REVIEW

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Advances in personalized medicine – medicinal chemistry and pharmacology of vemurafenib and ivacaftor

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Dedicated to Prof. Dr. Theo Dingermann, Frankfurt, on the occasion of his 65th birthday.

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Pharmacogenomics offers an entrance in the field of personalized medicine. This form of adapted therapy is going to be the future concerning the reduction of side effects and efficacy of the treatment of severe diseases. Vemurafenib and Ivacaftor are the first FDA approved drugs specially addressing mutated proteins. Both substances showed promising results in all clinical trials combined with relatively mild side effects by vemurafenib and placebo-like side effects by ivacaftor. The efficacy in addressing the specific mutation of each compound was confirmed in preclinical and clinical development.

1. Introduction

Over the last few years, the growing knowledge of pharmacogenomics has found introduction in clinical practice and therapy in several fields. Considerable interest has focused on genetic variability in pharmacokinetics but also the influence of polymorphisms on pharmacodynamics is intensively investigated and there are even the first drugs that target a specifically mutated protein. With the possibility to predict changed activities or metabolism of drugs the pharmacotherapy can be optimized for the individual patient by improving the efficacy and lowering side-effects. And in addition to the benefit for the patient the therapy can be more economic and cost-effective. While much progress has been made in understanding the pharmacogenomics of several drugs, others still have to be investigated. In honor of Prof. Dingermann who has been very active in researching pharmacogenomics and personalized medicine as well as in promoting it on the executive and political sector we want to highlight two advanced approaches in individualizing and personalizing pharmacotherapy with small molecules. Ivacaftor and vemurafenib have emerged as the first approved drugs that specifically target mutated proteins. Their medicinal chemistry and pharmacology shall be reviewed here.

Pharmacogenomics have an important role especially in oncology. With the development of potent and highly selective chemotherapeutics genotyping of tumor cells is crucial for the selection of an appropriate therapy since some anti-tumor agents are only effective, when certain oncogenic mutations are present and others are not. Tyrosine kinase inhibitors e.g. can potently inhibit the activity of the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) which is in several tumors constitutively activated by oncogenic mutations. But when an oncogenic mutation in the small GTPase K-Ras a down-stream target of EGFR occurs, the tyrosine kinase inhibitors are ineffective.

2. Vemurafenib

One of the most advanced agents in targeted tumor therapy is vemurafenib. It is the first personalized drug for the therapy of $BRAF^{V600E}$ mutated malignant melanoma. After promising results in all clinical trials the FDA approved vemurafenib (Zelboraf®) in August 2011. It is a small molecule serine threonine kinase inhibitor of BRAF which inhibits the abnormal activation of the mitogen-activated protein kinase (MAPK) pathway (Fonkem et al. 2012). In Europe the approval was achieved in February 2012 (Bollag et al. 2012).

The incidence for melanoma in developed countries is 9.5/100000 for men and 8.6/100000 for women (Mello 2012). While melanoma has a good prognosis in early stages, metastatic melanoma is nearly incurable. The tendency of this cancer to develop brain metastases is high and in nearly half of the patients such CNS metastases can be found. The prognosis of these patients is poor. Melanoma is hardly sensitive for cytotoxic chemotherapy and brain metastases are poorly affected by systemic chemotherapy (Balakan et al. 2012). Conventional treatments such as radiotherapy and previous surgical resection are not very promising because of the radioresistance of melanoma and the multitude of metastases in the brain tissue (Fonkem et al. 2012). All that results in a 5-year survival rate of only about 3% of patients with brain metastases (Agarwala 2009; Balakan et al. 2012).

2.1. MAPK (mitogen-activated protein kinase) pathway

In healthy cells the MAPK pathway transfers extracellular signals from the cell membrane to the nucleus. This effect is achieved by several phosphorylation steps during the signaling cascade (Inamdar et al. 2010). The RAF (rapidly accelerated fibrosarcoma) kinase joins RAS (rat sarcoma) at the cell membrane after an extracellular signal occurs e.g. the epidermal

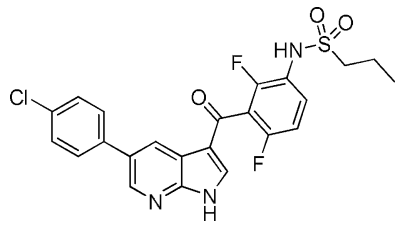

Fig. 1: Vemurafenib

growth factor (EGF) binds to its receptor EGFR. RAF is activated by phosphorylation and activates the downstream MEK/ERK cascade. There are three isoforms of RAF: ARAF, BRAF and CRAF which activate the MEK/ERK (ERK: extracellular signal-regulated kinase) pathway (Kaplan et al. 2010). In approximately 50% of the patients with malignant melanoma a mutation at codon 600 of the BRAF gene is detectable (Callahan et al. 2012). Thereby in 90% of the cases a single valine-to-glutamic acid substitution at codon 600 (V600E) of the BRAF kinase domain is existing. According to that mutation, BRAF is activated in a RAS-independent manner and leads to a downstream signal transduction in MAPK pathway. The result is a deregulated proliferation of tumor cells (Su et al. 2012). The remaining 10% of the mutations at codon 600 are in most instances valine-to-lysine substitutions ($BRAF^{V600K}$) (Callahan et al. 2012). Mutations in the BRAF, RAS and other genes can deregulate the pathway by increasing the signaling activity resulting in demanding cell proliferation, invasion, metastasis, migration, survival and angiogenesis (Inamdar et al. 2010).

2.2. *Crystal structure of vemurafenib*

In difference to other kinases the BRAF kinase region is folded into a conformation with a special intra-molecular protein-protein interaction between a glycine-rich loop and the activation segment to induce an inactive state of the kinase. Mutations resulting in a disruption of this interaction (e.g. V600E) are thought to simulate activation by phosphorylation (Wellbrock and Hurlstone 2010; Fisher and Larkin 2012). In $BRAF^{V600E}$ mutations the salt bridge between the Glu600 (instead of Val600 in the non-mutated protein) and Lys507 keeps the activation loop in a conformation that renders the mutated protein constitutively active (Fig. 3a, Bollag et al. 2012).

