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¹⁸FDG-PET predicts pharmacodynamic response to OSI-906, a dual IGF-1R/IR inhibitor, in preclinical mouse models of lung cancer

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Abstract

Purpose—To evaluate 2-deoxy-2-[¹⁸F]fluoro-D-glucose positron emission tomography imaging (¹⁸FDG-PET) as a predictive, non-invasive, pharmacodynamic (PD) biomarker of response following administration of a small-molecule IGF-1R/IR inhibitor, OSI-906.

Experimental Design—*In vitro* uptake studies of ³H-2-deoxy glucose following OSI-906 exposure were performed evaluating correlation of dose with inhibition of IGF-1R/IR as well as markers of downstream pathways and glucose metabolism. Similarly, *in vivo* PD effects were evaluated in human tumor cell line xenografts propagated in athymic nude mice by ¹⁸FDG-PET at 2, 4, and 24 hours following a single treatment of OSI-906 for the correlation of inhibition of receptor targets and downstream markers.

Results—Uptake of ³H-2-deoxy glucose and ¹⁸FDG was significantly diminished following OSI-906 exposure in sensitive tumor cells and subcutaneous xenografts (NCIH292) but not in an insensitive model lacking IGF-1R expression (NCI-H441). Diminished pharmacodynamic ¹⁸FDG-PET collected immediately following the initial treatment agreed with inhibition of pIGF-1R/pIR, reduced PI3K and MAPK pathway activity, and predicted tumor growth arrest as measured by high-resolution ultrasound imaging.

Conclusion—¹⁸FDG-PET appears to serve as a rapid, non-invasive, PD marker of IGF-1R/IR inhibition following a single dose of OSI-906 and should be explored clinically as a predictive clinical biomarker in patients undergoing IGF-1R/IR-directed cancer therapy.

Introduction

The insulin-like growth factor-1 receptor (IGF-1R) is a tetrameric transmembrane receptor tyrosine kinase that is widely expressed in normal human tissues and is up-regulated in a number of human cancers including colorectal, non-small cell lung, ovarian and pediatric cancers. The receptor is composed of two α and two β subunits linked by disulfide bonds in which the extracellular α subunit is responsible for ligand binding and the β subunit consists of a transmembrane domain and a cytoplasmic tyrosine kinase domain. Ligand binding activates the tyrosine kinase activity of IGF-1R and results in trans- subunit autophosphorylation and stimulation of signaling cascades that include PI3K-mTOR and MAPK pathways. Activation of IGF-1R has been reported to stimulate proliferation, survival, transformation, metastasis and angiogenesis, whereas inhibition of IGF-1R has been shown to impede tumorigenesis in several human xenograft models (1).

Increased expression of IGF-1R and its cognate ligands, IGF-I and IGF-II has been demonstrated in a wide range of solid tumors and hematologic neoplasias relative to normal tissue levels. Epidemiologic studies have shown an increased risk for the development of colon, lung, breast and bladder cancers with increased circulating levels of IGF-I (2–5). Additionally, IGF-1R expression levels have been correlated to poor prognosis in renal cell carcinoma (6,7). IGF-1R signaling mechanism has also been linked to resistance to various anti-tumor therapies including epidermal growth factor receptor inhibitors (1,6,8,9).

Similarly, the insulin receptor (IR) is composed of a heterotetramer consisting of two extracellular α -subunits and two transmembrane β -subunits. Binding of insulin to the IR extracellular α -subunit causes a conformational change bringing together the two β -subunits. Activated IR tyrosine kinase phosphorylates several intracellular substrates including IRS-1-4, Shc, Gab1 and Cbl. These phosphorylated proteins provide a docking site for effector proteins containing Src homology 2 (SH2) domains further linking IR to PI3-kinase (PI3K) via the regulatory p85 subunit. Homology between IR and IGF-1R ranges from 45–65% in the ligand binding domains to 60–85% in tyrosine kinase domains. Expression of IR is highest in adipose tissue and to a lesser extent in liver, heart and muscle (10). Overexpression of IR in breast, colon, lung, ovarian and thyroid cancers suggest a role of IR in tumor progression (10). More recently we have shown that forced overexpression of IR is tumorigenic in mice (11).

