Changes in ¹⁸F-Fluorodeoxyglucose and ¹⁸F-Fluorodeoxythymidine Positron Emission Tomography Imaging in Patients with Non-Small Cell Lung Cancer Treated with Erlotinib

Linda Mileshkin¹,², Rodney J. Hicks¹,², Brett G.M. Hughes³, Paul L.R. Mitchell²,³, Veena Charu⁴, Barbara J. Gitlitz⁷, David Macfarlane⁵, Benjamin Solomon¹, Lukas C. Amler⁸, Wei Yu⁸, Andrea Pirzkall⁸, and Bernard M. Fine⁸

Abstract

Purpose: Assessing clinical activity of molecularly targeted anticancer agents, especially in the absence of tumor shrinkage, is challenging. To evaluate on-treatment ¹⁸F-fluorodeoxyglucose (FDG) and/or ¹⁸F-fluorodeoxythymidine (FLT) positron emission tomography (PET) for this purpose, we conducted a prospective multicenter trial assessing PET response rates and associations with progression-free (PFS) and overall survival (OS) in 2nd/3rd-line non–small-cell lung cancer patients treated with erlotinib.

Experimental Design: PET/computed tomography (CT) scans were conducted at baseline, day (d)14 and d56 after the first daily erlotinib dose, with diagnostic CT at baseline and d56 (all scans centrally reviewed). PET partial metabolic response (PMR) was defined as a mean decrease (in ≤5 lesions/patient) of 15% or more maximum standardized uptake value. PFS was investigator-determined.

Results: Of 74 erlotinib-treated patients, 51 completed all imaging assessments through d56; 13 of 51 (26%) FDG-evaluable patients had PMR at d14, as did 9 of 50 (18%) FLT-evaluable patients. Four (7.8%) showed partial responses (PR) by d56 CT; all 4 had PMR by d14 FDG-PET with 3 PMRs by d14 FLT-PET. Three of the 4 patients with CT PR had evaluable archival tumor tissue; all 3 had epidermal growth factor receptor mutations. D14 and d56 PMRs by FDG or FLT were associated with improved PFS; HRs for PET responders versus nonresponders were 0.3 to 0.4. D14 FDG-PET PMR was associated with improved OS (P = 0.03) compared with FDG-PET nonresponders.

Conclusion: Early (d14) FDG-PET PMR is associated with improved PFS and OS, even in the absence of subsequent Response Evaluation Criteria in Solid Tumors response. These data support inclusion of FDG-PET imaging in clinical trials testing novel targeted therapies, particularly those with anticipated cytostatic effects. Clin Cancer Res; 17(10); 3304–15. ©2011 AACR.

Introduction

In cancer therapeutics, Response Evaluation Criteria in Solid Tumors (RECIST; ref. 1), based primarily on computed tomography (CT) scans, are widely used for response assessment, but are known to have a number of critical limitations. This is of particular concern in the clinical development of molecularly targeted therapies (2) that are expected to induce prolonged stable disease via cytostasis rather than objective morphological response (3). In lung cancer, use of RECIST is further confounded by frequent structural abnormalities, before and after treatment, which may not actually contain tumor (4).

A number of technologies, including CT volumetrics (5) and positron emission tomography (PET) imaging, offer the possibility of addressing these limitations. In particular, PET imaging with ¹⁸F-fluorodeoxyglucose (FDG; ref. 6; a measure of glucose metabolism) or ¹⁸F-fluorodeoxythymidine (FLT; ref. 7; an indirect measure of cell proliferation) has the potential to provide early and robust assessment of the clinical activity of molecularly targeted therapies by assessing biological changes in tumors that are likely to be of clinical relevance even in the absence of tumor shrinkage.

FDG is widely available for clinical use, is FDA approved, and is increasingly being used in PET imaging for the management of patients with cancer (8, 9). A number of organizations including the U.S. National Cancer Institute (NCI; ref. 10), the Society of Nuclear Medicine (11), and the European Association of Nuclear Medicine (12) have developed guidelines for the use of FDG-PET. In non–small-cell lung cancer (NSCLC), FDG-PET may provide...
FDG- and FLT-PET Imaging for Erlotinib-Treated Lung Cancer

Translational Relevance

Many cancer therapies in development may benefit patients through prolonged stable disease rather than tumor shrinkage. Hence, standard size-based response assessment per Response Evaluation Criteria in Solid Tumors (RECIST) likely underestimates therapeutic activity. To evaluate whether 18F-fluorodeoxyglucose (FDG) or 18F-fluorodeoxythymidine (FLT) positron emission tomography (PET) could address this limitation, we conducted a multicenter clinical study of these technologies by an established therapy: erlotinib in non–small-cell lung cancer.

We show that (i) using these technologies in a multicenter study is feasible; (ii) PET identifies a greater number of responders (including patients with epidermal growth factor receptor wild-type tumors) than RECIST; (iii) PET response is associated with improved progression-free and overall survival; and (iv) FDG-PET seems more informative than FLT-PET in this setting. These results strongly support the use of appropriate PET imaging as a clinical tool to evaluate investigational cancer therapies, and also to facilitate development of companion diagnostics by enabling identification of tumor characteristics in responder subpopulations.

the information at the time of initial staging or restaging, which can assist in therapeutic management decisions (13). In addition, prospective studies of patients with NSCLC showed that FDG-PET response to radiation and various chemotherapies was predictive of survival (14).

