Vemurafenib

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Abstract

The activating BRAF mutation V600E and related mutations in this codon are most important for the activation of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signalling pathway in melanoma. BRAF V600E mutations have been detected in ~40 % of melanoma patients and BRAF V600K mutations in ~5 % of melanoma patients. Activation of the MAPK pathway results in continuous stimulation of cell proliferation and inhibits programmed cell death. Vemurafenib (PLX4032) was developed as a low molecular weight molecule for the inhibition of the mutated serine threonine kinase BRAF, and it selectively binds to the ATP-binding site of BRAF-V600E kinase and inhibits its activity. The biochemical affinity of vemurafenib for mutated BRAF translates to potent inhibition of ERK phosphorylation and of cell proliferation exclusively in BRAF-mutant cell lines. In animal model experiments, it was demonstrated that vemurafenib achieved tumour regressions in cells harbouring the BRAF V600E mutation. The clinical trials with vemurafenib in unresectable metastatic melanoma in phase I, II, and III for patients harbouring BRAF V600E mutations demonstrated all unexpected high objective response rates ranging between 50 and 80 %. Median progression-free survival was prolonged from two months with dacarbazine to seven months with vemurafenib, and median overall survival was respectively prolonged from 9 to 14 months. A major problem that remains is the development of resistance to vemurafenib treatment after several months in the majority of patients, and multiple resistance mechanisms have already

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been described. Under vemurafenib treatment, about 25 % of patients developed cutaneous squamous cell carcinomas of the keratoacanthoma type with low invasive potential and without occurrence of metastasis. The overall tolerability of the drug was quite good, and a number of patients remained on treatment for long times. As other solid tumours like papillary thyroid cancer, colorectal cancer, non-small-cell lung cancer, and ovarian cancer likewise harbour BRAF mutation, vemurafenib is also tested in these entities. In future, combinations of vemurafenib with other kinase inhibitors and with immunotherapies will improve its therapeutic potential.

Keywords
Vemurafenib · BRAF mutation · Melanoma · BRAF inhibitor

Contents

1 Introduction

The prognosis for patient with distant melanoma metastasis (AJCC stage IV melanoma) is generally poor with a median survival ranging from 8 to 10 months after diagnosis depending on the number and the sites of metastatic spread and serum LDH (Chapman et al. 2011). The 5 year survival rate is 5–10 % in patients with metastatic melanoma (Eigentler and Garbe 2006). Treatment with single-agent chemotherapy or with combined schedules can produce palliative clinical response in a minority of patients (Pflugfelder et al. 2011). The discovery of activating BRAF mutations in approximately 50 % of patients with melanoma led to the development of a first targeted therapy of an activated oncogene in cutaneous melanoma, and clinical trials in other tumour entities are under way.

Mutations in the BRAF gene which substitute the valine at amino acid position 600 with glutamic acid (V600E) represent over 80 % of the BRAF mutations. Other variants of BRAF mutation are V600K with ~10 %, and less common V600R and V600D. Vemurafenib is also active in these less common BRAF V600

mutations, probably to a lower degree. BRAF mutations were mainly found in melanoma, colorectal cancer, papillary thyroid cancer, non-small-cell lung cancer, and ovarian cancer. Additionally, nearly all patients with hairy cell leukaemia carry the BRAF V600E mutation.

Targeted therapy represents nowadays a promising therapy for metastatic melanoma harbouring a drug-sensitive mutation. Vemurafenib was licensed for the treatment of non-resectable metastasised melanoma by the Food and Drug Administration Agency in the USA in August 2011 and by the European Medicines Agency in Europe in February 2012 on the basis of a phase III study for the treatment of patients carrying a BRAF V600 mutation (USFDA 2011; Hoffmann-La Roche Ltd 2012). With evaluated response rates ranging between 60 and 88 %, vemurafenib represents a therapeutic milestone in melanoma patients since decades (Flaherty et al. 2010; Schreck and Rapp 2006; Chapman et al. 2012). Additionally, an increase in overall survival up to 14 months compared to 9 months with standard chemotherapy treatment was reported, whereas some patients are still under treatment after 2 years (Chapman et al. 2012). Before treating patients with vemurafenib, patients must have a positive result from a BRAF mutational testing.