OSI-906 is a potent and highly selective tyrosine kinase inhibitor that exhibits similar biochemical potency against IGF-1R (8 nM) and IR (14 nM) and is greater than 4 orders of magnitude more selective for IGF-1R/IR compared to a wide number of other receptor and non-receptor kinases (12). Within a panel of >180 kinases only IGF-1R and IR were inhibited by greater than 50% at 1.0 μ M OSI-906. Inhibition of cell proliferation and induction of apoptosis following exposure to OSI-906 appears to be directly linked to inhibition of AKT in colorectal, lung, and pancreatic cancer cell lines (1,12). In addition, OSI-906 has shown potent antitumor activity *in vivo* in several xenograft models (1). Since IGF-1R and IR pathway signaling is linked to glucose metabolism, we asked whether 18 F-DG-PET could function as a surrogate pharmacodynamic marker for OSI-906. To this end, we employed *in vitro* cell culture assays and *in vivo* animal models measuring uptake of radioactive glucose analogues as a function of treatment by OSI-906. Our data demonstrate that glucose uptake is rapidly inhibited *in vitro* and *in vivo* and tracks with IGF-1R, IR and AKT inhibition after OSI-906 treatment in sensitive tumors. Moreover, reduced glucose uptake was readily observed after OSI-906 treatment in tumor tissues using 18 F-DG-PET imaging methodologies. Hence, 18 F-DG-PET may function as a rapid, non-invasive tumor specific pharmacodynamic (PD) marker for OSI-906 in the clinical setting where accurate assessment of PD effects is often times limited by the lack of readily

accessible tumor samples. As such ^{18}F FDG-PET may be a useful clinical tool in identifying active doses and patients potentially sensitive to this novel antitumor agent warranting further clinical investigation of this approach.

Materials and Methods

Cell Lines

Human non-small cell lung carcinoma cell lines (NCI-H292, NCI-H441) were obtained from American Type Culture Collection (Manassas, VA). All cell lines were maintained in RPMI 1640 media (Mediatech, Manassas, VA) supplemented with 10% FBS (Sigma, St. Louis, MO) and 1% sodium pyruvate (Mediatech, Manassas, VA) and maintained at 37°C and 5.0% CO_2 . Cells were propagated to 80–90% confluency prior to *in vitro* and *in vivo* assays.

^3H -2-Deoxy Glucose Uptake Assay

Cells were seeded in 12-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ) at a density of 9.0×10^5 cells per well in normal glucose (11.1 mM) media and allowed to attach for 6–8 hours at 37°C ($n = 3$ wells/group). The media was then changed to 5.5 mM glucose media and the cells were allowed to equilibrate overnight. Three hours prior to the assay, the media was again removed and replaced with media containing 0.0 mM glucose (glucose starvation). The cells were then treated with varying concentrations of OSI-906 (0.0 μM to 30 μM) and 0.15 mCi of ^3H -2-deoxy glucose (Perkin Elmer, Boston, MA). After 30 minutes the media was removed, the cells placed on ice and washed once with ice cold PBS (Mediatech, Manassas, VA). The PBS was then removed and the cells were lysed in RIPA buffer (Sigma, St. Louis, MO) for 15 minutes on ice. The lysates were harvested and counted in a Beckman LS6500 Liquid Scintillation counter (Fullerton, CA). ^3H -2-deoxy glucose uptake was calculated as raw counts and normalized to control samples (0.0 μM OSI-906). As a positive control of glucose uptake inhibition, NCI-H292 cells were treated with increasing concentrations (2.5 μM – 10 μM) of cytochalasin B (Sigma, St. Louis, MO), a known inhibitor of GLUT1 and GLUT4 glucose transporters.

Mouse Models

Studies involving mice were conducted in accordance with federal and institutional guidelines. NCI-H292 and NCI-H441 non-small cell human xenograft tumors were generated as described (13). Briefly, 4×10^6 cells were injected subcutaneously on the right flank of 5–6 week old female athymic nude mice (Charles Rivers, Wilmington, MA). Using this method, palpable tumors were typically observed within 2 weeks following injection of cells and were allowed to progress until approximately 150–200 mm^3 , and then randomized for treatment studies. Measurement of volume was performed using high resolution ultrasound imaging as described (14). Mice were treated when the tumors reached ~200 mm^3 in volume. Blood glucose was measured using a Freestyle digital glucose meter and test strips (Abbott) before and at 2, and 4 hours after treatment with 60 mg/kg OSI-906 or 25 mM tartaric acid vehicle.