Co-crystallization was an important part of the lead structure optimization to obtain vemurafenib as a potent and selective $BRAF^{V600E}$ inhibitor (Fig. 3b). The 7-azaindole scaffold was a result of a high-throughput screening with a scaffold-based approach of more than 20000 small molecules with selected chemical properties (molecular mass 150–350 Da, fewer than eight hydrogen bond donors and acceptors, few rotatable bonds and relatively high aqueous solubility). The hydrophobic cleft next to the hinge region of the BRAF kinase has kinase selective properties and offers chemical space for enlargement of the molecule. The arylsulfonic amide moiety was obtained during a drug design effort to find substitutions for optimal interaction between the deprotonated sulfonamide nitrogen and the backbone amide proton of Asp594. This is only possible when the BRAF activation loop is in DFG-in conformation (the DFG loop is apparent in the kinase protein family and consists of a characteristical asp-phe-gly motif, DFG-in renders the protein to be in active state) which is likely favored in $BRAF^{V600E}$ and leads to a up to 500-fold increased activity compared to wildtype BRAF (Zhang and Bollag 2010). The propyl chain of vemurafenib perfectly fits into an interior pocket of the mutant BRAF protein and causes an outward shift of the regulatory αC helix. This shift prevents the formation of a salt bridge between Lys483 and Glu501. The chloro-phenyl group was the result of an

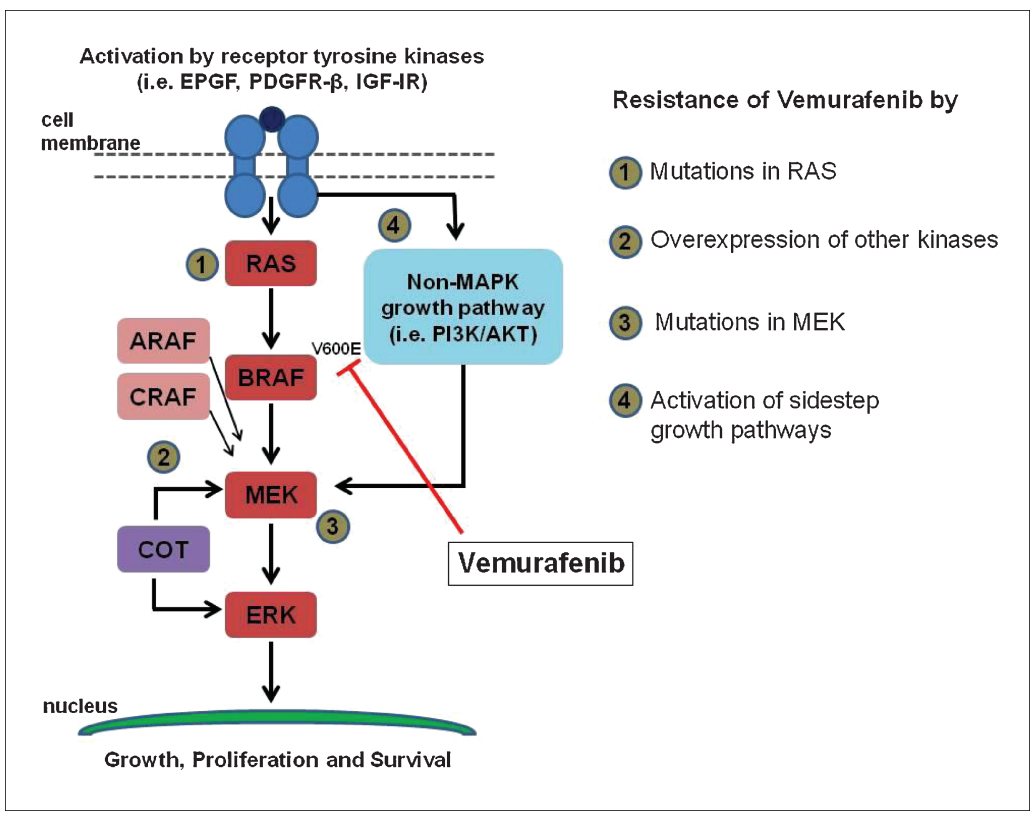

Fig. 2: *MAPK pathway (mitogen- activated protein kinase pathway)*is activated by different RTKs (receptor tyrosine kinases); the cascade is characterized by several activation steps by phosphorylation of RAS (Rat Sarcoma) followed by activation of BRAF (rapidly accelerated fibrosarcoma B), MEK and ERK (extracellular signal-regulated kinase); this activation cascade stimulates cell growth and proliferation processes in the nucleus; the $BRAF^{V600E}$ mutation renders the pathway in permanent activation and Vemurafenib can disrupt this ongogenic overactivation in a specific manner for this mutation; during Vemurafenib therapy resistances occur and are thought to be the result of additional mutations in RAS or MEK, overexpression of CRAF and COT or bypassing the MAPK pathway through other growth signaling cascades; according to (Flaherty et al. 2011; Luke and Hodi 2012)

