Plasma vemurafenib concentrations in advanced BRAFV600mut melanoma patients: impact on tumour response and tolerance†

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Background: Vemurafenib improves survival in advanced BRAFV600mut melanoma patients, but tolerance is often poor and resistance frequently occurs, without predictive factor. Our aim was to investigate for the first time a relationship between plasma vemurafenib concentration (PVC) and efficacy or tolerance.

Methods: Plasma samples from unresectable metastatic BRAFV600mut melanoma patients treated with vemurafenib monotherapy were prospectively collected at each tumour response evaluation (RECIST 1.1) or when adverse event occurred (CTCAE 4.0). PVC was measured with liquid chromatography–tandem mass spectrometry. Herein, we report on PVC at steady state (≥14 days after vemurafenib introduction or dose modification). Samples collected after first

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melanoma progression were excluded from the response analysis. All samples were analysed in the tolerance analysis. We kept the closest collected sample from the onset of each adverse effect or the one with the highest PVC in the absence of this adverse effect. Comparisons of means (Student’s t-tests and Wilcoxon rank sum tests) and of frequencies ($\chi^2$ tests) were carried out. A logistic regression analysis identified predictors of progression.

**Results:** We included 105 plasma samples in 23 patients (10M/13F). Initial vemurafenib dose was 960 mg b.i.d., reduced by 25% (8 patients) or 50% (2 patients) for intolerance in 10 patients (44%). PVC displayed high inter-individual variability (13.0–109.8 µg/ml, median 54.0). Mean PVC was lower at time of progression (38.8 ± 19.7 µg/ml) than mean PVC found when tumour was stable or in partial or complete response (56.4 ± 21.0 µg/ml, $P = 0.013$, 21 patients). Logistic regression revealed that having a low PVC ($P = 0.01$) or brain metastasis ($P = 0.01$) were both significantly and independently associated with tumour progression. High PVC was not statistically significantly associated with the occurrence of adverse effects.

**Conclusion:** PVC at steady state is highly variable and low PVC was associated with tumour progression, suggesting a new path to melanoma resistance to vemurafenib.

**Key words:** vemurafenib, pharmacokinetics, metastatic melanoma, melanoma progression, tumour response, tolerance

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**Introduction**

The incidence of cutaneous melanoma is rising in Caucasians. Vemurafenib, a recently approved BRAF-targeted therapy belonging to the rising class of kinase inhibitors, has been shown to improve disease-free and overall survivals in inoperable American Joint Committee on Cancer (AJCC) stage III–IV patients with melanoma harbouring a BRAFV600E mutation [1]. However, tumour escape frequently occurs and significant adverse effects have been observed with vemurafenib, such as arthralgia, skin rash, photosensitivity, alopecia, fatigue, nausea, diarrhoea, and cutaneous squamous cell carcinoma. No correlation has been shown between the occurrence of these adverse events and better or longer efficacy of vemurafenib on tumour control, as it has been reported for skin toxicity with EGFR inhibitors in some cancers [2]. There are to date no known predictive factors of either anti-tumour response or occurrence of adverse events in vemurafenib-treated melanoma patients.

According to the summary of product characteristics [3], the median elimination half-life of vemurafenib is 51.6 h, with the 5th and 95th percentile of individual half-life estimates ranging from 29.8 to 19.5 h. This suggests high inter-patient variability, which might also influence drug efficacy and tolerance. Only one study, carried out during the early development of vemurafenib when the exact formulation was not standardized, studied steady-state concentration needed for efficacy in melanoma [4]. Our aim was to investigate for the first time in the ‘real life’ a potential relationship between plasma vemurafenib concentration (PVC) at steady state and tumour control or occurrence of adverse events.

