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The Use of Dried Blood Spots for Pharmacokinetic Monitoring of Vemurafenib Treatment in Melanoma Patients

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Abstract

Pharmacokinetic monitoring is increasingly becoming an important part of clinical care of tyrosine kinase inhibitor treatment. Vemurafenib is an oral tyrosine kinase inhibitor that inhibits mutated serine/threonine protein kinase B-Raf (BRAF) and is approved for the treatment of adult patients with BRAF V600 mutation-positive unresectable or metastatic melanoma. The aim of this study was to establish the relationship between dried blood spot (DBS) and plasma concentrations of vemurafenib to enable the use of DBS sampling, which is a minimally invasive form of sample collection. In total, 43 paired plasma and DBS samples (in duplicate) were obtained from 8 melanoma patients on vemurafenib therapy and were analyzed using high-performance liquid chromatography–tandem mass spectrometry. Plasma concentrations were predicted from the DBS concentrations using 2 methods: (1) individual hematocrit correction and blood cell-to-plasma partitioning and (2) the calculated slope explaining the relationship between DBS and plasma concentrations (without individual hematocrit correction). Vemurafenib DBS concentrations and plasma concentrations showed a strong correlation ($r = 0.964$), and the relationship could be described by ($[\text{vemurafenib}]_{\text{plasma}} = [\text{vemurafenib}]_{\text{DBS}}/0.64$). The predicted plasma concentrations were within ±20% of the analyzed plasma concentrations in 97% and 100% of the samples for the methods with and without hematocrit correction, respectively. In conclusion, DBS concentrations and plasma concentrations of vemurafenib are highly correlated. Plasma concentrations can be predicted from DBS concentration using the blood cell-to-plasma partition and the average hematocrit value of this cohort (0.40 L/L). DBS sampling for pharmacokinetic monitoring of vemurafenib treatment can be used in clinical practice.

Keywords

dried blood spot, vemurafenib, therapeutic drug monitoring, pharmacokinetics, bioanalysis

Dried blood spot (DBS) sampling for therapeutic drug monitoring (TDM) is increasingly used.[1,2] DBS sampling is a minimally invasive form of sample collection. After collection, there is no need for centrifugation, and storage and shipment can usually be done at room temperature. For TDM, specific advantages are that sampling can be done easily at home by the patient at predefined times such as just before drug intake (trough concentration). TDM is increasingly becoming an important part of clinical care for many new drug classes.

With the introduction of tyrosine kinase inhibitors, which are taken orally on a continuous basis, TDM is now more frequently used in oncology.[3] Vemurafenib is an oral tyrosine kinase inhibitor that inhibits mutated serine/threonine protein kinase B-Raf (BRAF) and is approved for the treatment of adult patients with BRAF V600 mutation-positive unresectable or metastatic melanoma.

Recently, new data were published on the relationship between vemurafenib plasma concentrations and tumor response and toxicity.[4–7] Patients with disease progression had lower plasma concentrations compared with patients with stable disease and those who were partial or complete responders. Three independent groups suggested a TDM target concentration of 42 μg/mL.[4–7] Plasma concentrations exceeding 62 μg/mL have been associated with higher risk for

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the development of grade ≥ 2 rash.[7] These results emphasize the importance of early drug monitoring of melanoma patients using vemurafenib to identify patients with a higher risk for treatment failure or grade ≥ 2 skin rash.

Currently, plasma concentrations are the gold standard for TDM. DBS sampling is a patient friendly and simple alternative, but the pharmacokinetics of vemurafenib have only been explored in plasma so far. To be able to use DBS sampling to determine the vemurafenib plasma concentration, the relationship between plasma and DBS concentrations of vemurafenib has to be established.

Therefore, the objective of the current study was to compare vemurafenib concentrations in DBS and plasma using 2 high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) assays that were validated and previously described.[8,9] The correlation between DBS and plasma concentration was studied, and 2 methods to predict plasma concentrations from DBS concentration were evaluated.