Procurement of ^{18}F FDG

^{18}F FDG was synthesized in the Vanderbilt University Medical Center Radiopharmacy and distributed by PETNET. The average radiochemical purity of the product was 98.5% and specific activity was $>1,000$ Ci/mmol.

¹⁸F-DG-PET Imaging

Animal handling methods in preparation for and during ¹⁸F-DG-PET imaging were similar to published protocols (15–17). Briefly, prior to imaging, mice were fasted overnight and allowed to acclimate to the PET imaging facility environment for at least 1 hour while in a warmed chamber at 31.5 °C. Mice were administered a single dose of OSI-906 at 60 mg/kg in a 25 mM tartaric acid vehicle via oral gavage (n=8/group). ¹⁸F-DG was administered via a single retro orbital injection of ~200 µCi (100 µL) and imaged 2, 4 and 24 hours post dosing of OSI-906, or 4 hours after tartaric acid vehicle. Mice were conscious during the uptake period and maintained in a warmed chamber. Following a 50-minute uptake period, 10-minute static PET scans were collected on a Concorde Microsystems micro-PET Focus 220 (Siemens, Culver City, CA). Mice were maintained under 2% isofluorane anesthesia in 100% O₂ at 2 L/min and kept warm via a circulating water heating for the duration of the scan. Immediately following imaging, mice were sacrificed and tissues collected for molecular analysis. PET images were reconstructed using the ordered subsets expectation maximization (OSEM) algorithm. The percent injected-dose per gram of tissue (%ID/g) was calculated from analysis of tumor regions of interest using ASIPRO software (Concorde Microsystems Inc.).

Statistical Analysis of Data

Wilcoxon Rank Sum (Mann-Whitney U) tests were performed to compare each treatment time point to vehicle treated mice. Comparisons were unadjusted for the multiplicity of testing and were deemed significant if $p < 0.05$.

Pharmacokinetic Analysis *in vivo*

At 2, 4, and 24 hours after administration of OSI-906 blood was collected via cardiac puncture and placed in BD Microtainer EDTA collection tubes (Becton Dickinson, Franklin Lakes, NJ). The samples were centrifuged at 1500 × g for 10 minutes and plasma protein precipitated with methanol. Analysis of drug concentration was performed by HPLC-MS tandem mass spectroscopy (Applied Biosystems, Foster City, CA).

Immunoprecipitation/Western Blot Analysis

Phosphorylation of IGF-1R and IR in cells and tumor samples were analyzed by immunoprecipitation/Western blotting. Cells were lysed using NP-40 lysis buffer (Sigma, St. Louis, MO). Tumor samples were homogenized using Precellys 24 (MO BIO Laboratories Inc., Carlsbad, CA) in tumor lysis buffer (1% Triton X-100, 10% glycerol, 50 mM HEPES (pH 7.4), 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EDTA supplemented with fresh protease inhibitor cocktail (Sigma, St. Louis, MO), phosphatase inhibitor cocktail (Sigma, St. Louis, MO), 10 mM NaF and 1 mM sodium orthovanadate). After pre-clearing by centrifugation (14,000 rpm for 15 minutes), 1 mg of total protein was immunoprecipitated with anti-phosphotyrosine antibody (pY20, Exalpha, Shirley, MA) at 4 °C overnight. The immunoprecipitates were separated on SDS-PAGE and immunoblotted with a total IGF-1R antibody (Cell Signaling, Danvers, MA) followed by detection using enhanced chemiluminescence (GE Healthcare Life Sciences, Piscataway, NJ). The blots were re-probed with total IR antibody (Cell Signaling, Danvers, MA). Phosphorylated IGF1-R and IR bands were quantified using an Image Quant LAS 4000 with Image Quant TL 7.0 software (GE Healthcare Life Sciences, Piscataway, NJ).

