Dose Selection, Pharmacokinetics, and Pharmacodynamics of BRAF Inhibitor Dabrafenib (GSK2118436)

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Abstract

**Purpose:** Dabrafenib is a selective, potent ATP-competitive inhibitor of the BRAFV600-mutant kinase that has demonstrated efficacy in clinical trials. We report the rationale for dose selection in the first-in-human study of dabrafenib, including pharmacokinetics, tissue pharmakodynamics, 2[18F]fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) pharmakodynamics, and dose–response relationship.

**Experimental Design:** Dabrafenib was administered orally, once, twice (BID), or three times daily (TID). Selected dose cohorts were expanded to collect adequate data on safety, pharmakokinetics, or pharmakodynamics. A recommended phase II dose (RP2D) was chosen based on safety, pharmakokinetic, pharmakodynamic, and response data.

**Results:** One hundred and eighty-four patients were enrolled and treated with doses ranging from 12 mg once daily to 300 mg BID in 10 cohorts. Pharmakokinetic assessment of dabrafenib demonstrated a less-than-dose-proportional increase in exposure after repeat dosing above 150 mg BID. Similar to parent drug concentrations, exposure for all metabolites demonstrated less-than-dose-proportional increases. Predicted target inhibition of pERK (>80%) was achieved at 150 mg BID, with a similar magnitude of inhibition at higher doses in BRAFV600 mutation melanoma biopsy samples. Although there was large variability between patients, FDG uptake decreased with higher daily doses in patients with BRAFV600 mutation–positive melanoma. A favorable activity and tolerability profile was demonstrated at 150 mg BID. There was no improvement with TID dosing compared with BID dosing, based on FDG-PET and tumor response analyses in patients with melanoma.

**Conclusion:** The RP2D of dabrafenib was determined to be 150 mg BID after considering multiple factors, including pharmakokinetics, tissue pharmakodynamics, FDG-PET pharmakodynamics, and the dose–response relationship. A maximum tolerated dose for dabrafenib was not determined. *Clin Cancer Res; 20(17); 4449–58. © 2014 AACR.*

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Translational Relevance

In the current era of targeted anticancer agents, phase I investigators face new challenges to define acceptable criteria for selecting the optimal dose for subsequent phase II and III investigations. Historically, the recommended phase II dose has been determined after establishing a toxicity profile and identifying the maximum tolerated dose (MTD), based on the presumption of a dose–response relationship. However, dose-related toxicities may not be relevant surrogates for efficacy in less toxic, targeted drugs. In this report, we describe the rationale for dose selection in the first-time-in-human phase I trial of dabrafenib, in which multiple endpoints were considered, including pharmacokinetics, tissue- and 2[18F]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET)-assessed pharmacodynamics, and dose–response relationship. Our trial contributes to the experience that the MTD is not always equal to the optimal biologic dose, reflecting a paradigm shift in which efficacy is not compromised at doses below the MTD.

Introduction

Dabrafenib (GSK2118436) is a selective, potent ATP-competitive inhibitor of rapidly accelerated fibrosarcoma (RAF) kinases, including the BRAFV600 mutation, in kinase panel screening, cell lines, and xenografts (1). A randomized phase III trial demonstrated a 50% objective response rate in patients with BRAFV600 mutation–positive metastatic melanoma and improved progression-free survival with dabrafenib compared with dacarbazine (2).

Historically, the recommended phase II dose (RP2D) for an anticancer drug has been determined after establishing a toxicity profile and identifying the maximum tolerated dose (MTD), based on the presumption of a dose–response relationship (3). Targeted agents, however, may have more favorable toxicity profiles because they are designed to inhibit molecular aberrations specific to cancer cells, and high doses that induce toxicity may not correlate with greater efficacy. Targeted drugs, such as kinase inhibitors, may require an alternative metric to MTD that defines the dose–response relationship using suitable pharmacodynamic or other endpoints (4, 5).

Here, we report the rationale for dose selection in the first-time-in-humans phase I study of dabrafenib (6). To determine the RP2D, we considered multiple endpoints, including pharmacokinetics, tissue- and 2[18F]fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET)-assessed pharmacodynamics, and dose–response relationship.

