

Differences in the Biologic Activity of 2 Novel MEK Inhibitors Revealed by ^{18}F -FDG PET: Analysis of Imaging Data from 2 Phase I Trials

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Two mitogen-activated protein kinase kinase (MAPK2, also known as MEK) inhibitors were assessed with ^{18}F -FDG PET in separate phase I clinical studies, clearly illustrating the potential of metabolic imaging for dose, dosing regimen, and compound selection in early-phase trials and utility for predicting nonresponding patients. **Methods:** ^{18}F -FDG PET data were collected during 2 independent, phase I, dose-escalation trials of 2 novel MEK inhibitors (RO5126766 and RO4987655). PET acquisition procedures were standardized between the 2 trials, and PET images were analyzed centrally. Imaging was performed at baseline; at cycle 1, day 15; and at cycle 3, day 1. A 10-mm-diameter region of interest was defined for up to 5 lesions, and peak standardized uptake values were determined for each lesion. The relationship between PET response and pharmacokinetic factors (dose and exposure), inhibition of extracellular-signal-regulated kinase (ERK) phosphorylation in peripheral blood mononuclear cells, and anatomic tumor response as measured by Response Evaluation Criteria in Solid Tumors was investigated for both compounds. **Results:** Seventy-six patients underwent PET, and 205 individual PET scans were analyzed. Strong evidence of biologic activity was seen as early as cycle 1, day 15, for both compounds. ^{18}F -FDG PET revealed striking differences between the 2 MEK inhibitors at their recommended dose for phase II investigation. The mean amplitude of the decrease in ^{18}F -FDG from baseline to cycle 1, day 15, was greater for patients receiving RO4987655 than for those receiving RO5126766 (47% vs. 16%, respectively; $P = 0.052$). Furthermore, a more pronounced relationship was seen between the change in ^{18}F -FDG uptake and dose or exposure and phosphorylated ERK inhibition in peripheral blood mononuclear cells in patients receiving RO4987655. For both investigational drugs, PET responses tended to be greatest in

patients with melanoma tumors. ^{18}F -FDG was able to identify early nonresponding patients with a 97% negative predictive value. **Conclusion:** These data exemplify the role of ^{18}F -FDG PET for guiding the selection of novel investigational drugs, choosing dose in early-phase clinical development, and predicting nonresponding patients early in treatment.

Key Words: ^{18}F -FDG PET; MEK inhibitor; biomarker

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The use of PET in the early stages of drug development is becoming increasingly popular. PET assessment of directly radiolabeled drugs has been used to determine drug biodistribution and receptor occupancy and can aid patient selection by identifying those expressing a particular molecular target (1,2). Microdosing studies allow the visualization of drug disposition in healthy volunteers using subpharmacologic doses (3), possibly permitting the early identification of those drug candidates who show the potential for activity and the early exclusion of those who do not. Thus, these studies could conceivably save clinical development costs. ^{18}F -FDG PET—because it is an established tool in clinical practice in oncology (4)—is easily implemented in clinical studies to identify proof of biologic activity.

Past generations of chemotherapeutics were cytotoxic and therefore well suited to traditional response evaluation based on morphologic changes. In contrast, many novel targeted therapies are not cytoreductive but instead work by inducing senescence or cell cycle arrest, resulting in disease stabilization that translates into long-term benefit in many tumor types (5,6). However, early-phase trials of novel, targeted, noncytotoxic compounds still tend to use anatomic response rates as endpoints, with dose escalation

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typically based on toxicity and pharmacokinetic endpoints. Although pharmacodynamic assessments are increasingly common components of phase I trials, they rarely influence the selection of the recommended dose for phase II trials (RP2D).

Mitogen-activated protein kinase kinase (MAPK2, also known as MEK) is a promising target for anticancer therapies because of its central position within the RAF-MEK-ERK signaling pathway, which communicates the signal from a variety of cell surface receptors to the nucleus of the cell (7). Dysregulation of this pathway is common in human cancers and affects multiple pathways involved in differentiation and proliferation. As the only known kinase capable of activating the downstream extracellular signal-regulated kinase (ERK), inhibition of MEK can potentially inhibit multiple downstream oncogenic signals. In addition, proof of mechanism for MEK inhibitors can readily be determined by measuring the level of phosphorylated ERK (pERK), either directly in tumor biopsy specimens or indirectly in surrogate tissues such as peripheral blood mononuclear cells (PBMCs).

We hypothesized that ^{18}F -FDG PET could be used as an early pharmacodynamic biomarker to distinguish the biologic effects of different doses of investigational new drugs and to guide the selection of appropriate dose and regimens for investigation in phase II trials. To test this hypothesis, we compared ^{18}F -FDG PET data from 2 phase I, dose-escalation trials of 2 novel inhibitors of MEK in patients with advanced solid tumors and correlated findings with various endpoints including pharmacokinetics, efficacy, and pharmacodynamic biomarkers of activity.

In clinical practice, it is of particular importance to identify patients who are not responding to treatment early, thereby avoiding exposure to ineffective therapy and associated adverse events and costs. Taking this into consideration, we investigated the ability of ^{18}F -FDG PET to predict the absence of response early and identify patients who are underdosed or who will fail to benefit from the projected phase II dosing regimen.

MATERIALS AND METHODS

Study Participants

Eligible subjects were identified from patients enrolled in 2 phase I, open-label, multicenter dose-escalation trials of 2 novel MEK inhibitor molecules (RO5126766 and RO4987655; F. Hoffmann–La Roche Ltd.) (8,9). Patients recruited to both trials had advanced or metastatic solid tumors of any type, which were not amenable to standard therapy. Inclusion criteria for ^{18}F -FDG PET mandated that patients have at least 1 tumor measuring 2 cm or more, a fasting serum glucose of 180 mg/dL or less, and the ability to lie still in the scanner for more than 45 min. Patients with diabetes mellitus or with a fasting glucose greater than 180 mg/dL were not scanned with ^{18}F -FDG PET. PET was performed at 7 study sites, using the following PET/CT equipment: Discovery 690 and Discovery ST (GE Healthcare); Biograph 1 LSO and Biograph mCT (Siemens); and Gemini GXL 16, GS, and TF (Philips). Written informed consent was obtained from all

patients, and the study was approved by multiple institutional review boards and conducted in accordance with good clinical practice.

Study Drug Dose-Escalation

In study NO21895 (NCT00773526), the dual Raf and MEK inhibitor RO5126766 was administered orally once daily for 28 d in 4-wk cycles. Doses were escalated according to an accelerated titration design from 0.1 to 2.7 mg once daily. Two intermittent treatment regimens were also assessed, 7 d on/7 d off (7/7 regimen; from 2.7 to 5.0 mg) and 4 d on/3 d off (4/3 regimen; from 2.7 to 4.0 mg).

