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Maximising the potential of AKT inhibitors as anti-cancer treatments

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Abstract:

PI3K/AKT signalling is commonly disrupted in human cancers, with AKT being a central component of the pathway, influencing multiple processes that are directly involved in tumourigenesis. Targeting AKT is therefore a highly attractive anti-cancer strategy with multiple AKT inhibitors now in various stages of clinical development. In this review, we summarise the role and regulation of AKT signalling in normal cellular physiology. We highlight the mechanisms by which AKT signalling can be hyperactivated in cancers and discuss the past, present and future clinical strategies for AKT inhibition in oncology.

Keywords: AKT; PKB; PI3K/AKT signalling; AKT inhibitors; cancer

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Abbreviations: AE, adverse event; CRPC, castrate resistant prostate cancer; DLT, dose limiting toxicity; DNA-PK, DNA-dependent protein kinase; DSB, double strand break; GSK3β, glycogen synthase kinase beta; INPP4B, polyphosphate 4-phosphatase type II; MTD, maximum tolerated dose; OS, overall survival; PD, pharmacodynamics; PDK1, 3-phosphoinositide-dependent kinase-1; PFS, progression free survival; PH domain, plektrin homology domain; PHLPP, PH domain and leucine rich repeat protein phosphatase 1; PI3K, phosphatidylinositol 3-kinases; PIP3, phosphatidylinositol-3,4,5-trisphosphate

PK, pharmacokinetics; PP2A, protein phosphatase 2A; PR, partial response; PTEN, phosphatase and tensin homolog; RP2D, recommended phase II dose; RTKs, receptor tyrosine kinases.

1. Introduction

The AGC kinases, named after the protein A, G and C kinases, are an evolutionarily conserved group of proteins that share a hydrophobic motif at the c-terminus of their catalytic core. This motif, composed of F-X-X-F/Y-S/T-Y/F, is known as the PIF-pocket and regulates catalytic activity (Arencibia et al., 2013; Manning and Cantley, 2007). The AGC kinase family comprises 14 family members, of which AKT (also known as PKB; protein kinase B) is a key member.

There are three AKT isoforms, transcribed from separate genes, which share three highly conserved domains: a central catalytic domain and two regulatory domains: a lipid-binding N-terminal PH (plektrin homology) domain, and the hydrophobic motif (**Figure 1**). The PH domain contains a lipid-binding module that promotes the interaction of AKT with the plasma membrane, an important step in AKT activation. The hydrophobic motif contains an important docking site for the activating kinase PDK1 (3-phosphoinositide-dependent kinase-1) and also provides allosteric regulation of catalytic activity (Scheid & Woodgett, 2003; **Figure 1**). There is approximately 80% sequence homology between the isoforms with most variability occurring in the linker region between the PH and catalytic domains (Brodbeck et al., 1999; Cheng et al., 1992; Hanada et al., 2004; Jones et al., 1991).

2. AKT activation

Mechanisms of AKT activation have been reviewed previously (Liao and Hung, 2010; Scheid and Woodgett, 2003), but essentially, AKT activity is regulated downstream of receptor tyrosine kinases (RTKs), such as those within the EGF

(epidermal growth factor), Insulin, PDGF (platelet derived growth factor), FGF (fibroblast growth factor) and VEGF (vascular endothelial growth factor) families. RTKs activate class I phosphatidylinositol 3-kinases (PI3K), either directly, or in conjunction with adaptor proteins such as IRS-1/2 (insulin receptor substrate -1/2; **Figure 2**). The PI3Ks phosphorylate phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3). AKT binding to PIP3 at the plasma membrane induces a conformational change that results in phosphorylation of AKT, predominantly on two highly conserved residues, Thr^{308} and Ser^{473} leading to AKT activation (**Figure 1**). Phosphorylation of Thr^{308} in the activation T-loop of the catalytic domain by PDK1, results in a conformational change that enhances substrate affinity and promotes AKT kinase activity (Alessi et al., 1997). Phosphorylation of Ser^{473} within the PIF pocket of AKT by mTORC2 (mammalian target of rapamycin complex 2) is thought to promote AKT activity by increasing the affinity of AKT to PDK1 (Sarbassov et al., 2005). In fact, multiple different kinases for Ser^{473} have been described in the literature and it's likely that mechanisms determining full activation of AKT are context dependent. It has been accepted, for example, that following DNA damage, the PI3K-like kinase (PIKK) DNA-PK (DNA-dependent protein kinase) is responsible for AKT Ser^{473} phosphorylation and that AKT activation prevents apoptosis following ionizing radiation (Bozulic et al., 2008). Multiple other phosphorylation sites on AKT have been described, although the physiological importance of these is not yet fully understood (Risso et al., 2015) and mechanisms of constitutative activation of AKT signalling in cancer are discussed further below.

Important negative regulators of the PI3K/AKT signalling pathway include the tumour suppressor genes and phosphatases PTEN (phosphatase and tensin homolog), PP2A (protein phosphatase 2A) and PHLPP (PH domain and leucine rich repeat protein phosphatase 1; Gao et al. 2005), which dephosphorylate PIP3, AKT pThr^{308} and AKT pSer^{473} respectively (Toker & Marmiroli, 2014; **Figure 2**). PTEN hydrolyses the 3′- phosphate on PIP3 to terminate PI3K signalling. The SH2 domain-containing inositol phosphatases (SHIP-1/2) are able to hydrolyse the 5′-phosphate on PIP3 to generate PI(3,4)P2, the function of which is not clear, although some studies suggest that like PIP3, PI(3,4)P2 is able to facilitate PDK1 activation of AKT (Gewinner et al., 2009). Recently, a fourth putative tumour suppressor of the PI3K/AKT pathway has been described namely, polyphosphate 4-phosphatase type II (INPP4B; Gewinner et al. 2009). This is able to de-phosphorylate PI(3,4)P2 to PI(3)P *in vitro*, resulting in attenuation of AKT signalling (Gewinner et al., 2009).

Of all the tumour suppressors, PHLPP in particular is a key regulator of AKT activity and it's ability to fine-tune the PI3K/AKT pathway is discussed in greater detail below.

3. Downstream signalling of AKT

A consensus phosphorylation motif has been described for AKT substrates: R-X-R-X-X-S/T-B where X represents any amino acid and B represents bulky hydrophobic residues (Alessi et al., 1996). Numerous AKT substrates have been published in the literature, with several being extensively validated that impact on multiple cellular processes including: cell growth, proliferation and survival,

cellular metabolism, glucose uptake and angiogenesis (**Table 1**; Manning & Cantley 2007). Importantly there is great overlap and crosstalk between substrate functions, with many substrates being regulated directly by AKT phosphorylation, as well as indirectly, through AKT-mediated phosphorylation of transcription factors. AKT signalling therefore plays a central role in multiple pathways involved in tumourigenesis and hyperactivation of the PI3K/AKT pathway is a common occurrence in cancer (Liu et al., 2009).

4. AKT in normal physiology

As well as playing a central role in cancer, AKT signalling is also essential for normal cellular physiology. In adults, AKT1 expression is ubiquitous, expression of AKT2 is elevated in insulin-responsive tissues and expression of AKT3, whilst found at low levels in all tissues, is more highly expressed in the brain and endocrine tissues (http://www.proteinatlas.org/ ;Uhlen et al. 2010; Uhlén et al. 2015).

AKT plays an essential role in glucose homeostasis and a deleterious effect of pharmacological inhibition of AKT is hyperglycaemia and hyperinsulinaemia which correlates with peak plasma level drug concentrations (Crouthamel et al., 2009). The AKT substrate GSK3β (glycogen synthase kinase beta) phosphorylates and inhibits glycogen synthase, preventing glycogen synthesis. AKT activation results in phosphorylation and inhibition of GSK3β relieving the inhibitory effect on glycogen synthase, resulting in glycogen synthesis. As well as stimulating glycogen synthesis, AKT also promotes cellular uptake of glucose by prompting translocation of the glucose transporter GLUT4 to the plasma

membrane. The AKT substrate directly involved in this process has not been well-defined and several candidate proteins are described in the literature (Manning and Cantley, 2007).

