Unlocking Pandora's box: personalising cancer cell death in non-small cell lung cancer

Dean A Fennell1,3* and Charles Swanton2,3

Abstract
Evasion of apoptosis is a hallmark of tumorigenesis and a recognised cause of multidrug resistance. Over the last decade, insights into how apoptosis might be exploited in non-small cell lung cancer (NSCLC) and how cancer therapeutics might be used to engage apoptotic signalling in a personalised manner have changed markedly. We are now in the wake of a paradigm shift in stratified therapeutic approaches related to NSCLC. At the heart of this shift in thinking is the emerging knowledge that even the most drug-resistant cancers exhibit a functional death pathway and, critically, that this pathway can be efficiently engaged, leading to clinical benefit. This review will summarise current knowledge of mitochondrial apoptotic pathway dysfunction in NSCLC and how the next generation of targeted therapeutics might be used to exploit deficiencies in apoptotic signalling in a personalised manner to improve clinical outcome and predict therapeutic benefit.

Keywords: Personalised medicine, Lung cancer, Somatic mutations, Apoptosis, Mitochondria, Targeted therapy

Review
The mitochondria collectively represent a cellular Pandora’s box. The key to effective treatment of non-small cell lung cancer (NSCLC) should be the identification of personalised therapeutic approaches capable of selectively engaging mitochondrial cell death. An approach that holds the greatest promise for achieving this may lie in the use of genetics to identify ‘keys to unlock’ Pandora’s box in NSCLC.

Pandora’s box and mitochondrial apoptosis
The organelles termed mitochondria constitute a major component of the canonical death pathway which initiates apoptosis. During life, these organelles play a critical role in maintaining bioenergetic homeostasis, predominantly through the synthesis of adenosine trisphosphate. However, following a fatal cellular insult, mitochondria commit irreversibility to ensuring the death of the cell. To achieve this, mitochondria release into the cytoplasm death-signalling proteins, which are normally harboured, safe out of reach, within the intermembrane space or cristae. This occurs as a result of mitochondrial outer membrane permeabilisation or MOMP. Upon release, these factors which include cytochrome C [1,2], OMI, smac [3], and apoptosis-inducing factor [4,5] lead to the activation of cellular demolition machinery comprising zymogens termed caspases, which systematically cause the non-inflammatory elimination of the cell via apoptosis and immune cell engulfment [6]. MOMP occurs as a result of the activation of proapoptotic BCL2 family proteins.

The multidomain family members BAX and BAK exhibit genetic redundancy and undergo homo- and hetero-oligomerisation [7,8] to promote pore formation in the outer mitochondrial membrane — essentially the key to unlocking Pandora’s box [9]. To trigger MOMP, BAX/BAK must first be activated. This requires a subset of proapoptotic BCL2 family proteins which harbour a single BCL-2 homology domain 3 (BH3) [10-13]. This alpha helical amphipathic domain is sufficient to activate BAX and BAK and may do so by two mechanisms. The BH3-only proteins BIM, BID and PUMA directly activate BAX/BAK, leading to their oligomerisation and MOMP [14,15]. BIM is activated by post-translational modification through phosphorylation, causing its stabilisation [16-19]. In contrast, BID is a substrate for caspase 8 cleavage induced by death receptor ligation [20], whereas PUMA is transcriptionally activated [21]. A second group of five BH3-only proteins (BAD, NOXA,
BNIP3, BFK and BMF) activates BAX and BAK by disrupting their interaction with the prosurvival members of the BCL2 family, which comprise BCL2, BCLXL, MCL1, BCLW and BCLB [22-25]. The apparent redundancy in BH3-only proteins may be explained by their role as functionally distinct cell damage sensors.

