



Year: 2015

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DOI: 10.1002/prp2.113

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <http://doi.org/10.5167/uzh-109838>

Published Version



Originally published at:

Goldinger, Simone M; Rinderknecht, Jeannine; Dummer, Reinhard; Kuhn, Felix Pierre; Yang, Kuo-Hsiung; Lee, Lucy; Ayala, Ruben C; Racha, Jagdish; Geng, Wanping; Moore, David; Liu, Mei; Joe, Andrew K; Bazan, Selby Patricia Gil; Grippo, Joseph F (2015). A single-dose mass balance and metabolite-profiling study of vemurafenib in patients with metastatic melanoma. *Pharmacology Research Perspectives*, 3(2):e00113. DOI: 10.1002/prp2.113

ORIGINAL ARTICLE

A single-dose mass balance and metabolite-profiling study of vemurafenib in patients with metastatic melanoma

Simone M. Goldinger¹, Jeannine Rinderknecht¹, Reinhard Dummer¹, Felix Pierre Kuhn², Kuo-Hsiung Yang^{3,5}, Lucy Lee^{3,7}, Ruben C. Ayala^{3,8}, Jagdish Racha³, Wanping Geng^{3,6}, David Moore³, Mei Liu^{3,9}, Andrew K. Joe^{3,10}, Selby Patricia Gil Bazan⁴ & Joseph F. Grippo³

¹Department of Dermatology, University Hospital Zurich, Gloriastrasse 31, 8091, Zurich, Switzerland

²Department of Medical Radiology, University Hospital Zurich, Raemistrasse 100, CH-8091, Zurich, Switzerland

³Roche Innovation Center New York, 430 East 29th Street, New York, New York, 10016

⁴Roche Innovation Center Basel, Grenzacherstrasse 124, 4070, Basel, Switzerland

⁵Current address: UNC Eshelman School of Pharmacy, Chapel Hill, North Carolina

⁶Current address: EMD Serono R&D Institute, Billerica, Massachusetts

⁷Current address: Eisai Inc., Woodcliff Lake, New Jersey

⁸Current address: Rutgers University, New Brunswick, New Jersey

⁹Current address: Revlon, Edison, New Jersey

¹⁰Current address: Novartis Pharmaceuticals Corporation, East Hanover, New Jersey

Keywords

BRAF inhibitor, disposition, elimination, mass balance, metabolism, metastatic melanoma, Vemurafenib

Correspondence

Joseph F. Grippo, Roche Innovation Center New York, 430 East 29th Street, New York, NY 10016. Tel: +1-973-262-6220; Fax: +1-646-461-5175; E-mail: joseph.grippo@roche.com

Funding Information

This study was sponsored by F. Hoffmann-La Roche, Ltd.

Received: 18 June 2014; Revised: 15 October 2014; Accepted: 27 October 2014

Pharma Res Per, 3(2), 2015, e00113, doi: 10.1002/prp2.113

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Abstract

Vemurafenib, a selective inhibitor of oncogenic BRAF kinase carrying the V600 mutation, is approved for treatment of advanced BRAF mutation-positive melanoma. This study characterized mass balance, metabolism, rates/routes of elimination, and disposition of ¹⁴C-labeled vemurafenib in patients with metastatic melanoma. Seven patients with metastatic BRAF-mutated melanoma received unlabeled vemurafenib 960 mg twice daily for 14 days. On the morning of day 15, patients received ¹⁴C-labeled vemurafenib 960 mg (maximum 2.56 MBq [69.2 μCi]). Thereafter, patients resumed unlabeled vemurafenib (960 mg twice daily). Blood, urine, and feces were collected for metabolism, pharmacokinetic, and dose recovery analysis. Within 18 days after dose, ~95% of ¹⁴C-vemurafenib-related material was recovered from feces (94.1%) and urine (<1%). The parent compound was the predominant component (95%) in plasma. The mean plasma elimination half-life of ¹⁴C-vemurafenib-related material was 71.1 h. Each metabolite accounted for <0.5% and ≤6% of the total administered dose in urine and feces, respectively (0–96 h postdose). No new metabolites were detected. Vemurafenib was well-tolerated. Excretion of vemurafenib via bile into feces is considered the predominant elimination route from plasma with minor renal elimination (<1%).

Abbreviations

ABA, absolute bioavailability; AUC, area under the plasma concentration–time curve; CL_{cr}, creatinine clearance; cuSCC, cutaneous squamous cell carcinoma; CV, coefficient of variation; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LSC, liquid scintillation counting; MBP, microprecipitated bulk powder; MBq, megabecquerel; NCI CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; PK, pharmacokinetic; RECIST, Response Evaluation Criteria In Solid Tumors; SPE, solid-phase extraction; T_{max} , time to the maximum plasma concentration after drug administration; λ_z, apparent elimination rate; μCi, microcurie.

Introduction

Metastatic melanoma is an aggressive disease associated with poor prognosis, a 5-year survival rate of approximately 15%, and median overall survival of 8–10 months (Falkson et al. 1998; Tsao et al. 2004; Atkins et al. 2008; Patel et al. 2011). Until recently, treatment options were limited. Response rate with dacarbazine, a chemotherapeutic agent typically used for treatment of metastatic disease, is 7–12%, with median overall survival between 5.6 and 7.8 months (Chapman et al. 1999; Middleton et al. 2000; Avril et al. 2004; Dummer et al. 2012a).

Recent advances in the treatment of metastatic melanoma have seen vemurafenib (RG7204; PLX4032; RO5185426)—a selective inhibitor of oncogenic BRAF kinase carrying a V600 mutation (BRAF^{V600} kinase) (Tsai et al. 2008; Bollag et al. 2010; Søndergaard et al. 2010)—introduced as a treatment option. Approximately 50% of all melanomas have an oncogenic BRAF mutation (Thomas et al. 2007), which results in constitutive activation of the BRAF kinase and downstream signaling.

Results of phase 2 and phase 3 studies of vemurafenib and other BRAF inhibitors have shown improved response rates, progression-free survival, and overall survival in patients with BRAF^{V600}-mutated metastatic melanoma (Chapman et al. 2011, 2012; Dummer and Flaherty 2012; Hauschild et al. 2012; Sosman et al. 2012; McArthur et al. 2014). Progression-free and overall survival data formed the basis for approval of vemurafenib as monotherapy for adult patients with unresectable or metastatic BRAF^{V600}-mutation positive melanoma in the United States in August 2011 (Genentech, Inc. 2014).

Vemurafenib was also approved in Europe in February 2012, and has been approved or submitted for approval in several other countries.

The successful introduction of vemurafenib as a treatment option for metastatic melanoma warranted a better understanding of the disposition of orally administered vemurafenib. Preclinical studies characterized routes of elimination in rats and indicated several metabolites predominantly eliminated with excretion via bile into feces (F. Hoffmann-La Roche, data on file). In addition, preclinical studies identified a number of potential minor metabolites of vemurafenib in human liver microsomes and hepatocytes (Fig. 1) (F. Hoffmann-La Roche, data on file). The current study aimed to characterize the mass balance, metabolism, rates and routes of elimination, and disposition of ¹⁴C-labeled vemurafenib in patients with metastatic melanoma. Population pharmacokinetic (PK) modeling suggests that the median elimination half-life for vemurafenib was 57 h with continual multiple-dose vemurafenib (Genentech, Inc. 2014) and considerably shorter after a single dose (~25 h; Ribas et al, 2014 reference). Vemurafenib exhibits extensive accumulation at steady state, and characterization of single and multiple-dose PK suggests that drug disposition is different at steady state than after a single dose (Grippio et al. 2014). This study was conducted accordingly at steady state by administering multiple doses of unlabeled vemurafenib to patients for 14 days followed by a single dose of ¹⁴C-vemurafenib. Patients received the potential benefit of a therapeutic dose, and the drug disposition properties were characterized at a time more relevant to a long-term treatment regimen.

