Monitoring Reversal of MET-Mediated Resistance to EGFR Tyrosine Kinase Inhibitors in Non–Small Cell Lung Cancer Using 3′-Deoxy-3′-[18F]-Fluorothymidine Positron Emission Tomography

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Abstract

Purpose: MET amplification is one of the mechanisms underlying acquired resistance to EGFR tyrosine kinase inhibitors (TKI) in non–small cell lung cancer (NSCLC). Here, we tested whether 3′-deoxy-3′-[18F]-fluorothymidine ([18F]FLT) positron emission tomography/computerized tomography (PET/CT) can detect MET-mediated resistance to EGFR TKIs and monitor the effects of MET inhibitors in NSCLC.

Experimental Design: H1993 and H820 NSCLC cells with high and low levels of MET amplification, respectively, and HCC827-expressing MET, but without gene amplification, were tested for the effects of MET inhibitors on the EGFR pathway and proliferation both in vitro and in vivo. Nude mice bearing NSCLCs with and without MET amplification were subjected to [18F]FLT PET/CT before and after treatment with crizotinib or erlotinib (50 mg/kg and 100 mg/kg p.o. for 3 days).

Results: H1993 cells showed high responsiveness to MET inhibitors and were resistant to erlotinib. Conversely, HCC827 cells showed high sensitivity to erlotinib and were resistant to MET inhibitors. Accordingly, H1993 tumors bearing MET amplification showed a mean reduction in [18F]FLT uptake of 28% and 41% after low- and high-dose treatment with crizotinib for 3 days, whereas no posttherapy changes of [18F]FLT uptake were observed in HCC827 tumors lacking MET amplification. Furthermore, a persistently high [18F]FLT uptake was observed in H1993 tumors after treatment with erlotinib, whereas HCC827 tumors showed up to 39% reduction of [18F]FLT uptake following erlotinib treatment. Imaging findings were confirmed by Ki67 immunostaining of tumor sections.

Conclusions: [18F]FLT PET/CT can detect MET-mediated resistance to EGFR TKIs and its reversal by MET inhibitors in NSCLC.

Introduction

Targeting epidermal growth factor receptor (EGFR) is one of the most promising treatment strategies in patients with advanced non–small cell lung cancer (NSCLC), and several EGFR tyrosine kinase inhibitors (TKI) are currently approved or are under clinical development for the treatment of NSCLC. Previous studies showed that the presence of activating mutations of EGFR in NSCLC is strongly associated with a high sensitivity to EGFR TKIs and candidate patients with advanced disease to first-line therapy with these drugs (1, 2). When EGFR-mutant NSCLCs are exposed to inhibitors such as gefitinib or erlotinib, EGFR signaling pathways are suppressed because they are under the exclusive control of EGFR. This will result in growth arrest and apoptosis induction and accounts for the high responsiveness of NSCLCs bearing activating EGFR mutations.

Unfortunately, despite this initial and marked response, most tumors may become resistant to EGFR TKIs and two different molecular mechanisms of acquired resistance were identified and confirmed in patients with NSCLC (3, 4). About half of resistant tumors develop secondary mutations in EGFR (T790M), which prevent an effective inhibition by EGFR TKIs due to steric hindrance or an increased binding affinity for ATP (5). An additional 15% to 20% of tumors from refractory patients undergo amplification of MET receptor tyrosine kinase, which causes a HER3-dependent activation of signaling cascade downstream the EGFR despite its inhibition by TKIs (6, 7).
Translational Relevance

Patients with advanced non–small cell lung cancer (NSCLC) bearing activating mutations of EGFR are currently assigned to first-line therapy with EGFR tyrosine kinase inhibitors (TKI). Despite initial high responsiveness, virtually all patients develop resistance to EGFR TKIs, and one of the mechanisms causing treatment failure is MET amplification. Here, noninvasive imaging with 3'-deoxy-3'-[18F]-fluorothymidine ([18F]FLT) and positron emission tomography (PET) were used to detect MET-mediated resistance in NSCLC and to monitor its reversal by MET inhibitors. We provided consistent evidence that imaging of proliferation with [18F]FLT before and after treatment with MET inhibitors may reveal the functional cross-talk between EGFR and MET occurring in the presence of a high copy number of the MET gene. The major translational relevance of this study is to provide a tool for noninvasive functional assessment of MET modulation in EGFR-driven NSCLCs that became resistant to EGFR TKIs, thus serving as an adaptive imaging biomarker for personalized therapy.

