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Authors: Jake S. O'Donnell[1,2,3], Daniela Massi[4], Michele W.L. Teng[1,3°], Mario Mandala[5*°]

Affiliations:

[1]Cancer Immunoregulation and Immunotherapy Laboratory, QIMR Berghofer Medical Research Institute, Herston, 4006, Queensland, Australia.

[2]Immunology in Cancer and Infection Laboratory, QIMR Berghofer Medical Research Institute, Herston 4006, Queensland, Australia.

[3]School of Medicine, The University of Queensland, Herston 4006, Queensland, Australia.

[4]Unit of Medical Oncology, Department of Oncology and Haematology, Papa Giovanni XXIII Cancer Center Hospital, Piazza OMS 1, 24100 Bergamo, Italy

[5]Department of Oncology and Hematology, Papa Giovanni XXIII Hospital, Bergamo, Italy

*Corresponding author: Mario Mandala, Unit of Medical Oncology, Department of Oncology and Haematology, Papa Giovanni XXIII Cancer Center Hospital, Piazza OMS 1, 24100 Bergamo, Italy. E-mail address: mariomandala@tin.it or mmandala@asst-pg23.it Tel: +39 (035) 2673687, Fax: +39 (035) 2674985.

° Michele W.L. Teng and Mario Mandala share senior authorship

Abstract

Cancer therapies will increasingly be utilized in combination to treat advanced malignancies so as to increase their long-term efficacy in a greater proportion of patients. In particular, much attention has focused on developing targeted therapies that inhibit the PI3K-AKT-mTOR signaling network which is dysregulated in many cancer types. In addition, there is now a growing appreciation that targeting of these pathways can impact not only on cancer cells, but also host immunity. The clinical success of cancer immunotherapies targeting T-cell immune checkpoint receptors PD-1/PD-L1 has demonstrated the importance of immunoevasion as a hallmark of cancer. In this review, we discuss how PI3K-AKT-mTOR inhibitors target cancer cell biology, attenuate immune cell effector function and modulate the tumor microenvironment. We next discuss how the immunomodulatory potential of these inhibitors can be exploited through rational combinations with immunotherapies and targeted therapies.

Keywords: PI3K, AKT, mTOR, inhibitions, targeted therapy, immunotherapy, combination therapy, tumor microenvironment, immunotherapeutic resistance.

Introduction

In the past five years, cancer immunotherapy has achieved remarkable clinical efficacy in the treatment of many advanced cancer types [1]. This success represents the culmination of two decades of key research studies and conceptual developments that have occurred in the cancer immunology field. In particular, it is now appreciated that an immune response against tumor-specific neoantigens can be generated but the efficacy of these tumor-specific T cells can be limited by the immunosuppressive microenvironment fostered by many tumors [1, 2]. To date, therapies that relieve T cell suppression such as monoclonal antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or programmed cell death protein 1 (PD-1) receptor expressed on T cells or its primary ligand programmed cell death ligand 1 (PD-L1) expressed on tumors or immune cells, have demonstrated the greatest clinical benefit [3-5]. In addition, a further increase in clinical efficacy was obtained by co-targeting CTLA-4 and PD-1 in the treatment of advanced melanoma [6, 7], demonstrating multiple non-redundant immunosuppressive pathways exist in the tumor microenvironment (TME). Nevertheless, only 50% of patients responded to anti-CTLA-4 and anti-PD-1 combination treatment, and treatment is in some cases associated with severe toxicity [8]. Although single agent anti-PD-1/PD-L1 therapy have demonstrated promising clinical activity in diverse tumor types including melanoma, renal cell carcinoma and lung cancer, there are still a significant proportion of patients who display primary resistance to these therapies [5]. More recently, a proportion of melanoma patients who previously were responsive to anti-PD-1 relapsed, demonstrating that like targeted therapies, acquired resistance to immunotherapies can develop [9]. Thus, identifying strategies to further increase the efficacy of cancer immunotherapies in a wider proportion of cancer patients is a key research focus. Given the safety profile of agents targeting the PD-1/PD-L1 pathway [3, 5, 10], these agents are currently being evaluated in combination with other therapies such as targeted therapies.

The signaling network defined by the phosphoinositide 3-kinase (PI3K), AKT (also referred to as protein kinase B (PKB) [11]) and mammalian target of rapamycin (mTOR), controls most hallmarks of cancer including cell cycle, survival, metabolism, motility and genomic instability; it was this understanding that prompted the development of therapeutic reagents to inhibit this pathway in oncology [12]. It is becoming increasingly apparent that this network also regulates many features of the immunosuppressive microenvironment. Interestingly, recent data derived from clinical trials and pre-clinical mouse models suggests that therapeutic inhibition of the PI3K-AKT-mTOR signaling network may have the dual benefit of staving tumor progression by limiting proliferation, migration and survival, and also augmenting tumor immunosurveillance by preventing activation of immunosuppressive pathways and enhancing anti-tumor immune-intrinsic properties [13]. While targeted therapies can induce high initial response rates, most patients relapse due to acquired resistance [14]. In this review, we first discuss how PI3K-AKT-mTOR inhibitors not only impact directly upon cancer cells, but also have the capacity to affect immune cell effector function and to modulate the tumor microenvironment. We next discuss how PI3K-AKT-mTOR inhibitors can be rationally used in combination with immunotherapies and targeted therapies to improve outcomes for patients with a variety of cancers.

PI3K-AKT-mTOR dysregulation in cancer

Over the past 30 years, the PI3K-AKT-mTOR signaling pathway has been robustly characterized. Much is now understood regarding the molecular mechanisms by which this network regulates the activities of the cell cycle, cellular proliferation, growth, survival, protein synthesis and glucose metabolism [15, 16]. Schematically, growth factors, cytokines or chemokines can stimulate the activation of receptor tyrosine kinases (RTK). This allows for the down-stream activation of important signaling intermediates capable of activating

PI3K molecules. These kinases catalyse the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3) [17]. These lipid products are capable of recruiting pleckstrin homology (PH) domain-containing proteins such as AKT, mTOR complex 2 (mTORC2), and phosphoinositide-dependent kinase 1 (PDK1); the interaction of either PDK1 or mTORC2 with AKT can trigger its activation [11, 18]. Finally, AKT activates the mTOR complex 1 (mTORC1) [19], which promotes cell growth and protein synthesis (**Figure 1**) [20, 21].

Consistent with its physiological role, the PI3K-AKT-mTOR pathway has been found to be hyperactivated in many types of cancer. Overall, this pathway is dysregulated via several genetic mechanisms in approximately 30% of solid cancers (**Table 1**). As such, this represents one of the most frequently dysregulated signaling cascades in human cancers [12]. Oncogenic activation of the PI3K pathway can occur though several mechanisms such as, mutation and/or amplification of genes encoding RTKs [e.g., EGFR (*ERBB1*) and Her2 (*ERBB2*)], subunits of the PI3K (e.g., p110α, p110β, p85α, and p85β), AKT (*AKT1*), or activating isoforms of the RAS GTPases [22-24]. Additionally, components of the PI3K pathway that negatively regulate its function such as the phosphate and tensin homologue (PTEN) are amongst the most frequently occurring tumor suppressor mutations in human cancers [25-27].

