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Vemurafenib treatment for melanoma: Efficacy, toxicity, and resistance

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Abstract. Melanoma is a malignant form of melanocytes, which has abnormal genetic alternation on the MAPK signalling pathway. Vemurafenib is a targeted therapy agent of melanoma, which is approved to target BRAF V600E-mutant melanoma in the US and EU. As an analogue of PLX4720, it has a promoted efficacy but maintain the selectivity to BRAF V600E kinase. Although vemurafenib has demonstrated its strong effects in clinical, potential toxicity of cutaneous lesions is a serious side effect problem in vemurafenib treatment. To avoid such serious adverse event, refrain from sun exposure and combination therapy with vemurafenib are suggested. Reactivation of the MAPK signalling pathway is the main acquired resistance of BRAF inhibitors, combinatorial therapies are suggested but with limited effects, new small molecules are undergoing development. DNA-based diagnostic test is a priority to determine gene types and detect BRAF mutations for patient stratification.

Keywords: melanoma, vemurafenib, BRAF, drug resistance, patient stratification.

1. Introduction

Melanoma, a prevalent skin cancer in western countries, has a high survival rate for localised tumours but significantly drops for metastatic cases [1, 2]. Surgery is the primary therapy of localised melanoma, achieving a 70-90% cure rate [1]. For patients with metastatic unresectable or recurrent melanoma, who are ineligible for surgery, rely on therapies such as chemotherapy, photodynamic therapy (PDT), immunotherapy, and targeted therapy to control disease progression [3].

Dacarbazine is the only chemotherapeutical drug licensed for melanoma, which is commonly used in clinical trials as a comparative drug [1]. PDT is an adjuvant therapy [3], which is often combined with dacarbazine for improved outcomes.

Targeted therapy agents involve small molecule inhibitors that target mutations in key signalling pathways. While sun exposure plays a role in melanoma, genetic alterations strongly associated with oncogenic mutations are found in approximately 70% of melanoma patients [4], aligning with malignant phenotypes and cell proliferation [4].

Vemurafenib is the first approved small molecule inhibitor of treating malignant melanoma with BRAF V600E-mutant in the US and Europe [1], which is a disruptive drug and worthy to be discussed. This review provides an overview of vemurafenib, including the model of action, effectiveness, adverse events and toxicity, resistance, and current companion diagnostic tests for patient stratification.

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2. Mode of action

Melanoma is a malignant form of melanocytes, which can be found throughout endogenous and indigenous of the body [5]. Under the normal condition of melanocytes, the RAS-RAF-MEK-ERK signalling pathway, also called MAPK signalling pathway, projects signals from the cell surface to the transcription factors to regulate gene expressions, such as cellular proliferation, differentiation, and surviva [l, 5]. RAS binds to the receptor of growth factor on cellular surface and sends signals to RAF, RAF phosphorylates and activates MEK, followed by ERK phosphorylation [1]. The activated ERK will then send signals to the nucleus and activates transcription factors, which then enables cell proliferation, differentiation, and survival. It is also worth to notice that each component in this pathway has many isoforms. For example, RAF has three isoforms (ARAF, BRAF, and CRAF), and the BRAF is highly expressed in the neural crest cells, such as melanocytes [1].

BRAF-mutant is the most common genetic alternation, which represents 50%-60% of melanomas [5], and over 90% of BRAF-mutant are happened at amino acid position 600, presented as a substitution of glutamic acid for valine (BRAF V600) [6]. This mutation activates BRAF proteins and overstimulates the MAPK signalling pathway, allowing MEK and ERK kinases can be independently activated without the activation of RAS, results in sustaining cellular proliferation and differentiation of melanocytes, and causes melanoma [1]. Vemurafenib selectively inhibits phosphorylation of MEK and ERK by binding to the BRAF V600-mutant and reduces signalling through MEK and ERK to downregulate transcription factors to stop the overexpression of cellular differentiation and proliferation (Figure 1).