Materials and Methods

Study design and patients

The primary objectives were to determine the safety and RP2D of dabrafenib. Secondary objectives included objective tumor response assessment and determination of the pharmacokinetic/pharmacodynamics profile. Dabrafenib was administered as mesylate salt in a gelatin capsule shell. The study was conducted in two parts: Part 1 (dose escalation with pharmacokinetic/pharmacodynamic expansions) and part 2 (RP2D and low-dose expansions). Dabrafenib was administered orally once, twice (BID), or three times (TID) daily during dose escalation. Selected dose cohorts were expanded to up to 20 patients to collect data on safety, pharmacokinetics, and/or pharmacodynamics. The MTD was defined as the highest dose at which no more than 1 of 6 patients experienced a dose-limiting toxicity (DLT; Supplementary Materials and Methods). The protocol was approved by the Institutional Review Boards of participating institutions and is registered with Clinicaltrials.gov (NCT00880321). All patients provided written informed consent.

Study assessments, pharmacokinetics, and pharmacodynamics

Toxicity, response, and BRAF genotyping were assessed as previously reported (Supplementary Materials and Methods; refs. 6, 7). Safety data and pharmacokinetics were evaluated for all enrolled patients. Tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST; v1.0; ref. 8). Response data were reported for patients with BRAFV600 mutation–positive melanoma with measurable disease at baseline without untreated brain metastases, and who had not previously received a BRAF or MEK inhibitor.

Blood samples for determination of plasma concentrations of dabrafenib and its metabolites, including hydroxy-, carboxy-, and desmethyl-dabrafenib, were collected at multiple time points (Supplementary Materials and Methods). Because preclinical data suggested that dabrafenib may induce CYP3A4 enzymes, urine was collected to measure 6β-hydroxycortisol and cortisol concentrations at baseline and after repeat dosing (day 15) in part 1. Urinary 6β-hydroxycortisol-to-cortisol ratio was compared between days 1 and 15 to determine potential for CYP3A4 induction (9).

The study included a 12-patient drug–drug interaction cohort at the RP2D to assess the effect of dabrafenib on single-dose pharmacokinetics of midazolam, a CYP3A4 probe. Single 3-mg doses of midazolam were administered orally under fasting conditions at baseline (day 1) and day 15, 1 hour after the morning dose.

Tumor biomarkers were evaluated from biopsies collected at baseline and within the first 2 weeks of dosing in selected patients. Following hematoxylin and eosin staining to assess morphology and routine diagnostic IHC, semi-quantitative IHC for pERK, Ki-67, and p27 was performed on paraffin-embedded tumor samples to assess the pharmacodynamic response to dabrafenib (Mosaic Laboratories LLC). The staining intensity (1+, 2+, or 3+) and extent (expressed as percentage of tumor cells at each intensity) were assessed in the nucleus, cytoplasm, and combined (nucleus/cytoplasm or total). A composite score (H score)
was calculated on the basis of the sum of the products of intensity and extent.

FDG-PET scanning was performed at baseline and week 2 in selected patients with BRAFV600 mutation–positive melanoma. Conditions were standardized for patient weight and height, start of fasting time before scan (at least 4 hours), time of injection of FDG, and PET scan start and end times, all of which were recorded. PET scans were not performed if serum glucose levels were higher than 200 mg/dL (11 mmol/L). The same protocol and specifications were to be used at both time points. FDG-PET–anonymized images with noncontrast CT were uploaded via AGMedNet and read by an independent reviewer (Perspective Informatics, Inc). The regions of interest were located on the image by the independent reviewer, who was able to refer to the RECIST assessment of contrast-enhanced CT imaging, as available, to aid in selecting lesions at the screening time point, although additional lesions may have been selected if they showed significant FDG uptake. The reviewer calculated standardized average (SUVmean) and peak (SUVmax) uptake values based on the pixel values within each region of interest of the baseline and treatment digital FDG-PET images.

