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The PI3K pathway in human disease

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Abstract

Phosphoinositide 3-kinase (PI3K) activity is stimulated by diverse oncogenes and growth factor receptors, and elevated PI3K signaling is considered a hallmark of cancer. Many PI3K pathway-targeted therapies have been tested in oncology trials, resulting in regulatory approval of one isoform-selective inhibitor (idelalisib) for treatment of certain blood cancers, and a variety of other agents at different stages of development. In parallel to PI3K research by cancer biologists, investigations in other fields have uncovered exciting and often unpredicted roles for PI3K catalytic and regulatory subunits in normal cell function and in disease. Many of these functions impinge upon oncology by influencing the efficacy and toxicity of PI3K-targeted therapies. Here we provide a perspective on the roles of class I PI3Ks in the regulation of cellular metabolism and in immune system functions, two topics closely intertwined with cancer biology. We also discuss recent progress developing PI3K-targeted therapies for treatment of cancer and other diseases.

Introduction and Historical Context

Reversible phosphorylation of inositol lipids controls diverse functions in cells. The head group of phosphatidylinositol can be phosphorylated on three of the free hydroxyls to form seven different phosphoinositide species with distinct roles in vesicle trafficking and signal transduction. Studies from several laboratories in the 1980s established that activated growth factor receptors and oncoproteins associate with an enzyme that phosphorylates PtdIns (Sugimoto et al., 1984; Whitman et al., 1985). At that time, only two phosphoinositides were known to exist: phosphatidylinositol-4-phosphate (PtdIns-4-P) and phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P_2). In 1988 the enzymatic activity that associated with oncoproteins (specifically polyoma middle T antigen) was shown to phosphorylate the 3′-

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hydroxyl substituent of the inositol ring to produce phosphatidylinositol-3-phosphate (PtdIns-3-P) (Whitman et al., 1988) and a follow up paper (Auger et al., 1989) revealed that platelet-derived growth factor (PDGF) stimulates this enzyme to produce phosphatidylinositol-3,4-bisphosphate (PtdIns-3,4-P_2) and phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P_3) in smooth muscle cells. These findings led to the proposal that the bioactive product of phosphoinositide 3-kinase (PI3K) activity is important for cellular responses to growth factors and for malignant transformation. This prediction has been confirmed by thirty years of research showing that elevated PI3K signaling can contribute to tumorigenesis and is a hallmark of human cancer. Driven by this discovery, medicinal chemistry efforts have yielded a large toolbox of PI3K pathway inhibitors with varied selectivity profiles, many of which are being tested in clinical trials for cancer (Table S1). Along the way, we have learned that PI3K transmits important signals that regulate a variety of physiological processes in virtually all tissue types studied to date. Consequently, it comes as no surprise that the development of PI3K inhibitors to treat cancer has been challenged by the emergence of dose-limiting, on-target adverse effects. Inhibitors specific to mutated forms of PI3K that are commonly found in a wide variety of cancers could circumvent the on-target toxicities and lead to far better efficacy/toxicity profiles. Furthermore, the increasingly refined view of how various PI3K enzymes function in different cell types continues to unveil new opportunities for therapeutic intervention in cancer and in other diseases.

The PI3K field provides a prime example of the importance of basic research to understanding a family of proteins with relevance to human disease. Indeed, studies of PI3K genetics in model organisms have provided some of the most fundamental insights into the function of PI3K enzymes and their lipid products. The first PI3K gene to be cloned was *S. cerevisiae Vps34*, which is required for vacuolar protein sorting in yeast and is the only PI3K gene in that organism (Herman and Emr, 1990). Similarly, the human ortholog, hVPS34 (encoded by *PIK3C3*), is required for vesicle trafficking and for autophagy (Backer, 2016). These findings highlight that the most evolutionarily conserved function of 3′-phosphoinositides is to direct traffic of cargo between cellular organelles. An elegant chemogenomic strategy in budding yeast identified the target of rapamycin (TOR) and established its role in nutrient sensing (Heitman et al., 1991) before the mammalian target of rapamycin (mTOR; also known as the mechanistic target of rapamycin) was discovered and shown to integrate signals from nutrients and PI3K. In another example, a genetic screen in *C. elegans* provided the first clue that PI3K controls metabolism and aging (Dorman et al., 1995; Morris et al., 1996), conclusions that were supported by later studies of the PI3K/mTOR pathway in mice (Foukas et al., 2013; Selman et al., 2009; Wu et al., 2013). Studies in *D. melanogaster* also revealed critical roles for this pathway in growth control of cells and organs and reinforced the connection of PI3K with FOXO transcription factors first identified in worms (Hay, 2011). The first direct demonstration that PI3K genes have transforming potential was provided by a study of chicken cells infected with an avian retrovirus encoding an activated PI3K catalytic subunit (Chang et al., 1997), although much earlier mutational studies of polyoma middle T antigen had shown that binding and activation of PI3K was critical for the transforming function of this oncoprotein (Whitman et al., 1985). Later cancer genomic analyses revealed that activating mutations in PI3K genes

(most commonly the *PIK3CA* gene encoding p110α) occur frequently in human tumors (Samuels et al., 2004).

Generation of mice with deletion or mutation of PI3K genes has been instrumental in delineating the unique and redundant functions of PI3K isoforms in mammalian cells and tissues (Okkenhaug, 2013; Vanhaesebroeck et al., 2010). The complexity of PI3K signaling is well illustrated by studies of the immune system. Indeed, one of the most important themes arising from mouse genetic models has been that the signaling outputs from the various PI3K isoforms must be carefully balanced for proper immune cell development and to optimize responses to pathogens. In accordance with these preclinical observations, it is now appreciated that human immunodeficiencies can result from either loss- or gain-of-function mutations in certain PI3K-encoding genes (Lucas et al., 2016). Additionally, knowledge gained from mouse genetics has led to the concept that drug-mediated inhibition of PI3K isoforms expressed in immune cells (p110γ and p110δ) can reprogram the immune system to combat solid tumor cells more effectively (Okkenhaug et al., 2016).

The knowledge accumulated during the past three decades of lipid kinase research indicates that the PI3K family members participate in an extraordinarily broad range of cellular regulatory processes, including cell growth and proliferation, metabolism, migration, and secretion. Moreover, aberrations in PI3K signaling contribute to an equally broad spectrum of human diseases, such as cancer, immunological disorders, neurological disorders, diabetes, localized tissue overgrowth and cardiovascular disease. This review will highlight recent advances in our understanding of the molecular mechanisms that underpin the PI3K signaling network, with particular emphasis on the contributions of this network to cellular metabolism and immune regulation – two complex processes that offer both challenges and opportunities for the development of PI3K pathway targeted agents.

The PI3K signaling network

Class I PI3K enzyme structure and activation

Human cells express three classes of PI3K enzymes. This review focuses on the class I PI3Ks, their mechanisms of activation, and the signaling networks in which they participate. There are three class II PI3Ks (PI3K-C2α, β, γ) and a single class III PI3K (hVPS34). The reader is referred to other recent reviews concerning class II and III PI3K function (Backer, 2016; Falasca and Maffucci, 2012; Hawkins and Stephens, 2016; Okkenhaug, 2013).

Mammals express four class I catalytic isoforms (p110α, β, γ, and δ encoded by *PIK3CA*, *PIK3CB*, *PIK3CG*, and *PIK3CD*) that catalyze the phosphorylation of PtdIns-4,5-P_2 to generate PtdIns-3,4,5-P_3 (Figure 1). This phospholipid acts as a second messenger to recruit cytoplasmic proteins to specific plasma membrane or endomembrane locations. The p110α and p110β proteins are expressed ubiquitously, whereas expression of p110γ and p110δ is enriched in immune cells. Each catalytic isoform forms a dimer with a regulatory subunit that modulates the activity and subcellular localization of the complex (Figure 1). In normal cells, PtdIns-3,4,5-P_3 is induced transiently by growth factor stimulation and is rapidly metabolized by lipid phosphatases, including the tumor suppressor PTEN, which terminates PI3K signaling via removal of the 3′-phosphate from PtdIns-3,4,5-P_3. Cancer cells

frequently contain elevated amounts of PtdIns-3,4,5-P_3 due to increased activity of oncogenic signaling proteins residing upstream of PI3K, or to mutational activation of PI3K itself. Many cancers also exhibit loss of PTEN function, which elevates basal and stimulated PtdIns-3,4,5-P_3 abundance by reducing the turnover rate of this second messenger. In a meta-analysis of cancer genome sequencing studies, *PIK3CA* and *PTEN* were found to be the second and third most highly mutated genes in human cancers (Lawrence et al., 2014).

Activation of Class I PI3Ks occurs through multiple upstream pathways that couple a broad range of cell surface receptors to specific PI3K isoforms. Generally, PI3Ks are capable of being activated by receptor-coupled tyrosine kinase activities, small Ras-related GTPases, and heterotrimeric G proteins. Each class I isoform has a domain that interacts with members of the Ras GTPase superfamily (Figure 1). For p110α, p110γ and p110δ, this domain binds to Ras or R-ras subfamily members whereas p110β interacts with the Rac/cdc42 subfamily. Three of the class I catalytic isoforms (For p110α, β and δ; collectively known as the class IA subgroup) associate with regulatory subunits whose SH2 domains bind to phosphotyrosyl residues on growth factor receptors or adaptor proteins such as IRS1. The other catalytic isoform (p110γ; known as class IB) associates with regulatory subunits (p101, p87) that mediate binding to βγ subunits of heterotrimeric G proteins following activation of G protein-coupled receptors (GPCRs). Adding to this complexity, the p110β isoform contains a Gβγ binding site that enables this isoform to be a coincidence detector for GPCR and tyrosine kinase signaling (Houslay et al., 2016). Through an unknown mechanism, p110δ in B lymphocytes is activated by chemokine receptors, which are members of the GPCR family. In murine macrophages, p110γ can be activated downstream of tyrosine kinases as well as GPCRs (Schmid et al., 2011). In summary, GPCRs and RTKs exhibit considerable plasticity in terms of coupling to the various Class I PI3Ks, determined in part by the cellular context.

Structural and biophysical studies have clarified the mechanisms of activation of different class I isoforms (Backer, 2010; Burke and Williams, 2015). As an example, we will discuss the p110α isoform and its activation by physiological signals as well as by cancer-associated *PIK3CA* mutations. p110α associates with one of five different regulatory subunits (p85α, p55α, p50α, encoded by *PIK3R1*; p85β, *PIK3R2*; p55γ, *PIK3R3*). Each of these subunits contains two SH2 domains (N-SH2, C-SH2) flanking a coiled-coil region known as the inter-SH2 (iSH2) domain (Figure 1). The catalytic and regulatory subunits make additional contacts that maintain the enzyme in a low activity state under basal conditions. The helical, kinase and C2 domains of the catalytic subunit contact the p85-N-SH2 domain; the C2 domain also contacts the p85-iSH2 domain. Binding of the regulatory subunit's SH2 domains to phosphotyrosines relieves these inhibitory contacts and positions the dimer near the membrane where it can access substrate and receive further inputs from Ras and other signaling components.

Activating PI3K mutations in cancer, immune deficiency and tissue overgrowth

Many distinct *PIK3CA* activating mutations have been identified in human tumors. The two most common mutation "hotspots" are H1047R and E542K/E545K (Figure 1). The H1047R mutation enhances interaction of the kinase domain with membranes and bypasses the

requirement for association with Ras (Burke and Williams, 2015). In contrast, E542K and E545K mutations disrupt the inhibitory interface with the N-SH2 domains of the regulatory subunits (Burke et al., 2012; Miled et al., 2007). Other less common *PIK3CA* mutations (e.g. N345K, C420R) disrupt the interface of the C2 domain with iSH2. Tumor-associated mutations in other class I PI3K genes are very rare. However, C-terminal truncation and deletion mutants that disrupt part of the iSH2 domains of regulatory subunits (encoded by *PIK3R1* and *PIK3R2*) are oncogenic and frequently occur in brain and endometrial cancers (Figure 1). Transformation by these regulatory subunit variants requires activation of the p110α catalytic isoform (Sun et al., 2010).

Some cancer-associated *PIK3CA* mutations can also occur during development, and result in mosaic tissue overgrowth syndromes, venous malformations and brain malformations associated with severe epilepsy (Kurek et al., 2012). Analogous activating mutations in *PIK3CD* encoding p110δ (E1021K, E525K, N334K) have been identified in approximately 100 patients worldwide (Lucas et al., 2016), with distinct mutations affecting other domains discovered recently (Heurtier et al., 2017). Affected individuals suffer from a dominant immunodeficiency disorder termed Activated PI3K-Delta Syndrome (APDS). Disease-causing *PIK3CD* mutations elevate basal activity and membrane binding of p110δ, the dominant class I isoform in lymphocytes. Another subgroup of patients harbor *PIK3R1* germline deletions and develop an immunodeficiency termed APDS2, based on its clinical similarity to the syndrome in patients with activating *PIK3CD* mutations. Interestingly, the most common *PI3KR1* deletion in APDS2 selectively enhances basal activity of p85α/p110δ complexes, relative to p85α/p110α complexes (Dornan et al., 2017). This finding helps to explain why APDS2 patients do not have elevated risk of solid tumors associated with expression of mutationally-activated p110α.

PI3K effectors

The most proximal outcome of PtdIns-3,4,5-P_3 production by class I PI3Ks is the recruitment of specific proteins to membrane signaling complexes. The shared property of these PI3K effectors is a pleckstrin homology (PH) domain selective for PtdIns-3,4,5-P_3 and/or PtdIns-3,4-P_2. Within the family of PI3K effectors are subsets with distinct enzymatic or signaling functions. These include serine/threonine kinases of the AGC kinase family, tyrosine kinases of the TEC (tyrosine kinase expressed in hepatocellular carcinoma) family, and modulators of small GTPase activities, termed guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) (Figure 1). In this way, multiple, diverging downstream pathways can be simultaneously triggered by PI3K activation. A few canonical examples are discussed below.

Compared to other effectors, members of the AKT sub-family of AGC serine/threonine kinases (AKT1, AKT2, AKT3) seem to be activated more universally downstream of receptor-mediated PI3K activation. In fact, AKT phosphorylation often serves as a surrogate readout of class I PI3K activation. This tight coupling of PI3K to AKT is likely the result of two factors. First, phosphorylation of the AKT activation loop (Thr 308 on AKT1) occurs through a relatively straightforward mechanism, involving dual recruitment to the plasma membrane of AKT and its upstream activating kinase, phosphoinositide-dependent kinase-1

(PDK-1) activation (Manning and Toker, 2017). Membrane colocalization of the constitutively active PDK-1 with AKT facilitates PDK1-mediated phosphorylation of AKT. Second, the PH domains of both AKT and PDK-1 have affinity for both PtdIns-3,4,5-P_3 and PtdIns-3,4-P_2. The latter lipid can be produced from PtdIns-3,4,5-P_3 by SHIP-1 and SHIP-2 (Figure 1), is often sustained after a transient peak of PtdIns-3,4,5-P_3, and may promote AKT activation at endomembranes (Manning and Toker, 2017).

Although AKT phosphorylation on Thr 308 is both necessary and sufficient to mediate many downstream events, additional phosphorylation sites control substrate selectivity, stability and possibly subcellular localization (Manning and Toker, 2017). mTOR complex-2 (mTORC2) phosphorylates Ser 473 of the AKT hydrophobic motif (Sarbassov et al., 2005); this modification promotes maximal AKT activity and seems particularly important for a subset of substrates including Forkhead Box, subgroup O (FOXO) transcription factors (Jacinto et al., 2006). The mechanisms by which mTORC2 is activated to phosphorylate AKT have not been fully resolved (Ebner et al., 2017; Liu et al., 2015).

AKT phosphorylates many substrates involved in cell proliferation, metabolism, survival and motility (Manning and Toker, 2017). Mutations in the PH domain that promote membrane localization occur frequently in cancer (e.g. AKT1-E17K in 4–8% of breast cancer patients), supporting the idea that AKT is an important PI3K effector in oncogenic signaling. Notably, AKT plays an evolutionarily conserved role in growth factor signaling downstream of PI3K. In *C. elegans*, two AKT orthologs act downstream of an insulin receptor homolog (DAF2) and PI3K (AGE1) to suppress activity of DAF-16, a transcription factor homologous to human FOXO proteins. Likewise, the response to insulin in mammalian cells involves AKT-mediated inactivation of FOXO-dependent transcription.

TEC family tyrosine kinases are key PI3K effectors in lymphocytes. BTK, ITK and TEC all possess PH domains with exquisite selectivity for PtdIns-3,4,5-P_3. A key function is to phosphorylate phospholipase C to promote hydrolysis of PtdIns-4,5-P_2. Although a TEC homolog exists in flies (Tec29), the expansion of this kinase family in vertebrates and prominent expression in lymphoid cells is consistent with crucial roles in adaptive immunity. Indeed, humans lacking the *BTK* gene or with mutations affecting the BTK PH domain have a profound block in B cell development and fail to produce antibodies, a genetic immunodeficiency known as X-linked agammaglobulinemia. There is a strong link between PI3K and BTK function in B cells, first shown by knockout studies in mice where deletion of *Pik3r1* or *Pik3cd* caused defects in B cell development and survival similar to those in mice lacking BTK (Deane and Fruman, 2004). More recently, pharmacological inhibitors of BTK (ibrutinib; Imbruvica® and ACP-196) and p110δ (idelalisib; Zydelig®) have shown a strong convergence of clinical activity in cancer, with best responses in malignancies of mature B cells (Fruman and Cantley, 2014).

