Cerebrospinal fluid concentrations of vemurafenib in patients treated for brain metastatic BRAF-V600 mutated melanoma

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Anti-BRAF agents, including vemurafenib, have modified the prognosis for patients with melanoma. However, a difference can still be observed between extracerebral and cerebral responses. The aim of this study was to investigate the diffusion of vemurafenib in cerebrospinal fluid (CSF) from patients treated for brain metastatic BRAF-V600 mutated melanoma. Six patients treated with vemurafenib 960 mg twice daily were included. These patients had undergone a lumbar puncture because of suspicions of leptomeningeal metastasis, along with simultaneous blood sampling to measure vemurafenib level. The concentrations of vemurafenib in the CSF and the plasma were measured by high-performance liquid chromatography. The mean plasma and CSF concentrations of vemurafenib were 53.4 ± 26.2 and 0.47 ± 0.37 mg/l, respectively. The mean ratio of the CSF : plasma concentration was 0.98 ± 0.84%. No relationship was found between plasma and CSF concentrations ($P = 0.8$). In conclusion, our preliminary results highlight for the first time a low CSF vemurafenib penetration rate associated with a large interindividual variability in patients treated for metastatic BRAF-V600 mutated melanoma and brain metastases. Further investigations with larger cohorts are required to study the relationship between CSF vemurafenib concentrations and cerebral response. *Melanoma Res* **00:000–000**

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Introduction

Melanoma is one of the most common diagnoses in patients with brain metastases (BM) [1]. BM are diagnosed in more than 50% of melanoma patients with metastatic disease and are found in 70% of autopsies series. BM are correlated with a very poor prognosis. The median survival from the time of diagnosis of cerebral metastases is usually limited to several months [2].

Recently, vemurafenib, a BRAF kinase inhibitor, has shown efficacy [3]. The median progression-free survival was similar between patients previously treated by stereotactic radiation therapy (SRT), whole-brain radiotherapy or surgery ($n = 90$, 4.0 months) and those who had not ($n = 56$, 3.7 months). Similarly, the best overall response rate was not statistically different in the two groups (20 vs. 18%, respectively). Finally, the median overall survival was 6.7 months in the two groups.

Besides, Peuvrel *et al.* [4] reported in a retrospective cohort of 86 patients that the extracerebral disease was well controlled in 59% of patients during brain progression, suggesting that a difference could still be observed between extracerebral and cerebral responses to vemurafenib.

The penetration of antineoplastic agents is often limited in the central nervous system by the intact blood–brain barrier (BBB). A disruption of the BBB can be observed in BM, allowing a better distribution of systemic agents into the brain [5].

In terms of oral targeted therapies, data on cerebrospinal fluid (CSF) penetration are only available in patients with lung cancer and treated with epidermal growth factor receptor tyrosine kinase inhibitors such as erlotinib and gefitinib. The CSF penetration rate ranged from 1.1 to 1.3% for gefitinib [6,7] and from 2.8 to 5.1% for erlotinib [6,8–10].

In terms of vemurafenib, two studies explored its intracerebral diffusion in mice [11,12]. However, to the best of our knowledge, no data from patients treated for metastatic BRAF-V600 mutated melanoma are currently available.

In this context, the aim of this present exploratory study was to investigate the diffusion of vemurafenib in CSF from patients treated for metastatic BRAF-V600 mutated melanoma and BM.

Patients and methods

This study included six consecutive adult patients from the Lille Regional Teaching Hospital treated for melanoma with vemurafenib initiated for BM. It was carried out in conjunction with the search for a BRAF-

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positive mutation. These patients had been treated with twice-daily 960 mg doses of vemurafenib at a set time. Before study entry, all patients provided written informed consent for the assessment of vemurafenib concentrations in both plasma and CSF. All patients had received a lumbar puncture as part of the search for tumoral meningitis held between February 2012 and June 2013 in accordance with instructions provided by a neuro-oncologist on the basis of clinical and radiological criteria. Standard cytological and biochemical analyses were carried out. At the same time, a blood sample was obtained to determine blood glucose levels and vemurafenib concentration. CSF and plasma concentrations of vemurafenib were assessed by liquid chromatography with ultraviolet detection as described previously [13]. The standard calibration of the method was adapted to determine the CSF concentration and the specificity of the analytical method in linear calibration range was checked. The CSF penetration rate of vemurafenib as well as the CSF : plasma concentration ratio of vemurafenib were defined. The relationship between plasma and CSF concentrations of vemurafenib was assessed using the Pearson correlation coefficient. A *P*-value below 0.05 was considered significant. The statistical analysis was carried out using software R Foundation For Statistical Computing (Vienna, Austria).