Analytical method

Plasma dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib concentrations were analyzed using validated ultra high-performance liquid chromatography with tandem mass spectrometric detection (UHPLC/MS-MS) methods over the range of 1 to 1,000 ng/mL. Only dabrafenib and hydroxy-dabrafenib were measured originally but the method was later modified and revalidated to include desmethyl-dabrafenib concentrations. A separate UHPLC/MS-MS method was required to analyze carboxy-dabrafenib plasma concentrations. Midazolam plasma concentrations were determined using a validated analytical HPLC/MS-MS method validated over 0.1 to 100 ng/mL concentration range.

Statistical analysis

Pharmacokinetic parameters were calculated after single and repeat dosing using standard noncompartmental methods for dabrafenib and its three metabolites. Dose proportionality was assessed using a power model. To estimate the accumulation after repeat dosing and to assess time invariance, ratios of area under the concentration–time curve over the dosing interval (AUC0-¥) on days 8 or 15 to AUC0-¥ and area under the concentration–time curve from time zero extrapolated to infinity (AUC0-¥) on day 1, respectively, were calculated.

The logarithmic-transformed urinary 6-ß-hydroxycortisol-to-cortisol ratio was analyzed using a mixed effect model with the patient as a random effect and day (day 1 or 15) as a fixed effect. Logarithmic-transformed midazolam pharmacokinetic parameters (AUC0-¥, AUC0-¥, and maximum observed concentration; Cmax) were analyzed using a mixed-effect model with the patient as a random effect and treatment (with or without dabrafenib) as a fixed effect. Point estimate and 90% confidence intervals (CI) were back calculated to provide geometric mean ratios of midazolam with dabrafenib versus midazolam alone.

Changes from baseline were calculated for biopsy results using H scores, for FDG-PET data using sum of maximum standardized uptake value (SUVmax), and for tumor size using sum of longest diameter of RECIST target lesions. Summary statistics were provided by cohorts.

Dabrafenib’s effect on tumor pERK inhibition, FDG-PET, and tumor size was evaluated with a pharmacokinetic-pharmacodynamic model using nonlinear mixed-effect analysis. Different exposure–response models were evaluated, including an inhibitory maximum effect (Emax) model (Supplementary Materials and Methods).

Inhibition of pERK was correlated on the basis of total concentration of effective drug and metabolites adjusted for relative potency. The relative potency of dabrafenib metabolites relative to parent drug was based on the ratio of IC50 from two in vitro cellular activity assays, including protein-shifted SKMEL28 cellular pERK inhibition assay and a protein-shifted proliferation assay using Colo205 cells (data not shown). The 95% CIs around parameter estimates of pERK, FDG-PET, and tumor size were generated on the basis of nonparametric bootstrapping (N = 500 bootstrap datasets).

Results

Study population

One hundred and eighty-four patients with solid tumors were enrolled and treated with doses ranging from 12 mg once daily to 300 mg BID (Table 1 and Supplementary Table S1). Most patients had poor prognostic characteristics, such as stage M1c disease. Ten percent of patients had received >2 prior lines of systemic therapy in the metastatic setting.

Safety

As previously reported (6), doses were escalated to 300 mg BID without identification of an MTD. DLTs were observed in 3 of 20 patients given 200 mg BID (grade 3 cutaneous squamous-cell carcinoma, grade 3 syncope, grade 2 pyrexia) and in 2 of 10 patients given 300 mg BID (grade 4 hyponatremia, grade 3 cutaneous squamous-cell carcinoma; Supplementary Results and Supplementary Table S1). No DLTs were observed in patients given <200 mg BID.

Efficacy

Among the 130 patients with BRAFV600 mutation–positive melanoma with measurable disease at baseline without untreated brain metastases, and who had not previously received a BRAF or MEK inhibitor, objective responses were observed at week 9 (part 1) or week 6 (part 2) at doses of 35 to 300 mg BID (Tables 2 and 3).

Pharmacokinetics

After single-dose oral administration, parent drug plasma concentrations peaked 1.0 to 2.5 hours postdose and decreased thereafter following a biexponential decline.
Median terminal half-life ranged from 4.0 to 6.8 hours after single-dose administration. Increases in C_{max} and AUC were generally dose-proportional with single doses up to 300 mg (Fig. 1A and Supplementary Table S2). The power model’s mean slope (90% CI) was 0.780 (0.523–1.036) for C_{max} (n = 54) and 0.897 (0.734–1.061) for AUC_{0–t} (n = 42); a slope of 1.0, with 90% CI including 1.0, indicates dose-proportional pharmacokinetics.