Methods

Patients

The current study was a substudy of a longitudinal follow-up cohort study of patients of the Antoni van Leeuwenhoek/Netherlands Cancer Institute (AvL-NKI) on vemurafenib therapy. Patients in this substudy were sampled (plasma and DBS) in the outpatient clinic during their monthly routine follow-up (while on steady state). According to the guideline for method comparison and bias estimation using patient samples from the Clinical and Laboratory Standards Institute (CLSI), at least 40 patient samples were needed.[10] Patients were recruited from March 2013 to March 2014. This study was approved by the medical ethics committee of the AvL-NKI, and informed consent was obtained from all patients.

Sampling

Whole blood (by venipuncture) and finger-prick blood samples were collected within 10 minutes of each other by the same nurse. The whole-blood samples were collected in K_2EDTA tubes centrifuged for 10 minutes at 1700*g* to isolate plasma, which was stored at nominally −20°C pending analysis. After sterile cleaning of the skin, 4 DBS samples were obtained using a 1.8−mm contact-activating lancet (Becton, Dickinson and Company, Franklin Lakes, New Jersey) on a Whatman FTA DMPK-A card (Whatman, GE Healthcare, Buckinghamshire, United Kingdom). The DBS samples were dried for at least 3 hours at room temperature and were stored at room temperature in a foil bag with a desiccant package pending further analysis.

Bioanalysis

Bioanalysis of plasma[9] and DBS[8] was performed using 2 HPLC-MS/MS methods that were described previously and were validated according to the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines for bioanalytical method validation.[11,12] Briefly, the sample pretreatment for plasma included liquid–liquid extraction using tert-butyl methyl ether. Vemurafenib was separated on a C18 column (Gemini C18 column, 110 Å, 50 × 2.0 mm ID, particle size, 5.0 μm; Phenomenex, Torrance, California) with gradient elution and analyzed with triple quadrupole mass spectrometry (Finnigan, TSQ Quantum Ultra; Thermo Fisher Scientific, Waltham, Massachusetts). Vemurafenib proved to be stable for at least 424 days in plasma at −20°C.[9]

DBS samples were first visually inspected to ensure the spots were at least 3 mm in diameter on both sides of the paper. Prior to processing, a 3-mm-diameter disc was punched (hand punch, Fiskars, 3-mm circle) from the sample collection card. Vemurafenib was extracted from this punched sample by methanol-acetonitrile (50:50, v/v). The same HPLC-MS/MS settings as described above were used for analysis. The DBS samples were analyzed in duplicate according to the guidelines for method comparison of the CLSI to determine the difference between the 2 spots.[10] Additional stability results showed that vemurafenib was stable for at least 827 days on the FTA DMPK-A cards because QC low (3 μg/mL) and QC high (75 μg/mL) both had a deviation within ±15% of the nominal concentration. The average of the 2 samples was compared with the plasma concentration.

The range of both assays was from 1 to 100 μg/mL, and in both assays, $^{13}C_6$-vemurafenib was used as the internal standard.

Comparison of DBS Versus Plasma Concentrations

DBS concentrations and plasma concentrations were compared with 2 methods. In method 1, plasma concentrations were predicted from the DBS concentration using the following equation, which accounts for the individual hematocrit values (Hct) and the red blood cell-to-plasma partition ratio:

$$[\text{Analyte}]_{\text{plasma}} = \frac{[\text{Analyte}]_{\text{DBS}}}{(1 - \text{Hct}) + K_{\text{BC:plasma}} \cdot \text{Hct}} \quad (1)$$

where $[\text{Analyte}]_{\text{plasma}}$ is the predicted analyte concentration in plasma, $[\text{Analyte}]_{\text{DBS}}$ is the analyte concentration in DBS, Hct is the individual hematocrit value, and $K_{\text{BC:plasma}}$ is the blood cell-to-plasma partitioning

coefficient, which indicates the affinity for plasma and its cell components. This equation was used previously by Jager et al.[13] The value for $K_{BC:plasma}$ can be calculated with the following equation:

$$[Analyte]_{BC} = K_{BC:plasma} \cdot [Analyte]_{plasma} \quad (2)$$

where $[Analyte]_{BC}$ is the analyte concentration in red blood cells, $[Analyte]_{plasma}$ is the plasma concentration, and $K_{BC:plasma}$ is the blood cell-to-plasma partitioning coefficient. A value of 11.40% of vemurafenib bound to red blood cells has been described.[14] If this fraction is used for $[Analyte]_{BC}$ and the remaining 88.6% for $[Analyte]_{plasma}$ the $K_{BC:plasma}$ is 0.129, which was used to predict the plasma concentration in equation 1.