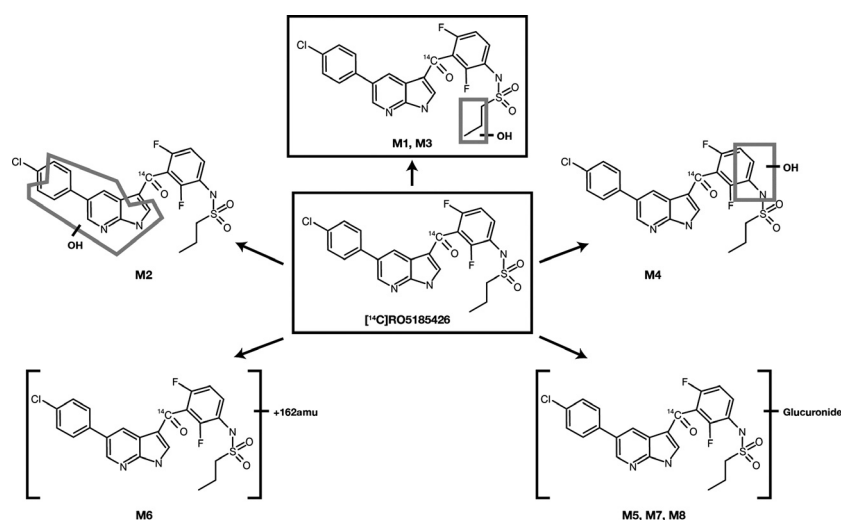


Figure 1. Potential vemurafenib metabolites identified from in vitro studies in human liver microsomes and hepatocytes (F. Hoffmann-La Roche, data on file).

Material and Methods

Study design

This was an open-label, nonrandomized, single-center, phase 1 study in patients with metastatic melanoma. Owing to occurrence of cutaneous squamous cell carcinoma (cuSCC) and secondary primary melanomas in other vemurafenib clinical studies (Flaherty *et al.* 2010; Lacouture *et al.* 2010; Robert *et al.* 2011; Oberholzer *et al.* 2011; Zimmer *et al.* 2012), this study was conducted in metastatic melanoma patients rather than healthy volunteers.

After screening and enrollment, patients were dosed at home with unlabeled vemurafenib 960 mg twice daily (microprecipitated bulk powder [MBP] formulation consisting of four 240-mg tablets) (Hoffmann-La Roche, Nutley, NJ) from day 1 to day 14. Patients returned to the clinic on the evening of day 14. On the morning of day 15, patients received a single oral 960-mg dose of ^{14}C -vemurafenib after ≥ 8 h of fasting. Patients fasted for an additional 4 h after dosing (light snacks were allowed) to allow full characterization of the fasted state and were then given a meal. The radiolabeled test compound was delivered in capsule formulation and consisted of six 120-mg capsules of unlabeled and four 60-mg capsules of radiolabeled vemurafenib. Each 60-mg capsule contained a maximum of 17.3 μCi of radiolabeled material to yield a maximum dose of radioactivity of 69.2 μCi (2.56 MBq).

^{14}C -vemurafenib was prepared by the isotope labeling group at Hoffmann-La Roche, with specific activity of 111.4 $\mu\text{Ci}/\text{mg}$ and radiochemical purity of 99.2%, as determined by radio-high-performance liquid chromatography (HPLC). ^{14}C isotope labeling was incorporated on the carbonyl carbon of vemurafenib (Fig. 1).

Collection of blood, urine, and feces samples for radioactivity counting began on the evening of day 14 before administration of the radioactive dose and continued until the level of radioactivity recovered from excreta was $\leq 1\%$ of radioactivity in the administered dose between any two successive 48-h interval assessments (recovery criterion).

On the evening of day 15, patients resumed their regular dose of unlabeled vemurafenib indefinitely until disease progression, unacceptable toxicity, or other criteria for withdrawal from the study. Beginning on day 16 and throughout the PK collection period up to day 36, patients fasted overnight for ≥ 8 h preceding the morning PK sample collection and for 4 h after dosing and then were given a meal. Patients were also required to drink ≥ 1.5 L of fluid daily to ensure proposed urine sample amounts were collected.

The study was conducted in accordance with accepted standards of Good Clinical Practice and conformed to the principles of the Declaration of Helsinki. An independent ethics committee for the Department of Dermatology of the University Hospital Zurich approved the study, and all patients provided written informed consent before study enrollment. This study is registered with ClinicalTrials.gov, number NCT01164891.

Patients

Patients aged ≥ 18 years with untreated or previously treated metastatic melanoma (surgically unresectable stage IIIc or stage IV) that was $\text{BRAF}^{\text{V600}}$ -mutation positive (cobas[®] 4800 BRAF V600 Mutation Test; Roche Molecular Diagnostics, Pleasanton, CA) were eligible for enrollment. Eligible patients had an Eastern Cooperative Oncology Group performance status of 0 or 1; evaluable disease (measurable by Response Evaluation Criteria In Solid Tumors [RECIST] criteria, version 1.1); and adequate hematologic, renal, and liver function. A negative pregnancy test result in premenopausal women and use of adequate contraception was necessary before the first dose. All patients underwent a baseline skin examination, and preexisting cuSCC lesions were to have been excised with adequate wound healing before dosing.

Patients were not eligible if they had active central nervous system lesions; had a history of known spinal cord compression or carcinomatous meningitis; were expected to need additional anticancer therapy; were breast-feeding; or had any medical condition capable of altering absorption, metabolism, or elimination of the study drug.

Sample preparation for pharmacokinetic assessments and metabolic profiling

Plasma and blood samples were collected to analyze total radioactivity and to identify unchanged ^{14}C -vemurafenib and the presence of metabolites. Urinary and fecal samples were collected to analyze total radioactivity at particular time points, to analyze percentage and fraction of dose recovered as total radioactivity, and for metabolite profiling. Plasma pools were prepared by taking equal aliquots (by weight) of each individual sample. Urine and feces were pooled by taking a fixed proportion (%) of each sample or homogenate weight.

Sample pretreatment

Plasma

A suitably sized aliquot (approximately 2 g) of each pooled plasma sample was vortex-mixed with ~ 3 vol

acetonitrile (6 mL), then centrifuged (3000 rpm for 10 min at 4°C). The resulting supernatant (extract 1) was transferred to a vial. The pellet was then reextracted with an additional ~3 vol acetonitrile, and the supernatant (extract 2) was combined with extract 1. The combined supernatant was concentrated under a stream of nitrogen gas (35°C), and the residue was reconstituted in 200 μ L of HPLC mobile phase. Recovery of radioactivity in plasma was determined in the final extract by liquid scintillation counting (LSC) and ranged from 83% to 128% across each pool for individual patients. Chromatograms presented here from HPLC analysis of metabolite profiles represent the majority of radioactivity in the collected plasma sample.

Feces

A suitably sized aliquot (approximately 2.5 g) of each pooled feces sample was vortex-mixed with ~3 vol acetonitrile (approximately 7.5 mL), then centrifuged (3000 rpm for 10 min at 4°C). The resulting supernatant (extract 1) was transferred to a vial. The pellet was then reextracted with an additional ~3 vol acetonitrile, and the supernatant (extract 2) was combined with extract 1. The combined supernatant was concentrated under a stream of nitrogen gas (35°C) to approximately 4 mL. Recovery of radioactivity in feces was determined in the final extract by LSC and ranged from 87% to 111% across each pool for individual patients. Chromatograms presented here from HPLC analysis of metabolite profiles represent the majority of radioactivity in the collected feces sample.

Urine

Pooled urine samples were concentrated by solid-phase extraction (SPE). Oasis HLB 3-mL cartridges (60 mg; Waters Corporation, Milford, MA) were preconditioned with methanol (2 mL), then water (2 mL). Urine samples (approximately 100 mL) were applied to the cartridges and washed with 20 mL water. Samples were then eluted using acetonitrile (4 \times 1 mL). Eluates were reapplied to preconditioned cartridges, washed with 10 mL water, and eluted with acetonitrile (4 \times 1 mL). Combined eluates were concentrated under a stream of nitrogen gas (30°C). Residues were reconstituted in 500 μ L acetonitrile/water 1:1 vol/vol. Because of the low level of radioactivity in urine samples, it was not possible to accurately determine recovery of radioactivity after sample preparation procedures in each sample. Therefore, to demonstrate no significant loss of radioactivity during SPE, a single sample of urine (pool 6007, patient 1001) was spiked with radio-labeled RO5185426 reference standard. The spiked sample was subjected to the same SPE method as study samples.