**Methods**

Data were prospectively collected in our specialized skin cancer department between April 2012 (date of vemurafenib early access programme and early commercialization in France) and December 2013 in unresectable stage IIIIC or IV BRAFV600E mutated melanoma patients treated with vemurafenib monotherapy and not otherwise included in any clinical trial. BRAFV600E mutational status was assessed as previously reported [5]. These patients were followed and monitored in the department according to our standard procedures. They had a consultation with one member of our team 1 and 2 times after beginning vemurafenib treatment, and then every 2 months until progression. Additional visits could be required in case of adverse event. During these consultations, patients were instructed repeatedly to adhere strictly to the manufacturer instruction brochure, which requires ingesting vemurafenib tablets during a meal. Just before or after these visits, blood was collected for standard blood tests (blood cell count, renal, and liver function tests). Efficacy was assayed every 2 months using thoracic and abdominopelvic computed tomographic (CT) scans, cerebral CT scan or magnetic resonance imaging, carried out by experienced radiologists. All images were collectively re-analysed on weekly basis during a joint meeting with the radiologists and tumour response classified according to RECIST version 1.1 [6] as complete response, partial response, stability, or progression in comparison with images obtained just before beginning vemurafenib therapy. Vemurafenib treatment could be continued when a patient on treatment experienced a progression of one or few lesions that could be treated with a local treatment such as radiotherapy or surgery.

This tumour evaluation was carried out without knowing PVCs and stored prospectively in a database. Adverse events were also routinely graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, without knowing PVCs.

After informing patients and obtaining oral consent, we prospectively collected an additional blood sample of 10 ml in patients during their regular follow-up or for unscheduled visits provided that blood had to be collected for usual blood tests. Blood was centrifuged, plasma frozen, and kept at −20°C until assayed. The following information was collected prospectively when blood was drawn: dose of vemurafenib, time of vemurafenib ingestion, and time of blood collection, declared compliance to therapy, and drug co-prescriptions. PVC was assessed without knowing tumour response or tolerance status using a validated liquid chromatography–tandem mass spectrometry method with electrospray ionization (precision: 13%; accuracy: >93%) and vemurafenib-D$_3$ as internal standard [7].

For this study, we searched in our database the records of vemurafenib-treated patients who had at least one PVC measured at steady state. Steady state was defined as no introduction nor modification of vemurafenib dose within 14 days before blood collection. To study the relationship between PVC and tumour response, we searched in our database, for each assay of PVC, the result of tumour response assessment carried out around blood collection date. Only assays carried out until first progression were analysed to study the relation between PVC and tumour response. To study the relationship between PVC and tolerance, we analysed the result of vemurafenib assay carried out closest to the onset of each adverse effect. In the absence of each adverse effect, we kept for this analysis, for each patient, the highest PVC measured.

Quantitative data were expressed as mean ± standard deviation (SD), median and range; qualitative data as frequency and percent. Comparisons of quantitative data were carried out using the Student’s t-test and the
Wilcoxon rank sum test as appropriate. Comparison of frequencies was carried out using the $\chi^2$ test. A linear regression analysis was used to determine the trend of PVC s over time. A logistic regression analysis was conducted to identify the predictive factors of progression. A $P$ value <0.05 was considered statistically significant. Statistical analysis was carried out using SAS software version 9.3 (SAS Institute, Inc., Cary, NC). PVCs were not normalized for the administered dose.

This study was approved by the local ethics committee (CPP Ile de France XI), and considered to be standard of care and, according to the French Law, did not require a written informed consent. However, consent was obtained orally from all patients. Study was conducted according to the principle of the declaration of Helsinki [8].

results

We analysed 105 plasma samples (range 1–8 per patient) from 23 vemurafenib-treated patients (10 M/13 F) with metastatic, $BRAF^{V600E}$ mutated, unresectable melanoma of either AJCC stage IIIC ($N=3$) or IV ($N=20$). Among AJCC stage IV patients, 12 (60%) had brain metastases, and 2 had been treated with stereotaxic cerebral radiotherapy during vemurafenib treatment.

Vemurafenib was used as first-line therapy in 17 patients (74%), second-line in 2 patients (9%) and third-line or more in 4 patients (17%). Among the 12 patients with brain metastasis, 8 received vemurafenib as first-line therapy (67%). Previous treatments were immunotherapy with ipilimumab ($N=5$) or vaccination protocol ($N=1$), or chemotherapy as dacarbazine ($N=3$), fotemustine ($N=2$), and cisplatine ($N=1$).