Direct effects of PI3K-AKT-mTOR inhibitors on cancer cells

In oncology, targeting of the PI3K-AKT-mTOR pathway was seen as an opportunity to overcome tumor complexity and genomic heterogeneity through a central oncogenic driver, essential to many cancers [28]. To date, there are six classes of compounds targeting the PI3K-AKT-mTOR pathway at different regulatory levels. These include mTOR inhibitors,

active site mTOR inhibitors, pan-class I PI3K inhibitors (PI3Ki), isoform-selective PI3Ki, pan-PI3K-mTOR inhibitors and AKT inhibitors (**Table 2**). These agents are currently being tested within clinical trials at different stages (**Table 3**) [29].

Rapamycin and its analogues (rapalogues) are recognized as the first generation of mTOR inhibitors which inhibit the activity of mTORC1 by binding to FRBP-12 (12 kDa FK506-binding protein; also known as FKBP1A), to form a ternary complex with mTOR [30, 31]. Rapalogues such as everolimus, temsirolimus, deforolimus, and ridaforolimus are currently undergoing a number of clinical trials for therapeutic efficacy in a variety of human malignancies [30]. To date, temsirolimus and everolimus have been FDA approved for the treatment of advanced renal cell carcinoma, subependymal giant cell astrocytoma and for progressive neuroendocrine tumors of pancreatic origin; however, only modest therapeutic effects have been observed [32, 33].

Several pan-class I PI3K inhibitors target all four class I PI3K isoforms (PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ) with similar potencies such as CDC-0941 [34], BKM120 [35], XL147 [36], PX-866 [37], BAY806946 [38], and CH5132799 [39]; however, all remain within clinical trials. The primary argument in support of this modality is that most cancer cells express multiple PI3K isoforms with redundant functions [40]. The main concern associated with their use, is that doses needed to fully block all class I PI3Ks for extended periods might not be well-tolerated. For example, at the concentration needed to fully inhibit PI3K using the pan-PI3K inhibitor, BKM120 (buparsilib), off-target effects upon tubulin, resulting in cellular toxicity are observed [35]. For this reason, isoform-selective PI3K inhibitors with the capacity to completely block their relevant target, while limiting toxicities associated with the use of more broad inhibition have been developed [12]. Of these, the most impressive results

have been achieved using the p110δ-selective inhibitor, GS-1101 (idelalisib), which causes dramatic responses in chronic lymphocytic leukaemia, without any major off-target effects. As such GS-1101 has been approved as a first-in-class agent for the treatment of relapsed chronic lymphocytic leukaemia, follicular B cell non-Hodgkin's lymphoma and small lymphocytic lymphoma [41, 42].

As one of the key effector nodes in the PI3K pathway, AKT was also seen as an attractive therapeutic target. To date, two classes of pan-AKT inhibitors have been developed: i) allosteric inhibitors (e.g. MK-2206) which bind to the PH domain and prevent plasma membrane localisation, and phosphorylation at Thr308 and Ser473 by PDK1 and mTORC2, respectively; ii) adenosine triphosphate (ATP)-competitive inhibitors (e.g. AZD5363 [43], ipatasertib [GHC-0068] [44], afuresertib [45], and GSK2141795 [46]) which limit AKT kinase activity. Early phase, single-agent clinical trials with AKT inhibitors have generally shown underwhelming efficacy, as having anti-proliferative, rather than antitumor activity, with stable disease identified as the best overall response [27, 47].

Overall, few PI3K-ATK-mTOR pathway inhibitors including small molecule inhibitors and biological reagents have reached later phases of drug development and approval. This is due to a few conceptual concerns which have hindered developmental progress such as: i) the challenge of targeting enzymes utilized by tumors that are also active, and play crucial roles in the homeostasis of most tissues; ii) pathway blockade generally fails to induce cancer cell death and can lead to selection of compensatory pathways that maintain survival which can eventually restore tumor growth; iii) oncogene addition to PI3K-AKT-mTOR signaling is not absolute; iv) the delivery of suboptimal dosing schedules due to toxicity; v) the presence of compensatory pathways, such as growth factor receptor signaling, which is capable of

bypassing the effects of targeted blockade. In any case, a large number of clinical trials are ongoing evaluating PI3K-AKT-mTOR inhibitors as monotherapies or in combination with other agents in (**Table 3**). Further investigation will almost certainly be needed to determine the mechanisms of actions of these therapies, and more specifically, to investigate their immunomodulatory effects within the tumour microenvironment in order to critically plan biomarker-enriched clinical trials.

Effects of PI3K-AKT-mTOR inhibitors on the tumor microenvironment

While the therapeutic effects of PI3K-AKT-mTOR inhibition are generally underwhelming, a body of recent research suggests that they can have significant biological effects in modulating both sides of the tumor-immune interface. Inflammation, while positive in some context, is a critical component of tumor progression [48]. The tumor microenvironment contains a variety of immune cell types which can either promote or limit tumor development and progression [49]. Interestingly, the activity of most immune cell types is at least, to some extent, affected by the PI3K-AKT-mTOR pathway [50]. As such, the PI3K-AKT-mTOR pathway can regulate tumor-intrinsic and immune-intrinsic features of the immunosuppressive tumor microenvironment. For example, *PTEN*-mutations within mouse models of melanoma have been shown separately to promote the expression of immunosuppressive cytokines and chemokines such as CCL20, CXCL1, IL6 and IL-23 [51], IL-6 and IL-10 [52], and also angiogenic factors such as VEGF [53]. Encouragingly, inhibition of the PI3K pathway using the selective PI3Kβ (p110β) isoform inhibitor, GSK2636771 [53], or the pan-PI3K inhibitor, LY29002 [54] in various pre-clinical tumor models, has been shown to enhance $CD8^+$ T cell infiltration within tumor tissue, resulting in reduced tumor burden and significant survival benefit [53, 54]. As well as promoting tumor vascularization, VEGF is important for promoting the infiltration of regulatory immune cell

subsets including immature dendritic cells, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs); each capable of inhibiting functional anti-tumor immune responses [55, 56]. Therefore inhibition of VEGF secretion would likely promote anti-tumor immune responses.

