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SHORT COMMUNICATION

FDG-PET as a pharmacodynamic biomarker for early assessment of treatment response to linifanib (ABT-869) in a non-small cell lung cancer xenograft model

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Abstract Linifanib (ABT-869) is a multitargeted receptor tyrosine kinase inhibitor. This work aims to evaluate F-fluorodeoxyglucose-positron emission tomography (FDG-PET) as a pharmacodynamic (PD) biomarker for linifanib treatment utilizing the Calu-6 model of human non-small cell lung (NSCLC) cancer in SCID-beige mice. Animals received either vehicle or 12.5 mg/kg linifanib orally twice a day for the duration of the study. Imaging was performed at −1, 1, 3, and 7 days after beginning treatment ($n = 12$–14 per group). Linifanib inhibited tumor growth and suppressed tumor metabolic activity. Changes in tumor FDG uptake were observed as early as 1 day after beginning linifanib treatment and were sustained for the duration of the study. This study confirms that linifanib is efficacious in this xenograft model of human NSCLC and confirms FDG-PET is a potential PD biomarker strategy for linifanib therapy.

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Introduction

Inhibition of angiogenesis is a valid target for many cancers such as renal cell carcinoma, gastrointestinal stromal tumors, and hepatocellular carcinoma and is undergoing evaluation in many others [3, 14, 15]. Multiple growth factors are involved in angiogenesis including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF). A multitargeted approach to inhibiting angiogenesis is especially promising because multiple signaling pathways are involved in angiogenesis, and targeting only one may allow the tumor to circumvent inhibition by using an alternate pathway [5]. Several such multitargeted tyrosine kinase inhibitors have already been approved [4].

Linifanib, ABT-869, is a structurally novel multitargeted receptor tyrosine kinase inhibitor against VEGF and PDGF families and Flt3 kinase [1]. Additionally, it has been shown to inhibit proliferation and induce apoptosis via Akt and glycogen synthase kinase 3beta-dependent pathways [7]. Efficacy was observed in vivo in xenograft models of human fibrosarcoma, breast, colon, glioma, and small cell lung carcinoma cancer when given orally [1, 8]. Compared to other multitargeted receptor kinase inhibitors that have undergone clinical development, linifanib is selective against a broader range of relevant kinases and has lower activity against off-target kinases [20]. In the phase I study, linifanib proved tolerable and showed evidence of antiangiogenic activity by DCE-MRI, measurement of circulating endothelial cells, and plasma VEGF levels [19]. Linifanib is currently in phase 2 and 3 clinical studies in colorectal carcinoma, non-small cell lung cancer, and hepatocellular carcinoma.

Translational strategies are becoming increasingly more important due to the high attrition rates of late phase trials. This is especially true for oncology drug development where failure rates are particularly high [2]. Imaging can be part of the translational strategy and may allow for earlier identification of drugs that are or are not having their intended effects [12]. ^{18}F-fluorodeoxyglucose (FDG), a glucose analogue, is a positron emission tomography (PET) tracer used for quantitative imaging of glucose uptake. After entering the cell, FDG is phosphorylated by hexokinase causing it to be trapped in the cell; the lack of oxygen at the C-2 position prohibits FDG from undergoing further catabolism. FDG-PET imaging takes advantage of tumors' increased utilization of glucose [6, 18]. It has been used for diagnosing, staging, and evaluating treatment in oncology preclinically and clinically [9, 16].

We aimed to preclinically evaluate FDG-PET as a potential, translational pharmacodynamic (PD) biomarker for linifanib therapy. Tumor FDG uptake was evaluated longitudinally to determine when a PD response was observable in a human non-small cell lung cancer (NSCLC) xenograft model.

Materials and methods

Cell culture

The human non-small cell lung cancer cell line Calu-6 was purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured using Eagle's Minimum Essential Medium (Invitrogen, Carlsbad, CA) supplemented with 10% HyClone fetal bovine serum (Thermo Fisher Scientific Inc., Waltham, MA, USA). Cells were incubated at 37°C in 5% CO_2 and 95% relative humidity.