In a previous study (8), we showed that the noninvasive visualization of proliferation with 3'-deoxy-3'-[18F]-fluorothymidine ([18F]FLT) and positron emission tomography/computerized tomography (PET/CT) can successfully identify NSCLCs that are resistant due to the occurrence of T790M secondary mutation and the reversal of such resistance by last-generation EGFR TKIs such as CL-387,785 and WZ4002. The aim of this article is to test whether [18F]FLT PET/CT is able to detect MET-mediated resistance to EGFR TKIs in NSCLCs and to monitor the reversal of such resistance by MET inhibitors.

MET is a receptor tyrosine kinase, also known as the hepatocyte growth factor (HGF)/scatter factor receptor, whose aberrant activation and expression occurs in many types of cancer (9). Binding of HGF to MET causes receptor dimerization and autophosphorylation of tyrosine residues in the kinase domain that leads to the activation of downstream signaling cascades mainly mediated by the RAS–MAPK and PI3K–AKT pathways, which ultimately result in cell-cycle progression, survival, and increased cell motility (10). Furthermore, several studies reported the occurrence of signal cooperation and functional cross-talk between MET and other receptor pathways, including EGFR, ERBB2, and IGF1R (11). Engelman and colleagues (6) reported that MET amplification in lung cancer cell lines can cause EGFR TKI resistance through HER3-dependent activation of the PI3K–AKT pathway. This redundant lateral signaling allows maintaining the activation of the EGFR pathways even in the presence of EGFR inhibitors (3, 6). Interestingly, the expression of relatively high levels of MET without gene amplification or moderate MET amplification does not imply the occurrence of a functional cross-talk with EGFR (12). Furthermore, it is not clear how many copies of the MET gene are needed to cause EGFR TKI resistance in NSCLC. Therefore, to identify MET-mediated resistance to EGFR TKI, it is essential to perform a functional test revealing the existence of a cross-talk between MET and EGFR. We hypothesized that imaging of proliferation with [18F]FLT and PET/CT may functionally assess whether MET-mediated signaling can activate the EGFR pathway even in the presence of EGFR TKIs, and whether MET inhibitors by modulating downstream mediators of the EGFR pathway can overcome resistance to EGFR TKIs due to MET amplification. To this end, we selected three NSCLC cell lines with or without MET gene amplification showing different levels of MET expression and tested the effects of MET inhibitors on the EGFR pathway and proliferation both in vitro and in vivo.

Materials and Methods

Cell lines and culture conditions

NSCLC cells with or without MET gene amplification showing different levels of MET expression were obtained from and authenticated by American Type Culture Collection. In particular, H1993 cells are reported to have a high level of MET amplification (15 copy number; refs. 13, 14) that is known to confer resistance to EGFR TKIs (6). H820 cells have a low level of MET amplification (6 copy number; refs. 14, 15) and are also reported to contain an activating drug-sensitive EGFR mutation (deletion in exon 19, delE746_E749) and the drug-resistant T790M mutation, although the latter occurs at a very low frequency (7%; refs. 16, 17). HCC827 cells do not have MET amplification although MET protein is expressed on the plasma membrane of those cells and they also bear activating drug-sensitive EGFR mutation (deletion in exon 19, delE746_A750; ref. 18). All cells were grown in RPMI medium containing 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 50 μg/mL streptomycin in a humidified incubator in 5% CO2 at 37°C.

Cell treatment and toxicity assay

Drug-induced toxicity was assessed by using the MTS assay (Promega) as previously described (19). Briefly, NSCLC cells were seeded in 96-well flat-bottomed plates at a density of 5,000 to 10,000 per well and allowed to attach for 24 hours. Cells were then treated for 72 hours at 37°C with increasing concentration (range, 0.01–10 μmol/L) of PHA-665,752 (Selleck Chemicals), a selective MET inhibitor, crizotinib (PF-02341066), a dual MET and related anaplastic lymphoma kinase (ALK) inhibitor (Selleck Chemicals), erlotinib, or vehicle. After the addition of MTS, the number of viable cells was determined spectrophotometrically and expressed as a percentage of viable cells, considering the untreated control cells as 100%. At least three independent experiments were performed in triplicates and data were pooled.