With method 2, plasma concentrations were predicted without individual hematocrit correction to investigate whether DBS sampling can also be used without individual hematocrit determination (see equation 3). In this method the slope (m), which describes the relationship between the analyzed plasma concentration and the analyzed DBS sample concentration, was used to predict the plasma concentration.

$$[Analyte]_{plasma} = \frac{[Analyte]_{DBS}}{m} \quad (3)$$

Statistics

The difference between the in-duplicate analyzed DBS samples was compared using Bland-Altman plots. The plasma and DBS concentrations were compared using weighted Deming regression. The slope of the regression line was used to describe the relationship between the plasma and DBS concentration in method 2. The predicted and analyzed concentrations were compared using Bland-Altman plots. Acceptance criteria for the agreement between predicted and analyzed plasma concentrations were based on the guidelines for bioanalytical method validation of the FDA and EMA (incurred samples reanalysis)[11,12]; the difference in concentration should not exceed ±20% of the mean for at least 67% of the samples. All calculations were performed with the R statistical software package (version 3.1.0; hppt://cran.r-project.org).

Results

Patients and Sampling

In total, 43 duplicate DBS samples and plasma samples were collected from 8 patients during multiple hospital visits. Two DBS sample duplicates were not suitable for analysis because the spot size was too small. For 32 of the adequate DBS samples, a hematocrit value was available from a corresponding venous blood sample that was used for individual hematocrit corrections. The mean hematocrit of the venous samples was 0.40 L/L (0.27 to 0.49 L/L). Vemurafenib doses ranged from 480 mg twice daily to 960 mg twice daily.

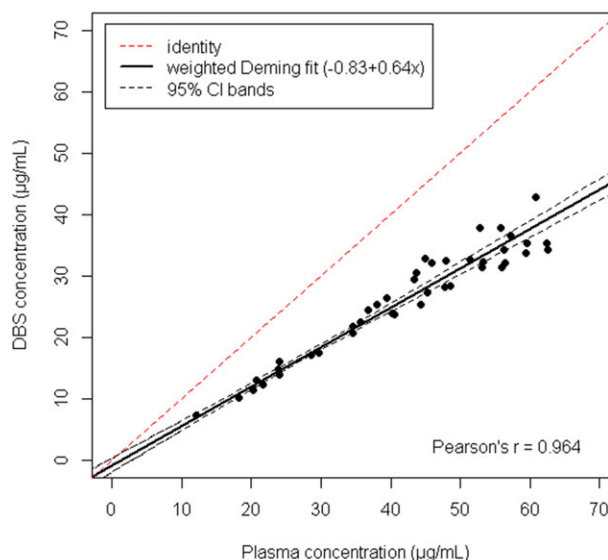

Figure 1. Correlation of plasma concentrations of vemurafenib and the corresponding DBS sample concentrations. Weighted Deming regression was used describe the correlation between vemurafenib plasma and DBS sample concentrations. The broken red line is the line of identity, the solid line is the line of regression, and the 2 broken black lines indicate the 95% confidence interval. The slope is 0.64 (95%CI, 0.60 to 0.68), and the intercept is −0.83 (95%CI, −1.97 to 0.31).

Dried Blood Spot Versus Plasma Concentrations

Figure 1 shows the relationship between the analyzed plasma concentration and the analyzed DBS concentration. A strong correlation was found, with a correlation coefficient of $r = 0.964$. The concentrations found in DBS samples were consistently lower than the corresponding plasma concentration. The slope was determined to be 0.64 (95%CI, 0.60 to 0.68) with an intercept of −0.83 µg/mL (95%CI, −1.97 to 0.31) and was used to predict the plasma concentration using method 2.

The DBS samples were analyzed in duplicate to determine the difference between the 2 spots. Figure 2 shows a Bland-Altman plot of the mean concentration of the 2 DBS samples and the difference between the 2 DBS samples, which shows the excellent reproducibility of DBS sampling.