Initial vemurafenib dose was 960 mg b.i.d. for all patients, reduced by 25% ($N=8$) or 50% ($N=2$) for grade CTCAE $\geq$2 adverse effects in 10 patients (44%). Dose was increased by 25% in one patient with a very slowly progressive melanoma and excellent tolerance of vemurafenib. Adverse effects that led to dose reduction were widespread maculopapular skin rash ($N=5$), diarrhoea ($N=1$), fatigue ($N=2$), acute renal failure ($N=1$), and uveitis ($N=1$). All patients declared high compliance to treatment.

Figure 1 displays PVCs for each patient according to the dose ingested at time of blood sampling. We observed a high inter-individual variability of PVC (13.0–109.8 $\mu$g/ml, median 54.0). When considering only samples taken while patients were taking full-dose vemurafenib, variability was still high, with a median concentration of 55.0 $\mu$g/ml and a range of 13.0–109.8 $\mu$g/ml (data not shown). We did not observe significant decrease of PVCs with time ($r=−0.20$, $P=0.09$). We did not find any impact of body mass index on PVCs (data not shown). Patients were instructed to take vemurafenib according to manufacturer instruction. Only one patient declared to take repeatedly vemurafenib at distance of meals: mean PVC of this patient was $56.2 \pm 5.0 \mu$g/ml.

Among the 105 plasma samples, 32 were drawn after first progression; thus, a total of 73 plasma samples, taken from 21 patients (range 1–7 per patient), were available to study the relation between PVC and tumour response. Mean delay between tumour response evaluation and blood collection date was 10.4 days. Clinical characteristics of the 21 patients included in this response-concentration analysis are summarized in Table 1 and Figure 2. Twelve patients had first tumour progression during study, after a mean 35 weeks on vemurafenib (Figure 2). Mean PVC was lower in patients at time of first progression.
(38.8 ± 19.7 µg/ml; number of samples N = 12, from 12 patients) compared with mean PVC measured when tumour was considered stable (N = 3, from 3 patients), or in partial (N = 36, from 14 patients) or complete (N = 22, from 5 patients) response (56.4 ± 21.0 µg/ml; N = 61 from 18 patients; P = 0.013; Figure 3). Progression was also significantly more frequently observed at evaluations of patients who exhibited brain metastasis before beginning vemurafenib than for patients without prior brain metastasis (P = 0.02). Logistic regression revealed that having a low vemurafenib concentration [P = 0.01, odds ratio (OR) = 1.053 (1.010–1.100) for each 1 µg/ml decrease] or brain metastasis (P = 0.01, OR = 7.1 (1.5–32.8)) were both significantly associated with tumour progression.

These results were not influenced by the number of plasma samples taken into consideration in each patient. When considering only the first plasma sample harvested, mean PVC in the 12 patients at time of first progression (38.8 ± 19.7 µg/ml) was still significantly lower than in the patients with complete or partial response or who were considered as stable (59.8 ± 20.0 µg/ml; P = 0.009). In another sensitivity analysis, we defined steady state as no introduction nor modification of vemurafenib dose within 28 days before blood collection. Using this definition, only 6 samples (of 73) would have been removed from the efficacy analysis, which did not change our results (data not shown).

To study the relationship between PVC and tolerance, we included all 105 samples. No significant difference was observed between mean PVC and the occurrence of each adverse effect, such as alopecia (N = number of patients with, N = 16; P = 0.61), palmoplantar keratoderma (N = 13; P = 0.48), maculopapular rash (N = 5; P = 0.11), panniculitis (N = 5; P = 0.97), squamous cell carcinoma (N = 5; P = 0.36), arthromyalgia (N = 10; P = 0.95), diarrhoea (N = 11; P = 0.12), uveitis (N = 1), increase >30% of creatinine level (N = 5; P = 0.1), transaminitis (N = 3; P = 0.11). We observed only a trend towards an increase of the mean PVC for two adverse effects: maculopapular rash and creatinine-level increase >30%.

