Targeted Drug Therapies for BRAF Mutated Tumours

Michael McCrohan
Fourth year Medicine, Trinity College Dublin

Abstract

Mutations in the Ras/Raf/MEK/ERK pathway are frequently present in human cancer. Following extracellular signaling, the G protein Ras becomes activated which further leads to activation of a member of the Raf kinase family. Subsequent activation of other cascade members, such as MEK (MAP/ERK kinase) and ERK (extracellular signal-regulated kinase) eventually results in the activation of transcription factors, which regulate key cellular processes such as growth, differentiation, and apoptosis. The v-raf murine sarcoma viral oncogenes homolog B1 (BRAF) is frequently mutated in a range of human cancers including melanoma, papillary thyroid carcinoma, and colorectal carcinoma. Consequently, BRAF has been identified as a therapeutic drug target with the development of BRAF inhibitors in recent years. Researchers are focusing on understanding the resistance mechanisms present in BRAF-mutated cancers and have identified combination therapy as a potential treatment option that may overcome resistance to BRAF inhibitors. In this review of the literature, the development of BRAF inhibitors and their clinical use is investigated. In addition, resistance to BRAF inhibitors and combination therapy are discussed.

Introduction

The Ras/Raf/MEK/ERK pathway (figure 1) is involved in multiple cellular processes including cell differentiation, proliferation, cell cycle-arrest, and apoptosis, which are initiated following extracellular signaling[1]. The v-raf murine sarcoma viral homolog B1 (BRAF) belongs to the Raf (Rapidly accelerated fibrosarcoma) family of serine/threonine kinases and is the most dominant activator of the MAPK/ERK kinase (MEK) pathway[2, 3]. BRAF mutations are found in approximately 8% of human cancer cases with a significant number harboring the BRAF-V600E mutation. Thus, BRAF mutations and, in particular, the BRAF-V600E mutation have become a focal point of research into targeted therapies to treat specific cancer types. In this article, BRAF inhibitors will be reviewed, focusing mainly on the findings of preclinical and clinical trials. Resistance to BRAF inhibitors and the potential and efficacy of combination therapy in combating this resistance will also be discussed.

CLINICAL POINTS

BRAF mutations are found in approximately 8% of human cancer cases

Vemurafenib and dabrafenib are BRAF inhibitors used for the treatment of BRAF-V600E mutated unresectable or metastatic melanoma

BRAF inhibitor resistance can be divided into multiple subtypes based on the mechanism of the resistance

MAP/ERK kinase (MEK) is one of the most successful drug targets utilised in combination therapy targeting BRAF mutations to date

Trametinib/dabrafenib combination therapy is used for the treatment of unresectable or metastatic melanoma harbouring a BRAF V600E or V600K mutation

Future research should concentrate on innovative combination therapies to target resistance mechanisms more aggressively

BRAF inhibitors

Vemurafenib (Zelboraf) was developed to treat mutant-BRAF melanomas as well as other solid tumours with BRAF mutations5. Trunzer and colleagues demonstrated that vemurafenib initially works by inhibiting the MAPK signalling pathway, resulting in a substantial decrease in ERK phosphorylation after 15 days of therapy being given to the patient. This leads to downstream suppression of cyclin D1, expression of the cell-cycle inhibitor p27, and eventually cell-cycle arrest[6]. Vemurafenib is effective in xenograft models of melanoma harbouring a