TEC family kinases including BTK are expressed in various leukocyte subsets (mast cells, macrophages) where they function downstream of Fc receptors. Elucidation of these functions has led to an appreciation that BTK inhibitors have potential utility in solid tumors by disrupting the supportive roles of macrophages (Gunderson et al., 2016).

GEFs for Rho/Rac/cdc42 family GTPases are less widely appreciated, but nonetheless critical effectors of class I PI3K signaling. These small GTPases are regulated by many GEFs, of which only a subset bear PH domains with selectivity for PtdIns-3,4,5-P_3. In neutrophils, P-Rex1 is a signal integrator activated by βγ subunits of heterotrimeric G proteins together with PtdIns-3,4,5-P_3 produced by p110γ. P-Rex1 stimulates a GTPase cascade involving RhoG and Rac, leading to activation of the NADPH oxidase as well as neutrophil migration (Damoulakis et al., 2014; Welch et al., 2002) (Figure 1). In cancer cells and growth factor-stimulated fibroblasts, PI3K activation drives Rac-mediated actin re-organization. In addition to modulating cell morphology and motility, this PI3K/Rac signaling axis drives increases in glycolytic flux through the release of aldolase from actin filaments (Hu et al., 2016) (Figure 1).

The last PI3K effector we will discuss in this section is mTOR. This serine-threonine kinase forms two cellular complexes known as mTORC1 and mTORC2, with distinct subunit composition and substrate selectivity (Figure 2) (Saxton and Sabatini, 2017). Apart from AKT-Ser473, established substrates of mTORC2 include analogous sites in serum- and glucocorticoid regulated kinases (SGKs) and protein kinase C (PKC) isoforms. mTORC1 phosphorylates numerous substrates that promote anabolic metabolism to support cell growth and proliferation. mTORC1 activity can be increased by mitogenic signals through PI3K/AKT, RAS/ERK and other pathways (Dibble and Cantley, 2015), but also requires coordinate signals delivered through nutrient sensing pathways (Saxton and Sabatini, 2017). Thus, mTORC1 represents a key signaling node that coordinates anabolic metabolism and cell mass accumulation with growth factor receptor stimulation and nutrient availability.

Rapamycin is a bacterially-derived product that binds to intracellular FKBP12, thereby generating a complex that binds to mTORC1 at an allosteric site, termed the FKBP12-rapamycin binding (FRB) domain. Importantly, although rapamycin is exquisitely selective for mTORC1, this drug has differential effects on the phosphorylation of distinct mTORC1 substrates (Figure 2). ATP-competitive mTOR kinase inhibitors (TORKi) fully suppress kinase activity of both mTORC1 and mTORC2 without affecting integrity of the complexes (Figure 2). Comparisons of rapamycin and TORKi have provided valuable insights into the function of mTOR complexes and their substrates.

The direct mTORC1 substrate S6 kinase-1 (S6K1) contributes to metabolic reprogramming by increasing glycolysis and protein, lipid, and nucleotide biosynthesis (Figure 2) (Magnuson et al., 2012). mTORC1 also initiates powerful negative feedback regulation of growth factor receptor signaling, such that inhibition of mTORC1 or S6K1 leads to elevated activation of PI3K, AKT and the ERK pathway (Carracedo et al., 2008; Saxton and Sabatini, 2017). S6K1 is highly sensitive to inhibition by rapamycin, and the disruption of S6K1-mediated negative feedback might contribute to limited efficacy of rapamycin and its derivatives (termed rapalogs) in cancer.

The eukaryotic initiation factor-4E (eIF4E)-binding proteins (4E-BPs) are key mTORC1 substrates that control cell proliferation and survival. Phosphorylation of 4E-BPs by mTORC1 inhibits their binding to eIF4E, enabling assembly of the latter with eIF4G and eIF4A to form an active, cap-binding translation initiation complex known as eIF4F. Among

cap-dependent mRNA transcripts, those that are more sensitive to decreased eIF4F activity are enriched in cell cycle and survival factors. Pharmacological and genetic studies have validated eIF4F as an oncogenic node and targetable vulnerability in cancer cells (Malka-Mahieu et al., 2017). Importantly, 4E-BP phosphorylation is inhibited to a greater extent by TORKi than by rapamycin (Figure 2), and the more penetrating inhibition of translation initiation by TORKi contributes to the more profound inhibition of cell growth and proliferation by these agents.

The PI3K pathway in cellular and organismal metabolism

PI3K signaling is evolutionarily conserved among multicellular organisms as a mechanism to respond to external growth cues. In mammals PI3K signaling is activated downstream of a myriad of growth factor receptors, including PDGF receptor (PDGFR) and epidermal growth factor receptor (EGFR), which drive proliferation and migration, insulin-like growth factor receptor (IGFR) which stimulates growth and survival, and insulin receptor (INSR) which regulates metabolic homeostasis. To coordinate responses to extracellular queues, the effectors of PI3K need to alter multiple facets of the cell, e.g. signaling that drives cell cycle progression also generates increased demand for metabolic programs to produce the energy and macromolecular synthesis to support cell growth and mitotic cell division. In order to meet these biosynthetic requirements, the PI3K/AKT/mTOR network must orchestrate a complex set of metabolic responses in the host cell.

Upon growth factor stimulation, receptor tyrosine kinases undergo conformational changes allowing them to autophosphorylate and become active. INSR autophosphorylation recruits the insulin receptor substrate (IRS) proteins, which the INSR phosphorylates on several sites to generate an optimal binding motif for the SH2 domains of p85 (Cantley and Songyang, 1994).

The immediate effect of insulin-driven PI3K signaling in muscle and fat cells is an increase in glucose uptake, attributable to enhanced glucose transporter translocation to the membrane (Huang and Czech, 2007) as well as increases in transcription and translation of the genes encoding these transporters (Lien et al., 2016). In muscle and adipose tissue, AKT2 is the primary isoform that phosphorylates and inhibits the function of the RabGAP, AS160, which allows intracellular vesicles containing the glucose transporter GLUT4 to migrate to the plasma membrane (Yuasa et al., 2009). This sequence of events supports enhanced glucose uptake into these tissues within minutes of serum insulin elevation. Most other tissues rely on increased insulin or IGF1-dependent transcription and translation of GLUT1 or other glucose transporters to increase glucose uptake, a much slower process. PI3K signaling has also been implicated in GLUT1 translocation to the cell membrane; however the mechanism for this translocation has not been fully elucidated (Bentley et al., 2003; Rathmell et al., 2003). Since GLUT1 is the major glucose transporter in many cancers, understanding how PI3K contributes to GLUT1 translocation may have clinical significance as oncologists attempt to modulate cancer cell metabolism as a therapeutic strategy. PI3K signaling controls transcription of GLUT1 through multiple mechanisms, including activation of mTORC1 that indirectly elevates the expression of HIF1α (Thomas et al., 2006; Wieman et al., 2007), and c-Myc (Osthus et al., 2000), transcription factors that

drive the expression of genes involved in glucose metabolism, including *SLC2A1* encoding GLUT1. AKT can also acutely stimulate glucose uptake by phosphorylating the adaptor protein TXNIP, reducing endocytosis of glucose transporters GLUT1 and GLUT4 (Waldhart et al., 2017).

AKT also enhances glucose metabolism by phosphorylating hexokinase 2 to facilitate its association with voltage-dependent anion channels at the mitochondrial membrane (Roberts et al., 2013) and indirectly by activating PFKFB2 which generates fructose 2,6-bisphosphate, an allosteric activator of PFK1 (Deprez et al., 1997). PI3K and AKT regulate other aspects of cellular metabolism through the activation of mTORC1. The activation of S6K1/2 and inhibition of 4EBP1 by mTORC1 (Figure 2) drives anabolic processes including protein and nucleotide synthesis as well as transcriptional activation of genes encoding enzymes of glycolysis and the pentose phosphate pathway (Dibble and Cantley, 2015). Both PI3K/AKT and mTORC1 promote lipid synthesis through activation of SREBP1 and SREBP2 transcription factors (Düvel et al., 2010; Porstmann et al., 2008).

A variety of negative feedback loops have evolved to maintain homeostasis of the PI3K/mTOR signaling pathway, to ensure that cells do not attempt to grow under conditions of energy stress or nutrient starvation and to protect multicellular organisms from localized tissue overgrowth. For example, when mTORC1 is highly active, it phosphorylates and stabilizes the adaptor protein GRB10, which binds and down-regulates the insulin receptor (Hsu et al., 2011; Yu et al., 2011). Variants of the *GRB10* gene were implicated in type 2 diabetes (Prokopenko et al., 2014).

PI3K activity also initiates AKT-independent signaling cascades to impact cellular metabolism. In a study examining the mechanism by which PI3K inhibitors impact the progression of *BRCA1/TP53* mutant tumors, Juvekar et al. demonstrated that synthesis of ribose and a set of glycolytic intermediates were impaired in response to PI3K inhibition, with lesser effects of AKT inhibitors (Juvekar et al., 2012). This result was dependent on the release of aldolase from F-Actin filaments mediated by PI3K-dependent Rac activation and consequent remodeling of the cytoskeleton. The aldolase-dependent increase in glyceraldehyde-3-phosphate provides a mechanism for increased ribose synthesis via the non-oxidative pentose phosphate pathway, allowing cells to generate the RNA and DNA needed for cell growth and proliferation (Hu et al., 2016). Thus, cytoskeletal remodeling driven by the PI3K-Rac axis drives not only cell motility but also metabolic reprogramming downstream of growth factor receptors. The role of PI3K signaling in effecting cytoskeletal remodeling is further highlighted by the recent identification of NT5C as a novel AKT substrate with a role in cytoskeletal remodeling that is mediated through interaction with ARP2/3 (Moniz et al., 2017).

p110α mediates most tissue responses to insulin and IGF1, driving tissue growth and maintaining glucose homeostasis throughout development (Engelman et al., 2006; Foukas et al., 2006). Early studies in mice demonstrated that the genes encoding class IA regulatory subunits (*Pik3r1* and *Pik3r2*, encoding p85α and p85β) or the p110 catalytic subunits (*Pik3ca*, encoding p110α) play complex roles in insulin response in muscle and liver (Engelman et al., 2006). These studies reveal that insulin-dependent growth of heart and

skeletal muscle is mediated by p110α (Engelman et al., 2006). Together the data show that the p85 regulatory subunit has both positive and negative regulatory functions in insulin signaling, as p85 is required for p110 stability and function, but suppresses insulin signaling in some tissues when in excess over p110 (Luo et al., 2005a). Suppression of insulin signaling by p85 subunits appears to occur by multiple mechanisms, including through the sequestration of IRS-1 and via activation of PTEN and JNK (Luo et al., 2005b, 2006; Taniguchi et al., 2006).

Keeping the complex PI3K-AKT-mTOR network homeostatically balanced is critical to prevent aberrant cellular proliferation and to maintain glucose homeostasis. This is highlighted by the deleterious impact of sporadic activating mutations in *PIK3CA*, as well as other genes in the PI3K-AKT-mTOR pathway, which arise during early embryonic development and give rise to mosaic tissue overgrowth syndromes including CLOVES (congenital lipomatous overgrowth, vascular malformations, and epidermal nevi) (Kurek et al., 2012), as discussed below. Reciprocally, inhibitory point mutations affecting the C-SH2 domain of p85α (primarily R649W; Figure 1) result in a dominant growth defect known as SHORT (short stature, hyperextensibility of joints, ocular depression, Rieger anomaly, and teething delay) Syndrome. In addition to developmental abnormalities and short stature, these patients have characteristics of Type 1 diabetes, without defects in insulin production or the insulin receptor, but as the result of impaired ability of insulin to activate PI3K (Chudasama et al., 2013). The underlying mechanism for this has not yet been established; however, the contrast of the severe phenotypes of these patients with the earlier mouse models of complete p85α deletion that demonstrated increased insulin sensitivity (Fruman et al., 2000a; Terauchi et al., 1999) highlights our incomplete understanding of the intricacies of this signaling network. The existence of patients with these phenotypes confirms the critical role of PI3K signaling downstream of IR/IGFR1 both for regulating metabolism as well as controlling cellular growth and proliferation.

Alterations leading to dysregulated insulin/PI3K signaling result in highly complex pathologies at the organismal level. In a normal setting, increases in blood glucose (typically from eating) will induce the pancreas to release insulin, thereby signaling to muscle and fat to take up more glucose, and to the liver to suppress glucose release until the system is brought back into homeostasis (Hopkins et al., 2016). If this pathway is perturbed, as is the case in patients with insulin resistance, the resulting, persistent hyperglycemia can lead serious, multi-organ pathology and even death. Obesity, which is usually caused by excessive food intake, frequently results in insulin resistance and is associated with an array of other metabolic changes correlated with increased cancer risk. However, it is difficult to draw direct connections between any one of the multiple concurrent changes that occur with obesity as many of them - increased inflammation, hyper-insulinemia, and changes in hormone signaling - have been shown to promote cancer. Although excessive PI3K signaling is a hallmark of cancer cells, too little PI3K signaling in the liver and muscle can lead to insulin resistance and type 2 diabetes. Understanding at the molecular level how PI3K signaling is maintained in tumors at the same time that PI3K signaling in muscle and liver is suppressed (insulin resistance) could facilitate the development of new therapies that target the dysregulation of PI3K/insulin signaling in tumors without disrupting normal tissues, such as the development of drugs that specifically target mutant isoforms of oncogenic

proteins (e.g. p110α with mutant H1047R) thus sparing endogenous signaling molecules which are critical for the maintenance of normal homeostasis.

One key consideration in targeting PI3K or AKT for cancer treatment is how to manage the on-target toxicity to systemic metabolism. The PI3K gene that is most commonly mutated in human cancers, *PIK3CA*, is also the gene that encodes the isoform of PI3K (p110α) that mediates insulin responses in muscle, liver and fat. Because most p110α inhibitors that have entered clinical trials for solid tumors inhibit both the mutant and wild type p110α at therapeutic doses, these drugs induce acute insulin resistance, resulting in severe hyperglycemia, which, in turn, leads to severe hyperinsulinemia. In tumors that express IR, this systemic feedback may play a significant role to limit the therapeutic efficacy of these compounds (Figure 3), particularly in the setting of patients who are already insulin resistant (Gallagher et al., 2012). In the clinic, prolonged hyperglycemia caused by long term treatment with PI3K inhibitors is typically managed with biguanides (Bendell et al., 2012) that increase systemic insulin sensitivity and reduce basal blood glucose and insulin levels, though systemic insulin is typically still elevated, and is likely to compromise therapeutic responses to PI3K inhibitors. Moving forward, the clinical success of PI3K targeted therapies may be dependent on either identifying patient populations for whom the systemic metabolic impact of these compounds will not inhibit the therapeutic efficacy, perhaps patients whose tumors do not express IR/IGFR, or implementing new ways to limit the hyperinsulinemia through diet and drug combinations. Alternatively, drugs that have higher selectivity for mutant versus wild type PI3K could circumvent this systemic feedback. Both of these approaches would be expected to reduce the hyperglycemia and systemic metabolic disruptions that occur due to the on-target effects of compounds that inhibit the PI3K signaling cascade.

PI3K in innate and adaptive immunity

General concepts

Host defense in vertebrates is mediated by secreted proteins (including antibodies, complement, anti-microbial peptides) and by a diverse array of leukocytes with distinct functions. Each cell type of the immune system expresses receptors that elicit cellular responses in part through activation of class I PI3Ks. Notably, the study of PI3K signaling in leukocytes has revealed a number of important distinctions from other cellular systems. A central difference is that p110γ and p110δ are the dominant class I isoforms that produce PtdIns-3,4,5-P_3 following receptor engagement (Okkenhaug, 2013). p110α and p110β are expressed in immune cells but their roles are restricted (Kulkarni et al., 2011; Ramadani et al., 2010). A second distinct feature is that, unlike growth factor receptor signaling, PI3K activation in leukocytes is not always an “on switch” that promotes a more powerful immune response. Depending on the receptor, the cell type, and the degree of PI3K activation, this pathway can activate or dampen responses, or skew cellular differentiation fates. A third key difference is the wiring of signaling cascades downstream of PtdIns-3,4,5-P_3 production. For example, for some lymphocyte responses TEC family kinases seem to play a greater role than AKT in PI3K signaling output, and mTORC1 is more dependent on nutrient inputs than on PI3K/AKT activity. Each of these distinctions has important implications when

considering the action of PI3K-mTOR pathway inhibitors on the immune system. Before elaborating further on the complexity of PI3K signaling in leukocytes, we will summarize some general concepts in host defense and tumor immunity.

Immune responses are generally categorized in terms of two broad sets of responses, denoted as innate immunity or adaptive immunity. Cells of the innate immune system provide a first line of defense that acts quickly via receptors with invariant ligand specificity, but lacks specificity or memory for pathogens. The adaptive immune system involves a diverse repertoire of lymphocyte clones (T and B cells) each with a unique antigen receptor; clonal expansion and differentiation provides delayed but powerful antigen-specific immunity that can last a lifetime. Adaptive immunity requires prior activation of innate immune cells, usually dendritic cells (DCs), by pathogen-associated molecular patterns that induce the DCs to migrate to lymph nodes where they present antigen and provide costimulation to T cells. In turn, lymphocytes differentiate and produce factors (cytokines and antibodies) that enhance pathogen destruction by innate immune cells and complement proteins. In addition to clonally diverse T and B cells, there exist multiple lymphocyte subsets with innate immune cell-like properties, including natural killer (NK) cells, γδ T cells, B-1 B cells, and innate lymphoid cells (ILCs). Conversely, mast cells are usually categorized as an innate immune cell yet these cells are activated to degranulate by diverse antigens, recognized by cell surface-bound IgE antibodies.

