REVIEW

The pharmacological impact of ATP-binding cassette drug transporters on vemurafenib-based therapy

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Abstract Melanoma is the most serious type of skin cancer and one of the most common cancers in the world. Advanced melanoma is often resistant to conventional therapies and has high potential for metastasis and low survival rates. Vemurafenib is a small molecule inhibitor of the BRAF serine-threonine kinase recently approved by the United States Food and Drug Administration to treat patients with metastatic and unresectable melanomas that carry an activating BRAF (V600E) mutation. Many clinical trials evaluating other therapeutic uses of vemurafenib are still ongoing. The ATP-binding cassette (ABC) transporters are membrane proteins with important physiological and pharmacological roles. Collectively, they transport and regulate levels of physiological substrates such as lipids, porphyrins and sterols. Some of them also remove xenobiotics and limit the oral bioavailability and distribution of many chemotherapeutics. The overexpression of three major ABC drug transporters is the most common mechanism for acquired resistance to anticancer drugs. In this review, we highlight some of the recent findings related to the effect of ABC drug transporters such as ABCB1 and ABCG2 on the oral bioavailability of vemurafenib, problems associated with treating...
melanoma brain metastases and the development of acquired resistance to vemurafenib in cancers harboring the BRAF (V600E) mutation.

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1. Introduction

Melanoma is the most serious type of skin cancer. It originates in pigment-producing melanocytes. Melanoma has become one of the most common cancers in the world. Due to its high potential for metastasis, individuals with this disease have a poor prognosis and low survival rates. Melanoma at advanced stages is often resistant to conventional radiation therapy and chemotherapy as a result of multiple mechanisms, including increased DNA repair and alterations of several key regulatory genes or proteins. Therefore, therapeutic approaches directed at specific signaling pathways or mutations in melanoma have been employed. One of the targets is the RAS-activated serine-threonine protein kinase B-raf (BRAF). It plays a central role in the regulation of the mitogen-activated protein kinase (MAPK) signaling pathway that regulates cell division, proliferation and differentiation in melanoma. The consequence of mutations is the constitutive activation of the BRAF kinase and downstream MAPK signaling that promotes unregulated cell proliferation and cell invasion. In melanoma patients, the BRAF(V600E; valine to glutamate) substitution is the most common mutation, which is associated with poor clinical outcome and brain metastases. Since this mutation is found in approximately 40–60% of melanoma patients, improved clinical outcome is expected for melanoma patients with inhibition of BRAF(V600E) signaling.

2. Vemurafenib treatment for BRAF (V600E) mutation patients with advanced or metastatic melanoma

Vemurafenib (PLX4032, Zelboraf) is a small molecule inhibitor of the cytoplasmic BRAF serine-threonine kinase (chemical structure given in Fig. 1), which in 2011 was approved by the US Food and Drug Administration (FDA) for treatment of metastatic and unresectable melanomas that carry an activating BRAF(V600E) mutation. Moreover, in addition to treat unresectable BRAF(V600E) mutant melanomas, studies on evaluating the effectiveness of vemurafenib in brain metastases of melanoma (ClinicalTrials.gov identifier NCT01378975), colorectal cancer (ClinicalTrials.gov identifier NCT00405587) and thyroid cancer (ClinicalTrials.gov identifier NCT01709292) are ongoing. Unfortunately, acquired drug resistance to vemurafenib and relapse among patients were reported frequently within months of therapy. Identifying and overcoming mechanisms that lead to acquired clinical resistance to vemurafenib presents a significant therapeutic challenge.

3. The impact of ATP-binding cassette transporter-mediated drug transport on cancer chemotherapy

Generally, the success of cancer chemotherapy depends on several key factors. For an anticancer agent to be effective, a sufficient amount of the drug must be distributed to the target site(s), which is dependent on the chemical and biological properties of the therapeutic agent, as well as the location of the target site(s). Cancer cells can often acquire resistance through adaptation or spontaneous induction of certain key regulatory genes during the course of chemotherapy, which is dependent on the patient, cancer type, stage of the disease and treatment strategy. Collectively, drug absorption, distribution and acquired resistance may result in poor response to chemotherapy and unfavorable patient outcome. Among various adverse factors in cancer chemotherapy, energy dependent drug efflux and drug compartmentalization are the most common ways that cancer cells evade drug absorption and drug penetration.