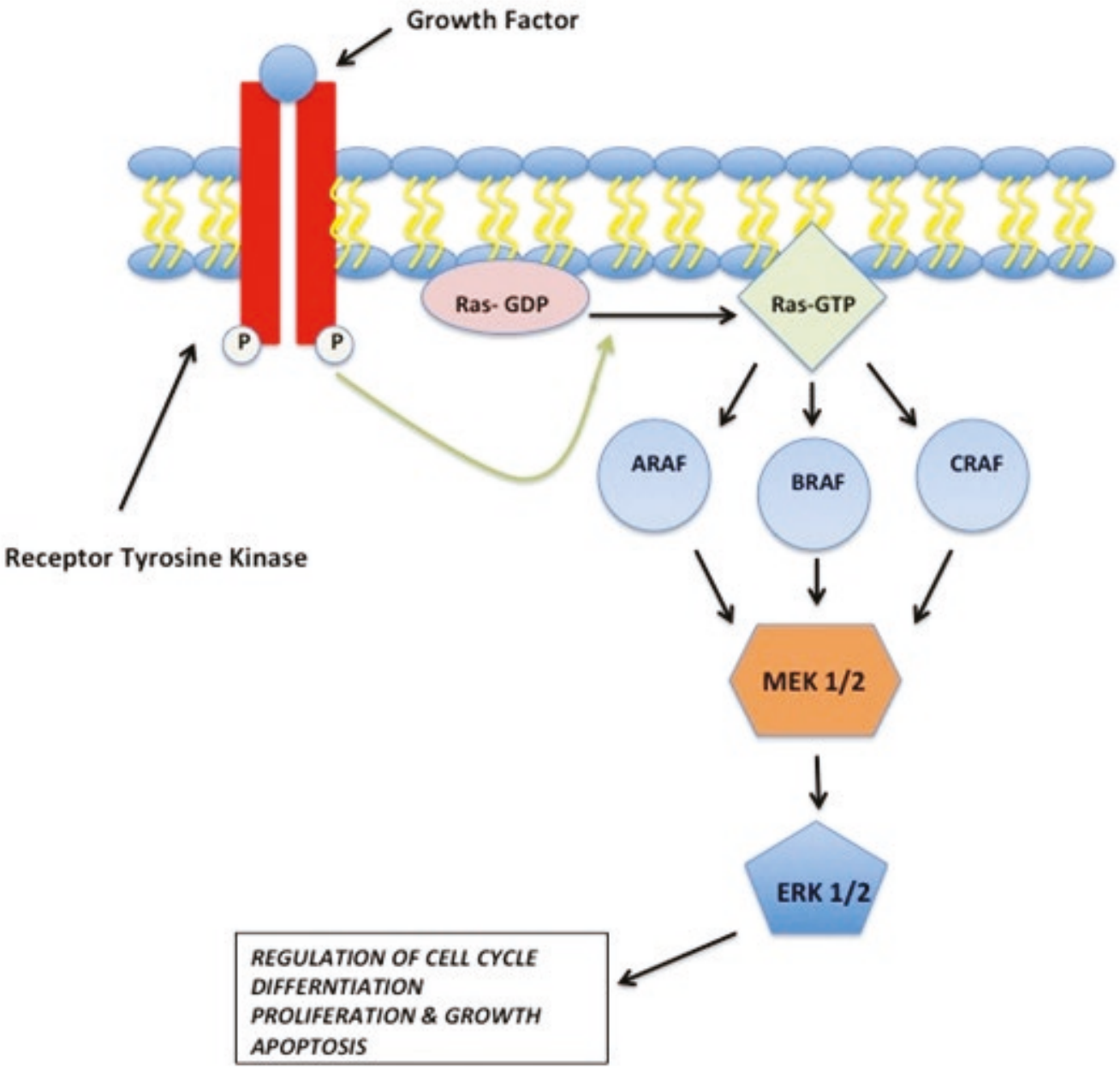

figure 1 The Ras/Raf/MEK/ERK pathway: Tyrosine kinase receptors are stimulated by growth factors resulting in the activation of the G protein Ras. Ras activates members of the RAF family (ARAF, BRAF, CRAF), which subsequently activate MEK 1/2. Following the activation of ERK 1/2 by MEK 1/2, transcription factors are activated leading to an array of key cellular processes[4]

BRAF-V600E mutation. It inhibits proliferation in BRAF-V600E melanoma cells and other melanoma cells with BRAF mutations at residue 600 such as V600R, V600D and V600K[7]. A randomised phase 3 clinical trial compared vemurafenib to dacarbazine: the only current chemotherapeutic drug with U.S Food and Drug Administration (FDA) approval for treating metastatic melanoma. This trial demonstrated that single-agent vemurafenib resulted in an improvement in response rate, progression-free survival (PFS), and overall survival in comparison to dacarbazine in patients with metastatic melanoma harbouring a BRAF-V600E mutation[8]. Subsequently in 2011, the FDA approved the oral BRAF inhibitor, vemurafenib, for the treatment of BRAF-V600E mutated unresectable or metastatic melanoma.