In study BO21189 (NCT00817518), the MEK inhibitor RO4987655 was administered orally for 28 d. A classic 3 + 3 dose-escalation design was used to investigate once-daily (1.0 escalating to 2.5 mg) and twice-daily (3.0 escalating to 21.0 mg total daily dose) dosing regimens.

Treatment in both trials was administered until disease progression, unacceptable toxicity, or patient refusal, whichever occurred first. Any patient withdrawn from either trial was excluded from further PET analysis.

On the basis of the safety and efficacy data from these 2 trials, the RP2D was established as 2.7 mg once daily (4/3) for RO5126766 and 8.5 mg twice daily (total daily dose, 17 mg) for RO4987655 (8,9).

^{18}F -FDG PET Procedure

All PET was performed in accordance with consensus recommendations for the use of ^{18}F -FDG PET in National Cancer Institute trials (10). ^{18}F -FDG PET was performed at baseline; at cycle 1, day 15 (+5 d), for all patients; and again at cycle 3, day 1 (+5 d), in those patients who had not progressed according to Response Evaluation Criteria in Solid Tumors (RECIST) assessment. In a small number of patients (4/76), at the site's discretion, additional ^{18}F -FDG PET scans were obtained at later time points. Patients fasted for a minimum of 6 h before tracer administration and were required to have serum glucose levels of 180 mg/dL or less before undergoing scanning. PET scans covering the area from the base of the skull to the mid thigh (vertex to toe for melanoma patients) were acquired at 60 min (± 10 min) after the intravenous injection of an individualized activity of ^{18}F -FDG (5.2–7.8 MBq/kg to give a final activity of 300–600 MBq). Administered activity was dependent on local practice and scanner type, and the activity administered for all PET scans was kept within 10% of the calculated activity prescribed for each patient at baseline. Patients were positioned, and PET was performed according to local procedures, with the same method used for both baseline and follow-up scans in individual patients. Particular emphasis was placed on using the same PET/CT equipment for each patient and on maintaining the same conditions for uptake time and scanning time. Scans were obtained in either 2-dimensional or 3-dimensional mode, and low-dose attenuation CT (typically ≤ 80 mAs) was performed for all PET scans for attenuation correction. Images were reconstructed with full correction for attenuation, scatter, and randoms, with the reconstruction algorithm adjusted to the acquisition mode and local guidelines. If the tumor-to-surrounding background ratio of the baseline scan was less than 2, follow-up scans were not obtained and the patient was excluded.

PET Data Processing and Image Analysis

All PET images were analyzed centrally by an independent nuclear medicine specialist masked to the clinical data. All images

were analyzed using the same software (Leonardo; Siemens). Up to 5 lesions with maximal focal ^{18}F -FDG uptake were visually selected for quantitative analysis at baseline. A circular region of interest (ROI) with a 10-mm diameter, centered on maximum standardized uptake value (SUVmax), was used to define peak SUV (SUVpeak) (11). All lesions selected at baseline were assessed at follow-up visits. Lesions present at baseline but not selected for SUV measurement were assessed qualitatively for a change in ^{18}F -FDG intensity and extent. Lesions detected at follow-up but not present at baseline were recorded as new lesions.

Metabolic Response (MR) Assessment

PET response or nonresponse was classified using the guidelines of the European Organization for Research and Treatment of Cancer (12). MR was classified in relation to the percentage change from baseline in the sum of the SUVpeak for up to 5 lesion values for each individual patient.

Complete MR was defined as the complete resolution of ^{18}F -FDG uptake in all lesions, which becomes indistinguishable from surrounding normal tissue.

Partial MR (PMR) was defined as a reduction of 15% or more in the sum of the SUVpeak after 1 cycle of therapy and greater than 25% after more than 1 treatment cycle, with no new lesions detected and no progression of those lesions not selected for SUVpeak measurement. A reduction in the extent of tumor ^{18}F -FDG uptake was not necessary for PMR.

Progressive metabolic disease was defined as any of the following: a greater than 25% increase, compared with baseline, in the sum of the SUVpeak for lesions selected for measurement in any follow-up scan; at least 1 individual lesion showing a greater than 25% increase, compared with baseline, in SUVpeak and greater than 1.0 in absolute value; a visible and unequivocal increase in the extent of ^{18}F -FDG uptake (20% in the longest dimension); a significant increase in ^{18}F -FDG intensity or extent in lesions not selected for SUVpeak measurement; or the appearance of a new ^{18}F -FDG-avid lesion.

Stable metabolic disease was defined as an increase in tumor ^{18}F -FDG uptake not sufficient for progressive metabolic disease or a decrease not sufficient for PMR.

Correlation of ^{18}F -FDG Uptake with Pharmacokinetic and Pharmacodynamic Factors and RECIST Morphologic Response

The relationship between efficacy as measured by ^{18}F -FDG PET (i.e., the change in SUVpeak from baseline to cycle 1, day 15, or to cycle 3, day 1) and pharmacokinetic parameters, inhibition of ERK phosphorylation in PBMCs, and clinical benefit as measured by RECIST 1.0 was investigated.

Detailed pharmacokinetic sampling was performed at cycle 1, day 15 (before the dose and at 1, 3, 7, and 12 (± 2) hours after the dose in the study with RO4987655 and before the dose and 0.5, 1, 2, 4, 8, 12, and 24 h after the dose in the study with RO5126766), and study-drug exposure was estimated using the area under the time-concentration curve.

Blood samples for the assessment of ERK phosphorylation in PBMCs were collected alongside those for pharmacokinetics. Target inhibition of 4 β -phorbol 12-myristate 13-acetate-induced pERK in PBMCs was assessed using flow cytometry, and pERK inhibition was calculated as the percentage decrease in mean fluorescent intensity between the pre- and postdose samples.

Tumors were assessed according to RECIST 1.0 criteria (13) using either CT or MRI at the baseline evaluation performed after inclusion in the clinical trial and every 8 wk (± 3 d) on day 1 of the cycle, beginning at cycle 3.

Statistical Considerations

The positive predictive value (PPV) and negative predictive value (NPV) of ^{18}F -FDG PET, as well as the sensitivity and specificity, were evaluated. For this purpose, patients were categorized according to their metabolic response (complete MR + PMR vs. stable metabolic disease + progressive metabolic disease) and their RECIST response (complete response + partial response [PR] vs. stable disease + progressive disease). A confidence interval (CI) was obtained using the Clopper-Pearson (exact) method.