Normally, the first line of cellular defense against xenobiotics is to rapidly reduce the intracellular concentration of xenobiotics by means of a transporter-mediated efflux system. Unfortunately, cancer cells can utilize the same protective mechanism by up-regulating some of the drug transporters that reduce drug sensitivity in patients, many of whom eventually relapse with multidrug-resistant forms of cancer. One of the most common causes of acquired drug resistance in cancer is energy-dependent drug efflux by members of the human ATP-Binding Cassette (ABC) protein superfamily. Human ABC proteins are subdivided into seven families (ABCA-ABC), based on structural and sequence similarities. Several ABC proteins are

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**Figure 1** Chemical structures of vemurafenib, dabrafenib and sorafenib.
transplanters that can utilize energy derived from ATP to mediate direct drug efflux. These ABC transporters are membrane proteins, consisting of transmembrane domains (TMDs) and distinctive nucleotide-binding domains (NBDs). The TMDs form substrate-binding pockets, while the NBDs generate energy from ATP hydrolysis to actively transport a wide range of substrates, including anticancer agents, across biological membranes, reducing intracellular drug concentration and eventually resulting in multidrug resistance (MDR)\(^\text{32}\). ABCA9, ABCB1, ABCB5, ABCB8, ABCG2, ABCD1 and ABCG2 are some of the ABC proteins that have been identified in melanoma cells\(^\text{23–28}\). In this review, we focus mainly on the potential roles of ABCB1, ABCG2 and ABCB5 in limiting the absorption, distribution and penetration of vemurafenib, as well as in the development of resistance to this drug in cancer cells expressing a BRAF(V600E) mutation.

3.1. ABCB1

The 170 kDa cell membrane ABCB1 (also known as P-glycoprotein, P-gp) was the first member of the mammalian ABC protein family to be identified\(^\text{20}\). ABCB1 consists of two transmembrane domains, each containing six \(\alpha\)-helices, both linked to ATP-binding domains that provide energy by hydrolyzing ATP to transport drug substrate across cell membranes. A large number of classical anticancer agents including taxanes, Vinca alkaloids, etoposide, teniposide, camptothecins, methotrexate, colchicines, actinomycin D, anthracyclines and mitoxantrone are well-known drug substrates of ABCB1. More importantly, many of the newly developed targeted therapy drugs such as tyrosine kinase inhibitors (TKIs), have been identified as substrates of ABCB1 as well\(^\text{33}\). ABCB1 is expressed in endothelial cells at the blood–brain barrier (BBB) sites in normal brain tissue and also in primary brain tumors, and it functions to limit penetration of the brain by many chemotherapeutics\(^\text{31–32}\). In addition, ABCB1 is highly expressed in many normal tissues, including those of the liver and intestinal walls, signifying the physiological and pharmacological importance of ABCB1\(^\text{32}\). Moreover, ABCB1 is known to be overexpressed in many types of cancer and is linked to the MDR phenotype\(^\text{33}\). Considering the wide tissue distribution and substrate specificity of ABCB1, it is not surprising that ABCB1 plays a key role in limiting the oral bioavailability of anticancer drugs, preventing drug distribution and penetration through the blood–brain barrier and affecting therapeutic outcome in patients\(^\text{34}\). In terms of melanomas, endogenous ABCB1 mRNA has been detected in the melanoma cell lines SK-MEL-28, SK-MEL-5 and M16\(^\text{23,34}\), as well as noncutaneous melanomas\(^\text{35,36}\). ABCB1 was also detected in a subpopulation of human melanoma cells that co-express ABCB5, hTERT, and Nanog, and has high self-renewal capacity, representing characteristics of melanoma stem cells\(^\text{37}\). Interestingly, though the MDR phenotype has been shown in human BRO melanoma cells transfected with human ABCB1\(^\text{38}\), the relevance of endogenous ABCB1 in conferring drug resistance in melanomas has not been demonstrated yet.