Recovery of radioactivity from the spiked sample was determined in the final extract by LSC.

Plasma and blood samples for PK profiling were collected before dosing (days 14 and 15) and at 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 168, 216, 312, and up to 504 h after the 14 C-vemurafenib dose. Plasma samples for metabolic profiling were collected at 1, 2, 4, 6, 12, 24, 36, 48, and 72 h after the 14 C-vemurafenib dose. Three pooled samples were used in profiling: 4 plus 6 h, 12 plus 24 h, and 36 plus 48 h. Urine samples were collected before the dose and in quantitative fractions on day 15 (0–6, 6–12, and 12–24 h) and during 24-h intervals thereafter (24–48, 48–72, and 96–120 h after dosing) until the recovery criterion was met (defined previously herein). Because total radioactivity in urine was low, a single pool from 0–6 to 72–96 h was prepared for metabolic profiling. Fecal samples were collected before dosing and during 24-h intervals after dosing until the recovery criterion was met. Two sample pools were prepared for metabolic profiling: 0–24 plus 24–48 h and 48–72 plus 72–96 h.

Concentrations of vemurafenib in human plasma from the study were measured using a validated liquid chromatographic-tandem mass spectrometry (LC-MS/MS) method. The linear quantitation range of the assay was from 25 to 50,000 ng/mL, using plasma volumes of 0.050 mL.

Pharmacokinetic analysis

PK analysis was conducted using data from only those patients who met the recovery criterion. All PK data were summarized using descriptive statistics. No formal statistical analyses were applied. Standard noncompartmental methods (WinNonlin version 5.2.1, Pharsight Corp., Mountain View, CA) were used to determine PK parameters in blood and plasma, including time to maximum observed plasma concentration; maximum observed plasma concentration; apparent elimination rate (λ_z), computed as the magnitude of the slope from log-linear regression of the apparent terminal elimination phase of the plasma concentration–time curve; elimination half-life, computed as $(\ln 2)/\lambda_z$; and area under the plasma concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$), or $AUC_{0-\tau}$, if appropriate.

Metabolite identification

Relative proportions of RO5185426 and its 14 C-labeled metabolites were characterized in human plasma, feces, and urine samples obtained from seven patients after a single oral dose of 14 C-RO5185426 followed by 960 mg twice daily for 15 days. Although samples were concentrated before radio-HPLC profiling analysis, levels of radioactivity in the samples were low, and detected

metabolite peaks were generally close to the limit of detection. Analytic conditions used for LC-MS/MS are summarized in Table 1. Tentative structures of radiolabeled metabolites were assigned based on mass spectral analysis (Fig. 1), and chemical structures were not confirmed because no metabolite standards were available. Data were summarized using descriptive statistics where appropriate.

Sample analysis

Radioactivity was measured for 5 min using Packard Tri-Carb liquid scintillation counters with facilities for computing quench-corrected disintegrations per minute. Efficiency correlation curves were prepared and routinely checked using [¹⁴C]-toluene and Ultima Gold™ quenched standards (PerkinElmer LAS U Ltd., Beaconsfield, UK). The limit of quantification was taken as twice the mean background disintegration rate obtained from vials containing liquid scintillant. Liquid scintillation counts and weighing data were entered manually.

All data from HPLC radio detectors were captured using Laura software (version 3.4.7.52; LabLogic Systems,

Sheffield, UK). Radiolabeled components in each chromatogram were evaluated to determine retention times and peak area values (% regions of interest). The limit of quantification for analysis of each sample type was taken as three times the background (peak height).

LC-MS/MS data were analyzed for presence of metabolites based on accurate masses of potential metabolites using Xcalibur 2.0. software (Thermo Scientific, Waltham, MA). Data from urine and fecal samples were summarized as proportions (%) and fraction of dose recovered as total radioactivity. Data for ¹⁴C-vemurafenib and its metabolites were summarized over time intervals using descriptive statistics where appropriate.

Safety assessments

Safety was determined by the reporting of adverse events; assessment of routine laboratory values; physical examination; assessment of vital signs; electrocardiography assessments; and evaluation of dermatology, head/neck, and chest computed tomography during the study and a 28-day follow-up period. Patients who had SCC during treatment were assessed for safety until death, withdrawal of consent, or loss to follow-up. Severity of adverse events and changes in laboratory data were reported and summarized using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.0). Safety was evaluated in all patients who received at least one dose of study drug; descriptive statistics are presented for safety data.

Results

At the clinical cutoff date (November 2010), seven patients with metastatic melanoma were enrolled at the dermatology unit of the Department of Dermatology, University Hospital Zurich, Switzerland. All seven patients were included in the safety population. Data for two patients were excluded from the analysis of ¹⁴C-vemurafenib PK because predose radioactive counts were detected in the plasma samples—sample contamination is a likely explanation for these data. Data from one patient were excluded from urinary metabolite profiling because of sample contamination.

All patients (three men; four women) were white; median age was 55 years (range, 39–75 years); and mean weight (\pm SD) was 75.5 \pm 20.1 kg. Identified target lesions were lymph nodes (six patients), liver (two patients), soft tissue (two patients), and pleura (one patient).

All patients had received systemic treatment for melanoma (one to six prior systemic treatments) and had a metastatic diagnosis for >1 year (range, 38.2–133.8 months).

Table 1. Summary of the analytical conditions used for LC-MS/MS analysis.

Instrumentation	ThermoFisher LTQ Orbitrap hybrid mass spectrometer connected to a ThermoFisher Accela HPLC system via an API interface		
Ionization mode	Positive ion electrospray		
Column	Waters, YMC ODS-AQ, 120Å, 5- μ m packing, 4 \times 150 mm		
Column temperature	30°C		
Solvent system			
Solvent A	Water + 0.1% acetic acid		
Solvent B	Acetonitrile + 0.1% acetic acid		
Gradient elution system	Time (min)	% A	% B
	0	95	5
	8	95	5
	13	75	25
	26	5	95
	28	5	95
	28.1	95	5
	33	95	5
Flow rate	1.0 mL/min (split approximately 10:1 radiodetector:ms)		
Scan range	Full scan <i>m/z</i> pos 100–900, MS/MS scan ranges were data dependent		
Resolution	Full scan 30,000, MSn 7500		
Source voltage	4.0 kV		
Capillary temperature	310C		

API, application programming interface; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MSn, multistage MS.

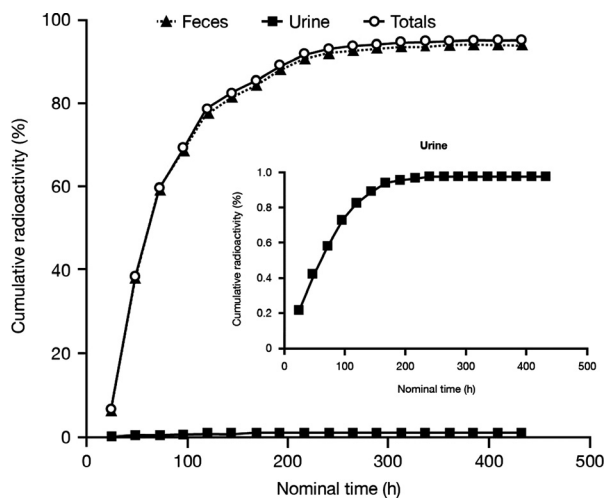


Figure 2. Cumulative excretion of ^{14}C -vemurafenib via feces and urine.

Four patients had elevated lactate dehydrogenase levels; four patients had two metastatic sites, including liver metastasis; and three patients had one metastatic site (pleura and lymph).