Markers of altered glycolysis were analyzed by Western blot analysis. Tumor or cell lysate samples were separated on SDS-PAGE, immunoblotted and detected using enhanced chemiluminescence (GE Healthcare Life Sciences, Piscataway, NJ). The antibodies included pAKT (Ser473), total AKT, pS6 (Ser235/236), pERK 1/2, total ERK 1/2, (Cell Signaling,

Danvers, MA) and β -actin (Sigma, St. Louis, MO). The phosphorylated to total signal intensities were quantified as above.

RTK Analysis

Tumor lysates were prepared according to manufactures protocol (Proteome Profiler, R&D Systems, Minneapolis, MN) in NP-40 lysis buffer and clarified by centrifugation. The samples were incubated with the Human Phospho-RTK Array at 2000 μ g total protein overnight at 4 °C with rocking. The arrays were developed using Super- Signal FEMTO ECL detection (Pierce, Rockford, IL). The phospho-spots on the RTK blot were quantified using Image Quant LAS 4000 with Image Quant TL 7.0 software (GE Healthcare Life Sciences, Piscataway, NJ)

Results

Sensitivity of NCI-H292 and NCI-H441 to OSI-906

Non-small cell lung cancer is a potentially attractive indication for OSI-906 due to the implication of IGF1R/IR as a driver in this, as well as drug resistance in this setting. We established sensitivity of the NCI-H292 and NCI-H441 xenograft models to OSI-906 *in vivo* by measuring tumor volumes longitudinally with high resolution ultrasound imaging. Daily treatment with 60 mg/kg OSI-906 over 10 days resulted in tumor growth inhibition in the NCI-H292 xenografts compared to controls (Fig. 1A), but no growth changes were observed in the non-responsive NCI-H441 xenografts (Fig. 1B). We found that NCI-H292 tumors had considerably higher levels of pIGF-1R and pIR than NCI-H441 tumors (Fig. 1C).

Inhibition of ^3H -2-Deoxy glucose uptake *in vitro*

We assessed the effect of OSI-906 treatment on uptake of ^3H -2-deoxy glucose in NCI-H292 and NCI-H441 cells *in vitro*. Cells were treated for only 30 minutes with OSI-906 in order to avoid potential anti-proliferative effects of the drug to interfere with this endpoint analysis. OSI-906 treatment resulted in a rapid and dose dependent inhibition of uptake of the radiotracer in the NCI-H292 cell line (Fig. 2A). The percent inhibition ranged from 12% to 60% as the dose increased from 1.0 μM to 30 μM OSI-906. In comparison the NCI-H441 cell line demonstrated a reduced sensitivity to OSI-906. For the NCI-H292 cell line a 35% decrease in uptake of ^3H -2-deoxy glucose was achieved at 10 μM OSI-906 whereas in the NCI-H441 cell line the same decrease of the radiotracer was observed at only 30 μM OSI-906 (Fig. 2B). Analysis for cell death by FACS using the Invitrogen Live/Dead assay determined no significant cell death at all OSI-906 concentrations (1.0 μM -30 μM) tested compared to 0.05% DMSO controls (data not shown). As a positive control, cytochalasin B (2.5 μM -10 μM) was administered to the NCI-H292 cells and evaluated for ^3H -2-deoxy glucose uptake in an analogous manner. Figure 3C shows that cytochalasin B significantly inhibits uptake of the radiotracer by 85–90% in this cell line, and that the inhibition of ^3H -2-deoxy glucose by OSI-906 in NCI-H292 cells represents a rapid PD effect.

Correlation with target-pathway inhibition *in vitro*

NCI-H292 cell lysates were treated with an increasing concentration of OSI-906 (0.0 μM -10 μM) for 30 minutes and then analyzed for pIGF-1R, pIR, pERK 1/2, pAKT, pS6 and β -actin as shown in Figure 2D. We observed a significant decrease in phosphorylation of AKT and S6 suggesting a correlation between decreased glucose uptake and inhibition of targets downstream of IGF-1R and IR. NCI-H292 cells treated at lower concentrations (10 nm – 5 μM) over 2, 12 and 24 hours, demonstrated target inhibition at all concentrations at 2 hours, and sustained inhibition of pIGF-1R at both 12 and 24 hours for all concentrations except 10 nM (Fig. 3).