Dabrafenib is an ATP-competitive RAF kinase inhib-

itor which inhibits the BRAF-V600E mutant kinase. A phase 3 trial showed that patients responded positively to dabrafenib with better PFS in comparison to dacarbazine. The trial reported a median PFS for patients treated with dabrafenib for 5.1 months, which was higher than the 2.7 months observed for the dacarbazine patient group[9]. Dabrafenib has also shown efficacy in inhibiting the MAPK pathway in melanoma cell lines with BRAF-V600D/R mutations. Significant inhibition of cell proliferation was observed in melanoma cell lines harbouring a BRAF-V600D or BRAF-V600R mutation. In addition, melanoma cells with BRAF-V600D/R mutations exhibited a stronger and more rapid inhibition of phosphorylated ERK when compared to wildtype BRAF control cells[10]. In May 2013, dabrafenib was approved by the FDA for the treatment of patients with unresectable or metastatic melanoma encompassing a BRAF-V600E mutation[11].

LGX818 is a potent RAF inhibitor with apoptotic and anti-proliferative properties in cells expressing a BRAF-V600E mutation. In the BRAF-V600E human melanoma cell line, LGX818 causes a decrease in the phosphorylation of ERK ultimately resulting in downstream inhibition of proliferation. Studies in human melanoma xenograft models harbouring a BRAF-V600E mutation reported that low oral doses of LGX818 led to a substantial and sustained decrease in the phosphorylation of MEK. In addition, several mutant-BRAF human tumour xenograft models grown in rats and mice showed that low doses of LGX818 can cause tumour regression[12]. At present, LGX818 is undergoing multiple clinical trials.

Sorafenib is a multi-kinase inhibitor with FDA approval for the treatment of hepatocellular carcinoma and renal cell carcinoma. As of November 2013, sorafenib also gained approval for the treatment of "locally recurrent or metastatic, progressive, differentiated thyroid carcinoma (DTC) refractory to radioactive iodine treatment"[13]. In preclinical studies, Sorafenib was active against a number of kinases including BRAF, CRAF, VEGFR-2, PDGFR-h, CKIT, and FLT314. Therefore, it inhibits cancer growth via two mechanisms: inhibition of tumour angiogenesis and, secondly, inhibition of cancer cell proliferation. It has exhibited anti-tumour effects, accounted for by its inhibition of the Ras/Raf/MEK/ERK pathway, in preclinical models of pancreatic, breast, colon, ovarian and lung carcinoma[15].

Regorafenib is a type-2 kinase inhibitor with FDA approval for the treatment of advanced gastrointestinal stromal tumours (GIST) that cannot be treated by means of surgical removal and that are unresponsive to other FDA-approved drugs. It is also an approved treatment for patients with metastatic colorectal cancer (mCRC) who have undergone specific therapies such as anti-VEGF therapy. Regorafenib is an oral multi-kinase inhibitor with potent anti-tumour activity. It has been shown to inhibit both wildtype and BRAF-V600E; However, other studies suggest no correlation between the mutation status of BRAF and regorafenib's effectiveness in inhibiting in vitro tumour cell proliferation or in vivo tumour growth[16]. These findings suggest that BRAF is not the primary target kinase of importance in terms of regorafenib efficacy.

RAF265 and XL281 are BRAF inhibitors currently under evaluation in clinical trials. RAF265 is an oral inhibitor of wildtype BRAF, BRAF-V600E, and CRAF. In addition, it also has inhibiting effects on c-Kit, VEGFR2 and PDGFRβ[5]. RAF265 is currently being evaluated in a phase 2 trial (NCT00304525) on patients with locally advanced or metastatic melanoma. XL281 is an orally bioavailable RAF inhibitor that has displayed a greater than 250-fold selectivity for RAF compared to other kinases[5]. A phase 1/2 trial (NCT01086267) involving XL281 treatment has recently been completed, with results pending. This study investigated XL281 alone or combined with the EGFR inhibitor, cetuximab, in patients with ad-

vanced or metastatic colorectal cancer harbouring mutations in KRAS or BRAF[17].

Mechanisms of resistance to BRAF inhibitors

There are multiple mechanisms in which resistance can be developed against BRAF inhibitors. In particular, evidence of resistance is seen in melanoma and the drugs used to treat it: vemurafenib and dabrafenib. For example, a substantial amount of variation exists in the amount of tumour reduction observed in patients responding to these treatments. In clinical trials with dabrafenib and vemurafenib, approximately 80-90% of patients who respond to treatment initially exhibit progression of their cancer within a year, demonstrating that BRAF inhibitor resistance is established quite rapidly[8, 9].