AKT function (especially AKT1 and AKT2) is also important for regulating cardiac growth, contractile function and coronary angiogenesis. It is essential for maintaining normal endothelial function and endothelial nitric oxide synthesis, with increased atherosclerosis and peripheral vascular disease demonstrated in *Akt1* knockout mice (Hers et al., 2011). AKT also has a pro-thrombotic effect, promoting platelet activation and aggregation, highlighting the need for tight control of AKT signalling (Hers et al., 2011). As well as its role in the cardiovascular and endocrine systems, AKT has also been shown to be neuro-protective and plays a role in synaptic transmission between neurons (Hers et al., 2011). Taken together therefore, given the wide-ranging effects of AKT signalling in normal human physiology, there is vast scope for significant on-target as well as off-target effects of AKT inhibitors in the clinic.

5. Selectivity within the AKT pathway

The phenotypes of *Akt* null mice have suggested non-redundant roles for the three isoforms, with *Akt1* knockout being embryonic lethal, *Akt2* null mice developing insulin-resistant diabetes and *Akt3* knock-out resulting in microcephaly (Toker and Marmiroli, 2014). In keeping with this, findings from pre-clinical studies, are consistent with AKT isoforms having partially overlapping, but distinct functions in cancer cells (Clark and Toker, 2014; Toker and Marmiroli, 2014). Phosphoproteomic screens have demonstrated unique

and common substrates for each of the AKT isoforms and functional studies have started to highlight the physiological relevance of these findings, for example during RNA processing in lung cancer (Sanidas et al., 2014). Isoform-mediated specificity has been best studied in breast cancer models however, where differential functions of AKT1, -2 and -3 in both inhibiting and promoting cell migration and proliferation have been demonstrated (Clark and Toker, 2014).

AKT1 has been shown to be important for G1-S checkpoint transition and proliferation, whereas AKT2 regulates cell-cycle exit through its interaction with p21 (Héron-Milhavet et al., 2006). In a recent study in triple negative breast cancer, AKT3, rather than AKT1 activity was most important for cellular proliferation (Chin et al., 2014a), suggesting a degree of context specificity for these findings. Interestingly, it has been observed that although activating mutations in AKT1 result in accelerated tumourigenesis, metastatic dissemination is decreased due to inhibitory effects of AKT1 on tumour cell invasion and migration (Chin and Toker, 2010; Hutchinson et al., 2004). By contrast, AKT2 activity promotes tumour invasion and metastatic dissemination in mouse breast cancer models (Dillon et al., 2009; Maroulakou et al., 2007). In addition, it has recently been shown that INPP4B inhibition of AKT2 is important for metastatic spread of follicular thyroid cancer (Chew et al., 2015). A number of different AKT1/2 substrates have been described that may account for the differential phenotype of cellular migration, which play a role in epithelial-mesenchymal transition (EMT), including the NFAT transcription factor (Yoeli-Lerner et al., 2005), palladin (Chin and Toker, 2010), the integrin β subunit (Arboleda et al., 2003) and the miR-200 family of micro-RNAs (Iliopoulos et al.,

2009). There are likely to be many more (Clark and Toker, 2014) and the relative importance of each of these is likely to depend on tumour subtype, as well as perhaps tumour stage and genetic background. An example of this is that PTEN deficient tumour cells have recently been shown to be dependent, specifically on AKT2 for survival (Chin et al., 2014b). This was shown to be true for prostate cancer, breast cancer and glioblastoma PTEN deficient cells lines and was at least in part, mediated through AKT2 dependent up regulation p21 (Chin et al., 2014b).

Several lines of evidence suggest that AKT does not need to be fully activated in order to phosphorylate all substrates. This suggests that different AKT substrates and therefore different cellular functions of AKT depend on varying levels of AKT activation. For example AKT Ser^{473} phosphorylation appears to be dispensable for phosphorylation of the AKT targets TSC2 and GSK3, as well as the TORC1 effectors, S6K and 4E-BP1, but not for FOXO1/3a (Jacinto et al., 2006). Interestingly, in patient non-small cell lung cancer samples, AKT Thr^{308} correlates with phosphorylation of AKT substrates PRAS40, TSC2 and TBC1D4, whereas AKT Ser^{473} phosphorylation does not (Vincent et al., 2011). This raises an important point clinically, when trying to determine whether or not a tumour might be dependent on AKT signalling for survival, suggesting the AKT Thr^{308} is a more suitable biomarker of AKT activation than AKT Ser^{473}.

Relative levels of the negative regulators of the PI3K/AKT pathway will also ultimately affect amplitude of AKT activation. For example, the PHLPP isoforms -1 and -2 have been shown to preferentially hydrolyse Thr^{308} on AKT2 and AKT3 respectively (Brognard et al., 2007). Cellular location and post-translational

modification of AKT isoforms also contribute to specificity within the pathway (Clark and Toker, 2014) and untangling all of these factors within the context of cancer is proving to be a considerable challenge.

6. Feedback loops within the PI3K/AKT signalling pathway

The PI3K/AKT signalling pathway is far from linear with multiple negative feedback loops fine-tuning overall activation levels (Carracedo and Pandolfi, 2008). The activity of the S6 kinases (S6K1/2), which are phosphorylation targets of mTORC1 play a pivotal role in negatively regulating AKT activity. IRS-1 (insulin receptor substrate-1) is an adaptor protein required for binding of the class IA PI3Ks with insulin and insulin growth factor (IGF) receptors. Phosphorylation of IRS-1 by S6K1 inhibits its interaction with insulin and IGF receptors and also promotes proteasomal degredation of IRS-1 (Harrington et al., 2005), thereby dampening PI3K signalling and reducing AKT activity. In addition to its effects on AKT activity via upstream inhibition of PI3K signalling, the mTORC1 complex also inhibits AKT by up-regulating PHLPP1 translation (Liu et al., 2011), the phosphatase responsible for dephosphorylating AKT pSer^{473} (**Figure 3**).

AKT activity is also self-limiting through its substrate GSK3β ; the kinase activity of which is negatively regulated by AKT phosphorylation (**Figure 3**; Li et al. 2009). GSK3β activation results in phosphorylation and subsequent ubiquitin mediated degredation of PHLPP (Li et al., 2009). AKT activation therefore results

in GSK3β □□□□□□□□□□□□□□ and high levels of PHLPP, which in turn, puts the brakes on the AKT signalling pathway.

Opposing the above mechanisms that limit the activity of the PI3K/AKT pathway, AKT inhibition results in up-regulation of RTKs, in part through loss of mTORC1 signalling, but also due to FOXO-dependent transcriptional increases in RTK expression (Chandarlapaty et al., 2011). This up-regulation of upstream pathway components in part will compensate for pharmacological inhibition of more downstream players and has been shown to contribute to resistance to AKT inhibitors (Garrett et al., 2011b).

In the context of normal growth factor signalling, it's easy to appreciate why it's advantageous that in 'times of plenty', chronic activation of mTORC1, leads to attenuation of AKT signalling (Manning, 2004). As discussed below, multiple components of the PI3K pathway are mutated or deleted in cancer cells and the level of the aberration within the pathway will have profoundly different effects on AKT activity depending on whether or not the negative regulatory feedback loops are intact. Equally, chronic vs acute inhibition of the pathway may result in very different signalling patterns. This makes targeting the pathway a significant challenge in oncology and also has implications for dosing schedules of AKT inhibitors.

7. AKT activation in cancer

The PI3K/AKT pathway is the most commonly disrupted signalling pathway in human cancers (Millis et al., 2016). PI3K/AKT pathway aberrations have been

identified in up to 40% of all tumour types, with PTEN loss by immunohistochemistry occurring most frequently (30%), followed by mutations in PIK3CA (13%), PTEN (6%) and AKT (1%; Millis et al. 2016). Whilst activating AKT mutations are relatively uncommon, AKT activation can arise as a result of several other genomic aberrations.