**Do prosurvival BCL2 proteins ‘lock’ Pandora’s box?**

Prosurvival BCL2 family proteins have long been considered as critical suppressors of apoptosis in cancer. This is supported by their high rate of gene amplification. For example, MCL1 encoded at the 1q21.2 locus frequently acquires copy number gain in cancer, followed by BCLX (BCLL2) [26]. The basis for this may be as a countermeasure to circumvent the proapoptotic activity of critical oncoproteins during transformation. For example, c-myc-driven transformation has been shown to require obligatory upregulation of BCL2 to overcome CD95-mediated apoptosis [27,28]. More recently, it has been shown that H-RAS-mediated transformation leads to non-apoptotic cell death through induction of autophagy with upregulation of the BH3-only protein NOXA and disruption of the MCL-1-beclin-1 complex [29]. Overexpression of prosurvival BCL2 family members may therefore reflect a required defence against constitutively generated ‘death signals’, which would otherwise induce apoptosis. Growing evidence suggests that constitutive activation of BH3 death signals probably induced by ‘insufficient oncogenes’ results in addiction to prosurvival BCL2 family proteins. It has been proposed that prosurvival BCL2 members, by forming constitutive complexes with activated BH3-only proteins, are primed for death [30]. Accordingly, BH3 domain peptides, capable of competitively inhibiting heterodimerisation of prosurvival BCL2 family members, can induce apoptosis by freeing BAX and BAK to oligomerise [31]. These BH3-only domains exhibit restricted specificity for prosurvival BCL2 members. For example, NOXA interacts only with MCL1 and A1, whereas BAD interacts with BCL2, BCLXL and BCLW. In contrast, BIM and PUMA are promiscuous in their prosurvival BCL2 protein interactions [32,33].

BH3 peptidomimetics are a new class of drug which were developed to mimic the interaction of BH3 domains. The prototype inhibitors navitoclax (ABT-263) [34] and ABT-737 were identified as BAD BH3 peptidomimetics using NMR-based screening, with high affinity for BCL2, BCLX and BCLW [35]. ABT-737 is a potent apoptosis sensitisier in preclinical models and can induce the regression of xenografts [36], which are primed for death such as small cell lung cancer. ABT-263 exhibits some evidence of efficacy in the clinical setting [37]; however, in common with other targeted agents, its efficacy may be limited in unselected populations. Identifying which cancers will respond to ABT-263 may be feasible. Because of the prosurvival BCL2 family restriction associated with ABT-263/737, there is robust preclinical evidence showing that MCL1 expression is a major resistance factor [38-42]. However, even in the presence of MCL1, ABT737 can exhibit preclinical activity in some cancers such as CLL, possibly due to constitutive occupancy due to priming for death. This possibility is reflected in gene expression analysis, which has shown a correlation between sensitivity and the expression of NOXA [43]. In preclinical models of lung cancer and mesothelioma, cell lines grown in three dimensions acquire apoptosis resistance [44] due to an altered expression of prosurvival BCL2 family members conferring sensitivity to ABT737, and this is associated with priming for death [45,46].

**Growth factor suppression of the core apoptosis machinery**

The mitochondrial apoptosis pathway is directly suppressed by survival signalling. BIM expression is regulated by phosphorylation in response to growth factors [16-19,47-50]. This occurs in a mitogen-activated protein kinase extracellular signal-regulated protein kinase 1/2 (MAPK-ERK)-dependent manner, leading to its polyubiquitination and destabilisation through proteasomal degradation [16]. The dissociating BH3-only protein BAD is regulated by serine phosphorylation, which causes its inactivation through sequestration to 14-3-3 [51].

The prosurvival BCL2 protein MCL-1 is also regulated by survival signals. The phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway, which signals downstream of receptor tyrosine kinases such as the epidermal growth factor receptor, stabilises MCL-1 via AKT signalling. Glycogen synthase kinase 3 (GSK-3) is a substrate for and is inhibited by AKT. Upon growth factor withdrawal, GSK-3 is de-repressed and phosphorylates MCL-1 at S159. This phosphorylation leads to ubiquinylation and degradation of MCL-1 via the proteasome. Collectively therefore, survival and proliferation are driven in parallel with suppression of apoptosis. Recently, it has emerged that dominant survival pathways exist in subsets of NSCLC, which, if targeted, can unleash BH3-only proteins and mediate effective apoptosis both at the bench and at the bedside.

