REVIEW

That which does not kill me makes me stronger; combining ERK1/2 pathway inhibitors and BH3 mimetics to kill tumour cells and prevent acquired resistance

Matthew J Sale and Simon J Cook

Signalling Laboratory, The Babraham Institute, Babraham Research Campus, Cambridge, UK

Correspondence

Matthew J Sale and Simon J Cook, Signalling Laboratory, The Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK. Email: matthew.sale@babraham.ac.uk; simon.cook@babraham.ac.uk

Keywords

acquired resistance; apoptosis; cancer; BCL2; BRAF; ERK1/2; MEK1/2; RAS; targeted therapeutics

Received

24 January 2013

Revised

10 April 2013

Accepted

25 April 2013

Oncogenic mutations in *RAS* or *BRAF* can drive the inappropriate activation of the ERK1/2. In many cases, tumour cells adapt to become addicted to this deregulated ERK1/2 signalling for their proliferation, providing a therapeutic window for tumour-selective growth inhibition. As a result, inhibition of ERK1/2 signalling by BRAF or MEK1/2 inhibitors is an attractive therapeutic strategy. Indeed, the first BRAF inhibitor, vemurafenib, has now been approved for clinical use, while clinical evaluation of MEK1/2 inhibitors is at an advanced stage. Despite this progress, it is apparent that tumour cells adapt quickly to these new targeted agents so that tumours with acquired resistance can emerge within 6–9 months of primary treatment. One of the major reasons for this is that tumour cells typically respond to BRAF or MEK1/2 inhibitors by undergoing a G1 cell cycle arrest rather than dying. Indeed, although inhibition of ERK1/2 invariably increases the expression of pro-apoptotic BCL2 family proteins, tumour cells undergo minimal apoptosis. This cytostatic response may simply provide the cell with the opportunity to adapt and acquire resistance. Here we discuss recent studies that demonstrate that combination of BRAF or MEK1/2 inhibitors with inhibitors of pro-survival BCL2 proteins is synthetic lethal for ERK1/2-addicted tumour cells. This combination effectively transforms the cytostatic response of BRAF and MEK1/2 inhibitors into a striking apoptotic cell death response. This not only augments the primary efficacy of BRAF and MEK1/2 inhibitors but delays the onset of acquired resistance to these agents, validating their combination in the clinic.

LINKED ARTICLES

This article is part of a themed section on Emerging Therapeutic Aspects in Oncology. To view the other articles in this section visit <http://dx.doi.org/10.1111/bph.2013.169.issue-8>

Abbreviations

A1, BCL2-related protein A1; AP1, activator protein 1; APAF1, apoptotic peptidase activating factor 1; ARAF, v-raf murine sarcoma 3611 viral oncogene homologue; BAD, BCL-X_L/BCL2-associated death promoter; BAK, BCL2 homologous antagonist/killer; BAX, BCL2-associated x protein; BCL2, B-cell lymphoma 2; BCL-X_L, B-cell lymphoma extra large; BH3, BCL2 homology domain 3; BID, BH3 interacting domain death agonist; BIK, BCL2-interacting killer; BIM, BCL2-interacting mediator of cell death; BMF, BCL2-modifying factor; BRAF, v-raf murine sarcoma viral oncogene homologue B1; CCND1, cyclin D1; CDC25A, cell division cycle 25 homologue A; CDK, cyclin-dependent kinase; CIP1, CDK-interacting protein 1; CRAF, v-raf-1 murine leukaemia viral oncogene homologue 1; CREB, cAMP responsive element binding protein; DKO, double knockout; EGFR, epidermal growth factor receptor; CRC, colorectal cancer; ELK1, ETS-like gene 1; EMT, epithelial-mesenchymal transition; ETS, v-ets erythroblastosis virus E26 oncogene homologue; FOXO3, forkhead box O 3; G1, growth phase 1; GSK3, glycogen synthase kinase 3; HIV, human immunodeficiency virus; HRAS, v-Ha-ras Harvey rat sarcoma viral oncogene homologue; HRK, harakiri, BCL2

interacting protein (contains only BH3 domain); KIP1, kinase inhibitory protein 1; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue; MCL1, myeloid cell leukaemia 1; MDM2, Mdm2, p53 E3 ubiquitin protein ligase homologue (mouse); MEK, MAPK or ERK kinase; MOMP, mitochondrial outer membrane permeabilization; MSK, mitogen- and stress-activated protein kinase; mTOR, mammalian target of rapamycin; MYC, v-myc myelocytomatosis viral oncogene homologue (avian); NRAS, neuroblastoma RAS viral (v-ras) oncogene homologue; NSCLC, non-small cell lung cancer; OMM, outer mitochondrial membrane; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide 3-kinase; PUMA, p53-up-regulated modulator of apoptosis; RAS, rat sarcoma virus oncogene; RB, retinoblastoma protein; RNAi, RNA interference; RSK, ribosomal protein S6 kinase; RTK, receptor tyrosine kinase; SCLC, small cell lung cancer; ZEB1, zinc finger E-box binding homeobox 1

Introduction

The RAS-regulated RAF-MEK1/2-ERK1/2 signalling cascade is frequently hyperactivated in human cancers due to mutations in *BRAF*, *RAS* or receptor tyrosine kinases (RTKs). Activating *BRAF* mutations, typically *BRAF*^{V600E}, are found in 60% of melanomas, 30% of thyroid cancers, 10% of colorectal cancers (CRCs) (Davies *et al.*, 2002; Xing, 2005) and almost all hairy cell leukaemias (Tiacchi *et al.*, 2011). *RAS* is the most commonly mutated oncogene in human cancers, being detected in around 90% of pancreatic cancers, 40% of CRC, 20% non-small cell lung cancers (NSCLCs) and 15% of melanomas (Downward, 2003). Tumour cells with mutations that activate ERK1/2 frequently exhibit a high dependence upon, or addiction to, this signalling cascade for proliferation and tumourigenesis (Solit *et al.*, 2006; Davies *et al.*, 2007), and thus targeting components of this pathway is an attractive therapeutic strategy. Accordingly, numerous small molecule inhibitors of RAF and MEK1/2 have been developed. The most advanced of these is the RAF inhibitor vemurafenib (Zelboraf, PLX4032, RG7204), which has been approved for the treatment of melanoma (Bollag *et al.*, 2012). Vemurafenib is effective at inhibiting ERK1/2 in cells expressing mutant *BRAF* (typically *BRAF*^{V600E}); however, in cells expressing wild-type *BRAF* or mutant *RAS*, RAF inhibitors actually activate ERK1/2 signalling (Hatzivassiliou *et al.*, 2010; Heidorn *et al.*, 2010; Joseph *et al.*, 2010; Poulikakos *et al.*, 2010). Mutant *BRAF*^{V600E} signals as a RAF-inhibitor sensitive monomer, but conditions that promote RAF dimerization, such as *RAS* activation, induce 'paradoxical activation' of ERK1/2 signalling. Binding of one protomer to a RAF inhibitor within a RAF dimer has been postulated to allosterically transactivate the other protomer, resulting in activation of ERK1/2 (Hatzivassiliou *et al.*, 2010; Poulikakos *et al.*, 2010). This suggests that in *BRAF*^{V600E}-positive tumours, *RAS* activity is too low to support the formation of RAF dimers, and consequently *BRAF*^{V600E} signals as a RAF-inhibitor sensitive monomer (Lito *et al.*, 2012). This selectivity for mutant *BRAF* means that RAF inhibitors like vemurafenib have a broad therapeutic index but are limited to mutant *BRAF*-positive cancers. In addition, the activation of ERK1/2 signalling by vemurafenib in cells expressing wild-type *BRAF* is thought to be responsible for the frequent and often rapid development of previously unsuspected *RAS*-mutant cutaneous squamous cell carcinoma (SCC) in patients treated with vemurafenib (Su *et al.*, 2012a), as well as recently identified cases of *RAS*-mutant CRC, colonic adenomas, gastric polyps and leukaemia (Andrews *et al.*, 2012; Callahan *et al.*, 2012; Chapman *et al.*, 2012). These effects are not restricted to vemurafenib

and have been observed with other *BRAF* and *RAF* inhibitors, such as dabrafenib and sorafenib (Anforth *et al.*, 2012; Arnault *et al.*, 2012). Consequently, 'paradox breaker' RAF inhibitors that do not induce paradoxical activation of ERK1/2 in cells with high *RAS* activity are in development (Ma *et al.*, 2011; Le *et al.*, 2013). One such inhibitor, PLX7904 (or paradox breaker 04), inhibited ERK1/2 as effectively as PLX4720 (a close structural analogue of vemurafenib) in *BRAF*-mutant melanoma cells, but unlike PLX4720 did not hyperactivate ERK1/2 in melanoma cells expressing mutant *NRAS* or SCC cells expressing mutant *HRAS*. In addition, PLX7904 was effective against *BRAF*-mutant melanoma cells with acquired resistance to vemurafenib mediated by secondary mutation in *NRAS* (Le *et al.*, 2013).

In contrast to vemurafenib, MEK1/2 inhibitors, such as selumetinib (AZD6244; ARRY-142886), do not induce paradoxical activation of ERK1/2 signalling and are effective against a broader spectrum of tumours, including those driven by mutations in *BRAF*, *KRAS* or RTKs. However, the broader action of MEK1/2 inhibitors may result in a narrower therapeutic window when compared with RAF inhibitors that target mutant *BRAF* only. In contrast to the majority of kinase inhibitors, MEK1/2 inhibitors like selumetinib do not compete with ATP, but instead bind to an allosteric pocket within MEK1/2 (Davies *et al.*, 2007). This is thought to account for the exquisite selectivity of selumetinib for MEK1/2 in preclinical studies, in which ~40 other kinases (including the closely related MEK5) were not substantially inhibited at 10 µM (Yeh *et al.*, 2007).