Results

Six patients were enrolled from February 2012 to March 2013. The patients' characteristics are summarized in Table 1. The median age of the patients was 58 years. Five of these patients had a BRAF-V600E-type mutation and the other patient had a BRAF-V600K-type mutation. All six patients were diagnosed with parenchymal BM. Five out of six patients had from more than three BM and two had been treated previously by SRT. The lumbar puncture showed the presence of malignant cells in one patient. During the subsequent monitoring phase, two other leptomeningeal metastases were diagnosed. One diagnosis derived from imaging and typical clinical signs within 2 months and half of lumbar puncture. The second diagnosis was made from CSF cytology within 2 months of lumbar puncture.

Table 1 Patients' characteristics

Age	
Median (range) (years)	58 (27–64)
Sex	
Male	4
Female	2
Type of mutation	
BRAF-V600E	5
BRAF-V600K	1
Number of brain metastases (all of which are parenchymal)	
1	1
2–3	0
>3	5
Tumoral meningitis confirmed by the lumbar puncture	
Yes	1
No	5
Treatment lines for melanoma	
First	5
Second	1
Vemurafenib initiated for first BM	
Yes	5
No	1
Cause of death	
Primarily of CNS progression	5
Systemic disease progression	1

BM, brain metastases; CNS, central nervous system.

All patients died over the next year. The median survival from the primary diagnosis of melanoma was 24 months (range 15–128 months). The median interval after the first diagnosis of melanoma to BM was 19 months (range 0–238 months). The median survival after the first diagnosis of BM was 195 days (range 40–356 days).

The mean ± SD concentration of vemurafenib in CSF was 0.47 ± 0.37 mg/l (range 0.12–1 mg/l). The mean ± SD plasma concentration of vemurafenib was 53.4 ± 26.2 mg/l (range 28.9–96.1 mg/l). The median concentrations were 0.29 and 44.2 mg/l in plasma and CSF, respectively.

The mean ± SD CSF : plasma concentration ratio of vemurafenib was 0.98 ± 0.84% (range 0.28–2.5%). The median ratio was 0.70%. The two patients with the highest vemurafenib penetration in CSF (1.39 and 2.50%) were the same ones who had undergone previous BM treatment by SRT. One patient underwent surgery (ventriculoperitoneal derivation) before treatment of BM with a ratio equal to 0.34% (Table 2).

Finally, no relationship was found between the concentration in plasma and that in CSF ($P = 0.8$) (Fig. 1).

Discussion

To the best of our knowledge, the present exploratory study is the first to investigate vemurafenib concentration in CSF from patients treated with mutated BRAF-V600 melanoma and BM.

Our results proved that vemurafenib can penetrate the BBB. Vemurafenib was reported to be efficient in patients with melanoma and BM. In previous case reports [14–16], clinical and radiological responses were obtained about 1 month after vemurafenib treatment. Pathological examination of the resected specimen showed tumor necrosis [14,15].

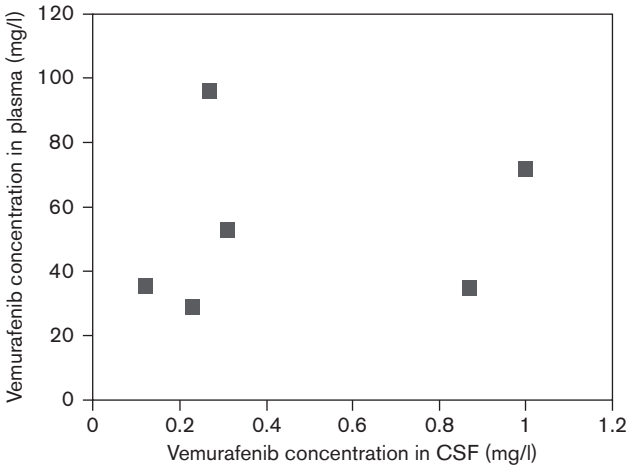
The mean CSF penetration rate of vemurafenib was in accordance with those reported in patients with lung cancer and treated with erlotinib or gefitinib [6–10]. Although all patients were treated with the recommended daily dose (960 mg twice daily), the present study found a high interindividual variability in vemurafenib diffusion to CSF, which may explain the lack of relationship between plasma and CSF concentrations (0.28–2.5%). In addition, the lack of a relationship between plasma vemurafenib concentration and CSF vemurafenib concentration suggests that the CSF penetration of vemurafenib may depend on multiple factors.