Urinary and fecal elimination

All seven patients met the recovery criterion after ^{14}C -vemurafenib dosing. Good recovery of ingested radioactivity was found in these patients, with a mean \pm SD of $95.0 \pm 2.4\%$ (range, 91.0–98.3%; coefficient of variation [CV]%, 2.5) of the ^{14}C -vemurafenib-related material recovered from urine and feces within 18 days after dosing. Most of this radioactive material was found in feces (Fig. 2). A mean of $94.1 \pm 2.7\%$ (range, 89.4–97.6%; CV %, 2.8) of radioactivity was recovered in feces and $<1.0\%$ (range, 0.56–1.63%; CV%, 43.7) was recovered in urine. The time course of cumulative ^{14}C -vemurafenib excretion in urine and feces showed that the majority of urinary

and fecal radioactivity was eliminated within 192 and 264 h, respectively.

Pharmacokinetics in blood and plasma

After administration of a single oral dose of ^{14}C -vemurafenib, absorption occurred with T_{max} (time to maximum plasma concentration after drug administration) between 5 and 8 h, followed by a biphasic mode of elimination with an inflection at 72 h (Fig. 3). PK parameters for ^{14}C -vemurafenib are detailed in Table 2. The mean C_{max} for ^{14}C -vemurafenib-related material was $\sim 7.8 \mu\text{g eq/mL}$. The mean plasma elimination half-life of ^{14}C -vemurafenib-related material was 71.1 h (range, 51–86 h) (Table 2).

Mean \pm SD unlabeled plasma vemurafenib trough concentrations ranged from 58.2 ± 27.1 to $67.4 \pm 22.2 \mu\text{g/mL}$ from days 15 through 17, representing steady-state vemurafenib plasma trough concentrations.

The mean \pm SD AUC ratio of blood/plasma was 0.72 ± 0.05 (range, 0.69–0.81; Table 2). Because hematocrit values ranged from 0.40% to 0.51% for men and from 0.36% to 0.47% for women, blood/plasma ratios would be ~ 0.45 – 0.50 if plasma accounted for all ^{14}C -vemurafenib-related material. In this case (assuming 40–50% of whole blood is cells and 50–60% is plasma), approximately 10–20% of ^{14}C -vemurafenib-related material might be bound to the cellular compartment in whole blood.

Metabolite characterization

Limited metabolites of ^{14}C -vemurafenib were identified in human plasma, and the parent compound was the predominant component in pooled plasma samples at 4–6 (mean \pm SD, $99.5 \pm 1.4\%$), 12–24 ($95.9 \pm 5.5\%$), and 36–48 h ($96.0 \pm 3.8\%$; Fig. 4A). Overall, potential metabolites in human plasma represented $<5\%$ of total chromatographic radioactivity and $<5\%$ of radioactivity

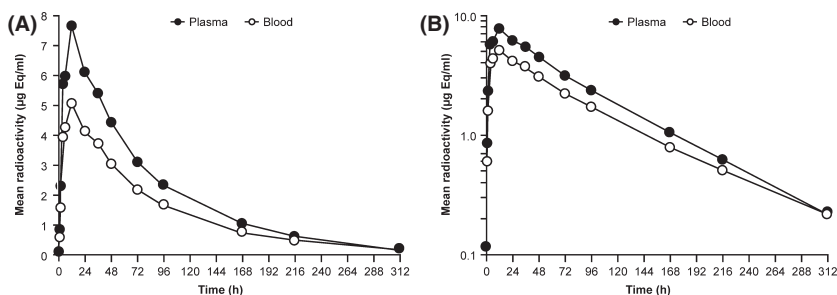


Figure 3. Mean ^{14}C -vemurafenib concentration-time profiles in plasma (closed circles) and blood (open circles) ($n = 5$). (A) linear scale, (B) logarithmic scale.

Table 2. ^{14}C -vemurafenib pharmacokinetics after a single oral dose.

Parameter, mean \pm SD [CV%]	Blood	Plasma
<i>N</i>	5	5
C_{\max} ($\mu\text{g eq/mL}$)	5.25 \pm 1.71 [32.5]	7.83 \pm 2.28 [29.1]
T_{\max} (h)	4.1 (4–12) ¹	4.1 (4–12) ¹
AUC_{last} ($\mu\text{g eq/mL}\cdot\text{h}$)	456 \pm 85.4 [18.7]	633 \pm 123 [19.4]
$t_{1/2}$ (h)	84.4 \pm 24.6 [29.1]	71.1 \pm 15.4 [21.6]
Blood/plasma AUC ratio	0.72 \pm 0.050 [6.83]	N/A

AUC, area under the plasma concentration–time curve; C_{\max} , maximum observed plasma concentration; T_{\max} , time to maximum observed plasma concentration; $t_{1/2}$, elimination half-life.

¹Median (minimum–maximum).

associated with the parent compound. The monohydroxylated species M3 (Fig. 1) was the only metabolite detected in plasma (Fig. 4A). The mean percentage of M3 increased with time from 0.5% to ~4% between 12 and 24 h and remained constant in the 48-h pool.

Limited metabolites of ^{14}C -vemurafenib were identified in human fecal samples. In the first 48 h, $\geq 94\%$ of all recovered radioactivity in feces was associated with the parent compound (mean \pm SD, 94.2 \pm 5.8%), with total metabolites accounting for $\leq 6\%$ of the total administered dose (2.6, 0.2, and 3.1% for the M6, M3, and M8 metabolites, respectively; Fig. 4B). Between 48 and 96 h, total metabolites accounted for a considerably higher proportion of radioactivity, with mean values of 55.5 \pm 20.1% for the parent compound and 18.8%, 13.7%, and 11.9% for M6, M3, and M8, respectively (Fig. 4B).

When calculated as mean \pm SD of the total radioactive dose in pooled fecal samples, ~55% of the total radioactive dose was found as a parent molecule, and 6.0%, 3.4%, and 4.1% as M6, M3, and M8, respectively, within the first 96 h. In the first 48 h, the parent molecule was 38% of the total input dose and M6, M3, and M8 were 1.0%, 0.1%, and 1.2%, respectively. From 48 to 96 h, the parent molecule was 17% of the total input radioactive dose and M6, M3, and M8 were 5.0%, 3.3%, and 2.9%, respectively. Due to limitations in the amount of radioactive material, metabolic profiling was not conducted at subsequent times.

Despite extensive sample collection, low levels of radioactivity were present in pooled urine sample extracts. Therefore, a single concentrated pool of urine was analyzed covering the whole observation period (0–96 h). Potential metabolites each accounted for $< 0.5\%$ of total administered dose in urine, and the parent compound accounted for ~1% of total input radioactive dose. M3 and a parent glycosylated metabolite M6 were detected

(Fig. 4C). Two additional regions of radioactivity were observed in chromatograms of some urine samples ($< 0.2\%$ of input radioactive dose on average, with approximate retention times of 15 and 20 min). However, levels of radioactivity in these regions were close to the limit of detection of the radio-HPLC system. Overall, no new metabolites were detected in plasma or feces. Representative full-scan data and MSn (multistage MS) data for metabolites M3 (from feces and plasma), M6 and M8 (both from feces) are presented in Figures 5A–C.

Safety

The seven patients had a mean duration of exposure to vemurafenib of 74.1 days (range, 28–112 days) and received a mean cumulative dose of 138.6 g (range, 53.8–215.0 g) (this includes the initial 14 days of unlabeled vemurafenib). All patients experienced at least one adverse event related to vemurafenib; most adverse events were mild or moderate (NCI CTCAE grade 1 or 2).

The most common adverse events (occurring in ≥ 2 patients) were fatigue (five patients), arthralgia (four patients), hyperkeratosis (five patients), maculopapular rash (four patients), and papilloma (three patients). Alopecia, erythema, photosensitivity reaction (Dummer et al. 2012a), pruritus, peripheral edema, abdominal distension, (Zimmer et al. 2012) diarrhea, and cuSCC were reported for two patients each. These adverse events are not uncommon and have been universally reported across vemurafenib studies (Flaherty et al. 2010; Chapman et al. 2011; Ribas et al. 2011; Sosman et al. 2012; McArthur et al. 2014). Adverse events of grade 3 severity were reported for five patients: cuSCC was reported for two patients; generalized rash, fatigue (resulting in dose interruption), and keratoacanthoma (see below) were reported for one patient each. No grade 4 adverse events were reported.