Relationships between the change from baseline in SUVpeak and dose, change from baseline in the longest tumor diameter, and pERK inhibition were investigated by linear regression using Mathematica (Wolfram Research). One- and 2-sided *t* tests (when appropriate) were used to investigate differences in ^{18}F -FDG decreases at cycle 1, day 15, and at cycle 3, day 1, and between the 2 MEK inhibitors at their respective R2PD. χ^2 tests were used to compare the frequencies of PMRs at the R2PD between the 2 MEK inhibitors. A *P* value of less than 0.05 was considered significant.

RESULTS

Patient Characteristics

One hundred and one patients were enrolled in 2 phase I trials; 52 patients received the dual Raf/MEK inhibitor RO5126766 in study NO21895, and 49 patients received the MEK inhibitor RO4987655 in study BO21189. Seventy-six patients who received the dual Raf and MEK inhibitor RO5126766 in study NO21895 (*n* = 42) or the MEK inhibitor RO4987655 in study BO21189 (*n* = 34) were scanned with ^{18}F -FDG PET. The baseline characteristics of those patients are detailed in Table 1. Almost half of the patients scanned with ^{18}F -FDG PET were patients with melanoma (33/75 [44%]). All patients showed at least 1 lesion with a tumor-to-surrounding background SUV ratio of the baseline scan of at least 2. The median number of lesions measured with SUVpeak per patient was 5 (mean, 4.6; range, 1–5). In total, 205 individual PET scans were analyzed (baseline: *n* = 76; cycle 1, day 15: *n* = 75; cycle 3, day 1: *n* = 48; after cycle 3, day 1: *n* = 6). For 1 patient, the baseline and follow-up scans were obtained using different PET camera systems; therefore, SUVpeak was not evaluated in this patient and the patient was excluded from further analysis. Four patients on study NO21895 received RO5126766, and 8 patients on study BO21189 received RO4987655, at the RP2D.

Metabolic Tumor Response

Metabolic response was evaluable in 75 patients at cycle 1, day 15, and in 48 patients at cycle 3, day 1 (Table 2). Figure 1A shows an example of ^{18}F -FDG PET/CT images obtained at baseline; cycle 1, day 15; and cycle 3, day 1, in a patient who was treated with 4.0 mg of RO5126766 on the 4/3 regimen. PET/CT suggested partial metabolic response

TABLE 1
Baseline Demographics of Study Subjects (Evaluable Patients)

| Demographic | Total patients (<i>n</i> = 75) | Study NO21895 (RO5126766) (<i>n</i> = 41) | Study BO21189 (RO4987655) (<i>n</i> = 34) |
|--|------------------------------------|---|---|
| Male (<i>n</i>) | 49 (65.3) | 27 (65.9) | 22 (64.7) |
| Age (y) | | | |
| Median | 52 | 52 | 50 |
| Range | 24–79 | 24–74 | 27–79 |
| Height (cm) | | | |
| Median | 174 | 171 | 180 |
| Range | 150–196 | 150–196 | 150–192 |
| Weight (kg) | | | |
| Median | 76 | 75 | 77 |
| Range | 44–128 | 52–128 | 44–119 |
| Baseline Eastern Cooperative Oncology Group performance status (<i>n</i>) | | | |
| 0 | 33 (44.0) | 18 (43.9) | 15 (44.1) |
| 1 | 42 (56.0) | 23 (56.1) | 19 (55.9) |
| Primary cancer site (<i>n</i>) | | | |
| Melanoma | 33 (44.0) | 17 (41.5) | 16 (47.1) |
| Colon or rectum | 18 (24.0) | 8 (19.5) | 10 (29.4) |
| Lung | 4 (5.3) | 2 (4.9) | 2 (5.9) |
| Ovary | 4 (5.3) | 2 (4.9) | 2 (5.9) |
| Other | 16 (21.3) | 12 (29.3) | 4 (11.8) |

Patients on both studies had received median of 3 prior anticancer therapies (BO21189: range, 1–9; NO21895: range, 1–11). Data in parentheses are percentages.

for this patient, in contrast to RECIST, which returned stable disease; these results reflect the low PPV for PET seen in this study when RECIST response is considered as the method of reference.

Figure 1B shows an example of ^{18}F -FDG PET/CT images at baseline (upper); at cycle 1, day 15 (middle); and at cycle 3, day 1 (lower), in a patient with colon carcinoma treated with RO4987655 (17 mg/day given as a twice-daily administration [8.5 mg by mouth, twice-daily regimen]). This case reflects the high negative predictive value of PET because this patient showed a metabolic progression at cycle 1 on day15, which was later confirmed to progress using RECIST.

A decrease in ^{18}F -FDG uptake by cycle 1, day 15, was seen in 29 of 41 (71%) patients treated with RO5126766

and 27/34 (79%) patients treated with RO4987655 (Fig. 2). The change from baseline (CB) in SUVpeak at cycle 1, day 15 (CB_{C1D15}), and cycle 3, day 1 (CB_{C3D1}), remained relatively stable in patients receiving RO5126766 (mean CB_{C1D15} , 0.84; mean CB_{C3D1} , 0.77; 1-sided *t* test, *P* = 0.25) (Fig. 2A); conversely, in most of the patients receiving RO4987655, the ^{18}F -FDG response appeared to subside between the first and second follow-up assessments (mean CB_{C1D15} , 0.67; mean CB_{C3D1} , 0.86; 1-sided *t* test, *P* = 0.061) (Fig. 2B). This phenomenon was not related to dose or treatment.

The 2 drugs appeared to exhibit a different ^{18}F -FDG response at the RP2D in the overall tumor population and in melanoma tumors alone. Within the limitations of a small sample size, we observed a markedly larger mean reduction

TABLE 2
Metabolic Response at Cycle 1, Day 15, and Cycle 3, Day 1, and Best Overall Response According to RECIST

| Response | Metabolic response (%) | | RECIST response (%) | Best overall response (<i>n</i> = 71)* |
|-------------------------------|-------------------------------------|------------------------------------|------------------------|--|
| | Cycle 1, day 15 (<i>n</i> = 75) | Cycle 3, day 1 (<i>n</i> = 48) | | |
| Complete MR | 1 | 0 | Complete response | 0 |
| PMR | 33 | 5 | PR | 5 |
| Stable metabolic disease | 6 | 2 | Stable disease | 13 |
| Progressive metabolic disease | 35 | 41 | Progressive disease | 53 |

*Four patients were withdrawn from trial because of disease progression before first scheduled RECIST assessment at cycle 3, day 1.