FLT-PET has been used in a number of clinical studies (15–19). In NSCLC, it has been shown that PET imaging of FLT uptake correlates with the rate of tumor proliferation as measured by Ki67 tumor staining (16, 17).

For molecularly targeted therapies, several small studies in selected NSCLC patients have evaluated FDG- or FLT-PET changes (20–25). However, for molecularly targeted therapies, it has not yet been established whether these imaging approaches provide a clinically meaningful assessment of therapeutic activity in NSCLC, particularly in the absence of tumor shrinkage. To specifically address this question, we conducted a clinical study of changes on FDG- and FLT-PET early during treatment and subsequent clinical outcomes in NSCLC patients receiving an established molecularly targeted therapy, erlotinib.

Erlotinib (Tarceva) is a small-molecule tyrosine kinase inhibitor (TKI) of the epidermal growth factor receptor (EGFR). Among its clinical uses, erlotinib is an established therapy as a single agent in patients with locally advanced or metastatic NSCLC who progressed on at least 1 prior chemotherapy regimen (26–29). In this clinical setting, the pivotal BR.21 study showed that despite a relatively modest objective CT-RECIST response rate of 8.9%, erlotinib treatment resulted in significant improvement in progression-free (PFS) and overall survival (OS) compared with best supportive care (27). These data suggest that clinical benefit is not limited to patients with CT-RECIST response. Consequently, erlotinib treatment is ideal to assess the clinical performance of FDG- and FLT-PET to identify clinically meaningful activity of a molecularly targeted therapy in the absence of tumor shrinkage.

The phase I/II pilot study described here assessed FDG- and FLT-PET response (on day 14 and day 56) and clinical outcomes (day 56 CT response, PFS and OS). The primary objective was to determine whether patients with FDG- or FLT-PET response on day 56 of erlotinib treatment had longer PFS and OS than patients without PET response. This was assessed by estimating the HRs for PFS and OS between PET responders and PET nonresponders. Secondary objectives included determining the proportion of patients who showed FDG- and FLT-PET response on day 14 and day 56 among all patients, and among those with CT stable disease, and evaluating the safety of FLT. Exploratory objectives included assessment of the relationship between FDG- and FLT-PET response and tumor characteristics, particularly EGFR- and KRAS-related markers (30–36).

Patients and Methods

Patients

Inclusion and exclusion criteria were in accordance with standard eligibility criteria for erlotinib treatment (26). Eligible patients were 18 years or older with histologically confirmed locally advanced or metastatic, recurrent, or refractory NSCLC after failure of 1 or 2 prior systemic treatment regimens. Patients had a life expectancy ≥3 months or more; an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2; provided archival diagnostic paraffin–embedded tumor tissue; and had adequate key organ function as reflected by standard blood tests. All patients had at least 1 measurable lesion on CT scan that was assessable by FDG- or FLT-PET. Evaluable lesions were defined as measurable by CT (≥1.5 cm on spiral CT) and detectable by FDG-PET and/or FLT-PET. For this study, mediastinal lymph nodes between 1 and 2 cm in diameter were acceptable for both CT and PET response evaluation, provided that such lymph nodes did not represent the primary sites of measurable disease. The same lesion did not need to be detectable by both FDG- and FLT-PET imaging because the aim of the study was to evaluate the independent value of these imaging techniques for response assessment.

Exclusion criteria included prior treatment with EGFR-directed therapy; chemotherapy, radiotherapy, or investigational treatment within 14 days or 5 half-lives of systemic therapy; uncontrolled diabetes (fasting serum glucose level > 200 mg/dL); unstable systemic disease; pregnancy; and history of another malignancy in the previous 2 years.

All patients in the study provided written informed consent following approval of the study by the ethics review board at each participating site.
Study design
This was a single-arm, open-label, multicenter, and international study (see schema in Fig. 1). Patients underwent FDG- and FLT-PET scans at separate days at baseline, day 14 and day 56 (±3 days) of treatment. Patients were followed for disease progression, including CT scans on day 56 and then every 56 days (±7 days) thereafter, for up to 1 year after administration of the first dose of erlotinib. Patients were followed for survival every 4 months after their discontinuation visit until study closure.

Study treatment
Patients received erlotinib orally at 150 mg/day. Toxicities resulting from erlotinib administration were managed by symptomatic treatment and/or erlotinib dose interruptions or reductions as appropriate on the basis of the erlotinib prescribing information (26).

The following therapies were excluded during the study: any other anticancer therapies or investigational agents; radiotherapy, unless there were CT-measurable and PET-detectable lesions outside of the radiation field that could be followed for response to erlotinib; and corticosteroids, except for patients entering the study on stable doses ≤25 mg/day of prednisone (or equivalent low doses of other corticosteroids).

Imaging
All PET imaging was conducted with combined PET/CT scanners according to an acquisition protocol stipulated in a detailed imaging charter that was designed to be consistent with the NCI guidelines (10). We previously described the image acquisition requirements and compliance across the clinical sites in Binns and colleagues (37). In brief, patients received an injected dose of FDG on the basis of their weight, benchmarked at 10 mCi for a 70-kg patient and a maximum of 15 mCi. The injected dose of FLT for each patient scan was 7 mCi. Radiotracer uptake times were specified as 75 and 60 minutes ±15 minutes at baseline for FDG and FLT respectively; on-treatment scans were within 5 minutes of baseline uptake time. A high degree of compliance with the imaging protocol in this study was observed across all time points (37).