2 Structure and Mechanism of Action

BRAF is a member of the RAF family of serine threonine kinases (ARAF, BRAF, and CRAF) which are part of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signalling pathway (Schreck and Rapp 2006).

Therapy targeting the MAPK pathway through inhibition of oncogenic mutations in the BRAF kinase has become a standard treatment for patients who have metastatic melanoma with activating BRAF mutations. Mitogen-activated protein kinase cascades are key signalling pathways involved in the regulation of normal cell proliferation, survival, and differentiation. The RAF/MEK/ERK signalling has implications in a wide variety of cellular functions. This pathway is central for cell proliferation, cell cycle arrest, terminal differentiation, and cell death. RAF activates the MAPK kinase MEK1/2 which subsequently phosphorylates ERK1/2 (Peyssonnaux and Eychene 2001). Mutated BRAF V600E has a critical role for the proliferation and survival of melanoma cells through activation of the MAPK pathway. The mutation in the V600 codon changes the molecular confirmation of BRAF to the activated (phosphorylated) status. In June 2002, Davies and colleagues reported mutations of the BRAF gene in human cancers. BRAF mutation-induced oncogenes are present in approximately 5–10 % of all human malignancies (Davies et al. 2002). BRAF is the most frequently mutated protein kinase in melanoma (Greenman et al. 2007) and was identified in $\sim$50 % of malignant melanomas, in 15 % of thyroid tumours, in 8 % of colon carcinomas, in 4 % of all solid tumours, and up to 100 % in hairy cell leukaemia (Davies et al. 2002; Tiacci et al. 2011).

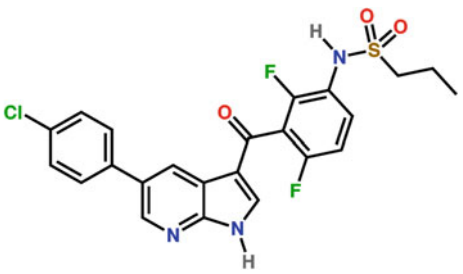

Fig. 1 Structure of vemurafenib: propane-1-sulphonic acid {3-[5-(4-chlorophenyl)-1H-pyrolo[2,3-b]pyridine-3-carbonyl]-2,4-difluoro-phenyl}-amide

Vemurafenib is a low molecular weight molecule, an orally available, selective inhibitor of BRAF with the V600E mutation but does not inhibit BRAF wild type; it selectively binds to the ATP-binding site of BRAF-V600E kinase and inhibits its activity (Fig. 1).

3 Preclinical Data

The discovery of the mutations in the BRAF gene, which represent approximately two-thirds of activating mutations in the oncogene protein kinases, was an important step in understanding the aetiology of metastatic melanoma.

The biochemical affinity of vemurafenib for mutated BRAF translates to cellular potent inhibition of ERK phosphorylation and of cell proliferation exclusively in BRAF-mutant cell lines. In preclinical cell line experiments, it was demonstrated that vemurafenib inhibited proliferation in cells harbouring the BRAF V600E mutation. Vemurafenib likewise caused tumour regressions of BRAF-mutant xenografts (Bollag et al. 2010).

4 Pharmacokinetics and Drug Interactions

After oral administration of a single 960 mg dose of vemurafenib, the substance was absorbed with a time needed to reach maximum concentration (tmax) of approximately 4 h. Mean maximum concentration achieved in the blood (Cmax) at the 960 mg dose level was approximately 4.8 ± 3.3 μg/ml. Clearance is approximately 30 L/day. The mean half-life time (t1/2) is 50 h, resulting in sixfold–ninefold accumulation between day 1 and day 15. Vemurafenib is excreted via faeces (94 %) and urine (1 %) (Shah et al. 2013; European Medicines Agency 2013).