Additional immunosuppressive microenvironment features in the tumor such as expression of immune checkpoint ligands appear to be regulated by the PI3K-AKT-mTOR pathway under certain circumstances. A variety of human and mouse tumors lacking expression of the tumor suppressor gene *PTEN*, have been shown to over-express PD-L1 [57-60]. Although usually induced in response to inflammatory cytokines such as T cell-derived interferons (IFNs) [61], PD-L1 can also be regulated (at least at the post-transcriptional level) by the PI3K-AKT-mTOR pathway [57]. The interaction of PD-L1 with PD-1 expressed by tumor-specific T cells can induce a state of functional exhaustion in which T cell proliferation, cytokine production, and migration are significantly limited [62]. This might provide one explanation as to why *PTEN*-mutant tumors are often veritable $CD8^+$ T cell deserts, and why in turn, such tumors are usually subject to poor immunological control [63]. Encouragingly, inhibition of the PI3K-AKT-mTOR pathway using either a pan-PI3K inhibitor wortmannin, pan-AKT inhibitor, MK-2206 or mTOR inhibitor, rapamycin, has been demonstrated to reduce expression of PD-L1 within *PTEN*-mutant triple-negative breast cancer cell lines *in vitro* [60]. Loss of *PTEN* within preclinical melanoma models has been demonstrated to inhibit T cell-mediated tumor killing and to decrease $CD8^+$ T cell infiltration within tumor tissue, which also correlated with poor outcomes to anticancer immunotherapies [53]. By contrast, it remains unlikely that inducible expression of PD-L1 by IFNs is regulated by the PI3K-AKT-mTOR network. Indeed, patient-derived melanoma cell lines with wild type (WT) *PTEN*,

cultured in the presence of a PI3K inhibitors LY294002, BKM120, or with a dual inhibitor of both PI3K and mTOR, BEZ235, failed to attenuate PD-L1 expression level [64].

Effects of PI3K-AKT-mTOR inhibitors on immune cells

Within the tumor microenvironment, immune suppression can be mediated not only by tumor cell-intrinsic effects, but also via recruitment of regulatory immune cell subsets such as MDSCs and Tregs into the tumor [13, 65]. While inhibiting components of the PI3K-AKT-mTOR pathway has been shown to reduce expression of immunosuppressive cytokines, chemokines, and checkpoint ligands, therapies such as these may also modulate the activity and viability of immunoregulatory cell types. For example, the differentiation of MDSCs appears to be heavily regulated by AKT; its inhibition has been demonstrated to limit MDSC differentiation *in vitro*. Nevertheless, the impact of AKT inhibition on MDSC differentiation within the tumor microenvironment remains poorly understood [66]. Additionally, it has been shown that a range of chemokines activate G-protein coupled receptors, tyrosine kinase, and toll-like receptors (TLRs) which signal via PI3Kγ (p110γ) within myeloid cells to promote their infiltration into tumor tissue [67]. Genetic and pharmacological inactivation of the p110γ was demonstrated to reduce α4β1 integrin-mediated adhesion of MDSCs and Tregs, limiting their infiltration and in turn reduced tumor growth and metastasis in murine models of melanoma, lung, pancreatic and breast cancers. Importantly, this reduction in tumor-associated inflammation and angiogenesis occurred without affecting systemic numbers of myeloid or lymphoid cells [67]. These data strongly support the findings of others who have demonstrated in pre-clinical models of colitis-associated cancer, mice lacking functional PI3Kγ (P110γ-/-) were found to harbour significantly fewer colon neoplasm in comparison to wild type controls [68, 69].

Tregs have been understood for some time to limit the activity of anti-tumor immune responses via a number of mechanisms including the secretion of IL-10 and sequestration of IL-2 [70, 71]. Interestingly, several reports have demonstrated that the utilization of the PI3K-AKT-mTOR signaling pathway differs between regulatory and conventional T cells, such that inhibition might tip the scales away from immune suppression, in favour of anti-tumour immunity [65, 72]. Using the pan-AKT inhibitors, IC87114, wortmannin, or MK-2206, it has been shown that Tregs are preferentially susceptible to inhibition; enhancing the number of $CD8^+$ T cells within tumor tissue, and improving tumor control [65]. To limit off-target effects associated with pan-inhibitors, isoform specific inhibitors of PI3Kδ (p110δ) have also been developed and shown to effectively reduce tumor burden and metastasis in murine models of melanoma, lung, breast, and pancreatic cancers [73]. Specifically, P110δ inactivation in Tregs has been demonstrated to impair maintenance and function of Tregs in the periphery. Indeed, these cells produce less IL10, and express lower levels of CD38, despite maintenance of a Treg gene signature. Importantly, $CD8^+$ T cell functions were preserved in the presence of this inhibitor [73]. Together, these data suggest that the PI3K-AKT-mTOR pathway is critical for maintaining the immunosuppressive function of Tregs, however, its precise mechanism remains to be fully characterised.

Following activation, $CD8^+$ T cells differentiate into effectors that proliferate and produce effector cytokines. To meet this increased bioenergetics demands, these cells undergo metabolic re-programming from quiescent mitochondrial oxidative phosphorylation (OXPHOS) to glycolysis [74]. This switch to glycolysis is directly linked to increased effector functions and is thought to occur in response to T cell receptor (TCR)-mediated signaling via the PI3K-AKT-mTOR pathway [75]. Following a productive immune response which overtime results in a decline in cognate antigen, the conversion to memory T cells is

characterised by a shift back to mitochondrial OXPHOS, fuelled by fatty acid oxidation. Especially in the context of cancer, chronic TCR signaling can induce tumor-specific $CD8^+$ T cell exhaustion. These cells upregulate PD1, which *in vitro* has been demonstrated to reduce PI3K-AKT-mTOR signaling which in turn results in decreased glycolysis [76]. Specifically, this occurred by repression of PPAR-gamma coactivator 1α (PGC1α) expression, which is essential for mitochondrial biogenesis and metabolic regulation. Within exhausted $CD8^+$ T cells this then leads to a state of energetic insufficiency. Blockade of the PD1 pathway was shown to increase the energetic metabolism of effector T cells and to promote their effector functions [77]. Together, these data might suggest that inhibition of the AKT-PI3K-mTOR signaling pathway could have a deleterious effect on exhausted effector T cell function; however, this possibility has not been robustly explored. As such the optimal schedule of when to administer these inhibitors will have to be determined. Notwithstanding these observations, inhibition of this signaling pathway has been demonstrated to improve the development of memory $CD8^+$ T cells with positive effects.

Recently it was shown that the progression-free survival of melanoma patients treated with anti-PD-1 was heavily associated with the development and persistence of memory T cells [9]. Indeed, a key feature of checkpoint inhibitors given as monotherapy is their ability to induce durable responses in ~20-40% of patients treated with anti-CTLA-4 or anti-PD-1 respectively [78, 79], in contrast with the immediate, but transient responses induced by conventional chemotherapy or by targeted therapies. The process of memory T cell differentiation appears to be influenced by a number of factors including collective input via the TCR, IL-2 and IL-12 receptors which feed signals into the PI3K-AKT-mTOR network [80]. Therefore a strong case can be made that AKT can govern the balance between terminal differentiation and the generation of $CD8^+$ T cell memory. In fact, this notion has been

strongly supported by studies in which mTOR inhibition using rapamycin significantly strengthened both the quality and quantity of $CD8^+$ T cells, enhancing memory T cell differentiation by promoting expansion of the memory precursor ($KLRG1^-CD127^+$) pool during the T-cell expansion phase, and accelerating memory T cell differentiation during the T-cell contraction phase [81]. Additionally, multiple studies have demonstrated in the context of cancer that inhibition of AKT can potently enhance memory T cell differentiation[82]. Mechanistically, it has been demonstrated that AKT can suppress the activity of memory-promoting transcription factors such as FOXO and TCR/LEF/β-catenin [83]. Inhibition therefore relieves this suppression and promotes memory differentiation. In the context of chronic antigen exposure, memory precursors have demonstrated a unique susceptibility to become exhausted, this perhaps provides a reason as to why T cell memory development in the context of cancer, and in chronic infections can be stunted [84].