3.2. ABCG2

ABCG2 (also known as breast cancer resistance protein, BCRP; or placenta-specific ABC transporter, ABCP; or mitoxantrone resistance protein, MXR) was identified in 1998\(^\text{39,40}\). In contrast to ABCB1, ABCG2 consists of a single ATP-binding domain followed by a transmembrane domain with six \(\alpha\)-helices in a reverse orientation\(^\text{41}\). A functional unit of ABCG2 is a dimer or a multimer. Similar to ABCB1, ABCG2 is overexpressed in many cancers, and is linked to reduced drug accumulation and to the development of MDR in patients with advanced non-small cell lung cancer or acute myeloid leukemia (AML)\(^\text{42,43}\). ABCG2 is capable of transporting a large variety of anticancer agents such as etoposide, docetaxel, topotecan, CPT-11, SN-38, methotrexate, flavopiridol, anthracyclines, mitoxantrone, and similar to ABCB1, many tyrosine kinase inhibitors including imatinib, nilotinib, saracatinib and ponatinib\(^\text{40,44,45}\). ABCG2 also has a physiological and pharmacological impact on drug bioavailability, drug distribution, protection of cells or tissues from xenobiotics and the transport of porphyrins and steroids\(^\text{46–47}\). Similar to ABCB1, ABCG2 has been detected at the luminal membrane of brain capillaries and the BBB, protecting the brain from xenobiotics and chemotherapeutics\(^\text{46–47}\). Studies have shown that both the protein expression and function of ABCG2 are upregulated in neuro-epithelial tumors, restricting penetration of chemotherapeutics and leading to the development of MDR\(^\text{48,49}\). ABCG2 is believed to play a protective role in cancer stem cells (CSCs) or “side population” cells, with self-renewal properties and critical roles in tumorigenesis, metastasis and relapse\(^\text{50}\). Since ABCG2 is expressed in a wide range of human stem cells, it is considered as a biomarker for stem cells. ABCG2, along with CD133 and nestin, have been detected in melanomas\(^\text{51–53}\), but the potential contribution of ABCG2 to chemoresistance in melanomas remains to be determined. Recently, ABCG2 has been linked to the disease gout, as mutations (for example Q141K) in this transporter result in decreased efflux of urate from kidney epithelial cells\(^\text{54,55}\).

3.3. ABCB5

ABCB5 is predominantly expressed in pigment-producing (melanogenic) melanoma cells\(^\text{53,54}\). The melanogenesis-related vesicles, called “melanosomes” are derived from lysosomes and represent a unique feature of melanomas\(^\text{56,57}\). Structurally, ABCB5 has 73% sequence homology with ABCB1 protein\(^\text{58,59}\). In contrast to ABCB1, which mediates drug efflux from cells, ABCB5 is thought to confer chemoresistance to cisplatin, doxorubicin and daunorubicin by intracellular drug sequestration\(^\text{50,52,57,58}\). Furthermore, studies have reported that ABCB5 protein expression is up-regulated upon exposure to the chemotherapeutic drugs dacarbazine (DTIC) and doxorubicin\(^\text{52,59}\). Both ABCB1 and ABCG2 are known to be present in cancer stem cells, and hence are used as stem cell markers\(^\text{50}\). Similar cancer stem cell properties were discovered in metastatic melanoma cells, in which ABCB5 was present\(^\text{55}\). These ABCB5-positive melanoma stem cells are not only drug-resistant, but also possess self-renewal, differentiation and tumorigenic capabilities\(^\text{58,59}\). Interestingly, a recent study showed that ABCB5-expressing cells are resistant to temozolomide, dacarbazine and vemurafenib, suggesting that ABCB5 may contribute to the drug resistance mechanism, and thus is a potential therapeutic target for melanoma chemotherapy\(^\text{50}\). However, ABCB5-mediated transport of these drugs in melanoma patient samples has not yet been demonstrated.