Clinical trials have been conducted, or are ongoing, with several MEK1/2 inhibitors, including selumetinib. Phase II trials assessing the efficacy of selumetinib in patients with advanced NSCLC, CRC and pancreatic cancer who had failed first line therapy showed little or no advantage to selumetinib monotherapy versus standard treatment course (Hainsworth *et al.*, 2010; Bennouna *et al.*, 2011; Bodoky *et al.*, 2012). In addition, no significant difference in progression free survival was observed in patients with chemotherapy-naïve advanced melanoma undergoing treatment with selumetinib versus temozolomide (Kirkwood *et al.*, 2012). However, this study and a study of the efficacy of selumetinib in iodine-refractory papillary thyroid cancer indicated that future trials should focus on patients with *BRAF* mutations (Hayes *et al.*, 2012). Greater success has been achieved using selumetinib in combination with other agents. In a phase II trial of *KRAS*-mutant advanced NSCLC, selumetinib in combination with docetaxel improved median overall and progression free survival relative to placebo plus docetaxel by approximately twofold (Jänne *et al.*, 2012). There is also evidence that the clinical

sensitivity of *BRAF*-mutant metastatic melanoma is enhanced when selumetinib is combined with either conventional chemotherapeutics or targeted drugs (Patel *et al.*, 2013). Thus, meaningful clinical responses to MEK1/2 inhibitors such as selumetinib are likely to depend on the development of effective combination therapies.

The enrolment criterion for phase II and phase III trials with the *BRAF* inhibitor vemurafenib included selecting patients with treatment-naïve *BRAF*-mutant metastatic melanoma. Both the phase II trial and interim analysis of the phase III trial comparing vemurafenib to dacarbazine reported confirmed clinical responses in around 50% of patients, compared with 5% for dacarbazine (Chapman *et al.*, 2011; Sosman *et al.*, 2012). Interim analysis of the phase III trial also reported superior median progression free survival with vemurafenib compared with dacarbazine (5.3 vs. 1.6 months). With these interim results and the conclusion of the phase II trial both demonstrating the substantial clinical efficacy of vemurafenib, the United States Food and Drug Administration recommended that the phase III trial be revised to assume the greater efficacy of vemurafenib over dacarbazine and independent data and safety monitoring board recommended patient crossover from the dacarbazine control arm to vemurafenib (Chapman *et al.*, 2011). This rapid clinical development led to the approval of vemurafenib for the treatment of *BRAF*-mutant metastatic melanoma in the United States in August 2011 and in the European Union in February 2012.

Activating *BRAF* mutations occur in around 8–10% of CRCs and correlate with poor prognosis (Richman *et al.*, 2009; Tol *et al.*, 2009; Fariña-Sarasqueta *et al.*, 2010). In comparison to the striking clinical responses seen in melanoma, the response to vemurafenib in a phase I study of patients with *BRAF*-mutant metastatic CRC was modest (Kopetz *et al.*, 2010). This is consistent with reports that vemurafenib treatment does not lead to sustained suppression of ERK1/2 phosphorylation in *BRAF*^{V600E}-mutant CRC cells due to epidermal growth factor receptor (EGFR)-mediated reactivation of ERK1/2 (Corcoran *et al.*, 2012; Prahallad *et al.*, 2012).

Despite these advances, the efficacy of *BRAF* and MEK1/2 inhibitors is limited by the development of acquired resistance that typically results in disease progression 6–7 months after treatment initiation (reviewed in Little *et al.*, 2013). Acquired resistance to *BRAF* inhibitors can arise through multiple mechanisms, such as switching to *ARAF* or *CRAF* (Montagut *et al.*, 2008; Villanueva *et al.*, 2010); up-regulation of alternative MEK1/2 activators (Johannessen *et al.*, 2010); activating *MEK1* mutation (Wagle *et al.*, 2011; Greger *et al.*, 2012); expression of *BRAF*^{V600E} splice variants that preferentially dimerize (Poulikakos *et al.*, 2011); *BRAF* amplification (Shi *et al.*, 2012); and activating *RAS* mutations (Nazarian *et al.*, 2010; Greger *et al.*, 2012; Su *et al.*, 2012b) or up-regulation/activation of RTKs (Nazarian *et al.*, 2010; Villanueva *et al.*, 2010; Yadav *et al.*, 2012; Girotti *et al.*, 2013). Interestingly, mutations in *BRAF* itself, such as those encoding 'gatekeeper' mutations that block drug binding, have not been observed in cell lines or patients with acquired resistance to *BRAF* inhibitors, despite the observation that engineering such mutations within *BRAF* can confer resistance *in vitro* (Whittaker *et al.*, 2010). Acquired resistance to MEK1/2 inhibitors may arise through amplification of *BRAF*^{T1799A}

(encodes *BRAF*^{V600E}) (Corcoran *et al.*, 2010; Little *et al.*, 2011), amplification of *KRAS*^{G38A} (encodes *KRAS*^{G13D}) (Little *et al.*, 2011) or mutations within *MEK1* or *MEK2* (Emery *et al.*, 2009; Wang *et al.*, 2011; Hatzivassiliou *et al.*, 2012). The majority of these mechanisms serve to reinstate ERK1/2 signalling and cell proliferation in the presence of drug (reviewed in Little *et al.*, 2013). Interestingly, we observed that acquired resistance to selumetinib resulting from *BRAF* amplification was reversible (Little *et al.*, 2011). Culturing these resistant cells in the absence of drug for several months led to their complete re-sensitization to the anti-proliferative effects of selumetinib, with *BRAF* expression and phospho-ERK1/2 levels returning to those observed in selumetinib-naïve cells. The mechanisms underlying resistance reversibility are currently unclear and are under investigation, but these results nevertheless suggest that staggered treatment strategies may delay or overcome acquired resistance in this context. A recent study using primary human *BRAF*^{V600E}-positive melanoma cells that acquire resistance to vemurafenib through elevated *BRAF* expression has supported this hypothesis (Das Thakur *et al.*, 2013). Dosing these cells intermittently with vemurafenib in xenograft models markedly stalled tumour growth relative to continual treatment. Together, these studies (Little *et al.*, 2011; Das Thakur *et al.*, 2013) suggest that such 'drug holiday' strategies with ERK1/2 pathway inhibitors may warrant evaluation in clinical trials.

Inhibition of ERK1/2 signalling in tumour cells addicted to this pathway typically results in G1 cell cycle arrest (Davies *et al.*, 2007; Sale and Cook, 2013). This reflects the pivotal role that ERK1/2 signalling plays in cell cycle progression (Figure 1). Through the activation of AP1 and ETS transcription factors, ERK1/2, in conjunction with ERK1/2-dependent ribosomal protein S6 kinase (RSK) activation, drives transcription of *CCND1* (cyclin D1) during the G1 phase of the cell cycle (Meloche and Pouysségur, 2007). *CCND1* binds to and promotes activation of CDK4 and CDK6, which in turn phosphorylate and inactivate retinoblastoma protein (RB). RB inactivation alleviates repression of E2F-mediated transcription, thereby permitting expression of many genes important for entry into, and progression through, S phase (Cobrinik, 2005). In addition, ERK1/2-mediated phosphorylation stabilizes MYC (Sears *et al.*, 2000), which in turn increases expression of cyclin D2, CDK4 and CDC25A, and may repress transcription of the cyclin-dependent kinase (CDK) inhibitor p21^{CIP1}, all of which act to promote progression into S phase (Galaktionov *et al.*, 1996; Bouchard *et al.*, 1999; Hermeking *et al.*, 2000; Gartel *et al.*, 2001). Finally, ERK1/2 and RSK may down-regulate the expression and/or promote inactivation of the CDK-inhibitor p27^{KIP1} (Rivard *et al.*, 1999; Delmas *et al.*, 2001; Fujita *et al.*, 2003). Thus, blockade of ERK1/2 signalling in tumour cells that are addicted to this pathway for proliferation typically promotes G1 cell cycle arrest through the down-regulation of positive cell cycle regulators and the accumulation of negative cell cycle regulators.

ERK1/2-mediated regulation of the BCL2 protein family

ERK1/2 signalling has been implicated in the regulation of many members of the BCL2 protein family. This regulation

Figure 1

ERK1/2-mediated regulation of the G1-S phase transition. Nuclear ERK1/2 phosphorylates and stabilizes/activates members of ETS and AP1 transcription factor families, which can induce transcription of *CCND1* (cyclin D1). The transcription factor MYC is stabilized by ERK1/2-mediated phosphorylation, and MYC can up-regulate the expression of cell cycle regulators such as cyclin D2 *CCND2* (cyclin D2) and *CDC25A*. *CCND1* and *CCND2* bind to and activate CDK4 and CDK6 (CDK4/6). Phosphorylation of RB by CDK4/6 frees the E2F transcription factors from RB-mediated repression, allowing E2F-induced transcription of genes such as *CCNE* (cyclin E), *CCNA* (cyclin A) and *MYC*. Newly synthesized *CCNE* binds and activates CDK2, which can also phosphorylate RB in a feedforward loop. *p27^{KIP1}*, an endogenous inhibitor of CDK2, is down-regulated and inactivated during cell cycle entry by a variety of mechanisms mediated by cyclin E-CDK2, ERK1/2 and RSK. CDK2, initially in complex with *CCNE* and later with *CCNA*, and the E2F transcription factors regulate many target factors to drive progression into, and through, S phase. ERK1/2 signalling is frequently hyperactivated in tumour cells as a result of mutations in RTKs, *RAS* or *BRAF* (shown, yellow star), providing validation for selective inhibitors of mutant *BRAF* (e.g. vemurafenib) or MEK1/2 (e.g. selumetinib).

typically promotes tumour cell survival through the up-regulation of pro-survival factors and down-regulation of pro-apoptotic BCL2 family members. Consequently, inhibition of ERK1/2 signalling using MEK1/2 or RAF inhibitors generally induces expression of pro-apoptotic BCL2 proteins in tumour cells.