PET and CT response assessment
PET and CT responses for all patients were determined at a central reading site (Peter MacCallum Cancer Center, Melbourne, Australia).

PET response assessment was based on the maximum standardized uptake value (SUVmax) of up to 5 regions of interest (ROI). SUVmax was measured by utilizing a 3-dimensional cluster in which averaging is done over a sphere with a radius of 1 pixel (a 7 voxel average, corresponding to a diameter of 6–9 mm) and was corrected for lean body mass. Tumor ROIs were identified for each patient on pretreatment scans on the basis of qualitative evaluation. Lesions with an uncorrected SUVmax of greater than 1.5 for FDG and less than 1.0 for FLT were analyzed for response. ROIs for FDG-PET versus FLT-PET did not have to be identical. However, whenever possible, lesions that were suitable for RECIST measurement and analysis with both tracers were selected as target lesions. The SUVmax of each ROI for on-treatment scans was compared with its SUVmax on the corresponding pretreatment scan, and the percent change was determined. If there was more than 1 ROI, a mean SUVmax (mSUVmax) was determined. On the basis of definitions by the European Organization for Research and Treatment of Cancer (EORTC), an objective PET response [partial metabolic response (PMR)] was defined as an mSUVmax decrease of 15% or more (6). PET stable metabolic disease (SMD) was defined as a mean change of 0% to 15% in SUVmax, and PET progressive metabolic disease (PMD) was defined as a mean increase of ≥15% or 1 or more new lesions on PET. Recognizing that the optimal definition of PET response is still under investigation, we also applied an exploratory analysis of a ≥25% decrease in mSUVmax threshold to define PET response on day 56; this is consistent with the EORTC recommendations for assessing tumor response after more than 1 cycle of chemotherapy.

CT response was assessed according to modified RECIST criteria (version 1.0); among preidentified CT RECIST target lesions, only those lesions found to be FDG avid were chosen as target lesions.

Safety assessments
Patients were evaluated clinically and with standard laboratory tests at screening and regular intervals during
the study. All adverse events (AE) were evaluated for possible relation to FLT, FDG, and erlotinib and graded according to the NCI Common Toxicity Criteria for AEs (NCI CTCAE; Version 3.0). FLT was the only investigational agent in this study.

**Biomarker assessments**

At study entry, archival tumor tissue was collected and subsequently tested for selected molecular markers. EGFR FISH analyses were conducted with a PathVysion(R) Kit (Abbott Molecular). High EGFR copy number was defined as high polysomy (≥4 gene copies in ≥40% of cells) or amplification (≥2 genes/chromosome or ≥15 gene copies in ≥10% of cells; ref. 38). Immunohistochemical analysis of EGFR was conducted by PharmDx kits (DAKO); positive EGFR expression was defined as EGFR staining of ≥10% of tumor cells. Highly sensitive analyses of EGFR somatic gene mutations in exons 18 to 21 and KRAS mutations in exons 2 and 3 were conducted by denaturing high-performance liquid chromatography (Transgenomics, Inc.).

**Statistical analysis**

Patients were considered evaluable for efficacy analysis if they underwent all study-required CT and PET imaging studies through day 56.

To compare PET with CT RECIST responses, the proportion of patients with FDG- and FLT-PET responses (PMR) after the initial 14 and 56 days of erlotinib treatment was estimated and compared with the response rate on day 56 per CT RECIST. PET/CT response rates on day 14 and/or day 56 were also estimated relative to selected tumor characteristics. The Kaplan-Meier survival methodology was used to estimate the median survival time and PFS for patients with or without PET responses for FDG- and FLT-PET on days 14 and 56. A Cox proportional hazard model was used to estimate HRs and 95% CI for PFS and survival in patients with PET response versus patients without PET response for FDG- and FLT-PET on day 14 and day 56. Differences between these groups were assessed by 2-sided log-rank test.

**Sensitivity analyses**

To assess the robustness of results about the association of PET response and clinical outcome, sensitivity analyses were conducted as follows: (i) exclusion of patients whose PET tracer uptake time violated protocol-defined criteria; (ii) exclusion of patients whose assessments violated protocol-defined window for PET scanning on days 14 and 56, respectively; and (iii) exclusion of patients whose PFS assessments may have been affected by PET scan findings on day 56.

To further test the association of PET response with clinical outcomes, subset analyses were carried out for all patients who completed the day 14 assessments but discontinued before day 56, and for patients with EGFR wild-type tumors.

In addition, a Cox proportional hazards model was applied by using mSUVmax as a continuous measurement to further explore the relationship between mSUVmax and efficacy outcomes.

Furthermore, baseline FDG uptake and an alternate PET response definition of mSUVmax decrease of 25% or more for the assessment of day 56 PET response were analyzed, and their relationship with clinical outcomes was tested.