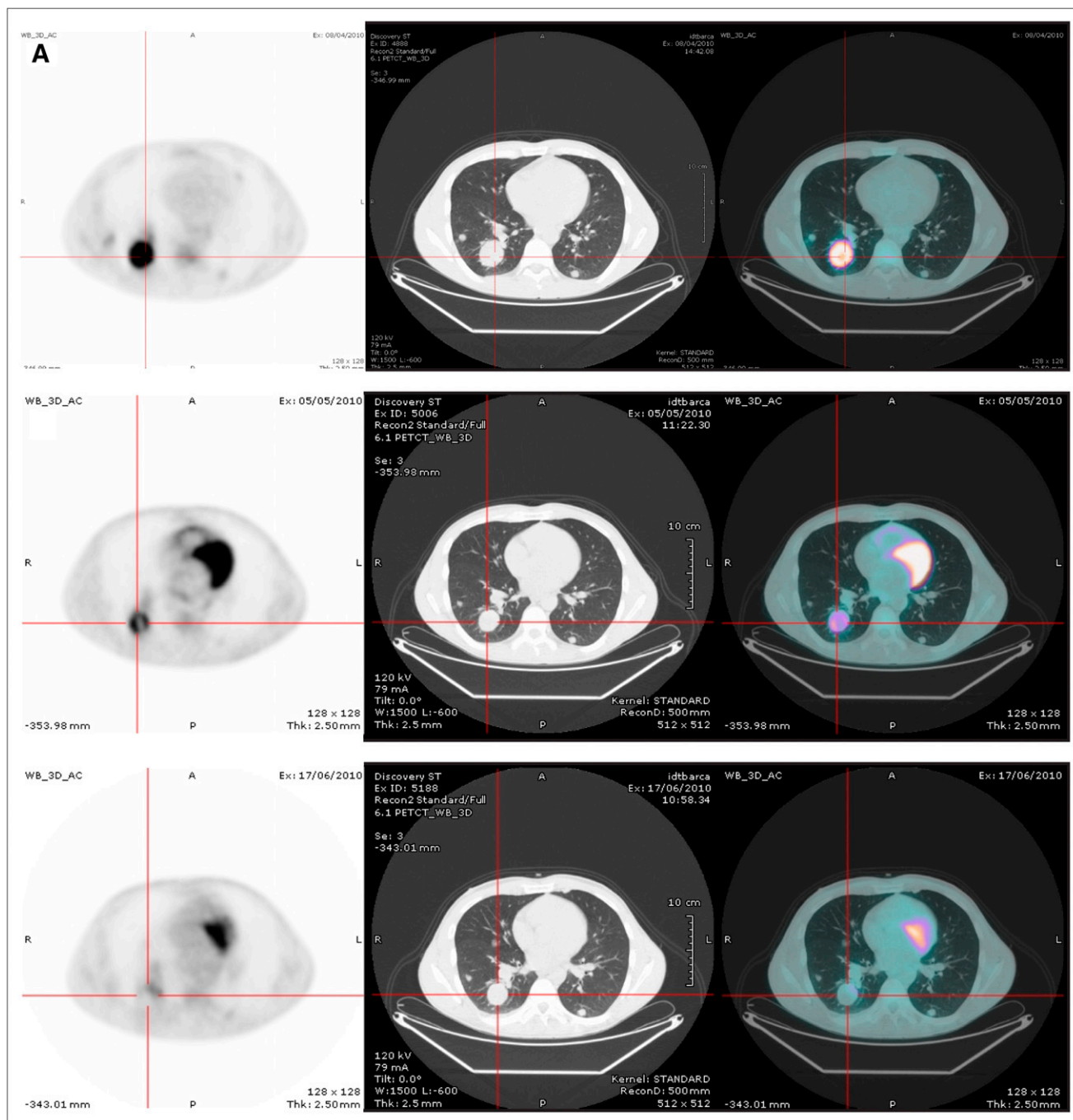


FIGURE 1. (A) Example of ^{18}F -FDG PET/CT images at baseline (top), at cycle 1, day 15 (middle); and at cycle 3, day 1 (bottom), in patient with metastatic skin melanoma who was treated with 4.0 mg of RO5126766 on the 4/3 regimen. PET/CT suggested partial metabolic response for this patient, in contrast to RECIST, which returned stable disease. These results reflect low PPV for PET seen in this study when RECIST response is considered as method of reference. (B) ^{18}F -FDG PET/CT images at baseline (top); at cycle 1, day 15 (middle); and at cycle 3, day 1 (bottom), in patient with colon carcinoma treated with RO4987655 (17 mg/day given as twice-daily administration [8.5 mg by mouth twice daily]). This case reflects high negative predictive value of PET because this patient showed metabolic progression at cycle 1 on day15, which was later confirmed to progress using RECIST.

in SUVpeak between baseline and cycle 1, day 15, with RO4987655 (8.5 mg twice daily), compared with RO5126766 (2.7 mg on the intermittent 4/3 regimen; Table 3), and this difference approached significance at the 95% level ($P = 0.052$) in the overall population.

Comparison of ^{18}F -FDG PET Data with RECIST Tumor Response

Of the 75 patients scanned with ^{18}F -FDG PET at cycle 1, day 15, 71 were also evaluable for tumor response at cycle 3, day 1, according to RECIST (Table 2). Treatment with