Vemurafenib is metabolised by CYP3A4, and the metabolites make up 5 % of the components in plasma. The parent compound makes up for the remaining 95 %. Results from an in vivo drug–drug interaction study in patients with cancer

demonstrated that vemurafenib is a moderate CYP1A2 inhibitor, a weak CYP2D6 inhibitor and a CYP3A4 inducer. Ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, and voriconazole are potent CYP3A4 inhibitors; concomitant administration of strong CYP3A4 inhibitors increases plasma concentration of vemurafenib (Shah et al. 2013; European Medicines Agency 2013).

Phenytoin, carbamazepine, rifampicin, rifabutin, rifapentine, and phenobarbital are CYP3A4 inhibitors; concomitant administration of strong CYP3A4 inhibitors decreases plasma concentration of vemurafenib. Co-administration of vemurafenib increased the AUC of caffeine (CYP1A2 substrate) 2.6-fold and increased the AUC of dextromethorphan (CYP2D6 substrate) by 47 %, while it decreased the AUC of midazolam (CYP3A4 substrate) by 39 %. Co-administration of vemurafenib resulted in an 18 % increase in AUC of S-warfarin (CYP2C9 substrate) (Shah et al. 2013; European Medicines Agency 2013).

5 Vemurafenib in Melanoma

The clinical trials with vemurafenib in phase I, II, and III for patients harbouring BRAF V600E mutations demonstrated all unexpected high objective response rates and improvement in progression-free and overall survival. The tolerability of the drug was quite good, and many patients remained on treatment for long times (Chapman et al. 2011; Flaherty et al. 2010; Sosman et al. 2012).

The results from a phase I study for the first time reported a high objective response rate in patients with metastatic melanoma harbouring BRAF mutations. Data of the phase I trial (BRIM1) were published In August 2010 (Flaherty et al. 2010). This trial had a two-phase design (dose escalation phase and an extension phase). Complete or partial tumour responses occurred in 26 of 32 patients within the extension phase (81 %). The response duration for the patients in dose escalation phase ranged from 2 to more than 18 months. The median progression-free survival among all patients was more than 7 months. In the dose escalation phase, 11 of 16 patients (69 %) with BRAF-V600E mutant melanoma had a complete or partial response, who were treated with doses of 240 mg twice daily or higher. Forty percentage of the extension cohort had dose reduction from 960 mg twice daily to 720, 600 or 480 mg twice daily due to the side effects which were demonstrated to be proportional to the dose of the drug. Predominantly cutaneous side effects have been observed as rash, photosensitivity, cutaneous squamous cell carcinoma, and palmar–plantar dysaesthesia. Furthermore, fatigue and arthralgia were reported in nearly one-third of patient in the extension phase. Well-differentiated cutaneous squamous cell carcinoma was diagnosed in more than 20 % of patients with low invasive potential and no metastatic course. The maximum tolerated dose was found to be 960 mg orally twice daily (Flaherty et al. 2010).

A multicentre, open-label phase II trial was conducted in patients with metastatic melanoma who had previously been treated with one or more prior systemic therapy (BRIM2) (Sosman et al. 2012). In this trial, 132 patients with melanoma harbouring a BRAF V600 mutation were treated with vemurafenib at a dose of 960 mg until the development of unacceptable toxic effects or disease progression. The median duration of response was 6.8 months (95 % CI: 5.6—not reached). The confirmed overall response rate was 53 %. Adverse events (AEs) were generally reversible (with dose modification or interruption). The most common adverse events (all grades) were arthralgia (seen in 59 % of patients), rash (52 %), and photosensitivity reactions (52 %). The most common grade 3 adverse event was cutaneous squamous cell carcinoma (seen in 26 % of patients), the majority of which were centrally reviewed as keratoacanthoma type. Forty-five percentage of patients required dose reductions, most commonly for rash, arthralgia, and liver function test abnormalities (Sosman et al. 2012).