**Results**

**Study population and patient disposition**

The study was conducted at 8 sites in Australia and the United States; 74 patients were enrolled and treated with erlotinib between November 2006 and April 2009. Among these 74 patients, 51 completed all protocol-mandated CT and FDG scans on days 14 and 56 (FDG evaluable patients), and 50 completed CT and FLT scans on days 14 and 56 (FLT evaluable patients). (Two patients completed all FDG scans but not all FLT evaluations; one completed all FLT scans but not all FDG studies.) Sixty-six and 63 patients completed FDG- and FLT-PET scans, respectively, on day 14 and were evaluable for sensitivity analyses. Of 186 lesions selected as target lesions on both RECIST and molecular imaging, 185 were suitable for FDG analysis and 168 for FLT analysis. Due to high adjacent background, 1 lesion was unsuitable for FDG analysis, but could be analyzed on FLT. Of 18 lesions deemed unsuitable for analysis on FLT, 16 had uptake that was too low to be evaluable, whereas 2 lesions could not be demarcated due to high physiological uptake in adjacent tissue (liver and spleen, respectively).

Twenty-one patients discontinued from study prior to completing all d56 study assessments. Reasons for discontinuation included: disease progression (n = 6), death (due to disease progression; n = 5), AEs (n = 4), and others (n = 6).

Baseline and demographic characteristics are shown in Table 1. Among 51 FDG evaluable patients, the most frequent histology was adenocarcinoma in 37 (72.5%) patients. Archival tumor tissue was obtained from 38 (74.5%) patients, and was evaluable for EGFR IHC, EGFR FISH, EGFR mutation, and KRAS mutation analysis in 38, 33, 35, and 35 patients, respectively. Sensitizing EGFR mutations were present in 4 (11.4%) patients and KRAS mutations were present in 7 (20.0%) patients with evaluable tissue. The majority of FDG- and FLT-PET avid lesions evaluated quantitatively for response were located in the lung (46.9% and 46.6%, respectively), followed by lymph nodes (24.5% and 26.7%), and mediastinum (13.3% and 13.7%). Lesions located in other organs were assessed rather infrequently (<10%) and included bone, abdomen, liver, skin/soft tissue, and spleen. Because only CT RECIST-evaluable PET avid lesions were chosen, the anatomic distribution was similar for CT RECIST target lesions. Information on lesion number, size, and baseline uptake is summarized in Table 1.

**Correlation of CT and PET responses**

CT responses on day 56 per RECIST 1.0 and PET PMR on days 14 and 56 are shown in Table 2. Of 51 FDG evaluable
Table 1. Demographics and baseline characteristics

<table>
<thead>
<tr>
<th>Patients with CT and FDG-PET evaluable lesions</th>
<th>( n = 51 )^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age (range)</strong></td>
<td>61 (47–78)</td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (58.8)</td>
</tr>
<tr>
<td>Female</td>
<td>21 (41.2)</td>
</tr>
<tr>
<td><strong>Race, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>44 (86.3)</td>
</tr>
<tr>
<td>Asian or Pacific islander</td>
<td>3 (5.9)</td>
</tr>
<tr>
<td>Black</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Not available</td>
<td>3 (5.9)</td>
</tr>
<tr>
<td><strong>ECOG PS, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15 (29.4)</td>
</tr>
<tr>
<td>1</td>
<td>29 (56.9)</td>
</tr>
<tr>
<td>2</td>
<td>7 (13.7)</td>
</tr>
<tr>
<td><strong>Smoking history, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>Previously smoked</td>
<td>35 (68.6)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>37 (72.5)</td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>8 (15.7)</td>
</tr>
<tr>
<td>Large-cell carcinoma</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Other (including NOS)</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td><strong>Prior Therapies</strong></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>32 (62.7)</td>
</tr>
<tr>
<td>Surgery</td>
<td>50 (98.0)</td>
</tr>
<tr>
<td>Systemic</td>
<td>51 (100)</td>
</tr>
<tr>
<td><strong>Time since initial diagnosis, median (range) in months</strong></td>
<td>15.0 (2–98)</td>
</tr>
<tr>
<td><strong>EGFR mutation status, n (%)</strong></td>
<td>( n = 35 )^b</td>
</tr>
<tr>
<td>Sensitizing mutant</td>
<td>4 (11.4)</td>
</tr>
<tr>
<td>Wild type</td>
<td>30 (85.7)</td>
</tr>
<tr>
<td><strong>KRAS mutation status, n (%)</strong></td>
<td>( n = 35 )^b</td>
</tr>
<tr>
<td>Mutated</td>
<td>7 (20.0)</td>
</tr>
<tr>
<td>Wild type</td>
<td>27 (77.1)</td>
</tr>
<tr>
<td><strong>CT target lesions, median (range)</strong></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td>Size, mm</td>
<td>24 (11–78)</td>
</tr>
<tr>
<td><strong>FDG-PET target ROIs, median (range)</strong></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td>FDG uptake (SUVmax)</td>
<td>5.63 (1.6–31.7)^c</td>
</tr>
<tr>
<td><strong>FLT-PET target ROIs, median (range)</strong></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td>FLT uptake (SUVmax)</td>
<td>2.5 (1.0–12.2)^c</td>
</tr>
</tbody>
</table>

Abbreviations: IHC, immunohistochemistry; NOS, not otherwise specified

^aTwo patients received FDG without FLT and one received FLT without FDG. Hence, the total number of patients with or without FLT scans is 50.