4. The pharmacological impact of ABC drug transporters on the bioavailability and distribution of vemurafenib

Reports have shown a high incidence of melanoma metastases in the brain\(^\text{63,64}\). Prior to the discovery of vemurafenib, a patient’s response to the standard therapy of interleukin-2 and dacarbazine was extremely poor\(^\text{4,6,67}\). However, in order for vemurafenib to be effective against brain metastases of melanoma, sufficient amounts of vemurafenib must first be absorbed in the gastrointestinal (GI)
tract (Fig. 2A), be distributed, and also penetrate the BBB and accumulate in the brain (Fig. 2B). The vasculature structure of the BBB consists of tightly sealed tight-junction protein complexes combined with overexpression of several ABC transporters that actively transport chemotherapeutics back into the bloodstream (Fig. 2B), making drug penetration of the brain a major obstacle in chemotherapy.

A recent study by Mohammed et al. reported that the delivery of vemurafenib to the brain is restricted due to its direct transport by human ABCB1 and mouse Abcg2 at the blood–brain barrier. In their in vitro experiments, the intracellular accumulation of vemurafenib was reduced in MDCKII cells transfected with ABCB1.

![Figure 2](image)

**Figure 2** The potential role of multidrug resistance-associated ABC drug transporters in the oral bioavailability, brain penetration and therapeutic efficacy of vemurafenib in melanoma and other cancer cells harboring V600E mutation in BRAF kinase. (A) Highly active ABCB1 and ABCC2 transporters in intestinal epithelial cells can significantly limit the absorption of vemurafenib into the blood stream, reducing its bioavailability. (B) The presence of both ABCB1 and ABCC2 at the blood–brain barrier restricts vemurafenib penetration of the brain, reducing its effectiveness in patients with brain metastatic melanoma. (C) The presence of ABCC2 confers resistance to vemurafenib in BRAF(V600E) mutant A375 melanoma cells. The role of the ABCB5 transporter in melanoma remains to be evaluated.

or ABCC2, as a direct result of ABCB1 and ABCC2-mediated transport of vemurafenib. Moreover, the ABCB1 and ABCC2-mediated transport of vemurafenib can be inhibited by zosuquidar and Ko143, respectively. Furthermore, in their knockout mouse model, the brain-to-plasma ratios of vemurafenib were increased significantly when Abcla1b and Abcg2 were both absent. The authors concluded that vemurafenib is a substrate of both ABCB1 and ABCG2, and both transporters play a significant role in limiting the central nervous system (CNS) distribution of vemurafenib. The findings by Mohammed et al. were later supported by an independent group. Durmus et al. reported that inhibition of both ABCB1 and ABCG2 could significantly improve the bioavailability (Fig. 2A) and brain penetration (Fig. 2B) of vemurafenib. In their in vitro experiments, vemurafenib transport mediated by either ABCB1 or ABCC2 was demonstrated by using MDCK II cells transduced with either human ABCB1 or ABCG2. The ABCB1- and ABCC2-mediated transport of vemurafenib was inhibited completely by the ABCB1 inhibitor zosuquidar and the ABCC2 inhibitor Ko143. In vivo, the dual Abcla1b/Abcg2 inhibitor elacridar significantly elevated the plasma levels of vemurafenib and brain accumulation in WT mice to the same levels as in Abcla1b−/−; Abcg2−/− mice. Interestingly, Durmus et al. found that Abcg2 is responsible for reducing the intestinal uptake of vemurafenib, but limited to a lower oral dose. In contrast, Abcla1b is accountable for reducing plasma levels of vemurafenib at later stages. This particular observation is in accordance with findings by Chapman et al., that in BRAF(V600E) mutant A375 melanoma cells, ABCC2 behaves as a high-affinity but low capacity transporter of vemurafenib.

5. The potential impact of ABC drug transporters on vemurafenib-based treatment of advanced or metastatic melanoma

Initial success at using vemurafenib to treat patients with metastatic and unresectable melanomas or other cancers that carry an activating BRAF(V600E) mutation was short lived. The rapid development of acquired resistance to vemurafenib is now becoming a major obstacle in the treatment of patients diagnosed with BRAF(V600E)-positive cancer. Multiple mechanisms involving the reactivation of the mitogen-activated protein kinase (MAPK) pathway have been reported in vemurafenib-resistant BRAF(V600E) mutant cancer cells. Up-regulation of CRAF, Tpl2/COT, RAS activation, enhanced activation of the FGFR3/RAS pathway, pathways that lead to reactivation of ERK signaling and activation of RTK signaling pathways such as IGF-1R or PDGFRβ have all been shown to contribute to acquired resistance to vemurafenib, depending on the cancer type.