In June 2011, the results from an open-label phase III study with a total of 672 patients with previously untreated melanoma with the BRAF V600E mutation were reported (Chapman et al. 2011). This study has been performed in order to evaluate the efficacy of vemurafenib as a monotherapy in comparison with dacarbazine chemotherapy. Vemurafenib treatment showed remarkable tumour responses in approximately 48 % of patients with vemurafenib treatment compared with 55 % for those on dacarbazine chemotherapy. Vemurafenib was associated with a relative reduction of 63 % in the risk of death and of 74 % in the risk of tumour progression (Chapman et al. 2011).

Vemurafenib is associated with a significant improved overall survival and progression-free survival in comparison with dacarbazine chemotherapy in patients with previously untreated, V600E BRAF-mutated metastatic melanoma.

6 Toxicity

Vemurafenib is generally well tolerated with manageable side effects. The adverse events reported in vemurafenib clinical studies were demonstrated to be proportional to the dose and exposure to the drug. The toxic effects were largely related to the skin, joints, liver, and CNS.

Only few patients needed to discontinue treatment permanently in the clinical studies due to adverse events. The frequency of adverse events leading to permanent discontinuation of treatment in phase I and phase II trials was 7 % and 3 %. Most common adverse reactions (in $\geq$30 % treated patients) were the following: arthralgia, rash, alopecia, fatigue, photosensitivity reactions, nausea, pruritus, and skin papillomas including squamous cell cancer. Prolongation of the QT interval was also reported. Thirty-one percentage of patients in the extension phase developed well-differentiated SCC with low invasive potential and without development of metastases (Flaherty et al. 2010).

In the phase II study, the most common adverse events reported were arthralgia, rash, mild to moderate photosensitivity reactions, fatigue, and alopecia (Sosman et al. 2012). Transient elevations of liver-enzyme levels were likewise reported. Three patients had transient palsies of the seventh cranial nerve, one patient had retinal-vein occlusion, and another patient had acute renal failure. Twenty-six percentage of patients developed SCC or keratoaconthoma; the median time to development of the first cutaneous squamous cell carcinoma or keratoacanthoma lesion was 8 weeks. The most common grade 3 adverse reactions were cutaneous SCC and rash. The possible mechanisms of developing SCC appears to be paradoxically increasing signalling of the MAPK pathway in cancer cells with wild-type BRAF that carry upstream RAS mutations, through signalling via CRAF (Oberholzer et al. 2012).

7 Vemurafenib in Colorectal Cancer

Approximately 10 % of all patients with colorectal cancer have BRAF(V600E) mutation (Tanaka et al. 2006). Patients with metastatic colorectal BRAF V600E mutation had poorer survival as compared with BRAF wild-type patients (Tie et al. 2011). The clinical responses to the vemurafenib in clinical trials were only 5 % in BRAF-mutant colorectal cancer (Kopetz et al. 2010). The low response rate for vemurafenib treatment in patients harbouring BRAF mutation is possibly explained by resistance to the kinase therapy. Therefore, the parallel blockade of the epidermal growth factor receptor (EGFR) may be a successful strategy in colon cancers, as this showed a strong synergy with BRAF(V600E) blockade. Inhibition of the activity of EGFR with cetuximab, erlotinib, or gefitinib and combination with BRAF inhibitor may be more effective in those patients (Prahallad et al. 2012). Another resistance mechanism to BRAF inhibitors has been reported in BRAF-mutant colon cancer which is the activation of the PI3K/AKT pathway. Therefore, inhibiting the PI3K pathway in combination with vemurafenib in BRAF-mutant CRC cell lines provided an improved anti-tumour action (Mao et al. 2013).

8 Vemurafenib in Papillary Thyroid Cancer

Forty-five to fifty percentage of patients with papillary thyroid cancers were reported to have activating BRAF mutations (Xing 2007). The incidence rate of BRAF mutation in recurrent or metastatic PTCs was approximately 80 %. A phase I study with vemurafenib showed a partial response and prolonged stabilization of disease in all patients with PTC treated with vemurafenib (Kim et al. 2013).

