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Inhibition of PI3K Signaling Spurs New Therapeutic Opportunities in Inflammatory/Autoimmune Diseases and Hematological Malignancies

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***Abstract*——The phosphoinositide 3-kinase/mammalian target of rapamycin/protein kinase B (PI3K/mTOR/Akt) signaling pathway is central to a plethora of cellular mechanisms in a wide variety of cells including leukocytes. Perturbation of this signaling cascade is implicated in inflammatory and autoimmune disorders as well as hematological malignancies. Proteins within the PI3K/mTOR/Akt pathway therefore represent attractive targets for therapeutic intervention. There has been a remarkable evolution of PI3K inhibitors in the past 20 years from the early chemical tool compounds to drugs that are showing promise as anticancer agents in clinical trials. The use of animal models and pharmacological tools has expanded our knowledge about the contribution of individual class I PI3K isoforms to immune cell function. In addition, class II and III PI3K isoforms are emerging as nonredundant regulators of immune cell signaling revealing potentially novel targets for disease treatment. Further complexity is added to the PI3K/mTOR/Akt pathway by a number of novel signaling inputs and feedback mechanisms. These can present either caveats or opportunities for novel drug targets. Here, we consider recent advances in 1) our understanding of the contribution of individual PI3K isoforms to immune cell function and their relevance to inflammatory/autoimmune diseases as well as lymphoma and 2) development of small molecules with which to inhibit the PI3K pathway. We also consider whether manipulating other proximal elements of the PI3K signaling cascade (such as class II and III PI3Ks or lipid phosphatases) are likely to be successful in fighting off different immune diseases.**

I. Introduction

Phosphoinositide 3-kinase (PI3K[1]) is an evolutionarily conserved family of lipid kinase enzymes, the first member of which was discovered in the late 1980s (Courtneidge and Heber, 1987; Whitman et al., 1988). PI3Ks are known to play nonredundant functions in multiple cellular processes ranging from cell growth and proliferation to migration and cytokine production. Discovery of PI3Ks and subsequent mapping of the signaling pathway heralded an intense effort by the pharmaceutical industry to develop inhibitors targeting components of this pathway. This was driven by the recognition that dysregulation of the PI3K pathway is associated with numerous cancers (Yuan and Cantley, 2008; Liu et al., 2009b) as well as inflammatory and autoimmune diseases (Rommel et al., 2007). Although PI3K is a compelling target for therapeutic intervention in cancer, several other kinases within the PI3K signaling cascade also offer opportunities, such as mammalian target of