Apoptosis is regulated by the BCL2 protein family

The mitochondrial pathway of apoptosis is regulated by members of the BCL2 protein family (Chipuk *et al.*, 2010). Interactions between these factors ultimately control the integrity of the outer mitochondrial membrane (OMM), thereby determining whether a cell survives or commits to apoptosis. Pro-apoptotic signals converge to promote mitochondrial outer membrane permeabilization (MOMP), which allows the release of soluble proteins resident in the intermembrane space into the cytosol. Most notably, cytochrome *c* released from mitochondria binds to APAF1, promoting its oligomerization and assembly into the apoptosome. The apoptosome acts as a caspase activation platform by first recruiting pro-caspase-9 and promoting its activation. Active caspase-9 is then able to cleave and activate the executioner caspases, caspase-3 and

caspase-7, which cleave a large number of cellular substrates resulting in apoptosis (Tait and Green, 2010).

BCL2 family members are classified as either pro-apoptotic or pro-survival. A1/BFL1, BCL2, BCL-w, BCL-X_L and MCL1 are the major pro-survival (or anti-apoptotic) members, and contain four BCL2-homology domains (BH1–4). They largely associate with the OMM and act to inhibit apoptosis by binding to pro-apoptotic factors (Chipuk *et al.*, 2010). The pro-apoptotic members of the BCL2 family are further divided into the BH3-only proteins and the effector proteins. BH3-only proteins are induced by a variety of cellular stresses and include BAD, BID, BIK, BIM, BMF, HRK, NOXA and PUMA. Upon induction, these bind to and inhibit their target pro-survival BCL2 family members. Whereas BIM and PUMA are thought to bind to and inhibit all five major pro-survival factors, most other BH3-only proteins exhibit more restricted binding preferences (Chipuk *et al.*, 2010). The pro-survival proteins are also able to bind and inhibit the effector proteins BAX and BAK. Thus, by binding to the pro-survival factors, BH3-only proteins can displace the effector molecules BAX and BAK, which are then free to undergo further activation events that lead to MOMP. In addition to inhibiting the action of pro-survival factors, certain BH3-only

BJP

BJP

BJP

BJP

BJP

BJP

BJP

BJP

catalysed by the ERK1/2-regulated kinases RSK and MSK1 (Bonni *et al.*, 1999; Tan *et al.*, 1999; Shimamura *et al.*, 2000; She *et al.*, 2002). Phosphorylation of this site, as well as phosphorylation of Ser136 by PKB (also known as Akt) (Zha *et al.*, 1996; Datta *et al.*, 1997; del Peso *et al.*, 1997), is proposed to inhibit BAD by facilitating binding to 14-3-3 proteins that sequester BAD in the cytosol away from pro-survival BCL2 proteins at the mitochondria (Zha *et al.*, 1996; Tan *et al.*, 1999).

Recently, ERK1/2 was proposed to regulate the stability of the BH3-only protein BIK, in a manner analogous to BIM (Lopez *et al.*, 2012). Direct phosphorylation of BIK on Thr124 by ERK1/2 was suggested to promote ubiquitination and subsequent proteasome-mediated degradation of BIK. Consistent with these observations, the authors demonstrated that MEK1/2 inhibition in tumour cells with *BRAF* and *RAS* mutations caused a striking up-regulation of BIK protein. However, we and others have observed little change in BIK expression upon perturbation of ERK1/2 signalling in tumour cells, and the reason for this discrepancy is currently unclear (Sheridan *et al.*, 2008; Sale and Cook, 2013).

In contrast to the above examples, the BH3-only protein NOXA is induced rather than repressed by ERK1/2 signalling (Sheridan *et al.*, 2010; Elgendy *et al.*, 2011; Basile and Aplin, 2012). Inhibition of ERK1/2 down-regulates NOXA protein and mRNA expression, but the underlying mechanisms are unknown (Basile and Aplin, 2012). Why NOXA should exhibit this opposing reciprocal regulation by ERK1/2 signalling is unclear but it may be relevant to the onset of autophagy during oncogene-induced senescence. Strong ERK1/2 signalling induced by conditional overexpression of mutant HRAS increased NOXA expression, which appeared to be involved in the induction of autophagy under these conditions (Elgendy *et al.*, 2011).

ERK1/2-mediated regulation of pro-survival BCL2 family members

The pro-survival factor MCL1 has a short half-life due to the presence of a PEST [peptide sequence that is rich in proline (P), glutamic acid (E), serine (S) and threonine (T)] domain. MCL1 turnover is regulated by phosphorylation of sites within the PEST domain. ERK1/2-mediated phosphorylation of Thr163 within this domain is proposed to stabilize MCL1 (Domina *et al.*, 2004) whereas GSK3-catalysed phosphorylation within the PEST domain enhances MCL1 turnover (Maurer *et al.*, 2006). Thus, inactivation of GSK3 by the ERK1/2-dependent RSK kinases is a further mechanism by which ERK1/2 may promote MCL1 expression. In addition, *MCL1* transcription is postulated to be positively regulated by ERK1/2-mediated activation of the transcription factor ELK1 (Townsend *et al.*, 1999; Vickers *et al.*, 2004; Booy *et al.*, 2011).

cAMP responsive element binding protein (CREB) plays a central role in promoting transcription of *BCL2*, *BCL-X_L* and *MCL1* in response to ERK1/2 signalling (Wilson *et al.*, 1996; Wang *et al.*, 1999; Boucher *et al.*, 2000). This is likely mediated by the ERK1/2-dependent kinases RSK and MSK, which can phosphorylate and activate CREB (Bonni *et al.*, 1999).

Tumour cell responses to ERK1/2 pathway inhibitors are typically cytostatic

Broadly, with the exception of NOXA, tumour cell ERK1/2 signalling promotes cell survival by increasing expression of

pro-survival proteins while repressing and inactivating pro-apoptotic proteins. Inhibition of ERK1/2 with MEK1/2 or *BRAF* inhibitors invariably promotes the expression or activation of multiple BH3-only proteins. However, despite strong induction of even the most potent pro-apoptotic proteins such as BIM and PUMA, the predominant response of tumour cells in culture or xenografts to ERK1/2 pathway inhibition is cytostatic rather than cytotoxic; that is, cells undergo G1 cell cycle arrest rather than apoptosis (Davies *et al.*, 2007; Sale and Cook, 2013). This is exemplified by the observation that CCND1 overexpression alone can confer resistance to ERK1/2 pathway inhibitors (Smalley *et al.*, 2008). Pro-survival BCL2 proteins are frequently up-regulated in tumour cells (Kirkin *et al.*, 2004) and, in comparison to the regulation of pro-apoptotic factors such as BIM or BMF, ERK1/2 pathway inhibition often has only modest effects on pro-survival protein levels and/or activity (Sale and Cook, 2013). Thus, in this setting, pro-survival proteins may provide residual buffering capacity against the BH3-only proteins that accumulate upon ERK1/2 inhibition. This provides a rationale for co-targeting ERK1/2 signalling and the pro-survival BCL2 family members, in an attempt to push tumour cells over the threshold required for considerable (i.e. clinically relevant) apoptosis. Over the past 10–15 years, several agents have been developed that inhibit the pro-survival BCL2 family members, with varying degrees of success.

Targeting pro-survival BCL2 family members in cancer

Given that the deregulation of apoptosis in tumour cells typically occurs upstream of BAX and BAK, altering the balance between pro-apoptotic and pro-survival BCL2 family members is an attractive strategy for promoting tumour cell apoptosis. Several molecules have been developed along these lines (reviewed in Chonghaile and Letai, 2008) including the antisense oligonucleotide oblimersen that targets pro-survival BCL2 (Klasa *et al.*, 2002), BH3 peptides, and small molecule mimics of the pro-apoptotic BH3 domain (BH3 mimetics). Oblimersen and the BH3 mimetics obatoclax (GX-15-070) and ABT-263 are the most clinically advanced.

Despite promising preclinical and early clinical trial data, the performance of oblimersen in phase III multiple myeloma and chronic lymphocytic leukaemia (CLL) clinical trials was unconvincing (Chanan-Khan *et al.*, 2009; O'Brien *et al.*, 2009b). These results have been attributed to the inability of oblimersen to reduce BCL2 protein expression in tumours *in vivo*.

Obatoclax is a putative BH3 mimetic small molecule that exhibits activity against all the pro-survival BCL2 family members, but has modest potency (Zhai *et al.*, 2006). Obatoclax can induce cell death through various mechanisms, some of which are consistent with BH3 mimetic activity but some of which do not require BAX and BAK (Konopleva *et al.*, 2008). Thus, the principal mechanism by which obatoclax induces cell death is uncertain. Obatoclax has shown modest clinical benefit in early stage trials of CLL and small-cell lung cancer (SCLC) (O'Brien *et al.*, 2009a; Paik *et al.*, 2010). However, a phase II study of obatoclax plus topotecan in

SCLC showed no improvement in overall response rate compared with that observed with topotecan monotherapy (Paik *et al.*, 2011).

