

# Downregulation of $^{18}\text{F}$ -FDG Uptake in PET as an Early Pharmacodynamic Effect in Treatment of Non-Small Cell Lung Cancer with the mTOR Inhibitor Everolimus

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Everolimus downregulates glucose metabolism-associated genes in preclinical models. Inhibition of glucose metabolism measured by  $^{18}\text{F}$ -FDG PET was postulated to serve as a pharmacodynamic marker in everolimus-treated non-small cell lung cancer (NSCLC) patients. **Methods:** In 8 NSCLC patients treated with everolimus, the percentage change in  $^{18}\text{F}$ -FDG PET uptake (days 8 and 28 relative to baseline) was determined using a variety of summed standardized uptake value (SUV) measures. Both maximum and mean SUVs were used, with normalizations to body surface area and body weight and with and without correcting for plasma glucose levels. **Results:** In 5 patients, a reduction of  $^{18}\text{F}$ -FDG PET uptake on day 8 was observed with all methods, ranging from  $-12.8\%$  to  $-72.2\%$ . **Conclusion:** These observations demonstrate that inhibition of glucose metabolism is an early effect of everolimus treatment in NSCLC patients and can be assessed using  $^{18}\text{F}$ -FDG PET.

**Key Words:** everolimus; positron emission tomography;  $^{18}\text{F}$ -FDG; lung cancer

**J Nucl Med 2009; 50:1815–1819**

DOI: 10.2967/jnumed.109.065367

**E**verolimus (RAD001) is a novel oral inhibitor of the mammalian target of rapamycin (mTOR), a downstream

component of the PI3K/AKT pathway involved in the regulation of cell proliferation, angiogenesis, and metabolism. Recently, everolimus has been approved by the Food and Drug Administration for advanced renal cell carcinoma after failure of first-line treatment (1). In relapsed non-small cell lung cancer (NSCLC), everolimus also showed clinical activity and was well tolerated (2).

In a murine model of prostate intraepithelial neoplasia, treatment with everolimus resulted in the downregulation of genes involved in glucose metabolism through the downregulation of the transcription factor hypoxia-inducible factor 1 (3). It was postulated that a rapid response in  $^{18}\text{F}$ -FDG PET analysis might reflect mTOR inhibition as a pharmacodynamic response.

The purpose of the present pilot study was to demonstrate the downregulation of glucose metabolism in everolimus-treated NSCLC patients as an early pharmacodynamic effect using  $^{18}\text{F}$ -FDG PET.

## MATERIALS AND METHODS

### Patient Selection and Treatment

Eighty-five patients were recruited into a multicenter phase II study investigating everolimus monotherapy in advanced relapsed NSCLC (2,4). A subgroup of 8 patients from 2 study centers (Cologne and Amsterdam) was evaluated by sequential  $^{18}\text{F}$ -FDG PET. Standard CT scans were obtained of all patients. The study was approved by the ethics committees of both centers, and informed consent was obtained from all patients. Everolimus was

Received Apr. 28, 2009; revision accepted Jul. 29, 2009.  
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**TABLE 1.** Demographic Data and Pathology

Subject	Age (y)	Sex	WHO performance status	Pathology	Smoking status	Prior antineoplastic chemotherapy (CT) or tyrosine kinase inhibitor (TKI) therapy
0701-0002	63	Male	0	Large cell carcinoma	Ever	1 CT regimen
0701-0003	47	Male	1	Adenocarcinoma	Current	2 CT regimens
0701-0004	58	Male	1	Squamous cell carcinoma	Ever	1 CT regimen
0701-0005	65	Male	1	Adenocarcinoma	Ever	2 CT regimens + 1 EGFR-TKI regimen
0601-0001	57	Female	1	Adenocarcinoma	Ever	1 CT regimen
0601-0003	56	Female	2	Squamous cell carcinoma	Ever	2 CT regimens
0601-0007	52	Female	0	Adenocarcinoma	Current	1 CT regimen
0601-0009	74	Male	2	Adenocarcinoma	Ever	1 CT regimen

Smoking status: ever smokers were subjects who had smoked at least 100 cigarettes or cigars in their lifetime.