Table 2 Vemurafenib concentrations in plasma and CSF

Patient	BRAF-V600 mutation	Previous systemic therapy	Treatment of BM before time *t*	Plasmatic albumin (g/l)	Vemurafenib in plasma (mg/l)	Vemurafenib in CSF (mg/l)	Ratio vemurafenib CSF : plasma (%)	Cerebral response at time *t*	Tumor cells in CSF
1	V600E	Temozolomide	No	32	28.9	0.23	0.80	PD	Yes
2	V600E	No	SRT	42	72.1	1.00	1.39	PD	No
3	V600E	No	no	44	96.1	0.27	0.28	PD	No
4	V600E	No	SRT	41	34.8	0.87	2.50	PD	No
5	V600E	No	No	40	52.9	0.31	0.59	PD	No
6	V600K	No	Ventriculoperitoneal derivation	48	35.5	0.12	0.34	PD	No

BM, brain metastases; CSF, cerebrospinal fluid; PD, progression disease; SRT, stereotactic radiation therapy.

Fig. 1



No correlation between plasma concentration of vemurafenib and the corresponding CSF concentration ($P = 0.8$, $R^2 = 1.61\%$). CSF, cerebrospinal fluid.

First, two studies carried out in mice showed that P-gp (P-glycoprotein) and breast cancer resistance protein (BCRP), which are two efflux proteins, restricted the central nervous system diffusion of vemurafenib and confirmed the role of the efflux pumps in actively extruding vemurafenib from the brain and the CSF [11, 12]. After a constant intraperitoneal infusion of vemurafenib (2.5 mg/kg) for 48 h, the rate of brain penetration was significantly increased in Mdr1a/b(−/−)Bcrp1(−/−) mice (by factor 80) compared with wild-type mice [11]. These results suggest that the expression level of P-gP and BCRP at the choroid plexus of our patients could explain the interindividual variability in the penetration of vemurafenib into CSF.

Another study by Durmus *et al.* [12] showed that P-gP and BCRP restricted in-vivo vemurafenib accumulation in both the serum and the brain. After the coadministration of a P-gP and BCRP inhibitor (elacridar 100 mg/kg), the concentration of vemurafenib increases by factors 2.5 and 9.4 in serum and brain, respectively, compared with the administration of only vemurafenib.

Second, several clinical factors such as the presence of leptomeningeal metastasis or BM, previous treatment by surgery or radiotherapy are known to increase BBB permeability to drug treatment [17,18].

Indeed, a study by Lockman *et al.* [18] analyzed BM from two models (human and murine). Greater than 89% of BM statistically increased blood–tumor barrier permeability compared with the normal brain.

In the present study, the highest vemurafenib concentrations in CSF were observed in two patients who were treated by SRT. Two patients had a diagnosis of leptomeningeal metastasis within 3 months after the study with ratios equal to 1.39 and 0.28%.

Finally, vemurafenib is extensively bound to albumin (>99%). Given that only drug-free concentration can cross BBB, one can expect that penetration of vemurafenib in CSF would be greater in patients experiencing hypoalbuminemia. However, the limited number of patients included in our study does not allow us to confirm this hypothesis. Overall, it clearly appears that there is an interindividual variability in CSF vemurafenib penetration and a lack of relationship between plasma and CSF concentrations. Therefore, assessment of vemurafenib concentrations in CSF in future clinical trials might be useful/essential to show the strong link that exists between the cerebral response and CSF vemurafenib concentration.

In the present study, the mean plasma vemurafenib concentration in patients treated with the recommended daily dosage (960 mg twice daily) is in accordance with that reported previously (42.0 ± 15.0 mg/l) in a phase 2 study [19]. The low CSF vemurafenib penetration rate reported in the present study may explain in part the difficulty in achieving a sustained response over time. However, the present study has two major limitations: (a) the limited number of patients included and (b) the CSF drug concentration is not known to be the best surrogate marker for brain extracellular fluid concentration [20–22]. Indeed, the intracerebral microdialysis is the benchmark for this purpose. Therefore, future clinical investigations using intracerebral microdialysis in larger cohorts are essential to confirm our hypothesis. However,

it is interesting to know the CSF concentration because leptomeningeal metastases occur very frequently in melanoma (22 to 46% of cases) [23]. Furthermore, surgical treatment of BM is rarely indicated because BMs are often multiple and associated with progressive extracerebral disease. For instance, the blood–tumor barrier permeability varies in magnitude within and between metastases [18].

Conclusion

Our preliminary results highlight for the first time a low CSF vemurafenib penetration rate associated with a large interindividual variability in patients treated for metastatic BRAF-V600 mutated melanoma and BM. Further investigations with larger cohorts are required to assess the relationship between CSF vemurafenib concentrations and cerebral response.

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Conflicts of interest

Professor Mortier reports non-financial support from Roche, BMS, GSK and MSD outside the work submitted. Dr Le Rhun reports personal fees, non-financial support and financial support for clinical research from Mundipharma, personnal fees and non-financial support from Roche, and financial support for clinical research from Amgen outside the work submitted. For the remaining authors there are no conflicts of interest.

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