Therapeutic combinations to improve anti-tumor immunity

As single agent therapies, the efficacy of PI3K-AKT-mTOR inhibitors in the treatment of a variety of cancers has generally been underwhelming [12, 85]. There are several possible reasons as to why; most notably: i) administration of therapies at tolerated concentrations can fail to have biological and therapeutic efficacy; ii) these therapies are targeting a signaling pathway that is integral for the homeostasis of most tissues. For example, the PI3K-AKT-mTOR pathway is important for patterning the development and activities of lymphocytes [86, 87]; a great deal of early preclinical work conducted within this field took advantage of *in vitro* systems, or immunodeficient animal models. While such analyses might have been useful for modelling the cytostatic features of PI3K-AKT-mTOR inhibitors, they are unfortunately not useful for dissecting potentially harmful immunocidal off-target effects. More recently, these potential effects have been taken into account and it was demonstrated

quite effectively that therapies targeting PI3Kγ, AKT, mTOR, or PI3Kδ were capable of augmenting tumor immune surveillance without broad toxicity [65, 67, 72, 81]. Tolerability notwithstanding, generally their efficacy within preclinical models remains modest. Given their immunomodulatory capacity, it is highly likely that the full potential of PI3K-AKT-mTOR inhibitors could be best realized when used in combination therapies with either immunotherapies, or targeted therapies as discussed in the next section (**Figure 3**).

Combination with immunotherapies

It has been firmly established that the efficacy of cancer immunotherapies will depend on priming and generation of tumor neoantigen-specific T cells [88], that can migrate into tumor tissue [89], and mediate effector functions (to which tumor cells are sensitive) [62, 90], and develop stable immunological memory [9]. To achieve the most effective outcomes for patients treated with a variety of anti-cancer therapies, combination therapeutic strategies involving PI3K-AKT-mTOR inhibition may be of benefit (**Figure 2**) [1]. To enhance priming and activation of tumor antigen-specific T cells, various cancer vaccination strategies have been developed. These include more traditional approaches in which immunogenic, tumor-specific peptides are combined with adjuvant and administered systemically, as well as more innovative strategies which involve the isolation of autologous DCs and loading them with tumor antigen *ex vivo* to be administered as a cellular vaccine [91, 92]. While such strategies are capable of generating systemic, tumor-specific T cell responses, their magnitude does not necessarily correlate with tumor regression. Mechanistically, this form of resistance has in some instances been demonstrated to occur as a result of T cell resistance. Upregulation of the PI3K-AKT-mTOR network can potently induce signaling via NF-κB, capable of driving transcription of a variety of anti-apoptotic molecules including cellular inhibitors of apoptosis (cIAPs), caspase-8/FADD (FAS-associated death domain)-like IL-1β-converting enzyme

(FLICE), inhibitory protein (c-FLIP) and members of the Bcl-2 family such as A1/BFL1 and BCL-XL [92, 93]. By therapeutically targeting up-stream PI3K-AKT signaling, sensitivity to tumor-specific $CD8^+$ T cell-mediated cytotoxicity was shown to be enhanced [92]. These data suggest that the combination of peptide vaccine with PI3K-AKT inhibition would likely improve the sensitivity of tumors to immune-mediated assault (**Figure 3**). Broader characterisation might, however, be warranted to identify whether specific genetic variations were responsible for the upregulation in AKT which could be useful for predicting outcome.

To enhance the effector function (migration, proliferation and cytotoxicity) of tumor-specific T cells, immunotherapies targeting the PD-1 and CTLA-4 immune checkpoints have demonstrated the greatest clinical benefit. The general consensus is that anti-CTLA4 therapy functions by promoting proliferation of tumor-specific $CD8^+$ T cells within secondary lymphoid organs, while anti-PD1 therapy relieves exhaustion of tumor-specific $CD8^+$ T cells within tumor tissue; enhancing the number and function of tumor-specific effectors [1]. Since their clinical inception, it has become apparent that therapies such as these are also often subject to primary or acquired resistance [94]. Our understanding of the mechanisms underlying resistance to these therapies is improving. Now, limited evidence suggests that defects in antigen presentation [88], T cell resistance [90], the secretion of immunosuppressive cytokines from tumor cells or immune cells, the presence and activity of MDSCs and Tregs within tumor tissue, and the failure to generate protective immunological memory can all contribute to failure to anti-cancer therapies [95]. As discussed, PI3K-AKT-mTOR inhibition can augment the secretion of immunosuppressive cytokines [51-53], the infiltration of MDSCs and Tregs into tumor tissues [67-69], and promote the development of memory T cells [9, 96]. For these reasons, it is highly likely that the combining therapeutic strategies involving PI3K-AKT-mTOR inhibition with checkpoint blockade would be effective (**Figure 3**). Importantly however, several questions remain: i) would PI3K-AKT-

mTOR inhibition complement anti-PD-1/anti-CTLA4 immunotherapies within resistant tumors in which β-catenin signaling is dysregulated? One study reported its dysregulation limited the chemotaxis of antigen presenting cells capable of priming tumor-specific immunity into tumor tissue [88], ii) it was recently shown that acquired resistance to anti-PD1 therapy can occur as a result of T cell resistance to IFNγ (due to *JAK 1* and *2* mutations) or due to defective presentation of cognate antigen (due to *β2M* mutations) [90]. iii) Would PI3K-AKT-mTOR inhibition be useful to sensitize tumors in which acquired resistance to anti-PD1 therapy has developed to alternative immunotherapies?