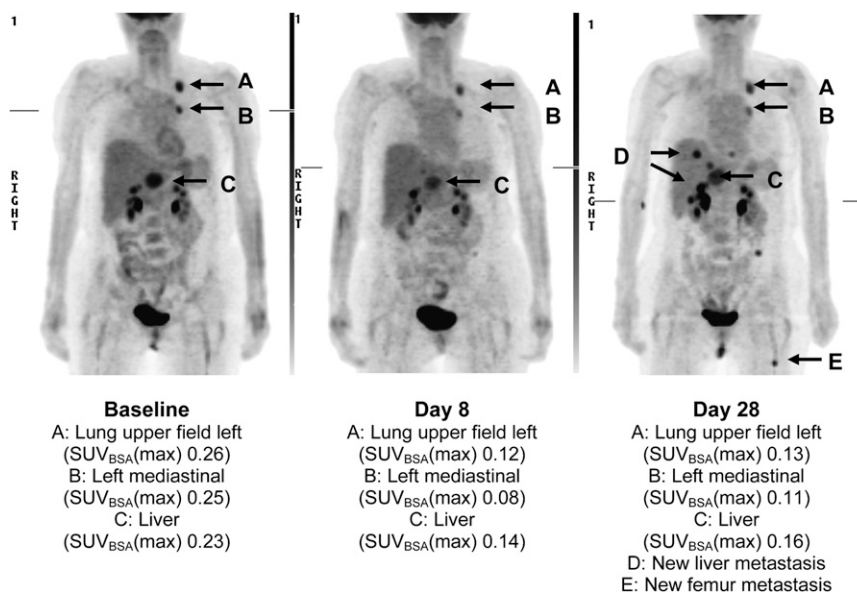
administered orally as a continuous once-daily dose of 10 mg until disease progressed or unacceptable toxicity developed.

### Imaging Procedures

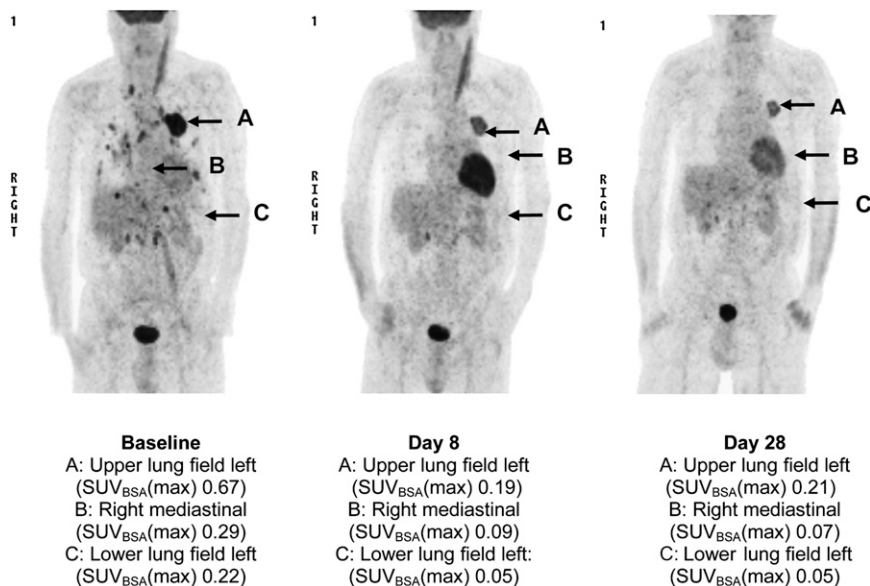
<sup>18</sup>F-FDG PET was performed before the start of therapy (baseline) and on days 8 and 28 of treatment. ECAT EXACT 47 (CTI/Siemens) and ECAT EXACT HR+ (CTI/Siemens) scanners were used in Cologne and Amsterdam, respectively.