RNA interference

MET-targeted siRNA pool (ON-TARGETplus SMARTpool siRNA MET) and control nontargeting siRNA pool
(scrambled) were purchased from Dharmacon, Inc. and used according to the manufacturer's instructions. Briefly, H1993 cells were plated at 40% confluence in medium supplemented with 10% FBS and allowed to attach for 24 hours. Cells were then transfected with 100 nmol/L siRNAs using Dharmafect reagent (Dharmacon). After 72 hours, cells were treated with 0.5 and 1 μmol/L erlotinib or vehicle for 6 hours and then lysed for Western blot analysis.

Antibodies and Western blotting
Whole-cell lysates were prepared as previously described (8). Briefly, cells were treated with 1 μmol/L PHA-665,752, crizotinib, or erlotinib for 6 hours and then lysed in 200 μL of a buffer containing 1 mmol/L EDTA, 1% Nonidet P-40, and protease and phosphatase inhibitors (Sigma-Aldrich). The suspension was homogenized by passages through a 26-gauge needle and centrifuged at 13,000 × g for 30 minutes at 4°C. Western blot analysis of proteins from whole-cell lysates was carried out using a standard procedure. Polyvinylidene difluoride (PVDF) membranes were probed by using monoclonal antibodies against phospho-EGFR (Cell Signaling Technology; 0.1 mg/mL), MET (25H2), Cell Signaling Technology; 0.1 mg/mL), p42/44 MAPK (Cell Signaling Technology; 0.1 mg/mL), phospho-p42/44 MAPK (Thr202/Tyr204; Cell Signaling Technology; 0.1 mg/mL), actin (Sigma; 1 μg/mL), α-tubulin (Sigma; 1 μg/mL), and rabbit polyclonal antibodies against EGFR (Santa Cruz Biotechnology; 1:1,000), phospho-AKT (Ser473; Santa Cruz Biotechnology; 1:1,000), phospho-HER3 (Tyr-1289; Cell Signaling Technology; 1:1,000), phospho-MET (Tyr 1234/1235; Cell Signaling Technology; 1:1,000), HER3 (Santa Cruz Biotechnology; 1:500), AKT (Cell Signaling Technology; 1:1,000), and cyclin D1 (Cell Signaling Technology; 1:1,000).

Animal tumor models and treatment
Female BALB/c (nu/nu) mice, 6-week-old, weighing 15 to 20 g were purchased from Charles River Laboratories. All animal experimental procedures were approved by the Italian Ministry of Health-Animal Welfare Direction (Protocol No. 22813-A-24/12/2010). H1993 and HCC827 cells (5–10 × 10⁶) were resuspended in 200-μL RPMI medium and injected s.c. into the flank of nude mice. Cells were then allowed to grow for 2 weeks and when tumors reached a mean volume of approximately 100 mm³ [volume = 0.5 × greatest diameter × (shortest diameter)²] animals were randomized into treatment groups (of at least 4 animals for each cell line, for each treatment and dose) and subjected to imaging studies or long-term tumor response assessment. For imaging studies, tumor-bearing animals were treated daily for 3 days by oral gavage with 50 and 100 mg/kg crizotinib (PF-02341066) or erlotinib. PHA-665,752, despite being a selective MET inhibitor, could not be used for in vitro treatment due to its poor pharmaceutical properties and oral bioavailability (20). Crizotinib was indeed designed from the structure of PHA-665,752 (20) and, having a high affinity for MET and ALK (21), is currently under investigation in clinical trials (9, 22). For the assessment of long-term tumor response and for longitudinal imaging studies, tumor-bearing animals were subjected to treatment with 100 mg/kg/d crizotinib or erlotinib from days 0 to 9 and tumor volume was measured daily by a caliper.