Conceptual advances leading to the understanding that endogenous tumor-specific immune responses can be generated, prompted the development of immunotherapeutic strategies in which autologous T cells are isolated and expanded, or manipulated from patient *ex vivo* before reinfusion, to bolster tumor-specific immunity [97]. More traditional adoptive cell therapy (ACT) approaches involve the isolation of tumor-infiltrating lymphocytes (TILs) extracted from biopsy material [97]. One of the main features of TILs that has been associated with objective response is their long-term persistence after transfer. Unfortunately, TILs isolated from tumor material are usually terminally differentiated and unable to develop a memory phenotype [82, 98]. To overcome this limitation, AKT inhibition was shown in a mouse model of ACT, to reprogram the transcriptional profile of $CD8^+$ T cells into that of memory cells. Functionally, this was shown to enhance their anti-tumor efficacy and to improve overall survival [82, 98]. More recently, ACT has been improved upon to allow for the *ex vivo* manipulation of CD8 T cells transduced with chimeric antigen receptor (CAR), specific for tumor antigens that are not restricted by MHC Class I (major histocompatibility complex I) [99]. While demonstrated to be effective in the treatment of a number of malignancies, the presence of IL-2 within *ex vivo* cultures, necessary to maintain viability,

has, paradoxically been shown to reduce CAR T cell activity [99]. Similarly, it was recently demonstrated that the efficacy of CAR T cells in the treatment of solid tumours *in vivo*, could be improved with the addition of PI3K inhibitors during *ex vivo* culturing, however, these were preliminary findings and the underlying mechanism remains to be fully elucidated [99, 100].

Combination with targeted therapies

BRAF and MEK inhibitors have demonstrated broad efficacy in treatment of *BRAF*-mutant melanoma where tumor proliferation and survival is promoted by oncogenic activation. Like inhibitors of the PI3K-AKT-mTOR signaling pathway, the efficacy of these therapies have also been shown to depend on host-derived immunity in the form of $CD8^+$ T cells and also NK cells; the infiltration of which into tumour tissue is enhanced following therapy [101]. Interestingly, the efficacy of these therapies is often limited due to the development of resistance [102]. A major mechanism by which resistance develops is via activation of the PI3K-AKT-mTOR pathway [102, 103]. The combination of therapies targeting BRAF/MEK and the PI3K-AKT-mTOR pathway have demonstrated additive or synergistic efficacy. For example, vemurafenib in combination with inhibitors of mTORCs 1 and 2 has been shown to be synergistic in the treatment of BRAF-mutant melanoma (**Figure 3**) [104-106]. In addition, the efficacy of therapies which inhibit epidermal growth factor receptor (EGFR) family member signaling such as erlotinib (which targets EGFR) and lapatinib (a dual inhibitor of EGFR and Her2) has also been shown in some cases to be limited by signaling via the PI3K-AKT-mTOR network. Encouragingly, the combination of lapatinib with the AKT inhibitor MK-2206 has demonstrated efficacy in recent early phase clinical trials for treatment of various solid tumors [107].

Conclusions

The heterogeneity and adaptability of many tumors strongly supports the need to utilize robust therapies which target the disease on multiple fronts. Throughout this review, we have detailed how inhibition of components of the PI3K-AKT-mTOR network can have dichotomous functions; they can halt tumor cell proliferation, and augment tumor immune surveillance. The divergent responses to PI3K-AKT-mTOR inhibition to date have been caused by a variety of factors including cellular heterogeneity, dynamic interactions and cross-talk between different nodes of the PI3K-AKT-mTOR pathway, off-target effects upon immune cells and cellular plasticity. To release their broad potential, PI3K-AKT-mTOR inhibitors require further optimization of their dosage and scheduling with respect to their use in various combinations therapies. By defining the optimal approaches to combine PI3K-AKT-mTOR inhibitors with other cancer therapies, particularly those targeting PD-1/PD-L1, it is hoped that the rates of both primary and acquired resistance could be reduced. It is probable that such therapeutic combinations could be associated with increased risk of inducing severe immune-related adverse events (irAEs). When used as single-agents, some PI3K inhibitors including idelalisib, are associated with what appear to be mild irAEs; this is seen with increased T cell infiltration within tissues, occurs more frequently within first-line patients who are more immunocompetent, and can be alleviated by administration of steroids [108]. Additionally, biallelic loss of *PIK3CD* within humans has been associated with similar symptoms, suggesting that these are likely to be irAEs. In fact, these effects are similar to irAEs observed in response to checkpoint blockade against PD-1and/or CTLA-4 [109]. Fortunately, such conditions are usually reversible following treatment withdrawal [109]. Moving forward, an increased understanding of the immuno-modulatory effects of PI3K-AKT-mTOR inhibitors may allow its rationale combination with other therapies to improve

clinical outcomes for patients although safety of each combination will have to be throughly assessed.

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. The PI3K-AKT-mTOR signaling network.

The PI3Ks are family of 8 members, grouped into three classes. In the context of cancer, four class I enzymes, termed PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ appear to be of significance. These are heterodimers of a 110 kDa catalytic subunit (p110α, p110β, p110γ and p110δ) and a p85 regulatory subunit. Activated adaptor proteins bind via YXXM motifs of PI3Ks and relieve p85-mediated inhibition of p110. This recruits p85-p110 heterodimers to their substrate, the lipid phosphatidylinositol-4,5-bisphosphate (PIP2), at the plasma membrane. PI3K (p110), then phosphorylate PIP2 to produce the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). Phosphate and tensin homologue (PTEN), dephosphorylates PIP3 in position 3 inositol ring, thereby negatively regulating PI3K signaling outputs. Several pleckstrin homology (PH) domain-containing proteins, including SGK, PDK1 and most importantly, AKT, bind to PIP3 at the plasma membrane. The phosphorylation of AKT at Thr308 by PDK1 and at Ser473 by a complex involving mTOR/Rictor complex (mTORC2) results in full activation of this enzyme. AKT phosphorylates a host of cellular proteins including GSK3α, GSKβ, the FOXO transcription factors, MDM2, BAD, and p27KIP1 to facilitate survival and cell cycle entry. Additionally, AKT phosphorylates and inactivates Tuberin, a GTPase-activating protein (GAP) for the Ras homologue, Rheb. Inactivation of Tuberin allows GTP-bound Rheb to accumulate and activate the mTOR/Raptor complex (mTORC1), which ultimately regulates protein synthesis, RNA translation, cell growth, and autophagy.

Figure 2. Suppressive mechanism mediated by the PI3K-AKT-mTOR network in the tumor microenvironment.A. Upregulation of AKT within tumor cells as a result of PTEN mutations, or other methods of abberant pathway activation can lead to the upregulation of

anti-apoptotic molecules and prevent tumor cells from undergoing apoptosis in response to $CD8^+$ T cell-mediated assault. **B.** Additionally, some PTEN-mutant, and some PTEN wild type tumors with upregulated AKT signaling often secrete VEGF, a potent chemoattractant of MDSCs and Tregs. The presence of these cell types within tumor is negatively correlated with response to a variety of anti-cancer therapies; especially immunotherapies as they are capable of inhibiting the activity of a variety of immune cell types, particularly $CD8^+$ T cells. **C.** PTEN-mutant tumours are often reported to constitutively express PD-L1, the ligand for the immune checkpoint receptor PD-1. The interaction of PD-L1 with PD-1 expressed on the surface of activated $CD8^+$ T cells can induce a state of functional exhaustion and inhibit their anti-tumor activity. **D.** Immunosuppressive cytokines secreted from tumors as a result of PI3K-AKT-mTOR signaling can suppress tumor-specific $CD8^+$ T cell activity (migration, proliferation and cytotoxicity). **E.** Together, these immunosuppressive effects can prevent the induction of protective immunological memory. Tem = effector memory, Tcm = central memory, Trm = tissue resident memory.