Before being scanned, patients fasted for at least 6 h. The acquisition of PET scans was started 60 min after injection of 370 MBq of <sup>18</sup>F-FDG. The attenuation-corrected scan trajectory covered 90 cm (6 bed positions, 5-min emission, 3-min transmission). All scans were normalized; corrected for decay, dead time, scatter, and randoms; and reconstructed using ordered-subset expectation maximization. To allow exchangeability of standardized uptake value (SUV) measures, reconstruction settings and image resolution need to be matched (5). Therefore, datasets collected at both sites were reconstructed using the same

reconstruction algorithms and settings, that is, ordered-subset expectation maximization using 2 iterations and 16 subsets, zoom 1, an image matrix size of 128 × 128, and gaussian postsmoothing of 5 mm in full width at half maximum (FWHM). Using these settings, we determined final image spatial resolution primarily by voxel size and postreconstruction filtering. For both scanners, the resulting image resolution was approximately 7 mm FWHM near the center of the field of view. CT results were assessed using a 16-slice multidetector CT scanner (Brilliance 16; Philips Medical Systems) at baseline, every 4 wk until week 16, and every 8 wk thereafter. CT scans (collimation, 16 × 1.5 mm) were obtained 60 s after the start of a 100-mL intravenous contrast material injection (Accupaque 350; GE Healthcare Buchler GmbH und Co.). Disease status was assessed by local radiologic evaluation of all lesions. All <sup>18</sup>F-FDG PET studies and CT scans were additionally evaluated centrally in a masked fashion by an independent nuclear medicine physician and radiologist, respectively.



**FIGURE 1.** SUV<sub>BSA</sub>(max) at baseline and on days 8 and 28 for patient 0601-0001.



**FIGURE 2.** SUV<sub>BSA</sub>(max) at baseline and on days 8 and 28 for patient 0701-0004.

### **<sup>18</sup>F-FDG PET Evaluation and Response Assessment**

Up to 5 evaluable lesions with a baseline tumor-to-background ratio greater than or equal to 2 were selected and analyzed. Local background values were determined as follows. First, an initial estimate of the edge of the metabolic tumor volume was made using a 70% of maximum uptake 3-dimensional isocontour (i.e., a 3-dimensional volume of interest [VOI]). Next, all voxels located between 1.5 and 2.0 cm from this edge were assigned to a local background VOI. The average voxel value of the latter VOI was then defined as local background.

For each lesion, both localization and whether <sup>18</sup>F-FDG uptake was homogeneous or heterogeneous were noted. The longest diameter of the lesion (on the PET image) and the orthogonal diameter were measured and reported. For each lesion, the maximum SUV normalized to injected activity and body surface area (SUV<sub>BSA</sub>[max]) was determined. This is the measured tracer concentration of the hottest voxel within the lesion, normalized to injected dose and body surface area, as recommended by the European Organization for Research and Treatment of Cancer (EORTC) (6). The hottest voxel within each lesion was defined using dedicated standardized in-house (VUmc)-developed software (7). In addition, maximum SUV normalized to body weight (SUV<sub>BW</sub>[max]) was determined. Furthermore, mean SUV estimates based on a 70% isocontour (SUV<sub>mean</sub>) were determined, again normalized to both body surface area and body weight, respectively. All SUV parameters were calculated both with and without a correction for plasma glucose level. For each patient, all SUV parameters were summed (sSUV) for all identified lesions, resulting in a total of 8 sSUV estimates per patient (sSUV<sub>BSA</sub>[max], sSUV<sub>BSA-Glu</sub>[max], sSUV<sub>BW</sub>[max], sSUV<sub>BW-Glu</sub>[max], sSUV<sub>BSA</sub>[mean], sSUV<sub>BSA-Glu</sub>[mean], sSUV<sub>BW</sub>[mean], and sSUV<sub>BW-Glu</sub>[mean]). For each method, metabolic response was defined as percentage change of sSUV at days 8 and 28 compared with baseline.

