tumor regressions were observed in any of the lung xenograft models tested at any dose schedule, with the best effect observed being tumor stabilization.
Lucitanib Activity in FGFR1/2 Deregulated Systems

In gastric and endometrial cancer models, lucitanib treatment caused a dose-dependent tumor growth inhibition in all xenografts. The antitumor activity observed in FGFR2 non-amplified gastric xenograft model (MNK45) was comparable to the one in the one FGFR2 amplified (SNU16) (Figure 3C and Table 2). Similarly, lucitanib exerted a dose-dependent antitumor activity in endometrial cancer xenografts models regardless of FGFR2 mutational status (Figure 3B, and Table 2). Tumor regressions were observed at the highest tested dose of 20 mg/kg in HEC1A and MNK45 bearing wt FGFR2, in 2 and 3 out of 10 mice respectively (data not shown).

**Lucitanib Pharmacokinetics After Chronic Drug Treatment**

To better characterize the pharmacological properties of lucitanib, mice bearing H1299, H1581 and DMS114 lung xenografts were treated for 12 consecutive days with different doses of lucitanib. Twelve days of continuous drug treatment was predicted to achieve the steady-state plasma concentration based on previous studies (35, 36). At 4 (Figure 4, A and B) and 24 hours (Figure 4, C and D) after the last administered dose, animals were sacrificed and drug concentrations were measured in plasma and tumors. At both time points, lucitanib concentrations were similar in the different tumor models (Supplementary Table 1), were correlated to the administered dose (Figure 4). No difference in drug levels was observed in regards to FGFR1 amplification status (Supplementary Table 1). The drug accumulated in tumors after repeated treatments, with tumor/plasma ratios increasing from 2.5 ± 0.7 at 4 hours to 24.8 ± 7.4 at 24 hours. Comparable plasma and tumor drug levels were found in the endometrial and gastric xenograft models (Supplementary Figure 3).

**Discussion**

Lucitanib is a novel small molecule that selectively binds VEGFR1–3, PDGFRα/β and FGFR1–3 receptor tyrosine kinases with Kd <100 nM in biochemical assays. Our previous data have shown that lucitanib is highly active as a single agent in diverse human tumor xenografts with tantumor activity being related to its anti-angiogenic effects [34]. In addition, a synergistic effect of the combination lucitanib plus paclitaxel was demonstrated in triple negative breast cancer human xenografts [35]. In the present study, we explored the ability of lucitanib to inhibit the FGFR pathway in different experimental systems characterized by deregulation of the pathway due to increased levels of FGFR1/2 mRNA, amplified FGFR1, and/or expression of mutated or amplified FGFR2.

Although FGFR1 has been identified as a potential driver for cell proliferation in breast and lung cancer cell lines [36,37], it remains to be established whether FGFR1 gene copy number is a predictive biomarker for response to FGFR1 inhibition in terms of tumor growth and survival. Data reported on NSCLC cells suggest that FGFR1 amplification does not always predict sensitivity to FGFR1 inhibition [15]. Only the H1581 cell line, harboring a focal FGFR1 amplification, was growth-inhibited when treated with shRNA targeting FGFR1. An association between FGFR1 amplification and response to NVP-BGJ398, a selective pan-FGFR inhibitor, was found when a large panel of cell lines were tested [27]. However, in the same study, 54% of the over-expressing FGFR1 mRNA cell lines were not sensitive to the drug suggesting that additional genetic alterations may play a role in FGFR dependency [27]. FGFR1 mRNA was shown to be a better biomarker for ponatinib response in lung cancer than FGFR1 gene copy number [38]. A discrepancy between FGFR1 amplification level and FGFR1 protein expression was reported in a panel of lung cell lines, and low FGFR1 expression was associated with resistance to the different FGFR inhibitors [39]. In the panel of cell lines used in the present study, we found an in vitro correlation between FGFR1 gene copy number and FGFR1 mRNA, and showed that lucitanib inhibition of cellular proliferation was higher in lung cancer cells harboring the higher FGFR1 gene copies. Lucitanib mainly induced cytotastic effect in the FGFR1-aberrant DMS114 SCLC cell line. Drug treatment of these cells caused a clear G1 block and no induction of apoptosis. In the H1299 NSCLC cell line, wt for FGFR1, a late S-G2 block and no induction of apoptosis was observed, suggesting a different cytostatic mechanism. Lucitanib was also extremely active in FGFR2 amplified or mutated cell lines with a 10-fold lower drug IC50 than in FGFR2 wt cells.

The marked activity of lucitanib in vitro in cells where the FGFR1/2 pathway is aberrant is consistent with the activity reported in preclinical models for other anti-FGFR compounds [24,40]. However for lucitanib, the translation of this antitumor effect in vitro is complicated by the fact that the drug has a more potent inhibitory activity on VEGFRs/PDGFRs than FGFRs [34,35] and that the VEGFRs have a direct role in tumor angiogenesis. In order to dissect the relative contribution of the inhibition of these receptors (VEGFRs and FGFRs) to the antitumor activity of lucitanib, the compound was tested at a range of doses in wt and FGFR1 amplified lung cancer cells, in wt and mutated FGFR2 endometrial cancer cells and in wt and FGFR2 amplified gastric cancer cells transplanted subcutaneously into nude mice. Lucitanib showed a dose dependent antitumor activity, with activity observed even at the lowest dose tested (2.5 mg/kg). In FGFR1 amplified xenografts lucitanib had greater antitumor activity as compared to FGFR1 wt xenograft, especially at the 5 mg/kg dose.
Pharmacokinetic analysis clearly showed that lucitanib tumor levels at 4 and 24 hours after the last dose intake following 12 days treatment were correlated to the administered dose and were comparable in wt (H1299) and amplified FGFR1 (H1581 and DMS114) xenografts. Tumor drug concentrations at 4 hours were similar to the in vitro IC50 values for FGFR1 wt H1299, but much higher (from 3 to 26 fold) than the IC50 values for FGFR1 amplified H1581 and DMS114 tumors. Interestingly, the drug accumulated in the tumor with high tumor/plasma ratios that increased with time from 2.5 at 4 hours to 24.8 at 24 hours post-dosing. In agreement with these data, a high lucitanib tumor distribution has recently been reported in a breast cancer biopsy from a woman participating in the First in Human Study of lucitanib. After 21 days of treatment, just before the daily oral dose of 10 mg, lucitanib achieved a tumor drug concentration of 11 μM, 25 fold higher than the concomitant plasma drug concentration of 0.43 μM [33].

In conclusion, the current study provides evidence that lucitanib is able to potently inhibit the FGF/FGFR pathway and FGFR-dependent tumor cell proliferation in vitro. Our in vivo data in FGFR1 deregulated systems suggest that simultaneous inhibition of VEGF and FGF receptors in FGFR1 dependent tumors can be therapeutically advantageous. Interestingly, while lucitanib showed enhanced inhibition of FGFR1 amplified lung xenografts, lucitanib was highly active in all endometrial and gastric xenografts models tested, irrespective of FGFR2 status. The role of FGFR aberrations as a predictive biomarker for response to lucitanib should be further evaluated and validated in prospective clinical studies.

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