Although the primary role of vertebrate immune systems is to detect and destroy pathogens, both innate and adaptive arms also protect from cancer. Cytotoxic T cells can recognize and kill tumor cells presenting neo-antigen peptides on class I major histocompatibility complex (MHC) molecules. NK cells can kill tumor cells that downregulate class I MHC and/or upregulate stress ligands, or tumor cells coated with antibodies bound to cell surface tumor antigens. There is ample evidence from both mouse and humans that intrinsic or drug-induced immunosuppression increases cancer incidence. Furthermore, anti-cancer immune responses exert selective pressure on heterogeneous tumor cells, a process known as immunoediting, such that cells capable of evading innate and/or adaptive immune responses are selected during tumor evolution. Escape mechanisms include loss of tumor antigens, expression of checkpoint receptor ligands, secretion of suppressive cytokines, sequestration of nutrients, recruitment of various immunoregulatory cell types, and creation of physical barriers. In recent years multiple strategies have emerged to boost the immune system's ability to detect and destroy tumor cells. Several immunotherapies have been approved for clinical use in a broad range of cancers, with many more in clinical trials. To broaden and deepen clinical responses, there is considerable interest in combining immunotherapies with small molecule targeted agents. Thus, defining the impact of PI3K-mTOR pathway inhibitors on immune cell subsets is essential to designing effective combinations for cancer treatment, and for expanding the application of these agents to immune disorders.

PI3K in innate immunity

Inflammation is an immediate response to pathogen detection by the innate immune system. Production of chemokines by macrophages in the infected tissue, together with complement fragments C3 and C5, increase local vascular permeability and attract neutrophils. Both

resident macrophages and infiltrating neutrophils phagocytose bacteria via a variety of cell surface receptors. Neutrophils activate an intracellular NADPH oxidase that produces reactive oxygen species (ROS) to kill engulfed bacteria. Each of these processes requires class I PI3K activation (Hawkins and Stephens, 2015).

Most chemoattractants bind G protein-coupled receptors (GPCRs) that activate p110γ (Hawkins and Stephens, 2015). PtdIns-3,4,5-P_3 recruits the guanine nucleotide exchange factor p-Rex-1 to activate a G protein cascade of Rho and Rac GTPases leading to cytoskeletal remodeling and ROS production (Damoulakis et al., 2014). Evidence that p110γ inactivation suppresses these responses led to initial excitement about developing p110γ inhibitors for inflammatory disease (Rückle et al., 2006). However, initial challenges for discovery of highly selective compounds and concerns about maintaining host defense have slowed progress for p110γ inhibitors in this therapeutic area. Instead, exciting evidence that p110γ inhibitors can reprogram the immune milieu in tumors (discussed below) has led to clinical trials in cancer of a selective p110γ inhibitor (IPI-549) combined with immunotherapy (De Henau et al., 2016). Given the key functions of p110γ in neutrophils, it will be important to carefully monitor frequencies of bacterial infections in cancer patients enrolled in p110γ inhibitor trials.

The p110δ and p110β catalytic isoforms also contribute to cellular responses promoting inflammation. After GPCR triggering of p110γ in human neutrophils, the subsequent activation of p110δ is needed to sustain NADPH oxidase activity (Condliffe et al., 2005). Both p110δ and p110β regulate the spreading of neutrophils and macrophages on extracellular matrix and the engulfment of IgG-opsonized particles. p110β plays a dominant role in ROS production by neutrophils recognizing immobilized immune complexes, and p110β-deficient mice were resistant to immune complex-mediated inflammation *in vivo* (Kulkarni et al., 2011). In microglia, p110δ is expressed and required for efficient release of TNFα in response to glucose deprivation and restoration, an *in vitro* correlate of ischemic stroke. In an *in vivo* mouse model of ischemia and reperfusion, selective inhibition of p110δ reduced cerebral damage and improved neurological outcome (Low et al., 2014). There is also evidence that p110δ inhibition is effective in mouse models of chronic obstructive pulmonary disease (Marwick et al., 2010), and clinical trials of a p110δ inhibitor (GSK2269557) are underway in this disease.

An evolutionarily conserved mechanism by which innate immune cell recognize pathogen-associated molecular patterns (PAMPs) is via Toll-like receptors (TLRs). TLR engagement activates NFκB and interferon-regulatory factors to induce transcriptional changes needed for immune responses. TLRs also augment PI3K-mTOR pathway activity, with either positive or negative regulatory consequences in different TLR signaling contexts. During innate antiviral responses triggered by TLR7 and TLR9 in plasmacytoid dendritic cells, the PI3K-mTOR pathway has a primarily positive role in type I interferon (IFNα and IFNβ) production (Costa-Mattioli and Sonenberg, 2008). Selective inhibitors of p110δ or mTORC1 suppress type I IFN production, and both S6Ks and 4E-BPs have been implicated in this process. Despite these findings, it is not yet apparent whether human patients treated with PI3K or mTOR inhibitors have specific impairments in virus-induced interferon production.

Innate receptors for bacterial cell wall components include TLR2 and TLR4; TLR5 recognizes the conserved flagellin protein complex. Engagement of these TLRs on myeloid cells (macrophages, monocytes and conventional DCs) promotes production of pro-inflammatory cytokines including IL-1, TNFα, and IL-12 that are balanced by production of anti-inflammatory products including IL-10. Interestingly, in many contexts PI3K-mTOR pathway activation by TLRs serves to attenuate the inflammatory response (Weichhart et al., 2015). Consequently, PI3K-mTOR pathway inhibitor treatment of mouse and human myeloid cells increases transcription of genes encoding inflammatory cytokines, decreases IL-10 production, and enhances their capacity to prime T cells. The PI3K-mTOR pathway antagonizes TLR signaling, in part by promoting STAT3 activity while suppressing the pro-inflammatory NFκB-mediated transcriptional program. In addition, p110δ has an isoform-specific role in LPS responses by promoting internalization of TLR4 and dissociation of the adaptor protein TIRAP (Aksoy et al., 2012). Interestingly, hyperactivation of mTORC1 signaling in macrophages lacking TSC2 suppresses NFκB function and drives metabolic reprogramming, loss of quiescence, and macrophage proliferation leading to formation of granulomas (Linke et al., 2017).

The pro-inflammatory effect of PI3K-mTOR pathway inhibitors has several important implications. First, it likely contributes to common long-term side effects of rapamycin or rapalog treatment, namely mucositis and pneumonitis. Mucositis is also one of the main dose-limiting toxicities of the TORKi compounds (Table S1) AZD2014 (Basu et al., 2015) and CC-223 (Bendell et al., 2015a), and occurred frequently in a phase I trial of TAK-228 (Ghobrial et al., 2016). Mucositis was reported in phase I studies of pan-PI3K inhibitor buparlisib (BKM120) (Bendell et al., 2012; Ragon et al., 2017) and the dual PI3K/mTOR inhibitor BEZ235 (Bendell et al., 2015b; Carlo et al., 2016). The p110δ inhibitor idelalisib is associated with autoimmune colitis as discussed below, but also with lung and liver inflammation (Coutré et al., 2015) that might arise from increased innate immune stimulation. Thus, a significant impediment to clinical application of PI3K-mTOR pathway inhibitors is the development of inflammatory conditions.

On the other hand, the pro-inflammatory potential of PI3K-mTOR pathway inhibitors is potentially advantageous for the immunotherapy of cancer. Many solid tumors have a resident population of tumor-associated macrophages (TAMs), and a pro-inflammatory gene expression profile (high IL-12, interferon-γ) in several tumor types correlates with extended patient survival (Kaneda et al., 2016a). The PI3K catalytic isoform p110γ is abundantly expressed in TAMs and promotes a more immune suppressive phenotype characterized by expression of IL-10 and TGFβ (Kaneda et al., 2016a). Gene expression signatures associated with high p110γ expression in TAMs are associated with reduced patient survival (Kaneda et al., 2016a). Notably, genetic or pharmacological inhibition of p110γ delayed tumor growth in several mouse models, stimulated anti-tumor T cell responses and enhanced the efficacy of immune checkpoint blockade (De Henau et al., 2016; Kaneda et al., 2016a). In a separate study of pancreatic cancer, p110γ inhibition opposed tumor progression by augmenting CD8 T cell responses and reducing tumor cell invasion and the protective fibrosis (known as desmoplasia) characteristic of this disease (Kaneda et al., 2016b). It should be stressed that the p110γ isoform is not expressed in most solid tumors, and adoptive transfer studies confirmed the TAM-intrinsic role of p110γ in tumor

immunosuppression (Kaneda et al., 2016a, 2016b). Thus, the anti-tumor effect of p110γ inhibition occurs entirely via reprogramming the immune microenvironment. Another point of emphasis is that the anti-tumor effect of p110γ inhibition is relatively modest, but more dramatic when combined with immune checkpoint blockade.

Another scenario where the pro-inflammatory action of PI3K-mTOR pathway inhibitors has potential utility for cancer control is in the development of DC-based tumor vaccines. In murine DCs stimulated with flagellin in the presence of killed tumor cells, PI3K inhibitors suppressed production of IL-10 and TGFβ while preserving or enhancing IL-12 release (Marshall et al., 2012). In a tumor vaccine model, adoptive transfer of PI3K inhibitor-treated DCs enhanced anti-tumor efficacy and fostered the expansion of effector T cells secreting inflammatory cytokines IFNγ and IL-17 (Marshall et al., 2012). This type of approach holds promise for improving efficacy of DC-based vaccines while avoiding systemic administration of PI3K-mTOR pathway inhibitors. mTORC2 might play a key role downstream of PI3K in the programming of DC function, as injection of rictor-deficient DCs into B16 melanoma tumors stimulated T cell responses that slowed tumor growth (Raïch-Regué et al., 2016).

PI3K in adaptive immunity

The adaptive immune system is important for antigen-specific immune responses and for immunological memory to pathogens and vaccines. In addition, the balance of anti-tumor versus immunosuppressive lymphocyte subsets is a key factor in tumor progression and response to immunotherapies. Class I PI3K signaling is activated by antigen receptors expressed by T and B cells, and by other inputs including costimulatory molecules and cytokine receptors. Several comprehensive reviews have detailed how PI3K signaling is engaged by different receptors to regulate a variety of lymphocyte responses, and how genetic deficiency or hyperactivity of PI3K isoforms can both lead to immunodeficiency (Hawkins and Stephens, 2015; Lucas et al., 2016; Okkenhaug and Vanhaesebroeck, 2003). The roles of mTORC1 and mTORC2 in the metabolic programming and differentiation of effector lymphocytes is another topic that has been thoroughly reviewed (Chi, 2012; Jellusova and Rickert, 2016; Waickman and Powell, 2012). Here we will emphasize and discuss the distinct wiring of PI3K signaling networks in lymphocytes. Some of these differences help to explain the remarkable efficacy of p110δ-targeted inhibitors in B cell tumors, as well as the immunosuppressive mechanism of rapamycin.

The TEC family proteins BTK, ITK and TEC have key roles in PI3K signaling responses downstream of antigen receptors in T and B cells (Figure 4). These tyrosine kinases possess a PH domain for PtdIns-3,4,5-P_3-dependent membrane recruitment, along with SH2 and SH3 domains for protein-protein interactions. In B cells, engagement of the antigen receptor (B cell receptor; BCR) and co-receptor (CD19/CD21/CD81) triggers formation of signaling microclusters, also known as signalosomes, that drive activation of phospholipase Cγ, leading to production of diacylglycerol and inositol trisphosphate. These second messengers ultimately trigger Ca^{2+} mobilization and the activation of NFκB as well as the Ras-Raf-Mek-Erk pathway. Within the BCR signalosome, BTK and the PI3K p85α/p110δ dimer are required for maximal signaling output as measured by Ca^{2+} flux, IκB degradation and Erk

phosphorylation. Notably, BTK inhibition can reduce PI3K signaling output in B cells (Bojarczuk et al., 2016; Compagno et al., 2017; Saito et al., 2003), supporting the concept that PI3K and BTK cooperate in the signalosome rather than operating in a simple linear fashion. The functional link between BTK and p85α/p110δ is strongly supported by genetic evidence. Humans lacking BTK have X-linked agammaglobulinemia, an immunodeficiency syndrome associated with few mature B cells and a profound defect in antibody production. Similarly, rare autosomal recessive loss-of-function mutations in *PIK3R1* (p85α) or *PIK3CD* (p110δ) result in agammaglobulinemia (Conley et al., 2012; Zhang et al., 2013). In mice, loss of p85α or p110δ causes similar defects in B cell development and activation as those observed in B cells lacking BTK and TEC (which is partially redundant with BTK in mouse B cells). Consistent with the "signalosome" model of activation, inactivation of other BCR signaling components causes similar B cell phenotypes in mice and germline mutations cause agammaglobulinemia in humans (Fruman et al., 2000b).

p110δ and BTK also function in shared signaling pathways in human B cell tumors. This is supported by convergent clinical responses to selective inhibitors of p110δ (idelalisib, duvelisib) and BTK (ibrutinib, acalabrutinib) in chronic lymphocytic leukemia (CLL), as well as by *in vitro* studies of B cell leukemia and lymphoma cells. In CLL and activated B cell-type diffuse large B cell lymphoma, chronic BCR signaling drives cell survival that is reduced by p110δ or BTK inhibitors. PI3K and BTK also function downstream of other functionally important receptors including CD40 and receptors for chemokines. Chemokine signaling via PI3K and BTK drives migration towards stromal cells secreting pro-survival factors such as BAFF, and increased adhesion to these supportive cells and to extracellular matrix. As a consequence, most CLL patients treated with p110δ or BTK inhibitors experience rapid lymph node shrinkage that is mainly due to impaired chemokine-dependent homing and reduced retention of leukemia cells in lymph node niches.

In T cells, antigen receptor (T cell receptor; TCR) signaling also involves formation of signalosomes containing p110δ and TEC family kinases ITK and TEC. In mice, loss of ITK or p110δ causes some similarities in T cell phenotypes including reduced TCR-mediated Ca^{2+} flux and adhesion. CD4 T cells lacking p110δ or ITK display impaired Th2 responses (Miller et al., 2004; Nashed et al., 2007; Okkenhaug et al., 2006; Soond et al., 2012) and are resistant to Th2-driven asthma. However, an inhibitor of ITK kinase activity failed to protect in an asthma model and actually enhanced Th2-mediated inflammation (Sun et al., 2015). In addition, it has not been firmly established that PI3K activation by the TCR or other receptors on T cells is required for ITK/TEC activation, or vice versa. Notably, p110δ and TEC kinases also serve important functions downstream of Fc receptors in innate leukocytes including mast cells and macrophages. These findings suggested possible applications of p110δ inhibitors to ameliorate symptoms of allergy (Ali et al., 2004) and BTK inhibitors to reprogram the myeloid compartment of pancreatic tumors (Gunderson et al., 2016).

Another prominent feature of class I PI3K signaling in B and T cells is the importance of FOXO regulation by AKT (Figure 4). Combined loss of Akt1 and Akt2 recapitulates some B cell development phenotypes associated with loss of p85α or p110δ, particularly reduced numbers of marginal zone (MZ) and B-1 cells (Calamito et al., 2010). Conversely, deletion of *Foxo1* increased MZ B cell numbers and corrected the MZ B cell deficiency in mice

lacking CD19 (Chen et al., 2010). It is also important for AKT activity to be attenuated at various decision points in B cell development to allow FOXO-dependent transcriptional programming. For example, Foxo1 is required for expression of the IL-7 receptor in pro-B cells and *Rag* genes at the pre-B cell stage (Amin and Schlissel, 2008; Dengler et al., 2008). On the other hand, some level of PI3K activation by the pre-BCR is required for extinction of *Rag* gene expression and further developmental progression (Ramadani et al., 2010). Intermediate levels of PI3K/AKT signaling output also seem to be required for survival of B-cell acute lymphoblastic leukemia cells, as deletion of PTEN caused hyperactivation of AKT leading to p53-mediated cell death, in a process analogous to the physiological deletion of autoreactive immature B cells (Shojaee et al., 2016).

When B cells are activated by antigen, they undergo clonal expansion and differentiate to secrete antigen-specific antibodies of various classes. Some B cells quickly differentiate into plasmablasts, which mainly produce low-affinity IgM, while others adopt a germinal center fate to undergo class switch recombination (CSR) and somatic hypermutation (SHM), resulting in secretion of higher affinity class-switched antibodies. This differentiation decision depends in part on the level of PI3K/AKT signaling versus the activity of FOXO transcription factors (Limon and Fruman, 2012), which control expression of the *Aicda* gene encoding activation-induced cytidine deaminase (AID) (Dengler et al., 2008). Elevated PI3K signaling through the loss of PTEN strongly suppresses class switching while increasing the plasmablast fate (Omori et al., 2006). Class switching can be restored *in vitro* by expression of constitutively active Foxo1 or AID (Omori et al., 2006). Conversely, PI3Kδ inhibition increases AID expression and CSR while reducing plasmablast differentiation (Omori et al., 2006). Similarly, inactivation of mTORC2 or AKT promotes class switching in a FOXO-dependent manner (Limon et al., 2014).

Within the germinal center B cell compartment, cyclical changes in the activity of PI3K/AKT versus FOXO are essential for proper trafficking and differentiation. The architecture of germinal centers (GCs) includes dark and light zones defined by histological staining. GCB cells undergo cycles of movement between the dark zone where they proliferate rapidly and undergo CSR and SHM, and the light zone where they are selected for antigen binding affinity. In mouse GCs, PI3K activity is restricted to the light zone while nuclear Foxo1 is largely absent. A fraction of cells in the light zone that do express Foxo1 are destined for dark zone re-entry as Foxo1 is needed to instruct the dark zone gene program including the chemokine receptor *Cxcr4* (Dominguez-Sola et al., 2015; Sander et al., 2015). *Foxo1* deletion or increased PI3K activity lead to loss of architectural polarity and lack of dark zones while impairing SHM and class switching.