AKT amplification

In keeping with its role in promoting EMT transition and promoting metastatic disease, *AKT2* is frequently amplified in cancer. Analysis of the cBioPortal database for cancer genomics demonstrates that *AKT2* amplification is found in up to 16% of pancreatic cancers, 16% of uterine cancers, 13% of breast cancers and 5-10% of ovarian, lung and bladder cancers (**Figure 4**A). Early work also demonstrated that siRNA depletion of *AKT2* resulted in reduced cell growth and invasiveness only in cells overexpressing *AKT2*, consolidating its role in tumourigenesis (Cheng et al., 1996). By contrast, *AKT1* amplification is found less frequently across tumour types, occurring in 20% of neuroendocrine prostate cancers, 10% of pancreatic cancers and 3-5% of breast and serous ovarian cancers. To date, high levels of *AKT3* amplification have been identified most often in breast cancers and as discussed, AKT3 may be the predominant isoform responsible for growth and proliferation of triple negative breast cancers (Chin et al., 2014a). Interestingly, high levels of *AKT3* expression have also been demonstrated in over 25% of neuroendocrine prostate cancers (**Figure 4**A).

AKT mutations

In contrast to amplifications, mutations in *AKT2* are found much less frequently and to our knowledge, no commonly occurring functionally activating mutations

in *AKT2* have been described. *AKT1* is the most frequently mutated isoform in tumours and this occurs at low frequency (approximately 1% of all cancers; **Figure 4**B; www.cbioportal.org; Cerami et al. 2012; Gao et al. 2013). Several functionally transforming mutations of *AKT1* have been described however (Yi et al., 2013), the predominant mutation is *AKT1*^{E17K} (Carpten et al., 2007). This glutamic acid to lysine substitution within the lipid-binding pocket of AKT1 results in pathological localisation of AKT1^{E17K} to the plasma membrane and subsequent constitutative activation of AKT1 signalling (Carpten et al., 2007). In contrast to physiological AKT1 activation that occurs upon binding of AKT1 to PIP3 at the plasma membrane, the *E17K* mutation in AKT1 alters PIP specificity, resulting in high affinity binding to highly abundant PIP2 within the plasma membrane (Landgraf et al., 2008). AKT1^{E17K} therefore utilises both PIP3 as well as PIP2 for its activation and as a result, remains constitutively active at the cell membrane (Landgraf et al., 2008).

Both allosteric and catalytic inhibitors of AKT are capable of suppressing AKT1^{E17K} activity and subsequently inhibiting growth of tumours harbouring AKT1^{E17K} in breast cancer models (Davies et al., 2015). *AKT1*^{E17K} mutations are found most frequently in breast, bladder, cervix and prostate cancers (**Figure 4**B). Inhibition of AKT1^{E17K} tumours in a clinical setting has also now been demonstrated and will be discussed in more detail below.

Alterations of other proteins of the PI3K/AKT signalling pathway

Over-expression or activating mutations of proteins upstream of AKT in the PI3K/AKT pathway result in AKT activation, as do loss of the tumour suppressor

proteins: PTEN, PHLPP, PP2A and INPP4B (Liu et al., 2009). Germline mutations in PTEN result in PTEN hamartoma syndromes, of which Cowden's syndrome is the best described; characterised by the development of multiple hamartomas and with increased risk of carcinomas of the breast, thyroid, endometrium and kidney. Somatic PTEN loss is most commonly identified in endometrial carcinoma, prostate carcinoma and glioblastoma and can arise due to mutations, insertions and deletions throughout the *PTEN* gene (Hollander et al., 2011).

8. The clinical development of AKT inhibitors

Inhibiting PI3K/AKT signalling has long been an attractive therapeutic approach in oncology. Numerous compounds that inhibit the pathway at all levels are now in clinical development, including those targeting AKT (Yap et al., 2008). AKT inhibitors fall predominantly into two separate classes. Allosteric inhibitors of the AKT PH-domain prevent localisation of AKT to the plasma membrane, thereby blocking AKT phosphorylation and activation. The second class of inhibitors comprise ATP- competitive inhibitors of AKT, of which there are several in the early stages of clinical development (**Table 2**).

Monotherapy with AKT inhibitors

The AKT inhibitors that have entered clinical evaluation have been discussed in alphabetical order.

AFURESERTIB (GSK2110183) AND GSK2141795

Afuresertib (GSK2110183) and GSK2141795 (GlaxoSmithKline) are both orally bioavailable, ATP-competitive inhibitors of AKT1-3 which have anti-tumour effects *in vitro* and *in vivo* as monotherapy and in combination with the MEK inhibitor trametinib (GSK1120212; Dumble et al. 2014). In a phase I trial of

afuresertib in patients with haematological malignancies, DLTs consisted of deranged liver function tests and the MTD was established as 125mg daily. The most frequent (>10% of patients) treatment-related adverse events (AEs) were nausea (23.3%), diarrhoea (20.5%), dyspepsia (19.2%), fatigue (16.4%), gastrointestinal reflux disease (15.1%), and anorexia (13.7%). Promising anti-tumour activity was demonstrated in patients with multiple myeloma (Spencer et al., 2014). Preliminary data from a phase I study of GSK2141795 demonstrated an MTD of 75mg daily with DLTs of G3 hyperglycaemia, G4 hypoglycaemia, G3 hypoglycaemia and G2 stomatitis with promising tumour responses seen in patients with an activated PI3K/AKT pathway (Burris et al., 2011).

ARQ 092 and ARQ 751

ARQ 092 and ARQ 751 (ArQule) are both highly potent allosteric inhibitors of wild type and AKT^{E17K}, with ARQ 751 having enhanced PK properties and potency (**Table 2**; Lapierre et al., 2016; Yu et al., 2015). Structural studies have shown that allosteric inhibitors bind to a region between the PH-domain and kinase domain on AKT (Wu et al., 2010). This stabilises the PH-domain in a 'closed' conformation which blocks ATP binding to the kinase site, obstructing the phospholipid binding site, and thereby inhibiting AKT recruitment to the plasma membrane (Wu et al., 2010).

Preliminary data from a phase Ia dose escalation trial of ARQ 092 has demonstrated common drug-related adverse events (≥10%) of hyperglycaemia (30%), macular-papular rash (28%), nausea (20%), diarrhoea (19%), pruritis (16%), fatigue (15%_ and decreased appetite (14%; Tolcher et al., 2016). Early

evidence of anti-tumour activity has been shown with two durable partial responses in patients with activating *PIK3CA* mutations (Tolcher et al., 2016) and promising clinical activity in patients with AKT^{E17K} mutations has also now been demonstrated in the phase Ib expansion cohort (Abbadessa et al., 2015).

Interestingly, AKT inhibition using ARQ 092 is also being explored for the treatment of Proteus syndrome (Lindhurst et al., 2015). Rarely a somatic $AKT1^{E17K}$ mutation can arise during development that results in Proteus syndrome; a progressive, mosaic, segmental overgrowth syndrome that can affect any part of the body (Biesecker, 2001). The severity and extent of tissue involvement is unique to each patient, with bone, fat, skin and connective tissue being most commonly involved (Biesecker, 2001). Affected patients have an increased risk of malignancy, including mesothelioma and breast cancer, and are at high risk of life-threatening venous thromboembolism (Biesecker, 2001). In preclinical studies, ARQ 092 has been shown to suppress AKT signalling in cell lines and tissues from patients with Proteus syndrome (Lindhurst et al., 2015). In contrast to the treatment of AKT-mutated cancers, the aim of such treatments is to restore physiological levels of AKT signalling in cells rather cytotoxicity. A phase I trial of ARQ 092 is currently recruiting children and adults with Proteus syndrome (NCT02594215) and preliminary data suggest that doses of ARQ 092 that are significantly lower than the MTD for cancer will be sufficient to treat patients with Proteus syndrome (Lindhurst et al., 2015).