^bThirty-five patients had available tissue. However, for 1 patient, tissue was deemed unevaluable for KRAS mutation status.

^cMedian SUVmax values reflect values corrected for lean body mass in each patient, whereas the range for individual lesions reflects the raw SUVmax value on the basis of the mean value obtained from clustering of the most intense voxel and the 6 voxels surrounding it. The median uncorrected SUVmax for lean body mass was 8.8 for FDG-PET and 4.2 for FLT-PET.
patients who received daily oral erlotinib and completed all assessments through day 56, 4 (7.8%) patients had a partial response (PR) by day 56 CT evaluation.

Eight patients had a PMR on day 56 by FDG- and FLT-PET scan. All 4 patients with CT-PR on day 56 also had a PMR on day 56 on FDG-PET, whereas 2 had a PMR on day 56 on FLT-PET.

The early PMR rates by FDG- and FLT-PET on day 14 were higher than the day 56 rates. Of 51 FDG evaluable patients, 13 (26%) had a PMR on day 14, and of 50 FLT evaluable patients, 9 (18%) had a PMR on day 14. All 4 patients with a CT-PMR on day 56 also had a PMR on day 14 on FDG-PET, whereas 3 had a PMR on day 14 FLT-PET.

Waterfall plots of mSUVmax for days 14 and 56 FDG- and FLT-PET are shown in Figure 2A and Supplementary Appendix Figure 1A, respectively, ordered by CT response (PR, SD, and PD) and relative change in mSUVmax. FDG-PET PR, SMD, and PMD are indicated, as are FLT-PET response defined as mean decrease of 15% or more in SUVmax across all ROIs.

Association of PET response with PFS and OS

Both days 14 and 56 PMR by either FDG or FLT were positively associated with clinical outcomes. There was a significant association between FDG- and FLT-PET response and improved PFS at both days 14 and 56 (Table 2, Fig. 2B and Supplementary Appendix Fig. 1B). However, day 14 FDG-PET PMR was the only measurement significantly associated with improved OS: HR 0.44 [P = 0.03; 95% CI = (0.21, 0.94)] compared with non-responders.

FDG-PET responses and corresponding patient and tumor characteristics

Figure 2C shows a waterfall plot of observed changes in FDG uptake by day 14, ordered by FDG-PET mSUVmax and indicating corresponding CT responses and patient and tumor characteristics, and new lesions identified by FDG-PET. Of the 35 patients with tumor tissue evaluable for mutations, 4 had sensitizing EGFR mutations and 7 had KRAS mutations.

The 4 sensitizing EGFR mutations were: 1 in exon 18, 1 in exon 19 (deletion), and 2 in exon 21 (L858R). All 3 of the 4 patients with CT PR, who had evaluable archival tumor tissue, had EGFR mutations (2 with L858R and 1 exon 19); these 3 patients were females with adenocarcinoma (2 white and 1 Asian; Table 3). All exhibited a greater decrease in mSUVmax on day 14 (median: –55.3%, range: –72.3%, –43.9%) than the 6 patients with day 14 PMR and confirmed EGFR wild-type tumors (median: –19.9%, range: –36.4%, –19.1%). Furthermore, these 3 patients with EGFR mutant tumors had a sustained reduction of mSUVmax on day 56, whereas only 2 of 6 patients with EGFR wild-type tumors maintained the same degree of mSUVmax reduction on day 56 compared with day 14 (see Fig. 2A and C). The patient with an exon 19 deletion had both CT stable disease on day 56 and stable disease by PET on both days 14 and 56. In addition to these 4 patients, 1 patient was found to have an EGFR exon 20 insertion. This mutation is associated with resistance to EGFR TKIs (37) and indeed, this patient had CT progressive disease (PD) on day 56 and PET progression on days 14 and 56. All 7 patients with KRAS mutant tumors had CT stable disease on day 56. Interestingly, 1 of these patients had a

Table 2. CT and PET assessments of response rates, OS, and PFS

<table>
<thead>
<tr>
<th></th>
<th>CT (n = 51)</th>
<th>FDG-PET (n = 50)</th>
<th>CT (n = 51)</th>
<th>FLT-PET (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 56</td>
<td>Day 14</td>
<td>Day 56</td>
</tr>
<tr>
<td>PR, n (%)</td>
<td>4 (8)</td>
<td>13 (26)</td>
<td>8 (16)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>SD, n (%)</td>
<td>26 (51)</td>
<td>17 (33)</td>
<td>12 (23)</td>
<td>26 (62)</td>
</tr>
<tr>
<td>PD, n (%)</td>
<td>21 (41)</td>
<td>21 (41)</td>
<td>31 (61)</td>
<td>20 (40)</td>
</tr>
<tr>
<td>PFS HR for responders</td>
<td>0.29</td>
<td>0.28</td>
<td>0.32</td>
<td>0.29</td>
</tr>
<tr>
<td>P value, 95% CI</td>
<td>-</td>
<td>&lt;0.001 (0.13, 0.6)</td>
<td>0.01 (0.14, 0.77)</td>
<td>-</td>
</tr>
<tr>
<td>OS HR for responders</td>
<td>0.32</td>
<td>0.44</td>
<td>0.49</td>
<td>0.32</td>
</tr>
<tr>
<td>P value, 95% CI</td>
<td>0.11 (0.08, 1.32)</td>
<td>0.03 (0.21, 0.94)</td>
<td>0.13 (0.19, 1.25)</td>
<td>0.11 (0.08, 1.32)</td>
</tr>
<tr>
<td></td>
<td>0.74 (0.38, 1.97)</td>
<td>0.62 (0.34, 1.92)</td>
<td>0.87</td>
<td>0.80</td>
</tr>
</tbody>
</table>