Inhibition of ^{18}F FDG uptake *in vivo*

^{18}F FDG-PET images of mice bearing the NCI-H292 and NCI-H441 xenografts are shown in Figure 4A. The NCI-H292 xenografts (sensitive to OSI-906 treatment) show a significant decrease ($p < 0.05$) in ^{18}F FDG uptake at 2, 4 and 24 hours post dosing with OSI-906 compared to vehicle treated controls. NCI-H441 xenografts (insensitive to OSI-906 treatment) did not demonstrate a significant change in uptake of ^{18}F FDG at any time point evaluated. Graphically, these results are shown in Figures 4B and 4C. The decreased %ID/g in the NCI-H292 xenografts is suggestive of a rapid PD effect observed by ^{18}F FDG imaging mediated by the inhibition of IGF-1R and IR pathways by OSI-906. Conversely, for the NCI-H441 xenograft model no difference in uptake of the radiotracer was observed in the tumor samples between vehicle controls and the OSI-906 treatment group.

Correlation with target pathway inhibition

Target inhibition of both pIGF-1R and pIR by a single dose of OSI-906 at 60 mg/kg *in vivo* in NCI-H292 xenograft tumors is shown in Fig. 5A. The data show that at 2 and 4 hours post treatment target inhibition of pIGF-1R is $> 80\%$ with 30% inhibition observed at 24 hours (Fig. 5B). The effect on pIR is equally pronounced, demonstrating significant target inhibition of this receptor. Target inhibition of pIR was $> 80\%$ at 4 hours post treatment with 40% inhibition observed at 24 hours. Inhibition of both target receptors correlated with decreased uptake of ^{18}F FDG in the same tumor samples analyzed. Figure 5C shows the results of a Western blot from tumor lysates at selected time points from mice bearing the NCI-H292 xenografts that were treated with 60mg/kg OSI-906 ($n = 4/\text{group}$). We found reduced activation levels of targets involved in glycolysis that are downstream of IGF-1R and IR, including pAKT, pS6 and pERK 1/2 as measured four hours post treatment with OSI-906 compared to untreated control lysates. Importantly, Western blot analysis of OSI-906 treated NCI-H441 tumor xenografts, which do express very low levels of the target receptor, showed no reduction in pAKT levels at any time point compared to control (Fig. S1)

Pharmacokinetic Analysis

Table S1 shows the drug concentration in the plasma samples from the NCI-H292 xenografts remained at a constant concentration $\sim 20 \mu\text{M}$ for 2 to 8 hours post dosing. By 24 hours post-dosing, the level of OSI-906 in the plasma had decreased by $\sim 60\%$ to approximately $6.5 \mu\text{M}$, resulting in some potential loss of target coverage with time.

Discussion

Catabolism of glucose through the TCA cycle in normal cells is the preferred method of ATP production leading to cell proliferation and survival. It is now well known that many cancer cells avidly consume glucose and produce lactic acid for ATP production despite the inefficiency of this metabolic pathway. The reason why cancer cells utilize a less efficient means of ATP production remains elusive; however, recent studies suggest that in cancer cells an increase in glycolysis, in addition to respiration, can generate energy more quickly than normal cells that rely on respiration alone. As a result, this high rate of glucose metabolism by cancer cells has resulted in the wide use of ^{18}F FDG PET to image and diagnose rapidly dividing cells including tumors (18).

Both IGF-1R and IR signal through the PI3K signaling pathway. PI3K is linked to both growth control and glucose metabolism. PI3K directly regulates glucose uptake and metabolism via AKT mediated regulation of glucose transporter activation and expression (GLUT1 and GLUT4), enhanced glucose capture by increased hexokinase activity and stimulation of phosphofructokinase activity (19–22). PI3K activation thus renders cells