Table 1. Clinical characteristics of 21 patients in whom blood samples were drawn before first melanoma progression on vemurafenib

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years ± SD)</td>
<td>51 ± 17</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>11/10</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23.2 ± 3.5</td>
</tr>
<tr>
<td>AJCC staging</td>
<td></td>
</tr>
<tr>
<td>IIIc</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>IV</td>
<td>18 (86%)</td>
</tr>
<tr>
<td>M1a</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>M1b</td>
<td>0</td>
</tr>
<tr>
<td>M1c</td>
<td>17 (81%)</td>
</tr>
<tr>
<td>Line of treatment</td>
<td></td>
</tr>
<tr>
<td>First line</td>
<td>16 (76%)</td>
</tr>
<tr>
<td>Second line</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Third line or more</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Subcutaneous metastasis</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Node metastasis</td>
<td>18 (86%)</td>
</tr>
<tr>
<td>Visceral metastasis</td>
<td>16 (76%)</td>
</tr>
<tr>
<td>Cerebral metastasis</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>N patients with vemurafenib dose reduction</td>
<td></td>
</tr>
<tr>
<td>for adverse effects</td>
<td></td>
</tr>
<tr>
<td>−25%</td>
<td>7 (33%)</td>
</tr>
<tr>
<td>−25% then −50%</td>
<td>6 (28%)</td>
</tr>
<tr>
<td>−25% then −50%</td>
<td>1 (5%)</td>
</tr>
</tbody>
</table>

Data are numbers (%).

Figure 2. Swimmer plot presenting plasma vemurafenib concentrations in µg/ml. Each bar represent one patient, from the beginning of vemurafenib (0) to end of treatment in weeks or end of study. Each numerical value represents the result, its position on the bar represent the date of plasma drawn, the shape of the point the dosage received, and the colour the result of tumoral evaluation carried out in simultaneously (light grey (pink online), progression, black (green online) partial or complete response, or stabilization).
Moreover, we prospectively lysis that took into account major prognostic criteria for meta-
cluded. However, we were able to conduct a multivariate ana-
results of PVCs.
Figure 3. Mean plasma vemurafenib concentration in patients with disease progression (number of samples N = 12 in 12 patients) and in patients with partial or complete response (N = 61 in 18 patients) (using RECIST 1.1 criteria: stability, partial, or complete response). Bar = SD.

discussion
This study showed high variability of steady-state vemurafenib plasma concentrations in patients with advanced melanoma treated with vemurafenib. Multivariate analysis showed that, at the time of evaluation of tumour response, progression was significantly associated with two parameters: lower plasma steady-state vemurafenib concentration and presence of cerebral metastasis before vemurafenib treatment. The poorer prognosis of brain metastasis, even in the BRAFinh era [9], has already been shown. To our knowledge, our study is the first one showing that tumour progression is associated with lower vemurafenib plasma concentration.

One limitation of our study is the small number of patients included. However, we were able to conduct a multivariate analysis that took into account major prognostic criteria for metastatic melanoma in the BRAFinh era. Moreover, we prospectively collected clinical information, using standardized criteria for tumour response evaluation and reporting of adverse events, and the analyses were conducted completely blinded to the results of PVCs.

The advantages of the vemurafenib mass spectrometry-assay we used should also be emphasized: (i) its high specificity, with no interference with endogenous products, vemurafenib metabolites, or with co-administered medications or their metabolites; (ii) its higher sensitivity when compared with other published techniques which use UV detection; (iii) a 10 times lower detection threshold. Long-term stability in plasma of vemurafenib was tested for 18 months, showing no degradation of vemurafenib. Since samples were frozen for <12 months in this study, duration of storage could not have impacted our results.

The high inter-patient variability of vemurafenib concentration found herein despite the fact that we meticulously collected blood samples at steady state may be explained by some parameters, which were not examined in our study. Intake of vemurafenib on an empty stomach may lead to significantly lower steady-state exposure than intake of vemurafenib with or a short time after a meal [3, 10]. No influence of meal on vemurafenib exposure was demonstrated in our series, but we did not record for each drug intake, the time of corresponding meal.

Mean PVC is lower upon melanoma progression. Our result suggests nevertheless a relationship between PVC and efficacy as has been showed in another study [11], and presumably a new resistance mechanism to vemurafenib that is independent of intra-tumour resistance mechanisms. This study, in a limited number of patients, did not show that higher PVCs were significantly associated with a greater risk of experiencing some adverse effects. The poor tolerance of vemurafenib, with 44% of patients requiring vemurafenib dose reduction in the BRIM-3 study because of adverse effects, is well known [12]. Surprisingly, we found that, when patients took reduced doses of vemurafenib, the mean PVCs were only slightly lower than when they were taking full doses (data not shown). This observation, which may be due to a better adherence to treatment in the absence of adverse effects, suggests that dose reduction of vemurafenib in intolerant patients could yield slightly lower PVCs that could still be within the ‘therapeutic range’.