**Dominant oncogenes as Achilles’ heels for unleashing BH3-only proteins**

Although there is overwhelming evidence now to implicate genomic instability and temporal acquisition of complex somatic gene alterations as causal factors during carcinogenesis, the identification of critical oncogenic drivers has had major implications for the development of therapeutics in NSCLC. The paradigm in this solid tumour mirrors the discovery and targeting of the BCR-ABL fusion protein, the dominant oncogenic driver in
chronic myelogenous leukaemia [52-55]. Prior to 2004, a therapeutic plateau had been reached in the management of NSCLC. Platinum-based therapy was considered the gold standard with no identifiable superior regimen, associated modest response rates and impact on overall survival [56] even with the addition of novel agents [57,58]. Clinical trials then focused on what was considered a homogeneous NSCLC population. Although this approach led to the approval of the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) erlotinib in second and third line NSCLC [59,60], this was not reflected in the negative, front-line pivotal phase III trials combining EGFR TKIs gefitinib and irressa with chemotherapy in unselected populations [61-64]. The seminal discovery that a subset of patients harbouring somatic mutations of the EGFR exhibits dramatic responses to the orally bioavailable receptor tyrosine kinase inhibitor gefitinib or erlotinib [65-67] spearheaded the shift in thinking about how to target NSCLC more effectively. The superior efficacy of oral TKI therapy for treating mutant EGFR about how to target NSCLC more effectively. The superior efficacy of oral TKI therapy for treating mutant EGFR mutations of the EGFR exhibits dramatic responses to the orally bioavailable receptor tyrosine kinase inhibitor gefitinib or erlotinib [65-67] spearheaded the shift in thinking about how to target NSCLC more effectively. The superior efficacy of oral TKI therapy for treating mutant EGFR

Somatic mutations of EGFR, of which the most common are deletions of exon 19 and nucleotide substitutions in exon 21 [70], have been shown to confer an increase in anti-apoptotic signalling capacity [71] and confer a cell survival advantage driving Darwinian selection. Mutation of EGFR causes an increase in enzyme activity and suppression of autoinhibitory function [72] and confers resistance to conventional cytotoxic drugs [71]. Inhibition leads to dramatic activation of apoptosis via the mitochondrial pathway [73] involving activation of BIM in common with the apoptosis that results following the inhibition of bcr-abl kinase in CML [55,74]; this is consistent with a common link between driver oncogene addiction, survival and suppression of critical death signals.

Upon inhibition of EGFR, there is a dramatic increase in the level of BIM through transcriptional and post-translational mechanisms (Figure 1). Activation of BIM requires the MAPK-ERK signalling pathway, but not JNK nor PI3K/AKT signalling. Silencing this BH3-only protein alone effectively rescues from EGFR inhibition in vitro and in vivo [75-78], ABT-737 confers sensitivity to erlotinib in EGFR mutant cells [57,77], implicating a potential for BH3 peptidomimetics as enhancers of EGFR TKIs efficacy in common with BIM-mediated apoptosis in bcr-abl-inhibited CML [55]. However, one caveat to this approach could be the rapidly emerging resistance and failure to activate critical death signals.

**BIM mediates apoptosis following ALK inhibitor in EML4-ALK-rearranged NSCLCs**

Within 3 years of the initial discovery of EGFRMUT addiction in NSCLC, a small proportion of NSCLCs (around 4 % to 6 %) was reported to harbour a small inversion within chromosome 2p, resulting in the fusion of the echinoderm microtubule-associated protein-like 4 (EML4) gene and the anaplastic lymphoma kinase (ALK) gene. As with EGFRMUT NSCLC, these NSCLCs are restricted to a specific histological subset of NSCLCs, namely non-squamous tumours. Consistent with EML4-ALK being a driver mutation, NSCLCs harbouring this gene rearrangement are mutually exclusive of EGFR or KRAS mutations. The mechanism underlying induction of apoptosis following inhibition of ALK involves the upregulation of BIM and downregulation of the inhibitor of apoptosis protein survivin [79]. EML4-ALK drives the ERK pathway, and this is involved in the suppression of BIM, in contrast to surviving which is regulated by the STAT pathway in response to EML4-ALK. Consequently, targeting EML4-ALK appears to induce apoptosis, in common with EGFR inhibition, through activation of intrinsic mitochondrial pathway. Based on the dramatic responses seen in the clinical setting [80], the ALK inhibitor crizotinib (PF-02341066) received approval in record time [80].