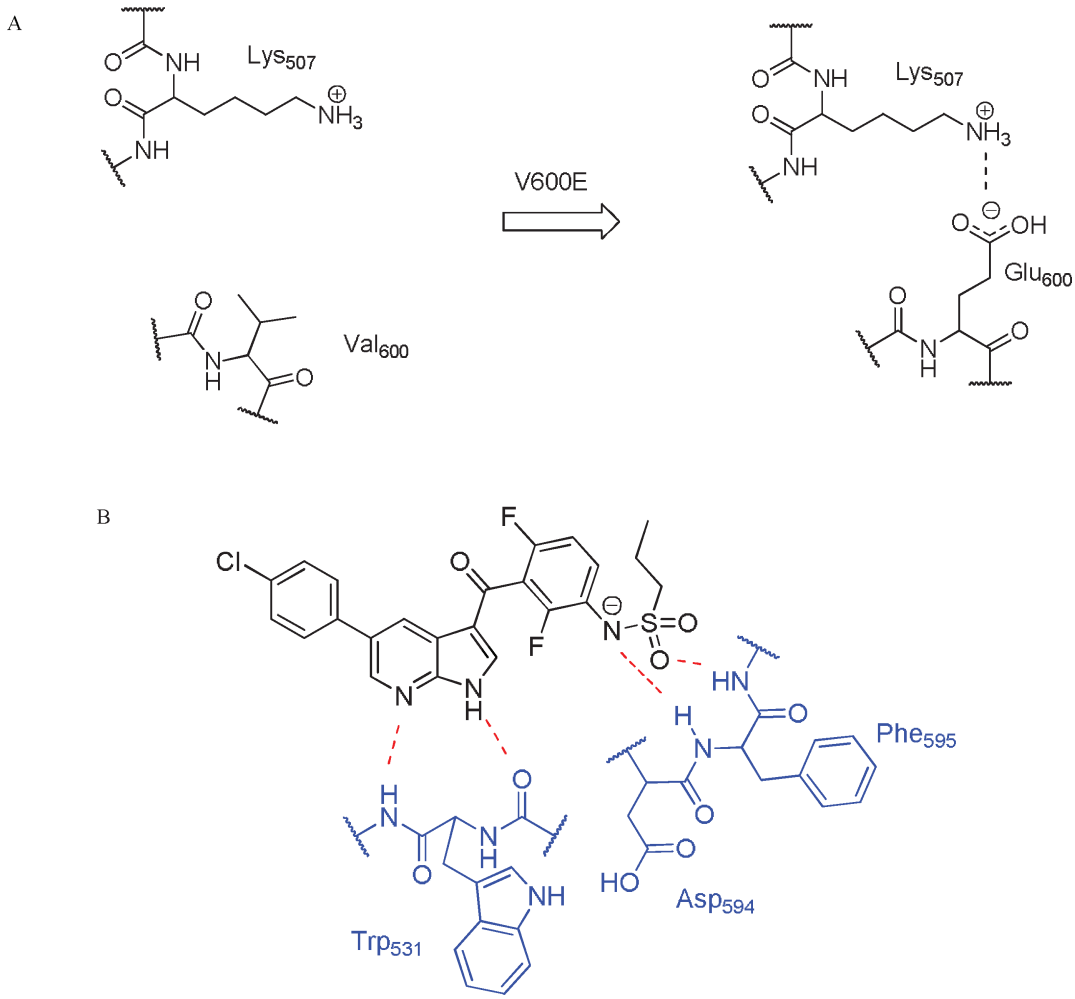

Fig. 3: (a) *Consequence of single amino acid change in BRAF^{V600E}*, the BRAF^{V600E} mutation leads to an additional salt bridge between Lys507 and Glu600 (right) which is not present in unmutated BRAF (left), this salt bridge renders the kinase active resulting in enhanced proliferation, cell growth and survival (Bollag et al. 2010; PDB). (b) *Known interactions of vemurafenib with mutated BRAF^{V600E}* obtained by co-crystallization (Bollag et al. 2010; PDB).

independent optimization for improved potency and pharmaceutical properties (Bollag et al. 2012).
Vemurafenib has a greater affinity than endogenous ATP to bind in the nucleotide-binding pocket of the activated BRAF^{V600E} in an ATP –mimetic manner resulting in a reduced activity of the whole kinase (Davis and Schlessinger 2012).

2.3. *Preclinical and clinical development of vemurafenib*

Initially sorafenib, a multiple tyrosine kinase inhibitor, was tested as a BRAF-inhibitor after the BRAF^{V600E} mutation was discovered in the year 2002. It had no beneficial effects and failed in phase II and III trials because of missing clinical efficacy for the treatment of malignant melanoma (Finn et al. 2012). Vemurafenib was therefore developed to be a more selective and highly potent inhibitor of mutated BRAF. *In vitro* the agent also shows inhibitory effects against other kinases (e.g. ARAF, CRAF and wildtype BRAF) after they were activated by other mechanisms (Halaban et al. 2010). Potent anticancer characteristics were observed in cellular and animal models as well as in first phase I trials showing a complete or partial tumor regression (Flaherty et al. 2011). Vemurafenib has a ten-fold greater efficacy in all BRAF mutated cells (IC_{50} range 60–450 nM) compared to wildtype BRAF cells ($IC_{50} > 2.4$ μM). In purified BRAF^{V600E} cells the IC_{50} value was actually 44 nM (Halaban et al. 2010; Inamdar et al. 2010).
Vemurafenib is orally available, metabolized by CYP3A4 in the liver and cleared primarily via the faeces. It has a high plasma protein binding and a half life time of about 57 h (Heakal et al. 2011, http://reference.medscape.com/drug/zelboraf-vemurafenib-999679#10)

2.3.1. *Phase I*

Clinical investigation of vemurafenib started with a multicenter dose-escalation trial on 55 patients (49 with melanoma). The starting oral dose was 160 mg vemurafenib daily and increased to 1120 mg to confirm a maximum tolerated dose of 960 mg twice-daily. The dose was limited by grade 2 or 3 rash, fatigue and arthralgia. Another phase I trial was performed afterwards with 32 melanoma patients at this maximum dose. The response rates were impressive with 81% complete or partial tumor regression and 56% confirmed response in patients with BRAF^{V600E} melanoma. Responses were also detectable using lower doses denoting that there were partial to total responses in some patients at doses of 240 to 720 mg twice daily in the dose-escalation phase of the trial. The progression-free survival (PFS) was more than 7 months and the duration of response ranged from 2 to 18 months (Lemech and Arkenau 2012; Ascierto et al. 2012a; Flaherty et al. 2010).

2.3.2. *Phase II*

For the phase II trial of the selective kinase inhibitor 132 patients with BRAF V600 mutations (122 BRAF^{V600E} and 10 BRAF^{V600K}) were enrolled. Main enrollment criteria were a pro-

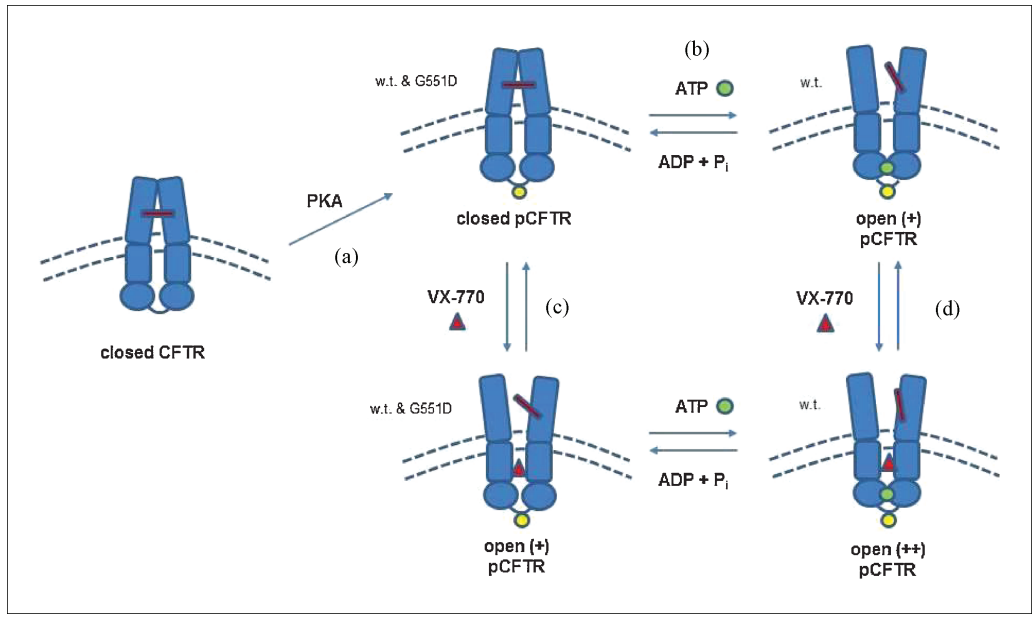

Fig. 4: *Postulated mechanism of action of ivacaftor (VX-770)*: (a) w.t. and G551D-CFTR are phosphorylated by protein kinase A (PKA) or other kinases; (b) binding of ATP then induces gating in w.t. CFTR but not in G551D-CFTR; (c) VX-770 directly binds to phosphorylated w.t. and G551D-CFTR and can open (potentiate) the channel pore without binding of ATP by unknown conformational changes; (d) VX-770 can also potentiate phosphorylated and ATP-activated w.t. CFTR and enhance gating. according to Eckford et al. (2012)