Typically, these mechanisms of resistance to BRAF inhibitors can lead to the MAPK pathway becoming reactivated or alternatively result in activation of other pathways supporting proliferation and survival. Thus, BRAF inhibitor resistance is probably a multifactorial process[18].

BRAF inhibitor resistance can be divided into multiple subtypes based on the mechanism of the resistance. These subtypes include changes in BRAF itself, resulting in reactivation of the MAPK pathway, as well as alterations occurring in constituents other than BRAF in this pathway, such as MEK1 or NRAS. The PI3K-Akt pathway has also been implicated in BRAF resistance with particular emphasis on loss of PTEN in this process. In addition, alterations in the components regulating the cell cycle have been linked to BRAF resistance[18].

A RAF kinase switch has been implicated in BRAF resistance in melanoma patients. This involves cancer cells relying less on BRAF for signalling and instead utilising the other RAF isoforms, ARAF and CRAF. Inhibition of BRAF forces melanoma cells to rely on a different signalling pathway. This kinase switch enables the tumour to continue utilising the MAPK pathway to support its growth and proliferation[19]. Montagut and associates also observed this switch from BRAF to CRAF dependency in cancer cells and proposed that it accounted for resistance to BRAF inhibitors. The potential of drugs combating CRAF was also highlighted in this study. Geldanamycin, a Heat Shock Protein 90 (HSP90) inhibitor, was observed to promote the degradation of CRAF and revealed the potential of targeting CRAF to reduce resistance to BRAF inhibition[20]. Ganetespib is also a HSP90 inhibitor and its activity has been studied in BRAF-V600E mutated melanoma lines. It has shown promising results in tackling intrinsic and acquired resistance to the BRAF inhibitor vemurafenib[21].

Activation of the PI3K/AKT/mTOR pathway has been linked to malignancy and there is evidence for its role in melanoma, which typically occurs alongside activation of MAPK signalling[22]. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a tumour suppressor gene that decreases Akt activity and its mutant form is associated with BRAF mutated melanoma[23]. The PI3K-AKT pathway and PTEN may be implicated in the mechanisms of BRAF resistance. In particular, crosstalk between the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathway following treatment with a BRAF inhibitor is important in cell survival and resistance. There is mounting evidence to suggest an important role for PTEN in this mechanism of resistance with studies showing that PTEN-null melanoma lines display increased resistance to the inhibitor PLX4720[24, 25].

Combination therapy and its potential to overcome resistance

BRAF resistance can occur through multiple mechanisms. Therefore, molecular inhibitors within various pathways are being studied to decipher the best combination of drugs to counteract resistance. The PI3K/AKT/mTOR pathway is being evaluated as a

possible pathway to co-target with BRAF inhibitors. A study conducted on BRAF-V600E mutated melanoma cells demonstrated that combining inhibitors of both the MAPK and AKT pathways decreases the growth rate of a number of cell lines resistant to vemurafenib. The study suggested that adding an AKT or mTOR inhibitor to vemurafenib therapy might enhance overall inhibition[26].

Other combination strategies being investigated include the use of HSP90 inhibitors and selective inhibitors of ERK 1/2, located downstream of BRAF. HSP90 is a molecular chaperone that is important for the stability and function of multiple signaling proteins. Moreover, it stabilizes many proteins, for example BRAF, that are involved in tumour growth[27]. The importance of the HSP90 inhibitor Ganetespib in combating BRAF resistance was discussed above. XL888 is an additional HSP90 inhibitor that has demonstrated positive results in overcoming BRAF resistance. A recent study reported that in vitro and in vivo, XL888 was successful in inhibiting and inducing tumour regression and apoptosis in melanoma cell lines resistant to vemurafenib[28].

MAP/ERK kinase (MEK) is possibly the most successful drug target utilised in combination therapy targeting BRAF mutations to date. MEK 1/2 catalyses the phosphorylation of threonine and tyrosine residues located on ERK 1/2, allowing ERK 1/2 to translocate to the nucleus and activate key transcription factors essential for cell growth and proliferation[29]. BRAF mutations rely on the MEK/ERK pathway more than ras mutations and this reliance appears to increase the sensitivity of BRAF for MEK inhibition[30].