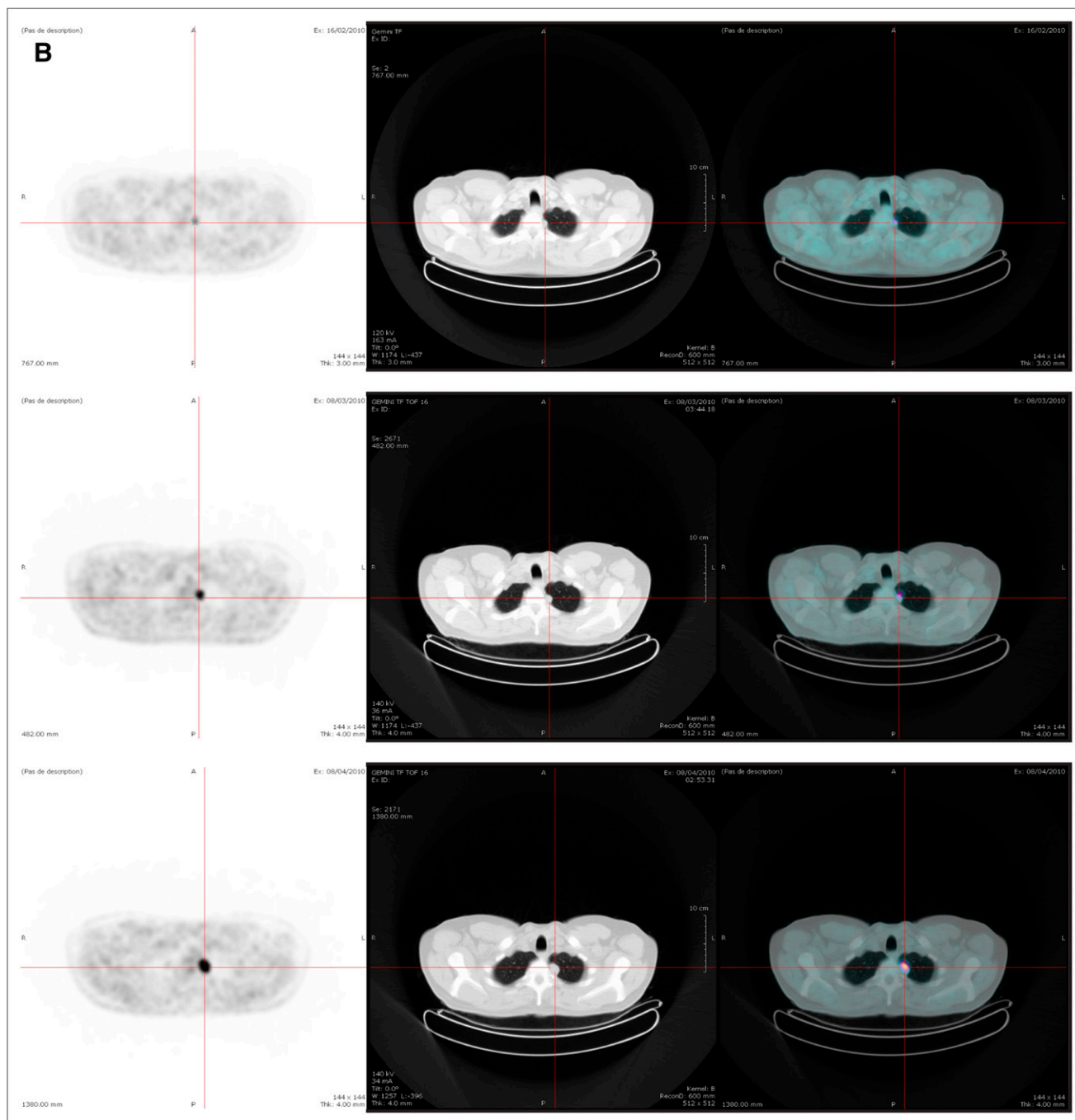


FIGURE 1. (Continued).

both RO5126766 and RO4987655 showed encouraging antitumor activity as measured by RECIST (8,9). For RO5126766, 7 patients (including 4 with melanoma) experienced stable disease (lasting >16 wk), and 3 patients achieved PR (all melanoma) as the best overall response. For RO4987655, 6 patients (including 4 with melanoma) experienced stable disease (lasting >16 wk) and 2 achieved PR (both melanoma).

For both compounds, a weak relationship was observed in the overall tumor population between the change in ^{18}F -

FDG uptake between baseline and cycle 1, day 15, and the change in the longest diameter of target lesions as measured by RECIST on cycle 3, day 1 ($R^2 = 0.16$ and 0.04 for RO4987655 and RO5126766, respectively; Fig. 3A). This relationship was more pronounced in the population with melanoma ($R^2 = 0.27$ and 0.16 for RO4987655 and RO5126766, respectively; Fig. 3B). Overall, the relationship between ^{18}F -FDG and RECIST data appeared to be stronger for RO4987655, but statistical significance was not reached (Fig. 3 legend). Comparable results were obtained

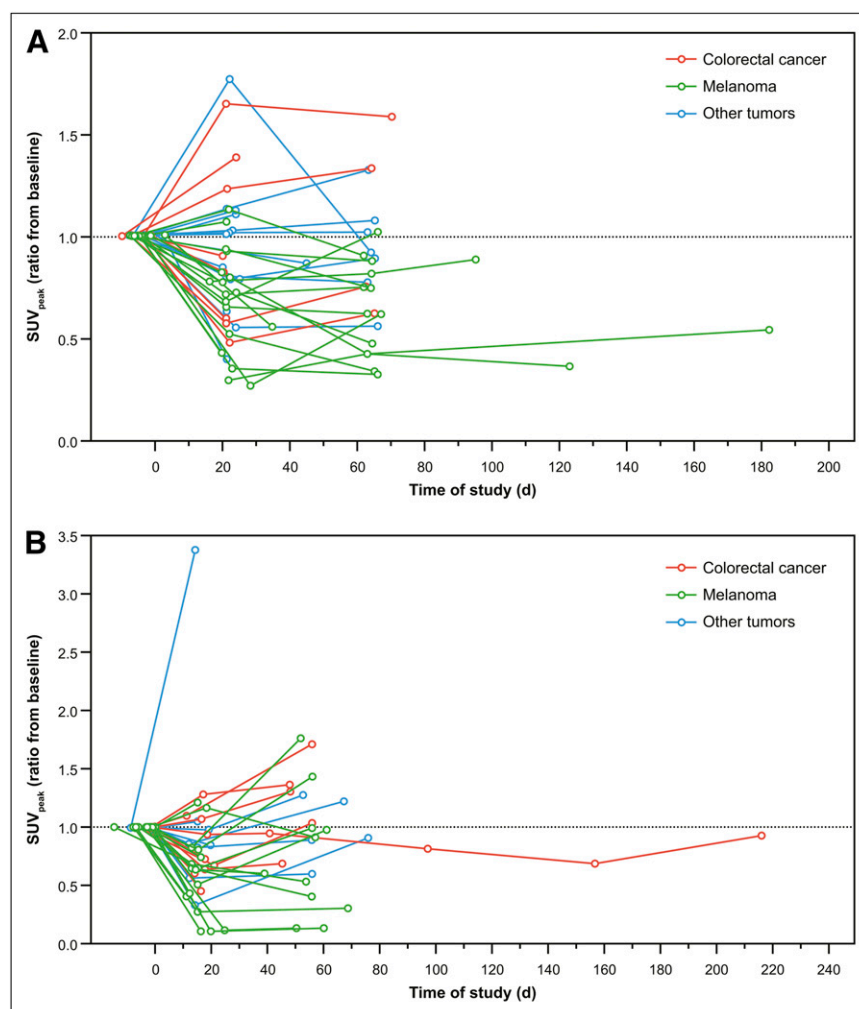


FIGURE 2. Change from baseline in SUV_{peak} as function of time for all patients receiving RO5126766 (A) and RO4987655 (B). ¹⁸F-FDG PET was performed at baseline (day 0 to -7) and at follow-up visits on cycle 1, day 15 (+5 d), and cycle 3, day 1 (+5 d). Treatment cycle length in both studies was 28 d.

using ¹⁸F-FDG data collected on cycle 3, day 1 (for all tumors: $R^2 = 0.06$ and 0.11 for RO4987655 and RO5126766, respectively; for melanoma: $R^2 = 0.07$ and 0.15 for RO4987655 and RO5126766, respectively).