AZD5363

AZD5363 (Astrazeneca) is a potent competitive kinase domain inhibitor of AKT1-3, which in pre-clinical studies has been shown to inhibit phosphorylation of AKT substrates (PRAS40, GSK3β and S6) and tumour growth in xenograft models (Davies et al., 2012). The presence of an activating *PIK3CA* mutation, $AKT1^{E17K}$ mutation or PTEN loss, in the absence of a significant *KRAS* (or other activating mutation in an alternative pro-survival pathway) significantly correlated with sensitivity to AZD5363 across a range of tumour cell lines (Davies et al., 2012, 2015). Interestingly, continuous low doses of AZD5363 had a predominantly anti-proliferative effect on cells when given as monotherapy and significant levels of apoptosis were only observed using an intermittent high dose schedule, suggesting that intermittent high dose schedules might be a more effective clinical strategy for the treatment of AKT dependent tumours (Davies et al., 2012).

A phase I trial of AZD5363 monotherapy in patients with solid tumours has investigated the toxicity, pharmacokinetics (PK) and PD of intermittent vs continuous dosing (Banerji et al., 2015). Three schedules were explored: continuous dosing (7/7), four days a week (4/7) and two days a week (2/7) of which the MTDs were 320mg BID, 480mg BID and 640mg BID respectively. The DLTs were rash and diarrhoea for 7/7, and hyperglycaemia for 2/7 with no DLTs identified for 4/7. As expected following AKT inhibition, the most common causally related adverse events ≥ CTC Grade 3 were hyperglycaemia (20%), diarrhoea (10%), rash (10%), nausea (3%) and fatigue (1%). PK profiles at 480mg BID (4/7) resulted in higher steady state levels of AZD5363 compared to

continuous dosing at lower drug concentrations and were consistent with exposures that resulted in tumour regression in preclinical models. Pre- and post-treatment biopsies confirmed target engagement in tumour tissue, with an increase in pAKT and reductions in pGSK3β and pPRAS40. Based on PK, PD and toxicity data, 480mg BID (4/7) was determined as the RP2D of AZD5363. Early data from an expansion cohort at this dose in patients with breast or gynaecological tumours harbouring *PIK3CA* mutations demonstrated anti-tumour activity in approximately 50% of patients with *PIK3CA* mutations although the magnitude of response in most patients was not deemed sufficient to go forward with monotherapy in a *PIK3CA* mutant population. Preliminary expansion data at the RP2D in patients with AKT1 ^{E17K} mutations (without concomitant RAS/RAF mutations) has shown very promising anti-tumour activity. At the time of reporting, 13/17 (76%) ER+ve breast, 8/10 (80%) gynaecological and 10/12 (83%) other solid tumour patients demonstrated target lesion responses with 4 (24%), 2 (20%) and 3 (25%) partial responses (PRs) respectively (Hyman et al., 2015). A phase I trial of AZD5363 monotherapy in Japanese patients with solid tumours has also determined 480mg BID (4/7) to be the RP2D and demonstrated 2 PRs in patients with *AKT1*^{E17K} mutations (Tamura et al., 2016). *AKT1*^{E17K} is therefore a promising predictive biomarker for response to AZD5363 monotherapy across a range of tumour types and the full results of this trial are awaited with interest.

IPATASERTIB (GDC-0068)

Optimisation of ATP-competitive AKT inhibitors using structure based design led to the discovery of ipatasertib (GDC-0068; Genentech), a potent and highly selective inhibitor of AKT1-3 that causes dose-dependent AKT signalling

inhibition in cancer cells and xenograft tumour models (Blake et al., 2012; Lin et al., 2013). In pre-clinical models, markers of hyperactive AKT signalling correlated with sensitivity to ipatasertib, including high basal phospho-AKT levels, PTEN loss and *PIK3CA* mutations (Lin et al., 2013). Preliminary data from a phase I study established the MTD as 600mg QD of ipatasertib, with two DLTs of fatigue at higher dose levels and a low incidence of clinically significant hyperglycaemia (Yan et al., 2011). A dose- and time-dependent PD response was demonstrated through inhibition of pGSKβ in platelet rich plasma in doses above 200mg (Yan et al., 2011, 2013). As with AZD5363, the clinical focus for ipatasertib is now on combination studies (see below).

MK-2206

MK-2206 (Merck; MSD) is the most clinically advanced allosteric inhibitor of AKT, although other allosteric inhibitors of AKT include ARQ092 (ArQule; Lapierre et al., 2016), ARQ751 (ArQule; Yu et al., 2015) and BAY1125976 (Bayer; Politz et al., 2016; **Table 2**).

MK-2206 is a predominantly AKT1/2 inhibitor with reduced potency against AKT3 (Yan, 2009) and has been shown to have single agent activity against a range of cell lines harbouring RTK activation, PTEN loss/mutation and/or *AKT2* amplification (Lu et al., 2009). A phase I trial of MK-2206 monotherapy in patients with solid tumours demonstrated a MTD of 60mg on alternate days with DLTs of skin rash and stomatitis (Yap et al., 2011). Drug related AEs included rash (51.5%), nausea (36.4%), pruritus (24.2%), hyperglycaemia (21.2%), and diarrhoea (21.2%). MK-2206 had a long half life of 40-100 hrs and plasma concentrations declined in a biphasic manner (Yap et al., 2011). On-target PD

tumour effects were also demonstrated (decrease in AKT pSer^{473}). Similarly to other AKT inhibitors, intermittent dosing schedules seem to be better tolerated and equally, if not more effective. Compared to the alternate day schedule, once weekly dosing of MK-2206 was equally as well tolerated up to a recommended phase II dose (RP2D) of 200mg with pulsatile inhibition of AKT demonstrated in tumour biopsies (Yap et al., 2014). Given the limited anti-tumour activity seen with MK-2206 monotherapy in phase II trials across a range of tumour types, attention has focused on combination trials (**Table 2** and see below).

PERIFOSINE

The first AKT inhibitor to enter clinical development was in fact an alkylphospholipid, perifosine (D-21266; KRX0401; Aeterna Zentaris). Alkylphospholipids accumulate in the plasma membrane and inhibit AKT activation by disrupting the interaction between AKT and the phospholipids (Kondapaka et al., 2003). As such, these compounds are relatively non-selective (Hideshima et al., 2006). In clinical studies, doses of oral perifosine are limited by gastro-intestinal (GI) toxicity when administered daily and several studies have tested different loading and maintenance schedules in order to achieve administration of biologically active doses without intolerable toxicity (Crul et al., 2002; Figg et al., 2014; Van Ummersen et al., 2004; Unger et al., 2010). Multiple Phase II trials have been conducted using a loading dose/maintenance dose schedule (**Table 2**) and of those reported, perifosine monotherapy has shown modest anti-tumour activity in soft tissue sarcomas and renal cell carcinomas (Bailey et al., 2006; Cho et al., 2012; Knowling et al., 2006).

Combination strategies with AKT inhibitors

Activation of the PI3K/AKT pathway has been implicated in resistance to a number of anti-cancer agents, providing a strong rationale for the clinical exploration of AKT inhibitor combinations.

CHEMOTHERAPY

AKT activation has been linked to both chemotherapy and radiation resistance. DNA-PK is one of the apical kinases involved in DNA double strand break (DSB) repair and is essential for classical non-homologous end-joining, the predominant repair pathway of DSBs in human cells (Jette and Lees-Miller, 2015). Following the generation of DNA DSBs, AKT is activated in a DNA-PK – dependent manner and promotes survival through anti-apoptotic mechanisms that are not well described (Bozulic et al., 2008; Brown et al., 2015; Wendel et al., 2004). In pre-clinical studies, AKT inhibition has been shown to hypersensitise cells to a number of chemotherapeutic agents (Davies et al., 2012; Hirai et al., 2010; VanderWeele et al., 2004).

Continuous low dose perifosine has been combined safely with a number of chemotherapies (**Table 2**). A randomised phase II trial of capecitabine +/- perifosine as second or third line treatment for metastatic colorectal cancer showed a significant OS benefit of the combination (17.7 v 7.6 months; P = .0052; Bendell et al. 2011), however these results were not validated in the phase III setting (Bendell et al., 2012). Interestingly, the presence of a *PIK3CA* mutation or PTEN loss did not predict for response to the capecitabine/perifosine combination which may in part be due to the high levels of *KRAS* mutations in colorectal cancers (Eng et al., 2014).