Figure 3. Rationale for combining immunotherapies with PI3K-AKT-mTOR inhibitors. PI3K-AKT-mTOR (A) or BRAF/MEK (B) inhibitors can have both tumor cell-intrinsic and immune cell-intrinsic effects. By combining these targeted therapies with various immunotherapeutic approaches (C), it is likely that anti-tumor efficacy can be further improved.

TABLE 1 – PI3K/AKT/mTOR aberrations in main solid tumors*			
PI3K pathway aberrations	**Chromosome Location**	**Protein Function**	**Histopathology (Prevalence %)**
***PIK3R1/2* mutation**	PIK3R1 (5q13.1) PIK3R2 (19q13.2-q13.4)	Genes encoding p85α and p85β, the regulatory subunits of PI3K	Prostate: 22% Endometrial Carcinoma: 20% (PIK3R1); 5% (PIK3R2) GBM: 8-10% Breast: 2% Colorectal: < 1% Skin SCC: 11% Melanoma 1-2%
***PIK3CA* mutation**	3q26.3	Gene encoding p110α, the catalytic subunit of PI3K	Breast: 20%-50% Endometrial, ovarian: 25-30% SCLC: 20-23% HNSCC: 20% Colorectal: 14% GBM: 10% Prostate: 6% NSCLC): 5% Melanoma 3%
***PIK3CA* amplification**	3q26.3	Gene encoding p110α, the catalytic subunit of PI3K	NSCLC: 10-30% HNSCC: 20% Breast: 10% Esophageal SCC: 10%
***AKT1/2* amplification**	AKT1 14q32.32 AKT2 19q13.1-q13.2	Genes encoding AKT1 and 2 protein isoforms	HNSCC: 5% Breast, ovarian, pancreatic, Gastric cancer: < 5% Colorectal cancer: < 1%

***AKT1* mutation**	14q32.32	Gene encoding for AKT1 protein isoform	Colorectal: 6% Breast (TNBC): 3% NSCLC: 1-2% Endometrial: 1%
PTPN12 loss	7q11.23	Gene encoding the tumor suppressor PTPN12, a non-receptor tyrosine phosphatase leading to enhanced tyrosine phosphorylation of multiple growth factor receptors, that leads to PI3K pathway activation	Breast: 23% Breast (TNBC): 60%
INPP4B loss	4q31.1	Gene encoding INPP4B, which dephosphorylates phosphatidylinositol 3,4-bisphosphate (PI(3,4)P2) on the D4 position generating phosphatidylinositol 3-phosphate (PI(3)P)	Breast (TNBC): 30%-56% Lung (NSCLC): 47% Ovarian: 40% Prostate: 8%
PTEN loss	10q23.3	Gene coding PTEN, that negatively regulates this pathway by dephosphorylating PIP_3 at its D3 position, thereby inhibiting downstream kinase activation	NSCLC: 75% Endometrial: 55% Ovarian: 45% Colorectal: 20%-40% Breast, HNSCC: 30% GBM: 60%-80% Prostate: 20%
***PTEN* mutation**	10q23.3	Gene coding PTEN, which negatively regulates this pathway by dephosphorylating PIP_3 at its D3 position, thereby inhibiting downstream kinase activation	Endometrial: 44% Breast: < 5% Colorectal: < 5% GBM: 5%-40%

* PI3K-AKT-mTOR aberrations in haematopoietic malignancies is reviewed by Gao et al. [110]

TABLE 2 - PI3K/AKT/mTOR pathway inhibitors: clinical characteristics, genomic context and toxicity				
PI3K/AKT/mTOR pathway inhibitors	**Biomarkers of response**	**Potential advantages**	**Limitation**	**Toxicity**
Dual PI3K/mTOR Inhibitors [111, 112]	Tumor types with loss-of-function in the negative regulators PTEN, TSC1/2, and STK11	Drugs with broadest activity profile	Multikinase blockade leads to increased toxicity, this class of agents may not be as well suited to combination therapy as other agents	Hyperglycemia, astenia, nausea/vomiting, diarrhea, rash, mucositis
Isoform-Specific PI3K Inhibitors [113, 114]	PIK3A mutation	To be investigated in selective patients according to biomarkers of response	Drugs with narrowest activity profile	α – Hyperglycemia, astenia, nausea/vomiting, diarrhea β – Astenia, nausea/vomiting, diarrhea, anemia δ – Astenia, nausea/vomiting, diarrhea, rash, liver dysfunction, pneumonitis, pyrexia, hematologic toxicities
AKT inhibitors [115, 116]	PTEN loss or *PIK3CA* mutations	AKT inhibition counteracts its tumor-driven immunosuppressive effects through remodeling of the tumor microenvironment and restoring T cell metabolism.	The response rate as single agent is 5% , hence combination is needed [85]	Hyperglycemia, astenia, nausea/vomiting, diarrhea, rash
mTORC1/2 Inhibitors [117, 118]	Unknown	mTORC1 and mTORC2 inhibition	Genomic profiling of responsive tumors has not	Hyperglycemia, astenia, nausea/vomiting, diarrhea, rash, pneumonitis,

			been reported	hepatoxicity
Pan-class I PI3K inhibitors [119, 120]	Tumor types that lack *PIK3CA* mutations	Better suited to combination therapy than dual PI3K/mTOR inhibitors The efficacy of pan-PI3K inhibitors are not restricted to tumors harboring PIK3CA mutations	Drugs with narrower activity profile than dual PI3K/mTORi	Hyperglycemia, astenia, nausea/vomiting, diarrhea, rash

TABLE 3 – Current clinical trials investigating PI3K-AKT-mTOR inhibitors in solid tumors*				
Drug(s)(clinical trial number)	**Phase**	**Histopathology**	**Primary Endpoint(s)**	**Secondary Endpoint(s)**
Monotherapy				
PI3K inibithors				
BYL719 (NCT02145312)	2	SCCHN	DCR	OR, PFS, OS, TTP, Toxicity, Safety, Quality of life
BKM120 (NCT01629615)	2	Breast cancer	Clinical benefit	PFS, OS, AEs, Safety, PK
BKM120 (NCT01830504)	2	Thyroid cancer	PFS	OR, OS, Safety
BKM120 (NCT01737450)	2	Head and neck cancer	DCR	PFS, OS, Safety, OR, DOR, TTP
BKM120 (NCT01806649)	2	Esophageal Squamous cell Carcinoma	DCR	Safety, PFS, OS
CUDC-907 (NCT02307240)	1	NUT midline carcinoma Breast cancer Solid tumors	MDT	PK, Safety, Tolerability
ZSTK474 (NCT01682473)	1	Solid tumors	DLT	PK
BYl719 (NCT02506556)	2	Breast cancer	OR	CBR, PFS, Safety, Tolerability
Lenvatinib (NCT02860936)	2	Carcinomas of the Salivary Glands	OR	PFS, OS, Safety, Tolerability, DOR