Selumetinib is a potent MEK 1/2 inhibitor that is showing promising results in clinical trials[31]. Patel and colleagues conducted a phase 1 trial on melanoma patients assessing the efficacy of selumetinib in combination with other drugs. Results demonstrated improved response rates and a prolonged time to progression in patients with BRAF mutated tumours when compared to patients with wildtype BRAF tumours[32]. Another recent phase 2 trial found that selumetinib effectively induced tumour regression in melanoma patients harbouring a BRAF mutation[33].

In terms of MEK inhibition integration into combination therapies to combat BRAF resistance, trametinib (Mekinist) has been the most promising MEK 1/2 inhibitor developed. In January 2014, the FDA approved the combination treatment consisting of trametinib and the BRAF inhibitor, dabrafenib for the "treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation as detected by an FDA-approved test"[34]. This combination treatment was approved following the results of an open-label trial (NCT01072175) investigating trametinib/dabrafenib combination therapy in patients with BRAF-mutated melanoma[34]. Patients receiving the combination therapy of trametinib and dabrafenib had complete or partial response rates of 76% and response duration of 10.5 months. This was compared to patients receiving dabrafenib monotherapy who had complete or partial response rates of 54% and response duration of 5.6 months. Overall response rates were similar for patients with V600E and V600K mutations[35, 36]. Unfortunately, resistance is still a problem even with trametinib/dabrafenib combination therapy. Further research is necessary to investigate the resistance patterns causing the decreased efficacy of this combination of drugs in an effort to develop more successful drug combination strategies[36].

Conclusion

There has been much success in the development of BRAF targeted drug therapies in recent years. However, this recent success underscores the importance of drug resistance in combating BRAF-mutated cancers. It is clear that combination therapy is a promising method to effectively treat cancers harbouring BRAF mutations. BRAF inhibitor monother-

apy has improved survival rates for cancer patients but, as discussed, is not able to overcome resistance. The fact that resistance to BRAF inhibitors, like vemurafenib or dabrafenib, can occur through multiple mechanisms highlights the need for treatment options that target multiple components of different pathways involved in tumour progression. The FDA approval of dabrafenib and trametinib combination therapy for the treatment of metastatic and unresectable melanoma harbouring BRAF-V600E/K mutations is very promising. However, resistance is inevitable even with the use of this effective combination of drugs and future research should concentrate on innovative combination therapies to target resistance mechanisms more aggressively.

In summary, BRAF is a promising drug target that has been at the centre of recent clinical treatment successes. Since the discovery of BRAF as an oncogene in 2002, our knowledge of its role in cancer, its resistance mechanisms and its potential as a drug target have improved greatly. In the future, we hope to see combination therapy at the forefront of BRAF-mutated tumour treatment with the hope of improved survival rates amongst this group of cancer patients.

References

1 Peyssonnaux, C. and A. Eychene, The Raf/MEK/ERK pathway: new concepts of activation. Biol Cell. 93(1-2): p. 53-62 (2001).

2 Robinson, M.J. and M.H. Cobb, Mitogen-activated protein kinase pathways. Curr Opin Cell Biol. 9(2): p. 180-6 (1997).

3 Improta, G., et al., Biological insights into BRAF mutations in melanoma patient: Not mere therapeutic targets. Oncoimmunology 2(8): p. e25594 (2013).

4 Pratilas, C.A. and D.B. Solit, Targeting the mitogen-activated protein kinase pathway: physiological feedback and drug response. Clin Cancer Res 16(13): p. 3329-34 (2010).

5 Zambon, A., et al., Small molecule inhibitors of BRAF in clinical trials. Bioorg Med Chem Lett 22(2): p. 789-92 (2012).

6 Trunzer, K., et al., Pharmacodynamic effects and mechanisms of resistance to vemurafenib in patients with metastatic melanoma. J Clin Oncol 31(14): p. 1767-74 (2013).

7 Yang, H., et al., RG7204 (PLX4032), a selective BRAFV600E inhibitor, displays potent antitumor activity in preclinical melanoma models. Cancer Res 70(13): p. 5518-27 (2010).

8 Chapman, P.B., et al., Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364(26): p. 2507-16 (2011).

9 Hauschild, A., et al., Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 380(9839): p. 358-65 (2012).

10 Gentilcore, G., et al., Effect of dabrafenib on melanoma cell lines harbouring the BRAF(V600D/R) mutations. BMC Cancer 13: p. 17 (2013).