### **CT Scan Response Assessment**

In the independent radiologic review, longest diameters in the horizontal plane of up to a maximum of 5 lesions per organ and a maximum of 10 lesions in total (including the lesions noted for <sup>18</sup>F-FDG PET) were measured using Response Evaluation Criteria

in Solid Tumors (8). The sum of the longest diameters (sCT) of all selected patient lesions and the percentage change in the sCT on day 28, compared with baseline, were calculated.

### **Time to Progression (TTP)**

Time to progression was defined as time from the date of start of treatment to the date of an event defined as the first documented progression of the disease or death due to underlying cancer. If a patient had no event, time to progression was censored at the date of the last adequate tumor assessment.

## **RESULTS**

### **Patient Characteristics**

Eight patients with advanced NSCLC were evaluated by sequential <sup>18</sup>F-FDG PET (patient characteristics are shown in Table 1). All patients received 10 mg of everolimus continuously, once daily. Everolimus was well tolerated, and only mild to moderate adverse events were observed (2).

### **<sup>18</sup>F-FDG PET**

In all 8 patients, <sup>18</sup>F-FDG PET studies were performed at baseline and day 8 of treatment, and in 5 patients also on day 28. Assessment on day 28 in the remaining 3 patients was not performed, either because of progressive disease and discontinuation from the study (2 patients) or because of refusal by the patient (1 patient). At baseline, patients had between 2 and 5 lesions that qualified for evaluation (total, 28 lesions). Lesions were localized in lung (15), mediastinum (6), and extrathoracic sites (total, 7; 3 bone and 4 soft tissue). Examples of <sup>18</sup>F-FDG scans are shown in Figures 1 and 2.

In 5 patients, a reduction in <sup>18</sup>F-FDG uptake on day 8 was observed using all methods, ranging from -12.8% in sSUV<sub>BW-Glu</sub>(max) (patient 0601-0009) to -72.2% in sSUV<sub>BW</sub>(mean) (patient 0701-0004) (Table 2). In 1 patient (0601-0007) increased <sup>18</sup>F-FDG uptake was observed with all methods, ranging from +29.3% in sSUV<sub>BW</sub>(max) to +77.1% in sSUV<sub>BSA-Glu</sub>(mean).