Across the 2 trials, the PPV of ¹⁸F-FDG at cycle 1, day 15, was 12% (95% CI, 3%–28%), and the NPV was 97% (95% CI, 86%–100%). The sensitivity was 80% (95% CI, 28%–100%), and the specificity was 55% (95% CI, 43%–68%).

TABLE 3
¹⁸F-FDG PET Response at Recommended Phase II Dose for RO4987655 and RO5126766 on Cycle 1, Day 15

| Response | RO4987655 (8.5 mg twice daily) | <i>n</i> | RO5126766 (2.7 mg 4/3*) | <i>n</i> | <i>P</i> |
|-------------------------------------|--------------------------------|----------|-------------------------|----------------|--|
| SUV_{peak} reduction | | | | | |
| All tumors | –47% | 8 | –16% | 4 [†] | 0.052 [‡] |
| Mean | –55% | | –13% | | |
| Range | –90% to 29% | | –48% to 11% | | |
| Melanoma tumors | –63% | 5 | –28% | 2 | 0.152 [‡] |
| Mean | –61% | | –28% | | |
| Range | –90% to –36% | | –48% to –8% | | |
| Metabolic response | | | | | |
| All tumors | 75% PMR | 8 | 25% PMR | 4 | Not significant ($P > 0.3$) [§] |
| Melanoma tumors | 83% PMR | 5 | 50% PMR | 2 | Not significant ($P > 0.8$) [§] |

*4/3 = 4 days on treatment, 3 d off.

[†]One patient escalated from 2.25 to 2.7 mg after single run-in dose.

[‡]One-sided *t* test.

[§] χ^2 independence test.

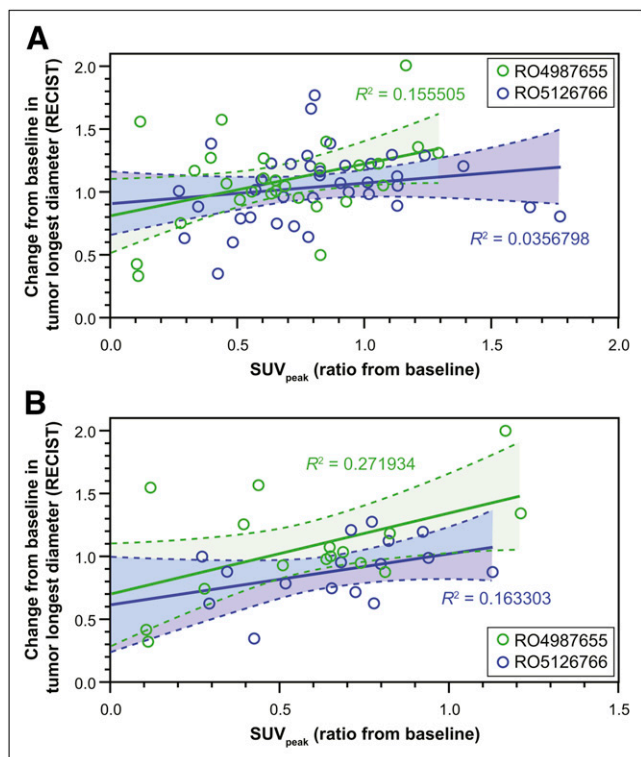


FIGURE 3. Best change from baseline for longest diameter as measured per RECIST as function of percentage change from baseline on cycle 1, day 15, for SUVpeak: overall tumor population ($R^2 = 0.16$ [slope = 0.41, $P = 0.03$] and $R^2 = 0.04$ [slope = 0.151, $P = 0.25$] for RO4987655 and RO5126766, respectively) (A) and melanoma tumors only ($R^2 = 0.27$ [slope = 0.65, $P = 0.03$] and $R^2 = 0.16$ [slope = 0.41, $P = 0.12$] for RO4987655 and RO5126766, respectively) (B). Solid line represents tendency curve (linear regression), and shaded region represents 90% confidence interval around tendency curve.

Correlation of ^{18}F -FDG PET Data with Dose of MEK Inhibitor and Serum Exposure

There was no apparent correlation between the PET data and drug dose of MEK inhibitors in the overall population of patients receiving either RO5126766 or RO4987655. However, for RO4987655, the insignificance of the correlation ($R^2 \gg 0$, slope $\gg 0$, $P = 1$) was driven by a single data point. This data point corresponded to a patient with a clear cell sarcoma of the left femur who received RO4987655 at a total daily dose of 21 mg for a duration of 18 d, after which this patient died of an unrelated intestinal perforation due to an abdominal metastasis. When this patient was omitted from the analysis, an apparent relationship between change in SUVpeak and dose was observed ($R^2 = 0.22$, slope = -0.025 , $P = 0.006$; Fig. 4).

Furthermore, a clear relationship between ^{18}F -FDG PET data and study drug dose was observed for both compounds in patients with melanoma, with larger decreases in SUVpeak occurring with increasing dose level ($R^2 = 0.42$ and 0.44 for RO4987655 and RO5126766, respectively; Fig. 5).

When the change in SUVpeak versus exposure to study drug was investigated, a similar pattern was seen (data not

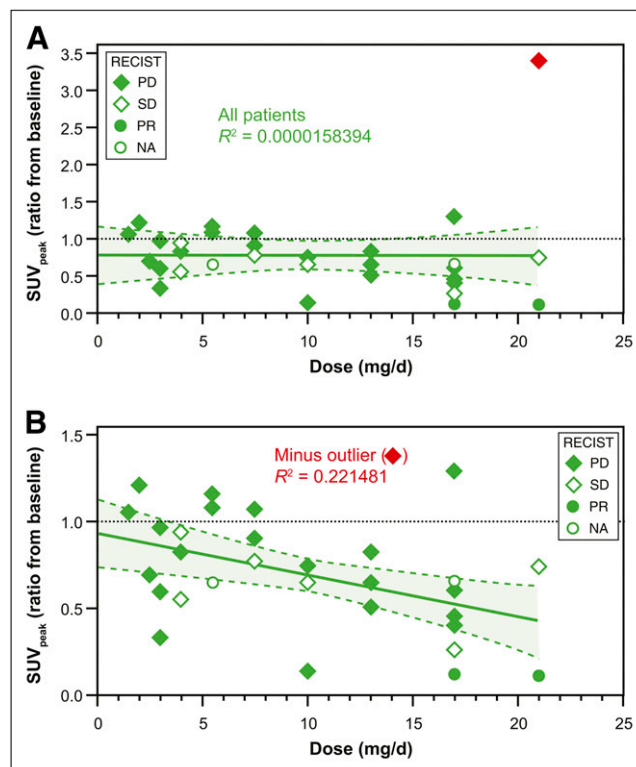


FIGURE 4. Change from baseline in SUVpeak at cycle 1, day 15, as function of RO4987655 dose: overall tumor population (A) and overall tumor population after removal of outlier at 21 mg (B). Data points are categorized according to RECIST best overall response. Patients with RECIST partial response also had largest change in ^{18}F -FDG uptake between baseline and cycle 1, day 15. Solid line represents linear regression, and shaded region represents 90% CI around tendency curve. NA = patient not assessable by RECIST; PD = progressive disease; SD = stable disease.

shown), with the largest ^{18}F -FDG PET effects found in the subpopulation of patients with melanoma. The relationship was again more pronounced in patients receiving RO4987655.