Figure 3 shows the regression of the observed and the predicted plasma concentrations (using both methods). For method 1, the slope was 1.03 (95%CI, 0.96 to 1.09), and the intercept was −2.75 µg/mL (95%CI, −4.61 to −0.89 µg/mL). For method 2 (without individual hematocrit correction) the slope was 1.00 (95%CI, 0.94 to 1.07), and the intercept was −1.42 µg/mL (95%CI, −3.31 to 0.48 µg/mL). For both methods the theoretical optimal values for slope 1 and intercept

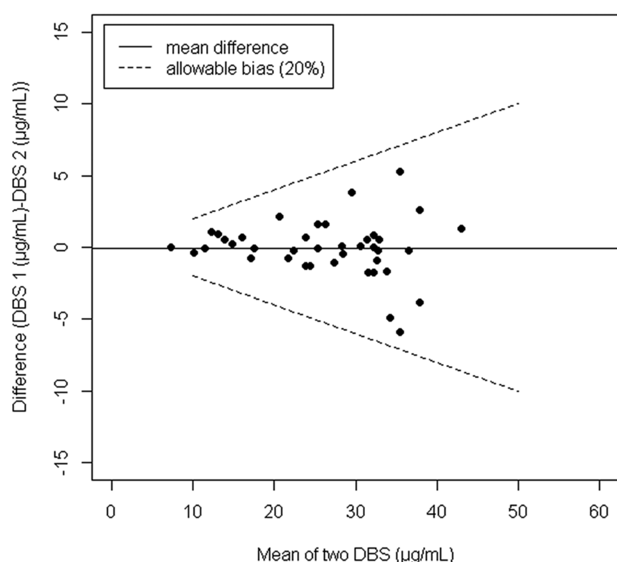

Figure 2. The difference between the 2 analyzed DBS samples from 1 collection card. The differences between the 2 DBS samples are all within ±20% of the mean of both spots.

(0 μg/mL) are in the confidence intervals, indicating that both methods adequately predict the observed plasma concentrations.

Figure 4 shows a Bland-Altman plot of the predicted plasma concentration and the analyzed plasma concentration. With method 1, the differences between the predicted plasma concentrations and the analyzed plasma concentrations were within ±20% of the analyzed plasma concentrations for 97% of the samples (using individual hematocrit correction). With method 2, the differences between the predicted plasma concentrations and the analyzed plasma concentrations were within ±20% of the analyzed plasma concentrations for all samples (without individual hematocrit correction).

Discussion and Conclusions

In this study we showed that plasma concentrations of vemurafenib can adequately be predicted from DBS concentrations, which makes DBS sampling a practical alternative for plasma sampling. Differences between predicted and observed plasma concentrations were well within the criteria used in the guidelines for bioanalytical method validation of the FDA and EMA (incurred samples reanalysis).

We showed that the difference of the in-duplicate analyzed DBS samples was within ±20% of their mean, indicating adequate reproducibility of this sampling technique. The difference between DBS concentrations and plasma concentrations could be explained by the blood cell-to-plasma partition ($K_{BC:plasma}$) and the individual and the average hematocrit value of this cohort (0.40 L/L). When using the mean hematocrit of this patient group (0.40 L/L) and the $K_{BC:plasma}$ of 0.129, the denominator of equation 1 is equal to 0.65, which is almost equal to the calculated slope, which was 0.64 (95%CI, 0.60 to 0.68).