gressive disease after a previous systemic therapy for advanced melanoma and the absence of brain metastases. An overall response of 53% was observed with a total response in 8 of the patients (6%) and a partial response in 62 patients (47%). The mean duration of response was 6.7 month. The median PFS was 6.8 months and 62 of the 132 patients were alive at the cut-off date (median follow-up of 12.9 months). The median overall survival (OS) was 15.9 months (Fisher and Larkin 2012; Mello 2012; Sosman et al. 2012).

2.3.3. *Phase III*

In a randomized phase III trial, the former standard therapy and only drug that was previously approved for the treatment of metastatic melanoma by the FDA, dacarbazine was compared to vemurafenib therapy. 675 patients with BRAF V600 mutated, metastatic melanoma (including 19 patients with $BRAF^{V600K}$ and one with $BRAF^{V600D}$), who had no former therapy, were enrolled. The patients had unresectable tumors, were more than 18 years old and had a life expectancy of > 3 months. Patients were excluded if there was a history of cancer in the last 5 years or uncontrolled brain metastases.

The ratio to obtain either dacarbazine (1000 mg per square meter of body-surface area by intravenous infusion every 3 weeks) or vemurafenib (960 mg twice-daily, orally) was 1:1 and was randomly assigned. Dose reductions were accomplished for both therapies when intolerable grade 2 or higher toxic effects occurred (38% of patients).

After 6 months the overall survival in the vemurafenib group was 84% compared to 64% in the dacarbazine group. PFS was assessed in 549 patients with an estimated duration of 5.3 months under vemurafenib therapy compared to 1.6 months for dacarbazine. 439 patients were evaluated for tumor response. In the vemurafenib group 106 patients of 219 (48%) showed partial (104 patients) or complete (2 patients) response after a median response time of 1.45 months. In the dacarbazine group only 12 of 220 patients (5%) showed an obvious partial response after a median response time of 2.7 months. Four of ten $BRAF^{V600K}$ patients had a partial response to vemurafenib (Chapman et al. 2011; Fisher and Larkin 2012; Mello 2012).

2.3.4. *Side effects of vemurafenib*

In clinical trials the most common grade 2 and 3 side effects were: arthralgia, rash, nausea, photo-sensitivity, fatigue, cutaneous squamous-cell carcinoma, pruritus, alopecia and palmar-plantar dysesthesia. Some patients showed asymptomatic, transient elevations in liver-enzyme levels. Most side effects (89%) however, were grade 1 or 2. Cutaneous sqamous-cell carcinoma occurred in ∼30% of the patients but could be resected in the majority of the cases and did never lead to a discontinuation of treatment (Davis and Schlessinger 2012; Flaherty et al. 2010; Sosman et al. 2012).

2.4. *Resistances*

Unfortunately, most patients developed a resistance against vemurafenib 2–18 months after treatment initiation. While the onset of response to vemurafenib is quite fast, the median duration of this response was only 6.2 months. In FDG-PET(^{18}F-Fluor-desoxyglucose positron-emission-tomography) scans of some patients first effects were observable after only two weeks of vemurafenib therapy. However, when resistance occurred after some months, the disease exhibited strong progression again (Finn et al. 2012).

Most surprisingly, no secondary mutation in BRAF is responsible for this resistance although the main mechanisms of resistance are new mutations in a target. Resistance might be the result of mutations in RAS and MEK (Fig. 2: resistances (1) and (3)) which lead to an activation of downstream processes (Luke and Hodi 2012). Melanoma cells are able to use additional kinase proteins (like CRAF and COT: cancer Osaka thyroid = MAP3K8, Fig. 2: (2)) and non-MAPK growths pathways to sidestep BRAF and reactivate the MAPK pathway (Fig. 2: (4)).

There are several concepts to abrogate resistances of vemurafenib with the aim to exploit the excellent initial efficacy over a longer period of time. The most promising strategies are highlighted here.

Deactivation of BRAF is responsible for a negative feedback that leads to a proven paradox activation of the MEK/ERK cascade by other mechanisms. This effect is thought to be a possible

reason for resistance. The most important approach to reduce resistances is the intervention in the MAPK pathway at the subsequent point of MEK activation which is thought to eliminate the actual reason of activation. This attempt unfortunately shows a cross resistance with BRAF inhibitors when used as a single agent except for the co-presence of a NRAS mutation (Atefi et al. 2011).

The dual application of BRAF and MEK inhibitors shows synergistic effects that lead to a complete drawdown of ERK phosphorylation and therefore block proliferation. Hence, MEK inhibitors can restore the sensitivity to vemurafenib (Su et al. 2012).

Another explanation for the development of resistances is that the activation of the insulin-like growth factor receptor (IGFR) and the platelet-derived growth factor receptor (PDGFR) stimulates the PI3K (phosphoinositide 3-kinase)-AKT(also known as protein kinase B) pathway which plays an additional role in the formation of melanoma. This pathway circumvents the BRAF inhibition of vemurafenib. PI3K-AKT signaling is increased in vemurafenib resistant cell lines (Shi et al. 2011). *In vitro* investigations showed a synergistic effect of vemurafenib co-administered with an AKT inhibitor. ERK and AKT phosphorylation is abrogated when vemurafenib is combined with an AKT inhibitor (Su et al. 2012; Tuma 2011). The combination of a MEK and a AKT inhibitor may therefore provide additional beneficial effects (Atefi et al. 2011).

A third strategy to attenuate the development of resistances against the BRAF inhibitor could be a combination of vemurafenib with the approved human monoclonal antibody ipilimumab that allows durable responses for long-term follow-up therapy. In this combination vemurafenib might provide a fast onset and Ipilimumab an extention of response (Ascierto et al. 2012b).