Imaging studies with [18F]FLT and small-animal PET/CT
Each animal underwent a baseline and a posttreatment scan. The baseline scan on day 0 was performed 3 hours before any treatment, whereas the posttreatment scan on day 2 was performed 3 hours after the last drug administration. The whole synthesis of [18F]FLT was performed using the commercially available TRACERlab FX F-N synthesis module (GE Healthcare). Briefly, (5′-O-DMT-2′-deoxy-3′-O-nosyl-β-D-threo-pentofuranosyl)-3-N-BOC-thymine was used as precursor and subjected to radiofluorination according to a previously described procedure with slight modifications (23). The resulting labeled products had >99% radiochemical purity as assessed by high-performance liquid chromatography.

A small-animal PET/CT scanner (eXplore Vista Pre-Clinical PET Scanner GE Healthcare) was used for PET/CT studies. Briefly, animals were i.v. injected with 7.4 MBq of [18F]FLT and after 50 minutes were anesthetized and firstly subjected to CT scan for 10 minutes. One bed position including the tumor was scanned (axial field of view, 68 mm), and images were acquired with the X-ray source set at 35 kVp and 200 μA. PET images were then acquired at 1 hour after injection for a PET acquisition time of 20 minutes. Body temperature of animals was held constant during tracer biodistribution and imaging using a heating pad or heat lamp. After acquisition, the images were reconstructed by a combined algorithm based on Fourier rebinning (FORE) followed by 2D iterative image reconstruction using ordered-subsets expectation maximization (OSEM). PET and CT images were automatically coregistered and fused images were obtained. PET images were corrected for decay and converted to standardized uptake value (SUV). No statistically significant change of animal weight was observed after treatment. Three-dimensional regions of interest were drawn around the tumor on transaxial PET images of the baseline and posttreatment scans and a volume of interest was determined using an automated isocounting program (8, 24). The maximum voxel value of SUV (SUVmax) within the tumor volume of interest was then registered for each study. Biodistribution study in a subgroup of untreated animals (n = 7) showed that SUVmax values were indeed significantly correlated to %ID/g of tumor determined by gamma counting (r = 0.97; P = 0.0003; data not shown). Therefore SUVmax values were used to calculate the percentage change of [18F]FLT uptake in the posttreatment study as compared with baseline scan in each animal. Two additional subgroups of H1993 tumor-bearing animals underwent longitudinal imaging studies and scans were repeated after 6 and 9 days of treatment with crizotinib or erlotinib (100 mg/kg/d). All quantitative data from animal imaging studies were expressed as mean ± SE.
Levels of EGFR signaling mediators and rate of proliferation in tumor xenografts

At the end of imaging studies, tumors were surgically removed, immediately frozen in liquid nitrogen, and stored at −80°C until studied.

Tumor samples obtained from animals treated for 3 days with 100 mg/kg crizotinib or erlotinib were homogenized on ice in RIPA lysis buffer containing 50 mmol/L Tris–HCl pH 7.5, 150 mmol/L NaCl, 1% NP40, 0.1% SDS, 0.5% sodium deoxycholate, protease, and phosphatase inhibitors (Sigma-Aldrich) using a dounce homogenizer followed by passages through a 26-gauge needle. The suspension was clarified by centrifugation at 13,000 × g for 30 minutes at 4°C and subjected to Western blot analysis.

The rate of proliferation was determined by Ki67 immunostaining of tumor section. Briefly, 10 consecutive 5-μm adjacent sections were cut in a cryomicrotome corresponding to the largest cross-sectional area of the tumor. The rate of proliferation of tumor cells was evaluated using the rabbit polyclonal antibody directed against Ki67 antigen (Abcam) dilution at 1:100 and goat polyclonal secondary antibody to rabbit IgG-HRP dilution at 1:1,000. Tumor sections were immunostained using a standard procedure, with diaminobenzidine used as chromogen, and then counterstained with hematoxylin.

After mounting cover slips, tumor sections were examined by light microscopy at ×400 magnification. The observer was not aware of the treatment group or [18F]FLT PET/CT findings of the particular specimen examined. Each section was divided in 4 to 10 regions randomly selected along the two major diameters. A minimum of 100 tumor cells were counted in every region and the results expressed as the mean percentage of positively stained tumor cells in a section (8, 25). Data from treated tumors (three for each cell line and for each treatment) were averaged and compared with data from untreated tumors (n = 3) by an unpaired Student t test.