Three serious adverse events (all grade 3) were recorded for two patients. One patient had cuSCC and one had cuSCC and keratoacanthoma. Lesions were excised and resolved with no sequelae. All three events were considered related to vemurafenib; however, none resulted in dose modification/interruption or discontinuation of vemurafenib.

At data cutoff, two patients were alive (> 2 years after study start). Five patients died of progressive disease. No patient discontinued treatment because of an adverse event.

In terms of laboratory evaluations, grade 3 lymphopenia was recorded for four patients, low white blood cell count was recorded for three patients, elevated low-density lipoprotein cholesterol level was noted for three patients, and elevated γ -glutamyl transferase levels were seen in one patient. No clinically significant changes in electrocardiography or vital signs were reported.

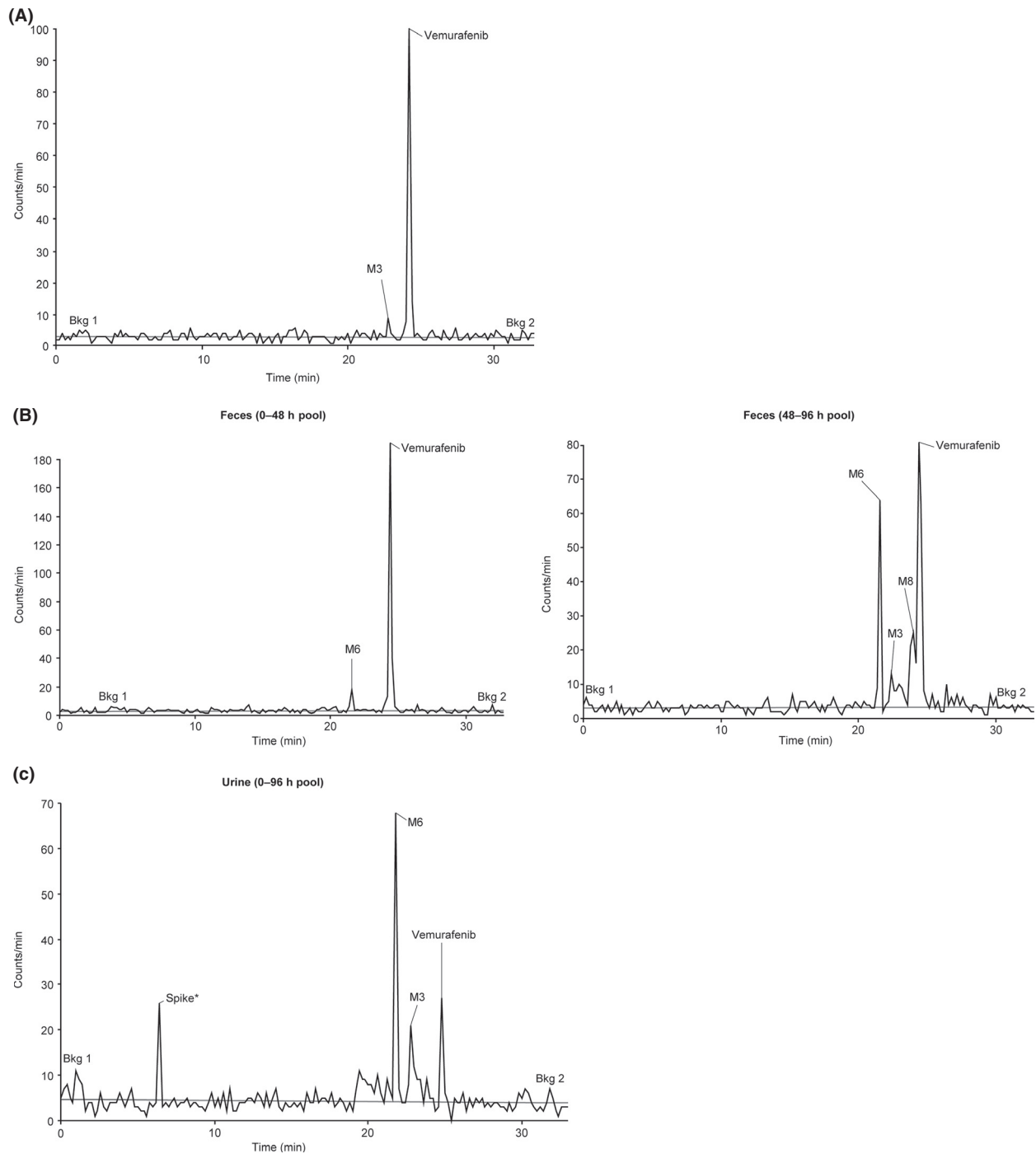


Figure 4. Metabolic profiling after a single dose of ^{14}C -vemurafenib in (A) plasma (36–48 h), (B) feces (0–48 h; 48–96 h), and (C) urine (0–96 h). *Electrical spike caused by static (excluded from profile as not a metabolite).

Discussion

The human mass balance of vemurafenib in this study in adults with metastatic melanoma was adequately represented because mean recovery of total radioactivity administered as ^{14}C -vemurafenib across the seven patients was

~95.0% (range, 91.0–98.3%). Results from this study show that vemurafenib was primarily excreted unchanged in feces, with minimal renal excretion. Approximately 94% of input radioactivity was recovered in feces, with $\leq 1\%$ in collected urine. Limited metabolism of vemurafenib was evident in plasma, feces, and urine. Approximately 55% of the

(A)

Type of fragmentation	Precursor ion	Observed m/z	Elemental composition*
Full scan	--	506.0738	$C_{23}H_{19}ClF_2N_3SO_4$
MS/MS	506	488.0641	$C_{23}H_{17}ClF_2N_3SO_3$
MS/MS	506	384.0709	$C_{20}H_{13}ClF_2N_3O$
MS ³	384	255.0320	$C_{14}H_8ClN_2O$
MS ³	384	363.0576	$C_{20}H_{11}ClFN_3O^+$

*protonated species

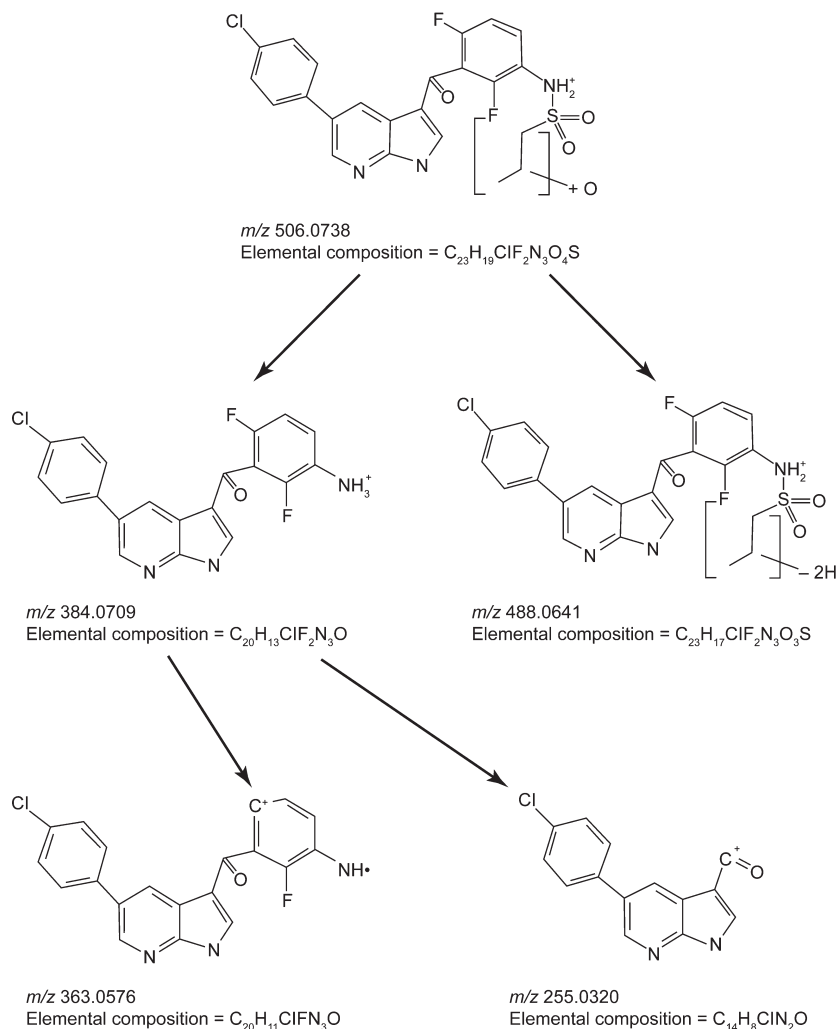


Figure 5A. Metabolite M3 (m/z 506.0738). Monohydroxylation. Representative full-scan data and MSⁿ data were obtained from feces and plasma samples. The observed m/z values and proposed elemental compositions are summarized. A fragmentation scheme is also shown.