dependent on glucose leading to glucose addiction. In normal cells, activation of PI3K/AKT is highly controlled by dephosphorylation of phosphatidylinositol by PTEN. However, in many cancers, PTEN is lost leading to constitutive activation of the PI3K pathway (23). Moreover, activation of this pathway can be enhanced by other mechanisms which, when combined, can constitute some of the more prevalent classes of mutations in human malignancy (e.g. PI3CA, AKT2, BCR-ABL, HER2/neu, etc.). Therefore, activation of AKT is likely the most important signaling event in relation to cellular metabolism, because AKT is sufficient to drive glycolysis and lactate formation and suppress macromolecular degradation in cancer (23,24). It has been shown that various therapeutic agents that disrupt the PI3K/AKT pathway, either directly or upstream of PI3K/AKT lead to decreased glucose uptake in tumors as measured by ^{18}F FDG-PET (25). Furthermore, the ability to inhibit FDG uptake in tumors has been shown to correlate well with treatment response in a number of cancers. As a consequence, ^{18}F FDG-PET has been used clinically in cancer patients to predict response to various therapies via the ability of agents to disrupt glucose metabolism and glucose uptake in tumors (22,26–28)

The primary purpose of these studies was to determine if ^{18}F FDG-PET could be used as an early, non-invasive PD biomarker for the dual kinase inhibitor OSI-906. We first determined *in vitro* using the sensitive cell line, NCI-H292 that a rapid decrease in ^3H -2-deoxy glucose uptake was observed in a dose dependent manner after treatment with pharmacologically relevant concentrations of OSI-906. In the NCI-H441 cell line reduced sensitivity to equimolar concentrations of OSI-906 was observed for the same assay. NCI-H292 cell lysates were then probed for markers of altered glycolysis by Western blot analysis and showed a significant reduction in pIGF-1R, pIR, pAKT, pS6, and pERK 1/2. Target inhibition of these markers strongly link IGF-1R and IR to the PI3 kinase and AKT pathways and resultant changes in metabolic activity of cultured cells when exposed to OSI-906.

In vivo, decreased uptake of ^{18}F FDG was observed rapidly at 2, 4, and 24 hours after administration of an efficacious dose of 60 mg/kg of OSI-906 in NCI-H292 tumor bearing animals. In comparison, the insensitive NCI-H441 xenografts demonstrated no change in uptake of the radiotracer at the same time points and same dosage. Analysis of target inhibition of pAKT, pS6, pERK 1/2, pIGF-1R and pIR from NCI-H292 tumor lysates was performed by Western blot and RTK array analysis. The results showed strong target inhibition of these markers at 4 hours post administration of a single 60 mg/kg dose of OSI-906, further corroborating the link of metabolic activity of tumors with IGF-1R and IR signaling pathways. Specific target inhibition of pIGF-1R and IR by RTK array analysis resulted in significant ($p < 0.05$) reduction of these phospho-targets ($> 80\%$) at 2 and 4 hours post administration of the agent, and correlated to reduced uptake of ^{18}F FDG. Blood glucose levels of non-tumor bearing mice appeared elevated from a baseline, fasted level following 2 and 4 hours of 60mg/kg OSI-906 treatment, however, the increased levels were not statistically significant ($p > 0.5$). As expected, similarly evaluated vehicle treated mice did not exhibit elevated glucose levels when evaluated at 2 hrs and 4 hrs (Fig. S2A). Importantly, ^{18}F FDG uptake in NCI-H441 tumors, which are insensitive to OSI-906, was similar in both OSI-906-treated and vehicle-treated tumors. The fact that post-treatment ^{18}F FDG uptake in these mice was not decreased when compared to baseline imaging suggests that the somewhat elevated circulating glucose levels had no detectable impact on ^{18}F FDG uptake in this study. As further evidence, no change in ^{18}F FDG uptake was seen in skeletal muscle following OSI-906 (Fig. S2B), and only a slight increase in liver ^{18}F FDG uptake was seen at 2 and 4 hours before returning to baseline at 24 hours (Fig. S2C). Nonetheless, it is possible that human trials incorporating ^{18}F FDG PET as a biomarker of response to OSI-906 may benefit from measurement of blood glucose levels, as the effects on ^{18}F FDG uptake in patient studies could be larger than we observed in mice.