**TABLE 2. Results of sSUV(max) and sSUV(mean)**

Patient no.	PET BW/BSA	Baseline		Day 8		Day 28		Day 8 % change		Day 28 % change		sCT (day 28 % change)	TTP (d)
		sSUVmax	sSUVmean	sSUVmax	sSUVmean	sSUVmax	sSUVmean	sSUVmax	sSUVmean	sSUVmax	sSUVmean		
0701-0002	BW	48.5	41.7	38.73	31.41	42.96	35.62	-20.14	-24.68	-11.42	-14.58	3.3	22
	BW-Glu	45.57	39.18	36.41	29.52	43.81	36.33	-20.1	-24.66	-3.86	-7.27		
	BSA	1.29	1.12	1.03	0.83	1.14	0.95	-20.16	-25.89	-11.63	-15.18		
0701-0003	BSA-Glu	1.22	1.04	0.97	0.8	1.17	0.97	-20.49	-23.08	-4.1	-6.73		
	BW	18.51	14.32	15.05	11.88	NA	NA	-18.69	-17.04	NA	NA	NA	26
	BW-Glu	16.29	12.6	12.94	10.22	NA	NA	-20.56	-18.89	NA	NA		
0701-0004	BSA	0.57	0.45	0.46	0.37	NA	NA	-19.29	-17.78	NA	NA		
	BSA-Glu	0.51	0.39	0.4	0.32	NA	NA	-21.57	-17.95	NA	NA		
	BW	44.06	36	12.29	10	12.47	9.99	-72.11	-72.22	-71.7	-72.25	-26.6	191
0701-0005	BW-Glu	49.34	40.32	14.25	11.6	13.22	10.58	-71.12	-71.23	-73.21	-73.76		
	BSA	1.18	0.97	0.33	0.27	0.33	0.27	-72.03	-72.16	-72.03	-72.16		
	BSA-Glu	1.33	1.09	0.39	0.31	0.35	0.29	-70.68	-71.56	-73.68	-73.39		
0601-0001	BW	54.19	43.99	43.88	34.77	NA	NA	-19.03	-20.96	NA	NA	NA	12
	BW-Glu	128.95	104.68	124.63	98.62	NA	NA	-3.35	-5.79	NA	NA		
	BSA	1.22	0.99	1	0.78	NA	NA	-18.03	-21.21	NA	NA		
0601-0003	BSA-Glu	2.89	2.35	2.85	2.26	NA	NA	-1.38	-3.83	NA	NA		
	BW	29.31	25.65	13.41	11.22	26.36	21.34	-54.25	-56.26	-10.06	-16.80	77.9	28
	BW-Glu	32.24	28.23	14.21	11.89	39.01	31.57	-55.92	-57.89	21	11.83		
0601-0007	BSA	0.74	0.65	0.34	0.28	0.67	0.55	-54.05	-56.92	-9.46	-15.38		
	BSA-Glu	0.82	0.72	0.36	0.31	0.98	0.8	-56.1	-56.94	19.51	11.11		
	BW	10.83	8.67	10.63	8.67	NA	NA	-1.84	0	NA	NA	12.7	32
0601-0009	BW-Glu	10.19	8.15	13.82	11.28	NA	NA	35.62	38.41	NA	NA		
	BSA	0.3	0.24	0.29	0.24	NA	NA	-3.33	0	NA	NA		
	BSA-Glu	0.29	0.23	0.39	0.32	NA	NA	34.48	39.13	NA	NA		
0601-0007	BW	16.43	13.49	21.25	18.08	24.06	20.48	29.34	34.03	46.44	51.82	11.1	31
	BW-Glu	16.1	13.22	26.78	22.78	28.86	24.58	66.34	72.31	79.25	85.93		
	BSA	0.44	0.36	0.58	0.49	0.65	0.56	31.82	36.11	47.73	55.56		
0601-0009	BSA-Glu	0.42	0.35	0.72	0.62	0.78	0.66	71.43	77.14	85.71	88.57		
	BW	8.17	6.47	6.17	4.87	7.39	5.71	-24.48	-24.73	-9.54	-11.75	-16.7	100
	BW-Glu	17.98	14.23	15.68	12.38	19.96	15.42	-12.79	-13.00	11.01	8.36		
0601-0009	BSA	0.21	0.17	0.16	0.13	0.19	0.15	-23.81	-23.53	-9.52	-11.76		
	BSA-Glu	0.46	0.37	0.4	0.32	0.52	0.4	-13.04	-13.51	13.04	8.11		

BSA = body surface area; BW = body weight; Glu = glucose; NA = not applicable.



Inconsistent results between data with and without plasma glucose correction were observed in 2 patients. This finding can be explained by altered or elevated plasma glucose levels (patient 0701-0005, 11.9 mmol/L at baseline and 14.2 mmol/L on day 8; patient 0601-0003, 4.7 mmol/L at baseline and 6.5 mmol/L on day 8).

A reduction in  $^{18}\text{F}$ -FDG uptake on day 28 was shown in 2 patients (0701-0004 and 0701-0002) using all methods. Two patients (0601-0001 and 0601-0009) showed inconsistent results between methods on day 28, because of elevated glucose plasma levels (7.4 and 13.5 mmol/L, respectively). The fifth patient (0601-0007) showed a further increase of  $^{18}\text{F}$ -FDG uptake already observed on day 8.