ABT-737 and ABT-263 (navitoclax) are small molecule BH3 mimetics developed by Abbott Laboratories (Abbott Park, IL, USA) which inhibit BCL2, BCL-X_L and BCL-w (Oltersdorf *et al.*, 2005; Tse *et al.*, 2008). Although used in the majority of preclinical studies, ABT-737 is not orally bioavailable and so the closely related molecule ABT-263 is being evaluated in clinical trials. ABT-263 is orally bioavailable and has similar affinities and specificities for the pro-survival proteins as ABT-737. In contrast to other putative BH3 mimetics, ABT-737/263-induced cell death absolutely requires BAX or BAK (van Delft *et al.*, 2006), and ABT-737/263 can displace pro-apoptotic factors from pro-survival proteins (Del Gaizo Moore *et al.*, 2007), indicating that ABT-737 and ABT-263 act 'on target'. In addition, these compounds exhibit 100–10 000-fold greater affinity for their target BCL2 family members than other small molecule inhibitors (Oltersdorf *et al.*, 2005; Zhai *et al.*, 2006; Tse *et al.*, 2008). However, ABT-737 and ABT-263 have low affinity for MCL1, A1/BFL1 and BCL-B. Thus, although there are exceptions, these small molecules are typically most effective at killing cells expressing higher BCL2 and BCL-X_L, whereas tumour cells expressing higher MCL1 exhibit intrinsic resistance (Konopleva *et al.*, 2006; van Delft *et al.*, 2006). Consistent with this, acquired resistance to ABT-737 can arise through increased expression of MCL1 and/or A1 (Yecies *et al.*, 2010).

ABT-263 has shown efficacy in early stage clinical trials, particularly for the treatment of CLL. In a phase I study of relapsed or refractory lymphoid malignancies (including CLL) 22% of patients had partial responses (Wilson *et al.*, 2010), and in a phase I study of relapsed or refractory CLL 35% of patients exhibited partial responses (Roberts *et al.*, 2012). In contrast, the results of trials targeting SCLC and other solid tumours have been modest. In a phase II trial assessing the efficacy of ABT-263 against advanced or recurrent SCLC, 1 patient of 39 had a partial response (Rudin *et al.*, 2012). This suggests that, for solid tumours at least, ABT-263 may be best employed as combination therapy with other agents.

Harnessing the apoptotic potential of ERK1/2 pathway inhibitors using BH3 mimetics

Response duration to targeted cancer monotherapies is often short, with acquired resistance rapidly emerging. This is reminiscent of early strategies that targeted rapidly mutating or heterogeneous infectious diseases such as human immunodeficiency virus (HIV) or tuberculosis, in which responses to monotherapies were transient and quickly gave rise to resistant variants (Bock and Lengauer, 2012; Glickman and Sawyers, 2012). However, the use of targeted combination therapies has changed this: antibiotic combinations cure tuberculosis, and treatment of HIV with anti-viral combinations indefinitely suppresses the virus to a chronic low level (Fox *et al.*, 1999; Clavel and Hance, 2004). HIV combination therapy works by rapidly and synergistically reducing viral

load relative to single agent therapies, thereby minimizing the pool of viruses from which resistance can develop (Clavel and Hance, 2004; Bock and Lengauer, 2012). In the case of tuberculosis, similar initial responses are achieved regardless of whether treated with a single antibiotic or combination (Fox *et al.*, 1999); importantly, however, combination therapy halts the emergence of resistance that occurs with single agents. Clearly, human cancer biology is far more complex than that of HIV and tuberculosis, but based on this experience, combinations of targeted cancer therapeutics that achieve rapid tumour elimination and suppress the development of acquired resistance will be required for durable clinical responses.

ABT-263 synergizes with ERK1/2 pathway inhibitors to induce substantial tumour cell apoptosis

As discussed above, despite the induction of potent pro-apoptotic BH3-only proteins such as BIM, BMF and PUMA, MEK1/2 and BRAF inhibitors typically induce minimal apoptosis with the predominant response being a G1 cell cycle arrest. However, the BH3-only proteins induced by ERK1/2 inhibition may prime tumour cells for apoptosis. Thus, combining an ERK1/2 pathway inhibitor with other agents that take advantage of this priming event may tip the balance of pro-apoptotic and pro-survival factors in favour of commitment to apoptosis. One such approach is to combine an ERK1/2 pathway inhibitor, such as selumetinib or vemurafenib, with the BH3 mimetic ABT-737 or ABT-263. Scott and colleagues first demonstrated the potential for MEK inhibitors to combine synergistically with BH3 mimetics and induce tumour cell apoptosis (Cragg *et al.*, 2008). Recent studies have now extended these findings using the MEK1/2-selective inhibitor selumetinib in both *BRAF*- and *RAS*-mutant cancers, and the *BRAF* selective inhibitor PLX4720 in *BRAF*-mutant cancers. Results from our work demonstrated that while treating a variety of CRC tumour cells with selumetinib or ABT-263 alone, induced little cell death (typically < 20% at 48 h post-treatment), strong synergistic cell death and inhibition of clonogenic survival were observed upon treatment with combined selumetinib and ABT-263 (~60–80% cell death at 48 h). We observed similar results in melanoma cell lines expressing mutant *RAS* or *BRAF*, and others have demonstrated that combined MEK1/2 and BCL2/BCL-X_L inhibition is frequently effective against *KRAS*-mutant lung and pancreatic tumour cells (Corcoran *et al.*, 2013; Tan *et al.*, 2013). In all cases examined, cell death required caspase activity and was confined to tumour cells addicted to ERK1/2 signalling; tumour cells with high PI3K-PKB signalling known to be intrinsically resistant to MEK1/2 inhibitors also exhibited resistance to this combination (Cragg *et al.*, 2008; Balmanno *et al.*, 2009; Corcoran *et al.*, 2013; Sale and Cook, 2013). Several reports have demonstrated that PI3K/mTOR inhibitors can also interact synergistically with BH3 mimetics to induce apoptosis in certain contexts (Qian *et al.*, 2009; Spender and Inman, 2012; Rahmani *et al.*, 2013). Thus, combining such agents may be a valid approach for tumour cells with high PI3K-PKB activity and intrinsic resistance to MEK1/2 inhibitors. Furthermore, a recent study has shown that while the novel MEK1/2 inhibitor G-963 synergized to promote apoptosis in the majority of NSCLC and pancreatic

cancer cell lines tested, these effects were enhanced by the addition of the PI3K inhibitor GDC-0941 (Tan *et al.*, 2013).

Engelman and colleagues observed that sensitivity to selumetinib plus ABT-263 correlated with the expression of markers of epithelial differentiation, such as E-cadherin (Corcoran *et al.*, 2013). Furthermore, knock-down of ZEB1 in the mesenchymal KRAS-mutant lung cancer cell line A549 promoted expression of epithelial markers and sensitized these cells to both ABT-263 and selumetinib plus ABT-263. This is consistent with many reports demonstrating that epithelial-mesenchymal transition (EMT) promotes resistance to various apoptotic stimuli (Vega *et al.*, 2004; Robson *et al.*, 2006; Arumugam *et al.*, 2009), which has frequently been attributed to PKB activation (Escrivà *et al.*, 2008; Tiwari *et al.*, 2012).

The BRAF inhibitor PLX4720, a close preclinical analogue of vemurafenib (PLX4032), also combined synergistically with ABT-737/263 to induce caspase-dependent cell death and inhibit clonogenic survival of colorectal and melanoma tumour cell lines (Sale and Cook, 2013; Wroblewski *et al.*, 2013). This combination was also effective against cells established from patients with BRAF inhibitor-naïve melanoma (Wroblewski *et al.*, 2013). In addition to these observations in solid tumour types, ERK1/2 pathway inhibitors have also been shown to combine synergistically with ABT-737 to induce the death of acute myeloid leukaemia cells (Zhang *et al.*, 2008; Konopleva *et al.*, 2012).

Combining a MEK1/2 inhibitor and BH3 mimetic also shows potent *in vivo* efficacy. Xenograft models of the KRAS-mutant CRC cell lines HCT116, SW620 and SW1463 yielded similar results: selumetinib treatment in isolation slowed tumour growth considerably relative to control but did not induce regressions; ABT-263 treatment had little effect on tumour expansion relative to control but combination of selumetinib with ABT-263 caused marked tumour regressions that were sustained for the entirety of the 21–27 day experiments (Corcoran *et al.*, 2013). In addition, generating true *in vivo* tumours using a genetically engineered KRAS-driven lung cancer mouse model revealed that while selumetinib or ABT-263 led to average tumour regressions of around 30–40% after 2 weeks of treatment, the combination caused 70–80% tumour regression (Corcoran *et al.*, 2013).

ERK1/2 pathway inhibitors combine with ABT-263 to induce BAX- and BIM-dependent apoptosis

The interplay between the pro-survival BCL2 proteins and pro-death BH3-only proteins ultimately serves to regulate the activation of the pro-death effector proteins BAX and BAK. Using isogenic HCT116 cells lacking BAK, BAX or both [double knockout (DKO)] (Wang and Youle, 2012), we found that those lacking BAX (BAX^{-/-} and BAK^{-/-}BAX^{-/-} DKO) were almost completely resistant to apoptosis induced by selumetinib, ABT-263 or selumetinib plus ABT-263, whereas those lacking only BAK remained sensitive. Thus, at least in HCT116 cells, apoptosis in response to these agents absolutely required BAX, whereas BAK was dispensable (Sale and Cook, 2013). Consistent with this, selumetinib and ABT-263 combined to synergistically activate BAX (Sale and Cook, 2013).