2.5. Conclusion and outlook

Malignant melanoma is the worst form of all skin cancer types and is in most cases incurable. Although BRAF is known since 1988 (Davis and Schlessinger 2012) the first established personalized therapy to target exclusively the $BRAF^{V600E}$ mutation is vemurafenib which was approved by the FDA in 2011. About 50% of the patients with melanoma are affected by this kind of mutation which causes over-activation of the kinase. The development of vemurafenib is a result of co-crystal structure analysis in combination with *in-silico* drug design and medicinal chemistry and gives an insight into the possibilities of actually personalized and targeted research approaches.

Vemurafenib showed impressive results in all clinical trials with response rates up to 80% and a short time until first effects can be observed (∼2 weeks). With its efficacy and relatively mild side effects the discovery of vemurafenib is a great progress in skin cancer therapy. Unfortunately, the majority of patients are developing resistances against the drug after an average of 6 months. Nevertheless vemurafenib increases the progression free time (5.3–6.8 months) and median overall survival (∼15.9 months) and therefore outmatches other available therapy options for malignant melanoma.

Actually, research is engaged to find possible combination therapies to circumvent the resistance mechanisms of vemurafenib and to develop new substances with similar efficacy and selectivity without the occurrence of resistances. Besides a multitude of possible combination therapies with vemurafenib, several other BRAF or $BRAF^{V600E}$ inhibitors are in clinical trials at present. Norvatis e.g. has two promising compounds for $BRAF^{V600E}$ melanoma and other advanced solid tumors in phase I - LGX818 - (clinical trials.gov ID: NCT01436656), also tested in combination with MEK162 (NCT01543698), and Phase II - RAF265 - (NCT00304525). The H. Lee Moffitt Cancer Center and Research Institute terminated a phase I trial of XL888 (NCT00796484) which is a selective inhibitor of HSP90, a key component of a molecular chaperone complex and therefore involved in sidestep growth pathways. The compound is investigated in a phase I trial (NCT01657591) in combination with vemurafenib. Another phase I trial for advanced $BRAF^{V600E}$ solid tumors is performed for the compound PLX3603 by Hoffmann-La Roche (NCT01143753). The most promising substance however is dabrafenib that is currently in phase III with the MEK inhibitor trametinib (NCT01584648).

Notably, all new research approaches in the field of BRAF mutations are based on the development of vemurafenib which illustrates the possibility of personalized therapy of such mutations.

3. Ivacaftor

3.1. Cystic fibrosis

Also the treatment of cystic fibrosis (CF) has entered personalized medicine recently. Cystic fibrosis is the number one recessively inherited disease in the western world affecting about 80.000 people worldwide (Lubamba et al. 2012). The disease depends on various mutations in an epithelial ion channel, the cystic fibrosis transmembrane conductance regulator protein (CFTR). These defects in the gene of CFTR lead to premature termination of translation, wrong splicing, misfolding of the protein and succeeding degradation or improper functionality of the channel. If CFTR is not present at the cell surface or fails to fulfil its physiological function the transport of chloride ions out of the cells of polarized epithelia is impaired. Amongst several other clinical symptoms the defect channel especially causes the accumulation of highly viscous mucus in the lung as the osmotic uptake of water into the mucus fails with the missing chloride ions. The mucus furthermore provides a breeding ground for various microorganisms and cystic fibrosis patients suffer from chronic infections that finally lead to end stage lung disease. Although cystic fibrosis is present in all ethnic groups it has a significantly higher incidence in caucasians since F508del as the most frequent mutation causing the disease distinguishes them from other ethnicities (Comer et al. 2009; Lubamba et al. 2012).

For decades the pharmacotherapy of cystic fibrosis has only addressed the secondary outcomes of CFTR defects such as mucus accumulation and infections along with their symptoms but was never able to treat the CF causing mutations themselves and 'repair' the defect channel. Recently this situation has changed and while several causal cystic fibrosis therapeutics are in clinical development the first causal drug has yet entered the marked (Davis et al. 2012).

However, it is not the frequent deletion of phenylalanine at position 508 (F508del) that is now causally treatable with a small molecule but the gating mutation G551D which is characterized by the substitution of glycin at position 551 by aspartic acid and leads to impaired activation and regulation of the channel. The

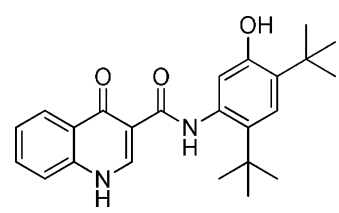

Fig. 5: VX-770 (Ivacaftor)

small molecule ivacaftor (formerly VX-770) is now the first approved causal therapy to treat cystic fibrosis that is dependent on the gating mutation G551D. The drug stabilizes and enhances the open period of physiologically activated G551D-CFTR and is therefore called a CFTR potentiator. Several other gating mutations as well as the deletion of phenylalanine at position 508 which also has characteristics of disturbed gating when the mutated channel reaches the surface might be treatable with CFTR potentiators as well but clinical data is not sufficient yet (Accurso 2010; Barrett et al. 2012; Bompadre et al. 2007; Comer et al. 2009; Davis et al. 2012; Galfrè et al. 2012).

3.2. Structure and activity of CFTR

The transmembraneous chloride ion channel CFTR is a member of the ATP-binding cassette (ABC) transporter family and composed of five distinct domains. At the N-terminal end a transmembrane domain (TMD1) containing six α-helices is located followed by a nucleotide binding domain (NBD1) and a regulatory region before α-helical transmembrane domain (TMD2) and nucleotide binding domain (NBD2) are repeated. CFTR is activated by binding of intracellular nucleotides such as ATP or cAMP at the NBDs and phosphorylation at the regulatory region by protein kinase A (PKA) or protein kinase C (PKC) (Eckford et al. 2012; Galfrè et al. 2012; Hudson et al. 2012; Lubamba et al. 2012; Wang et al. 2010).

The gating mutation G551D is located in the NBD1 of CFTR. *In silico* models and crystal structure analyses of the NBD1 suggest that glycin at position 551 is necessary for normal interaction of the wild-type NBD1 with ATP and that the ATPase activity of the protein is impaired when Gly551 is changed to aspartic acid since introduction of the large charged side chain of aspartic acid into the NBD1 completely disrupts its ATP binding pocket. In wild type CFTR binding of ATP to the NBDs leads to the formation of a head-to tail dimer of the NBDs which is thought to induce conformational changes in CFTR and promote opening of the channel pore. Subsequent ATP hydrolysis disrupts the NBD dimer and causes closure of the channel. With hindrance of ATP binding and hydrolysis by the mutation of Gly551 this gating cycle of the channel is impaired (Atwell et al. 2010; Bompadre et al. 2007; Eckford et al. 2012; Galfrè et al. 2012; Hudson et al. 2012; Lewis et al. 2010; Melin et al. 2006; Moran 2010; Pyle et al. 2011; Wang et al. 2010).