The sCT changes at day 28 ranged from  $-26.6$  to  $+77.9\%$ ; time to progression ranged from 12 to 191 d (Table 2).

## DISCUSSION

This study shows, for the first time to our knowledge, that the inhibition of glucose metabolism is an early event in everolimus treatment of NSCLC and can be measured by  $^{18}\text{F}$ -FDG PET already after 1 wk of therapy.

The use of  $^{18}\text{F}$ -FDG PET as a pharmacodynamic tool in cancer patients treated with mTOR inhibitors was suggested not only by preclinical experiments (3) but also by recent clinical data. In solid tumors treated with the mTOR inhibitor rapamycin,  $^{18}\text{F}$ -FDG PET response after 4 wk of therapy correlated with inhibition of Akt and glycolysis independent from antiproliferative activity (9). For the application of  $^{18}\text{F}$ -FDG PET as a pharmacodynamic tool, however, the evaluation of the pharmacodynamic response as early as possible after initiation of therapy, that is, after reaching the serum steady-state level, is preferable.

The present evaluation using sSUV revealed a reduction of glucose uptake in 5 of 8 patients on day 8 of everolimus monotherapy, independent of the method of normalization of SUV, of whether maximum or mean voxel values were used, and of whether a correction for plasma glucose levels was applied. Formally, EORTC criteria for a partial metabolic response in  $^{18}\text{F}$ -FDG PET assessment (6) were met in 4 of these patients. The goal of this proof-of-principle study, however, was to explore  $^{18}\text{F}$ -FDG PET as a pharmacodynamic tool for everolimus therapy in NSCLC rather than to evaluate early metabolic response as a predictor of outcome. No standardized criteria for assessing pharmacodynamic endpoints in such a setting exist. On the basis of the 28-h half-life of everolimus, the steady state is reached after 4–5 d of treatment (10). Thus, it cannot be excluded that  $^{18}\text{F}$ -FDG uptake on day 8 of treatment is already too late to assess the maximal pharmacodynamic effect. Increased  $^{18}\text{F}$ -FDG uptake on day 8 in 1 patient, as observed with all methods, may be a reflection of the contributions of both drug effect and tumor growth. Discrepancies in  $^{18}\text{F}$ -FDG changes between different methods observed in 2 patients are due to elevated and varying plasma glucose levels between baseline and follow-up scans.

The results presented show that inhibition of glucose metabolism is an early pharmacodynamic effect of everolimus treatment in lung cancer patients and can be assessed by  $^{18}\text{F}$ -FDG PET. Future confirmatory studies should be aimed at establishing  $^{18}\text{F}$ -FDG PET as a pharmacodynamic tool for optimizing everolimus therapy. In view of the present results, both SUV(max) and SUV(mean) seem to be appropriate for such analyses. A correction for plasma glucose level seems to be necessary in the case of elevated or varying plasma glucose levels. Further studies, however, should include a formal comparison between SUV and glucose metabolism using a dynamic scanning protocol to exclude the possibility that this relationship is perturbed by everolimus. In addition, future studies are needed to assess the optimal time point after the start of everolimus therapy.

The low patient number in this pilot study does not allow drawing any valid statistical conclusion on the predictive value of day-8 PET. However, the most pronounced sSUV<sub>BW</sub>(mean) on day 8 (patient 0701-0004,  $-72.22\%$ ) was maintained on day 28 ( $-72.25\%$ ) and correlated with the longest time to progression (191 d).

## CONCLUSION

Results of this study translate the observations from preclinical experiments on the effect of mTOR inhibition on glucose metabolism in cancer cells into a clinical context and suggest that  $^{18}\text{F}$ -FDG PET could be a valuable tool for evaluating early pharmacodynamic effects of mTOR inhibition in NSCLC patients.

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