Statistical analysis

Statistical analyses were done using the software MedCalc for Windows, version 10.3.2.0, (MedCalc Software). Unpaired and paired Student t tests were used when appropriate to compare means. In particular, a paired t test was
used to compare $[^{18}F]$FLT uptake in the same tumors before and after treatment, whereas an unpaired $t$ test was used to test differences between untreated controls and treated groups with respect to quantitative histopathologic data and toxicity assays. Differences between means were considered statistically significant for $P < 0.05$, indicated by the symbol *, and highly statistically significant for $P < 0.01$, indicated by the symbol **.

Results

Levels of MET expression in the three selected NSCLC cell lines are shown in Fig. 1A. The relative highest levels of MET and phospho-MET were found in H1993 cells containing 15 copies of the MET gene. H820 cells, containing six copies of the MET gene, showed considerable levels of MET and phospho-MET proteins that were lower than those found in H1993 cells and similar to those expressed by HCC827 cells lacking MET amplification.

The sensitivity of H1993, H820, and HCC827 cells to increasing concentrations of erlotinib, PHA-665,752, and crizotinib was preliminarily tested by MTS assay and the results are shown in Fig. 1B–D. As expected, H1993 were the most resistant cells to erlotinib, whereas HCC827 cells showed the highest responsiveness to the same drug. Furthermore, an intermediate grade of resistance to erlotinib was observed in H820 cells. Conversely, the highest sensitivity to MET inhibitors was found in H1993 cells, whereas HCC827 failed to respond to both PHA-665,752 and crizotinib treatment and H820 cells showed sensitivity to MET inhibitors only at high concentrations of the drug.

To test whether MET inhibitors were able to block the signaling pathway downstream the EGFR in a HER3-dependent manner, whole-cell lysates from untreated and treated cells were analyzed by Western blotting (Fig. 2A and B). All cell lines treated with PHA-665,752 showed a dramatic decrease of phospho-MET as compared with the corresponding untreated cells but only H1993 cells showed a concomitant decrease of phospho-HER3, phospho-AKT, phospho-ERK, and cyclin D1. Also, a strong inhibition of the EGFR pathway was observed in HCC827 cells when treated with erlotinib and not with MET inhibitor (Fig. 2A and B). Conversely, H820 cells, despite the reduction of phospho-MET and phospho-HER3 levels, did not show a significant concomitant decrease of phospho-AKT,

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Modulation of EGFR signaling by EGFR and MET inhibitors. A, representative Western blot analyses of samples obtained from NSCLC cells exposed to 1 $\mu$mol/L erlotinib or PHA-665,752 for 6 hours. Levels of total and phosphorylated forms of EGFR, MET, and HER3. B, representative Western blot analyses of samples obtained as described in A showing the levels of EGFR downstream signaling mediators. Protein samples obtained from untreated and treated cells were separated by SDS-PAGE and immunoblotted with specific antibodies (see Materials and Methods). Tubulin served to ensure equal loading.
phospho-ERK, and cyclin D1 in response to 1 μmol/L PHA-665,752, whereas EGFR signaling was weakly inhibited by 1 μmol/L erlotinib.

To test whether the resistance of H1993 cells to erlotinib was MET mediated, these cells were transfected with MET-targeted siRNA pool or control nontargeting siRNA pool and then were exposed to 0.5 and 1 μmol/L erlotinib for 6 hours. Figure 3A shows that downregulation of MET levels by MET-targeted siRNA transfection of H1993 cells results in a strong reduction of cyclin D1 levels only after treatment with erlotinib.

H1993 and HCC827 cells were then selected for in vivo imaging studies with [18F]FLT PET/CT with the aim to detect MET-mediated resistance to erlotinib and to monitor the reversal of such resistance by MET inhibitors. Furthermore, due to its poor pharmaceutical properties and oral bioavailability, PHA-665,752 could not be used for in vivo treatment, and tumor-bearing animals received crizotinib and were compared with erlotinib-treated animals. Figure 3B shows that the effects of crizotinib on MET phosphorylation and cyclin D1 levels were similar to those induced by PHA-665,752 in H1993 and HCC827 cells. Furthermore, long-term tumor response to crizotinib and erlotinib was preliminarily tested in animals bearing H1993 xenografts (Fig. 3C). Tumor volume was stably reduced by 50% in response to crizotinib treatment, whereas it increased up to 2.6- and 4-folds after treatment with erlotinib or vehicle, respectively.