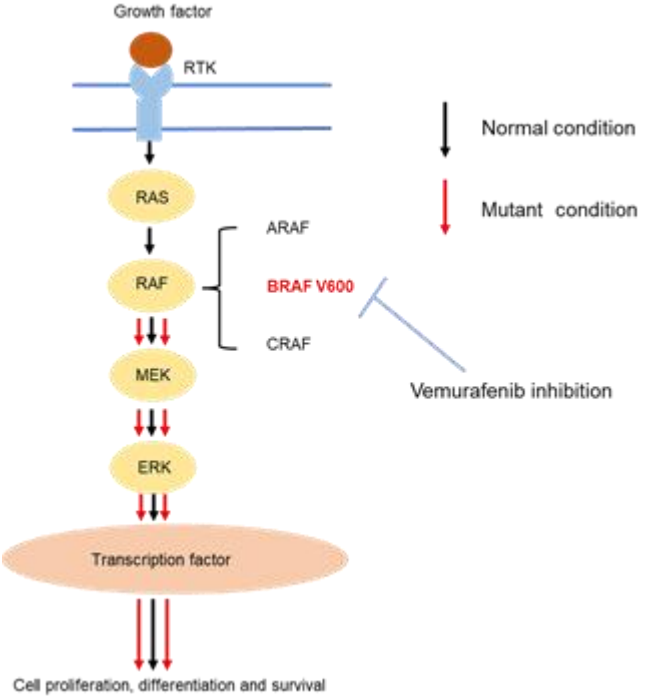

Figure 1. RAS-RAF-MEK-ERK signalling pathway (also called MAPK signalling pathway) in normal condition and mutant condition. Vemurafenib inhibits mutation at BRAF V600. Abbreviations: RTK, receptor tyrosine kinases; RAS, rat sarcoma viral oncogene homolog; RAF, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase.

3. Effectiveness

Vemurafenib is an analogue of PLX4720, a derivative of 7-azaindole that selectively binds within the RAF pocket and overlaps the ATP binding site [7]. PLX4720 significantly inhibits the activity of BRAF V600E kinase at a lower concentration than wild-type BRAF in vitro, and inducing cell

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apoptosis in melanoma models, especially in BRAF V600E positive cells [7]. Since vemurafenib was investigated by scaffold- and structure-based discovery approach and crystallography-guided optimisation, it maintains the selectivity to BRAF V600E kinase but has improved efficacy compared to the efficacy than PLX 4720 (IC50 of PLX 4720: 13 nmol/L, vemurafenib: 30 nmol/L) [1, 7, 8].

In vitro study of vemurafenib on melanoma cell lines shows prompted antiproliferative effects and apoptotic death in all BRAF-mutants (V600E, V600D, V600K, and V600R) [9]. In vivo animal studies in different BRAF V600E-expressing melanoma mice models show dose-dependent tumour regression and improved animal survival [8]. Both in vitro and in vivo studies show better effectiveness than PLX4720.

A phase III trial led by Chapman et al. in 2011 evaluated the efficacy of vemurafenib in patients with unresectable late-stage melanoma and positive BRAF V600E mutation has also demonstrated the advantages of vemurafenib The trial included 675 patients who received vemurafenib and dacarbazine. Vemurafenib was orally administered at a dose of 960 mg twice a day, while dacarbazine was given intravenously at a dose of 1000 mg per square meter of body surface area every 3 weeks [10]. The progression-free survival (PFS) and overall survival (OS) rate were primary endpoints of this study. The vemurafenib group demonstrated a PFS of 5.3 months compared to 1.6 months in the dacarbazine group. The OS rate after 6 months was 84% in the vemurafenib group and 64% in the dacarbazine group. Tumour size decreased in most patients in the vemurafenib group, but not in the dacarbazine group [10]. These findings indicate a significant increase in PFS with vemurafenib, a summary of this trial data on PFS and OS are shown in Figure 2.

Vemurafenib is also effective on other BRAF V600-mutant diseases. As BRAF V600 mutations are usually found in several glioma subtypes, a phase 2, open-label, non-randomised, multicentre study on positive BRAF V600 mutant but nonmelanoma patients has shown a meaningful activity of vemurafenib, but the efficacy varies with histologic subtypes, and the clinical benefits were not as significant as the treatment on BRAF V600E [11].