However, besides by binding and hydrolysis of ATP, CFTR activity is also regulated through phosphorylation at the unique R-domain by protein kinases. The mechanism of CFTR activation by this phosphorylation remains unclear but several experiments indicate that it is independent from ATP-mediated gating. G551D-CFTR therefore shows low activity although it cannot be activated by ATP (Eckford et al. 2012; Pyle et al. 2011; Wang et al. 2010).

3.3. Ivacaftor (VX-770) drug development

3.3.1. Preclinical development

The development of ivacaftor started with a high throughput screening based on a cellular membrane potential fluorescence assay. After structural optimization of the lead scaffold considering potency, selectivity and physico-chemical properties VX-770 was selected for preclinical and clinical development (van Goor et al. 2009).

In vitro characterization of VX-770 revealed an EC_{50} of about 0.1 μM for the increase of forskolin stimulated CFTR mediated I_T (as a marker for chloride flux) in Fisher rat thyroid cells that expressed human G551D-CFTR. I_T was increased by about 4-fold, but in the absence of forskolin VX-770 was inactive. Notably, even higher potentiating activity of VX-770 was found for F508del-CFTR that was previously corrected by incubation at low temperature ($EC_{50} \sim 25$ nM, 6-fold increase). Hence, the compound is a potentiator of G551D-CFTR and F508del-CFTR but not an activator since it increases the flow of ions through activated CFTR but is inactive without activating stimulus by forskolin e.g. For rescuing F508del-CFTR however, VX-770 is probably not sufficient since this mutation is not only characterized by impaired gating but predominantly by misfolding of the protein and degradation. This mutation might require a CFTR potentiator and a CFTR corrector (Eckford et al. 2012; van Goor et al. 2009).

The concrete molecular mechanism of action and the binding site of VX-770 remain unclear so far. It has been discussed, whether VX-770 interacts directly with CFTR or rather targets a kinase or phosphatase that in turn modifies CFTR. Recent studies have revealed that VX-770 also improves the gating of purified PKA-phosphorylated G551D-CFTR *in vitro* which suggests that the drug directly binds to the channel protein and does not interact with CFTR modifying enzymes. Notably, ivacaftor was also able to enhance gating of purified CFTR with the G551D mutation in absence of ATP or analogues to an extent of 30–50% of wt CFTR. VX-770 furthermore slightly enhanced the ATPase activity of G551D-CFTR but this was not sufficient to rescue the mutant. VX-770 alone was not able to enhance gating of unphosphorylated CFTR channel protein which contradicts other hypotheses that suggest an interaction of Ivacaftor with lipid membranes in which the channel is incorporated (Bompadre et al. 2007; Eckford et al. 2012; Pasyk et al. 2009; Wang et al. 2010).

Though the molecular binding site of the agent remains unknown, these results have proven that the defective channel G551D-CFTR and probably also the wild-type protein can be activated by an ATP-independent mechanism. It has been proposed that ivacaftor binds to an allosteric binding site of the channel protein and directly modifies structural elements that are important for gating. However, much more research is required to prove this hypothesis but the lack of serious side-effects of ivacaftor also indicates high selectivity which might be explicable by an interaction with an allosteric binding site that is not conserved amongst the ABC-transporter family (Eckford et al. 2012).

3.3.2. Clinical studies with ivacaftor

As potential agent for an orphan disease VX-770 entered clinical development with a randomized double-blind and placebo-controlled phase II trial inclosing 39 adult patients with cystic fibrosis that at least carried one allele of G551D-CFTR. The study was primarily designed to evaluate safety and adverse events of ivacaftor and secondarily to investigate CFTR ion channel function, pulmonary function and health-related life quality. In two stages patients received VX-770 at doses of 25, 75, 150 and 250 mg or placebo twice daily for 28 days overall (Accurso 2010).

Generally, the investigational drug was well tolerated and all subjects completed the study. Some moderate (elevated blood-glucose) and the only severe (pulmonary exacerbation) adverse events that were reported during the trial were not considered to be caused by the study drug and resolved without discontinuation of VX-770. The adverse events profile was similar in all study groups and VX-770 showed no notable side effects (Accurso 2010).

Besides this favorable safety profile, ivacaftor exhibited beneficial effects on CFTR function which was assessed by measurement of nasal potential difference and sweat chloride.

After 14 days of administration significant changes in nasal potential difference were observable within the subjects that had received at least 75 mg VX-770 twice daily while no changes were detected in the placebo group. In all verum groups a significant and dose-dependent reduction of sweat chloride also indicated improvement of CFTR function which was not found in the placebo group. For clinical efficacy FEV_1 (forced expiratory volume in one second) served as biomarker and in the VX-770 75 mg and 150 mg groups significant within-subject improvements from baseline were observed after 14 days of intake. Mean improvement of FEV_1 was significant for all verum groups and showed dose-dependency (Accurso 2010).

These first clinical results of ivacaftor showed good safety with an adverse events profile comparable to placebo and proved clinical efficacy of the drug. With these promising data several further studies have been conducted. Evaluation of VX-770 in 161 patients with the G551D mutation above 11 years of age in a randomized, placebo-controlled, double-blind phase III trial (Ramsey et al. 2011) revealed a significant clinical effect compared to placebo. The verum group received 150 mg ivacaftor twice daily and had improved pulmonary function after 2 weeks of treatment which was maintained through 48 weeks of the study. Compared to placebo the FEV_1 change was more than 10% greater in patients receiving VX-770 and subjects receiving the drug had 55% less pulmonary exacerbations than participants on placebo. In addition, beneficial effects on other biomarkers were observed after 48 weeks of treatment including sweat chloride and weight gain. Ivacaftor had furthermore a safety profile comparable with placebo (Ramsey et al. 2011). A comparable trial (NCT00909727) enrolling patients between 6 and 11 years of age with G551D-CFTR showed similar safety and efficacy. Since cystic fibrosis has a very early onset and especially medications for children with the disease are necessary ivacaftor offers great improvement to all patients with the CFTR gating defect G551D.

In addition to its efficacy in potentiating G551D-CFTR *in vivo* VX-770 was also studied in subjects homozygous for the F508del mutation (Flume 2012) but the results showed no significant efficacy of ivacaftor in patients with F508del-CFTR. Further studies are necessary to evaluate the utility of the drug to potentiate F508del-CFTR. Ivacaftor alone might not be sufficient to rescue this frequent mutation *in vivo* but could act synergistically with a CFTR corrector.