PQR309 (NCT02850744)	2	Glioblastoma multiforme	PFS OR	AEs, SAEs, Physical examination, Depression Test, PK, DOR
BKM120 (NCT02128724)	1	NSCLC	MTD	Changes in 18F-Misonidazole uptake as detected by PET-CT scans, PK
AKT inhibitors				
ARQ 092 (NCT01473095)	1	Solid Tumors Malignant Lymphoma	Safety Tolerability	PK, PD, RP2D
GSK2141795 (NCT00920257)	1	Solid Tumors Lymphoma	RP2D	Efficacy, Metabolite profile
MK2206 (NCT01283035)	2	Ovarian cancer Fallopian cancer Peritoneal Cancer	OR	OS, PFS, Toxicity
MK2206 (NCT01277757)	2	Breast cancer	OR	PFS, Median response duration, Safety
MK2206 (NCT01604772)	2	Adenoid Cyst Carcinoma	OR	PFS, OS, Toxicity
AZD5363 (NCT02077569)	2	Breast cancer	PhD	PK, Toxicity
MK2206 (NCT01349933)	2	Head and neck cancer	PFS OR	AEs, OS, PFS
GSK2110183 (NCT01531894)	2	Solid tumors Hematologic malignancies	AEs PK Vital signs	
MK2206 (NCT01260701)	2	Gastric cancer Gastroesophageal Junction	OS	PFS, OR, AEs

		Cancer		
MK2206 (NCT01425879)	2	Biliary Cancer	ORR	AEs, OS, PFS
MK2206 (NCT01239355)	2	Liver cancer	PFS	OR, OS
MSC2363318A (NCT01971515)	1	Solid tumors	DLT	PD, PK, BOR, CBR, AEs
AZD5363 (NCT01226316)	1	Solid tumors	Safety Tolerability	PK, OR
MK-2206 (NCT01186705)	2	Colorectal cancer	ORR	
PQR 309 (NCT02483858)	1	Solid tumors	Safety Tolerability	DLT, PK
Selumetinib (NCT00888134)	2	Solid tumors	OR in patients with cancer other than melanoma	AKT Pathway activity, or in NSCLC and colon cancer, PFS
mTOR inhibitors				
Everolimus (NCT01412515)	2	Kaposi Sarcoma	OR	
Everolimus (NCT01206764)	4	Renal cell carcinoma	PFS	ORR, OR, Safety
Everolimus *or* *or* Nilotinib Sorafenib *or* Lapatinib *or* Pazopanib (NCT02029001)	2	Solid tumors	12-week PFS PFS	OR, OS, Safety, Quality of life
Combination therapy				
PI3K inhibitors				
Everolimus + Tamoxifen	2	Breast cancer	Clinical benefit at 24 weeks	Response,

(NC T01298713)				Toxicity, OS
Everolimus + Octreotide LAR + Metformin (NCT02294006)	2	pWDNETs	PFS	Safety, Tolerability, OS, OR
BKM120 + Erlotinib (NCT01487265)	2	NSCLC	PFS	DLT, OS, DOR, OR
BKM120 + Cetuximab (NCT01816984)	1/2	SCCHN	Phosphorylated (p)-EGFR MTD	Apoptosis induction, OR, OS, PFS,
BYL719 + Letrozole (NCT01791478)	1	Breast cancer	MTD	Clinical benefit, PFS, OS, OR,
BYL719 + AUY922 (NCT01613950)	1	Gastric cancer	DLT MTD	AEs, BOR, PK, ORR, PFS, OS, SAEs
GDC-0941 + Cisplatin (NCT01918306)	1b/2	Breast cancer	MTD OR	CBR, TTP
BKM120 + Cisplatin + Etoposide (NCT02194049)	1	Small cell lung cancer	AEs	MTD, OR, OS, TTP, PK
WX-037 +/- WX-554 (NCT01859351)	1	Solid tumors	DLT	AEs, SAEs, PK
Taselisib + Enzalutamide (NCT02457910)	1/2	Breast cancer	CBR MTD	PFS, OR, PK
BYL719 + Nab-paclitaxel (NCT02379247)	1/2	Breast cancer	RP2D OR	CBR, PK, PFS, OS
Taselisib / Pictilisib + Palbociclib (NCT02389842)	1	Breast cancer	RP2D Safety CBR PFS	PK

			DOR	
Taselisib / Pictilisib + Palbociclib (NCT02389842)	1	Solid tumors Breast cancer	RP2D Safety Toxicity	PK
Copanlisib + Cetuximab (NCT02822482)	1/2	SCCHN	MTD RP2D	OR, OS, AEs, PK
GDC-0032 + Tamoxifen (NCT02285179)	1	Breast cancer Ovarian cancer Uterine Cancer	MDT	Safety, PK, OR
INC280 + Buparlisib (NCT01870726)	1b/2	Glioblastoma	PFS MTD	SAEs, Tolerability, PK, OR, OS
Letrozole +/- BYL719 *or* BKM120 (NCT01923168)	2	Breast cancer	pCR OR	AEs, PK
BKM120 + Weekly Cisplatin + Radiotherapy (NCT02113878)	1	SCCHN	MTD	OR, TTP, OS
AEB071 + BYL719 (NCT02273219)	1	Uveal Melanoma	MTD	AEs, PK, CBR, OS
Copanlisib + Trastuzumab (NCT02705859)	1	Breast cancer	MTD CBR	AEs/Toxicity, OS, PFS, DOR, Safety, Tolerability, TTP
Fulvestrant +/- Alpelisib (NCT02437318)	3	Breast cancer	PFS	OS, OR, Safety, Tolerability, PK, PFS
BKM120/ Placebo + Fulvestrant (NCT01633060)	3	Breast cancer	PFS	OS, OR, CBR, AEs, Overall health status
BKM120 *or*	1	Ovarian cancer	MTD	Safety, PK

BYL719 + Olaparib (NCT01623349)		Breast cancer	RP2D	
Gedatolisib + Palbociclib + Faslodex (NCT02626507)	1	Breast cancer	Safety MTD	pCR
GSK2636771 + Paclitaxel (NCT02615730)	1/2	Gastric cancer	RP2D PFS	DLT
BKM120 + Tamoxifen (NCT02404844)	2	Breast cancer	PFS	OS, OR, DCR, AEs, Incidence and severity of depressive episodes
Taselisib/Placebo + Fulvestrant (NCT02340221)	3	Breast cancer	PFS	OS, OR, AEs
Gedatolisib + Paclitaxel + Carboplatin (NCT02920450)	1/2	NSCLC	DLT OR	PFS, OS, AEs
GSK2636771 + Enzalutamide (NCT02215096)	1	Prostate cancer	AEs/SAEs DLT, Safety	PK, Response
Enzalutamide +/- LY3023414 (NCT02407054)	2	Prostate Cancer	PFS	TTP, PK, OR
LEE011 + BYL719 + Letrozole (NCT01872260)	1/2	Breast cancer	DLT	Safety, AEs/SAEs, PK, OR, DOR, PFS
PQR309+ Eribulin (NCT02723877)	1/2	Breast cancer	AEs/SAEs CBR OR	TTR, DOR, PFS, TTP, PK, OS
LEE011 + Fulvestrant + BYL719 *or* BKM120 (NCT02088684)	1/2	Breast cancer	DLT PFS	Safety, Tolerability, PK, OR, DOR, OS