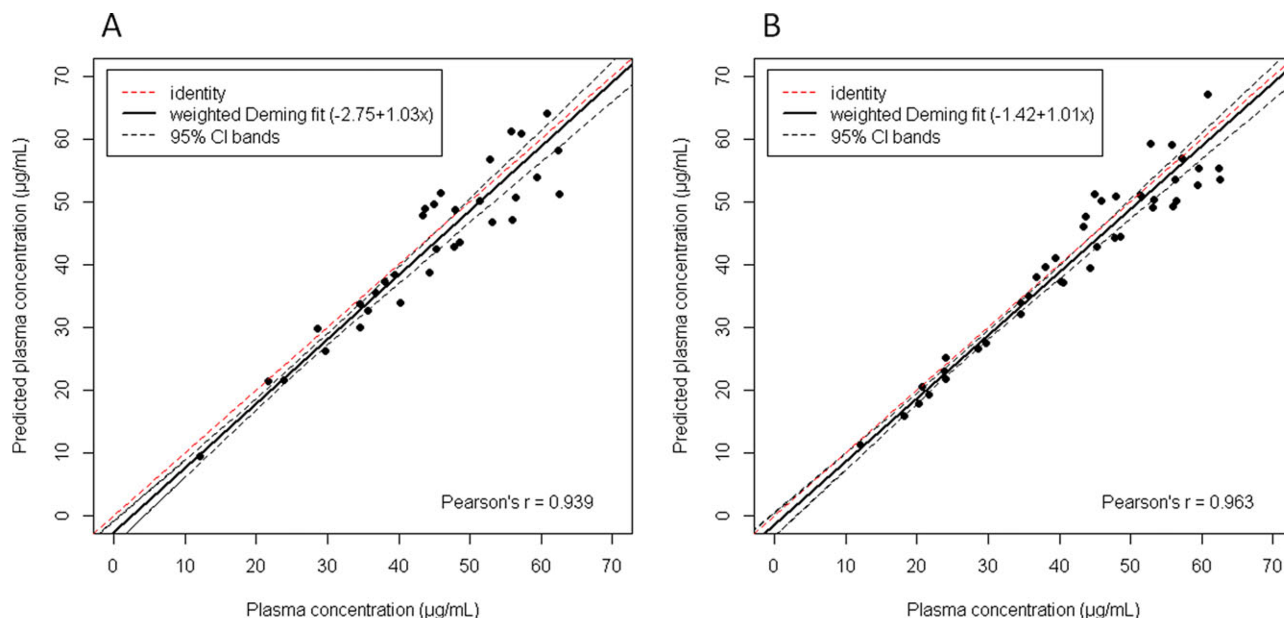

Figure 3. Correlation of the predicted plasma concentrations of vemurafenib and the corresponding DBS sample concentrations with hematocrit correction (A) and without hematocrit correction (B). Weighted Deming regression was used to describe the correlation between the predicted plasma concentrations and the determined plasma concentrations. The broken red line is the line of identity, the solid line is the line of regression, and the 2 broken black lines indicate the 95% confidence interval. The slope in (A) is 1.03 (95%CI, 0.96 to 1.09), and the intercept is −2.75 μg/mL (95%CI, −4.61 to −0.89 μg/mL). The slope in (B) is 1.00 μg/mL (95%CI, 0.94 to 1.07 μg/mL), and the intercept is −1.42 μg/mL (95%CI, −3.31 to 0.48 μg/mL).