**Resistance to targeted therapies through emergent secondary mutations blocks apoptosis**

In the case of mutant EGFR, clinical efficacy is frequently limited by secondary mutation. The most commonly described mutation T790M [81,82], which can also be transmitted in the germline, is also a susceptibility allele [83,84]. T790M is present as a minor clone in NSCLC [85,86] and may be selected for during therapy [87]. This mutation has been shown to prevent the activation of BIM in response to gefitinib but can be overcome by an irreversible inhibitor of EGFR [76]. Other critical resistance mechanisms have been identified to be linked to the inhibition of EGFRMUT and include MET amplification [88-91], PTEN loss [92], HER2 kinase domain [93] or PIK3CA, and transformation to small cell lung cancer [87]. Selection pressure mediated by dramatic apoptosis induction drives the emergence of resistant clones capable of abrogating apoptosis signalling. Strategies to overcome this problem include structure-based modelling and discovery of *bona fide* T790M EGFR-targeting inhibitors [94]. Bypassing the EGFR altogether and target downstream pathways is another possibility. Survival signals from EGFR diverge through the PI3K/AKT and MAPK/ERK pathways. In EGFRMUT cell lines, inhibition of PI3K/ERK pathway leads to selective downregulation of MCL-1. In contrast, inhibition of the MAPK/ERK pathway leads to BIM upregulation. Combining PI3K and MEK inhibition may therefore present a strategy for overcoming resistance [95], as might targeting dependence on NF kappa B, which has been identified by RNAi screening in the context of EGFRMUT [96].
In common with resistance mechanisms identified for EGFR (and indeed bcr-abl), ALK-rearranged cancers have been reported to develop secondary mutations [97,98]. Rearranged ALK is relatively unstable and is dependent upon heatshock protein 90 to prevent its spontaneous ubiquitination and targeting to the proteasome for degradation. Inhibition of the chaperone, heatshock protein 90 (HSP90), leads to a downregulation of EML4 ALK and inhibition of signalling even in the presence of secondary mutations, suggesting that this might be a potential strategy for overcoming resistance [99-101]. As with EGFR, structure-based modelling has potential to identify small-molecule inhibitors capable of overcoming resistance due to gatekeeper mutation of ALK [102].

Personalising NSCLC therapy: towards identifying the full complement of oncogenic drivers in NSCLC

Beyond EGFR and ALK rearrangement, several additional somatic gene alterations linked to oncogenic drivers have been and are continued to be identified across the genomic landscape of NSCLC. As with the prototypical somatic mutations in EGFR and ALK, many of these may provide real opportunities for achieving similarly dramatic therapeutic outcomes. Sequencing efforts have identified mutations of KRAS, B-RAF, Her2/erb2, PIK3CA, LKB1 and MET amplification [103-105]. These commonly occurring mutations can be screened in a clinical-practice-based setting to enable personalised therapy to be most effectively delivered [106]. For many or all of these oncogenic drivers, inhibition of the dominant signalling pathway may trigger BIM-dependent apoptosis, as shown for mutant B-RAF [107-109], Her2 [110] and MET amplification. KRAS is frequently mutated in around a quarter of NSCLCs, and its activation causes lung cancer in mice [111]. It has been widely recognised as a hard target to drug [112]. However, this situation is changing. For example, C-RAF, but not B-RAF, has been identified through gene ablation studies, and TBK1 through RNAi screening, to be essential for KRAS-dependent NSCLC. Stability of mutant KRAS may depend on HSP90 [113-115], implicating this chaperone as a potential molecular target. Finally, novel specific inhibitors of KRAS have been identified. There have been rapid advances in genome sequencing technology, which now provide an opportunity to systematically interrogate somatic gene alterations in NSCLC [116,117]. The International Cancer Genome Consortium has been developed to do this in NSCLC and 49 other cancers [118]. With this technological development, it is likely that there will be the ability to comprehensively annotate the full complement of somatic gene alterations involved in driving NSCLC. The translational implications for personalised therapeutics and predictive biomarker development are clear based on the previous successes: that early identification of these oncogenic drivers will reflect vulnerabilities that will allow stratification of patients to receive the appropriate and effective apoptosis-inducing therapy.