AZD5363 significantly enhances the activity of docetaxel in xenograft studies (Davies et al., 2012). Preliminary data from a phase I trial of AZD5363 in combination with docetaxel in patients with metastatic castrate resistant prostate cancer (mCRPC) has determined a RP2D of 320 mg BID 4 days on/3 days off in combination with full dose docetaxel (75mg/m^2 q3w) and prednisolone (5mg BID D1-21; Crabb et al. 2016). DLTs were G3 rash and G3 diarrhoea. The randomised phase II trial is currently recruiting (ProCAID, NCT02121639). Early results from a phase Ib study determined a RP2D of 400mg ipatasertib QD days 1-21 q4w in combination with weekly paclitaxel 90mg/m^2 with grade 3 AEs of diarrhoea, fatigue and hyperglycaemia at higher doses (Bendell et al., 2014). Promising anti-tumour activity was demonstrated in breast cancer patients with 6 PRs (22%) including 4 patients who had previously progressed on paclitaxel (Bendell et al., 2014). Similarly, phase I/II trials of AZD5363 in combination with weekly paclitaxel are on going in breast and gastric cancer populations with results awaited (**Table 2**).

Activation of AKT has been associated with platinum resistance in pre-clinical studies of ovarian cancer (Stronach et al., 2011). In a phase I/II study of afuresertib in combination with carboplatin (iv AUC5 q3w) and paclitaxel (iv 175mg/m2 q3w), the MTD of afuresertib was 150mg daily with DLTs of G3 rash, G2 febrile neutropenia and G2 lip swelling (Blagden et al., 2016). Promising anti-tumour activity of the combination was demonstrated and at the time of reporting, overall response rate (ORR) for platinum-resistant patients was

32.1% (95% CI: 15.9–52.4) and median progression free survival (PFS) was 7.1 months (95% CI: 6.3–9.0).

PARP INHIBITORS

Pre-clinical studies have demonstrated synergy between PI3K inhibition and PARP inhibition in BRCA1/2 mutant mouse models (Juvekar et al., 2012). The mechanism of this synergy has not yet been fully explained. Published data suggests that PI3K inhibition impairs DSB repair by homologous recombination through reduced expression of BRCA1 and BRCA2 (Ibrahim et al., 2012). It is also likely however, that as with chemotherapy, the anti-apoptotic/pro-survival effects of PI3K inhibition are important in this setting. Preliminary data have shown promising anti-tumour activity of AZD5363 in combination with olaparib across a range of tumour types (Michalarea et al., 2016). At the time of reporting, out of 37 evaluable patients, there were 10 RECIST partial or complete responses, including 3 patients with BRCA wild-type tumours and 1 patient who had previously been treated with a PARP inhibitor. The recommended phase II schedules of this combination are 300mg BID olaparib + 400mg BID 4/7 AZD5363 or 300mg BID olaparib + 640mg BID 2/7 AZD5363. Common G1-2 toxicities were nausea, fatigue, anaemia, diarrhoea, anorexia, mucositis and vomiting on both schedules.

RTK/PI3K/AKT/MTOR INHIBITORS

Given the multiple feedback loops within the PI3K-AKT signalling pathway, dual inhibition at different levels seems like a sensible strategy, although over-lapping toxicities limits dosing.

Pre-clinical studies have demonstrated synergy between EGFR and AKT inhibition in EGFR-wild type and mutant NSCLC cell lines (Puglisi et al., 2014) and a number of early phase studies of gefitinib or erlotinib in combination with MK-2206 have been completed (**Table 2**). The most common grade 3 AEs in a phase II trial of erlotinib 150mg orally OD with MK-2206 200mg weekly, in patients with erlotinib-resistant NSCLC, were rash, diarrhoea, fatigue and mucositis (Lara et al., 2015). Although partial response rates were low in both EGFR wild-type and -mutant tumours (9% and 3% respectively) a disease control rate of 43% in EGFR-wild type tumours met pre-specified levels of clinical activity to warrant further investigation. A phase I trial has also shown tolerability of gefitinib in combination with MK-2206 with a RP2D of 250mg gefitinib daily and 200mg MK-2206 weekly with early signs of clinical activity in EGFR resistant patients with NSCLC (Lin et al., 2014). A phase II trial exploring erlotinib in combination with MK-2206 as part of the BATTLE-2 program (NCT01248247) is on going.

PTEN loss and activating *PIK3CA* mutations have been associated with relative resistance to trastuzumab both in clinical and pre-clinical studies (Berns et al., 2007; Junttila et al., 2009; Nagata et al., 2004). Inhibition of HER2/ErbB2 with trastuzumab has been shown to activate PTEN and attenuate PI3K/AKT signalling (Junttila et al., 2009; Nagata et al., 2004). Trastuzumab-induced growth inhibition has also been shown to correlate with inhibition of AKT signalling in HER2 +ve cells and combined inhibition of HER2 and AKT signalling is synergistic (Junttila et al., 2009). MK-2206 has in particular, been shown to be well-tolerated in combination with trastuzumab and paclitaxel/trastuzumab in

phase I trials, demonstrating promising anti-tumour activity in HER2+ and paclitaxel refractory tumours (Chien et al., 2016; Hudis et al., 2013).

Results of a phase I trial of the mTOR inhibitor ridaforolimus in combination with MK-2206 demonstrated an MTD of 10mg ridaforolimus OD for 5/7 and 90mg MK-2206 weekly with 1/17 patients experiencing a DLT (G3 rash) at this dose (Gupta et al., 2015). Other common AEs included skin rash, stomatitis, diarrhoea and anorexia. Promising anti-tumour activity was demonstrated in a number of heavily pre-treated breast cancer patients in an expansion cohort comprising patients with a low 'RAS pathway signature score', a 147-gene signature that has been shown to correlate with tumour dependence on RAS signalling and resistance to PI3K pathway inhibition (Loboda et al., 2010).

MAPK PATHWAY INHIBITORS

There are now several lines of evidence showing that tumours harbouring activating mutations in both the PI3K/AKT and MAPK signalling networks, require inhibition of both to negatively affect tumour growth and survival, due to significant crosstalk and redundancy between the pathways (Davies et al., 2012; She et al., 2010). Doses of MK-2206 in combination with the MEK inhibitor selumetinib in a phase I trial of patients with solid tumours were limited predominantly by skin rash, diarrhoea and stomatitis (Tolcher et al., 2015a). The RP2D was defined as MK-2206 135mg weekly and selumetinib 100mg daily, with dose reductions of both drugs required due to overlapping toxicity. Significant tumour responses were seen in patients with *KRAS*-mutant NSCLC and low-grade ovarian cancer, but not in *KRAS*-mutant colorectal cancer, again suggesting differences in biology based on tumour context. Preliminary data from a phase II

trial of MK-2206 in combination with selumetinib in patients with pre-treated *KRAS* mutant NSCLC has shown a disease control rate of 61% (Papadimitrakopoulou et al., 2014) and the full results of this study are awaited with interest.

Preliminary results from a phase Ib study of ipatasertib in combination with the MEK inhibitor cobimetinib has shown early evidence of anti-tumour activity, but tolerability has been challenging (Bendell et al., 2014). Afuresertib has also been tested in combination with a MEK inhibitor, trametinib with only intermittent dosing being tolerable (Tolcher et al., 2015b). Continuous dosing schedules of GSK214795 in combination with trametinib have been achieved (Algazi et al., 2015; Shoushtari et al., 2016) and a number of trials exploring GSK2141795 in combination with trametinib are ongoing (**Table 2**). Preclinical data has clearly demonstrated that sub-maximal inhibition of the combination of MEK and AKT does not significantly increase growth inhibition of *BRAF* or *PIK3CA* mutant cells, compared to maximally inhibiting MEK or AKT alone (Stewart et al., 2015). This has significant implications for the clinical development of such combinations and requires intelligent phase I trial design that tests optimal schedules as well as doses (Lopez and Banerji, 2016).