In vivo xenografts

All animal studies were conducted in accordance with the guidelines established by the internal Institutional Animal Care and Use Committee. C.B.-17 SCID-bg mice (Charles River Laboratories, Wilmington, MA, USA) were inoculated with 5×10^6 cells subcutaneously in the right flank of the animal. Inoculation volume was 0.2 mL consisting of a 50:50 mixture of cells in growth medium and Matrigel (BD Biosciences, Franklin Lakes, NJ). Tumor volume was estimated by two to three weekly measurements of the length and width of the tumor by electronic calipers and applying the following equation: $V = L \times W^2/2$. Tumors were allowed to reach approximately 355 mm^3 and mice were size-matched (day 0) into treatment and control groups ($n = 12$–14 per group). All animals were monitored individually throughout the experiment. ABT-869 or vehicle (2% ethanol, 5% Tween 80, 20% polyethylene glycol 400, 73% saline) was administered orally at a dose of 12.5 mg/kg twice a day for 7 days. Tumor volumes were calculated on days 0, 3, and 7.

FDG-microPET/CT imaging and analysis

All images were acquired on a Siemens Inveon microPET/CT scanner (Knoxville, TN, USA). Mice were fasted for a minimum of 6 h prior to imaging. 300 μCi of FDG (IBA Molecular, Romeoville, IL, USA) in 100–200 μL per mouse was administered via lateral tail vein injection. During imaging, mice were anaesthetized using 2% isoflurane. CT images were acquired at 80 kVp, 500 μA with an exposure time of 210 ms, and 200 steps. CT images were reconstructed using filtered back projection with a Shepp–Logan filter. PET images were acquired for 7 min and were reconstructed using a 2D ordered subset-expectation maximization reconstruction algorithm. Animals were imaged 1 day prior to the beginning of treatment (day −1) and 1, 3, and 7 days after beginning treatment. Linifanib was administered approximately 40 min prior to FDG injection.

PET/CT images were analyzed using InVivoScope (Bioscan, Washington DC, USA) for tumor FDG uptake. Tumor regions of interest (ROIs) were defined based on both the PET and CT images for PET quantitation. The standardized uptake value (SUV) was calculated using the following formula:

$$\mathrm{SUV} = \frac{\text{Tumor FDG Concentration}}{\text{injected dose/Body weight}}$$

Changes in SUV for each animal were expressed as a percent of baseline and averaged for each treatment group.

Statistical analysis

Data are presented as the average with standard error (mean ± SEM). Differences between treated and control groups at all time points were compared using Student's t test and were considered significant when the p value was less than 0.05.

Results

The average tumor volumes when the treatment was started, day 0, were 360 ± 20 mm^3 for the vehicle-treated group and 350 ± 20 mm^3 for the linifanib-treated group. The tumor volumes of the vehicle-treated group continued to increase throughout the study as seen in Fig. 1. On days 3 and 7, the average tumor volumes for the vehicle-treated

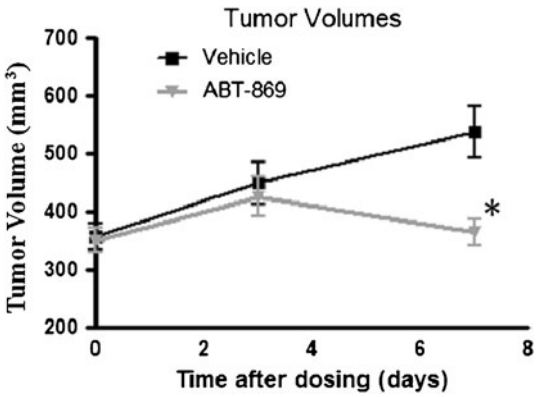

Fig. 1 Tumor volume measurements versus time for vehicle and linifanib treatment. Significant differences are indicated by an *asterisk*

group were 450 ± 40 and 540 ± 40 mm^3. The tumor volumes on days 3 and 7 of linifanib treatment were 430 ± 30 and 370 ± 20 mm^3 showing treatment resulted in a suppression of tumor growth. The difference in tumor volume between vehicle- and linifanib-treated groups was not significant until day 7 of treatment ($p = 0.015$).

