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Plasma vemurafenib exposure and pre-treatment hepatocyte growth factor level are two factors contributing to the early peripheral lymphocytes depletion in BRAF-mutated melanoma patients

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ABSTRACT

The therapeutic response to vemurafenib, a BRAF serine-threonine kinase inhibitor, exhibits large variations between patients. Evaluation of factors predicting the clinical efficacy of vemurafenib may help to identify patients at high risk of non-response in the early phase of treatment. The aim of this study was to analyze the pharmacokinetics of vemurafenib by a population approach and to evaluate the relationship between plasma drug exposure and pre-treatment plasma hepatocyte growth factor (HGF) levels with clinical effects (progression-free survival (PFS), peripheral lymphocytes depletion) in patients with metastatic $BRAF^{V600}$ mutated melanoma treated with single agent vemurafenib.

Concentration-time data ($n = 332$) obtained in 44 patients were analyzed using the NONMEM program. Pre-treatment plasma levels of HGF ($n = 36$) were assayed by ELISA method. A Cox model was used to identify prognostic factors associated with progression-free survival (PFS), and a linear regression to identify factors contributing to the depletion of peripheral lymphocytes at day 15.

Steady-state pharmacokinetics of vemurafenib was described by a one compartment model with first order absorption and first order elimination. None of the tested covariates explained the inter-patient variability in CL/*F*. A significant decrease in total lymphocytes count was observed within the first 15 days (median ratio Day15/Day0 = 0.66, $p < 0.0001$). Patients with Day15/Day0 ratio below 0.66 had longer PFS (14 *vs* 4 months, HR = 0.41, CI95% = [0.15–0.77], $p = 0.0095$). In the multivariate Cox model analysis, ECOG PS was the only parameter independently associated with PFS (grade 1 *vs* 0, HR = 3.26, CI95% = [1.29–8.22], $p = 0.01$ and grade ≥2 *vs* 0, HR = 4.77, CI95% = [1.52–14.95], $p = 0.007$). Plasma vemurafenib exposure ($p = 0.046$) and pre-treatment HGF levels ($p = 0.003$) were independently associated with the total lymphocyte ratio Day15/Day0.

Abbreviations: AAG, alpha-1 acid glycoprotein; AJCC, American Joint Committee on Cancer; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, Area Under the Curve; CI, Confidence Interval; CL/*F*, Apparent clearance; CRP, C Reactive Protein; EGFR, Epidermal Growth Factor Receptor; ECOG, Eastern Cooperative Oncology Group; F, Bioavailability; FOCE-I, First-Order Conditional Estimation with Interaction; HGF, Hepatocyte Growth Factor; HPLC, High Performance Liquid Chromatography; IOV, Inter-occasion variability; ka, Absorption rate constant; LDH, Lactate dehydrogenase; LLOQ, Lower Limit of Quantification; MDAs, Melanoma Differentiation Antigens; MAPK, Mitogen-Activated Protein Kinase; OFV, Objective Function Value; OS, Overall Survival; PD-1, Programmed Cell Death 1; PD-L1, Programmed Cell Death 1 Ligand; PFS, Progression Free Survival; PS, Performance Status; PI(3)K-AKT, phosphatidylinositol-3-OH kinase; $T_{1/2}$, Half-life; Vd/*F*, Apparent volume of distribution; VPC, Visual Predictive Check.

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These findings show that plasma vemurafenib exposure and pre-treatment HGF levels are two factors contributing to the early peripheral lymphocytes depletion which itself is associated with PFS.

1. Introduction

Vemurafenib (Zelboraf®) is one of two oral BRAF serine-threonine kinase inhibitors approved in the United States and European Union for first line treatment of metastatic $BRAF^{V600}$ mutant melanoma. Resistance to vemurafenib occurs within 4–6 months [1,2] after treatment initiation and might be partly explained by compensatory activity through alternative signaling pathways (N-RAS or COT) and subsequent activation of the mitogen-activated protein kinase (MAPK) pathway [3,4]. The mechanism of innate resistance is different. A recent study suggests that primary resistance is related to the secretion of hepatocyte growth factor (HGF) in the tumor micro-environment which results in activation of the HGF receptor MET, reactivation of the MAPK and phosphatidylinositol-3-OH kinase (PI(3)K-AKT) signaling pathways, and immediate resistance to RAF inhibition [3]. Conflicting results regarding the predictive role of HGF in the occurrence of resistance to BRAF inhibitors have been reported. Wilson et al. showed that increased pre-treatment plasma HGF levels were associated with worse progression-free survival (PFS) in patients with metastatic melanoma harboring BRAF mutation [5]. However, a recent study reported that detection of stromal HGF in pre-treatment biopsies of metastatic melanoma failed to predict response to RAF inhibitor therapy [6].