Analysing the expression of upstream regulators of BAX in COLO205 cells revealed that selumetinib (alone or in com-

bination with ABT-263) strongly induced the expression of the pro-apoptotic BH3-only proteins BIM and BMF. In these cells, RNAi-mediated knock-down of BIM inhibited apoptosis induced by selumetinib plus ABT-263 by 50–70%, demonstrating that cell death in response to this combination was in large part BIM dependent (Sale and Cook, 2013). However, in other cell types, including HCT116 (KRAS^{G13D}), the picture was more complex. As with COLO205 cells, MEK1/2 inhibition in HCT116 cells strongly induced BIM and BMF expression, but in this case PUMA expression also increased and robust knock-down of BIM and/or PUMA did not inhibit apoptosis induced by selumetinib and ABT-263 (Sale and Cook, 2013). Given that cell death was absolutely dependent on BAX, it is likely that alternative BH3-only proteins, such as BMF or BAD, or the reduced expression of pro-survival factors acts in a redundant fashion to promote apoptosis. Knock-down of BIM in the BRAF^{V600E}-positive melanoma cell lines Mel-RMu and SK-MEL-28 inhibited apoptosis induced by the combination of PLX4720 plus ABT-263 by 50–70% (Wroblewski *et al.*, 2013). In these cells, PLX4720 plus ABT-263 also promoted PUMA expression and altered the abundance of pro-survival factors. These effects, in addition to possible up-regulation of BMF, which was not assessed, may contribute to apoptosis in these melanoma cells.

ABT-263 promotes redistribution of selumetinib-induced BH3-only proteins from BCL-X_L to MCL1 resulting in stronger inhibition of pro-survival BCL2 proteins

To further define the mechanisms by which selumetinib and ABT-263 synergize to induce apoptosis, we immunoprecipitated BCL-X_L and MCL1 from cells treated with selumetinib, ABT-263 or the combination to assess their interaction with BH3-only binding partners. In COLO205 cells, MEK1/2 inhibition strongly promoted accumulation of BIM and BMF and their binding to both BCL-X_L and MCL1 (Sale and Cook, 2013). However, in the presence of the BH3 mimetic ABT-263, BIM and BMF no longer bound to BCL-X_L, but instead exhibited greater binding to MCL1. This is consistent with the high affinity of ABT-263 for BCL-X_L preventing binding of BIM and BMF to BCL-X_L, but not MCL1. Very similar effects were observed in HCT116 cells, except in this case the combination of selumetinib and ABT-263 promoted the redistribution of PUMA as well as BIM and BMF onto MCL1 (Sale and Cook, 2013). Thus, while ABT-263 alone can efficiently inhibit BCL-X_L but not MCL1, in the presence of selumetinib it promotes the redistribution of the BH3-only proteins BIM, BMF and PUMA from BCL-X_L to MCL1, resulting in greater overall inhibition of the pro-survival BCL2 proteins (Figure 3). In addition, while selumetinib only induced partial displacement BAX from BCL-X_L (we did not observe BAX binding to MCL1), ABT-263 robustly disrupted this interaction. Thus, in the presence of selumetinib plus ABT-263, BAX may undergo more efficient direct activation by any residual BIM freed up from BCL-X_L.

ABT-263 can delay acquired resistance to ERK1/2 pathway inhibitors

Tumour cells chronically exposed to ERK1/2 pathway inhibitors evolve to circumvent the G1 arrest induced by

Figure 3

Selumetinib and ABT-263 synergize to inhibit pro-survival BCL2 family proteins and activate BAX in ERK1/2-addicted tumour cell lines. Treatment of tumour cells with a MEK1/2 inhibitor such as selumetinib invariably induces strong expression of BH3-only proteins such as BIM and BMF (A, top). PUMA expression may also be induced. These BH3-only proteins then bind to pro-survival factors such as BCL-X_L and MCL1 (A, bottom). Despite this, little cell death occurs with MEK1/2 inhibition alone, likely due to residual pro-survival activity, including BCL-X_L and MCL1 (A, bottom). Addition of the BH3 mimetic ABT-263 (red triangle; B, top) causes a redistribution of selumetinib-induced BIM and BMF from ABT-263 sensitive BCL-X_L to ABT-263-resistant MCL1 (B, bottom). Thus, although ABT-263 cannot directly target MCL1, its combination with selumetinib results in indirect inhibition of MCL1 and consequently greater inhibition of pro-survivals (C, top). In addition, whereas selumetinib only resulted in partial displacement of BAX from BCL-X_L, ABT-263 efficiently disrupted this interaction. BAX could then potentially be directly activated by any residual BIM freed up from BCL-X_L (C, top). Activated BAX can subsequently oligomerize and insert into the outer mitochondrial membrane, resulting in MOMP (C, bottom). This allows cytochrome c release from the intermembrane space, followed by apoptosome formation, caspase activation and consequent apoptosis. (Reproduced with permission from Sale and Cook, 2013, *The Biochemical Journal*, 450, 285-294 © the Biochemical Society)

these agents, thereby acquiring resistance to their anti-proliferative effects (Little *et al.*, 2013). We reasoned that the weak cell death responses to ERK1/2 pathway inhibitors may simply allow tumour cells greater opportunity to adapt and evolve acquired resistance. Thus, using ABT-263 to boost primary cell death responses to ERK1/2 pathway inhibitors might minimize the residual population of cells from which resistant clones could emerge and/or increase the number of mutations required before a strong selective advantage is gained, in a manner analogous to drug combinations that rapidly reduce HIV viral load and inhibit

acquired resistance (Clavel and Hance, 2004; Bock and Lengauer, 2012).

We tested this hypothesis in CRC cells with *RAS* and *BRAF* mutations. Treating CRC cells transiently (72 h) with selumetinib plus ABT-263 inhibited the frequency of colonies that subsequently developed acquired resistance to selumetinib over 4–6 weeks by 90–95% (Sale and Cook, 2013). Continual treatment with selumetinib plus ABT-263 over a period of 2 weeks similarly inhibited the frequency of resistant colonies by 95%. This was observed in both *KRAS*- and *BRAF*-mutant tumour cells that otherwise rapidly

develop 100-fold resistance to selumetinib (Little *et al.*, 2011). These results are consistent with the *KRAS*-mutant CRC xenograft models discussed above, in which selumetinib plus ABT-263 induced sustained tumour regressions over the course of these 3–4 week experiments (Corcoran *et al.*, 2013). In addition, durable regressions of up to 7 weeks were observed in mutant *KRAS*-driven lung cancer mouse models (Corcoran *et al.*, 2013).

Thus, these studies provide substantial support for the clinical use of ERK1/2 pathway inhibitors in conjunction with ABT-263. This combination has the potential to augment primary responses and delay acquired resistance, while importantly continuing to harness tumour cell addiction to ERK1/2 signalling.

ABT-263 can overcome established acquired resistance to ERK1/2 pathway inhibitors in some cases

We also examined the effect of ABT-263 treatment on *BRAF*- or *KRAS*-mutant CRC cells that had already acquired resistance to selumetinib. In these cells, acquired resistance had arisen through an amplification of the driving oncogene, which acts to reinstate ERK1/2 signalling in the presence of drug (Little *et al.*, 2011). Under their normal growth conditions (in the presence of selumetinib), selumetinib-resistant COLO205 and HT29 cells (driven by *BRAF* amplification) remained sensitive to ABT-263. In fact, these resistant cells exhibited greater sensitivity to ABT-263 than parental COLO205 or HT29 cells by ~1.5–2-fold, suggesting they may be 'primed' for ABT-263-induced apoptosis. In the absence of selumetinib, however, these cells were resistant to ABT-263 treatment relative to parental cells. Selumetinib removal results in a rapid and sustained hyperactivation of ERK1/2 (Little *et al.*, 2011) which appears to abolish the priming event and diminish sensitivity to ABT-263. In contrast, HCT116 cells with acquired resistance to selumetinib (*KRAS* amplification) were cross-resistant to ABT-263, regardless of whether selumetinib was present. *KRAS* amplification in these cells activates PI3K-PKB signalling (Little *et al.*, 2011), which could potentially provide survival signals to promote ABT-263 cross-resistance. However, even when PI3K-PKB and ERK1/2 signalling in selumetinib-resistant HCT116 cells was inhibited to levels seen in parental HCT116, these cells remained less sensitive to ABT-263 than parental HCT116 cells; thus, *KRAS* amplification conferred resistance to ABT-263 under all conditions. This is consistent with our observations that alternative pathways contribute to acquired resistance in this setting (Little *et al.*, 2011) and underline the challenge faced by *KRAS* amplification as a mechanism of resistance.

Using melanoma cell lines established from patients pre- and post-vemurafenib treatment, Wroblewski *et al.* demonstrated that while vemurafenib-naïve cells underwent apoptosis in response to PLX4720 plus ABT-263, cells harvested post-treatment were refractory to this combination. It is currently unclear why some tumour cells with acquired resistance to ERK1/2 pathway inhibitors remain sensitive to ABT-263, while others are resistant. Nevertheless, ABT-263 has the potential to overcome acquired resistance to ERK1/2 pathway inhibitors in some settings.

Conclusions

Oncogene addiction and the therapeutic window that this can provide for tumour selective intervention holds a great promise for the development of new targeted anti-cancer agents. This has been most evident in the success of agents such as imatinib for the treatment of chronic myelogenous leukaemia (CML) (Capdeville *et al.*, 2002), gefitinib and erlotinib, for the treatment of NSCLC with mutant EGFR (Sequist and Lynch, 2008) and vemurafenib, and for the treatment of *BRAF*^{V600E}-positive melanoma (Chapman *et al.*, 2011). However, acquired resistance to these therapies is a major problem; indeed, the exquisite selectivity of some new targeted agents may actually provide a strong and very focused selection pressure for the rapid emergence of resistance through pathway remodelling (Little *et al.*, 2013). This may be exacerbated by a general failure to sufficiently engage or activate pro-death signalling pathways, including the BCL2 proteins. Indeed, the predominant response to *BRAF* and MEK1/2 inhibitors is cytostatic rather than cytotoxic. However, addiction to ERK1/2 signalling provides one arm of a synthetic lethal pair that, when combined with BH3 mimetics, results in a strong synergistic tumour cell death that is only observed in ERK1/2-addicted cells. This greatly improves primary efficacy and inhibits and delays the onset of acquired resistance. This strategy is also effective in tumours addicted to breakpoint cluster region/Abelson murine leukaemia viral oncogene and EGFR (Kuroda *et al.*, 2006; Cragg *et al.*, 2007), and so may merit more general consideration as a drug combination that can harness oncogene addiction and transform it into tumour cell-specific cell death.