Pharmacokinetic parameters obtained after repeat dosing are summarized in Supplementary Table S3. After repeated once-daily, BID, or TID dosing, there was no accumulation in plasma, and the mean AUC ratio of day 15 to day 1 was <1.0, suggesting that oral clearance changes with time. After administration of 150 mg BID, the geometric mean AUC was 47% lower on day 15 than on day 1. A 2-fold increase in dose (150 mg BID vs. 300 mg BID) resulted in only a 43% increase in AUC_{0–t} at day 15 and no increase in C_{max}. The dabrafenib mean pharmacokinetic profiles are shown in Fig. 1B. Exposure was greater on day 8 relative to day 15 following administration of 150 mg BID (Supplemental Materials and Methods). Increase in C_{max} AUC (Fig. 1A), and predose concentrations (C_{0}) were less than dose-proportional between 75 and 300 mg BID after repeat dosing.

For AUC_{0–t}, the mean (90% CI) slope of the relationship between day 15 AUC and daily dose was 0.605 h·ng/mL (0.329–0.881). There was overlap in individual exposure at total daily doses of 150 to 600 mg. The mean (90% CI) for the slope of C_{0} versus daily dose was 0.398 ng/mL (−0.115 to 0.910) with 90% CI including value of 0.

Dabrafenib is metabolized sequentially to three known metabolites that may contribute to clinical activity: hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib. Figure 1C demonstrates the mean concentration–time profile of dabrafenib and its metabolites after administration of 150 mg BID on day 15. Concentrations of dabrafenib and hydroxy-dabrafenib were in the same range, with a similar half-life. Both carboxy- and desmethyl-dabrafenib accumulated with repeat dosing with accumulation (AUC_{0–t} ratio of day 15:day 1) ranging from 2.78 to 8.77 for carboxy-dabrafenib and from 12.6 to 35.0 for desmethyl-dabrafenib across doses ≥70 mg BID. Although the metabolite half-lives could not be determined with the limited sampling period on day 1, they are predicted to be >24 hours based on accumulation ratios observed. After repeat-dose administration of 150 mg BID, the exposure for each of the three metabolites
relative to parent drug ($\text{AUC}_{\text{max}}$, of metabolite:parent, after adjusting for differences in molecular weight) was 0.8, 1.9, and 1.0, for hydroxy-, carboxy-, and desmethyl-dabrafenib, respectively. Similar to parent concentrations, exposure for all metabolites on day 15 was less than proportional to the increase in dose. The deviation from dose-proportionality was more pronounced in the following rank order: day 15 > day 8, desmethyl- > carboxy- > hydroxy-dabrafenib, and $C_{\text{t}} > C_{\text{max}} > \text{AUC}_{0-t}$.

An increase in the $6\beta$-hydroxy cortisol-to-cortisol ratio was observed after repeat dosing ($n = 93$ patients with paired samples), suggesting that the change in oral clearance over time may be due to CYP3A4 induction. The overall mean ratio (90% CI) of day 15:day 1 in patients receiving BID/TID regimens was 1.98 (1.75–2.23). To confirm the induction effect, 12 patients were enrolled in the midazolam assessment in part 2. A decrease in single-dose midazolam $C_{\text{max}}$, $\text{AUC}_{0-t}$, and $\text{AUC}_{0-\infty}$ with mean (90% CI) ratios of 0.388 (0.241–0.626), 0.234 (0.183–0.300), and 0.258 (0.210–0.318), respectively, was observed following repeat 150 mg BID dabrafenib dosing.

In part 2, median predose plasma concentrations in cycle 1 to 4 ranged from 53.6 to 77.3 ng/mL and from 30.7 to 56.3 ng/mL in patients receiving 150 mg BID and 50 mg BID, respectively. Parent drug and metabolite concentrations were similar across cycles and generally lower in patients receiving 50 mg BID compared with those receiving 150 mg BID, albeit in a nonproportional manner.