Recently, we have discovered that in addition to a RAF isoform switch and activation of various compensatory survival pathways, the overexpression of ABCG2 could also contribute to the development of acquired resistance to vemurafenib in BRAF(V600E) mutant cancer cells (Fig. 2C). This is not surprising since the overexpression of ABC drug transporters is one of the most common mechanisms of acquired resistance to anticancer agents. In our study, the interactions of vemurafenib with three major MDR-associated ABC drug transporters, ABCB1, ABCC1 and ABCG2 were investigated. Results showed that vemurafenib binds directly to the substrate binding pockets of ABCG2, inhibits its function and stimulates ATP hydrolysis. Similar interactions between vemurafenib and ABCB1 were observed, but the binding affinity and the
Role of ABC drug transporters in vemurafenib therapy

stimulation of ATP hydrolysis were significantly lower. We found that since vemurafenib binds to the drug binding site of human ABCG2 with relatively high affinity, it effectively inhibited ABCG2-mediated transport of other drug substrates. Moreover, at non-toxic concentrations, vemurafenib was able to restore chemosensitivity of ABCG2-overexpressing HEK293 cells to anticancer agents such as mitoxantrone and topotecan. Similarly, vemurafenib also restored the sensitivity of drug-resistant ABCG2-overexpressing and also expressing (V600E) mutant BRAF A375 melanoma cells to mitoxantrone. In contrast, no interaction was detected between vemurafenib and ABC1 protein. Moreover, 72 h of vemurafenib treatment had no significant effect on the expression of ABCB1, ABC1 or ABCG2 protein in cancer cells expressing wild-type BRAF. Surprisingly, while overexpression of human ABCG2 had no effect on the chemosensitivity of wild-type BRAF cancer cells to vemurafenib, the ectopic expression of human ABCG2 led to vemurafenib resistance in A375 melanoma cells harboring the BRAF(V600E) mutation. We found that in A375 melanoma cells, BRAF kinase inhibition by vemurafenib was significantly reduced in the presence of functional ABCG2, implicating ABCG2-mediated efflux as a mechanism of resistance for vemurafenib. Unfortunately, it is still unknown whether prolonged treatment with vemurafenib leads to overexpression of ABC drug transporters in BRAF(V600E) melanoma, thyroid or colorectal cancers. Furthermore, the potential impact of ABCB1 or ABCB5 or other MDR-associated ABC drug transporters on the therapeutic outcome using vemurafenib in melanomas or other cancers harboring the BRAF(V600E) mutation needs to be determined.