The tumor FDG uptake in the vehicle-treated group was increased throughout the experiment relative to baseline (Fig. 2). The average tumor FDG uptake as a percentage of baseline was 119 ± 8, 126 ± 8, and 104 ± 9 at days 1, 3, and 7 for vehicle treatment as seen in Fig. 2a. Treatment with linifanib resulted in decreased tumor uptake as early as 1 day after beginning treatment. The average tumor FDG uptake as a percentage of baseline was 84 ± 7, 64 ± 5, and 59 ± 6 at days 1, 3, and 7 days after beginning linfanib treatment. Differences in FDG uptake between vehicle- and linifanib-treated groups were significant on days 1, 3, and 7 ($p < 0.0002$). The difference in tumor FDG uptake between baseline and day 1 and the difference between day 1 and 3 of linifanib treatment were significant ($p < 0.05$). The difference in tumor FDG uptake between day 3 and day 7 of linifanib treatment was not significant ($p = 0.5$). Representative images can be found in Fig. 2b.

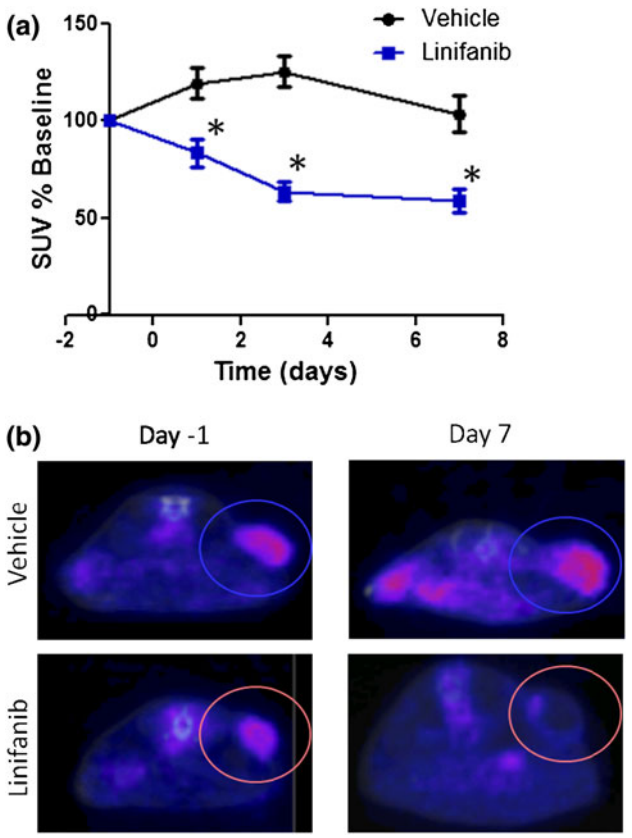

Fig. 2 Tumor FDG uptake for vehicle and linifanib treatment over time. **a** FDG uptake is represented as SUV as a % of baseline. Significant differences are indicated by an *asterisk*. **b** Representative FDG-PET images for vehicle and linifanib treatment for baseline imaging and day 7 after treatment

Discussion

The present study indicates that linifanib treatment quickly results in decreased tumor glucose utilization in the Calu-6 model of NSCLC. Changes in tumor FDG uptake with linifanib treatment compared to vehicle treatment were significant 1 day after beginning treatment, while changes in tumor volume were not significant until 7 days after beginning treatment. Thus, FDG-PET imaging is a potential early marker of drug effects compared to changes in tumor volume. The changes in tumor FDG uptake could be due to decreased metabolic activity or decreased perfusion.

Mixed results have been observed using FDG-PET for evaluating tyrosine kinase inhibitor treatment preclinically. FDG-PET was used to evaluate cediranib treatment in MCF-7 cells transfected with either non-VEGF or VEGF expression. The authors concluded that with both cell lines, the change in FDG uptake was associated with tumor size and not chronic or acute cediranib treatment, targeting VEGF [11]. However, a significant decrease in tumor FDG uptake was observed with vandetanib treatment, which targets VEGF, EGFR, and RET-tyrosine kinase, in a human medullary thyroid cancer model at 3 days after beginning treatment. The magnitude of decrease was sustained with treatment at 7 and 14 days [17].

Nevertheless, FDG-PET has been widely used to monitor tyrosine kinase inhibitor treatment response clinically. Specifically, it has been used to monitor sorafenib and sunitinib treatments [10, 13]. Based on the present FDG-PET findings and evidence of on-target effects clinically, it is anticipated that a response based on FDG-PET would be observed with linifanib therapy in patients responding to treatment.

Conclusions

Decreased tumor FDG uptake was observed as early as 1 day after beginning linifanib treatment and was maintained for the duration of treatment. Thus, FDG-PET may be a potential biomarker for PD response for linifanib. Our results confirm that linifanib is efficacious as a single agent in this xenograft model of human NSCLC.

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