Conclusions

The induction of apoptosis has long been cited as a key objective for achieving drug-induced tumour suppression;
however, evasion of this process has equally been known to limit the efficacy of conventional treatments. Now, in this era of genomics, it is clear that personalising drug therapy to most effectively target addiction to growth factor signalling pathways, by virtue of somatic mutations, provides a new exciting therapeutic opportunity, at least for initial disease control. However, combating resistance even in this era will present a major new challenge.

Competing interests
The authors declare that they have no competing interests.

Author details
1University of Manchester & Leicester University Hospitals, Hodgkin Building, Lancaster Road, PO Box 138, Leicester LE1 9HN, UK. 2Cancer Research UK. London Research Institute, Translational Cancer Therapeutics Laboratory, 44 Lincoln’s Inn Fields, London WC2A 3LY, UK. 3UCL Cancer Institute, Paul O’Gorman Building, Huntley St, London W1E, UK.

Authors’ contribution
DF and CW jointly co-authored, read and approved the final manuscript.

Received: 3 May 2012 Accepted: 3 May 2012
Published: 18 June 2012

References
1. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997, 91(4):479–489.
2. Cipolat S, Rudla T, Hartmann D, Costa V, Semeels L, Croes K, Metzger K, Frezza C, Annen W, D’Adamo L, Derko C, Dejaegere T, Pellegrini L, D’Hooge R, Scorrano L, De Strooper B: Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via APAF1-dependent cristae remodeling. Cell 2006, 126(1):163–175.
3. Du C, Fang M, Li Y, Li L, Wang X: Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000, 102(1):33–42.
4. Jooza N, Suisin SA, Daugas E, Stanford WL, Cho SK, Li CY, Saakia T, Elia AJ, Cheng HY, Ravagnan L, Ferri KF, Zamzami N, Wakeham A, Hakem R, Yoshida M, Kong YY, Mak TW, Zúñiga-Pflücker JC, Kroemer G, Penninger JM: Bcl-xL degradation via the proteasome pathway and regulates its proapoptotic function. Oncogene 2003, 22(4):6785–6793.
5. Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, Roth KA, Korsmeyer SJ: Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. Nature 1999, 400(6747):886–891.
6. Reynolds JE, Yang T, Qian L, Jenkinson JD, Zhou P, Eastman A, Craig RW: Mcl-1, a member of the Bcl-2 family, delays apoptosis induced by p53 overexpression in Chinese hamster ovary cells. Cancer Res 1994, 54(4):6348–6352.
7. Chen Y, Hibi M, Srinivasula SM, Wang X: Bim EL, a proapoptotic BH3 domain-only protein, promotes mitochondrial membrane permeabilization and cell death. J Cell Biol 1999, 146(6):891–898.
8. Certo M: Differential targeting of prosurvival Bcl-2 family members by their BH3-only ligands allows complementary apoptotic function. Nat Cell Biol 2009, 11(10):919–921.
9. Ren D, Tu HC, Kim H, Wang GX, Bean GR, Takeuchi O, Jeffers JR, Zambetti GP, Hsieh JJ, Cheng EH: BID, BIM, and PUMA are essential for activation of the BAK- and BAK-dependent cell death program. Science 2010, 330(6009):1390–1393.
10. Kim H, Tu HC, Ren D, Takeuchi O, Jeffers JR, Zambetti GP, Hsieh JJ, Cheng EH: Stepwise activation of BAX and BAK by BID, BIM, and PUMA initiates mitochondrial apoptosis. Mol Cell 2009, 36(3):487–499.