6. Impact of ABC drug transporters on treatment with other BRAF inhibitors (dabrafenib and sorafenib)

Dabrafenib (GSK2118436) is a new BRAF inhibitor (Fig. 1) designed to target melanomas expressing V600E and V600K mutant BRAF. Good clinical response rates have been observed in metastatic melanoma (including brain metastases) patients receiving dabrafenib, but cases of acquired resistance to dabrafenib have also been reported. Although the link between ABC drug transporters and acquired resistance to dabrafenib is still lacking, a recent study using MDCKII cells indicated that dabrafenib is a substrate of both ABCB1 and ABCG2. Moreover, Mittapalli et al. showed that in both in vivo and intact BBB models, the dabrafenib brain distribution is limited by the function of both ABCB1 and ABCG2. In contrast to vemurafenib and dabrafenib, sorafenib is a nonselective BRAF inhibitor (Fig. 1) that targets both BRAF and CRAF, and inhibits other multiple kinases. A phase I/II clinical trial reported that in metastatic melanoma patients, combination therapy of sorafenib, carboplatin and paclitaxel demonstrated a better response rate and also metastatic melanoma patients, combination therapy of sorafenib, carboplatin and paclitaxel demonstrated a better response rate and limit the penetration of the brain by vemurafenib (Fig. 2A and B), which is a major obstacle when treating patients with melanoma brain metastases. The clinical application of a dual ABCB1 and ABCG2 inhibitor such as elacridar could possibly provide a solution to increase the oral bioavailability and enhance brain penetration of vemurafenib in patients with brain metastatic melanoma. At the cellular level, the presence of MDR-associated ABC drug transporters may present new therapeutic challenges when treating cancers expressing the V600E mutant version of BRAF kinase. The ability of ABC drug transporters to effectively reduce the intracellular concentration of vemurafenib in cancer cells can potentially lead to acquired resistance to this drug (Fig. 2C). Moreover, the reported high affinity of vemurafenib for binding to ABCG2 suggests the potential use of vemurafenib as a chemosensitizer that would work alongside classical anticancer agents to target ABCG2-positive MDR cancers. Consistent with these findings, vemurafenib was found to dock in the drug-binding pocket of the homology model of human ABCB1 and ABCG2 and also modulate the function of the ABCCC10 (MRP 7) transporter. Thus, we propose that simultaneous administration of vemurafenib and protein kinase inhibitors targeting key signaling pathways that are involved in the development of acquired resistance to vemurafenib, as well as inhibiting the actions of ABC drug transporters in BRAF(V600E) mutant cancers, may offer great promise for effective treatment of melanoma patients.