INCB050465 + INCB039110 + Epacadostat (NCT02559492)	1	Solid tumors	DLT	OR, PFS, DOR
INCB050465 + Pembrolizumab (NCT02646748)	1	Solid tumors	Safety	OR, PFS, DOR
BKM120 + Trastuzumab + Paclitaxel (NCT01816594)	2	Breast cancer	pCR	OR, pCR, Safety
AKT inhibitors				
GSK2141795 + Dabrafenib + Trametinib (NCT01902173)	1/2	Colon carcinoma Melanoma Ovarian cancer	MTD	OS, PFS, Toxicity
MK2206 + Dinaciclib (NCT01783171)	1	Pancreatic cancer	MTD	DCR, AEs, OS, PFS
MK2206 + Lapatinib Ditosylate (NCT01281163)	1	Breast cancer	MTD	Safety, PK, Response
MK2206 + Lapatinib Ditosylate (NCT01245205)	1	Breast cancer	MTD AEs	OR, PFS, DLT, PFS
GSK1120212 + GSK2110183 (NCT01476137)	1	Solid tumors Myeloma	Safety OR	PK, PFS, DOR
AZD5363 + Olaparib (NCT02338622)	1	Solid tumors	Safety MTD	PK
MK2206 + Selumetinib (NCT01519427)	2	Melanoma	OR	Changes in Biomarker Expression, PFS, OS
GSK2141795 + Trametinib	2	Breast carcinoma	OR	CBR, DOR, AEs,

(NCT01964924)				SAEs, PFS
MK2206 + Selumetinib + Fluorouracil + Oxaliplatin (NCT01658943)	2	Pancreatic cancer	OS	AEs, PFS, OR
+/- GSK2141795 + Trametinib (NCT01979523)	2	Uveal melanoma	TTP	Toxicity, OS, PFS, OR
AZD5363 + Paclitaxel (NCT02423603)	2	Breast cancer	PFS	
MK-2206 + AZD6244 (NCT01333475)	2	Colorectal cancer	pERK and pAKT Levels in Tumor Biopsies	AEs
Triciribine + Carboplatin (NCT01690468)	1/2	Ovarian Cancer	MTD	OR, PFS, Duration of stable disease
MK-2206 + Paclitaxel + Trastuzumab (NCT01235897)	1	HER2-positive solid tumors	MTD	BOR
ARQ 092 + carboplatin + paclitaxel *or* ARQ 092 + paclitaxel *or* ARQ 092 + anastrozole (NCT02476955)	1	Solid tumors	AEs	PK, OR, DLT
Afuresertib + Paclitaxel (NCT02240212)	1	Gastric cancer	SAEs Lab: ECG, LVEF, blood pressure, vital signs assessment	PK, PFS, SAEs, AEs
AZD5363 +/- docetaxel and prednisolone	1/2	Prostate cancer	DLT PFS	Safety, Tolerability, PK, Biochemical OR

(NCT02121639)				
Paclitaxel +/- Ipatasertib (NCT02301988)	2	Breast cancer	pCR	OR, Efficacy, Safety, PK
Paclitaxel +/- Ipatasertib (NCT02162719)	2	Breast cancer	PFS	OS, OR, DOR
Ipatasertib + Cobimetinib (NCT01562275)	1	Solid tumors	DLT MTD	PK, OR, DOR, PFS
mTOR inhibitors				
Everolimus + Sorafenib (NCT01141309)	2	Thyroid cancer	OR	PFS, Safety, Toxicity
Temsirolimus + Sorafenib (NCT01025453)	2	Thyroid Cancer	OR	PFS, Safety, Tolerability
Paclitaxel followed by FEC *or* paclitaxel+RAD001 followed by FEC (NCT00499603)	2	Breast cancer	Inhibition of PI3K pathway	Response
Temsirolimus + Letrozole *or* Letrozole (NCT00062751)	2	Breast cancer	OR	BOR, TTP , PFS, DOR, Health Outcomes Assessment, Population with Response
PF-05212384 + Docetaxel, Cisplatin *or* Dacomitinib (NCT01920061)	1	Solid tumors	DLT	PK, Gene sequence data, OR
Everolimus + Eribulin (NCT02616848)	1	Breast cancer	MTD	PK, Safety
mTOR inhibitors + AKT inhibitors				

AZD2014 + AZD5363 + Olaparib (NCT02208375)	1/2	Endometrial cancer Ovarian Cancer	MTD	OR
Olaparib +/- AZD1775, AZD5363, *or* AZD2014 (NCT02576444)	2	Solid tumors	OR	
mTOR inhibitors + PI3K inhibitors				
Alpelisib + Everolimus *or* Alpelisib+ Everolimus + Exemestane (NCT02077933)	1	Breast cancer Renal cell cancer Pancreatic neuroendocrine tumors	MTD DLT AEs	Safety, Tolerability, PFS, DOR, CBR, OR, PFS
MLN0128 + MLN1117 (NCT01899053)	1	Solid tumors	Number of AEs PK	OR, DOR

***Source Document: Clinical Trial Gov. 2/10/2016.**

OR: Objective Response; **SCCHN:** Recurrent or Metastatic Squamous Cell Carcinoma of Head and Neck; **NSCLC:** Non small cell lung carcinoma; **NUT**: carcinomas characterized by chromosomal rearrangements that involve the gene encoding the nuclear protein of the testis (NUT); **DCR**: Disease control rate; **PFS**: Progression Free Survival; **OS**: Overall Survival; **TTP**: Time to progression; **PK**: Pharmacokinetics; **PD**: Pharmacodynamic; **pWDNETs:** well-differentiated neuroendocrine tumors; **RR:** Response rate; **RP2D:** Recommended phase 2 trial dose; **AEs**: Adverse Events; **MTD**: Maximum Tolerated Dose; **DOR**: Duration of response; **DLT**: Dose-limiting toxicity; **OR**: Overall response; **BOR:** Best Overall Response; **SAEs**: Serious Adverse Events; **CBR**: Clinical Benefit Rate; **TTP:** Time to disease progression; **pCR**: Pathological complete response**; ECOG**: Eastern Cooperative Oncology Group**; PS:** Performance status; **ECG**: Electrocardiogram; **LEVF**: Left ventricular ejection fraction; **FEC:** 5-Fluorouracil + Epirubicin + Cyclophosphamide