**Tumor biomarker pharmacodynamics**

Tumor biopsies were obtained from 15 patients with $\text{BRAF}^V600$ mutation-positive melanoma at baseline and during treatment. Because tumors often decreased in size rapidly after starting treatment, biopsy time was adjusted to an earlier date to reduce the risk of necrotic, nonevaluable tumor samples and to ensure there would be enough time for parent drug and metabolites to equilibrate with tissues and reach steady-state concentrations. Among the biopsies,

### Table 2. Clinical activity at week 2 FDG-PET and week 9 CT in patients with $\text{BRAF}^V600$ mutation-positive melanoma with measurable disease who had not previously received a BRAF or MEK inhibitor and without untreated brain metastases from part 1

<table>
<thead>
<tr>
<th>Dose level</th>
<th>$N$</th>
<th>Week 2 FDG-PET ($\text{SUV}_{\text{max}}$)</th>
<th>Week 9 tumor size (RECIST; ref. 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\leq$35 mg QD</td>
<td>1</td>
<td>29</td>
<td>Mean baseline (SE)</td>
</tr>
<tr>
<td>35 mg BID</td>
<td>5</td>
<td>13 (7)</td>
<td>–56 (21)</td>
</tr>
<tr>
<td>70 mg BID</td>
<td>7</td>
<td>36 (12)</td>
<td>–38 (14)</td>
</tr>
<tr>
<td>75 mg BID&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>56</td>
<td>–60</td>
</tr>
<tr>
<td>100 mg BID</td>
<td>7</td>
<td>44 (9)</td>
<td>–48 (8)</td>
</tr>
<tr>
<td>100 mg TID</td>
<td>14</td>
<td>52 (16)</td>
<td>–56 (4)</td>
</tr>
<tr>
<td>150 mg BID</td>
<td>12</td>
<td>48 (10)</td>
<td>–57 (6)</td>
</tr>
<tr>
<td>200 mg BID</td>
<td>8</td>
<td>63 (26)</td>
<td>–44 (12)</td>
</tr>
<tr>
<td>300 mg BID</td>
<td>5</td>
<td>100 (34)</td>
<td>–58 (17)</td>
</tr>
<tr>
<td>$\leq$35 mg QD</td>
<td>1</td>
<td>125 (56)</td>
<td>–2 (2)</td>
</tr>
<tr>
<td>35 mg BID</td>
<td>5</td>
<td>47 (13)</td>
<td>–44 (17)</td>
</tr>
<tr>
<td>70 mg BID</td>
<td>7</td>
<td>120 (27)</td>
<td>–19 (6)</td>
</tr>
<tr>
<td>75 mg BID&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>100 mg BID</td>
<td>9</td>
<td>138 (31)</td>
<td>–17 (9)</td>
</tr>
<tr>
<td>100 mg TID</td>
<td>14</td>
<td>85 (22)</td>
<td>–33 (8)</td>
</tr>
<tr>
<td>150 mg BID</td>
<td>16</td>
<td>92 (17)</td>
<td>–31 (6)</td>
</tr>
<tr>
<td>200 mg BID</td>
<td>16</td>
<td>150 (31)</td>
<td>–30 (9)</td>
</tr>
<tr>
<td>300 mg BID</td>
<td>10</td>
<td>91 (28)</td>
<td>–57 (10)</td>
</tr>
</tbody>
</table>

Mean % change (SE) RR (95% CI)

<table>
<thead>
<tr>
<th>Week 6 tumor size (RECIST)</th>
<th>Week 9 tumor size (RECIST; ref. 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\leq$35 mg QD</td>
<td>1</td>
</tr>
<tr>
<td>35 mg BID</td>
<td>5</td>
</tr>
<tr>
<td>70 mg BID</td>
<td>7</td>
</tr>
<tr>
<td>75 mg BID&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>100 mg BID</td>
<td>7</td>
</tr>
<tr>
<td>100 mg TID</td>
<td>14</td>
</tr>
<tr>
<td>150 mg BID</td>
<td>12</td>
</tr>
<tr>
<td>200 mg BID</td>
<td>8</td>
</tr>
<tr>
<td>300 mg BID</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.
<sup>a</sup>Pharmacokinetic cohort; patients were escalated from 75 mg BID to 150 mg BID after pharmacokinetic assessment on day 15. Only 1 patient had FDG-PET data.