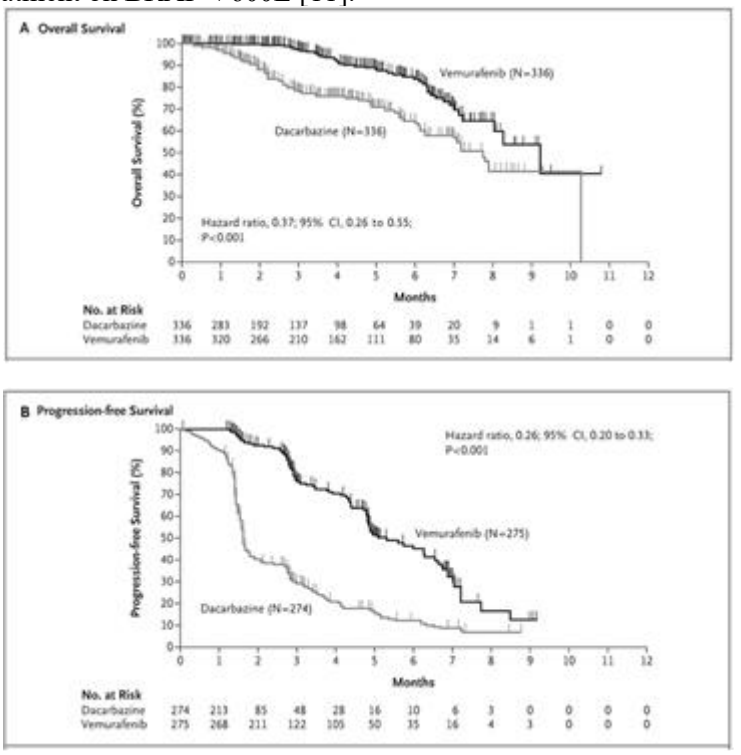

Figure 2. Overall survival (OS) and progression-free survival (PFS) data. 672 patients were measured for OS and 549 patients were measured for PFS [10].

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4. Adverse events and toxicity

The most frequent adverse events of vemurafenib group reported in the clinical trial of Chapman et.al. were cutaneous lesions, arthralgia, and fatigue, which were corresponded to clinical trial studies of vemurafenib in phase I and II [12, 13]. The potential toxicity of cutaneous lesions is a serious side effect problem in vemurafenib treatment and is also reported in many other literature [14-16], the most common dermatological toxic events related to the use of vemurafenib in patients with melanoma were cutaneous squamous cell carcinoma (SCC), keratoacanthoma (KA), rash, and photosensitivity [17].

SCC and KA are the most relevant side effect of vemurafenib, which occurs about 8 weeks after taking the drug [18]. KA can be considered as a lower graded SCC tumour [19], SCC is normally presented in two forms, a well-differentiated type and a KA type [18]. Rash develops through the whole body, including face/neck, trunk, and extremities, after 2 weeks of the drug initiation. There are many types of rashes reported, the maculopapular rash is the most common type of vemurafenib initiation [18]. Photosensitivity also develops within several days of the treatment, and it has been demonstrated that ultraviolet A (UVA) activates this toxic effect [20]. Therefore, avoiding sun exposure is essential for patients who are having vemurafenib therapy [18].

Furthermore, these skin lesions have been found that they are strongly related to the inhibition of BRAF expression alone [15]. This is due to the conflicting activation of the MAPK pathway induced by the inhibition of BRAF in cells that have no BRAF V600 mutation, such as keratinocytes, results in keratinocyte cell apoptosis and causes dermatologic toxicity [21]. Since an addition of MEK inhibition can downstream the BRAF to prevent the paradoxical activation and prolong the therapeutic effect [18], a combination of BRAF-MEK inhibitors is suggested.

5. Drug resistance

The emergence of drug resistance is a major problem in therapeutic success. The resistance to the MAPK pathway can be intrinsic and acquired resistance. Some intrinsic resistance includes loss of Phosphatase and TENsin homolog deleted on chromosome 10 (PTEN) and cyclin D1 amplification, which are accounted for 35% and 20% in BRAF-mutant melanomas [22]. Acquired resistances are mainly associated with the reactivation of the MAPK pathway, comprising alternative BRAF splicing, and amplification of BRAF or increasing gene copies of BRAF, which are considered as predominate resistance mechanism to bypass BRAF inhibition [22].

PTEN is a tumour suppressor gene, functioned by blocking PI3K pathway to weaken the ability of PI3K27. In cancers, the inactivation of PTEN is caused by genetic alterations including mutations, deletions, and hypermethylation in the PTEN promoter region [23]. Cyclin D1 positively regulates cell proliferation, and its expression can be enhanced by BRAF mutations. Therefore, tumour cells confer resistance from BRAF inhibitors [24].

Activating mutations in NRAS or KRAS, amplification of CRAF, or amplification of COT result in the reactivation of MAPK pathway and therefore resistance to BRAF inhibitors [25], bypass the inhibition of vemurafenib. Genetic alternation of activate MAPK/ERK activation are present in about 50% of melanoma resistance [22, 26]. In addition, increased gene copy number of BRAF induces ERK signalling reactivation and amplifies BRAF genes, which also results in upregulation of BRAF protein expression and reactivating ERK to trigger resistance [27] (Figure 3).