Acknowledgements

This work was supported by a sponsored research collaboration between AstraZeneca and the Babraham Institute in which AstraZeneca provided selumetinib, AZ628 and AZ12321046 for no charge. M. S. was supported by a BBSRC studentship.

Conflict of interest

Payments from AZ were confined to laboratory consumables and associated overheads. Neither S. C. nor M. S. received any personal financial remuneration of any sort from AstraZeneca.

References

- Andrews M, Behren A, Chiohn F, Tebbutt N, Do H, Dobrovic A *et al.* (2012). Colorectal cancer promoted in a melanoma patient receiving dabrafenib (GSK2118436) in combination with MEK1/2 inhibitor trametinib (GSK1120212). *Pigment Cell Melanoma Res* 25: 842.
- Anforth RM, Blumetti TC, Kefford RF, Sharma R, Scolyer RA, Kossard S *et al.* (2012). Cutaneous manifestations of dabrafenib

(GSK2118436): a selective inhibitor of mutant BRAF in patients with metastatic melanoma. *Br J Dermatol* 167: 1153–1160.

Arnault JP, Mateus C, Escudier B, Tomasic G, Wechsler J, Hollville E *et al.* (2012). Skin tumors induced by sorafenib; paradoxical RAS-RAF pathway activation and oncogenic mutations of HRAS, TP53, and TGFBR1. *Clin Cancer Res* 18: 263–272.

Arumugam T, Ramachandran V, Fournier KF, Wang H, Marquis L, Abbruzzese JL *et al.* (2009). Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. *Cancer Res* 69: 5820–5828.

Balmanno K, Cook SJ (2009). Tumour cell survival signalling by the ERK1/2 pathway. *Cell Death Differ* 16: 368–377.

Balmanno K, Chell SD, Gillings AS, Hayat S, Cook SJ (2009). Intrinsic resistance to the MEK1/2 inhibitor AZD6244 (ARRY-142886) is associated with weak ERK1/2 signalling and/or strong PI3K signalling in colorectal cancer cell lines. *Int J Cancer* 125: 2332–2341.

Basile KJ, Aplin AE (2012). Downregulation of Noxa by RAF/MEK inhibition counteracts cell death response in mutant B-RAF melanoma cells. *Am J Cancer Res* 2: 726–735.

Bennouna J, Lang I, Valladares-Ayerbes M, Boer K, Adenis A, Escudero P *et al.* (2011). A Phase II, open-label, randomised study to assess the efficacy and safety of the MEK1/2 inhibitor AZD6244 (ARRY-142886) versus capecitabine monotherapy in patients with colorectal cancer who have failed one or two prior chemotherapeutic regimens. *Invest New Drugs* 29: 1021–1028.

Bock C, Lengauer T (2012). Managing drug resistance in cancer: lessons from HIV therapy. *Nat Rev Cancer* 12: 494–501.

Bodoky G, Timcheva C, Spigel DR, La Stella PJ, Ciuleanu TE, Pover G *et al.* (2012). A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. *Invest New Drugs* 30: 1216–1223.

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

Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME (1999). Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* 286: 1358–1362.

Booy EP, Henson ES, Gibson SB (2011). Epidermal growth factor regulates Mcl-1 expression through the MAPK-Elk-1 signalling pathway contributing to cell survival in breast cancer. *Oncogene* 30: 2367–2378.

Bouchard C, Thieke K, Maier A, Saffrich R, Hanley-Hyde J, Ansorge W *et al.* (1999). Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. *EMBO J* 18: 5321–5333.

Boucher MJ, Morisset J, Vachon PH, Reed JC, Lainé J, Rivard N (2000). MEK/ERK signaling pathway regulates the expression of Bcl-2, Bcl-xL, and Mcl-1 and promotes survival of human pancreatic cancer cells. *J Cell Biochem* 79: 355–369.

Callahan MK, Rampal R, Harding JJ, Klimek VM, Chung YR, Merghoub T *et al.* (2012). Progression of RAS-mutant leukemia during RAF inhibitor treatment. *N Engl J Med* 367: 2316–2321.

Capdeville R, Buchdunger E, Zimmermann J, Matter A (2002). Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat Rev Drug Discov* 1: 493–502.

Chanan-Khan AA, Niesvizky R, Hohl RJ, Zimmerman TM, Christiansen NP, Schiller GJ *et al.* (2009). Phase III randomised study of dexamethasone with or without oblimersen sodium for patients with advanced multiple myeloma. *Leuk Lymphoma* 50: 559–565.

Chapman P, Metz D, Sepulveda A, Uehara T, Rustgi A, Nathanson KL *et al.* (2012). Development of colonic adenomas and gastric polyps in BRAF mutant melanoma patients treated with vemurafenib. *Pigment Cell Melanoma Res* 25: 847.

Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J *et al.* (2011). Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 364: 2507–2516.

Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR (2010). The BCL-2 family reunion. *Mol Cell* 37: 299–310.

Chonghaile TN, Letai A (2008). Mimicking the BH3 domain to kill cancer cells. *Oncogene* 27: S149–S157.

Clavel F, Hance AJ (2004). HIV drug resistance. *N Engl J Med* 350: 1023–1035.

Cobrinik D (2005). Pocket proteins and cell cycle control. *Oncogene* 24: 2796–2809.

Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA (2010). BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Sci Signal* 3: ra84.

Corcoran RB, Ebi H, Turke AB, Coffee EM, Nishino M, Cogdill AP *et al.* (2012). EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov* 2: 227–235.

Corcoran RB, Cheng KA, Hata AN, Faber AC, Ebi H, Coffee EM *et al.* (2013). Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell* 23: 121–128.

Cragg MS, Kuroda J, Puthalakath H, Huang DC, Strasser A (2007). Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. *Plos Med* 4: 1681–1689.

Cragg MS, Jansen ES, Cook M, Harris C, Strasser A, Scott CL (2008). Treatment of B-RAF mutant human tumor cells with a MEK inhibitor requires Bim and is enhanced by a BH3 mimetic. *J Clin Invest* 118: 3651–3659.

Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP *et al.* (2013). Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature* 494: 251–255.

Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y *et al.* (1997). Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 91: 231–241.

Davies BR, Logie A, McKay JS, Martin P, Steele S, Jenkins R *et al.* (2007). AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 kinases: mechanism of action in vivo, pharmacokinetic/ pharmacodynamic relationship, and potential for combination in preclinical models. *Mol Cancer Ther* 6: 2209–2219.

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S *et al.* (2002). Mutations of the BRAF gene in human cancer. *Nature* 417: 949–954.

van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE *et al.* (2006). The BH3 mimetic ABT-737 targets

- selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. *Cancer Cell* 10: 389–399.
- Delmas C, Manenti S, Boudjelal A, Peyssonnaud C, Eychene A, Darbon JM (2001). The p42/p44 mitogen-activated protein kinase activation triggers p27Kip1 degradation independently of CDK2/cyclin E in NIH 3T3 cells. *J Biol Chem* 276: 34958–34965.
- Dijkers PF, Medema RH, Lammers JW, Koenderman L, Coffey PJ (2000). Expression of the proapoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. *Curr Biol* 10: 1201–1204.
- Domina AM, Vrana JA, Gregory MA, Hann SR, Craig RW (2004). MCL1 is phosphorylated in the PEST region and stabilized upon ERK activation in viable cells, and at additional sites with cytotoxic okadaic acid or taxol. *Oncogene* 23: 5301–5315.
- Downward J (2003). Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 3: 11–22.
- Elgendy M, Sheridan C, Brumatti G, Martin SJ (2011). Oncogenic Ras-induced expression of Noxa and Beclin-1 promotes autophagic cell death and limits clonogenic survival. *Mol Cell* 42: 23–35.
- Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ *et al.* (2009). MEK1 mutations confer resistance to MEK and B-RAF inhibition. *Proc Natl Acad Sci U S A* 106: 20411–20416.
- Escrivà M, Peiró S, Herranz N, Villagrasa P, Dave N, Montserrat-Sentís B *et al.* (2008). Repression of PTEN phosphatase by Snail1 transcriptional factor during gamma radiation-induced apoptosis. *Mol Cell Biol* 28: 1528–1540.
- Ewings KE, Hadfield-Moorhouse K, Wiggins CM, Wickenden JA, Balmanno K, Gilley R *et al.* (2007). ERK1/2-dependent phosphorylation of BimEL promotes its rapid dissociation from Mcl-1 and Bcl-xL. *EMBO J* 26: 2856–2867.
- Faber AC, Corcoran RB, Ebi H, Sequist LV, Waltman BA, Chung E *et al.* (2011). BIM expression in treatment-naïve cancers predicts responsiveness to kinase inhibitors. *Cancer Discov* 1: 352–365.
- Fang X, Yu S, Eder A, Mao M, Bast JRC, Boyd D *et al.* (1999). Regulation of BAD phosphorylation at serine 112 by the Ras-mitogen-activated protein kinase pathway. *Oncogene* 18: 6635–6640.
- Fariña-Sarasqueta A, van Lijnschoten G, Moerland E, Creemers GJ, Lemmens VE, Rutten HJ *et al.* (2010). The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. *Ann Oncol* 21: 2396–2402.
- Fox W, Ellard GA, Mitchison DA (1999). Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. *Int J Tuberc Lung Dis* 3: S231–S279.
- Fujita N, Sato S, Tsuruo T (2003). Phosphorylation of p27Kip1 at threonine 198 by p90 ribosomal protein S6 kinases promotes its binding to 14-3-3 and cytoplasmic localization. *J Biol Chem* 278: 49254–49260.
- Del Gaizo Moore V, Brown JR, Certo M, Love TM, Novina CD, Letai A (2007). Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. *J Clin Invest* 117: 112–121.
- Galaktionov K, Chen XC, Beach D (1996). Cdc25 cell-cycle phosphatase as a target of c-myc. *Nature* 382: 511–517.
- Gartel AL, Ye X, Goufman E, Shianov P, Hay N, Najmabadi F *et al.* (2001). Myc represses the p21(WAF1/CIP1) promoter and interacts with Sp1/Sp3. *Proc Natl Acad Sci U S A* 98: 4510–4515.
- Girotti MR, Pedersen M, Sanchez-Laorden B, Viros A, Turajlic S, Niculescu-Duvaz D *et al.* (2013). Inhibiting EGF receptor or SRC family kinase signaling overcomes BRAF inhibitor resistance in melanoma. *Cancer Discov* 3: 158–167.
- Glickman MS, Sawyers CL (2012). Converting cancer therapies into cures: lessons from infectious diseases. *Cell* 148: 1089–1098.
- Greger JG, Eastman SD, Zhang V, Bleam MR, Hughes AM, Smitheman KN *et al.* (2012). Combinations of BRAF, MEK, and PI3K/mTOR inhibitors overcome acquired resistance to the BRAF inhibitor GSK2118436 dabrafenib, mediated by NRAS or MEK mutations. *Mol Cancer Ther* 11: 909–920.
- Hainsworth JD, Cebotaru CL, Kanarev V, Ciuleanu TE, Dmyanov D, Stella P *et al.* (2010). A phase II, open-label, randomized study to assess the efficacy and safety of AZD6244 (ARRY-142886) versus pemetrexed in patients with non-small cell lung cancer who have failed one or two prior chemotherapeutic regimens. *J Thorac Oncol* 5: 1630–1636.
- Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R *et al.* (2010). RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature* 464: 431–435.
- Hatzivassiliou G, Liu B, O'Brien C, Spörke JM, Hoeflich KP, Haverty PM *et al.* (2012). ERK inhibition overcomes acquired resistance to MEK inhibitors. *Mol Cancer Ther* 11: 1143–1154.
- Hayes DN, Lucas AS, Tanvetyanon T, Krzyzanowska MK, Chung CH, Murphy BA *et al.* (2012). Phase II efficacy and pharmacogenomic study of Selumetinib (AZD6244; ARRY-142886) in iodine-131 refractory papillary thyroid carcinoma with or without follicular elements. *Clin Cancer Res* 18: 2056–2065.
- Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvaz I, Dhomen N *et al.* (2010). Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* 140: 209–221.
- Hermeking H, Rago C, Schuhmacher M, Li Q, Barrett JF, Obaya AJ *et al.* (2000). Identification of CDK4 as a target of c-MYC. *Proc Natl Acad Sci U S A* 97: 2229–2234.
- Jänne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J, Barrios C *et al.* (2012). Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol* 14: 38–47.
- Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA *et al.* (2010). COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* 468: 968–972.
- Joseph EW, Pratilas CA, Poulikakos PI, Tadi M, Wang W, Taylor BS *et al.* (2010). The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. *Proc Natl Acad Sci U S A* 107: 14903–14908.
- Kirkin V, Joos S, Zörnig M (2004). The role of Bcl-2 family members in tumorigenesis. *Biochim Biophys Acta* 1644: 229–249.
- Kirkwood JM, Bastholt L, Robert C, Sosman J, Larkin J, Hersey P *et al.* (2012). Phase II, open-label, randomized trial of the MEK1/2 inhibitor selumetinib as monotherapy versus temozolomide in patients with advanced melanoma. *Clin Cancer Res* 18: 555–567.
- Klasa RJ, Gillum AM, Klem RE, Frankel SR (2002). Oblimersen Bcl-2 antisense: facilitating apoptosis in anticancer treatment. *Antisense Nucleic Acid Drug Dev* 12: 193–213.
- Konopleva M, Contractor R, Tsao T, Samudio I, Ruvoilo PP, Kitada S *et al.* (2006). Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer Cell* 10: 193–213.

- Konopleva M, Watt J, Contractor R, Tsao T, Harris D, Estrov Z *et al.* (2008). Mechanisms of antileukemic activity of the novel Bcl-2 homology domain-3 mimetic GX15-070 (obatoclax). *Cancer Res* 68: 3413–3420.
- Konopleva M, Milella M, Ruvolo P, Watts JC, Ricciardi MR, Korchin B *et al.* (2012). MEK inhibition enhances ABT-737-induced leukemia cell apoptosis via prevention of ERK-activated MCL-1 induction and modulation of MCL-1/BIM complex. *Leukemia* 26: 778–787.
- Kopetz S, Desai J, Chan E, Hecht JR, O'Dwyer PJ, Lee RJ *et al.* (2010). PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors. *J Clin Oncol* 28: Abstract 3534.
- Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC *et al.* (2006). Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. *Proc Natl Acad Sci U S A* 103: 14907–14912.
- Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR *et al.* (2005). BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. *Mol Cell* 17: 525–535.
- Le K, Blomain E, Rodeck U, Aplin AE (2013). Selective RAF inhibitor impairs ERK1/2 phosphorylation and growth in mutant NRAS, vemurafenib-resistant melanoma cells. *Pigment Cell Melanoma Res* doi: 10.1111/pcmr.12092
- Ley R, Balmanno K, Hadfield K, Weston C, Cook SJ (2003). Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. *J Biol Chem* 278: 18811–18816.
- Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M *et al.* (2012). Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. *Cancer Cell* 22: 668–682.
- Little AS, Balmanno K, Sale MJ, Newman S, Dry JR, Hampson M *et al.* (2011). Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK1/2 inhibitors in colorectal cancer cells. *Sci Signal* 4: ra17.
- Little AS, Smith PD, Cook SJ (2013). Mechanisms of acquired resistance to ERK1/2 pathway inhibitors. *Oncogene* 32: 1207–1215.
- Lopez J, Hesling C, Prudent J, Popgeorgiev N, Gadet R, Mikaelian I *et al.* (2012). Src tyrosine kinase inhibits apoptosis through the Erk1/2-dependent degradation of the death accelerator Bik. *Cell Death Differ* 19: 1459–1469.
- Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H *et al.* (2011). BRAF inhibitors upregulate EGFR ligands: a molecular link to RAF inhibitor-induced cutaneous squamous cell carcinomas. *Mol Cancer Ther* 10: Abstract A229.
- Maurer U, Charvet C, Wagman AS, Dejardin E, Green DR (2006). Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of MCL-1. *Mol Cell* 21: 749–760.
- Meloche S, Pouyssegur J (2007). The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene* 26: 3227–3239.
- Montagut C, Sharma SV, Shioda T, McDermott U, Ullman M, Ullman LE *et al.* (2008). Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. *Cancer Res* 68: 4853–4861.
- Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H *et al.* (2010). Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 468: 973–977.
- O'Brien SM, Claxton DF, Crump M, Faderl S, Kipps T, Keating MJ *et al.* (2009a). Phase I study of obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia. *Blood* 113: 299–305.
- O'Brien S, Moore JO, Boyd TE, Larratt LM, Skotnicki AB, Koziner B *et al.* (2009b). 5-year survival in patients with relapsed or refractory chronic lymphocytic leukemia in a randomized, phase III trial of fludarabine plus cyclophosphamide with or without Oblimersen. *J Clin Oncol* 27: 5208–5212.
- Oltschendorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA *et al.* (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 435: 677–681.
- Paik PK, Rudin CM, Brown A, Rizvi NA, Takebe N, Travis W *et al.* (2010). A phase I study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in solid tumor malignancies. *Cancer Chemother Pharmacol* 66: 1079–1085.
- Paik PK, Rudin CM, Pietanza MC, Brown A, Rizvi NA, Takebe N *et al.* (2011). A phase II study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in relapsed small cell lung cancer. *Lung Cancer* 74: 481–485.
- Patel SP, Lazar AJ, Papadopoulos NE, Liu P, Infante JR, Glass MR *et al.* (2013). Clinical responses to selumetinib (AZD6244; ARRY-142886)-based combination therapy stratified by gene mutations in patients with metastatic melanoma. *Cancer* 119: 799–805.
- del Peso L, González-García M, Page C, Herrera R, Nuñez G (1997). Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science* 278: 687–689.
- Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N (2010). RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* 464: 427–430.
- Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G *et al.* (2011). RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* 480: 387–390.
- Prahalad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D *et al.* (2012). Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 483: 100–103.
- Qian J, Zou Y, Rahman JS, Lu B, Massion PP (2009). Synergy between phosphatidylinositol 3-kinase/Akt pathway and Bcl-xL in the control of apoptosis in adenocarcinoma cells of the lung. *Mol Cancer Ther* 8: 101–109.
- Rahmani M, Aust MM, Attkisson E, Williams DC Jr, Ferreira-Gonzalez A, Grant S (2013). Dual inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism. *Cancer Res* 73: 1340–1351.
- Richman SD, Seymour MT, Chambers P, Elliott F, Daly CL, Meade AM *et al.* (2009). KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol* 27: 5931–5937.
- Rivard N, Boucher MJ, Asselin C, L'Allemain G (1999). MAP kinase cascade is required for p27 downregulation and S phase entry in fibroblasts and epithelial cells. *Am J Physiol* 277: C652–C664.
- Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL *et al.* (2012). Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J Clin Oncol* 30: 488–496.