total radioactive dose was found in feces as a parent molecule, with the three main metabolites showing $\leq 6\%$ each of the input dose within the first 96 hours post-dose. During this time, metabolites accounted for $\leq 0.5\%$ of the total administered dose in urine. Limited metabolism of vemurafenib was also evident in plasma, in which $>95\%$ of the recovered radioactivity was associated with the parent molecule over a 48-h interval. Metabolic profiling could not be conducted in pooled samples beyond 48 to 96 h because of a limited amount of radioactive material available.

The low level of metabolite formation in the general systemic circulation is consistent with previous studies in animal models (F. Hoffmann-La Roche, data on file), in which unchanged parent drug was the major component in rat and dog plasma after oral administration. In agreement with the present findings, studies in rats showed that vemurafenib-derived radioactivity (parent plus metabolites) was primarily recovered in feces (F. Hoffmann-La Roche, data on file), with most of the radioactivity related to unchanged parent drug

(B)

Type of fragmentation	Precursor ion	Observed <i>m/z</i>	Elemental composition*
Full scan	--	652.1325	C ₂₉ H ₂₉ O ₈ N ₃ ClF ₂ S
MS/MS	652	490.0798	C ₂₃ H ₁₉ O ₃ N ₃ ClF ₂ S
MS ³	490	383.0630	C ₂₀ H ₁₂ ON ₃ ClF ₂
MS ³	490	255.0319	C ₁₄ H ₆ ON ₂ Cl

*protonated species

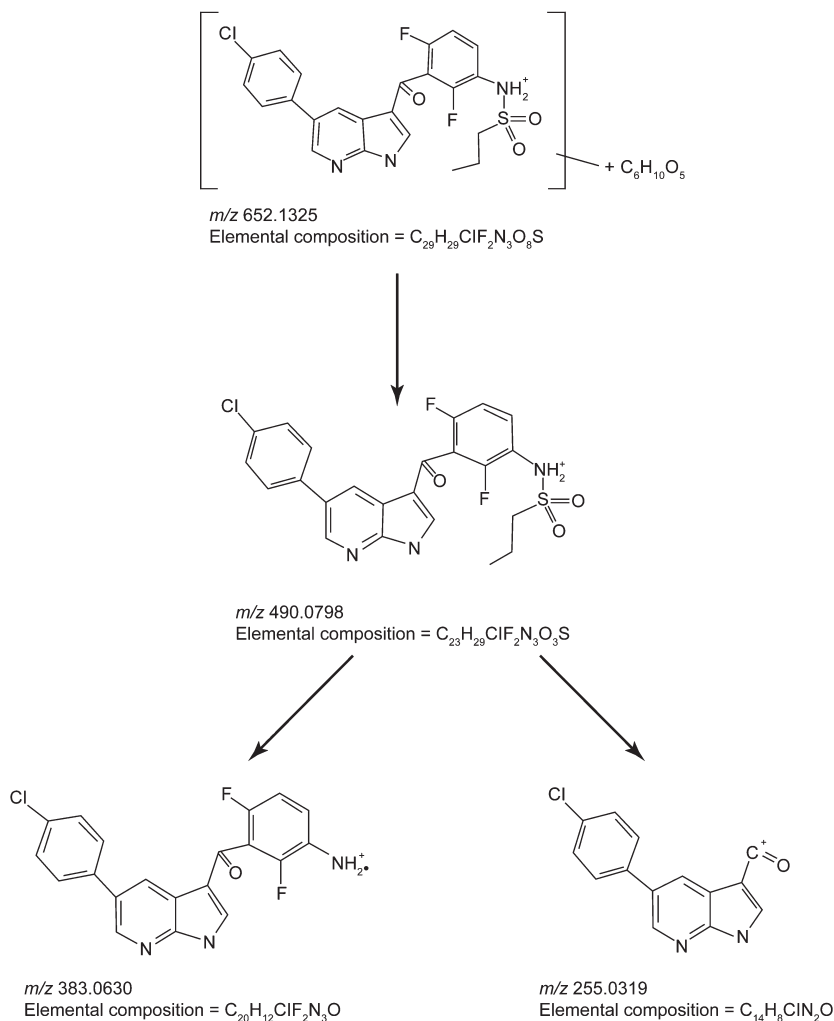


Figure 5B. Metabolite M6 (*m/z* 652.1325). Glucosylation. Representative full-scan data and MSn data were obtained from feces samples. The observed *m/z* values and proposed elemental compositions are summarized. A fragmentation scheme is also shown.

(F. Hoffmann-La Roche, data on file). Furthermore, metabolites detected in the current study are the same as those previously identified *in vitro* (hepatocytes and microsomes) and *in vivo* (rats and dogs) (F. Hoffmann-La Roche, data on file). After *in vitro* incubation of vemurafenib with mouse, rat, dog, monkey, and human liver microsomes and hepatocytes, unchanged parent compound RO5185426 was the major component (>91.5%), with formation of minor metabolites. No major metabolite was detected. Monohydroxylation

metabolites (M1–M4) were the minor metabolites detected in microsomes and hepatocytes; additionally, minor glucuronide metabolites (M5, M7, and M8) were also formed in hepatocytes. Metabolite M6, with addition of 162 mass units to RO5185426, formed with only human hepatocytes in small amounts (2.3%) and was characterized as a glucosylation metabolite of vemurafenib (Fig. 5B).

Metabolite identification was not the primary objective of this study. Previous investigations in human micro-