Dynamic changes in the balance between AKT and FOXO function are also important for the fate of $CD4^+$ and $CD8^+$ T cells. In resting T cells, FOXO transcription factors maintain expression of homing receptors that allow recirculation between blood and lymphoid tissue. Engagement of the TCR and costimulatory molecules activates PI3K/AKT to cause FOXO nuclear exit, thereby reprogramming homing receptor expression to favor lymph node exit and trafficking to infected tissue (Fabre et al., 2008; Kerdiles et al., 2009). In the context of T helper differentiation, the T follicular helper (T_{FH}) subset requires engagement of the costimulatory receptor, ICOS, which activates PI3K/AKT to suppress Foxo1-dependent gene

expression (Rolf et al., 2010; Stone et al., 2015). Foxo1 also plays a role in the differentiation of murine and human regulatory T cells (Tregs) (Hsu et al., 2015; Kerdiles et al., 2010); notably, Foxo1 activity must be finely tuned to preserve Treg trafficking and function (Luo et al., 2016). The decision of $CD8^+$ T cells to adopt effector or memory gene expression programs also depends on the balance of AKT and FOXO activity (Hess Michelini et al., 2013; Macintyre et al., 2011).

Accumulating evidence suggest that distinct differentiation fates are programmed at the first lymphocyte cell division via asymmetric partitioning of signaling proteins between daughter cells (Reiner and Adams, 2014). This was shown first in CD8+ T cells, where the first division results in one CD8-high daughter cell with high potential to proliferate and differentiate into cytotoxic effectors, and one CD8-low daughter cell destined to seed the memory CD8 T cell pool (Chang et al., 2007). A later study showed that the CD8-high daughter cells retain higher mTORC1 activity (Figure 5), which drives c-Myc expression to promote glycolytic metabolism required for the effector CD8 T cell fate (Verbist et al., 2016). Bifurcation of PI3K/mTORC1 activity also contributes to differentiation fates in B cells and CD4 T cells (Lin et al., 2015; Nish et al., 2017; Pollizzi et al., 2016) (Figure 5).

The wiring of mTORC1 signaling in lymphocytes has important differences from other frequently studied cell types. For example, activated lymphocytes frequently sustain mTORC1 signaling that is disconnected from PI3K/AKT activity (Figure 4). This was first observed in B-lymphoid tumor cells (Kharas et al., 2008; Wlodarski et al., 2005) and in mouse splenic B cell subsets (Donahue and Fruman, 2007), and later in $CD8^+$ T cells (Salmond et al., 2009). mTORC1 activity in B cells is highly dependent on nutrients *in vitro* (Donahue and Fruman, 2007; Wlodarski et al., 2005) and suppressed under hypoxic conditions in germinal centers (Cho et al., 2016; Jellusova et al., 2017). Similarly, in activated $CD8^+$ T cells, leucine uptake via the system L amino acid transporter Slc7a5 is required for mTORC1 activity, c-Myc translation and metabolic reprogramming (Sinclair et al., 2013) whereas PI3K/AKT signaling is dispensable for these outcomes (Macintyre et al., 2011). In cancer cells, ERK can phosphorylate TSC2 to promote mTORC1 activity but it is not clear whether this pathway is active in lymphocytes. However, ERK and RSK kinases provide a PI3K/AKT-independent input to S6 phosphorylation in TCR-activated $CD8^+$ cells (Salmond et al., 2009). In established IL-2-dependent $CD8^+$ effector T cells, mTORC1 activity is sustained by both JAK and SRC family tyrosine kinases, whereas PI3K/AKT activity is primarily dependent on Src family members (Ross et al., 2016).

Another indication of altered wiring of the mTOR network in lymphocytes is the role of mTORC1 effectors in cell growth and proliferation (Figure 4). In fibroblasts, mTORC1 promotes cell size increase mainly through S6Ks while driving cell cycle progression mainly through the 4E-BP/eIF4E axis (Dowling et al., 2010). In contrast, lymphocyte growth and proliferation are coupled through the 4E-BP/eIF4E axis while S6Ks are dispensable (So et al., 2016). The function of S6Ks in lymphocytes remains unclear but these kinases may regulate T helper cell differentiation (Kurebayashi et al., 2012; Pai et al., 2016; Sasaki et al., 2016). This convergence of signaling via 4E-BP/eIF4E might allow lymphocytes to more tightly couple cell mass accumulation to cell proliferation, to accommodate the extraordinarily rapid cell-doubling times of antigen-stimulated lymphocytes. Surprisingly,

eIF4E function is more rapamycin-sensitive in lymphocytes than in fibroblasts and many cancer cell types. This difference correlates with predominant expression of 4E-BP2, whose phosphorylation is more rapamycin-sensitive than 4E-BP1 on key mTORC1 phospho-sites (So et al., 2016). This 4E-BP isoform switch likely contributes to the stronger anti-proliferative effect of rapamycin in lymphocytes compared to other cell types. The central role of eIF4E in lymphocyte activation also is important to consider when developing eIF4E-targeted agents for cancer therapy.

While mTORC1 activity is essential for lymphocyte proliferation and effector subset differentiation, hyperactivation of mTORC1 in T or B cells lacking TSC1 impairs development, homeostasis and function (Jellusova and Rickert, 2016; Pollizzi et al., 2015). These observations support a unifying theme that PI3K/AKT/mTOR activation in lymphocytes is not an on/off switch for adaptive immunity. Instead, the degree of signaling output determines the outcome of B and T cell differentiation. A related concept is that the overall effect of PI3K inhibition *in vivo* is determined by opposing actions in different lymphocyte subsets. An informative example is provided by studies of the p110δ catalytic isoform. Inactivation of p110δ in T cells impairs differentiation of effector $CD4^+$ (Okkenhaug et al., 2006; Soond et al., 2010) and $CD8^+$ (Macintyre et al., 2011) T cells, yet also impairs Treg function (Ali et al., 2014; Patton et al., 2006). The Treg defect is likely responsible for autoimmune colitis that develops in p110δ-deficient mice (Okkenhaug et al., 2002; Patton et al., 2006). Likewise, diarrhea and colitis is a frequent side effect of idelalisib in human patients (Coutré et al., 2015). On the other hand, impaired Treg function has a beneficial outcome in the context of tumor immunity, where genetic or chemical inhibition of p110δ promoted tumor regression in several mouse models (Ahmad et al., 2017; Ali et al., 2014). The latter observation has raised interest in testing p110δ inhibitors to enhance immunotherapy response in solid tumors.

The phenotype of APDS patients also helps illustrate the opposing roles of p110δ in adaptive immune cells. Mutations in these patients elevate p110δ activity and promote lymphoproliferation. However, these persistently activated lymphocytes are prone to activation-induced cell death or senescence, and most patients have low IgG titers and poor vaccine responses consistent with impaired CSR and SHM. Thus, the overall outcome of hyperactivation of p110δ in lymphoid cells is a life-threatening immunodeficiency. Some APDS patients have been treated with rapalogs; there is hope that patients will benefit more from treatment with selective p110δ inhibitors, and clinical trials have been initiated (Table 1). Dosage and scheduling will need to be adjusted to minimize the inflammatory side effects of idelalisib described above. Additional concerns arose following regulatory approval of idelalisib, when some patients developed fatal infections. A phase 3 study of idelalisib with bendamustine and rituximab also reported an increased risk of infection including some deaths (Zelenetz et al., 2017). It is reasonable to propose that the infection risk is due to impaired $CD4^+$ and $CD8^+$ effector T cell differentiation.

Therapeutic targeting of the PI3K pathway in cancer

The recognition that PI3K signaling was aberrantly activated in the majority of human cancers, together with the presence of actionable target proteins in the PI3K/mTOR network,

spurred expectations that PI3K/mTOR pathway inhibitors would spawn a major paradigm shift in cancer therapy. It is now widely appreciated that the actual clinical results have fallen considerably short of this extremely high expectation. Three major factors have contributed to the underwhelming performance of the PI3K/mTOR pathway inhibitors. First, this pathway is activated via a myriad of cell surface receptors, and cancer cells have shown remarkable plasticity when it comes to amplifying upstream mechanisms to maintain signal flow through the PI3K/mTOR pathway and other compensatory pathways in the presence of pharmacological inhibitors. For example, exposure to the inhibitors themselves causes the disruption of negative feedback mechanisms that limit the activity of the pathway to a range compatible with normal cell physiology. Drug-induced interference with negative feedback regulation can reduce PI3K/mTOR pathway inhibitor therapeutic activities in the absence of genetic mutations, a phenomenon termed adaptive resistance. Second, intrinsic or acquired resistance to PI3K/mTOR pathway inhibitors is commonly associated with mutations or copy number alterations of regulatory genes within the pathway or parallel oncogenic pathways, or activation of growth factor receptors that stimulate both PI3K and MAPK signaling. Third, systemic administration of PI3K/mTOR pathway inhibitors is associated with dose-limiting toxicities that prevent sufficient target engagement in tumor tissues to maintain pathway suppression. To some extent, these challenges to effective therapy with PI3K/mTOR pathway inhibitors were anticipated based on the numerous roles that the PI3K/mTOR pathway plays in tissue growth, metabolism, and physiological functions.

Mechanisms of resistance to PI3K/mTOR pathway inhibitors

Adaptive resistance—Pharmacological inhibition of the PI3K pathway in cancer cell lines in culture is frequently followed, within hours to days, by the induction of adaptive (non-genetic) resistance mechanisms (Thorpe et al., 2015) (Figure 6). A well-established adaptive response that restores PI3K/mTOR pathway signaling in the presence of mTORC1-selective inhibitors involves the disruption of a S6K1-mediated feedback loop that destabilizes IRS-1. In the absence of mTORC2 inhibition, loss of this negative feedback leads to AKT hyperactivation (O'Reilly et al., 2006). Identification of this feedback loop and another involving adaptor protein Grb10 (Hsu et al., 2011; Yu et al., 2011) prompted support for the development of TORKi that target both mTORC1 and mTORC2. Cancer cells in culture also respond to PI3K/mTOR pathway inhibitors by "rebound" signaling driven in part by FOXO activity. One frequent mechanism involves increased transcription of genes encoding RTKs, most notably HER3, EGFR, and INSR/IGFR1 (Chakrabarty et al., 2012; Chandarlapaty et al., 2011; Muranen et al., 2012). These responses can, in principle, be addressed by combining PI3K pathway inhibitors with RTK inhibitors or blocking antibodies (García-García et al., 2012; Garrett et al., 2013); however, it will be challenging to predict and address the specific RTKs conferring PI3K inhibitor resistance in individual patients. RTK upregulation may be an especially challenging problem when using isoform-selective PI3K inhibitors, because one or more of the remaining class I PI3Ks may assume the signaling functions of the drug-inhibited PI3K isoform, thereby augmenting the resistance conferred by RTK upregulation. Another mechanism of FOXO-mediated rebound signaling is via upregulation of Rictor, leading to increased AKT phosphorylation in renal cancer cells (Lin et al., 2014).

A distinct mechanism of adaptive resistance was reported in triple negative breast cancer cells, which rapidly activated JAK2/STAT5 signaling during exposure to PI3K/mTOR pathway inhibitors (Figure 6). This adaptive response was addressed by co-treatment with a JAK inhibitor (Britschgi et al., 2012). A different nonreceptor tyrosine kinase, Src, which normally functions downstream of the EGFR and other RTKs, mediated adaptive resistance to a TORKi in a preclinical model of glioblastoma multiforme (GBM) (Wei et al., 2016). Src-mediated adaptive resistance is potentially targetable with the pan-Src family kinase inhibitor, dasatinib.

Bypass activation of mTOR and downstream targets—The mechanisms underlying primary and acquired resistance (Figure 6) are at least as diverse as those described above for adaptive resistance. Persistent mTORC1 activity in *PIK3CA* mutant breast cancer cell lines was responsible for resistance to the p110α-selective inhibitor alpelisib (BYL719; Table S1), and was reversed by combination with the mTORC1 inhibitor everolimus (Elkabets et al., 2013). An inverse correlation was also observed between the efficacy of alpelisib and phospho-S6 levels (a downstream target of mTORC1-S6K) after 28 days of treatment, indicating that inhibition of mTORC1 activity is pivotal for the antitumor activities of p110α inhibitors. Interestingly, persistent mTORC1 activity also predicts resistance to RAF or MEK inhibitors in *BRAF*-mutant melanoma models (Corcoran et al., 2013), indicating that drug-refractory mTORC1 activation confers broad-based resistance to both PI3K/mTOR pathway- and Ras pathway-targeted agents (Ilagan and Manning, 2016).

Although incomplete PI3K/mTOR pathway inhibition undoubtedly contributes to persistent mTORC1 activation, we now recognize that mTORC1 is also stimulated through PI3K-independent signaling mechanisms. Using a library screening approach, the protein serine-threonine kinase, Pim1, was shown to confer resistance to several PI3K/mTOR pathway inhibitors in breast cancer cells derived from the luminal A/B and HER2+ lineages (Le et al., 2016). Pim1 overexpression supported the activities of both AKT and mTORC1 in the setting of PI3K pathway inhibition. Elevated Pim1 expression correlated with alpelisib primary resistance in a panel of breast cancer cell lines, and a small molecule Pim1 kinase inhibitor conferred increased alpelisib sensitivity to three of the four breast cancer designated as high Pim1 expressors. Importantly, two of four breast tumor biopsies taken at the time of progression on alpelisib displayed upregulation of Pim1 expression relative to the pretreatment samples. Pim kinases may be significant contributors to resistance to PI3K/mTOR pathway inhibitors in breast cancer, as increased expression of mRNAs encoding Pim1, Pim2, or Pim3 were observed in more than ten percent of the nearly 1000 tumor samples analyzed in this study.

Aberrant activation of PDK1 or SGK1 has also been identified as mediators of resistance to p110α inhibitors in *PIK3CA* mutant breast cancer cells (Castel et al., 2016). Treatment of a *PIK3CA*-mutant, alpelisib-resistant breast cancer xenograft model with a combination of alpelisib and either a PDK1 or a SGK1 inhibitor caused significant tumor regression or stasis, respectively. Like AKT, SGK1 is activated by sequential phosphorylation by mTORC2 and PDK1, and shares with AKT the ability to phosphorylate TSC2, leading to mTORC1 activation. In the alpelisib-resistant cells, maximal mTORC1 inhibition was only achievable when alpelisib was combined with a PDK1 or SGK1 inhibitor. Analysis of 18

biopsies from patients treated with alpelisib showed that samples with high SGK1 mRNA and phospho-N-Myc downstream regulated 1 (NDRG1; a substrate for and biomarker of SGK1 activation) levels correlated with intrinsic resistance to alpelisib, whereas most phospho-NDRG1-negative tumors were from patients who attained partial responses or stable disease. Similarly, SGK3 overexpression and/or activation has been repeatedly linked to PI3K/mTOR inhibitor resistance in breast cancer cells (Bago et al., 2016; Gasser et al., 2014). These reports offer a common mechanism for intrinsic or acquired resistance to PI3K or AKT inhibitors.

Activation of parallel pathways—Amplification of *MYC* drives resistance to PI3K/mTOR pathway inhibitors in breast cancer cell lines (Figure 6), and *MYC* copy number and/or c-Myc expression is often elevated in breast cancer (Ilic et al., 2011; Liu et al., 2012) and lymphoid malignancies (Dang, 2012). Although direct targeting of c-Myc remains an elusive clinical goal, preclinical studies suggest that inhibition of the bromodomain and extra-terminal (BET) family protein, BRD4, antagonizes c-Myc-dependent PI3K pathway inhibitor resistance. BRD4 functions as an epigenetic reader that recognizes histones bearing acetylated lysine residues, and plays a key role in the regulation of transcriptionally-active chromatin. Inhibitors of BRD4 suppress the expression of super-enhancer-associated genes such as *MYC* (Delmore et al., 2011; Mertz et al., 2011; Zuber et al., 2011), and small molecule BET inhibitors are known to interfere with c-MYC-dependent transcriptional responses (Venkataraman et al., 2014). In ER+ breast cancer cells, BET inhibitors also overcame resistance to everolimus caused by c-Myc overexpression (Bihani et al., 2015), and exerted synergistic antitumor activity with PI3K inhibitors in mice with mammary tumors initiated by oncogenic PI3K-H1047R plus *MYC* transgene expression (Stratikopoulos et al., 2015). Interestingly, in breast cancer cell lines derived from either mutant *PIK3CA*-*Myc* double transgenic or *Pten*-null mice, the BET inhibitor, JQ1, attenuated PI3K pathway reactivation after treatment with the p110α/δ-selective inhibitor, pictilisib (GDC-0941; Table S1), by blocking upregulation of IGFR1 and insulin receptors. Moreover, rebound PI3K pathway activation by RTK expression was blocked by JQ1 in *PIK3CA* or *PTEN* mutant cell lines derived from breast, colorectal, prostate, brain and ovarian tumors (Stratikopoulos et al., 2015). In luminal breast cancer cell lines resistance to JQ1 was closely correlated with *PIK3CA* mutation, and JQ1 enhanced the anti-tumor efficacy of everolimus in a xenograft model, whereas *PTEN* loss was associated with increased sensitivity to BET inhibitors in basal breast cancer cells, suggesting that interplay between PI3K and BET signaling will be tumor cell context-dependent (Marcotte et al., 2016). Synergistic induction of cell death has also been observed in patient-derived ovarian cells treated with pictilisib and BET inhibitors (Kurimchak et al., 2016). The adverse event profile of the BET – PI3K inhibitor combination remains to be determined, but the evidence that BET inhibitors address multiple modalities of PI3K resistance supports further progression of this combination into clinical testing.