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References

1. Shukla S, Skoumbourdis AP, Walsh MJ, Hartz AM, Fung KL, Wu CP, et al. Synthesis and characterization of a BODIPY conjugate of the BCR-ABL kinase inhibitor Tasigna (nilotinib): evidence for transport of Tasigina and its fluorescent derivative by ABC drug transporters. Mol Pharm 2011;8:1292–302.
2. Balch CM, Gershenson JD, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199–206.
3. Chin L, Garraway LA, Fisher DE. Malignant melanoma: genetics and therapeutics in the genomic era. Oncogene 2003;22:3138–51.
4. Soengas MS, Lowe SW. Apoptosis and melanoma chemoresistance. Oncogene 2005;24:2149–82.
5. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004;351:998–1012.
6. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 2009;114:1537–44.
7. Wolf A, Bauer B, Hartz AM. ABC transporters and the Alzheimer’s disease enigma. Front Psychiatry 2012;3:54.
8. Wan PT, Garnet MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;116:655–67.
9. Hartz AM, Miller DS, Bauer B. Restoring blood-brain barrier P-glycoprotein reduces brain amyloid-beta in a mouse model of Alzheimer’s disease. Mol Pharmacol 2010;77:715–23.
10. Arkenau HT, Kefford R, Long GV. Targeting BRAF for patients with melanoma. *Br J Cancer* 2011;104:392–8.
11. Berghoff AS, Capper D, Preusser M. Lack of BRAF V600E protein expression in primary central nervous system lymphoma. *Appl Immunohistochem Mol Morphol* 2013;21:351–3.
12. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* 2010;467:596–9.
13. Facchetti F, Monzani E, La Porta CA. New perspectives in the treatment of melanoma: anti-angiogenic and anti-lymphangiogenic strategies. *Recent Pat Anticancer Drug Discov* 2007;2:73–8.
14. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011;364:2507–16.
15. Hartz AM, Bauer B. ABC transporters in the CNS
16. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Comparison of ATP-binding cassette transporter interactions with and without a muscle protein. *J Biol Chem* 2003;278:47156–65.
17. Ellison AM, Al-Hajj MA. ABCB8 mediates doxorubicin resistance in melanoma cells by protecting the mitochondrial genome. *Appl Immunohistochem Mol Morphol* 2013;21:351–3.
18. Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, et al. Principal expression of two mRNA isoforms (ABCB 5alpha and 5beta) of the ATP-binding cassette transporter ABCC5 in melanoma cells and melanocytes. *Exp Cell Res* 2007;329:662–7.
19. Frank NY, Pendse SS, Lapchak PH, Margaryan A, Shlain D, Doeing PK, et al. Different effects of interferon-alpha on melanoma cell lines: a study on telomerase reverse transcriptase, telomerase activity and apoptosis. *Br J Dermatol* 2003;148:1115–24.
20. Higgins CF. ABC transporters: from microorganisms to man.
21. Esiobu N, Green M, Echeverry A, Bonilla TD, Stinson CM, Hartz A, et al. High numbers of Staphylococcus aureus at three bathing beaches in South Florida. *Int J Environ Health Res* 2013;23:46–57.
22. Lincke CR, van der Veek J, Schuurhuis GJ, van der Velde-Koets T, Snait JI, Borst P. Multidrug resistance phenotype of human BJO melanoma cells transfected with a wild-type human mdr1 complementary DNA. *Cancer Res* 1990;50:1779–85.
23. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
24. McNama M, Clynes M, Dunne B, NicAmhlaibh R, Lee WR, Barnes C, et al. Multidrug resistance in ocular melanoma. *Br J Ophthalmol* 1996;80:1009–12.
25. Rouzaud F, Costin GE, Yamaguchi Y, Valencia JC, Berens WF, Chen KG, et al. Regulation of constitutive and UVR-induced skin pigmentation by melanocortin 1 receptor isoforms. *FASEB J* 2006;20:1927–9.
26. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011;364:2507–16.
27. Linn CE, Frank NY, Pendse SS, Lapchak PH, Margaryan A, Shlain D, Doeing PK, et al. Multidrug resistance phenotype of human BJO melanoma cells transfected with a wild-type human mdr1 complementary DNA. *Cancer Res* 1990;50:1779–85.
28. McNama M, Clynes M, Dunne B, NicAmhlaibh R, Lee WR, Barnes C, et al. Multidrug resistance in ocular melanoma. *Br J Ophthalmol* 1996;80:1009–12.
29. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
30. Rouzaud F, Costin GE, Yamaguchi Y, Valencia JC, Berens WF, Chen KG, et al. Regulation of constitutive and UVR-induced skin pigmentation by melanocortin 1 receptor isoforms. *FASEB J* 2006;20:1927–9.
31. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
32. Rouzaud F, Costin GE, Yamaguchi Y, Valencia JC, Berens WF, Chen KG, et al. Regulation of constitutive and UVR-induced skin pigmentation by melanocortin 1 receptor isoforms. *FASEB J* 2006;20:1927–9.
33. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
34. Rouzaud F, Costin GE, Yamaguchi Y, Valencia JC, Berens WF, Chen KG, et al. Regulation of constitutive and UVR-induced skin pigmentation by melanocortin 1 receptor isoforms. *FASEB J* 2006;20:1927–9.
35. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
36. McNamara M, Clynes M, Dunne B, NicAmhlaibh R, Lee WR, Barnes C, et al. Multidrug resistance in ocular melanoma. *Br J Ophthalmol* 1996;80:1009–12.
37. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
38. Rouzaud F, Costin GE, Yamaguchi Y, Valencia JC, Berens WF, Chen KG, et al. Regulation of constitutive and UVR-induced skin pigmentation by melanocortin 1 receptor isoforms. *FASEB J* 2006;20:1927–9.
39. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
40. McNamara M, Clynes M, Dunne B, NicAmhlaibh R, Lee WR, Barnes C, et al. Multidrug resistance in ocular melanoma. *Br J Ophthalmol* 1996;80:1009–12.
41. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
42. Rouzaud F, Costin GE, Yamaguchi Y, Valencia JC, Berens WF, Chen KG, et al. Regulation of constitutive and UVR-induced skin pigmentation by melanocortin 1 receptor isoforms. *FASEB J* 2006;20:1927–9.
43. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
44. McNamara M, Clynes M, Dunne B, NicAmhlaibh R, Lee WR, Barnes C, et al. Multidrug resistance in ocular melanoma. *Br J Ophthalmol* 1996;80:1009–12.
45. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
46. McNamara M, Clynes M, Dunne B, NicAmhlaibh R, Lee WR, Barnes C, et al. Multidrug resistance in ocular melanoma. *Br J Ophthalmol* 1996;80:1009–12.
47. Keshet GI, Goldstein I, Izrakh O, Cesarkas K, Shenhav L, Yakirevitch A, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008;368:930–6.
52. Grichnik JM, Burch JA, Schulteis RD, Shan S, Liu J, Darrow TL, et al. Melanoma, a tumor based on a mutant stem cell? J Invest Dermatol 2006;126:142–53.