- Robson EJ, Khaled WT, Abell K, Watson CJ (2006). Epithelial-to-mesenchymal transition confers resistance to apoptosis in three murine mammary epithelial cell lines. *Differentiation* 74: 254–264.
- Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR *et al.* (2012). Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. *Clin Cancer Res* 18: 3163–3169.
- Sale MJ, Cook SJ (2013). The BH3-mimetic ABT-263 synergises with the MEK1/2 inhibitor selumetinib/AZD6244 to promote BIM-dependent tumour cell death and inhibit acquired resistance. *Biochem J* 450: 285–294.
- Scheid MP, Schubert KM, Duronio V (1999). Regulation of bad phosphorylation and association with Bcl-x(L) by the MAPK/Erk kinase. *J Biol Chem* 274: 31108–31113.
- Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR (2000). Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev* 14: 2501–2514.
- Sequist LV, Lynch TJ (2008). EGFR tyrosine kinase inhibitors in lung cancer: an evolving story. *Annu Rev Med* 59: 429–442.
- Shao Y, Aplin AE (2010). Akt3-mediated resistance to apoptosis in B-RAF-targeted melanoma cells. *Cancer Res* 70: 6670–6681.
- Shao Y, Aplin AE (2012). ERK2 phosphorylation of serine 77 regulates Bmf pro-apoptotic activity. *Cell Death Dis* 3: e253.
- She QB, Ma WY, Zhong S, Dong Z (2002). Activation of JNK1, RSK2, and MSK1 is involved in serine 112 phosphorylation of Bad by ultraviolet B radiation. *J Biol Chem* 277: 24039–24048.
- Sheridan C, Brumatti G, Martin SJ (2008). Oncogenic B-RafV600E inhibits apoptosis and promotes ERK-dependent inactivation of Bad and Bim. *J Biol Chem* 283: 22128–22135.
- Sheridan C, Brumatti G, Elgendy M, Brunet M, Martin SJ (2010). An ERK-dependent pathway to Noxa expression regulates apoptosis by platinum-based chemotherapeutic drugs. *Oncogene* 29: 6428–6441.
- Shi H, Moriceau G, Kong X, Lee MK, Lee H, Koya RC *et al.* (2012). Melanoma whole-exome sequencing identifies (V600E) B-RAF amplification-mediated acquired B-RAF inhibitor resistance. *Nat Commun* 3: 724.
- Shimamura A, Ballif BA, Richards SA, Blenis J (2000). Rsk1 mediates a MEK-MAP kinase cell survival signal. *Curr Biol* 10: 127–135.
- Smalley KS, Lioni M, Dalla Palma M, Xiao M, Desai B, Egyhazi S *et al.* (2008). Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. *Mol Cancer Ther* 7: 2876–2883.
- Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A *et al.* (2006). BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 439: 358–362.
- Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS *et al.* (2012). Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 366: 707–714.
- Spender LC, Inman GJ (2012). Phosphoinositide 3-kinase/AKT/mTORC1/2 signaling determines sensitivity of Burkitt's lymphoma cells to BH3 mimetics. *Mol Cancer Res* 10: 347–359.
- Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O *et al.* (2012a). RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. *N Engl J Med* 366: 207–215.
- Su F, Bradley WD, Wang Q, Yang H, Xu L, Higgins B *et al.* (2012b). Resistance to selective BRAF inhibition can be mediated by modest upstream pathway activation. *Cancer Res* 72: 969–978.
- Tait SW, Green DR (2010). Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat Rev Mol Cell Biol* 11: 621–632.
- Tan N, Wong M, Nannini MA, Hong R, Lee LB, Price S *et al.* (2013). Bcl-2/Bcl-xL inhibition increases the efficacy of Mek inhibition alone and in combination with PI3 Kinase inhibition in lung and pancreatic tumor models. *Mol Cancer Ther* doi: 10.1158/1535-7163.MCT-12-0949
- Tan Y, Ruan H, Demeter MR, Comb MJ (1999). p90(RSK) blocks bad-mediated cell death via a protein kinase C-dependent pathway. *J Biol Chem* 274: 34859–34867.
- Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP *et al.* (2011). BRAF mutations in hairy-cell leukemia. *N Engl J Med* 364: 2305–2315.
- Tiwari N, Gheldof A, Tatari M, Christofori G (2012). EMT as the ultimate survival mechanism of cancer cells. *Semin Cancer Biol* 22: 194–207.
- Tol J, Nagtegaal ID, Punt CJ (2009). BRAF mutation in metastatic colorectal cancer. *N Engl J Med* 361: 98–99.
- Townsend KJ, Zhou P, Qian L, Bieszczyk CK, Lowrey CH, Yen A *et al.* (1999). Regulation of MCL1 through a serum response factor/Elk-1-mediated mechanism links expression of a viability-promoting member of the BCL2 family to the induction of hematopoietic cell differentiation. *J Biol Chem* 274: 1801–1813.
- Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S *et al.* (2008). ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res* 68: 3421–3428.
- VanBrocklin MW, Verhaegen M, Soengas MS, Holmen SL (2009). Mitogen-activated protein kinase inhibition induces translocation of Bmf to promote apoptosis in melanoma. *Cancer Res* 69: 1985–1994.
- Vega S, Morales AV, Ocaña OH, Valdés F, Fabregat I, Nieto MA (2004). Snail blocks the cell cycle and confers resistance to cell death. *Genes Dev* 18: 1131–1143.
- Vickers ER, Kasza A, Kurnaz IA, Seifert A, Zeef LA, O'donnell A *et al.* (2004). Ternary complex factor-serum response factor complex-regulated gene activity is required for cellular proliferation and inhibition of apoptotic cell death. *Mol Cell Biol* 24: 10340–103451.
- Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK *et al.* (2010). 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: 683–695.
- Wagle N, Emery C, Berger MF, Davis MJ, Sawyer A, Pochanard P *et al.* (2011). Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol* 29: 3085–3096.
- Wang C, Youle RJ (2012). Predominant requirement of Bax for apoptosis in HCT116 cells is determined by Mcl-1's inhibitory effect on Bak. *Oncogene* 31: 3177–3189.
- Wang H, Daouti S, Li WH, Wen Y, Rizzo C, Higgins B *et al.* (2011). Identification of the MEK1(F129L) activating mutation as a potential mechanism of acquired resistance to MEK inhibition in human cancers carrying the B-RafV600E mutation. *Cancer Res* 71: 5535–5545.
- Wang JM, Chao JR, Chen W, Kuo ML, Yen JJ, Yang-Yen HF (1999). The antiapoptotic gene mcl-1 is up-regulated by the phosphatidylinositol 3-kinase/Akt signaling pathway through a transcription factor complex containing CREB. *Mol Cell Biol* 19: 6195–6206.

- Wang YF, Jiang CC, Kiejda KA, Gillespie S, Zhang XD, Hersey P (2007). Apoptosis induction in human melanoma cells by inhibition of MEK is caspase-independent and mediated by the Bcl-2 family members PUMA, Bim, and Mcl-1. *Clin Cancer Res* 13: 4934–4942.
- Whittaker S, Kirk R, Hayward R, Zambon A, Viros A, Cantarino N *et al.* (2010). Gatekeeper mutations mediate resistance to BRAF-targeted therapies. *Sci Transl Med* 2: 35ra41.
- Wickenden JA, Jin H, Johnson M, Gillings AS, Newson C, Austin M *et al.* (2008). Colorectal cancer cells with the BRAF(V600E) mutation are addicted to the ERK1/2 pathway for growth factor-independent survival and repression of BIM. *Oncogene* 27: 7150–7161.
- Wilson B, Mochon E, Boxer L (1996). Induction of bcl-2 expression by phosphorylated CREB proteins during B- cell activation and rescue from apoptosis. *Mol Cell Biol* 16: 5546–5556.
- Wilson WH, O'Connor OA, Czuczman MS, LaCasce AS, Gerecitano JF, Leonard JP *et al.* (2010). Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. *Lancet Oncol* 11: 1149–1159.
- Wroblewski D, Mijatov B, Mohana-Kumaran N, Lai F, Gallagher SJ, Haass NK *et al.* (2013). The BH3-mimetic ABT-737 sensitizes human melanoma cells to apoptosis induced by selective BRAF inhibitors but does not reverse acquired resistance. *Carcinogenesis* 34: 237–247.
- Xing M (2005). BRAF mutation in thyroid cancer. *Endocr Relat Cancer* 12: 245–262.
- Yadav V, Zhang X, Liu J, Estrem S, Li S, Gong XQ *et al.* (2012). Reactivation of mitogen-activated protein kinase (MAPK) pathway by FGF receptor 3 (FGFR3)/Ras mediates resistance to vemurafenib in human B-RAF V600E mutant melanoma. *J Biol Chem* 287: 28087–28098.
- Yang JY, Zong CS, Xia WY, Yamaguchi H, Ding QQ, Xie XM *et al.* (2008). ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nat Cell Biol* 10: 138–148.
- Yecies D, Carlson NE, Deng J, Letai A (2010). Acquired resistance to ABT-737 in lymphoma cells that up-regulate MCL-1 and BFL-1. *Blood* 115: 3304–3313.
- Yeh TC, Marsh V, Bernat BA, Ballard J, Colwell H, Evans RJ *et al.* (2007). Biological Characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 Inhibitor. *Clin Cancer Res* 13: 1576–1583.
- You H, Pellegrini M, Tsuchihara K, Yamamoto K, Hacker G, Erlacher M *et al.* (2006). FOXO3a-dependent regulation of Puma in response to cytokine/growth factor withdrawal. *J Exp Med* 203: 1657–1663.
- Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ (1996). Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 87: 619–628.
- Zhai D, Jin C, Satterthwait AC, Reed JC (2006). Comparison of chemical inhibitors of antiapoptotic Bcl-2-family proteins. *Cell Death Differ* 13: 1419–1421.
- Zhang W, Konopleva M, Ruvolo VR, McQueen T, Evans RL, Bornmann WG *et al.* (2008). Sorafenib induces apoptosis of AML cells via Bim-mediated activation of the intrinsic apoptotic pathway. *Leukemia* 22: 808–818.