Tumor heterogeneity—Intratumoral heterogeneity is increasingly appreciated as an important contributor to tumor evolution and relapse following either chemotherapy or targeted therapy (McGranahan and Swanton, 2017). Increasingly sophisticated gene sequencing methodologies now support a branching model of tumor subclonal evolution,

indicating that solid tumor tissues comprise an intertwined ecosystem of sub-dominant and dominant clones that communicate with one another and normal host elements in the tumor microenvironment. Clonal dominance is determined in part by extrinsic selection pressures imposed on the tumor, by alterations in tumor location (in metastatic disease), host metabolism and immune responsiveness, and cancer therapy. It is now appreciated that the PI3K pathway can be oncogenically activated in a variable fashion at the subclonal level in primary lesions and metastatic lesions from the same patient. Parallel genomic analyses of 86 diverse, primary tumors and brain metastases from individual patients revealed that more than half of brain metastases contained actionable mutations not seen in the corresponding primary tumor (Brastianos et al., 2015). Mutations in *PTEN* and *PIK3CA* were featured prominently as heterogeneous drivers of PI3K/mTOR pathway activation in these brain lesions. These same two genes appear to be particularly prone to heterogeneous mutation patterns in primary tumor tissues. Subclonal mutations in *PIK3CA* were reported in several cancer subtypes, including NSCLC, colorectal cancer, breast cancer, and others (Harbst et al., 2016; de Bruin et al., 2014; McGranahan and Swanton, 2017; Uchi et al., 2016; Yates et al., 2015). Consistent with these findings, a study spanning nine cancer subtypes demonstrated that subclonal mutations in the PI3K-AKT-mTOR pathway were considerably more frequent than subclonal mutations in the RAS-MAPK pathway, which tended to be ubiquitously expressed in all subclones in the tumor (McGranahan et al., 2015). Convergent evolution of distinct subclonal mutations leading to loss of PTEN function have been documented in recent publications. Following acquired resistance to the p110α-selective inhibitor alpelisib in an ER^+ breast cancer patient, Juric and colleagues uncovered six distinct mutational events in *PTEN* across ten metastatic lesions, all on a common background of a clonal, mono-allelic *PTEN* deletion (Juric et al., 2015a). The *PTEN* deletion event occurred prior to PI3K inhibitor therapy, and it was striking that the majority of metastatic lesions impacted the remaining *PTEN* allele, albeit in different fashions, to achieve full resistance to alpelisib. Heterogeneous genetic alterations leading to PI3K pathway activation represent a potentially major contributor to the limited efficacy of PI3K/mTOR pathway inhibitors, as distinct subclonal alterations might translate into variable levels of sensitivity to these drugs, and in turn, shifts in clonal dominance during PI3K/mTOR pathway inhibitor therapy.

Systemic toxicity as a barrier to PI3K/mTOR pathway inhibitor development

A major hindrance to the broad development of PI3K pathway inhibitors has been the challenge of achieving sufficient depth of target inhibition in tumor tissue, while avoiding dose-limiting toxicities in the patient. Previous studies have established that deep (>90%) inhibition of hepatic PI3K/AKT signaling is required to generate a hyperglycemic response in mice (Taniguchi et al., 2006), and it is reasonable to expect that a similarly profound reduction in tumor tissue-associated p110α activity will be needed to achieve optimal therapeutic responses in most patients. PI3K/mTOR pathway inhibitors with the highest potentials for broad therapeutic activity are the pan-specific PI3K/mTOR and pan-PI3K inhibitors, but these also carry highest toxicity burdens in the PI3K/mTOR pathway inhibitor class. As is the case with the majority of targeted agents, deep, durable clinical responses will require combinations of PI3K/mTOR pathway inhibitors with chemotherapy, immunotherapy, and other targeted therapies. Many of these potential therapeutic partners

present their own set of adverse effects, which often overlap with those of PI3K pathway inhibitors.

The results of breast cancer clinical trials provide clear evidence that the toxicity of broad PI3K pathway inhibition is a significant barrier to optimal therapeutic effectiveness. The BELLE series of clinical trials assessed the efficacy and safety of various drug combinations with the pan-PI3K inhibitor buparlisib (BKM120; Table S1) in patients with ER+, HER2- breast cancer. Patients who had progressed on aromatase inhibitor monotherapy received fulvestrant plus either buparlisib or placebo in the Belle-2 phase 3 trial (Baselga et al., 2017). Although the primary endpoint of extended progression-free survival (PFS) was met, the overall response rate (ORR, equals PFS plus stable disease) was disappointingly low. However, in patients with *PIK3CA* hotspot mutations in circulating tumor cell DNA (ctDNA), PFS and ORR were significantly improved. Similar results were seen in BELLE-3, a phase 3 trial in patients who had progressed on everolimus receiving fulvestrant with buparlisib or placebo (Di Leo et al., 2017). Enthusiasm for the efficacy data was unfortunately dampened by the frequency of serious adverse events leading to frequent discontinuations in both BELLE trials, with elevation of liver transaminases as a dose-limiting toxicity. Buparlisib has off-target tubulin-binding activity, which prompted the identification of a close analog, PQR309, that is more selective for PI3K (Bohnacker et al., 2017) and is now in early clinical trials.

With respect to dual PI3K/mTOR inhibitors, a phase 1 trial of BEZ235 in advanced renal cell carcinoma was terminated early due to frequent dose-limiting toxicities without objective responses (Carlo et al., 2016). In a phase 2 trial of BEZ235 for pancreatic neuroendocrine tumors, 29 of 31 patients discontinued treatment due to adverse events and the primary PFS endpoint was not met, resulting in trial termination (Fazio et al., 2016). Apitolisib (GDC-0980, a pan-PI3K-mTOR inhibitor; Table S1) proved inferior to everolimus in a phase 2 trial in patients with renal cell carcinoma, and serious adverse events and trial discontinuations were more frequent in apitolisib-treated patients (Powles et al., 2016). The extent to which toxicities limited the dose intensity achievable in tumor tissues, but it is plausible that sub-optimal dosing has contributed to the generally disappointing results obtained with these agents.

Opportunities and challenges with isoform-selective PI3K inhibitors

Isoform-selective and isoform-sparing PI3K inhibitors that preferentially inhibit the activity of one or more PI3K isoforms might circumvent the intrinsic toxicity associated with pan-PI3K inhibition, and might be more permissive for exploration of combination therapies. Preclinical studies have shown that cancer cells bearing mutant *PIK3CA* or *HER2* amplification are frequently sensitive to p110α inhibition (Fritsch et al., 2014), whereas *PTEN*-mutant or -null tumors are more sensitive to p110β inhibition (Edgar et al., 2010; Ni et al., 2012). However, increased dependency on p110β is not an obligate outcome of loss of PTEN function. In a mouse model of ovarian endometrioid adenocarcinoma driven by *Pten* deletion and expression of oncogenic *Kras*, inhibition of p110α, but not p110β, was sufficient to prevent tumor growth (Schmit et al., 2014). Similarly, ovarian epithelial cells deficient for *PTEN* and p53 were either p110β or p110α-dependent, depending on the

absence or presence of an oncogenic *KRAS* allele (Schmit et al., 2014). Moreover, endometrial tumor cells deficient for *PTEN*, either with or without *KRAS* mutation, required dual p110α and p110β inhibition to decrease phospho-AKT levels and cell viability (Weigelt et al., 2013). NSCLC cell lines bearing either *PIK3CA* or *PTEN* mutation also exhibit resistance to isoform-selective inhibitors, but retain responsiveness to pan-PI3K inhibitors (Stamatkin et al., 2015). Therefore, both tissue of origin and genomic context influence PI3K isoform dependence in cancer cells, making the elucidation of robust, predictive biomarkers for PI3K/mTOR pathway inhibitor responsiveness a daunting challenge for translational oncologists.

PI3K isoform switching represents a well-documented mechanism of resistance to isoform-selective PI3K inhibitors. The Engelman group (Costa et al., 2015) found that inhibition of PI3K signaling in HER2-amplified or *PIK3CA*-mutant breast cancer lines by the p110α-selective inhibitor, alpelisib, was followed by a rebound in PtdIns-3,4,5-P_3 levels after 24 hours. Reversal of the drug effect was attributable to activation of p110β and was associated with increased p110β recruitment to HER3. Interestingly, in mutant *PIK3CA*-expressing cells, the rebound in PtdIns-3,4,5-P_3 level was not associated with a corresponding increase in phospho-AKT, suggesting that changes in PtdIns-3,4,5-P_3 levels are not obligatorily linked to downstream pathway activation. In breast cancer xenografts bearing overexpressed and mutationally activated PIK3CA, tumor regression was only observed after treatment with a combination of p110α and p110β-selective inhibitors. Similar results were reported by Baselga and colleagues (Schwartz et al., 2015), who found that treatment with a selective p110β inhibitor led to transient suppression of, followed by a significant rebound in phospho-AKT levels, which were attributed to the upregulation of the IGFR1-IRS1-p110α signaling cascade. Similarly, combined treatment with p110α- and p110β-selective inhibitors caused greater tumor growth inhibition in both a *PTEN*-null prostate cancer and ER^+ breast cancer models (Hosford et al., 2016). In *PTEN*-deficient, ER+ breast cancer xenografts, treatment with the triple combination of fulvestrant (a selective estrogen receptor degrader) together with inhibitors of p110α and p110β was required to trigger maximal, sustained tumor regressions. In contrast, treatment with the fulvestrant and p110β inhibitor doublet resulted in transient inhibition followed by a striking rebound in phospho-AKT, cyclin D1/3, and phospho-pRb levels. Collectively, these studies and others provide compelling support for the conclusion that the clinical activity of p110β-selective inhibitors is limited by the development of resistance due to isoform switching to p110α-mediated signaling. Parenthetically, it is noteworthy that an activating mutation in the human *PIK3CB* gene (encoding p110β D1067Y) leads to broad resistance to PI3K inhibitors; however, in contrast to *PIK3CA* mutations, this mutation is rarely observed in cancer patients (Nakanishi et al., 2016).

Despite the evidence for p110α/p110β redundancy in preclinical models, growing clinical evidence points to the potential of p110α-selective inhibitors in defined patient populations. Taselisib (GDC-0032; Table S1) inhibits p110α, p110γ and p110δ but not p110β, and furthermore has some selectivity for *PIK3CA* mutant proteins. A phase I dose-escalation trial of taselisib reported a 36% response rate for patients with *PIK3CA* mutant tumors, versus 0% in patients whose tumors lacked a *PIK3CA* hotspot mutation (Juric et al., 2017). In a phase 2 trial of fulvestrant plus taselisib, patients with ER+, mutant *PIK3CA*-expressing

breast cancer had substantially better overall responses than patients with wild-type *PIK3CA* tumors (Dickler et al., 2016). Although treatment discontinuation remained an issue in this study, the safety profile was considered sufficiently acceptable to warrant a phase 3 trial. In an open-label trial of the p110α-selective inhibitor alpelisib with fulvestrant, ER+ breast cancer patients with *PIK3CA* mutations displayed a significantly improved clinical benefit rate compared to patients with wild type *PIK3CA* (Juric et al, 2016 abstract), and a similar selectivity for mutant PIK3CA tumors was observed in a phase 1b trial of alpelisib with letrozole (Mayer et al., 2017). Importantly, the safety profile of alpelisib plus letrozole was superior to that of buparlisib plus fulvestrant in the BELLE-2 and 3 trials, and there were fewer discontinuations due to adverse events. Although more than half of patients manifested mild to moderate hyperglycemia, this issue was well managed with metformin. The phase 3 SOLAR-1 trial will study the effects of taselisib or placebo plus fulvestrant in greater that five hundred ER+ breast cancer patients with *PI3KCA* mutations and, in a separate cohort, patients with wild-type *PIK3CA*. This large study will go a long way toward establishing whether the PI3K isoform-selective inhibitors will deliver both efficacy and acceptable safety in patients with tumors expressing mutationally activated p110α.

Combination approaches

In some cases, resistance to PI3K inhibitors can be addressed with combination therapy involving other clinically established agents. In ER^+ positive breast cancer, PI3K and ER signaling mediate mutual antagonism, which results in pharmacological inhibition of one pathway driving enhanced signaling through the other. Inhibition of PI3K was previously reported to stimulate ER-dependent transcription (Bosch et al., 2015). A recent paper provided mechanistic insights into this phenomenon by demonstrating that inhibition of PI3K triggers activation of the lysine methyltransferase, KMT2D, which functions as an enhancer of ER-dependent transcription (Toska et al., 2017). The concept of mutual antagonism involving the PI3K and ER signaling pathways has led to a series of clinical trials testing PI3K inhibitors combined with ER degraders or aromatase inhibitors, as described in other sections of this review. It is hoped that these strategies will provide additional therapeutic options after the successful combination of mTORC1 inhibitor everolimus with exemestane (Baselga et al., 2012).

Activation of the Ras-Raf-Mek-Erk axis, which occurs in many preclinical tumor models following PI3K pathway blockade, has led to the clinical testing of several PI3K plus MEK inhibitor combinations (Britten, 2013; Juric et al., 2015b; LoRusso et al., 2012; Shimizu et al., 2012). However, clinical signs of efficacy were associated with serious adverse events, raising significant questions about the future development of this combination. The sensitivity of breast cancer models to PI3K inhibition often correlates with retinoblastoma protein (pRb) phosphorylation, with high phospho-pRb levels correlated with resistance (Vora et al., 2014). Key mediators of pRb phosphorylation are the G_1-phase-associated cyclin D-CDK4/6 complexes (Sherr and Bartek, 2017). The CDK4/6 inhibitor, ribociclib, sensitized resistant breast cancer cells to alpelisib, prevented the development of adaptive resistance, and provoked tumor regression in breast cancer xenograft models (Herrera-Abreu et al., 2016; Jansen et al., 2017). Combinations of PI3K pathway and CDK4/6 inhibitors have also shown promising activities in pancreatic, HNSCC and NSCLC models (Franco et

al., 2014; Gopalan et al., 2013; Ku et al., 2016). The therapeutic pairing of CDK4/6 inhibitors with PI3K inhibitors is particularly appealing in ER+ breast cancer, a cancer subtype with well-documented sensitivity to anti-estrogen plus CDK4/6 inhibitor therapy. Given that thirty percent of ER+ breast cancers bear activating *PIK3CA* mutations, triplet combinations of PI3K and CDK4/6 inhibitors with estrogen antagonists have attracted considerable interest, and clinical studies are now underway.

An intriguing combination opportunity for PI3K inhibitors has been uncovered in triple negative breast cancer (TNBC). Preclinical studies demonstrated that exposure of TNBC cell lines or patient-derived xenografts (PDXs) to the pan-PI3K inhibitor, buparlisib, resulted in reduced BRCA1 expression, and increased DNA damage (Ibrahim et al., 2012). Tumor growth inhibition in *PTEN*-null or *PIK3CA*-mutant PDX models was enhanced by the combination of buparlisib with the poly-ADP ribose polymerase (PARP) inhibitor, olaparib. PARP inhibitors have shown clinically significant activity in *BRCA1*- or *BRCA2*-mutated ovarian cancers (later-stage studies in *BRCA*-deficient TNBC patients are ongoing), due to a synthetic lethal mechanism in cells deficient in homologous combination-mediated DNA repair (Lord and Ashworth, 2016). Combinations of PI3K/mTOR pathway inhibitors and PARP inhibitors have also shown promising activity in prostate, ovarian and SCLC models (Cardnell et al., 2013; González-Billalabeitia et al., 2014; Rehman et al., 2012; Wang et al., 2016). Once again, results from an early clinical trial with buparlisib and olaparib suggest that toxicity will be a formidable obstacle to attaining the drug exposures needed to achieve an optimal therapeutic effect with this drug combination (Matulonis et al., 2016). The possibility that toxicity would be mitigated by combining a PARP inhibitor with a p110α-selective, rather than the pan-PI3K inhibitor, buparlisib, will undoubtedly be addressed in future clinical studies.

PI3K pathway activation and resistance to therapy

Activation of the PI3K/mTOR is a common mediator of resistance to numerous anticancer agents, including conventional chemotherapy and agents targeting other oncogenic nodes (Brown and Toker, 2015; Ilagan and Manning, 2016). Emerging evidences points to PI3K/mTOR signaling as a mechanism of resistance to cancer immunotherapy. Immuno-oncology is currently the most explosive area of cancer therapeutic development, due mainly to the unprecedented durability of the responses seen in patients with normally intractable metastatic cancers (Farkona et al., 2016; Pardoll, 2012). Despite the impressive responses seen in a subset of patients receiving immune PD1/L1- and CTLA4-targeted therapies, objective response rates are commonly in the 25–40% range, indicating that the majority of tumors are intrinsically resistant to these immune checkpoint inhibitors. Immunotherapy resistance was recently linked to tumor-cell autonomous PI3K pathway activation (Peng et al., 2016). *PTEN* silencing in BRAF-mutant melanoma cells led to impaired T cell tumor recruitment and anti-tumor immunity. In melanoma patients, reduced expression of PTEN was correlated with resistance to anti-PD1 therapy. Moreover, in melanoma patients with regional heterogeneity of PTEN expression in tumor tissues, T cell infiltration was consistently lower in sub-regions that lacked PTEN protein. In a spontaneous *Braf*-mutant, *Pten*-null mouse melanoma model, the combination of a selective PI3Kβ inhibitor with anti-PD1 significantly improved tumor growth inhibition and survival compared to each agent

alone. Although a pan-PI3K inhibitor impaired the vaccine-induced proliferation of gp100-specific T cells in a preclinical melanoma model, a p110α-selective inhibitor had no effect on this T-cell response, suggesting that isoform-selective inhibitors will be less likely to compromise anti-tumor T cell activity. PI3K/mTOR pathway activation in lung cancer models was associated with increased expression of the PD-1 ligand, PD-L1, and combination therapy with rapamycin plus anti-PD1 antibody significantly reduced tumor burden in a *KRAS*-driven mouse NSCLC model (Lastwika et al., 2016). However, in a separate study, the investigators observed no consistent effect of *PTEN* mutation or AKT amplification on the expression of PD-L1 by melanoma cell lines (Atefi et al., 2014). Additional studies are clearly needed to fully understand the mechanisms that regulate PD-L1 expression on tumor cells, and the complex interplay between tumor cell-intrinsic alterations in PI3K/mTOR pathway activity and antitumor immunity, before and during therapy with immunostimulatory agents, and in the absence or presence of traditional tumor cell-targeted therapeutics.