HORMONAL TREATMENTS

The development of castrate resistant prostate cancer (CRPC) is associated with reactivation of the androgen receptor axis as well as up-regulation of other pro-survival pathways including the PI3K pathway (Morgan et al., 2009). The PI3K/AKT pathway is activated in 30-50% of prostate cancers, most commonly through loss of PTEN, however inhibition of this pathway alone results in up-

regulation of AR signalling leading to resistance (Carver et al., 2011). In pre-clinical studies, synergistic inhibition of AKT and androgen receptor signalling using either bicalutamide or enzalutamide results in sustained tumour growth inhibition and PSA stabilisation in mouse models of CRPC (Thomas et al., 2013; Toren et al., 2015). Preliminary data from a phase I/II study of ipatasertib in combination with abiraterone, has shown promising clinical benefit in patients with CRPC (De Bono et al., 2016). Patients with mCRPC, previously treated with docetaxel, were randomized 1:1:1 to three arms: ipatasertib 400mg, ipatasertib 200mg, or placebo, all in combination with abiraterone 1000 mg and prednisone 10 mg daily. Compared to placebo, ipatasertib 400mg showed increased radiological PFS (median 8.2 vs 6.4 m; HR = 0.75; p= 0.17) and increased OS for ipatasertib 400mg vs placebo (median 18.9m vs 15.6m; HR = 0.72; p= 0.22) (De Bono et al., 2016). Patients with PTEN loss had a superior PFS benefit (De Bono et al., 2016). Full results of this trial are awaited with interest. A randomised phase II trial of enzalutamide +/- AZD5363 in patients with mCRPC (RE-AKT, NCT02525068) is currently recruiting. Similarly, in ER +ve breast cancer, hormone resistance is also associated with an increased dependence on PI3K/AKT signalling (Miller et al., 2010) and treatment of breast cancer cell lines and xenografts with AZD5363 in combination with fulvestrant is superior to AZD5363 alone (Fox et al., 2013; Ribas et al., 2015). FAKTION (NCT01992952), a randomised phase II trial of fulvestrant +/- AZD5363 is ongoing.

9. Future directions and conclusions

AKT remains a central player in the PI3K-AKT signalling network and promising data from a number of phase II combination trials look set to pave the way for

phase III studies and hopefully, clinical registration of some these compounds. Patient selection remains key, with AKT1^{E17K} looking like a particularly strong biomarker predictive of response to AZD5363 monotherapy. A basket trial approach to clinical trial design is becoming increasingly popular and suits collections of low frequency, but high potency mutations such as AKT1^{E17K}. Although potentially slow to complete, the clinical impact for patients harbouring such mutations should not deter licensing of compounds for such niche tumour populations.

The presence of AKT1^{E17K} and other genetic aberrations (such as activating *PIK3CA* mutations and PTEN loss) needs to be put in molecular context however as co-activation of other pro-survival pathways is likely to require combination treatment strategies as demonstrated with AKT and MEK inhibition. Other potential biomarkers such as the RAS pathway signature score and proteomic signatures predictive of response to AKT inhibitors attempt to look beyond the PI3K/AKT pathway itself and should be explored further (Cheraghchi-Bashi et al., 2015; Loboda et al., 2010).

Interestingly, intermittent high doses of AKT inhibitors have been shown to be a more effective strategy both clinically and pre-clinically. High doses appear to be required for induction of apoptosis and intermittent schedules overcome the low therapeutic index of these compounds. This is particularly important in combination studies where toxicity is often limiting (Shimizu et al., 2012). Current preclinical data suggests intermittent dosing that inhibits AKT signalling to a greater extent is more efficacious than chronically inhibiting AKT to a lesser

degree as a clinical strategy (Lopez and Banerji, 2016; Stewart et al., 2015). Interestingly due to feedback inhibition within the PI3K/AKT pathway, intermittent dosing may also delay treatment resistance. Despite the challenges, there remains intense enthusiasm in establishing AKT inhibitors within the future collection of personalised treatments for cancer and numerous clinical trials of AKT inhibitor combinations are actively recruiting.

The potential applications of AKT inhibitors are numerous. The scientific community has multiple drugs in the clinic with exciting potential to influence treatment paradigms in multiple cancers. Carefully thought out clinical trials with tolerable clinical schedules, appropriate combinations and patient selection biomarkers will be needed to maximally exploit the full potential of this class of agents.

Conflict of Interest

Dr Udai Banerji is employed by The Institute of Cancer Research. The Institute of Cancer Research was involved in the discovery of the AKT inhibitor AZD536 and has commercial interests related to the drug. The author is not named on the patent and does not receive payment as part of rewards to inventors scheme related to AZD5363.

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Cellular function	Substrate	Effect of AKT phosphorylation
Cell survival	BAD	Release of pro-survival Bcl-2 proteins from BAD.
	FOXO1 FOXO3a FOXO4	14-4-4 mediated export of FOXO transcription factors from the nucleus, blocking transcription of pro-apoptotic proteins such as BIM and FasL.
	MDM2	Nuclear translocation of MDM2 resulting in P53 ubiquitin-mediated degredation.
Cell growth	TSC2	Inhibits TSC2 function, resulting in mTORC1 activation.
	PRAS40	Phosphorylation of PRAS40 by AKT results in 14-3-3 mediated sequestration of PRAS40 and mTORC1 activation.
Cell proliferation	CDKN1B/p27 CDKN1A/p21	Prevents nuclear localisation of cyclin dependent kinases, blocking cell-cycle inhibitory effects and resulting in loss of the G1-S checkpoint.
Glucose homeostasis	Unknown	Results in translocation of glucose transporter 4 (GLUT4) to the plasma membrane.
	GSK3β	Inhibits glycogen synthase 3 (GSK3), thereby inhibiting glycogen synthesis.
Angiogenesis	eNOS	Activates endothelial nitric oxide synthase (eNOS), resulting in vasodilatation, vascular remodelling and angiogenesis.

Table 1: **The effect of AKT phosphorylation on well-described substrates** (Manning and Cantley, 2007). BAD (BCL2 Associated Agonist Of Cell Death); FOXO (Forkhead Box); MDM2 (MDM2 oncogene); TSC2 (Tuberous Sclerosis 2); PRAS40 (Proline Rich Akt substrate); CDKN (Cyclin-Dependent Kinase Inhibitor); GSK3β (Glycogen Synthase Kinase Beta).