The present findings support a strong link of rapidly altered metabolic activity in both cultured cells and *in vivo* tumors induced by target inhibition of the IGF-1R and IR signaling pathways. Though there is still much to be learned how cellular metabolism in proliferating cells is regulated, there is an ever increasing body of information supporting increased communication between signaling pathways and metabolic control of the cell. These studies suggest that ^{18}F FDG-PET has potential to serve as a rapid, non-invasive biomarker of pharmacodynamic effects of OSI-906 in patients treated with this dual IGF-1R/IR kinase inhibitor. This method may be most beneficial in early clinical development where accurate assessment of PD effects is often times limited by the lack of readily accessible tumor samples. As such ^{18}F FDG-PET may be a useful clinical tool in identifying active doses and patients potentially sensitive to this novel antitumor agent and perhaps other compounds of this target class. Currently, ^{18}F FDG-PET imaging is being employed in several clinical trials as a biomarker for early efficacy of OSI-906.

Statement of Translational Relevance

The development of inhibitors targeting the insulin-like growth factor-1 receptor (IGF-1R) and insulin receptor (IR) is a clinically important area of cancer research. OSI-906 is a potent and highly selective tyrosine kinase inhibitor now being evaluated in clinical studies that exhibits similar biochemical potency against IGF-1R (8 nM) and IR (14 nM), and is greater than 4 orders of magnitude more selective for IGF-1R/IR compared to a wide number of other receptor and non-receptor kinases. Objective means to assess pharmacodynamic response to OSI-906 therapy in tumors remains challenging. To this end, we evaluated ^{18}F FDG-PET as a clinically relevant molecular imaging metric to quantify and predict pharmacodynamic response to OSI-906 in preclinical mouse models of lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Daily treatment of mice bearing NCI-H292 xenografts with 60 mg/kg OSI-906 results in significant tumor growth inhibition (A) compared to analogously treated vehicle controls. In contrast, NCI-H441 xenografts (B) do not exhibit a difference in tumor growth when comparing OSI-906-treated and vehicle-treated cohorts. Receptor tyrosine kinase (RTK) arrays (C), illustrate that NCI-H292 cells possess relatively high levels of pIGF-1R and pIR compared to the barely detectable levels of pIGF-1R and pIR in NCI-H441 cells.

Fig. 2. ^3H -2-deoxy glucose uptake 30 minutes after OSI-906 treatment in NCI-H292 cells showed a dose-dependent decrease (A). Similar decreases in ^3H -2-deoxy glucose uptake were seen at higher doses of OSI-906 in the non responding NCI-H441 cells compared with the responding NCI-H292 cells (B). Treatment with cytochalasin B as a positive control in NCI-H292 cells demonstrated that ^3H -2-deoxy glucose uptake is directly affected by exposure OSI-906, and can be linked directly to cellular pathways associated with glucose metabolism (C). Western blot of NCI-H292 cells following 30 minutes of exposure to OSI-906 shows target inhibition of pIGF-1R and pIR at all doses as well as inhibition of downstream targets pAKT and pS6 (D).

Fig. 3.

Western blot of NCI-H292 cells treated with 10nM, 100 nM, 500 nM, 1 μ M, and 5 μ M OSI-906 show target inhibition over a 24 hour time course. All concentrations of OSI-906 induce a reduction in pIGF-1R at 2 hours, and inhibition remains through 24 hours in all but the lowest, 10 nM concentration.

Fig. 4. Representative transverse ^{18}F FDG-PET images of NCI-H292 and NCI-H441 tumor xenografts (A) show that ^{18}F FDG uptake is significantly reduced ($p < 0.05$) in the NCI-H292 xenografts at all time points following a single treatment of 60 mg/kg OSI-906 (B) while NCI-H441 xenografts show no changes in ^{18}F FDG uptake (C).

Fig. 5.

RTK array analysis demonstrates strong target inhibition of both pIGF-1R and pIR in NCI-H292 tumor lysates at 2, 4 and 24 hours after a single 60mg/kg treatment of OSI-906 (A,B).

In vivo Western blot of NCI-H292 tumor lysates at 4 and 24 hours shows inhibition of selected markers of altered glycolysis, pERK 1/2, pAKT and pS6 at 4 hours post-dose that return to baseline levels by 24 hours (C).