a number refers to the number of patients with available FDG-PET and CT data.
b PET response defined as mean decrease of 15% or more in SUVmax across all ROIs.
c refers to the number of patients with available FLT-PET and CT data.
d HR for responders compared with nonresponders.
Figure 2. FDG-PET responses on day 14 and day 56, and PFS and OS. A, waterfall plot of FDG-PET responses on days 14 and 56 ordered by day 56 CT responses. FDG-PET PMR, SMD, and PMD are color-coded within the bar graphs, and CT responses are color-coded underneath. B, PFS and OS in FDG-PET responders versus nonresponders. (FLT-PET showed a similar trend, but there were fewer FLT-PET responders). C, waterfall plot of day 14 FDG-PET changes, ordered by FDG-PET response, and corresponding patient demographics and tumor characteristics. For the "race" category, "other" refers to black (1 patient), Native Hawaiian or Pacific Islander (1 patient), or "not available" (2 patients). For EGFR, sensitizing mutations are shown as positive. In addition, 1 patient (3rd from right) had an EGFR mutation in exon 20 (which has been previously associated with resistance to EGFR TKIs).
day 56 PMR but day 14 PMD (28th from the left in Fig. 2A). This is the only patient who showed this reverse response. This white male patient is a current smoker with KRAS mutant adenocarcinoma who remained progression free for 32 weeks and survived for 12 months.

A summary of findings from the biomarkers analysis (EGFR and KRAS mutation status) is shown in Supplementary Appendix Table 1.

As examples, Figure 3 shows FDG-PET/CT scans for 2 patients with FDG-PET response, 1 with an activating mutation in exon 21 (L858R; Fig. 3A), and 1 with an EGFR wild-type, KRAS mutant tumor (Fig. 3B), illustrating the positive predictive value of FDG-PET for therapeutic activity, independent of the underlying molecular tumor profile.

In contrast to a recent study by Na and colleagues (21), we did not observe a significant difference in baseline FDG uptake values for PET responders versus nonresponders.

Sensitivity analyses
Sensitivity analyses were conducted that excluded: (i) data from 6 patients whose PET tracer uptake time violated protocol-defined criteria; (ii) data from 7 and 2 patients who violated protocol-defined window for PET scanning on days 14 and 56, respectively; and (iii) data from 4 patients whose PFS assessments may have been affected by PET scan findings on day 56. Each of these sensitivity analyses showed that the association between PET response and PFS and OS was maintained (data not shown).

In addition, the association of day 14 PET response with PFS and OS was tested in all patients who completed day 14 (n = 67), which includes 14 patients who discontinued between day 14 and day 56. The HRs for PFS and OS were 0.56 (P = 0.06) and 0.57 (P = 0.09) for FDG and 0.6 (P = 0.17) and 0.85 (P = 0.66) for FLT thereby showing a maintained positive trend of association of early PET response with clinical outcome.

The association of day 14 PET response with clinical outcome was also tested in the subset of patient with EGFR wild-type tumors; 6 of 31 and 4 of 30 patients with evaluable EGFR wild-type tissue had day 14 FDG and FLT PMR, respectively. Even though the numbers were small, there was a trend toward improved PFS in patients...
with day 14 PET response. The median PFS and OS were 5 and 10 months, respectively, for patients with EGFR wild-type tumors who had day 14 FDG response (PMR), compared with PFS of 2.8 months (HR = 0.45, P = 0.1) and OS of 7.6 months (HR = 0.7, P = 0.47) for nonresponders. Similarly, for day 14 FLT responders versus nonresponders, the median PFS and OS were 6 and 7.7 months versus 3.5 months (HR = 0.47, P = 0.2) and 7.6 months (HR = 1.04, P = 0.95), respectively.

When a threshold of a ≥25% decrease in mSUVmax was applied to define PET response on day 56, the FDG- and FLT-PET response rates are lower with 11.8% and 8% than those reported in Table 2. The corresponding HRs (P-value) for PFS and OS are 0.31 (0.02) and 0.36 (0.07) for FDG and 0.17 (0.01) and 0.38 (0.16) for FLT, indicating a similar correlation between PET response and clinical outcome as using a cutoff of 15% to define PET response.

Finally, to further explore the relationship between mSUVmax and efficacy outcomes, a Cox proportional hazards model by using mSUVmax as a continuous measurement showed that for FDG-PET, day 14 and day 56 mSUVmax were positively correlated with PFS (P < 0.001 value for both), and day 14 mSUVmax, but not day 56 mSUVmax (P = 0.12), was correlated with OS (P = 0.025). For FLT-PET, day 56 mSUVmax showed a positive correlation with PFS (P = 0.002) and OS (P = 0.05).

Potentially prognostic parameters were evaluated. Neither baseline SUVmax nor the number of FDG target lesions were found to be predictive of PET response.