Currently VX-770 is intensely investigated in further clinical studies with subjects bearing the F508del mutation or gating mutations distinct from G551D. The agent is either examined alone or in combination with other CFTR potentiators or correctors that might exhibit synergistic effects. Especially the F508del mutation could arise as the second CFTR defect which is causally treatable with a combination of a corrector that rescues the defect protein from degradation and a potentiator to improve its function.

3.4. Outlook

Ivacaftor as the first small molecule with clinical approval that is able to rescue a mutated protein might only be the precursor of a latter of other compounds that potentiate other mutated forms of CFTR. Besides being effective in potentiating G551D-CFTR, ivacaftor has proven that it is possible to modulate defective proteins and thereby restore their native function at least partly with small molecules.

4. Conclusion

Both vemurafenib and ivacaftor, are outstanding examples of modern drug discovery. Though their targets and their mechanisms of action strongly differ since vemurafenib is a kinase inhibitor while ivacaftor probably has characteristics of an allosteric agonist or modulator of a channel protein, both molecules have in common that they are the first approved small molecules that are specifically targeted to a certain mutation of a human protein. In the development of vemurafenib the structural and molecular properties of the targeted protein could be investigated in co-crystal structures and have helped to design a selective agent against the mutated kinase. The developmental program of ivacaftor however, had no access to crystal structures since the large transmembrane protein CFTR has not been crystallized yet. Instead, sophisticated *in vitro* assays with mutated CFTR proteins provided the data for required development.

Additionally, both molecules prove the possibility to target defect proteins. Ivacaftor partly restores the function of defective CFTR and vemurafenib selectively inhibits an oncogenically mutated kinase. The success of these drugs has evoked several approaches to develop similar agents such as CFTR potentiators for other gating mutations yet.

Referring to current research results, the FDA lists all compounds with special pharmacogenetic characteristics which require or advise genetic testing. In Germany such a list is compiled by the "*Verband der forschenden Pharmaunternehmen*". (www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm, www.vfa.de/de/arzneimittel-forschung/datenbanken-zu-arzneimitteln/individualisierte-medizin.html)

Hopefully this is only the beginning of personalized drug development and the future will see more drugs restoring the function of many more defective proteins or modulating the consequences of mutated proteins in signal cascades.

References

Accurso FJ, Rowe SM, Clancy JP (2010) Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. N Engl J Med 363: 1991–2003.

Agarwala SS (2009) Current systemic therapy for metastatic melanoma. Expert Rev Anticancer Ther 9: 587–595.

Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, Palmieri G, Testori A, Marincola FM, Mozzillo N (2012a) The role of BRAF V600 mutation in melanoma. J Transl Med 10: 85.

Ascierto PA, Simeone E, Giannarelli D, Grimaldi AM, Romano A, Mozzillo N (2012b) Sequencing of BRAF inhibitors and ipilimumab in patients with metastatic melanoma: a possible algorithm for clinical use. J Transl Med 10: 107.

Atefi M, Euw E von, Attar N, Ng C, Chu C, Guo D, Nazarian R, Chmielowski B, Glaspy JA, Comin-Anduix B, Mischel PS, Lo RS, Ribas A, Brandner JM (2011) Reversing melanoma cross-resistance to BRAF and MEK inhibitors by co-targeting the AKT/mTOR pathway. PLoS ONE 6: e28973.

Atwell S, Brouillette CG, Conners K, Emtage S, Gheyi T, Guggino WB, Hendle J, Hunt JF, Lewis HA, Lu F, Protasevich II, Rodgers LA, Romero R, Wasserman SR, Weber PC, Wetmore D, Zhang FF, Zhao X (2010) Structures of a minimal human CFTR first nucleotide-binding domain as a monomer, head-to-tail homodimer, and pathogenic mutant. Protein Engin Design Select 23: 375–384.

Balakan O, Süner A, Yiğiter R, Balakan T, Sirikçi A, Sevinç A (2012) Long-term survival in metastatic malignant melanoma: ipilimumab followed by vemurafenib in a patient with brain metastasis. Intern Med 51: 2819–2823.

Barrett PM, Alagely A, Topol EJ (2012) Cystic fibrosis in an era of genomically guided therapy. Human Mol Genet 21 (R1): R66–71.

Bollag G, Hirth P, Tsai J. Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, Burton EA, Wong B, Tsang G, West BL, Powell B, Shellooe R, Marimuthu A, Nguyen H, Zhang KYJ, Artis DR, Schlessinger J, Su F, Higgins B, Iyer R, D'Andrea K, Koehler A, Stumm M, Lin PS, Lee RJ, Grippo J, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, Chapman PB, Flaherty KT, Xu X, Nathanson KL, Nolop K (2010) Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature 467 (7315): 596–599.

Bollag G, Tsai J, Zhang J, Zhang C, Ibrahim P, Nolop K, Hirth P (2012) Vemurafenib: the first drug approved for BRAF-mutant cancer. Nat Rev Drug Discov 11: 873–886.

Bompadre SG, Li M, Hwang TC (2007) Mechanism of G551D-CFTR (cystic fibrosis transmembrane conductance regulator) potentiation by a high affinity ATP analog. J Biol Chem 283: 5364–5369.

Callahan MK, Rampal R, Harding JJ, Klimek VM, Chung YR, Merghoub T, Wolchok JD, Solit DB, Rosen N, Abdel-Wahab O, Levine RL, Chapman PB (2012) Progression of RAS-mutant leukemia during RAF inhibitor treatment. N Engl J Med 367: 2316–2321.

Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AMM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA (2011) Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364: 2507–2516.

Comer D, Ennis M, McDowell C, Beattie D, Rendall J, Hall V, Elborn J (2009) Clinical phenotype of cystic fibrosis patients with the G551D mutation. QJM 102: 793–798.

Davis MJ, Schlessinger J (2012) The genesis of zelboraf: targeting mutant B-Raf in melanoma. J Cell Biol 199: 15–19.

Davis PB, Yasothan U, Kirkpatrick P (2012) Ivacaftor. Nat Rev Drug Discov 11: 349–350.

Eckford PDW, Li C, Ramjeesingh M, Bear CE (2012) Cystic fibrosis transmembrane conductance regulator (CFTR) potentiator VX-770 (ivacaftor) opens the defective channel gate of mutant CFTR in a phosphorylation-dependent but ATP-independent manner. J Biol Chem 287: 36639–36649.