9 Vemurafenib in Non-Small-Cell Lung Cancer

BRAF mutations are reported in approximately 1–5 % of NSCLCs. The majority of the mutations were non-V600E (Naoki et al. 2002). Gautschi et al. (2012) reported one case with V600E mutation in NSCLC that responded to vemurafenib.

10 Vemurafenib in Hairy Cell Leukaemia

In 2011, Tiacci and colleagues reported of a 100 % detection rate of the BRAF V600E mutation in patients suffering from hairy cell leukaemia (Tiacci et al. 2011). Meanwhile, case reports were published indicating partial and complete remissions even for low doses of vemurafenib (Dietrich et al. 2012; Peyrade et al. 2013).

11 Biomarkers and Monitoring of Vemurafenib Treatment

Biomarkers in metastatic melanoma are used to assess the progression of disease, predict the response of treatment, and are part of staging examinations. In 2009, the American Joint Committee on Cancer (AJCC) included the serum lactate dehydrogenase (LDH) to classify stage IV into the M categories, M1a (soft tissue metastasis), M1b (pulmonary involvement), and M1c (involvement of other visceral organs or elevated LDH) (Balch et al. 2009). LDH is expressed ubiquitously in different healthy tissues. Elevated serum concentrations of the intracellular enzyme are mainly a result of cell lysis. Moreover, increased serum LDH levels occur in different tumour entities and indicate a high turnover of tumour cells as well as necrosis in fast-growing tumours. Increased LDH values are associated with high tumour burden and seem to be particularly elevated in liver metastases (Finck et al. 1983; Sirott et al. 1993).

Another prognostic factor for stage VI metastatic melanoma is serum S100B. In immunohistochemistry, routine staining with S100 polyclonal antibody is able to detect macrophages, monocytes, interdigitating reticulum cells, Langerhans cells, and cells from the neural crest including glia, Schwann cells, and melanocytes (Gogas et al. 2009; Hauschild et al. 1999). Serum S100B has been shown to be elevated at stage I/II in 0–12.0 %, at stage III in 8.7–31 %, and at stage IV in 48–100 % (Carlson et al. 2005). Weide et al. assessed the use of biomarker in melanoma patients with distant metastases. Serum markers LDH and S100B were found to be independent prognostic factors in melanoma patients with distant metastases, and both factors were associated with similar hazard ratios (Weide et al. 2012).

A retrospective study in 44 patients with stage IV melanoma who were treated with vemurafenib evaluated the potential of the tumour marker S100B as response and progression markers during vemurafenib treatment. Computed tomography scans and measurement of LDH and S100B levels were performed every

6–8 weeks. The correlation between response or progression and LDH and S100B levels was analysed. A good correlation between S100B and LDH decline and a RECIST-confirmed response was observed, especially when S100B and/or LDH were elevated at baseline. However, the correlation in case of tumour progression and S100B/LDH levels was low. Therefore, monitoring the course of the disease with tumour markers is thus not an alternative to monitoring with imaging examinations (Abusaif et al. 2013).

12 Summary and Perspectives

Vemurafenib is a very active drug in unresectable metastatic melanoma. Eighty-five percentage of patients develop tumour regressions up to objective responses in about 50 % of patients. The median progression-free survival time is 7 months; up to this duration, half of the patients have developed resistance to vemurafenib. A small percentage of patients of 10–15 % are now for 18–36 months on treatment and may develop late or no resistance. A major clinical challenge in vemurafenib treatment is the development of acquired vemurafenib resistance and the subsequent often rapid tumour progression. Several mechanisms of resistance to vemurafenib have been reported. The remarkable advances in the direct oncogene therapy in melanoma and the understanding of the mechanisms of vemurafenib resistance has led to the development of novel agents; particularly, the combination of BRAF and MEK inhibitors showed initial promising results. Several clinical trials are in progress using this combination with the different compounds of at least three international drug companies. Other kinase inhibitors probably of the PI3K-AKT signalling pathway will likewise be tested in combination with vemurafenib. Furthermore, these concepts of molecular targeted therapies will be combined with the new immunotherapies in melanoma, and it remains an open question whether simultaneous or sequential schedules will be used in future.

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