**Safety**

AEs were consistent with those previously reported in patients treated with erlotinib (26). No FLT-related AEs were identified.

**Discussion**

To our knowledge, this is (i) the largest prospective study of NSCLC patients evaluated with both FDG- and FLT-PET imaging in direct correlation with standard CT imaging and tumor tissue evaluation and (ii) the largest prospective study to evaluate PET imaging and its association with PFS and OS in the setting of a molecularly targeted therapy for NSCLC. Our results are consistent with and greatly expand on results from several smaller clinical studies of PET imaging in NSCLC patients treated with EGFR inhibitors, although none of these other studies directly compared FDG- and FLT-PET imaging (21–24, 39). For example, Sohn and colleagues found that an FLT-PET scan after 7 days of gefitinib treatment could predict the response to treatment in 28 nonsmokers with advanced adenocarcinoma of the lung (22). Sunaga and colleagues reported similar findings with FDG-PET scans at 2 and 4 weeks after treatment with gefitinib (21). Soto Parra and colleagues recently reported that FDG-PET–detected changes occurring as early as 2 days after starting erlotinib can predict response in NSCLC patients (24). Our results about the utility of FDG-PET are concordant with the results from studies in other cancer types and/or utilizing other molecularly targeted therapies. For instance, a recent study in patients with head and neck cancer showed that FDG-PET was reliable for detection of early responses to erlotinib (40). Similar results were seen in a study of imatinib for treatment of gastrointestinal stromal tumors, and FDG-PET scans are now commonly used to assess responses in this setting (41).

The primary purpose of this study was to assess whether FDG- and FLT-PET scans provide an early indication of therapeutic activity and subsequent clinical benefit in patients treated with an agent that has primarily cytostatic activity and for which standard imaging with CT is less informative. To focus our evaluation on the role of molecular imaging, we specifically evaluated NSCLC patients treated with the established molecularly targeted agent, erlotinib, which had already shown prolonged survival despite a low objective RECIST response rate (26, 27, 35). We have previously reported on the high degree of imaging protocol compliance across multiple clinical sites on this study (37), showing the feasibility of evaluating quantitative PET imaging in this setting. The results presented here indicate that both FDG- and FLT-PET imaging techniques provide additional information beyond standard CT imaging, especially when conducted early during erlotinib treatment.

We found that FDG- and FLT-PET scans identify more responding patients and more progressing patients than standard CT assessment. FDG imaging identified more responders than FLT imaging at both time points days 14 and 56. However, only FDG-PET scanning on day 14 was associated with both improved PFS and OS, irrespective of whether an RECIST response by CT was seen. Patients with FDG-PMR on day 14 had a median OS of 11.6 months compared with 7.6 months in those without a PMR (P = 0.03).

The likelihood of obtaining a response by FDG-PET did not seem to be associated with particular patient or tumor characteristics, although sample sizes were low in several subgroups. Nevertheless, those patients who eventually achieved a RECIST PR also had the most profound and sustained FDG PMR at the early time point and were found to have an underlying activating EGFR mutation. These results support the suggestion that cells with activating EGFR mutations are oncogene-addicted and decreased signaling through this pathway leads to a marked reduction in glucose utilization and subsequent cell death, possibly through apoptosis, leading to morphological regression. Preclinical studies with the EGFR kinase inhibitor gefitinib showed a dramatic decrease in FDG uptake in gefitinib-sensitive cell lines, also showing translocation of glucose transporters from the plasma membrane to the cytosol, reduced glucose transport rates, and reduced hexokinase activity (42). These metabolic alterations were found to precede changes in cell-cycle distribution, thymidine uptake, and apoptosis. In contrast, gefitinib-resistant cells exhibited no measurable changes in FDG uptake, either in cell culture or in vivo. Similarly, preclinical studies of cells...
transfected with a mutant tyrosine kinase, c-KIT, treated with a targeted kinase inhibitor, imatinib, have shown that a reduction in glucose utilization precedes cell death (43). Therefore, FDG-PET–based evidence of reduced glucose utilization may be associated with subsequent tumor regression and beneficial therapeutic effect. In contrast, early FDG-PET responses in patients with EGFR wild-type tumors seem to be predominantly associated with an SUVmax reduction of lesser magnitude. This might be interpreted as a less marked, yet clinically meaningful, treatment effect of erlotinib in a subset of patients with EGFR wild-type tumors.

Although prior studies have raised questions about benefit of EGFR TKIs in NSCLC patients with KRAS mutant tumors (30, 32), the FDG-PET responses seen in 2 patients in our study suggest that erlotinib may have clinically relevant effects on NSCLC tumors even in the presence of enhanced signaling through the mitogen-activated protein kinase pathway.