### Table 3. Clinical activity at week 6 FDG-PET and week 9 CT in patients with $\text{BRAF}^V600$ mutation-positive melanoma with measurable disease who had not previously received a BRAF or MEK inhibitor and without untreated brain metastases from part 2

<table>
<thead>
<tr>
<th>Dose level</th>
<th>$N$</th>
<th>Baseline mean (SE)</th>
<th>Mean % change (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 mg BID</td>
<td>20</td>
<td>146 (21)</td>
<td>–31 (4)</td>
</tr>
<tr>
<td>50 mg BID&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18</td>
<td>104 (21)</td>
<td>–20 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean % change (SE) RR (95% CI)

<sup>a</sup>An lower-dose cohort (50 mg BID) was included in part 2 of this study to better define the dose-efficacy relationship. Among the first 15 patients with $\text{BRAF}^V600E$ mutations treated at 50 mg BID, only three patients (20%) had a confirmed PR at the time of the interim analysis, which was less than the number of confirmed responses ($n = 4$) required to continue enrollment in this cohort.

<sup>b</sup>Two of the 18 patients did not have lesion measurement on week 6, so $n = 16$ for mean% change calculation.
10 paired samples from patients with no prior BRAF or MEK inhibitor treatment were considered evaluable because the pretreatment specimens showed adequate baseline expression (H score > 10) and the on-treatment specimens were obtained after 5 or more treatment days.

Of the 10 evaluable paired biopsies, eight were evaluable for pERK, 10 for Ki-67, and 9 for p27 (Fig. 2). As previously reported (6), the median (range) change in pERK expression from baseline was \(-83.9\% \text{ (} -38.0\% \text{ to } -93.3\%)\), with doses ranging from 70 to 200 mg BID, indicating evidence of enzymatic pathway inhibition. Of the eight evaluable biopsies for pERK, six showed evidence of \(-80\%\) inhibition of pERK expression. Changes in Ki-67 expression were not as sensitive with median change (range) of \(-66.5\% \text{ (} +14.4\% \text{ to } -81.1\%)\), whereas increases in p27 expression were noted in 6 of 9 patients, with a median change (range) of \(+28.6\% \text{ (} -90.6 \text{ to } +125\%)\).

The relationship between systemic exposure and percentage of pERK inhibition was characterized using an E_{max} model with 100% maximum inhibition and IC_{50} of 134 ng/mL (95% CI, 92.7–155) on the basis of parent drug and active metabolite concentrations including hydroxy-dabrafenib and desmethyl-dabrafenib (Fig. 2C, left). The potency of hydroxy-, desmethyl-, and carboxy-dabrafenib was 2-, 1/8th-, and 1/22nd-fold relative to the parent drug based on \textit{in vitro} assay, respectively. Because of its low potency, carboxy-dabrafenib is not believed to contribute to the clinical activity following dabrafenib administration. The percent change in pERK was predicted by total daily dose on the basis of the mean predose concentrations (C_{t}) observed on day 15 at that dose level (Fig. 2C, right). pERK inhibition reached a plateau at total daily doses more than 200 mg (>100 mg BID). A dose-related decrease in pERK was predicted with total daily doses <200 mg, with a plateau occurring beyond total daily doses of 200 mg thereafter. Administration of 150 mg BID was predicted to provide, on average, near maximum predicted possible target inhibition (\(\approx 80\%\)) based on the E_{max} model described.

**FDG-PET pharmacodynamics**

FDG-PET was performed at baseline and week 2 in 60 patients with BRAFV600 mutation melanoma receiving doses ranging from 35 mg once daily up to 300 mg BID. Four additional patients are included in this analysis who were not included in the previously reported preliminary analysis of 56 patients (6). Decreases in SUV_{max} were observed in 55 of 60 patients, with a median 60% decrease in SUV_{max} (range, −100% to +24%). The mean SUV_{max}...
percent change from baseline for each dose level ranged from $-19\%$ to $-58\%$ (Tables 2 and 3). The decrease from baseline in sum of SUV$\text{max}$ was generally dose related, except at 35 mg BID and 200 mg BID.