11 Goldenberg, M.M., Pharmaceutical approval update. P T 38(11): p. 705-7 (2013).

12 Stuart DD, L.N., Poon DJ, Aaralen K, Kaufman S, Merritt H et al, Preclinical profile of LGX818: A potent and selective RAF kinase inhibitor. Cancer Res 72(8 Suppl)(8) (2012).

13 FDA., U.S. Sorafenib (NEXAVAR). (2013); Available at: http://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm376547.htm. (Accessed: 25th January 2014)

14 Wilhelm, S.M., et al., BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64(19): p. 7099-109 (2004).

15 Clark, J.W., et al., Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43-9006, in patients with advanced, refractory solid tumors. Clin Cancer Res 11(15): p. 5472-80 (2005).

16 Wilhelm, S., et al., Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. International journal of cancer. Journal international du cancer 129(1): p. 245-255 (2011).

17 Clinicaltrials.gov., Safety and Efficacy Study of BMS-908662 Alone or in Combination With Cetuximab in Subjects With K-RAS or B-RAF Mutation Positive Advanced or Metastatic Colorectal Cancer. (2011); Available at: http://clinicaltrials.gov/ct2/show/NCT01086267?term=xl281&rank=2. (Accessed: 28th January 2014).

18 Bucheit, A. and M. Davies, Emerging insights into resistance to BRAF inhibitors in melanoma. Biochemical pharmacology 87(3): p. 381-389 (2014).

19 Villanueva, J., et al., Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell 18(6): p. 683-95 (2010).

20 Montagut, C., et al., Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res 68(12): p. 4853-61 (2008)

21 Acquaviva, J., et al., Overcoming Acquired BRAF Inhibitor Resistance in Melanoma via Targeted Inhibition of Hsp90

with Ganetespib. Mol Cancer Ther, (2014).

22 Davies, M.A., The role of the PI3K-AKT pathway in melanoma. Cancer J 18(2): p. 142-7 (2012)

23 Wu, H., V. Goel, and F.G. Haluska, PTEN signaling pathways in melanoma. Oncogene 22(20): p. 3113-22 (2003).

24 Deng, W., et al., Role and therapeutic potential of PI3K-mTOR signaling in de novo resistance to BRAF inhibition. Pigment Cell Melanoma Res 25(2): p. 248-58 (2012)

25 Paraiso, K.H., et al., PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. Cancer Res 71(7): p. 2750-60 (2011)

26 Atefi, M., et al., Reversing melanoma cross-resistance to BRAF and MEK inhibitors by co-targeting the AKT/mTOR pathway. PLoS One 6(12): p. e28973 (2011)

27 Neckers, L. and S.P. Ivy, Heat shock protein 90. Curr Opin Oncol 15(6): p. 419-24 (2003).

28 Paraiso, K.H., et al., The HSP90 inhibitor XL888 overcomes BRAF inhibitor resistance mediated through diverse mechanisms. Clin Cancer Res 18(9): p. 2502-14 (2012)

29 Roskoski, R., Jr., ERK1/2 MAP kinases: structure, function, and regulation. Pharmacol Res 66(2): p. 105-43 (2012).

30 Solit, D.B., et al., BRAF mutation predicts sensitivity to MEK inhibition. Nature 439(7074): p. 358-62 (2006).

31 Adjei, A.A., et al., Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol 26(13): p. 2139-46 (2008).

32 Patel, S.P., et al., Clinical responses to selumetinib (AZD6244; ARRY-142886)-based combination therapy stratified by gene mutations in patients with metastatic melanoma. Cancer 119(4): p. 799-805 (2013).

33 Catalanotti, F., et al., Phase II trial of MEK inhibitor selumetinib (AZD6244, ARRY-142886) in patients with BRAFV600E/K-mutated melanoma. Clin Cancer Res 19(8): p. 2257-64 (2013).

34 FDA., U.S. Trametinib and Dabrafenib. (2014); Available at: http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm381451.htm (Accessed: 2nd February 2014).

35 GlaxoSmithKline. Study ID 113220 results summary. (2013); Available at: http://www.gsk-clinicalstudyregister.com/study/113220-rs. (Accessed: 2nd February 2014).

36 Flaherty, K.T., et al., Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 367(18): p. 1694-703 (2012).

37 Davies, H., et al., Mutations of the BRAF gene in human cancer. Nature 417(6892): p. 949-54 (2002).

Michael McCrohan