A combination of vemurafenib with other therapies, such as another small molecular agent, immunotherapy, and PDT, may help to overcome these resistances, and also increase the efficacy and sensitivity. For example, combinatorial BRAF and MEK inhibitor therapies of cobimetinib/vemurafenib showed a limited clnical efficacyon previously developed resistance patients [28]. Next-generation therapies to overcome resistance to BRAF inhibitors are focused on developing small molecules to inhibit paradoxical activation of CRAF by BRAF inhibitors by interfering with isoform hetero- and homo-dimerisation [29].

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6. Companion diagnostic tests and stratification

Companion diagnostic tests are crucial for identifying specific genes or biomarkers in patients and stratifying them for targeted therapy. As vemurafenib is beneficial to patients with BRAF V600-mutant melanoma, a diagnostic test is necessary to determine the patients' gene types and stratify them accordingly. The current companion diagnostic test utilises RT-PCR to amplify BRAF gene copies and detect the nucleotide sequence. This assay employs two primers, one for detecting BRAF mutation and the other for wild-type BRAF. When the DNA polymerase combines with the primer into a new DNA strand, a fluorophore is emitted from the primer and be detected. Since the DNA synthesis in every cell cycle, the abundance of BRAF mutation is determined by comparing the number of cycles for mutation detection with the number of cycles for wild-type BRAF detection [30]. This test has a high sensitivity of 95% on the V600E mutation detection, but has limited sensitivity in detecting V600D and V600K mutations [30].

Validation studies have shown high accuracy and reliability with 0% invalid results reported in the cobas BRAF test conducted by Lopez-Rios et al. [31] and 4.8% in the study by Jurkowska et al [32].

In addition, there are some other techniques based on protein analysis to detect BRAF mutations. The monoclonal antibody VE1 is an antibody-based test by staining BRAF to the cytoplasm. Although some evidence shows its disadvantage on the possibility of false negatives, it has a higher sensitivity and selectivity than DNA-based test [30].

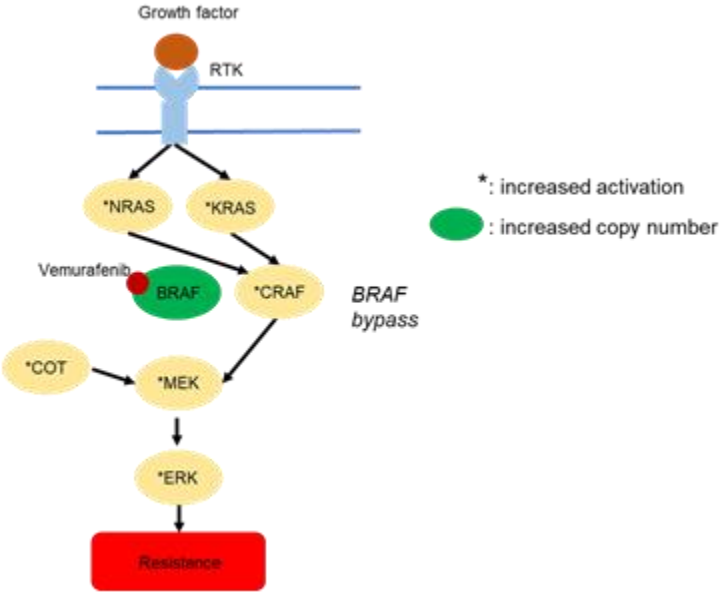

Figure 3. Mechanisms of BRAF inhibitor resistance in melanoma. Abbreviations: RTK, receptor tyrosine kinases; NRAS, neuroblastoma rat sarcoma viral oncogene homolog; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, serine/threonine-protein kinase B-raf; CRAF, RAF proto-oncogene serine/threonine-protein kinase; MEK, mitogen-activated protein kinase kinase; COT, cancer Osaka thyroid; ERK, extracellular signal-regulated kinase.

7. Conclusion

In conclusion, vemurafenib has been proven its efficacy targeting BRAF V600E mutation in the MAPK pathway in melanomas. The effectiveness has been established through various studies, including in vitro, in vivo, and clinical trials. Nevertheless, it is associated with toxicities such as cutaneous lesions, arthralgia, and fatigue, including squamous cell carcinoma (SCC), keratoacanthoma (KA), rash, and photosensitivity. Drug resistance, both intrinsic and acquired, is also a concern with vemurafenib. Combination therapies have limited benefits in overcoming resistance, and efforts are focused on developing small molecules to prevent paradoxical activation of CRAF by BRAF inhibitors. Companion diagnostic tests, such as the cobas 4800 BRAF V600 Mutation Test, are used

for patient stratification, while other antibody-based tests are emerging. Further research is needed to investigate resistance mechanisms, develop strategies to overcome resistance, and improve patient stratification tests.

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