Progress with mTOR-selective agents as anticancer drugs

Activation of mTOR, specifically mTORC1, is a key consequence of tumor-associated alterations that drive PI3K pathway activation. This observation, together with the early availability of rapamycin and its analogs (rapalogs) as clinically established mTORC1 inhibitors in organ transplantation, led to clinical oncology trials that resulted in the approval of two rapalogs for the treatment of ER+ breast, renal and pancreatic neuroendocrine tumors (Table S1). However, despite the positive PFS outcomes in these trials, rapalogs have shown limited efficacy in other trials, likely due to the incomplete inhibition of mTORC1, and compensatory upregulation of the PI3K signaling network (Figure 6). Subsequent medicinal chemistry efforts led to the discovery of a series of mTOR kinase inhibitors (TORKi) that, in contrast to the allosteric mechanism of inhibition employed by rapalogs, blocked mTOR signaling by interacting with the mTOR catalytic domain in an ATP-competitive fashion. As commonly seen with other similarly targeted protein kinase inhibitors, cancer cells acquired resistance to the TORKi through the outgrowth of clones bearing mTOR mutations that reduced the antiproliferative effects of these drugs. Genomic sequencing of ER+ breast cancer cells that had acquired resistance to mTOR inhibitors identified two mutations in the FKBP12-rapamycin binding (FRB) domain that induced high-level resistance to rapalogs, and a second mutation in the catalytic domain of mTOR that decreased sensitivity to a TORKi (Rodrik-Outmezguine et al., 2016). Interestingly, these mutations conferred drug resistance through hyper-activation of mTORC1, as opposed to interference with inhibitor binding to the target protein.

To overcome these mTOR-intrinsic mechanisms of resistance, Shokat and coworkers designed a bivalent inhibitor, termed RapaLink-1, containing a TORKi substituent that targets the catalytic domain, connected with a precisely designed linker to the second pharmacophore (a rapalog) to simultaneously engage the FRB domain of mTOR (Rodrik-Outmezguine et al., 2016). RapaLink-1 effectively blocked mTORC1 signaling by both FRB- and kinase domain-mutated mTOR proteins, and overcame resistance to single-agent rapamycin- or TORKi in these cells. Importantly, RapaLink-1 also effectively inhibited wild-type mTOR signaling. Thus, the RapaLink design may more effectively delay the

acquisition of mTOR resistance mechanisms. A follow up study highlighted two additional advantages of RapaLinks compared to TORKi (Fan et al., 2017). First, RapaLinks are somewhat selective for mTORC1 versus mTORC2 (Figure 2), possibly reducing toxicities associated with mTORC2 inhibition. Second, RapaLinks achieve a much longer duration of mTORC1 inhibition, correlating with greater anti-tumor activity in glioma models. Whether these third generation mTORC1 inhibitors will improve the efficacy-toxicity profile of mTOR-targeted therapies in cancer patients remains to be determined.

Optimizing therapeutic index through alternative dosing regimens

The vast majority of clinical studies with PI3K/mTOR pathway inhibitors have employed standard daily dosing regimens, with dose level and frequency based on maximal-tolerated dose (MTD) determinations. Although this approach ensures an acceptable safety profile, it does carry the risk that a safe dose may not be an effective dose due to insufficient target engagement in the tumor tissue. Strategies aimed at achieving optimal target modulation in the tumor, while sparing normal tissue, may be necessary for the PI3K/mTOR pathway inhibitors to achieve their full potential as anticancer agents. To this end, several groups are exploring alternative dosing strategies, particularly those involving high-dose, intermittent therapy as potential paths forward for the clinical applications of PI3K/mTOR pathway inhibitors. For example, the p110α/δ inhibitor copanlisib (BAY80-6946; Table S1), which has a half-life of only 0.7 hr after intravenous (IV) administration in mice, was far more effective in mice bearing BT474 breast cancer xenografts when administered intermittently (3 times per week) than when administered by continuous dosing (Liu et al., 2013). Similarly, intermittent dosing of the p110α/δ-selective inhibitor, AZD8835, was clearly superior to daily dosing of this drug in a preclinical breast cancer model (Hudson et al., 2016). A pharmacokinetic-pharmacodynamic model, based on measurements of caspase-3 cleavage, was used to derive simulations of apoptotic rates. This simulation revealed that intermittent dosing elicited repeated waves of intratumoral apoptosis with no loss of the peak apoptosis, whereas continuous dosing produced a lower level of apoptosis that waned with continued dosing. Higher levels of caspase 3 cleavage were correlated with more penetrating PI3K inhibition, as measured by phospho-AKT levels, during the intermittent, high-dose scheduling. The mTOR inhibitor, vistusertib (AZD2014; Table S1), caused similar growth inhibition of MCF7 ER+ breast cancer xenografts when dosed either continuously or intermittently, but higher rates of tumor cell apoptosis were observed during intermittent dosing (Guichard et al., 2015). These preclinical studies suggest that intermittent dosing schedules might allow higher dosing, and lead to more complete PI3K pathway inhibition and higher levels of apoptosis, even in the monotherapy setting. The clinical success of this proposal is contingent on the notion that normal tissues recover more adeptly during the inter-dose interval than does the tumor tissue.

Early clinical results with intermittent dosing of PI3K/mTOR pathway inhibitors are also yielding encouraging data. In a phase I trial of the pan-PI3K inhibitor, buparlisib, an intermittent five days on, two days off schedule produced fewer early onset adverse events, at the same daily dose of buparlisib (Ma et al., 2016). Intermittent dosing may also permit more pronounced pathway inhibition with equivalent toxicity for less toxic inhibitors such as the isoform-selective PI3K inhibitors. A phase 1 dose escalation trial of the p110α-selective

inhibitor, MLN1117 (now TAK-117; Table S1), compared two different, three day per week schedules at higher dose (900 mg maximum dose) with daily dosing (150 mg maximum) (Juric et al., 2015b). The intermittent dosing schedules allowed the investigators to achieve approximately 4-fold higher exposure than was attainable with daily dosing. Analysis of skin biopsies revealed enhanced inhibition of PI3K pathway, as measured by the pharmacodynamic biomarkers, phospho-4EBP1 and phospho-S6. Finally, gedatolisib (PKI-587; PF-05212384; Table S1), a potent inhibitor of both Class I PI3Ks and mTOR, is in early trials in combination with palbociclib and anti-estrogen therapy in women with metastatic ER+ breast cancer, and with *cis*-platinum in TNBC patients. The dosing protocol for gedatolisib in these study involves intravenous administration on a once-weekly basis (Mallon et al., 2011). The results of studies exploring intermittent dosing with PI3K/mTOR pathway inhibitors are eagerly anticipated, and the hope that these novel regimens will at least partially address the opposing requirements deep inhibition of the PI3K/mTOR pathway in tumor tissue, while minimizing toxic outcomes in normal tissues. Results of a phase 2 trial of copanlisib, presented at the 2017 AACR annual meeting (Martin Dreyling, personal communication), showed an approximate 70% response rate in patients with indolent lymphomas given once-weekly i.v. doses of copanlisib. Importantly, common toxicities associated with continuous inhibition of p110α (hyperglycemia) or p110δ (colitis) were transient or less frequent in the intermittently dosed cohorts.

Mitigation of toxicity is likely to be especially important in the development of combinations of PI3K pathway inhibitors with other targeted agents, particularly those with overlapping toxicity profiles. For instance, clinical trials conducted so far suggest that combinations of pan-PI3K inhibitors with MEK inhibitors will face considerable tolerability challenges (Shimizu et al, 2012; LoRusso et al, 2012). While the use of isoform-selective or isoform-sparing PI3K inhibitors will likely reduce toxicity burden of rational combinations, in many cases the design of an effective dose ratio and schedule may be as critical as the design of the combination itself. Intermittent dosing of either one or both combination partners may ameliorate toxicity and enhance therapeutic index. This concept was tested in a mouse breast xenograft model with combinations of the p110α/γ selective inhibitor, AZD8835, dosed intermittently, and partnered with either intermittently dosed fulvestrant, with continuously administered palbociclib (Hudson et al, 2016). These combinations produced robust tumor regressions; indeed, a triplet combination (AZD8835 plus fulvestrant plus palbociclib), with each drug delivered according to the above protocol, induced profound tumor regressions. Importantly, the MTD of each agent in the triplet combination was the same as that for monotherapy, suggesting that there is no combinatorial toxicity when two of three agents are administered intermittently.

Pulsatile dosing is also being investigated in an ongoing trial of the AKT inhibitor AZD5363 combined with the PARP inhibitor olaparib, in which AZD5363 is dosed according to three intermittent schedules (Michalarea et al., 2016). Although the rationale underlying the choice of an AKT inhibitor (versus a PI3K inhibitor) for this combination treatment protocol is not completely clear, published evidence suggests that PARP inhibition triggers AKT activation and tumor progression in certain settings (Cardnell et al., 2016). It may also be possible to reduce systemic toxicity by sequentially dosing combination partners, as in some chemotherapy combinations. The effectiveness of such a schedule would presumably be

optimized by partnering a PI3K-mTOR pathway inhibitor with an agent that left a sustained "mark" of the tumor; for example, a DNA-damaging cytotoxic agent, or a drug targeting the epigenome, such as a histone deacetylase inhibitor.

The encapsulation of PI3K pathway inhibitors in tumor-targeted nanoparticles represents an appealing strategy to promote asymmetric distribution of PI3K/mTOR pathway inhibitors in tumor versus other host tissues. In a preclinical study, P-selectin-binding, fucoidan-based nanoparticles served as delivery vehicles for alpelisib (Mizrachi et al., 2017). Once weekly dosing of the alpelisib-loaded nanoparticles in animals bearing head and neck cancer xenografts caused substantially improved survival, and sensitized the tumors to radiotherapy to a greater extent than daily free alpelisib dosing. In addition, nanoparticle-mediated drug delivery attenuated drug-induced hyperglycemia and hyperinsulinemia, and diminished the attrition of pancreatic islet beta cells seen after alpelisib treatment. These results support further exploration of tumor-targeted nanoparticles as promising tumor-selective delivery vehicles for PI3K/mTOR pathway inhibitors.

Biomarkers: Predicting response and resistance to PI3K pathway inhibitors

As with other targeted therapies, the success of PI3K pathway inhibitors in the clinical setting will be predicated on the accurate identification of those patients who are most likely to respond to treatment. As noted above, a phase 3 study (the BELLE-2 trial) of fulvestrant and buparlisib in letrozole-resistant patients revealed a clear difference in response to buparlisib depending on *PIK3CA* mutation status, as determined by circulating tumor (ct) DNA analysis (Baselga et al., 2017). In patients with *PIK3CA* mutations, response rates were clearly increased (18.4% versus 3.5%), as was the median PFS (7.0 v. 3.2 months) after buparlisib treatment relative to placebo. In contrast, patients lacking *PIK3CA* mutations realized no additional benefit from the fulvestrant plus buparlisib combination relative to fulvestrant alone (11.6% v. 10.6%), and experienced no PFS benefit (6.8 versus 6.8 months). Interestingly, no significant improvements in response rate or PFS were seen in patients with *PIK3CA* mutations or loss of PTEN expression when these parameters were assessed in archival tumor tissue. Similar increases in PFS benefit were seen in mutant *PIK3CA*-bearing patients in the BELLE-3 trial of fulvestrant plus buparlisib, which enrolled patients who were resistant to prior rapalog therapy (Di Leo et al., 2017). The BELLE trials confirmed that *PIK3CA* mutations predict sensitivity to PI3K inhibitors, and argue that analysis of real-time *PIK3CA* mutation status in ctDNA provides a more predictive biomarker of drug responsiveness than does analysis of archival tumor tissue. In contrast to archival samples from primary tumors, ctDNA analysis takes into account the dynamic changes in cancer genomes (and hence biomarker status) that occur during tumor evolution and in response to earlier therapies. The presence of *PIK3CA* mutations in ctDNA at the time of buparlisib treatment will logically identify drug-sensitive cell populations more accurately than a historical sample, in which potentially drug-responsive clones may have undergone attrition during tumor progression (McGranahan et al., 2015).

Recent evidence suggests that different *PIK3CA* mutations results have distinct consequences with regard to PI3K/mTOR pathway inhibitor therapy. Early clinical trials of the isoform-selective inhibitors, alpelisib and taselisib, in breast cancer have produced

results consistent with a previous meta-analysis of clinical trials of PI3K pathway inhibitors in patients with diverse cancers, revealing that patients with H1047R mutations had higher response rates than those with other *PIK3CA* mutations, or no mutations in this gene (Janku et al., 2013; Mayer et al., 2017). The p110β-sparing inhibitor, taselisib, (Table S1) was reported to induce degradation of mutant p110α (Friedman et al., 2017). It is conceivable that distinct activating mutations in p110α might confer differential susceptibilities drug-induced degradation; if this proves to be the case, perhaps drug-induced turnover of the p110α H1047R mutant underlies the increased taselisib sensitivity of tumors expressing this particular p110α oncoprotein. Nonetheless, activating mutations in *PIK3CA* are not sufficient to define the PI3K/mTOR pathway inhibitor-responsive population. Other positive or negative (e.g., Ras pathway alterations) biomarkers, preferably amenable to longitudinal measurements, are needed for optimal development of drug combinations containing the PI3K/mTOR pathway inhibitors.

The BOLERO-2 trial established the efficacy of everolimus plus exemestane in ER+/HER2- breast cancer (Baselga et al., 2012). In this trial, amplification of cyclin D1 had no impact on everolimus efficacy, whereas mutation or amplification of *FGFR1* or *FGFR2* resulted in less benefit from everolimus treatment, though the trial was not sufficiently powered to deliver definitive conclusions in this regard. A meta-analysis of the BOLERO-1 and BOLERO-3 phase 3 trials of everolimus or placebo in combination with trastuzumab and chemotherapy (paclitaxel or vinorelbine, respectively) in patients with HER2+ advanced breast cancer revealed a significant PFS benefit from everolimus in patients with either *PIK3CA* mutation or low PTEN expression (by IHC), whereas patients with neither of these alterations experienced no PFS benefit from the addition of everolimus (André et al., 2016).

The AKT E17K mutation causes constitutive membrane localization and pathway activation, and is found at a frequency of 1–4 percent across multiple tumor types. In early phase trials, the competitive AKT inhibitor AZD5363 showed promising activity in patients with tumors carrying the E17K mutation, as determined by tumor tissue and ctDNA analysis (Hyman et al., 2015; Tamura et al., 2016). In a trial of the AKT inhibitor ipatasertib (GDC-0068; Table S1) with abiraterone in metastatic castration-resistant prostate cancer (CRPC), an improved PFS response to ipatasertib in patients with *PTEN* loss (determined by FISH and NGS) but not in patients with intact *PTEN* (Hyman et al., 2015). Hence, *PTEN* loss and the AKT E17K mutation appear to be promising biomarkers of therapeutic sensitivity to AKT inhibitors.

Lessons learned from clinical experience with idelalisib

Idelalisib, a selective PI3Kδ inhibitor, was approved in 2014 for relapsed chronic lymphocytic leukemia (CLL) and two subtypes of non-Hodgkins lymphoma (NHL) in combination with rituximab. Clinical trials provided important insights into the mechanisms underlying the efficacy of idelalisib, as well as its toxicities (Furman et al., 2014; Gopal et al., 2014). Treatment of CLL patients with idelalisib leads to redistribution of transformed B cells from lymphoid compartments to the peripheral circulation, resulting in lymphocytosis. These responses are shared with those elicited by the BTK inhibitor ibrutinib (Byrd et al., 2013; Herman et al., 2014), consistent with the convergent roles of BTK and p110δ in the

BCR signalosome (Fruman and Cantley, 2014). The strong dependence on PI3Kδ for signaling through the BCR in CLL and indolent NHL explains the sensitivity of these transformed B cells to p110δ inhibition. Idelalisib-induced lymphocytosis deprives the malignant B cells of their protective niches in the peripheral tissues, and renders B cells susceptible to apoptotic signals provoked by rituximab immunotherapy or bendamustine chemotherapy. Thus, the predominant mechanism of action of idelalisib differs substantially from the presumptive direct actions of p110α on cancer cells; rather, p110δ inhibition suppresses host responses that nurture the survival and expansion of transformed B cell clones in CLL.