Then nude mice bearing H1993 and HCC827 xenografts were studied with [18F]FLT PET/CT before and after treatment. All tumors showed increased [18F]FLT uptake at the basal scan, whereas tracer uptake was reduced in H1993 xenografts after treatment with crizotinib in a dose-dependent manner (Fig. 4A and B) and remained persistently high after treatment with erlotinib (Fig. 4C). Conversely, HCC827 tumors showed no significant changes of [18F]FLT uptake after high-dose treatment with crizotinib (Fig. 5A) but tracer uptake was significantly reduced after treatment with erlotinib (Fig. 5B).

Quantitative analysis of percentage variations of [18F]FLT uptake in PET/CT studies performed after 3 days of treatment and compared with the corresponding baseline scans is shown in Fig. 6A. In the group of H1993 tumor-bearing animals treated with 50 mg/kg crizotinib, the mean SUVmax value was significantly reduced from 2.80 ± 0.43 at the baseline scan to 2.03 ± 0.24 (P < 0.05) in posttreatment scan, corresponding to a percentage reduction of 28%. After treatment with 100 mg/kg crizotinib, we observed a significant reduction of mean SUVmax value from 2.96 ± 0.07 to 1.76 ± 0.18 (P < 0.01), corresponding to a percentage reduction of 41%. Conversely, no significant reduction of tracer uptake was observed in response to low- (P = 0.85) and high-dose (P = 0.65) treatment with erlotinib. In the group of HCC827 tumor-bearing animals, the mean SUVmax value was significantly reduced from 2.96 ± 0.07 to 1.76 ± 0.18 (P < 0.01), corresponding to a percentage reduction of 41%. Conversely, no significant reduction of tracer uptake was observed in response to low- (P = 0.85) and high-dose (P = 0.65) treatment with erlotinib.

In the group of H1993 xenografts
levels of p-AKT, p-ERK 1/2, and cyclin D1 were dramatically reduced in H1993 tumors in response to crizotinib (100 mg/kg for 3 days) as compared with tumors from animals treated with erlotinib (100 mg/kg for 3 days). Conversely in HCC827 tumors, the levels of p-AKT, p-ERK 1/2, and cyclin D1 were strongly reduced after treatment with erlotinib (100 mg/kg for 3 days) as compared with tumors from animals treated with crizotinib (100 mg/kg for 3 days).

Figure 6D shows the rate of proliferation in tumors before and after 3 days of treatment. H1993 cells showed a statistically significant reduction of the rate of proliferation after low- (P = 0.01) and high-dose (P < 0.01) treatment with crizotinib, whereas no significant change was observed in response to erlotinib treatment at low-dose (P = 0.18) and high-dose (P = 0.65) regimens. Conversely, the rate of proliferation was significantly reduced in HCC827 cells after low- (P < 0.01) and high-dose (P < 0.01) treatment with erlotinib but it remained unchanged in response to crizotinib at low (P = 0.86) and high dosage (P = 0.48).

Discussion

Our study showed that [18F]FLT PET/CT is able to detect MET-mediated resistance to EGFR TKIs in NSCLC and to monitor the reversal of such resistance by MET inhibitors. The persistently high uptake of [18F]FLT in H1993 xenografts after treatment with erlotinib identified resistance to...
EGFR TKIs, whereas the significant reduction of tracer uptake in the same tumors after treatment with MET inhibitors indicated the reversal of MET-mediated resistance to EGFR TKIs. Conversely, HCC827 xenografts that do not bear MET amplification, despite the expression of relatively high levels of MET, do not undergo growth arrest in response to treatment with MET inhibitors as shown by unchanged [18F]FLT uptake in posttreatment imaging studies and by Ki67 immunostaining.