AFURESERTIB (GSK2110183)		
Monotherapy	**Phase I:** **Phase I/II:**	Multiple myeloma (NCT02177682); Haematological malignancies (NCT00881946) (Spencer et al., 2014); Healthy volunteers (NCT01827644, NCT02040480) Solid tumours (NCT01531894) (Yan et al., 2011)
Chemotherapy	Carboplatin + Paclitaxel Paclitaxel	Platinum resistant ovarian cancer (NCT01653912) (Blagden et al., 2016) Gastric cancer (NCT02240212)
MAPK	Trametinib	Solid tumours (NCT01476137) (Tolcher et al., 2015b)
Other	Bortezomib + Dex Ofatumumab	Multiple myeloma (NCT01428492) CLL (NCT01532700)
ARQ 751 and ARQ 092		
Monotherapy	**Phase I:**	Solid tumours (NCT01473095); Solid tumours with *PIK3CA* or *AKT1/2/3* activating mutations (NCT02761694,); Proteus syndrome (non-oncological indication; NCT02594215)
Combinations	Carboplatin +/- paclitaxel or anastrazole	Ovarian, endometrial, cervical and TNBC (NCT02476955)
AZD5363		
Monotherapy	**Phase I:** Solid tumours (NCT01226316, NCT01353781, NCT01895946) (Banerji et al., 2015; Dean et al., 2015; Tamura et al., 2016); mCRPC (NCT01692262). Expansion cohorts in *PIK3CA* and *AKT* mutant breast and gynaecological cancers. **Phase II:** Solid tumours with molecular match (NCT02465060); ER+ve breast cancer vs placebo (NCT02077569); NSCLC (NCT02664935, NCT02117167); Lymphoma (NCT02465060); metastatic breast cancer (NCT02299999)	
Chemotherapy/ Radiotherapy	Paclitaxel Docetaxel + Prednisolone	TNBC (NCT02423603); ER+ breast cancer (NCT01625286); Gastric cancer (NCT02449655, NCT02451956) CRPC (NCT02121639) (Crabb et al., 2016)
Targeted therapy/ hormonal therapy	Olaparib Olaparib + AZD2014 Enzalutamide Fulvestrant	Solid tumours (NCT02338622; NCT02576444) (Michalarea et al., 2016) Endometrial and ovarian cancers (NCT02208375) CRPC (NCT02525068) ER+ breast cancer (NCT01992952)
BAY1125976		
Monotherapy	**Phase I:**	Solid tumours (NCT01915576)
GSK2141795		
Monotherapy	**Phase I:**	Solid tumours (NCT00920257); Ovarian cancer (NCT01266954)
MAPK	Trametinib Trametinib/Dabrafenib	Solid tumours (NCT01138085, NCT01138085); AML (NCT01907815); Multiple Myeloma (NCT01989598); Endometrial cancer (NCT01935973, NCT02093546); TNBC (NCT01964924); Uveal melanoma (NCT01979523) (Shoushtari et al., 2016); BRAF WT melanoma (NCT01941927) (Algazi et al., 2015); Cervical cancer (NCT01958112) Solid tumours (NCT01902173)
GSK690693		
Monotherapy	**Phase I:**	Solid tumours (NCT00493818)
IPATASERTIB (GDC-0068)		
Monotherapy	**Phase I:** **Phase II:**	Solid tumours (NCT01090960); Healthy subjects (NCT02536391, NCT02063581, NCT02390492) GBM (NCT02430363)
Chemotherapy	Paclitaxel mFOLFOX6 Docetaxel	TNBC (NCT02301988, NCT02162719); solid tumours (NCT01362374) (Isakoff et al., 2014) Gastro-oesophageal cancer (NCT01896531); solid tumours (NCT01362374) Solid tumours (NCT01362374)
MAPK	Cobimetinib	Any cancer (NCT01562275) (Bendell et al., 2014)
Hormonal	Abiraterone + Pred Enzalutamide	CRPC (NCT01485861) (De Bono et al., 2016) Solid tumours (NCT01362374)

LY2780301		
Monotherapy	**Phase I:**	Single agent (NCT01115751) (Azaro et al., 2015)
Combinations	Paclitaxel Gemcitabine	Breast cancer (NCT01980277) Solid tumours (NCT02018874)
TRICIRIBINE (TCN-PM; VD-0002)		
Monotherapy	**Phase I:**	Solid tumours (NCT00363454) (Garrett et al., 2011a);Haematological malignancies(NCT00642031)
Combinations	Carboplatin	Ovarian cancer (NCT01690468)
MK2206		
Monotherapy	**Phase I:** Solid tumours (NCT00670488, NCT01071018) (Doi et al., 2015; Yap et al., 2011, 2014); Paediatric tumours (NCT01231919) (Fouladi et al., 2014) **Phase II: Solid tumours:** Adenoid cystic carcinoma (NCT01604772) (Ho et al., 2015); Breast cancer (NCT01277757); Early breast cancer (NCT01319539); Biliary cancer (NCT01425879) (Ahn et al., 2015); Colorectal (NCT01802320) (Dasari et al., 2016); KRAS WT PIK3CA mutant CRC (NCT01186705); Endometrial cancer (NCT01312753, NCT01307631) (Konstantinopoulos et al., 2014); Gastro-oesophageal (NCT01260701) (Ramanathan et al., 2015); Head and neck cancer (NCT01349933) (Ma et al., 2015); Liver cancer (NCT01239355); Nasopharyngeal carcinoma (NCT01370070); Neuroendocrine tumours (NCT01169649); Ovarian/primary peritoneal/fallopian tube cancers with *PI3K/AKT* mutations or PTEN loss (NCT01283035) **Haematological malignancies:** AML (NCT01253447) (Konopleva et al., 2014); Lymphoma (NCT01258998) (Oki et al., 2015); Diffuse large B-cell lymphoma (NCT01466868, NCT01481129)	
Chemotherapy	Paclitaxel Paclitaxel + Trastuzumab Carbo-taxol or Docetaxel	Solid tumours + breast cancer (NCT01263145) (Gonzalez-Angulo et al., 2015) HER2+ve tumours (NCT01235897) (Chien et al., 2016) Solid tumours (NCT00848718) (Molife et al., 2014)
HER2-directed	Lapatinib Trastuzumab +/- Lapatinib	HER2+ve breast cancer (NCT01281163, NCT01245205) (Wisinski et al., 2016) HER2+ve breast cancer (NCT01042379); HER2+ve solid tumours (NCT00963547, NCT01705340)(Hudis et al., 2013; Tripathy et al., 2015)
MAPK	Selumetinib Selumetinib + mFOLFOX	Solid tumours (NCT01021748) (Tolcher et al., 2015a); Colorectal (NCT01333475) (Do et al., 2015); Melanoma (NCT01519427); NSCLC (NCT01248247) (Papadimitrakopoulou et al., 2014) Pancreatic cancer (NCT01658943) (Chung et al., 2015)
PI3K/mTOR	Gefitinib Erlotinib Dalotuzumab Everolimus Ridaforolimus (MK8669)	NSCLC (NCT01147211) (Lin et al., 2014) NSCLC (NCT01248247) (Papadimitrakopoulou et al., 2014); Erlotinib resistant NSCLC (NCT01294306) (Lara et al., 2015); Solid tumours (NCT00848718) (Molife et al., 2014) Any cancer (NCT01243762) (Brana et al., 2014) Renal cancer (NCT01239342) Any cancer (NCT01295632) (Gupta et al., 2015)
Hormonal	Anastrozole +/or Fulvestrant Anastrozole +/- Goserelin Bicalutamide	ER +ve breast cancer (NCT01344031) (Ma et al., 2016) ER +ve HER2-ve breast cancer (NCT01776008) Prostate cancer (NCT01251861)
Other	Dinaciclib Bendamustine Hydrochloride, and Rituximab Hydroxychloroquine	Pancreatic carcinoma (NCT01783171) CLL or small lymphocytic leukaemia (NCT01369849) Solid tumours (NCT01480154)
PERIFOSINE		
Monotherapy	**Phase I:** All solid tumours (Crul et al., 2002; Figg et al., 2014; Van Ummersen et al., 2004; Unger et al., 2010) (NCT00019656, NCT00062387, NCT00005794; NCT00005794); **Phase II:** Breast cancer (Leighl et al., 2008)(NCT00054145); NSCLC (NCT00399789); Malignant glioma (NCT00590954); Head and neck (Argiris et al., 2006)(NCT00062387); Melanoma (Ernst et al., 2005) (NCT00053781); Pancreatic cancer (Marsh et al.,	

	2007) (NCT00059982, NCT00053924); Castrate resistant prostate cancer (Posadas et al., 2005)(NCT00060437); Biochemically recurrent prostate cancer (Chee et al., 2007) (NCT00058214); Renal carcinoma (Cho et al., 2012) (NCT00448721, NCT00498966); Soft tissue sarcoma (Bailey et al., 2006; Knowling et al., 2006) (NCT00053794, NCT00064324); Chemo resistant sarcoma (NCT00401388); Waldenstrom's macroglobulinemia (NCT00422656, NCT00398710); Leukaemia (NCT00391560).	
Chemotherapy/ Radiotherapy	Docetaxel	Epithelial ovarian cancer (Fu et al., 2012)(NCT00431054)
	Radiotherapy	Solid tumours (Vink et al., 2006)
	Capecitabine	Colorectal cancer (Bendell et al., 2011, 2012; Eng et al., 2014) (NCT01097018; NCT00398879)
Targeted therapy	Temsirolimus	Paediatric solid tumours (NCT01049841);Malignant Gliomas (NCT01051557,NCT02238496)
	Imatinib	GIST (NCT00455559)
	Bortezomib +Dex	Multiple myeloma (Richardson et al., 2011)(NCT00401011, NCT01002248)
	Lenalidomide +Dex	Multiple myeloma (Jakubowiak et al., 2012) (NCT00415064)
	Sorafenib	Lymphoprolierative diseases (Guidetti et al., 2014)
	UCN-01	Acute leukaemia, CML, high risk MDS (Gojo et al., 2013)(NCT00301938)

Table 2: **Table showing trials of AKT inhibitors in clinical development**. Trials that are currently recruiting or remain open are marked in red. Allosteric inhibitors are shaded blue. Competitive ATP inhibitors are shaded purple. Dex = dexamethasone. CRPC (castrate resistant prostate cancer). TNBC (triple negative breast cancer).