Finn L, Markovic SN, Joseph RW (2012) Therapy for metastatic melanoma: the past, present, and future. BMC Med 10: 23.

Fisher R, Larkin J (2012) Vemurafenib: a new treatment for BRAF-V600 mutated advanced melanoma. Cancer Manag Res 4: 243–252.

Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB (2010) Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 363: 809–819.

Flaherty KT, Yasothan U, Kirkpatrick P (2011) Vemurafenib. Nat Rev Drug Discov 10: 811–812.

Flume PA (2012) Ivacaftor in subjects with cystic fibrosis who are homozygous for the F508del-CFTR mutation Chest 142: 718–724.

Fonkem E, Uhlmann EJ, Floyd SR, Mahadevan A, Kasper E, Eton O, Wong ET (2012) Melanoma brain metastasis: overview of current management and emerging targeted therapies. Expert Rev Neurother 12: 1207–1215.

Galfrè E, Galeno L, Moran O (2012) A potentiator induces conformational changes on the recombinant CFTR nucleotide binding domains in solution. Cell Mol Life Sci 69: 3701–3713.

Halaban R, Zhang W, Bacchiocchi A, Cheng E, Parisi F, Ariyan S, Krauthammer M, McCusker JP, Kluger Y, Sznol M (2010) PLX4032, a selective BRAFV600E kinase inhibitor, activates the ERK pathway and enhances cell migration and proliferation of BRAFWT melanoma cells. Pigment Cell Melanoma Res 23: 190–200.

Heakal Y, Kester M, Savage S (2011) Vemurafenib (PLX4032): an orally available inhibitor of mutated BRAF for the treatment of metastatic melanoma. Ann Pharmacother 45: 1399–1405.

Hudson RP, Chong PA, Protasevich II, Vernon R, Noy E, Bihler H, An JL, Kalid O, Sela-Culang I, Mense M, Senderowitz H, Brouillette CG, Forman-Kay JD (2012) Conformational changes relevant to channel activity and folding within the first nucleotide binding domain of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 287: 28480–28494.

Inamdar GS, Madhunapantula SV, Robertson GP (2010) Targeting the MAPK pathway in melanoma: Why some approaches succeed and other fail. Biochem Pharmacol 80: 624–637.

Kaplan FM, Mastrangelo MJ, Aplin AE (2010) The wrath of RAFs: rogue behavior of B-RAF kinase inhibitors. J Investig Dermatol 130): 2669–2671.

Lemech C, Arkenau HT (2012) Novel treatments for metastatic cutaneous melanoma and the management of emergent toxicities. Clin Med Insights Oncol 6: 53–66.

Lewis H, Wang C, Zhao X, Hamuro Y, Conners K, Kearins M, Lu F, Sauder J, Molnar K, Coales S, Maloney P, Guggino W, Wetmore D, Weber P, Hunt J (2010) Structure and dynamics of NBD1 from CFTR characterized using crystallography and hydrogen/deuterium exchange mass spectrometry. J Mol Biol 396: 406–430.

Lubamba B, Dhooghe B, Noel S, Leal T (2012) Cystic fibrosis: Insight into CFTR pathophysiology and pharmacotherapy. Clin Biochem 45: 1132–1144.

Luke JJ, Hodi FS (2012) Vemurafenib and BRAF inhibition: a new class of treatment for metastatic melanoma. Clin Cancer Res 18: 9–14.

Melin P, Norez C, Callebaut I, Becq F (2006) The glycine residues G551 and G1349 within the ATP-binding cassette signature motifs play critical roles in the activation and inhibition of cystic fibrosis transmembrane conductance regulator channels by phloxine B. J Membrane Biol 208: 203–212.

Mello RA de (2012) Metastatic melanoma and vemurafenib: novel approaches. Rare Tumors 4: e31.

Moran O (2010) Model of the cAMP activation of chloride transport by CFTR channel and the mechanism of potentiators. J Theor Biol262: 73–79.

Pasyk S, Li C, Ramjeesingh M, Bear CE (2009) Direct interaction of a small-molecule modulator with G551D-CFTR, a cystic fibrosis-causing mutation associated with severe disease. Biochem. J. 418: 185–190.

PDB ID (2014) : 3OG7.

Pyle LC, Ehrhardt A, Mitchell LH, Fan L, Ren A, Naren AP, Li Y, Clancy JP, Bolger GB, Sorscher EJ, Rowe SM (2011) Regulatory domain phosphorylation to distinguish the mechanistic basis underlying acute CFTR modulators. AJP: Lung Cell Mol Physiol 301: L587.

Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Dřevínek P, Griese M, McKone EF, Wainwright CE, Konstan MW, Moss R, Ratjen F, Sermet-Gaudelus I, Rowe SM, Dong Q, Rodriguez S, Yen K, Ordoñez C, Elborn JS (2011) A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 365: 1663–1672.

Shi H, Kong X, Ribas A, Lo RS (2011) Combinatorial treatments that overcome PDGFR -driven resistance of melanoma cells to V600EB-RAF inhibition. Cancer Res 71: 5067–5074.

Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, Shyr Y, Ye F, Li J, Nolop KB, Lee RJ, Joe AK, Ribas A (2012) Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med 366: 707–714.

Su F, Bradley WD, Wang Q, Yang H, Xu L, Higgins B, Kolinsky K, Packman K, Kim MJ, Trunzer K, Lee RJ, Schostack K, Carter J, Albert T, Germer S, Rosinski J, Martin M, Simcox ME, Lestini B, Heimbrook D, Bollag G (2012) Resistance to selective BRAF inhibition can be mediated by modest upstream pathway activation. Cancer Res 72: 969–978.

Tuma RS (2011) Getting around PLX4032: studies turn up unusual mechanisms of resistance to melanoma drug. J Nat Cancer Inst 103: 170–177.

van Goor F, Hadida S, Grootenhuis PDJ, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, Zhou J, McCartney J, Arumugam V, Decker C, Yang J, Young C, Olson ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu P. (2009) Rescue of CF airway epithelial cell function *in vitro* by a CFTR potentiator, VX-770. Proc Natl Acad Sci USA 106: 18825–18830.

Wang W, Wu J, Bernard K, Li G, Wang G, Bevensee MO, Kirk KL (2010) ATP-independent CFTR channel gating and allosteric modulation by phosphorylation. Proc Nat Acad Sci 107: 3888–3893.

Wellbrock C, Hurlstone A (2010) BRAF as therapeutic target in melanoma. Biochem Pharmacol 80: 561–567.

Zhang C, Bollag G (2010) Scaffold-based design of kinase inhibitors for cancer therapy. Current Opin Genet Dev 20: 79–86.