Recent findings suggest that BRAF inhibitors could enhance immune responses and anti-tumor immunity in humans [7]. This rationale is based on evidence that MAPK pathway is implicated in downstream T cell receptor signaling [7]. Inhibition of MAPK pathway leads to increased expression of melanocyte differentiation antigens (MDAs) which occurs frequently in melanomas [8]. Indeed, Boni et *al.* reported that recognition and killing of tumor cells by T cells specific for MDAs was enhanced by BRAF inhibitor treatment which up-regulated MDAs expression. In the same study, no impact of selective BRAF inhibitors on proliferation and viability of T cells was found. In this context, the function of lymphocytes seems unaffected by BRAF inhibitors while antigenicity of melanoma cells is increased. Wilmott et al. reported an induction of tumor infiltration by $CD4^+$ and $CD8^+$ T cells under both vemurafenib and dabrafenib treatment [9]. Moreover, a recent study reported that vemurafenib induces a significant decrease in peripheral lymphocytes within the first 12 weeks of treatment, however this decrease was associated neither with PFS nor overall survival [10]. Interestingly, HGF secreted in the tumor microenvironment might be responsible for a reactivation of MAPK pathway and in turn, a decrease in anti-tumor immunogenic response. However, no data are available to clearly confirm this hypothesis. Thus factors contributing to immunomodulatory effects under vemurafenib treatment remain to be identified.

A large inter-individual variability in vemurafenib pharmacokinetics has been reported [1.11]. This variability could partly explain the inter-patient differences observed in terms of clinical outcomes (efficacy, safety). Recently, different pharmacokinetic-pharmacodynamics (PK/PD) studies have reported a relationship between plasma exposure to vemurafenib and clinical efficacy [11–13]. These findings suggest that a low drug plasma exposure could favor emerging resistance to vemurafenib. Thus, evaluation of factors influencing vemurafenib pharmacokinetics may help to identify patients at high risk of tumoral resistance in the early phase of treatment. To our knowledge, the only data investigating pharmacokinetics of vemurafenib by a population approach come from randomized clinical trial [14] and therefore do not reflect the wide variations in patients seen in routine practice. In this context, the identification of factors influencing the pharmacokinetic of vemurafenib in non-selected outpatients is necessary to better optimize the therapy in the daily clinical practice.

Overall, a better understanding of factors predicting the response to vemurafenib may help to identify patients at high risk of non-response in the early phase of treatment. In this context, the aim of the present work was to (A) evaluate vemurafenib pharmacokinetics by a population approach in unselected, adult patients treated for metastatic $BRAF^{V600}$ mutated melanoma and to identify demographic and biological covariates that might explain the large interindividual variability in plasma drug exposure, (B) to explore PK/PD relationship between plasma exposure and clinical effects of vemurafenib (PFS, peripheral lymphocytes depletion) taking into account pre-treatment plasma HGF levels.

2. Materials and methods

2.1. Patients and data collection

The study was carried out in Cochin University Hospital in Paris. The population represents a subgroup of a multicentric prospective study [13]. Forty-four patients for whom multiple blood collection over the treatment course was accepted for population pharmacokinetic analysis were included in the cohort. Patients received single-agent vemurafenib (240–960 mg) twice daily. All patients met the following inclusion criteria: age ≥18 years, presence of clinically and/or radiologically assessable disease. The investigational review board "Comité de Protection des Personnes – Ile de France" approved the study protocol (VEMUMELA); all patients provided written informed consent and agreed for the blood sampling in compliance with the ethical principles of the revised Declaration of Helsinki (2008) and with French regulations.

Adult outpatients started vemurafenib treatment at the recommended daily dose of 1920 mg (twice daily regimen) or less according to their Eastern Cooperative Oncology Group Performance Status (ECOG PS) and comorbidities, at the discretion of the attending physician. Physicians could decide on subsequent dose reductions based on safety profiles, since they were blind to the plasma drug assay results. In cases of grade 3 toxicity (except skin squamous cell carcinoma) or intolerable grade 2 toxicity, vemurafenib was reduced to 720 mg twice daily initially, then to 480 mg twice daily if worsening of toxicity occurred. If two dose reductions did not induce toxicity recovering, vemurafenib was interrupted until grade 0 or 1 toxicity was reached and then treatment was resumed with a dose reduction. Treatment discontinuation was recommended in case of grade 4 toxicity. Vemurafenib administration was continued until disease progression, patient refusal or intolerable adverse events or death.

During the treatment period, determination of plasma vemurafenib concentration, physical examination, complete blood cell count and serum chemistry were performed at days 0 (before the first dose), 15, 30 and then monthly. In case of dose modifications before day 15, plasma vemurafenib concentration was evaluated at least 7 days afterward and it was considered as a steady-state