Decreases in SUV$\text{max}$ correlated with the daily dose administered using an inhibitory $E_{\text{max}}$ model (Fig. 3, left). The inhibitory $E_{\text{max}}$ model was significantly better than a model with no dose-related decrease in SUV$\text{max}$ ($P = 0.001$). The median (95% bootstrap CI) total daily dose that resulted in a 50% maximum decrease in SUV$\text{max}$ ($ED_{50}$) was 214 mg (168–312). There was no significant difference in percent change of SUV$\text{max}$ when comparing TID regimens.
with BID ($P = 0.29$; Fig. 3, left). Changes in $SUV_{\text{max}}$ and $SUV_{\text{mean}}$ were similar and are not reported separately. There was no correlation between changes in tumor size at week 9 and changes in $SUV_{\text{max}}$ at week 2 ($P = 0.89$).

**Tumor size and response rate**

Because patients in part 1 were permitted to dose escalate after completing 9 weeks of treatment at the initial assigned starting dose, comparisons of unconfirmed clinical response in different cohorts of melanoma patients are presented at week 9 (Tables 2 and 3). The unconfirmed response rate in patients with $BRAF$ V600 mutation–positive melanoma at week 9 was 50% (95% CI, 24.7–75.3) at 150 mg BID; the response rate was lower at 200 mg BID (38%; 95% CI, 15.2–64.6) and higher at 300 mg BID (90%; 95% CI, 55.5–99.7). The response rate was 60% (95% CI, 14.7–94.7) at 35 mg BID; patients in this cohort had the smallest median sum of target lesions at baseline (Tables 2 and 3), with a mean value of 47 mm relative to an overall mean value of 112 mm. Baseline tumor size and change from baseline for each cohort are presented in Tables 2 and 3.

Data were described using an inhibitory Emax model as a function of average daily dose administered with a median (95% bootstrap CI) ED$_{50}$ of 801 mg (571–1217 mg) as shown in Fig. 3 (right). The model was statistically significantly better than a model where the response is not related to dose ($P = 0.002$). The estimated ED$_{50}$ was greater than the highest daily dose tested of 600 mg, suggesting that change in tumor size is very close to being dose linear. There was no significant difference when comparing TID with BID regimens ($P = 0.11$).

**Discussion**

In the current era of targeted anticancer agents, phase I investigators face new challenges to define acceptable criteria for selecting the optimal dose for subsequent phase II and III investigations (10). Historically, the selection of RP2D with cytotoxic medications has been based upon the principle that the highest dose tolerated by patients would likely be the most effective (11, 12). However, identifying an MTD and dose-related toxicities as surrogates for efficacy may not be necessary or relevant for the successful development of less toxic, targeted drugs.

Historically, therapeutic windows have been quite narrow with traditional chemotherapies, in which efficacious doses push the limits of toxicity. As newer, targeted agents are developed with improved safety profiles, the therapeutic window will hopefully increase. This seems to be the case with selective BRAF inhibitors, and in such situations, the optimal biologic dose may not necessarily equal the MTD.

In our first-in-human phase I study of dabrafenib, no MTD was identified in the range up to 300 mg BID. Pharmacokinetic and pharmacodynamics dose-response data were used to select the RP2D of 150 mg BID, which demonstrated a favorable activity-tolerability profile. The factors that supported the selection of this dose included pharmacokinetic characteristics, achievement of predicted target inhibition of pERK (>80%) at 150 mg BID with a similar magnitude of inhibition at higher doses, and evidence of exposure response observed with FDG-PET.