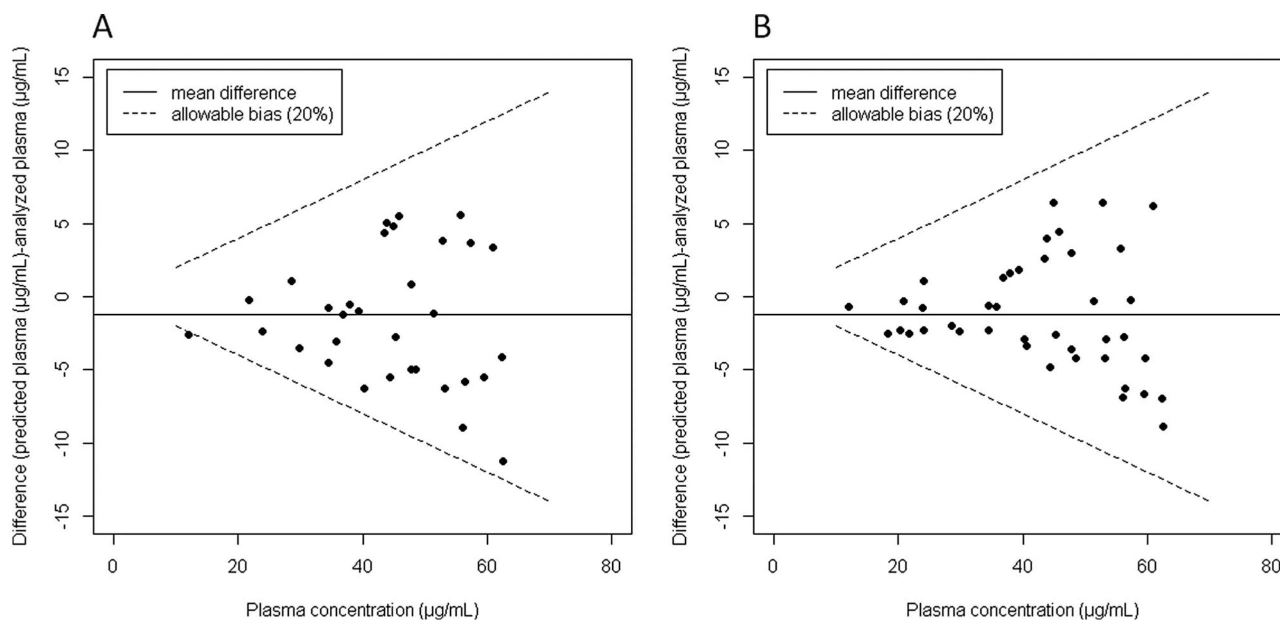

Figure 4. The difference between the predicted and analyzed plasma concentration with hematocrit correction (A) and without hematocrit correction (B). The differences between the predicted and the analyzed plasma concentration were within ±20% of the analyzed plasma concentration for 93% of the samples when using hematocrit correction (A). The differences from the predicted and the analyzed plasma concentrations of the results without hematocrit correction were all within ±20% of the analyzed plasma concentration.

In the analytical and clinical validation of DBS methods the effect of hematocrit can be profound for some analytes.[2,15,16] During validation of the bioanalytical method we have shown that for hematocrit values between 0.24 L/L (bias, −9.1%) and 0.45 L/L (bias, 11.4%), the method had acceptable accuracy and precision.[8] In this group of patients the average hematocrit value was 0.40 L/L (0.27 to 0.49 L/L). In the current study, we showed that no individual hematocrit correction is needed to predict plasma concentrations from analyzed DBS concentrations within the studied hematocrit range, as both methods to predict plasma concentrations provided similar results. Therefore, the easy-to-implement method 2 (without hematocrit correction) can be used to predict the plasma concentrations. However, in cases in which individual hematocrit values are outside the observed range in the current study, method 1 might give better results.

Determination of plasma concentrations is still the gold standard for TDM, although many DBS methods suitable for TDM are currently available. These results show that a conversion factor is needed to translate DBS concentrations to plasma concentrations. The recently proposed TDM target of at least 42 μg/mL in plasma would translate to a DBS target of at least 26.9 μg/mL.

The TDM target of 42 μg/mL was based on the plasma concentrations on day 14 or 15 of treatment, which was used as an early predictor of treatment outcome.[4–7] This DBS method would contribute to TDM of vemurafenib because patients are able to self-sample at home after, for instance, 2 weeks of treatment (at steady state). A rapid dose adaptation can be based on the described method. Usually patients visit the outpatient clinic once a month, but because of the rapid progression of many melanoma patients and the rapid inhibition of tumor growth by vemurafenib, earlier monitoring could be beneficial. However before this DBS method can be implemented for sampling at home by the patient, the feasibility of DBS self-sampling needs to be assessed. The feasibility of DBS self-sampling has previously been investigated at our institute in cancer patients receiving adjuvant tamoxifen treatment.[17] This study showed that 86% of the patients provided at least 1 DBS sample suitable for analysis. These results indicate that with sufficient instructions, patients should be able to self-sample at home. Currently, plasma samples are still drawn using venipuncture during the monthly clinical visits because venipuncture is still necessary for many other laboratory tests. However if pharmacokinetic monitoring is required outside these clinical visits, patients will be asked to self-sample at home after adequate instruction.

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There was a previous poster presentation of partial results at the Figon Dutch Medicine Days, Ede, The Netherlands, October 5–6, 2014.

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