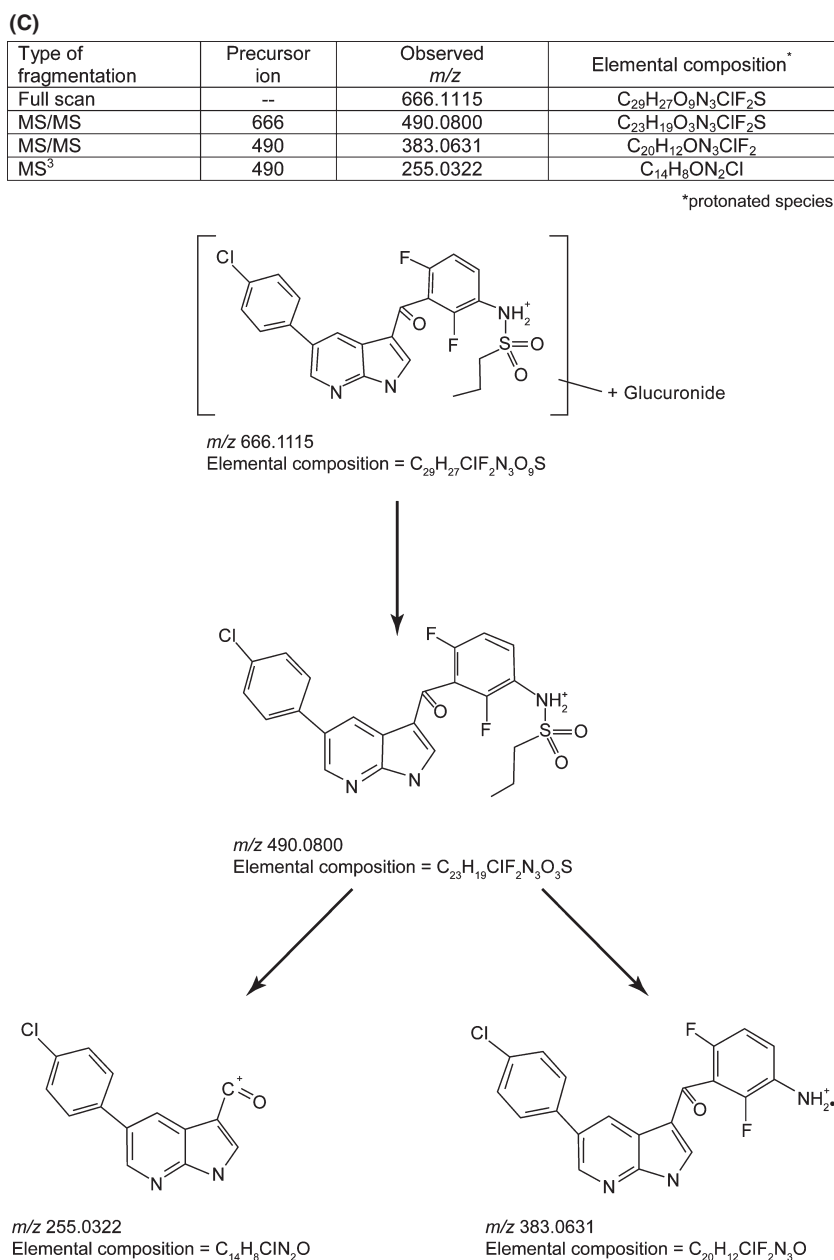


Figure 5C. Metabolite M8 (*m/z* 666.1115). Glucuronide. Representative full-scan data and MSn data were obtained from feces samples. The observed *m/z* values and proposed elemental compositions are summarized. A fragmentation scheme is also shown.

somes and in rats did not yield precise chemical structures of vemurafenib metabolites. Mass spectral analysis has been used to determine only the elemental composition of potential metabolites, providing clues to potential metabolite identification (Fig. 1). In the current study, low levels of metabolites were identified by LC-MS/MS: M3 representing <5% of radioactivity in each plasma sample, and M3 and M6 in urine and M3, M6, and M8 in feces, representing <0.5% and ≤6% of the input radioactive dose, respectively. Urine also contained very low

levels of two unidentified metabolites. Further characterization of potential metabolites was not possible because of difficulty separating peaks from background noise.

In vitro incubation studies of vemurafenib with human complementary cDNA-expressed enzymes and P450 isoform-specific chemical inhibitors showed that CYP3A4 was the primary enzyme responsible for metabolism of vemurafenib and formation of hydroxylated metabolites M1 to M4 in human liver microsomes. No further work has been done to characterize specific enzymes involved

in formation of glucuronidated (M5, M7, M8) or glycosylated (M6) metabolite species (F. Hoffmann-La Roche, data on file).

Overall, these observations support the view that the minor vemurafenib metabolism that occurs overall is hepatic, with excretion via bile into feces the predominant elimination route from plasma. The data show that the parent drug predominates in feces even in the 48- to 96-h pool post radioactive dose. It is interesting to speculate that predominance of the parent molecule found in the 48-h pooled sample partially represents unabsorbed drug, whereas the parent molecule found in the second pooled fraction from 48 to 96 h represents parent drug generated through hepatobiliary recirculation. This interpretation is consistent with data from bile-cannulated rat studies (F. Hoffmann-La Roche, data on file) and from recent data in which metabolites M6 and M8 predominated over the parent molecule in a bile sample from one patient receiving vemurafenib (manuscript in preparation). The predominant metabolites in feces seem to be glucuronidated (M8) and glycosylated (M6) species that might be substrates for gut flora, allowing for reconversion to the parent molecule in the intestine for subsequent reabsorption into the systemic circulation. Additional studies are planned to confirm the role of enterohepatic circulation.

Apparent clearance of vemurafenib in patients with preexisting mild and moderate renal impairment (mild: $n = 94$, creatinine clearance [CLcr] >60–89 mL/min, moderate: $n = 11$, CLcr 30–59 mL/min) was similar to that in patients with normal renal function (CLcr ≥ 90 mL/min), (Genentech, Inc. 2014) supporting the finding that a dose adjustment is not necessary for patients with mild or moderate renal impairment. Furthermore, in the population PK analysis using data from clinical trials in patients with metastatic melanoma, pre-existing mild and moderate renal impairment did not influence apparent clearance of vemurafenib (Genentech, Inc. 2014). Therefore, the finding that <1% of input radioactivity was recovered in urine suggests a minimal role for renal excretion in vemurafenib disposition.

It was not possible to accurately determine the elimination half-life of unchanged vemurafenib from plasma radioactivity because metabolite profiling was only conducted for the first 48 h and the amount of radiolabeled material was too low to characterize afterward. However, during the first 48 h, >95% of plasma radioactivity was accounted for by the parent compound. Elimination half-life of the radioactivity was 71 h (range, 51–86 h). This value lies within the range estimated from a population PK model (manuscript in preparation), in which the median of the individual elimination half-life estimate for vemurafenib was 57 h (the 5th and 95th percentile range was 30–120 h based on data predominantly taken from

multiple-dose kinetics of unlabeled material (Genentech, Inc. 2014). The elimination half-life after a single dose was considerably lower at ~25 h (Ribas *et al.* 2014). Vemurafenib reabsorption during multiple dosing through enterohepatic recirculation might account for increased apparent elimination half-life (Roberts *et al.* 2002; Shou *et al.* 2005).

Vemurafenib was well tolerated by the seven patients in the study. Reported adverse events are consistent with those observed in larger clinical studies of vemurafenib (Sosman *et al.* 2012; Chapman *et al.* 2011; McArthur *et al.* 2014; Oberholzer *et al.* 2011; Zimmer *et al.* 2012; Dummer *et al.* 2012b). Overall, no new safety signals were observed.

The vemurafenib dose selected for this study (960 mg twice daily) was based on phase 1 data, which identified this dosing regimen as the maximum tolerated dose and recommended dose for further clinical evaluation (Flaherty *et al.* 2010). Furthermore, 960 mg twice daily is the approved dose for treatment of advanced *BRAF* mutation-positive melanoma (Genentech, Inc. 2014). Manufacturing restrictions meant that the radioactive formulation of MBP could not be processed to the tablet formulation. Therefore, ^{14}C -vemurafenib was supplied as hand-filled capsules. No differences in vemurafenib pharmacokinetics were found in patients treated with hand-filled capsules or tablets in the phase 1 trial PLX06-02, and use of capsules containing radioactive vemurafenib in this study was not expected to materially alter the metabolic profile of the investigational drug. This theory is supported by an elimination half-life of ^{14}C -vemurafenib within the range of PK data for unlabeled compound (Genentech, Inc. 2014) and by a metabolite profile consistent with those reported in preclinical and in vitro studies (F. Hoffmann-La Roche, data on file).

In conclusion, this study determined the mass balance, metabolism, and rates and routes of elimination of vemurafenib in patients with metastatic melanoma. Vemurafenib was primarily excreted unchanged in feces. Renal excretion was minor, and limited metabolism of vemurafenib was evident in plasma. The limited metabolism that occurred was predominantly hepatic; therefore, excretion via bile into the feces is the predominant elimination route from plasma for parent vemurafenib. Furthermore, vemurafenib was well tolerated and no new safety signals were observed.

Acknowledgements

The authors thank Thomas Hartung, Roland Degan, Lucy Chen, and Peter Belica for contribution to the isotope work during this study. The authors thank Joy Hsu from the Roche Innovation Center New York for reference to the PK modeling work that appears in the Zelboraf

prescribing information. Support for third-party editing assistance for this manuscript was provided by F. Hoffmann-La Roche Ltd. This study was sponsored by F. Hoffmann-La Roche, Ltd.