For RO5126766, we observed a decrease in ^{18}F -FDG PET effect as a function of dose in patients on intermittent dosing, compared with daily dosing. For example, in melanoma patients receiving 2.25 mg once daily continuously, the mean change in SUVpeak at cycle 1, day 15, was -42% ($n = 4$; range, -73% to -11%), compared with -28% ($n = 2$; range, -49% to -8%) for patients receiving 2.7 mg on the 4/3 intermittent regimen and 0% in patients receiving 2.7 mg on the 7/7 intermittent regimen ($n = 2$; $+7\%$ change in 1 patient and -7% in the other; $P = 0.02$).

Correlation of ^{18}F -FDG PET Data with pERK Inhibition by RO5126766 and RO4987655

There was an apparent relationship between the ^{18}F -FDG PET data and the degree of pERK inhibition (maximum pERK inhibition during the observed period) in PBMCs in patients with melanoma ($n = 15$) treated with RO4987655 ($R^2 = 0.37$, slope = -0.02 , $P = 0.01$; Fig. 6). This relationship was not observed in patients with melanoma ($n = 9$)

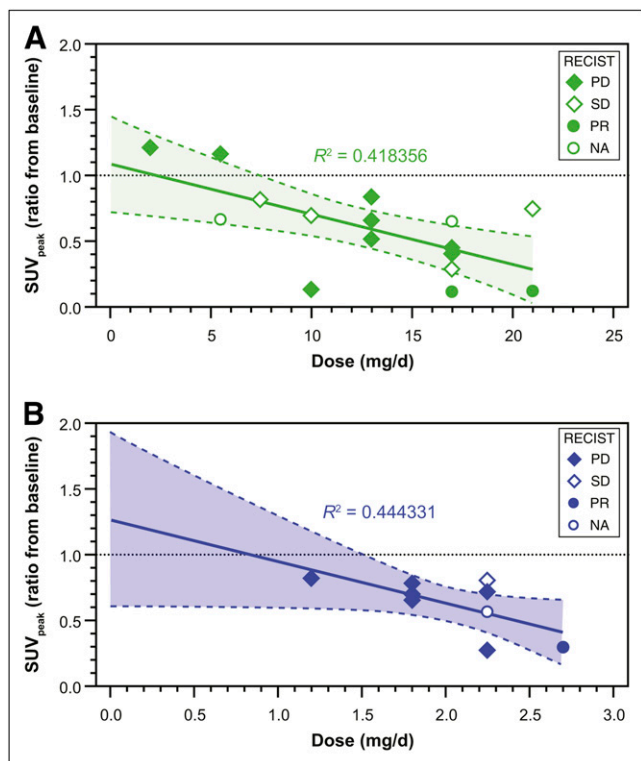


FIGURE 5. Change from baseline in SUVpeak at cycle 1, day 15, as function of dose in patients with melanoma: patients receiving RO4987655 (A) and patients receiving RO5126766 (only once-daily dosing shown) (B). Data points are categorized according to RECIST best overall response. Solid line represents tendency curve (linear regression), and shaded region represents 90% CI around tendency curve. NA = patient not assessable by RECIST; PD = progressive disease; SD = stable disease.

treated with RO5126766 once daily ($R^2 \gg 0.01$, slope $\gg 0$, $P \gg 0.7$). No apparent correlation between pERK inhibition and ^{18}F -FDG PET data was observed in the overall tumor population for either RO5126766 or RO4987655. However, for RO4987655, the insignificance of the correlation ($R^2 \gg 0$, slope $\gg 0$, $P \gg 0.8$) appeared to be driven by the same single data point mentioned earlier (clear cell sarcoma patient). When omitting this data point from the analysis, a weak relationship between pERK inhibition and the change in SUVpeak was seen ($R^2 = 0.14$, slope = -0.007 , $P = 0.03$).

DISCUSSION

We investigated the role of ^{18}F -FDG PET as a pharmacodynamic biomarker using data from 2 phase I trials of 2 novel MEK inhibitors. We observed strong evidence of biologic activity as measured by ^{18}F -FDG PET as early as cycle 1, day 15, with a decrease in ^{18}F -FDG uptake seen in most of the patients (71% for RO5126766 and 79% for RO4987655) and with the largest effect observed in patients with melanoma.

^{18}F -FDG PET allowed the early prediction of the absence of drug efficacy, with 97% NPV. PPV was low, suggesting

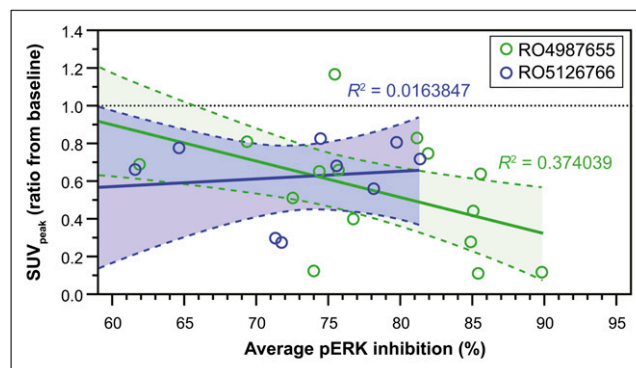


FIGURE 6. Change from baseline in SUVpeak at cycle 1, day 15, as function of average pERK inhibition during treatment period in patients with melanoma treated with RO4987655 and RO5126766 (only once-daily dosing shown). Solid line represents tendency curve (linear regression), and shaded region represents 90% CI around tendency curve.

that an early metabolic response was a necessary (but not in itself sufficient) indicator for later RECIST response. These high NPVs suggest that ^{18}F -FDG PET should be considered in patient management as a tool to select patients most likely to benefit from treatment with a MEK inhibitor, thereby avoiding the exposure of patients who are unlikely to respond to possible treatment-related adverse events and associated costs.

The low PPV tends to contradict results from previous studies showing the potential of PET for early assessment of the effectiveness of targeted therapy (14). However, our study was performed in patients in 2 phase I studies, many of them treated at low dose levels and therefore perhaps experiencing transient metabolic responses not sufficiently sustained for producing a subsequent RECIST response. Moreover, in our study, the reference method to determine predictive value was RECIST response. Stable disease according to RECIST was considered as no response in our statistical analysis. However, prolonged stable disease (without decrease of tumor masses) could also reflect efficiency. Furthermore, it is now also well established that morphologic evaluation using RECIST cannot always be considered as the more appropriate method to assess targeted therapy efficacy (14). Taking this into consideration, a large study in patients treated at recommended doses comparing PET response and survival would be necessary to confirm the performance of ^{18}F -FDG PET, in particular for PPV.