Figure 1: Structural domains of AKT.

Figure 2: Activation and negative regulation of AKT. See text for details.

Figure 3: Diagram illustrating feedback inhibition and activation within AKT signalling.

Figure 4: (A) Frequency of AKT1-3 amplification across a range of tumour types.. (B) AKT mutations are found in approximately 1% of all cancers. The frequency of AKT mutations vary between cancer types as shown. Data gathered from cbioportal.org database (Cerami et al. 2012; Gao et al. 2013). Only studies with n>100 included in analysis.

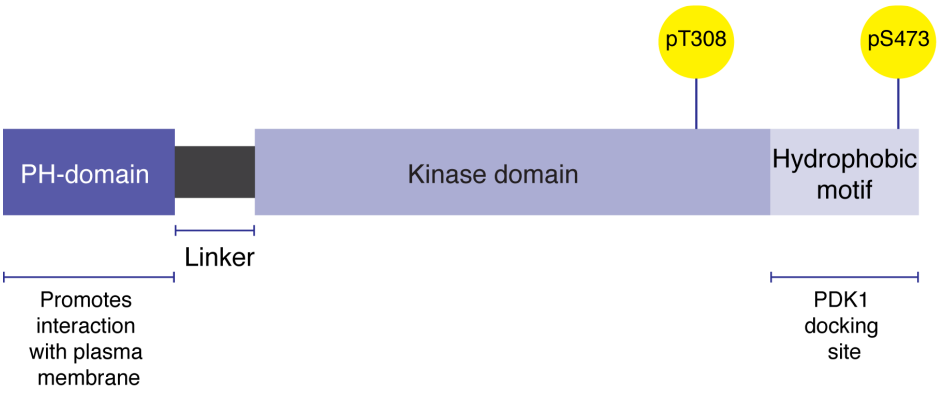

Fig 1

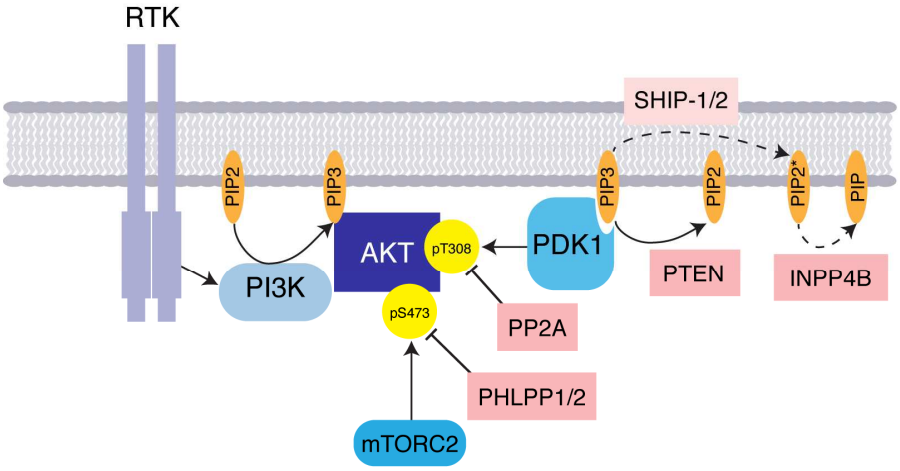

Fig 2

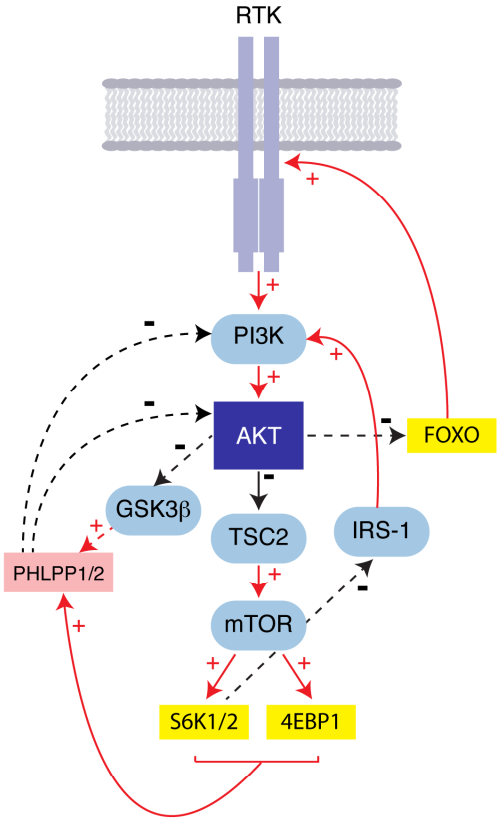

Fig 3

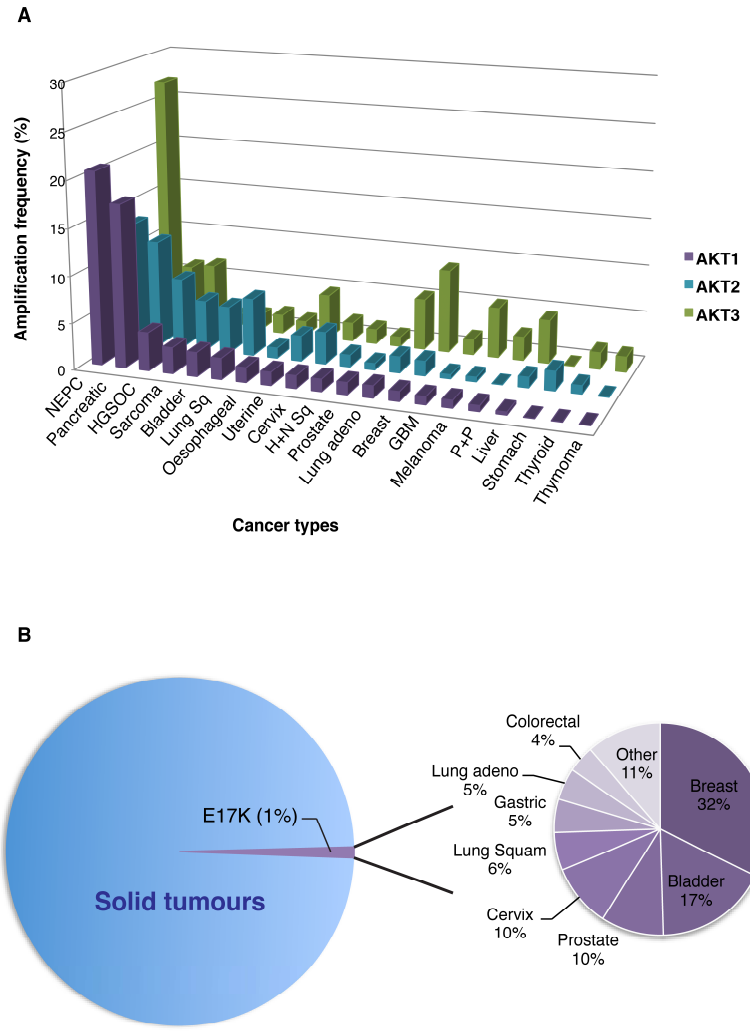

Figure 4A-B