Disclosures

Simone M. Goldinger has received research funding from the University of Zurich, has served on advisory boards for Bristol-Myers Squibb, and has received travel grants from Bristol-Myers Squibb, Roche, and Merck Sharp & Dohme. Reinhard Dummer has served on advisory boards and has been a consultant for Bristol-Myers Squibb, Roche, Merck Sharp & Dohme, GlaxoSmithKline and Novartis, and has been a consultant for Amgen, and has been an investigator for Bristol-Myers Squibb, Merck Sharp & Dohme, and Novartis. Lucy Lee is an employee of Eisai. Ruben Coronado Ayala is an employee of Hoffmann-La Roche. Jagdish Pacha is a former employee of Roche. Wanping Geng is a former employee of Hoffmann-La Roche and has stock options. David Moore is an employee of Roche Innovation Center New York. Mei Lui is a former employee of Roche and current employee at Revlon and provided uncompensated services for Roche and Revlon. Andrew K. Joe is a former employee of Hoffmann-La Roche and has stock options. Selby Patricia Gil Bazan is an employee of F. Hoffmann-La Roche Ltd. Joseph F. Grippo is an employee of Roche Innovation Center New York with company stock. Jeanine Rinderknecht and Felix Pierre Kuhn declare that they have no conflicts of interest. Kuo-Hsiung Yang did not declare any conflicts of interest.

References

- Atkins MB, Hsu J, Lee S, Cohen GI, Flaherty LE, Sosman JA, et al. (2008). Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2, and interferon alfa-2b with cisplatin, vinblastine, and dacarbazine alone in patients with metastatic malignant melanoma (E3695): a trial coordinated by the Eastern Cooperative Oncology Group. *J Clin Oncol* 26: 5748–5754.
- Avril MF, Aamdal S, Grob JJ, Hauschild A, Mohr P, Bonerandi JJ, et al. (2004). Fotemustine compared with dacarbazine in patients with disseminated malignant melanoma: a phase III study. *J Clin Oncol* 22: 1118–1125.
- Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, et al. (2010). Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* 467: 596–599.
- Chapman PB, Einhorn LH, Meyers ML, Saxman S, Destro AN, Panageas KS, et al. (1999). Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. *J Clin Oncol* 17: 2745–2751.
- Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. (2011). Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 364: 2507–2516.
- Chapman P, Hauschild A, Robert C, Larkin J, Haanen J, Ribas A, et al. (2012). Updated overall survival results for BRIM-3, a phase III randomized, open-label, multicenter trial comparing the BRAF inhibitor, vemurafenib with dacarbazine in previously untreated patients with BRAF^{V600E}-mutated metastatic melanoma. *J Clin Oncol* 30 (Suppl.): abstract 8502.
- Dummer R, Flaherty KT (2012). Resistance patterns with tyrosine kinase inhibitors in melanoma: new insights. *Curr Opin Oncol* 24: 150–154.
- Dummer R, Hauschild A, Guggenheim M, Keilholz U, Pentheroudakis G; ESMO Guidelines Working Group (2012a). Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 23 (Suppl. 7): vii86–vii91. doi:10.1093/annonc/mds229.
- Dummer R, Rinderknecht J, Goldinger SM (2012b). Ultraviolet A and photosensitivity during vemurafenib therapy. *N Engl J Med* 366: 480–481.
- Falkson CI, Ibrahim J, Kirkwood JM, Coates AS, Atkins MB, Blum RH (1998). Phase III trial of dacarbazine versus dacarbazine with interferon alpha-2b versus dacarbazine with tamoxifen versus dacarbazine with interferon alpha-2b and tamoxifen in patients with metastatic malignant melanoma: an Eastern Cooperative Oncology Group study. *J Clin Oncol* 16: 1743–1751.
- Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, et al. (2010). Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 363: 809–819.
- Genentech, Inc (2014). Zelboraf [prescribing information]. Genentech, Inc, South San Francisco, CA.
- Grippo JF, Zhang W, Heinzmann D, Yang KH, Wong J, Joe AK, et al. (2014). A phase I, randomized, open-label study of the multiple-dose pharmacokinetics of vemurafenib in patients with BRAF V600E mutation-positive metastatic melanoma. *Cancer Chemother Pharmacol* 73: 103–111.
- Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. (2012). Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 380: 358–365.
- Lacouture ME, McArthur GA, Chapman PB, Ribas A, Flaherty KT, Lee RJ, et al. (2010). PLX4032 (RG7204), a selective mutant RAF inhibitor: clinical and histologic characteristics of therapy-associated cutaneous neoplasms in a phase I trial. *J Clin Oncol* 28: abstract 8592.
- McArthur GA, Chapman PB, Robert C, Larkin J, Haanen JB, Dummer R, et al. (2014). Safety and efficacy of vemurafenib in BRAF^{V600E} and BRAF^{V600K} mutation-positive melanoma

(BRIM-3): extended follow-up of a phase 3, randomised, open-label study. *Lancet Oncol* 15: 323–332.

Middleton MR, Grob JJ, Aaronson N, Fierlbeck G, Tilgen W, Seiter S, et al. (2000). Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. *J Clin Oncol* 18: 158–166.

Oberholzer PA, Kee D, Dziunycz P, Sucker A, Kamsukom N, Jones R, et al. (2011). RAS mutations are associated with the development of cutaneous squamous cell tumors in patients treated with RAF inhibitors. *J Clin Oncol* 30: 316–321.

Patel PM, Suci S, Mortier L, Kruit WH, Robert C, Schadendorf D, et al. (2011). Extended schedule, escalated dose temozolomide versus dacarbazine in stage IV melanoma: final results of a randomised phase III study (EORTC 18032). *Eur J Cancer* 47: 1476–1483.

Ribas A, Kim KB, Schuchter LM, Gonzalez R, Pavlick AC, Weber JS, et al. (2011). BRIM2: an open-label, multicenter phase II study of vemurafenib in previously treated patients with BRAF V600E mutation-positive metastatic melanoma. *J Clin Oncol* 2(Suppl.): abstract:8509.

Ribas A, Zhang W, Chang I, Shirai K, Ernstoff MS, Daud A, et al. (2014). The effects of a high-fat meal on single-dose vemurafenib pharmacokinetics. *J Clin Pharmacol* 54: 368–374.

Robert C, Arnault JP, Mateus C (2011). RAF inhibition and induction of cutaneous squamous cell carcinoma. *Curr Opin Oncol* 23: 177–182.

Roberts MS, Magnusson BM, Burczynski FJ, Weiss M (2002). Enterohepatic circulation: physiological, pharmacokinetic and clinical implications. *Clin Pharmacokinet* 41: 751–790.

Shah N, Iyer RM, Mair H-J, Choi DS, Tian H, Diodone R, et al. (2013). Improved human bioavailability of vemurafenib, a

practically insoluble drug, using an amorphous polymer-stabilized solid dispersion prepared by a solvent-controlled coprecipitation process. *J Pharm Sci* 102: 967–981.

Shou M, Lu W, Kari PH, Xiang C, Liang Y, Lu P, et al. (2005). Population pharmacokinetic modeling for enterohepatic recirculation in Rhesus monkey. *Eur J Pharm Sci* 26: 151–161.

Søndergaard JN, Nazarian R, Wang Q, Guo D, Hsueh T, Mok S, et al. (2010). Differential sensitivity of melanoma cell lines with BRAFV600E mutation to the specific Raf inhibitor PLX4032. *J Transl Med* 8: 39.

Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, et al. (2012). Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 366: 707–714.

Thomas RK, Baker AC, DeBiasi RM, Winckler W, Laframboise T, Lin WM, et al. (2007). High-throughput oncogene mutation profiling in human cancer. *Nat Genet* 39: 347–351.

Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, et al. (2008). Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc Natl Acad Sci USA* 105: 3041–3046.

Tsao H, Atkins MB, Sober AJ (2004). Management of cutaneous melanoma. *N Engl J Med* 351: 998–1012.

Zimmer L, Hillen U, Livingstone E, Lacouture ME, Busam K, Carvajal RD, et al. (2012). Atypical melanocytic proliferations and new primary melanomas in patients with advanced melanoma undergoing selective BRAF inhibition. *J Clin Oncol* 30: 2375–2383.