11. Ley R, Balmanno K, Hadfield K, Weston C, Cook SJ: Activation of the ERK1/2 signaling pathway promotes phosphorylation and proapoptosome-dependent degradation of the BH3-only protein, Bim. J Biol Chem 2003, 278(21):18811–18816.
12. Biwas SC, Greene LA: Nerve growth factor (NGF) down-regulates the Bcl-2 homology 3 (BH3) domain-only protein Bim and suppresses its proapoptotic activity by phosphorylation. J Biol Chem 2002, 277(51):49511–49516.
13. Ley R, Ewings K, Hadfield K, Howes B, Balmanno K, Cook SJ: Extracellular signal-regulated kinases 1/2 are serum-stimulated “Bim(EL) kinases” that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. J Biol Chem 2004, 279(10):8837–8847.
14. Fennell and Swanton Bcl-2 family members. Nature 2010, 463(7283):899–905.
15. Fennell and Swanton Bcl-2 family members. Nature 2010, 463(7283):899–905.
16. Ley R, Ewings K, Hadfield K, Howes B, Balmanno K, Cook SJ: Extracellular signal-regulated kinases 1/2 are serum-stimulated “Bim(EL) kinases” that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. J Biol Chem 2004, 279(10):8837–8847.
17. Luciano F, Jacquel A, Golosetti P, Herrant M, Cagnol S, Pages G, Aubergier P: Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the prosome pathway and regulates its proapoptotic function. Oncogene 2003, 22(4):6785–6793.
18. Ley R, Ewings K, Hadfield K, Howes B, Balmanno K, Cook SJ: Extracellular signal-regulated kinases 1/2 are serum-stimulated “Bim(EL) kinases” that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. J Biol Chem 2004, 279(10):8837–8847.
19. Ley R, Ewings K, Hadfield K, Howes B, Balmanno K, Cook SJ: Extracellular signal-regulated kinases 1/2 are serum-stimulated “Bim(EL) kinases” that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. J Biol Chem 2004, 279(10):8837–8847.
20. Ley R, Ewings K, Hadfield K, Howes B, Balmanno K, Cook SJ: Extracellular signal-regulated kinases 1/2 are serum-stimulated “Bim(EL) kinases” that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. J Biol Chem 2004, 279(10):8837–8847.
21. Ley R, Ewings K, Hadfield K, Howes B, Balmanno K, Cook SJ: Extracellular signal-regulated kinases 1/2 are serum-stimulated “Bim(EL) kinases” that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. J Biol Chem 2004, 279(10):8837–8847.
22. Ley R, Ewings K, Hadfield K, Howes B, Balmanno K, Cook SJ: Extracellular signal-regulated kinases 1/2 are serum-stimulated “Bim(EL) kinases” that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. J Biol Chem 2004, 279(10):8837–8847.
42. Yecies D, Carlson NE, Deng J, Letai A, Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Brunicardi FC, Driscoll DJ, Driessen J, Hinds S, Liu C, Rabbitts T, Reuter S, Rowley JD, Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Fennell D, Swanton C, Ribeiro De Oliveira M, Bonomi P, Gandara D, Gandhi L, Camidge DR, Jacks T, Weinberg RA: Taking the study of cancer cell survival to a new level. Nature 2007, 449:1064-1066.

39. Lin X, Morgan-Lappe S, Huang X, Liu L, Zaculla DM, Vernetti LA, Fesik SW, Shen Y: Serine phosphorylation of off-target siRNAs reveals an essential role of m-1 in resistance to the small-molecule Bcl-2/Bcl-XL inhibitor ABT-737. Oncogene 2007, 26(27):3972-3979.

40. van Delft MF, Wei AH, Mason KD, Van Der Groep J, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, Adams JM, Roberts AW, Huang DC. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 2006, 10(3):389-399.