Interestingly, we found the results of FDG scans to be more informative than those of FLT scans. This is in contrast to preclinical observations that found FLT but not FDG-PET imaging to be a robust early indicator of erlotinib response in EGFR-dependent tumor models (43). Clinically, however, in NSCLC patients, FLT has relatively low uptake compared with FDG in many lesions. For instance, among the 217 lesions quantitatively assessed in our study, baseline FLT uptake uncorrected for lean body mass was lower (median SUVmax = 4.2, range: 1.0–12.2) than uptake of FDG (median SUVmax = 8.8, range: 1.6–31.7). FLT also has relatively high background uptake, particularly in liver and bone marrow. As a consequence, the degree to which changes in FLT uptake can be measured in response to erlotinib might be limited. However, given the desire to assess response by RECIST, bone lesions were seldom chosen in this study, thereby limiting potential discordance between FDG and FLT in assessing therapeutic response. Consequently, of 186 RECIST-evaluable lesions, 167 were assessed by both FDG and FLT; 18 FDG-avid lesions were not evaluable on FLT due to either low uptake or high physiologic background, whereas only 1 lesion was not evaluable on FDG (due to insufficient contrast to delineate the lesion), but was assessable on FLT. Including only lesions evaluated by both tracers did not alter the classification of any patient from responder to nonresponder on day 14 or day 56 FDG-PET.

It is important to note that the immediate downstream signaling target of EGFR is phosphatidylinositol-3-kinase, which regulates AKT, a serine/threonine protein kinase that plays a critical role in glucose metabolism. Accordingly, it is not surprising that FDG might provide an earlier readout of biological effects related to reduced EGFR signaling. Thus, it should not be assumed that FDG-PET will be a superior technique for therapeutic response assessment with all molecular-targeted therapies.

For FDG-PET, we used the EORTC definition of a PMR as a 15% or greater fall in mSUVmax that has been recommended for the assessment of early treatment changes (6). Other smaller studies investigating FDG-PET imaging following treatment with EGFR TKIs have utilized a cutoff of 15% arriving at similar results and showing an association of PET response with improved clinical outcome (25). The EORTC guidelines further recommend a cutoff of 25% as a more meaningful reduction at later time points, specifically after completion of more than 1 cycle of chemotherapy.

Although our exploratory analyses confirm our results even if a 25% cutoff is applied to measure PET response on day 56, it is important to recognize that the magnitude of posttreatment changes might be lower following molecularly targeted or cytokstatic therapy versus cytotoxic chemotherapy; hence, the appropriate definition of PET response may depend on the clinical setting and specific therapy.

Although our study showed an association between both FDG- and FLT-PET response and PFS/OS, the following question naturally arises: does this have implications for clinical practice? The current study was not designed to address this. While the data from our study are informative and support the use of PET imaging to detect clinically meaningful drug activity in the context of clinical drug development, they do not permit definitive conclusions pertaining to patient management. In particular, this study did not address whether a change in therapy, such as dose escalation or drug substitution, on the basis of (early) PET response would improve patient outcome. Additional clinical studies would be required to address this question. It will be important that future studies also assess how patient-reported outcomes correlate with PET imaging findings, given that symptomatic improvement with erlotinib may be seen very early in treatment for those who benefit (26, 27).

Nevertheless, we believe that these results strongly support the inclusion of PET imaging in clinical studies that test the clinical activity of novel targeted therapies, particularly for treatments anticipated to have primarily cytokstatic effects. In addition, by correlating early PET changes with tumor tissue biomarkers, PET may also prove useful for identifying underlying tumor characteristics in a responder subpopulation.

Disclosure of Potential Conflicts of Interest

B. Hughes, consultant, Roche; P. Mitchell, consultant, Roche; B. Solomon, consultant and speaker, Roche; V. Chanu, commercial research grant, Genentech, Inc., other commercial support, Roche, Ltd, consultant, Gentech; I. Amler, W. Yu, A. Pizkall, B. Fine, employees, Gentech, Inc. and hold stock with Roche, Ltd. The other authors disclosed no potential conflicts of interest.

Acknowledgments

We thank all the patients who participated in the study, and the clinical site teams: (in Australia) Peter MacCallum Cancer Centre, East Melbourne, Victoria; Austin Hospital, Heidelberg, Victoria; Royal Brisbane Women’s Hospital, Herston, Queensland; Prince Charles Hospital, Brisbane, Queensland; (in the USA) USC Medical Center Kenneth Norris Cancer Center, Los Angeles, CA; Pacific Cancer Medical Center, Inc., Anaheim, CA; St. Joseph Hospital, Regional Cancer Center, Orange, CA; and the Wilshire Oncology Medical Group Inc., Corona, CA. We acknowledge the site investigators M. Biron, T. Byun, P. Conti, J. Goh, F. Howard, S. Kipper, K. Lee, A. M. Scott, and H. Trung, as well as staff at the central reading site at the Peter MacCallum Cancer Centre, especially D. Binns and J. Callahan. We also thank A. Crain, Ph. D., of Genentech, Inc., for assistance in the preparation of this manuscript.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate payment of page charges. This article must therefore be hereby marked as prepublication. This article must therefore be hereby marked as prepublication.

References


Received October 15, 2010; revised January 31, 2011; accepted February 15, 2011; published OnlineFirst March 1, 2011.


Changes in $^{18}$F-Fluorodeoxyglucose and $^{18}$F-Fluorodeoxythymidine Positron Emission Tomography Imaging in Patients with Non−Small Cell Lung Cancer Treated with Erlotinib

Linda Mileshkin, Rodney J. Hicks, Brett G.M. Hughes, et al.

Clin Cancer Res 2011;17:3304-3315. Published OnlineFirst March 1, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-2763

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2011/05/19/1078-0432.CCR-10-2763.DC1

Cited articles
This article cites 40 articles, 21 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/17/10/3304.full#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/17/10/3304.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/17/10/3304.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.