Adverse events associated with idelalisib treatment included elevation of liver enzymes, diarrhea, pneumonitis and pneumonia (Furman et al., 2014; Gopal et al., 2014). Subsequent trials of idelalisib, which included trials of other combinations and trials in younger and treatment-naïve patients, encountered more severe adverse events, such as colitis, pneumonitis and high-grade liver enzyme elevations (Barr et al., 2016; Cheah et al., 2015; Lampson et al., 2016). Unfortunately, several drug-related deaths occurred as a result of *Pneumocystis jirovecii* or cytomegalovirus infection, leading to trial discontinuation. Currently idelalisib plus rituximab is used only in a salvage setting, with systemic immunosuppression and/or inflammatory complications managed by treatment interruption or corticosteroid treatment. Toxicities associated with idelalisib are considered mechanism-based, given the similarity to the pathologies seen in mice genetically engineered to lack p110δ activity. Patients with grade 3 or higher colitis or transaminitis after idelalisib treatment displayed higher plasma levels of chemokines and lower numbers of Tregs (Lampson et al., 2016), suggesting that the toxic effects of p110δ inhibition may stem in part from Treg depletion that relieves restraints on cytotoxic T cells. Interestingly *Pneumocystis jirovecii* infections have also been reported in cancer patients receiving the mTOR inhibitor, everolimus (Loron et al., 2015). The apparent suppression of Treg function in idelalisib-treated patients suggested that p110δ inhibition might enhance anti-tumor activity by inhibiting the functions of an important cellular contributor to the immunosuppressive tumor microenvironment. Inhibition of p110δ may interfere with Treg functions, in part, by suppressing the activation of AKT in antigen-responsive Treg cells. Monoallelic expression of an AKT-insensitive FOXO1 mutant in Treg cells resulted in decreased tumor infiltration of activated Tregs accompanied by enhanced activation of tumor-infiltrating cytotoxic T lymphocytes, and in turn increased inhibition of tumor growth in three different preclinical models (Luo et al., 2016). The potential of p110δ inhibitors to enhance antitumor immunity in mice via Treg inhibition was discussed above (Ahmad et al., 2017; Ali et al., 2014). The potential immunostimulatory effects of p110δ inhibitors are being explored in combination with anti-PD-1 antibody in ongoing clinical studies.

Tissue Overgrowth and Hamartoma Syndromes

Syndromes Caused by Deregulated mTORC1 Activation

In spite of the challenges experienced during the clinical development of PI3K/mTOR pathway inhibitors, some noteworthy success stories have emerged from the identification of patient populations bearing mutations, downstream of PI3K, which drive hyper-activation of

mTORC1 signaling (Table 1). A canonical example of mTORC1-driven disease pathology is tuberous sclerosis, an autosomal, dominantly inherited genetic disorder with a frequency of 1 in 6000 live births (Nathan et al., 2017; Switon et al., 2016). Consistent with the ubiquitous roles of mTORC1 in the stimulation of cell growth and proliferation, tuberous sclerosis is a multi-organ disease characterized by the outgrowth of benign tumors, termed hamartomas, in the brain, kidneys, heart, and other organs. Surprisingly, these patients have a relatively normal lifespan, but the propensity of tumors to develop in the central nervous system leads to cognitive defects and seizures that can be debilitating and even life threatening. Commonly observed brain lesions are cerebral hamartomas and subependymal giant cell tumors (SEGAs) (Franz et al., 2014; JóŸwiak et al., 2016).

Encouraging results obtained in TSC-deficient mouse models prompted clinical trials with rapalogs in tuberous sclerosis patients. Everolimus is now an established therapeutic agent for adults with high-risk renal angiomyolipomas associated with a diagnosis of tuberous sclerosis, and for the both young and adult patients with surgically non-resectable SEGAs (Bissler et al., 2008, 2013). Both rapamycin and everolimus have shown benefit in reducing the severity of epilepsy in tuberous sclerosis patients (Krueger et al., 2016; Switon et al., 2016). However, rapalog monotherapy is not curative, likely due in part to failure to completely block inappropriate mTORC1 signaling at clinically tolerable doses of these agents. In addition, chronic exposure to rapalogs often leads to serious complications, such as mucositis, pneumonitis, and increased risks of infection, which force discontinuation of therapy. In the absence of chronic exposure to the rapalog, the patient experiences tumor regrowth and symptomatic progression.

Lymphangioleiomyomatosis (LAM) is a syndrome that occurs almost exclusively in females, and is characterized by the emergence of smooth muscle-like cysts in the lung, and a gradual decline in pulmonary function (Henske and McCormack, 2012; Lam et al., 2017). Most women with LAM are diagnosed with a sporadic form of the disease, in which the parental, disease-causing clone has incurred biallelic loss of function mutations in *TSC1* or, in the majority of cases, *TSC2*. LAM is termed a “benign neoplasm”, because accumulating evidence suggests that LAM cells resemble, in phenotype and behavior, low-grade sarcoma cells. Indeed, the primary LAM cells that give rise to the cardinal symptom of cystic lung disease appear to be emigrants from distant organs, with the uterus suspected as a prominent source of pre-metastatic LAM cells. The overwhelming gender difference with regard to LAM incidence suggests that hormonal factors, specifically estrogen, collaborate with loss of TSC function during disease development and progression. Until recently, no effective treatment for LAM was available to the patients, who were left to suffer an inexorable decline in lung function, ultimately leading to the need for a life-saving lung transplant. However, the strong genetic linkage between loss of TSC function and LAM suggested that therapeutic targeting of mTORC1 might prove effective in halting or delaying LAM progression (Yates, 2016). A subsequent clinical study (the MILES trial) demonstrated that rapamycin (Sirolimus™) treatment significantly reduced the rate of decline in several parameters of pulmonary function (McCormack et al., 2011), leading to the drug’s approval by the FDA as the first therapy for LAM (Table 1 and Table S1). Unfortunately, as is the case for tuberous sclerosis patients, rapamycin only delays disease progression and does not reverse damage already done to the lungs. Recently, an important new sirolimus trial (the

MILED trial) was announced and is predicted to further increase the impact of rapalog therapy on the disease course of LAM (NCT03150914). This trial will focus on patients at a much earlier stage of the disease than those admitted to the MILES trial. Importantly, the dose of sirolimus selected for MILED (1 mg per day) will be half of the dose tested in the MILES trial. The intention is to reduce the drop-out rate due to adverse drug effects, and to allow these healthier, early-stage patients to derive greater benefit from the mTORC1 inhibitor, administered on a more chronic basis. Given the similarity of LAM lesions to overt cancerous tumors, it is anticipated that combinations involving rapalogs and/or other targeted agents will ultimately be required for optimal therapeutic benefit in this disease.

The efficacy of rapalog therapy in the setting of somatic loss of mutations involving TSC has also been observed in frank malignant disease. Solit and coworkers used deep genomic sequencing to identify somatic mutations in *TSC1* in patients with metastatic bladder cancer, and observed that these patients fared significantly better on everolimus therapy than did patients whose bladder tumors contained the wild-type *TSC1* gene (Iyer et al., 2012). A subsequent publication identified an advanced, anaplastic thyroid cancer patient bearing a nonsense mutation in *TSC1*, who also gave an extraordinary response to everolimus (Lim et al., 2016; Wagle et al., 2014). The patient's disease progressed after 18 months on drug, and re-sequencing of the tumor genome revealed a mutation in *MTOR* that encoded a single amino acid substitution in the FKBP12-binding domain. Although this alteration confers resistance to the rapalogs, the tumor should, in principle, retain sensitivity to ATP-competitive mTOR kinase inhibitors or the bidentate RapaLinks discussed above. Finally, a recent study identified a cohort of renal cancer patients with loss of function in *TSC1* and/or activating mutations in mTOR who were exceptional responders to rapalog therapy (Voss et al., 2014). Interestingly, the cancer-associated TSC mutations described above involve the *TSC1* gene, whereas the majority of alterations that underpin tuberous sclerosis and LAM target the *TSC2* subunit. It is tempting to speculate that in addition to activating mTORC1, these mutations disrupt an mTORC1-independent function(s) of TSC1, such as the regulation of transforming growth factor-β signaling (Thien et al., 2015).

Syndromes Caused by Deregulated PI3K Signaling

Heritable germline loss of function mutations in *PTEN*, a key negative regulator of the PI3K/mTOR pathway signaling network, give rise to a cluster of tissue overgrowth syndromes collectively termed *PTEN* hamartoma tumor syndrome (PHTS) (Gammon et al., 2016; Worby and Dixon, 2014). These *PTEN*-linked diseases are transmitted generationally in an autosomal dominant fashion. The archetypal member of this disease group is Cowden's syndrome (CS). Like the tuberous sclerosis complex syndrome described above, CS is a multi-organ system disorder characterized by anomalous tissue overgrowths. However, CS confers a considerably more significant risk of cancer development, with breast, kidneys, colon, thyroid, and endometrium being prime targets for tumorigenesis. Management of CS patients entails mostly supportive care, with particular attention paid to screening for various cancers. Sirolimus has been tested in CS patients (Table 1), and appears to ameliorate disease symptoms, but the response is only partial and escape from the drug has been observed after a few months of therapy (Munoz and Kurzrock, 2012). The less than optimal outcomes to date with rapalog therapy likely reflect the challenge associated

with treating the mTORC1 component of a disease stemming from inappropriate activation of the entire PI3K/mTOR pathway network.

CLOVES syndrome is a rare, non-malignant disease resulting from somatically-acquired, mosaic activating mutations in the *PIK3CA* gene (Kurek et al., 2012). This syndrome is characterized by regional tissue overgrowths and malformations affecting the epidermis, internal organs, skeleton, and central nervous system. CLOVES syndrome was recognized as a separate disease entity only ten years ago, and the main disease-modifying treatment is surgery. Both the disease itself and the surgery can be disfiguring and potentially life-threatening. In patients with vascular anomalies associated with elevated PI3K/AKT/mTOR signaling, rapamycin produced partial responses in 47 of 57 patients enrolled in a phase 2 study (Adams et al., 2016) (Table 1). Patients with *PIK3CA*-dependent vascular anomalies and other tissue overgrowths may achieve even greater benefit from treatment with a selective p110α inhibitor, which would block mTORC1-independent functions of p110α not covered by rapamycin. As expected, PI3K inhibitors strongly suppress the proliferation of CLOVES syndrome cells in culture (Loconte et al., 2015). We expect that p110α inhibitors will be tested in CLOVES syndrome patients in the near future, and, toxicity issues notwithstanding, it is hoped that these drugs will offer the possibility of complete remissions not achievable with rapalog therapy.

A recent report offered evidence for an alternative pathway of PTEN inactivation driven by somatic disruption of *PARK2*, which encodes Parkin, an E3 ligase involved in the elimination of dysfunctional mitochondria by mitophagy (Gupta et al., 2017; Pickrell and Youle, 2015). PARK2-deficient cancer cells exhibit elevated reactive oxygen species (ROS) and nitrogen oxide synthase activity, presumably due to defective mitochondrial function. These abnormalities result in loss of PTEN lipid phosphatase activity and reduced expression of the PTEN protein, attributable to the nitrosylation of a critical cysteine residue in the catalytic domain, and subsequent protein ubiquitination and degradation. The results of this study suggest that *PARK2* is a haploinsufficient tumor suppressor, and that as many as to two-thirds of human cancers exhibit reduced expression of the *PARK2* gene product. Extrapolation of these results to PTEN function argues that at least an equal proportion of human cancers are prone to PI3K/mTOR pathway hyper-activity due to impaired PtdIns-3,4,5-P_3 degradation. Moreover, these findings also offer a fascinating link to the mechanism of neurodegeneration in Parkinson's disease. Loss of function mutations in *PARK2* were first identified in individuals with early onset Parkinson's disease and might be an indication for testing PI3K/mTOR pathway inhibitors (Table 1). The beneficial effects of mTORC1 inhibition in Parkinson's disease may not be restricted to those cases associated with *PARK2* mutations. A study in *D. melanogaster* revealed that increases in 4E-BP1-dependent translational repression induced by rapamycin or genetic manipulation increased resistance of dopaminergic neurons to cell death in flies bearing disease-associated mutations in the homologs of *PARK*, *PINK1*, and *LRRK2* (Tain et al., 2009). Previous studies uncovered a link between Parkinson's disease and an increased risk of certain cancers (Feng et al., 2015). It would be interesting to learn whether reduced Parkin activity in the dopaminergic neurons of the substantia nigra also leads to loss of PTEN activity, and, in turn, whether an inappropriate increase in PI3K/mTOR signaling is causally related to the death of these neurons on Parkinson's patients.

Perspectives

Over three decades of intensive basic research and drug development have resulted in slow yet undeniable progress in targeting the PI3K/mTOR pathway for the treatment of cancer, along with renewed optimism for future success. The rapalogs have generally failed to deliver on expectations that these drugs would be broadly impactful anti-cancer agents, yet the combination of everolimus with aromatase inhibitors is now an approved second-line therapy in hormone-dependent breast cancer. Although early trials with PI3K/mTOR pathway inhibitors were not as successful as hoped, the next wave of clinical trial approaches is likely to improve the clinical outcome in many oncology settings. For example, the new generation of isoform-selective PI3K inhibitors shows great promise in combination with fulvestrant and/or CDK4/6 inhibitors in breast cancer. The long-sought goal of developing compounds that selectively inhibit mutationally activated forms of p110α has nearly been achieved, with taselisib in phase 3 trials for patients whose tumors harbor *PIK3CA* mutations. The p110δ inhibitor idelalisib is a clinical option in various blood cancers, and other p110δ-selective compounds with apparently improved safety are currently in development. Intermittent dosing schedules and nanoparticle-mediated drug delivery may broaden the therapeutic window for pan-PI3K inhibitors and TORKi. Development of treatment schemes that minimize systemic feedback through elevated glucose and insulin may further increase the efficacy-safety profiles of these compounds. Refinement of biomarkers for patient selection and prediction of relapse will undoubtedly improve the success rate of PI3K/mTOR pathway inhibitors. To produce even greater gains in application of PI3K/mTOR inhibitors, basic research is necessary to uncover synthetic lethality in different cancer types and leverage this knowledge for effective combinations.

A greater appreciation for the complex roles of PI3K/mTOR signaling in the immune system has uncovered unexpected strategies to modulate the tumor microenvironment for therapeutic benefit. Inhibition of p110δ or p110γ, neither of which is commonly expressed in solid tumor cells, can overcome immune tolerance and/or the immunosuppressive milieu to promote T cell anti-tumor responses and potentiate the efficacy of immunotherapies in preclinical models. Inhibition of PI3K and/or mTOR in dendritic cells also holds promise to enhance cancer vaccine approaches.

Another unexpected outcome of PI3K/mTOR research has been the identification of several disease indications outside of oncology that are likely to benefit substantially from PI3K/mTOR-targeted inhibitors (Table 1). Although relatively rare in incidence, these diseases share a common etiology, based on dysregulated PI3K/mTOR signaling, and cause immense suffering to patients and their families. The evolutionarily conserved role of PI3K and mTOR in organismal aging also provides new opportunities to extend lifespan and, more importantly, healthspan through partial pathway inhibition (Johnson et al., 2013; Pan and Finkel, 2017; Selman et al., 2009; Wu et al., 2013). In the coming years, studies of PI3K/mTOR biology will surely uncover additional surprises and new approaches to ameliorate human disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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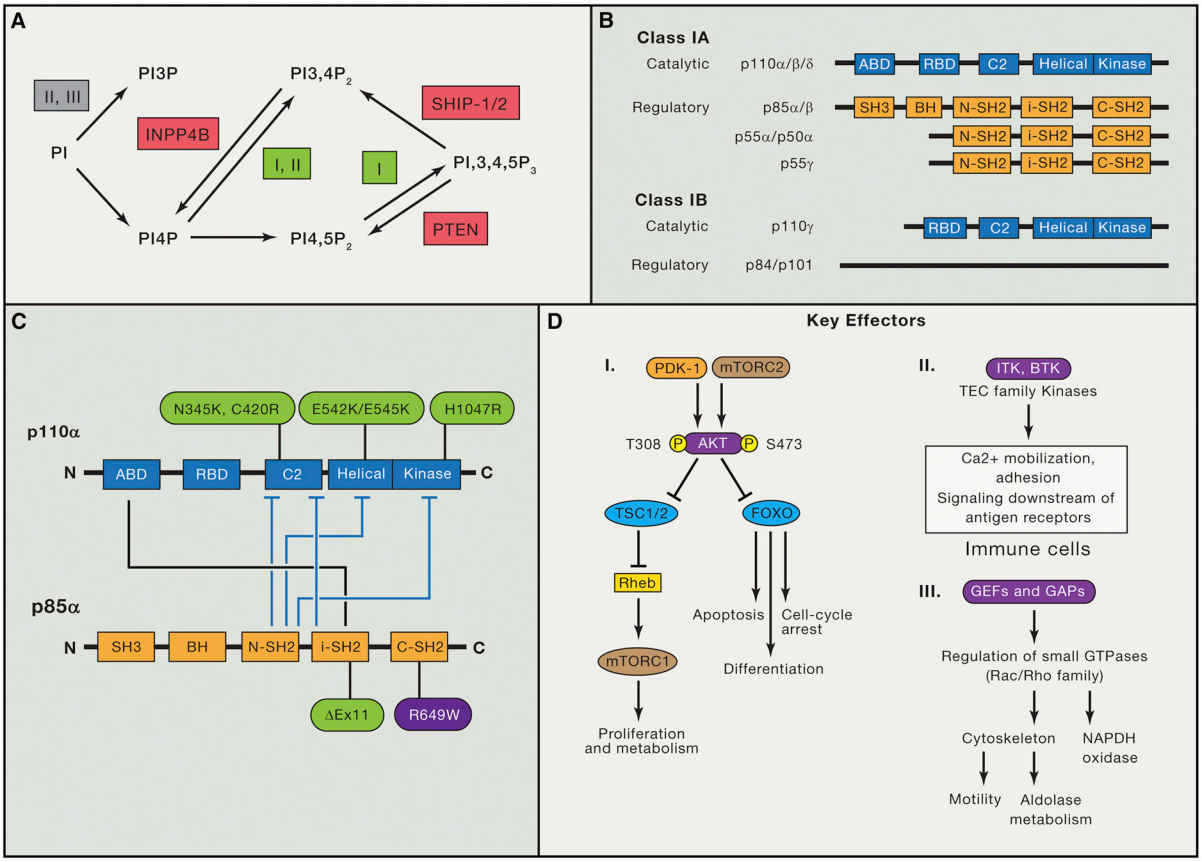

Figure 1. Overview of Phosphoinositides, Class I PI3K Protein Isoforms, p110α Activity Regulation, and PI3K Downstream Effectors

(A) Schematic overview of the major synthesis and degradation pathways for PtdIns-3-P (PI3P), PtdIns-3,4-P2 and PtdIns-3,4,5-P3. The classes of PI3K (I, II, or III) that mediate reactions are indicated. Lipid phosphatases are in red. INPP4B, inositol polyphosphate-4-phosphatase, type II.