41. Hauck P, Chao BH, Litz J, Krystal GW: ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 2008, 68(9):3421-3428.

42. Tse C, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Brunicardi FC, Driscoll DJ, Driessen J, Hinds S, Liu C, Rabbitts T, Reuter S, Rowley JD, Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Fennell D, Swanton C, Ribeiro De Oliveira M, Bonomi P, Gandara D, Gandhi L, Camidge DR, Jacks T, Weinberg RA: Taking the study of cancer cell survival to a new level. Nature 2007, 449:1064-1066.

43. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Marsh KC, Mitten MJ, Nimmer P, Roberts L, Tahir SK, Xiao Y, Yang X, Zang H, Fesik S, Rosenberg SH, Elmore SW. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 2008, 68(9):3421-3428.

44. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Brunicardi FC, Driscoll DJ, Driessen J, Hinds S, Liu C, Rabbitts T, Reuter S, Rowley JD, Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Fennell D, Swanton C, Ribeiro De Oliveira M, Bonomi P, Gandara D, Gandhi L, Camidge DR, Jacks T, Weinberg RA: Taking the study of cancer cell survival to a new level. Nature 2007, 449:1064-1066.

45. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Brunicardi FC, Driscoll DJ, Driessen J, Hinds S, Liu C, Rabbitts T, Reuter S, Rowley JD, Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Fennell D, Swanton C, Ribeiro De Oliveira M, Bonomi P, Gandara D, Gandhi L, Camidge DR, Jacks T, Weinberg RA: Taking the study of cancer cell survival to a new level. Nature 2007, 449:1064-1066.

46. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Marsh KC, Mitten MJ, Nimmer P, Roberts L, Tahir SK, Xiao Y, Yang X, Zang H, Fesik S, Rosenberg SH, Elmore SW. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 2008, 68(9):3421-3428.
smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib, Proc Natl Acad Sci USA 2004, 101(6):13306–13311.

68. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chow WYK, Ngan-Kwong Y, Jones EM, McDonald D, Szczesniak A, Bailey-Wilson JE, Amos CI, Pinney SM, Petersen GM, de Andrade M, Yang P, Wiest JS, Fain PR, Schwartz AG, Gazdar A, Gaba C, Rothschild H, Mandal D, Kuper T, Semencic D, Viswanath A, Govindan R, Minna J, Anderson MW, You M: EGFR-T790M is a rare lung cancer susceptibility allele with enhanced gefitinib and erlotinib resistances. Cancer Res 2007, 67(10):4665–4670.

69. Inukai M, Toyooka S, Ito S, Asano H, Ichihara S, Sol J, Suehisa H, Ouchi M, Aoe K, Aoe M, Kuura K, Shimizu N, Date H: Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small-cell lung cancer. Cancer Res 2006, 66(18):7854–7858.

70. Rosell R, Molina MA, Costa C, Simonetti S, Gimenez-Capitan A, Bertran-Alamillo J, Mayo C, Morán T, Mendez P, Cardenal F, Islas D, Procopio M, Cobos M, Insa A, Garcia-Campeiro R, Reguat N, Majem M, Viteri S, Canesqui E, Porta R, Massuti B, Queralt C, de Aguirre I, Sanchez JM, Sanchez-Ronco M, Mate JL, Ariza A, Benilchis S, Sanchez JJ, Bivaon TG, et al: Pretreatment EGFR T790M mutation and BCR1A1 mRNA expression in erlotinib-advanced non-small-cell lung cancer patients with EGFR mutations. Clin Cancer Res 2011, 17(5):1601–1606.

71. Sequist LV, Waltham MA, Dias-Santagata D, Dignamary S, Turke AB, Fidias P, Bergethon K, Shaw AT, Gettinger S, Cooper AK, Akihabandeh S, Heist RS, Tetalon J, Christensen JG, Wain JC, Lynch TJ, Vernovsky K, Mark EJ, Lanuti M, Iafarte AJ, Mino-Kenudson M, Engelman JA: Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011, 3(15):36.

72. Engelman JA, Zeijnallahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA: MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signalling. Science 2007, 316(5827):1039–1043.