Pharmacokinetic assessment revealed a less than dose-proportional increase in exposure after repeat dosing. Consistent with the decrease in midazolam, a CYP3A4 probe, observed during coadministration of dabrafenib, the decrease in dabrafenib AUC observed with repeat dosing is likely due to induction of its own metabolism. Because of their long half-lives, desmethyl- and carboxy-dabrafenib accumulated with repeat dosing, although exposure was also less than dose proportional on day 15. No increase in response rate was observed between 150 mg BID and 200 mg BID, whereas a higher percentage of responders was noted with 300 mg BID. However, differences in baseline tumor burden, relatively small sample size, and nonrandomization may have contributed to the variability observed. Pharmacokinetic analysis also revealed that there was low interpatient variability in exposure. The majority of patients were within the therapeutic range.
The lack of improvement with TID dosing compared with BID dosing may be explained by the contribution of metabolites, including the long half-life of desmethyl-dabrafenib. In vitro potency of each metabolite on cellular pERK inhibition and proliferation assays demonstrated ratios, which suggested that both hydroxy- and desmethyl-dabrafenib may contribute to clinical activity, whereas carboxy-dabrafenib is less likely to contribute despite its higher exposure relative to the parent drug after repeat dosing.

pERK analysis confirmed that dabrafenib inhibits the MAPK pathway. The results from 8 patients were used to model the expected pERK inhibition at doses above 150 mg BID, and the model predicted little added benefit above this dose. Ki-67 decreased with treatment, indicating that dabrafenib is antiproliferative.

We present the largest series of FDG-PET response analyses among patients treated with a selective BRAF inhibitor. Our study confirms dabrafenib’s antimitotic effects, as patients at all dose levels achieved substantial reduction in SUVmax. The observation that the degree of SUVmax reduction did not increase at doses above a total daily dose of 300 mg daily further supports 150 mg BID as the RP2D. Although FDG-PET is a good marker for metabolic uptake and a drug’s activity, the response measured using FDG-PET at week 2 did not correlate with tumor regression (percent change from baseline) assessed on week 9.

Dabrafenib is among a growing group of other effective anticancer agents that never reached an MTD, including bevacizumab (13), trastuzumab (14), and imatinib (15). The MTD for anticancer agents is not necessarily the optimal biologic dose, as demonstrated by low-dose decitabine, which has superior efficacy compared with high-dose decitabine (16). Our trial contributes to the experience that the MTD is not always equal to the optimal biologic dose and reflects a paradigm shift in which efficacy is not compromised at doses below the MTD (17).

Our study is limited by the small number of patients for whom tissue was available for pharmacodynamic analysis. Another limitation is patient population heterogeneity, in terms of tumor burden (Tables 2 and 3) and BRAF mutation subtype (V600E vs. V600K), which complicates the dose-response assessment. In addition, our study is limited by the absence of an analysis of durability of response and duration of tolerance. Such an analysis is not possible because patients who received doses of less than 150 mg were permitted to undergo dose escalation, and so any long-term endpoint would not truly reflect the effect of the starting dose.

In conclusion, the RP2D for dabrafenib was determined after considering many factors, including pharmacokinetics, tissue pharmacodynamics, FDG-PET pharmacodynamics, and dose–response relationship, without identification of an MTD. Investigators should consider a similar strategy in determining the RP2D in future first-time-in-human trials of targeted agents.

Disclosure of Potential Conflicts of Interest
G.S. Falchook reports receiving a commercial research grant from GlaxoSmithKline. G. Long reports receiving speakers bureau honoraria from GlaxoSmithKline and Roche and is a consultant/advisory board member for Bristol-Myers Squibb, GlaxoSmithKline, Merck, Novartis, and Roche. K. Kim reports receiving a commercial research grant from and is a consultant/advisory board member for GlaxoSmithKline. M. Brown and R. Kefford report receiving speakers bureau honoraria from and are consultants/advisory board members for GlaxoSmithKline. O. Hamid reports receiving a commercial research grant from GlaxoSmithKline. J. Infante is a consultant/advisory board member for GlaxoSmithKline. S. O’Day reports receiving commercial research grants from Bristol-Myers Squibb and Novartis. S. Blackman and B. Ma are employees of GlaxoSmithKline. D. Ouellet is an employee of and has ownership interests (including patents) in GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

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14. Slamon DJ. Final study report (FSR) for H0407g: A phase 1 safety and tolerance study of intravenous anti-p185 HER2 humanized monoclonal antibody (rhu MAb HER2) in patients with HER2 overexpressing tumors. Silver Spring, MD: US Food and Drug Administration; 1996.