(B) Domain structure of class I PI3K catalytic and regulatory subunits. ABD, adaptor-binding domain; RBD, Ras-binding domain; BH, breakpoint cluster region homology.

(C) Diagram of the intramolecular interactions between class IA catalytic and regulatory subunits (p110α and p85α are displayed as well studied examples). Tight binding of the ABD to iSH2 confers stability to p110α. The other contacts shown in blue block arrows diminish basal activity and are relieved upon regulatory subunits binding to pTyr. Cancer-associated activating mutations are shown in green. SHORT syndrome mutation in p85α (R649W) is in purple.

(D) Brief summary of key PI3K effectors: PDK-1, AKT, TEC family kinases, and GEFs/GAPs for small GTPases. AKT has many other important substrates not shown here (Manning and Toker, 2017). The specific GEFs that mediate PI3K-dependent Rac activation to promote motility and aldolase release are not known.

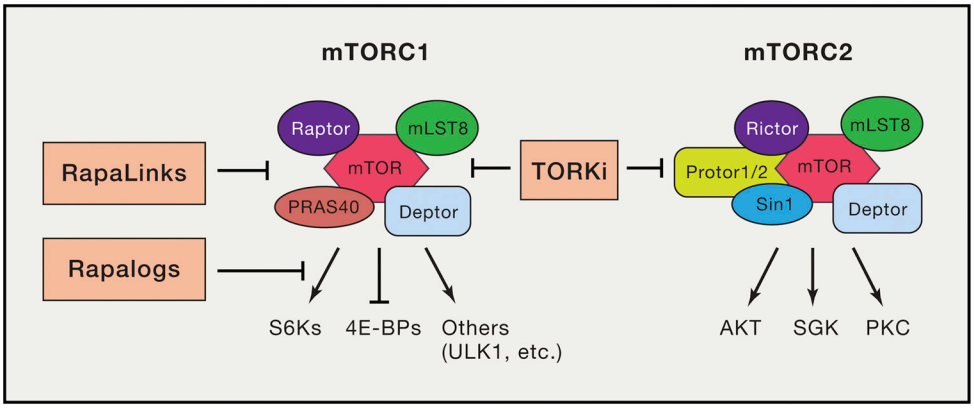

Figure 2. Overview of mTORC1 and mTORC2 complexes, key substrates, and inhibitors

The processes inhibited by different classes of mTOR inhibitor are shown. First generation rapalogs are partial inhibitors of mTORC1 that inhibit phosphorylation of S6Ks more than 4E-BP1. Second generation TORKi fully inhibit mTORC1 and mTORC2. Third generation RapaLinks fully but selectively inhibit mTORC1, and also overcome single resistance mutations to rapalogs and TORKi.

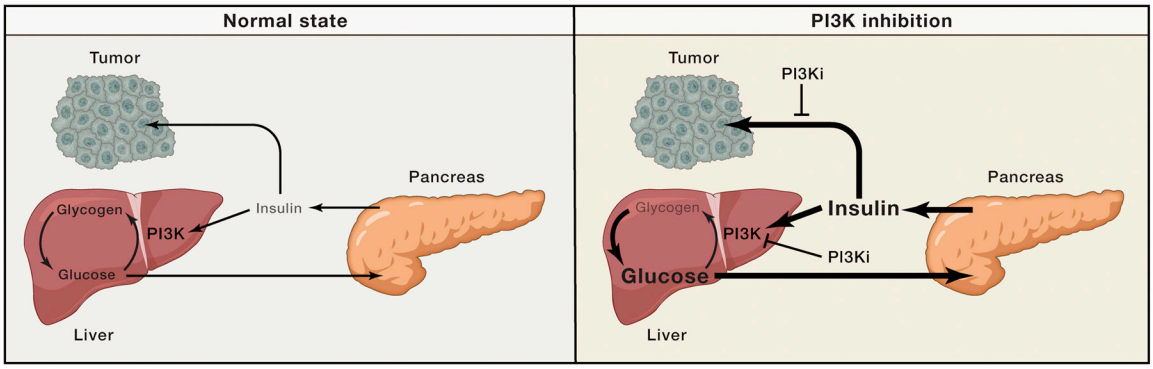

Figure 3. Cartoon of systemic glucose homeostasis in the normal state (left) and upon PI3K inhibitor treatment (right)

In the normal state blood glucose levels are maintained in homeostasis through the actions of insulin, which stimulates glucose uptake and glycogen storage thereby keeping the system balanced. Changes in blood glucose levels (such as increases upon eating) stimulate commensurate changes in insulin release which drive either increased glucose uptake (when insulin levels are high) or gluconeogenesis (when insulin levels are low). When PI3K inhibitors are used they perturb insulin signaling in cells, thereby pushing the systemic balance to favor glucose release. This causes blood glucose levels to acutely increase, which in turn signals to the pancreas to release a bolus of insulin. As indicated by the cartoon these high insulin levels have the potential to reactivate insulin signaling both in metabolic tissues, which is critical in order for the system to come back to homeostasis, as well as in tumors, where insulin has the potential to reactivate PI3K signaling thereby undercutting the efficacy of the PI3K inhibitors.

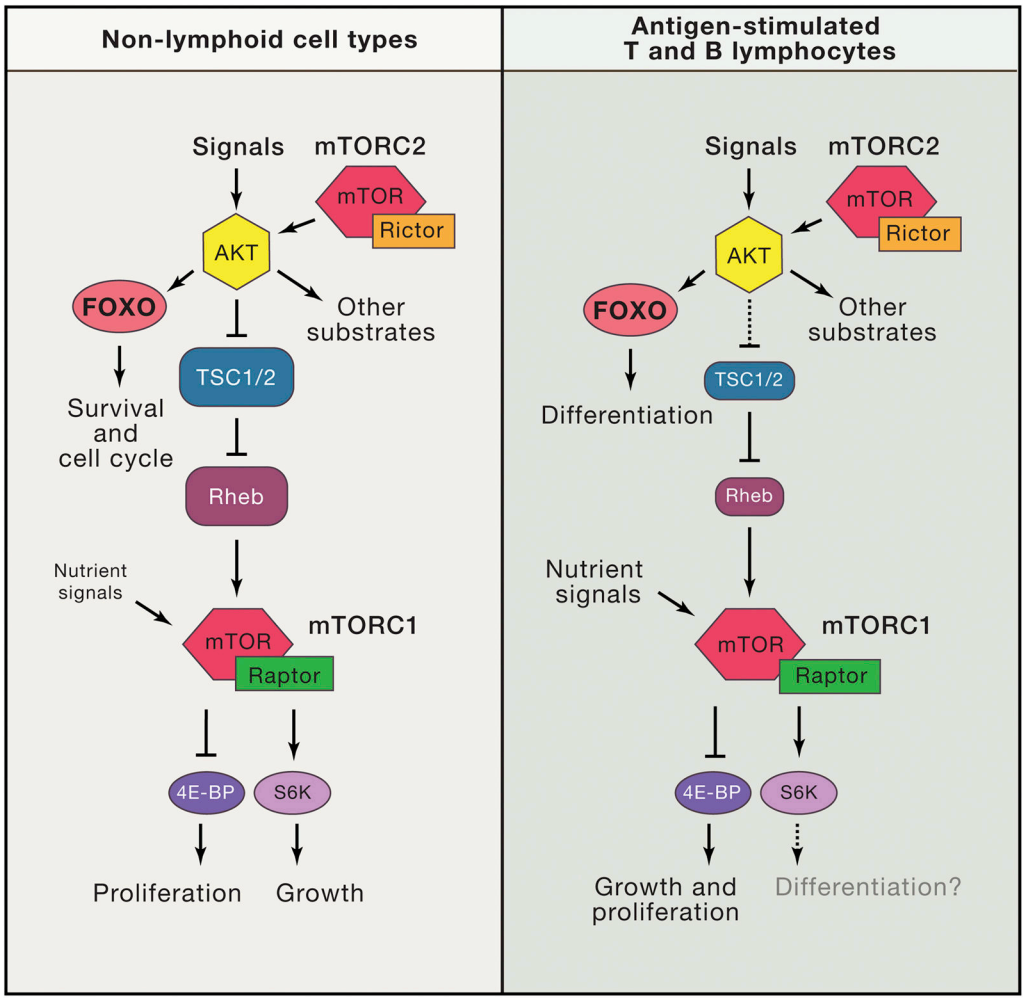

Figure 4. Distinct wiring of PI3K/mTOR network in lymphocytes (T and B cells) compared to other commonly studied non-lymphoid cell types such as fibroblasts

In lymphocytes, FOXO transcription factors have prominent roles in differentiation. mTORC1 is tightly coupled to nutrient access and often uncoupled from PI3K/AKT activity. Downstream of mTORC1, the 4E-BP/eIF4E axis controls both growth and proliferation whereas in other cell types, S6Ks are crucial for cell growth. S6Ks might contribute to lymphocyte differentiation but this is unproven.

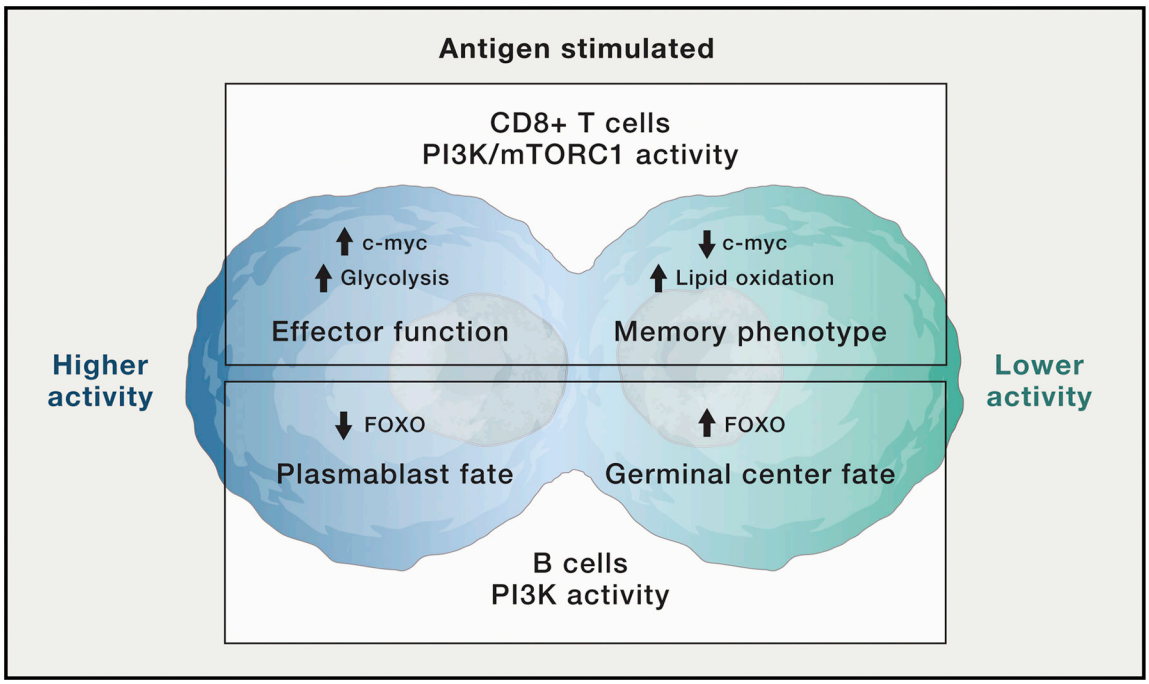

Figure 5. Asymmetric partitioning of PI3K/mTOR signaling during initial division of activated T and B cells results in distinct cell fates of daughter cells

This figure illustrates that the first division of activated lymphocytes produces two cells with differential levels of PI3K/mTOR signaling, which in turn drive distinct metabolic and differentiation programs. Top: CD8 T cells. Bottom: B cells.

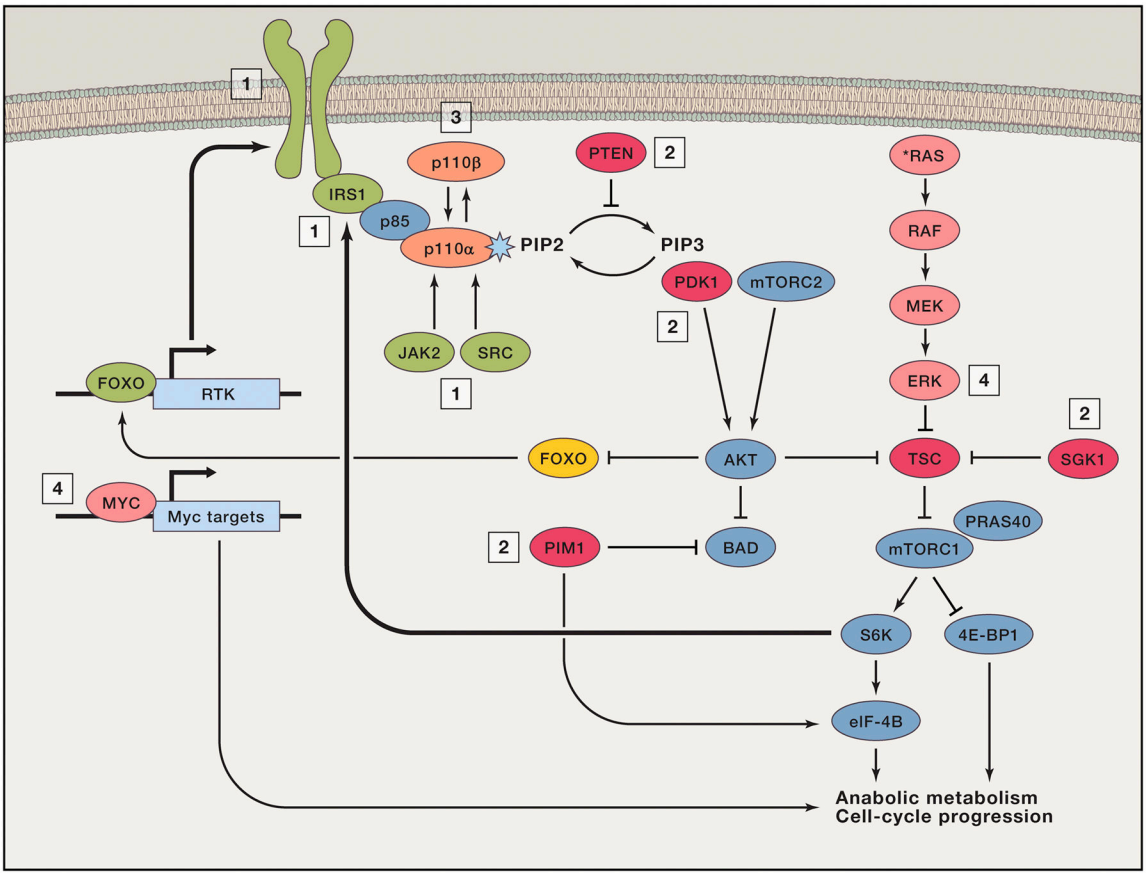

Figure 6. Mechanisms of Adaptive, Primary, and Acquired Resistance to PI3K/mTOR Pathway Inhibitors

Adaptive resistance (in green, also labeled “1”) often involves upregulation of upstream regulators, including RTKs (HER3, INSR, IGF-1R), IRS-1, JAK2, or SRC by disruption of negative-feedback loops. Primary or acquired resistance (in red, “2”) can arise by expression or activation of kinases with downstream targets in common with AKT or mTORC1 (SGK1, PDK1, PIM1), constitutive activation of mTORC1 signaling (e.g., due to loss of TSC function), or loss of PTEN expression. Primary or acquired resistance (in orange, “3”) can also arise by PI3K isoform switching; selective inhibition of p110α can lead to substitution by p110β or vice versa. Finally, primary or acquired resistance can also arise by activation of heterologous pathways leading to common endpoints; for instance, MYC-dependent transcriptional activation or ERK activity (in pink, “4”).

Table 1

Non-malignant diseases associated with hyperactive PI3K/mTOR signaling

Disease	Genetic defect	Targeted treatment (approved or tested)
Cowden Syndrome	*PTEN* haploinsufficiency	Rapalogs tested
CLOVES and other tissue overgrowth syndromes	Somatic *PIK3CA* mutation	Rapalogs tested; p110α inhibitors planned
APDS	Germline *PIK3CD* mutation	Rapalogs tested; p110δ inhibitors in trials
Tuberous sclerosis	*TSC* haploinsufficiency	Rapalogs approved
Lymphangioleiomyomatosis	Somatic *TSC* mutations	Rapalogs approved
Parkinson's Disease	*PARK2* mutations	Rapalogs *

* In patients with early-onset Parkinson's linked to *PARK2* or alpha synuclein mutations, mTORC1 inhibition might be beneficial if treatment were started early in disease. Two potentially beneficial actions in this setting are: (1) tempering inappropriate protein synthesis and growth signaling, and (2) increasing autophagy and the clearance of neurotoxic protein aggregates.