73. Turke AB, Zeijnallahu K, Wu YL, Song Y, Dias-Santagata D, Lilfihits E, Toschi L, Rogers A, Mok T, Sequist L, Lindeman NI, Murphy C, Akihabandeh S, Yeap BY, Xiao Y, Capelletti M, Iafrette AJ, Lee C, Christensen JG, Engelman JA, Janne PA: Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 2010, 17(1):107–88.

74. Cappuzzo F, Janne PA, Skolman O, Finocchiaro G, Rossi E, Ligozzi C, Zucali PA, Terracciano L, Toschi L, Roncalli M, Destro A, Incarnato M, Alosio M, Santoro A, Varella-Garcia M: MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol 2009, 20(2):298–304.

75. Benedetetini E, Sholl LM, Peyton M, Reilly J, Ware C, Davis L, Vena N, Bailey D, Yeap BY, Fiorentino M, Ligon AH, Pan BS, Richon V, Minna JD, Gazdar AF, Draetta G, Bosari S, Chiriauc LR, Lutterbach B, Loda M: Met activation in non-small-cell lung cancer is associated with de novo resistance to EGFR inhibitors and the development of brain metastasis. Am J Pathol 2010, 177(3):415–423.

76. Sos ML, Koker M, Wei BA, Heynck S, Robinovitsky R, Rander T, Seiger JM, Weiss J, Fischer F, Fromont P, Michel K, Pfeifer M, Mernel G, Giraud L, Peyton M, Gazdar AF, Minna JD, Garraway LA, Santoro A, Garcia-Echeverria C, Wong KK, Engelman JA: Differential induction of apoptosis in HER2 and EGFR addicted cancers following induction of apoptosis by activation of Akt and EGFR. Cancer Cell 2009, 69(3):526–536.

77. Wang SE, Narararima A, Perez Torres M, Xiang B, Wu FY, Yang S, Carpenter G, Gazdar AF, Mathuwsimvuyi SK, Arteaga CL: HER2 kinase domain mutation results in impaired phosphorylation and activation of HER2 and EGFR and resistance to tyrosine kinase inhibitors. Cancer Cell 2005, 11(6):295–306.

78. Zhou W, Ercan D, Chen L, Yen CH, Li D, Capelletti M, Cortez AB, Chiriac L, Jance R, Pideria R, Engen JR, Wong JK, Eick MJ, Gray NS, Yang JJ, Park JS, Amant AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA: HER2 kinase domain mutations results in impaired phosphorylation and activation of HER2 and EGFR and resistance to tyrosine kinase inhibitors. Cancer Cell 2005, 11(6):295–306.

79. Wang SE, Narararima A, Perez Torres M, Xiang B, Wu FY, Yang S, Carpenter G, Gazdar AF, Mathuwsimvuyi SK, Arteaga CL: HER2 kinase domain mutation results in impaired phosphorylation and activation of HER2 and EGFR and resistance to tyrosine kinase inhibitors. Cancer Cell 2005, 11(6):295–306.

80. Fennell and Swanton The EPMA Journal 2012, 3:6 Page 7 of 8 http://www.epmajournal.com/content/3/1/6

81. Vickers H, Sato M, James M, Wang D, Wang Y, Wang M, Jia D, Liu Y, Bailey-Wilson JE, Amos CI, Pinney SM, Petersen GM, de Andrade M, Yang P, Wiest JS, Fain PR, Schwartz AG, Gazdar A, Gaba C, Rothschild H, Mandal D, Kuper T, Semencic D, Viswanath A, Govindan R, Minna J, Anderson MW, You M: EGFR-T790M is a rare lung cancer susceptibility allele with enhanced gefitinib and erlotinib resistances. Cancer Res 2007, 67(10):4665–4670.

82. Bell DW, Gore I, Okimoto RA, Godin-Heymann N, Sordella R, Mullolly R, Sharma SV, Brannigan BW, Mohapatra G, Settleman J, Haber DA: Inherited susceptibility to lung cancer may be associated with the TP53D mutation resistance mechanism in lung cancer. Nat Genet 2005, 37(12):1315–1316.