MET overexpression resulting from transcriptional upregulation in the absence of gene aberrations has been found in a variety of epithelial malignant tumors, including NSCLC (26), and its constitutive activation has been reported to promote cell growth, invasion, and metastatic dissemination (9). An additional mechanism causing MET constitutive activation is gene amplification that has been observed in different human cancers, including NSCLC, where the increased gene copy number is an independent negative prognostic factor (12). Engelman and colleagues (3, 6) reported that MET amplification in EGFR-mutant NSCLC cells can cause resistance to EGFR TKIs by maintaining activation of the EGFR downstream pathway in the presence of EGFR inhibitors. MET amplification has been indeed confirmed in tumors of about 15% to 20% of patients with NSCLC refractory to erlotinib treatment (7, 27); but, at present, the copy number of the MET gene necessary to cause such resistance is undefined.

Therefore, we reasoned that in NSCLCs bearing a high copy number of the gene, MET inhibition would have resulted in EGFR downstream pathway inhibition and decreased proliferation. Our study showed indeed that noninvasive molecular imaging, by monitoring in vivo the rate of proliferation in response to MET inhibitors, can identify the functional cross-talk between EGFR and MET occurring in
the presence of a high copy number of the MET gene. It can be argued that MET inhibitors may modulate proliferation independently of the EGFR signaling pathway but this is not the case of patients with EGFR-driven NSCLC bearing activating mutations of EGFR. Our study showed indeed that proliferation cannot be modulated by MET inhibitors in HCC827 tumors expressing MET but showing a high responsiveness to EGFR TKIs due to the expression of mutant EGFR.

A number of MET-targeting agents are currently under evaluation in clinical trials either alone or in combination with conventional or molecularly targeted agents (28). In these studies, patient stratification according to MET expression, amplification, and activity is an essential requirement to ensure clinical benefit. However, in patients with refractory NSCLC, methods for assessing MET levels, gene copy number, or phosphorylation may require repeated tumor biopsy samples that cannot be easily obtained in all patients. The noninvasive detection of MET-mediated resistance to EGFR TKIs by $[^{18}F]$FLT PET/CT may be helpful for stratification of patients by identifying those that will benefit from treatment with MET inhibitors either alone or in combination with EGFR TKIs. $[^{18}F]$FLT PET/CT has been evaluated as a noninvasive imaging method to discriminate responders from nonresponders to MET-targeted therapy (29). In particular, $[^{18}F]$FLT PET/CT scans performed before and 7 days after gefitinib treatment in patients with advanced lung adenocarcinoma showed that the percentage changes of $[^{18}F]$FLT uptake were significantly different in responders from nonresponders (0.001).

Recently, simultaneous targeting of EGFR and MET by WZ4002 and crizotinib, respectively, has been reported to be effective in lung cancers bearing T790M mutation and concurrent MET amplification (30). We have previously demonstrated that $[^{18}F]$FLT PET/CT is able to detect T790M-mediated resistance to erlotinib and its reversal by WZ4002 treatment (8). Here, we showed that $[^{18}F]$FLT PET/CT may also identify tumors with a functional cross-talk between EGFR and MET. Because up to one third of patients with NSCLC who become refractory to reversible EGFR TKIs have tumors with concurrent T790M mutation and moderate MET amplification (7, 15); functional imaging with $[^{18}F]$FLT PET/CT may contribute to select the optimal treatment regimen in those patients by providing an adaptive imaging biomarker for therapy (31).

In conclusion, $[^{18}F]$FLT PET/CT is able to detect MET-mediated resistance to EGFR TKIs and to monitor the reversal of such resistance by MET inhibitors. Current clinical guidelines for treatment of advanced NSCLC recommend that newly diagnosed patients are assigned to specific first-line therapy on the basis of genetic profiling and mutational analysis of tumor samples (33). Our study indicates that $[^{18}F]$FLT PET/CT may be successfully used to identify during first-line therapy with EGFR TKIs patients who become refractory and to select those who may benefit from treatment with MET inhibitors either alone or in combination with EGFR TKIs, thus contributing to personalized therapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: F. Iommelli, S. Del Vecchio
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Grant Support
This work was partly supported by Ministry of University and Research, MERIT—Medical Research in Italy (project No. RBNE08/VN3_008) and AIRC, Associazione Italiana per la Ricerca sul Cancro (project No.11756).

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Received January 31, 2014; revised June 19, 2014; accepted July 10, 2014; published OnlineFirst July 22, 2014.

References


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