As many kinase inhibitors, vemurafenib undergoes hepatic metabolism, but its hepatocellular uptake remains poorly investigated [3]. After oral administration, vemurafenib is metabolized into several metabolites (essentially through CYP3A4) but it mainly circulates in plasma (95%) as the parent compound. During administration of 960 mg b.i.d. vemurafenib median Cmax at steady state is 56.7 ± 21.8 μg/mL, a figure consistent with our results.

In vitro studies have demonstrated that vemurafenib is an inhibitor of the efflux transporters P-glycoprotein (P-gp/ABCB1) and Breast Cancer Resistance Protein (BCRP/ABCG2). Biliary transporters could play a determinant role in vemurafenib elimination. Conjugation metabolites [glucuronidation and glycosylation controlled in part by UDP glucuronosyltransferase 1 (UGT1A1)] were also identified in humans. These transporters and phase II enzymes most probably contribute to vemurafenib inter-patient pharmacokinetic variability.

The high inter-patient variability of vemurafenib exposure could be explained by different genetic polymorphisms of P-gp and/or BCRP [13] and or UGT1A1 [13, 14]. It can be hypothe-
sized that patients who accumulate vemurafenib have at least one mutation on genes coding for these transporters or enzyme. Indeed, these mutations are very common in the general popu-
lation, ABCB1, non-functional mutations being observed in 25% of individuals and UGT1A1 mutations, responsible for Gilbert’s syndrome in 3%–7% of the population.

Our results suggest that vemurafenib concentration monitoring could be a new and relatively simple method to follow metastatic melanoma patients, as it has been shown for imatinib in patients with chronic myeloid leukaemia [15]. In melanoma, a study published in 2012 using sorafenib, a multi-kinase inhibitor, suggested a correlation between sorafenib exposure and efficacy [16]. Burger et al. have shown that chronic imatinib exposure decreased intracellular drug accumulation by induction of the ABCG2 (BCRP) and ABCB1 (P-gp/MDR1) drug transporters [17]. Overexpression of these efflux pumps could also be a mechanism of resistance to vemurafenib by limiting drug dis-
tribution at different tumour sites. An in vitro study has shown that brain distribution of vemurafenib is strongly restricted at the blood–brain barrier because of active efflux by both P-gp/ ABCB1 and BCRP/ABCG2 [18]. Elacridar, a specific inhibitor of P-gp/ABCB1 and BCRP/ABCG2, increases vemurafenib brain
penetration, in mouse models [19]. The presence of efflux transporters into the blood–brain barrier could explain the higher difficulty to obtain long-term anti-tumour response in melanoma brain metastasis [20]. Furthermore, tumour cells themselves could express these transporters. A study has shown, in vitro and in vivo in a xenograft model that melanoma therapy with dacarbazine, temozolomide, or vemurafenib might participate to the chemoresistance acquisition by selecting tumour cell sub-populations expressing ABCB5, which shares 73% of sequence homology with ABCB1 (MDR1) drug transport pumps. Cancer Biol Ther 2005; 4: 747–753.

These transporters could also be implicated in the safety of vemurafenib, as they are implicated in the safety of gefitinib in non-small-cell lung cancer patients [23].

UGT1A1 could also be implicated in the safety profile of vemurafenib, as it is strongly associated with severe irinotecan toxicity in colorectal patients [24]. Further studies are needed to examine the association between ABCB1, ABCG2, and UGT1A1 polymorphisms and clinical outcomes (efficacy and safety) of melanoma patients treated with vemurafenib.

Our preliminary findings should be confirmed in a prospective study in a larger series of patients, with repeated collection of samples, at steady state, at each tumour assessment and at each occurrence of new adverse effect. Should our findings be confirmed, another study exploring dose adjustments according to PVCs in order to increase vemurafenib anti-tumour efficacy and improve tolerance, is warranted. The role of genetic polymorphisms of crucial metabolism enzymes and transporters of vemurafenib on the occurrence of vemurafenib-induced adverse events should also be investigated.

disclosure

The authors have declared no conflicts of interest.

references