53. Klein WM, Wu BP, Zhao S, Wu H, Klein-Szanto AJ, Tahan SR. Increased expression of stem cell markers in malignant melanoma. Mod Pathol 2007;20:102–7.

54. Woodward OM, Tukaye DN, Cui J, Greenwell P, Constantoulakis LM, van de Locht LT, de Witte TJ, et al. Preferential expression of a high capacity urate exporter, ABCG2, in intractability of malignant melanomas. J Invest Dermatol 2006;127:9003–7.

55. Frank NY, Margaryan A, Huang Y, Schatton T, Waaga-Gassmer AM, Gasser M, et al. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. Cancer Res 2005;65:4320–33.

56. Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanomas. J Immunother 2008;31:2105–8.

57. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, et al. Adjuvant interferon alfa in malignant melanoma: an interdisciplinary and multinational expert review. Crit Rev Oncol Hematol 2013;85:149–61.

58. Flach EH, Rebecca VW, Herlyn M, Smalley KS, Anderson AR. Fibroblasts contribute to melanoma tumor growth and drug resistance. Mol Pharm 2011;8:2039–49.

59. Carlino MS, Saunders CA, Haydu LE, Menzies AM, Martin Jr. CC, Lebowitz PF, et al. 18F-labelled fluoroethylglucose-positron emission tomography (FDG-PET) heterogeneity of response is prognostic in dabrafenib treated BRAF mutant metastatic melanoma. Eur J Cancer 2013;49:395–402.

60. Wittmoe JS, Tembe V, Howle JR, Sharma R, Thompson IF, Rizos H, et al. Intratumoral molecular heterogeneity in a BRAF-mutant, BRAF inhibitor-resistant melanoma: a case illustrating the challenges for personalized medicine. Mol Cancer Ther 2012;11:2704–8.

61. Mittapalli RK, Vaidhyananathan S, Dudev AZ, Elmqquist W, Mechanisms limiting distribution of the threonine-protein kinase B-RafV600E inhibitor dabrafenib to the brain: implications for the treatment of melanoma brain metastases. J Pharmacol Exp Ther 2013;344:655–64.

62. Wilhelmsen SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAP/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099–109.

63. Flaherty KT, Schiller J, Schuchter LM, Liu G, Tuveson DA, Redlinger M, et al. A phase I trial of the oral, multikinase inhibitor sorafenib in combination with carboplatin and paclitaxel. Clin Cancer Res 2008;14:4836–42.

64. Abraham J, Edgerly M, Wilson R, Chen C, Rutt A, Bakke S, et al. A phase I study of the P-glycoprotein antagonist tariquidar in combination with vinorelbine. Clin Cancer Res 2009;15:3574–82.

65. Lagas JS, Fan L, Wagenaar E, Vlaming ML, van Tellingen O, Beijnen JH, et al. P-glycoprotein (P-gp,Abcb1), Abcc2, and Abcc3 determine the pharmacokinetics of etoposide. Clin Cancer Res 2010;16:130–40.

66. Agarwal S, Hartz AM, Elmqquist W, Baur B. Breast cancer resistance protein and P-glycoprotein in brain cancer: two gatekeepers team up. Neurosurg Rev 2012;35:109–14.

67. Hyatil F, Vergely C, Vignaud DP, Grand-Perret T. In vitro and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamido derivative. Cancer Res 1993;53:4595–402.

68. Vispute SG, Chen JJ, Sun YL, Sodani KS, Singh S, Pan Y, et al. Vemurafenib (PL4032, Zelboraf®), a BRAF inhibitor, modulates ABCB1-, ABCG2-, and ABCC10-mediated multidrug resistance. J Cancer Res Updates 2013;2:306–17.