Although further PET assessments were performed during cycle 3, we focused our analysis on the early PET measurements because of the greater relevance of early evaluation of biologic activity and the greater number of patients with PET data from day 15 of cycle 1, compared with day 1 of cycle 3 (98.7% vs. 63.2%, respectively). In that respect, detailed analysis of this population may have introduced bias, because patients who progressed before the second scheduled postbaseline PET assessment were withdrawn from the trials and did not undergo a cycle 3, day 1, scan.

Because of the ease of sample collection, there has been a lot of interest in using blood-borne markers to support decision making in early clinical trials. In these studies, the analysis of PBMC pERK inhibition focused on the detection of dose-dependent drug–target interaction and the correlation with pharmacokinetics, thus providing supporting evidence for the selection of the recommended phase II dose. The weak correlation between the biologic tumor effects as measured with ^{18}F -FDG PET and pERK activity in PBMC reflects the fact that drug–PBMC interactions and drug–tumor interactions are based on different stoichiometry and different diffusion of drug into these cell compartments and therefore do not support extending the use of PBMC beyond its intended use and as a surrogate tissues of tumor lesions.

Because patients were recruited from 2 multicenter trials, PET was performed at multiple centers and using different equipment. When PET scans obtained as part of a multicenter trial are compared, it is important that standardized PET protocols be followed to minimize variation. PET methodology in this study was performed in line with recommendations of the National Cancer Institute (10), and every effort was made to ensure that each patient was examined under the same conditions at the baseline and follow-up scans (specifically with respect to the time between injection of ^{18}F -FDG tracer and the scan). In addition, all PET images were analyzed centrally by a single nuclear medicine specialist. The reproducibility of ^{18}F -FDG PET data obtained in multicenter trials has been demonstrated by other authors, with centralized quality assurance and analysis producing the lowest intrasubject variation (15).

Most response-assessment studies measure the change in SUVmax, a single-pixel value that is adversely affected by noise, which leads to uncertainty in the quantification of treatment response. SUVpeak has been suggested as a more robust alternative, defined as the average SUV within a small, fixed-size region of interest centered on a high-uptake part of the tumor (16). Because of its larger volume, SUVpeak is less affected by image noise than SUVmax and therefore is expected to reduce uncertainties in the quantification of response to therapy. In our study, SUVpeak was determined using body weight and not lean body mass as recommended (16). However, on average, patient weight changed only 1% and 2% between the PET scans, suggesting an approximate difference of less than 1% between response determined using body weight SUVpeak and lean body mass SUVpeak (11). There is a wide variety of SUVpeak definitions in the literature, differing in the shape, size, and location of the fixed-size region of interest (11). The measurement of SUVpeak using a 1-cm ROI centered on SUVmax as performed in our study has been shown to correlate with SUVmax (11). As a result, patients were categorized in terms of metabolic responses based on the guidelines of the European Organization for Research and Treatment of Cancer (12). More recently, attempts have been made to formalize PET response assessment along the lines of the

RECIST commonly used in assessing anatomic response (16). Nevertheless, the criteria of the European Organization for Research and Treatment of Cancer are still the most widely used and therefore permit easier comparison with other clinical study results.

In addition to SUVpeak, measurement of SUVmean (mean value of all pixels in the tumor ROI) and total lesion glycolysis (TLG = total SUV \times tumor volume) were also obtained (data not shown). As expected, SUVmean was better correlated with SUVpeak than TLG; however, SUVmean and TLG values both strongly agreed with the results obtained with SUVpeak and did not affect the overall findings.

Standardized ^{18}F -FDG PET acquisition and centralized PET analysis by the single reader not only increased the quality and consistency of the output but also permitted head-to-head comparison of the 2 MEK inhibitors. For both compounds, a clear relationship was observed between ^{18}F -FDG PET and study drug dose. There was a markedly greater mean amplitude of ^{18}F -FDG decrease for RO4987655 (–47%) than for RO5126766 (–16%) at the RP2D of each drug in the overall tumor population, which approached statistical significance ($P = 0.052$). A similar pattern (albeit not statistically significant) was seen in melanoma tumors at the R2PD (–63% at 8.5 mg twice daily and –28% at 2.7 mg on the 4/3 regimen, respectively; $P = 0.15$) and with daily dosing (–63% at 8.5 mg twice daily and –42% at 2.25 once daily, respectively; $P = 0.1$). In addition, whereas the small number of samples available for analysis from these phase I studies limit the conclusions that can be drawn from these data, the relationship between change in ^{18}F -FDG uptake and dose/exposure and pERK inhibition in PBMC were nevertheless more pronounced in patients receiving RO4987655 than RO5126766.

^{18}F -FDG PET has been incorporated as a pharmacodynamic marker in several phase I trials of molecule-targeted drugs including the BRAF inhibitors GSK2118436 (17) and vemurafenib (18), the mTOR inhibitor everolimus (19), and lapatinib, a dual inhibitor of the ErbB1 and ErbB2 tyrosine kinases (20). ^{18}F -FDG PET data on MEK inhibitors in the literature are limited. Infante et al. demonstrated a reduction in SUVmax of 23%–48% in 4 evaluable melanoma patients treated with GSK1120212, a dual inhibitor of MEK1 and MEK2 (21). Similarly, Shapiro et al. reported that PMR was attained in 6 of 15 (40%) evaluable patients treated with a combination of the MEK inhibitor GDC-0973 and the PI3K inhibitor GDC-0941 in a dose-escalation trial (22). ^{18}F -fluoro-3'-deoxy-3'-L-fluorothymidine PET to investigate inhibition of proliferation by the MEK inhibitor PD0325901 has also been investigated (23,24). Although several other studies have confirmed the utility of PET in clinical trials, to our knowledge, this was the first study for which ^{18}F -FDG PET was used in a systematic and consistent manner over 2 phase I clinical trials with a view to comparing 2 compounds with similar mechanisms of action and guiding the most appropriate dose schedule to take forward into phase II.

CONCLUSION

The data presented here from 2 clinical studies with MEK inhibitors demonstrate that functional imaging with ^{18}F -FDG PET can be used to support the selection of dose, dosing regimen, and compounds early in phase I clinical trials.

We also observed clear evidence of the potential of ^{18}F -FDG PET to predict early nonresponding patients, which in turn, supports a wider adoption of this technique in clinical practice to support patient management.

DISCLOSURE STATEMENT

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