[1]Abbreviations: 3AC, 3α-aminocholestane; 3MA, 3-methyladenine; AQX-016A, (6aR,11aR,11bS)-4,4,6a,7,11b-pentamethyl-1,2,3,4a,5,6,11,11a-octahydrobenzo[a]fluorene-9,10-diol; AS-252424, 5-[[5-(4-fluoro-2-hydroxyphenyl)-2-furanyl]methylene]-2,4-thiazolidinedione; AS-605240, 5-(quinoxalin-6-ylmethylidene)-1,3-thiazolidine-2,4-dione; AZD8055, [5-[2,4-bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl]-2-methoxyphenyl]methanol; BCR, B-cell receptor; BKM120, 5-(2,6-dimorpholin-4-ylpyrimidin-4-yl)-4-(trifluoromethyl)pyridin-2-amine; BYL719, (*S*)-*N*1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl) pyrrolidine-1,2-dicarboxamide; CAL-101, 5-fluoro-3-phenyl-2-[(1S)-1-(7H-purin-6-ylamino)propyl]quinazolin-4-one; CCR, chemokine receptor; CdtB, cytolethal distending toxin subunit B; CLL, chronic lymphocytic leukemia; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; CZC24832, 5-(2-amino-8-fluoro-[1,2,4]triazolo[1,5-a]pyridin-6-yl)-N-*tert*-butylpyridine-3-sulfonamide; EAE, experimental autoimmune encephalomyelitis; EGF, epidermal growth factor; FcγR, Fc receptors for IgG; fMLP, *N*-formylmethionyl-leucyl-phenylalanine; GDC0941, 2-(1H-indazol-4-yl)-6-(4-methanesulfonylpiperazin-1-ylmethyl)-4-morpholin-4-ylthieno(3,2-d)pyrimidine; GPCR, G protein-coupled receptor; HL, Hodgkin lymphoma; IC-87114, 2-[(6-aminopurin-9-yl)-methyl]-5-methyl-3-(2-methylphenyl)quinazolin-4-one; IKBKE, inhibitor of nuclear factor κB kinase subunit ε; IL, interleukin; INK128, 3-(2-amino-5-benzoxazolyl)-1-(1-methylethyl)-1H-pyrazolo[3,4-d] pyrimidin-4-amine; IPI-145 (INK-055), *N*-(6-(4-amino-1-((8-methyl-1-oxo-2-*o*-tolyl-1,2-dihydroisoquinolin-3-yl)-methyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-yl)acetamide; LPS, lipopolysaccharide; LTB4, leukotriene B4; LY294002, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one; MDSC, myeloid-derived suppressor cell; MI, myocardial infarction; MS multiple sclerosis; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; NK, natural killer; OVA, ovalbumin; PDK, phosphoinositide lipid-dependent kinase; PH, pleckstrin homology; PI(3)P, phosphatidylinositol 3-phosphate; PI(3,4)P_2, phosphatidylinositol 3,4-bisphosphate; PI(3,4,5)P_3, phosphatidylinositol 3,4,5-trisphosphate; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PI-103, 3-(4-morpholin-4-ylpyrido[2,3]furo[2,4-b]pyrimidin-2-yl)phenol; PI3K, phosphoinositide 3-kinase; PIK75, *N*-[(*E*)-(6-bromoimidazo[1,2-a]-pyridin-3-yl)methylideneamino]-*N*,2-dimethyl-5-nitrobenzenesulfonamide hydrochloride; PKB/Akt, protein kinase B; PP242, (2Z)-2-(4-amino-1-propan-2-yl-2H-pyrazolo[3,4-d]pyrimidin-3-ylidene)indol-5-ol; PTEN, phosphatase and tensin homolog; PX-866, [(3aR,6E,9S,9aR,10R,11aS)-6-[[bis(prop-2-enyl)amino]methylidene]-5-hydroxy-9-(methoxymethyl)-9a,11a-dimethyl-1,4,7-trioxo-2,3,3a,9,10,11-hexahydroindeno[4,5-h]isochromen-10-yl] acetate; RA, rheumatoid arthritis; ROS, reactive oxygen species; RS, Reed-Sternberg; S1P, sphingosine-1-phosphate; SF1126, (8S,14S,17S)-14-(carboxymethyl)-8-(3-guanidinopropyl)-17-(hydroxymethyl)-3,6,9,12,15-pentaoxo-1-(4-(4-oxo-8-phenyl-4H-chromen-2-yl)morpholino-4-ium)-2-oxa-7,10,13,16-tetraazaoctadecan-18-oate; SHIP, SH2-domain containing inositol-5-phosphatase; SLE, systemic lupus erythematosus; TCR, T-cell receptor; TG-100-115, 3-[2,4-diamino-7-(3-hydroxyphenyl)pteridin-6-yl]phenol; TG-101-110, 6-(1H-indol-4-yl)-pteridin-2,4-diamine::6-(1H-indol-4-yl)pteridine-2,4-diamine; TGX-221, 9-(1-anilinoethyl)-7-methyl-2-morpholin-4-ylpyrido[1,2-a]pyrimidin-4-one; TLR, Toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; XL147, *N*-[3-(2,1,3-benzothiadiazol-5-ylamino)quinoxalin-2-yl]-4-methylbenzenesulfonamide; ZSTK474, 2-(2-difluoromethylbenzimidazol-1-yl)-4,6-dimorpholino-1,3,5-triazine.

rapamycin (mTOR), protein kinase B (PKB/Akt), and 3′-phosphoinositide lipid-dependent kinase-1 (PDK1), that are extensively reviewed elsewhere (Peifer and Alessi, 2008; Hers et al., 2011; Zask et al., 2011).

This review focuses on the potential of inhibiting PI3Ks in the immune system, where PI3K activation occurs in response to a diverse array of receptors that are expressed on leukocytes and are responsible for both innate and adaptive immune responses. PI3Ks are activated by antigen receptors, costimulatory receptors, Fc receptors, adhesion molecules, Toll-like receptors (TLRs) and cytokine receptors, as well as receptors for a variety of chemoattractants, including C5a, *N*-formylmethionyl-leucyl-phenylalanine (fMLP), chemokines, and sphingosine-1-phosphate (S1P) (Okkenhaug and Vanhaesebroeck, 2003; Vanhaesebroeck et al., 2005; Crabbe et al., 2007; Ward and Marelliberg, 2009). In this review, we consider the contribution of PI3Ks to the normal immune response and how dysregulation of this pathway can contribute not only to inflammatory and autoimmune diseases but also to hematological malignancies. Given their predominant expression in cells of hematopoietic linage, we will focus on the contribution of the γ and δ isoforms of class IA PI3K. We will also consider progress in development of selective pharmacological inhibitors, the influence of cancer in this process and how targeting the γ and δ PI3K isoforms has potential application in immune disease settings.

II. Phosphoinositide 3-Kinase Signaling Pathway

Four distinct PI3K subfamilies exist—commonly referred to as classes I, II, III, and IV—on the basis of their substrate specificities, primary structures, modes of regulation, and domain content (Fig. 1). Of these, the class I and the class IV PI3K-related kinase mTOR have been most extensively examined as targets for small-molecule–based therapies.

The class I PI3K enzyme family is composed of a regulatory subunit and a tightly associated catalytic subunit. The class IA enzymes comprise five regulatory subunits encoded by three genes: PIK3r1 encodes p85α and its alternative transcripts p55γ and p50α, PIK3r2 encodes p85β, and PIK3r3 encodes p55γ. Each of the three class IA p110 catalytic isoforms, PI3Kα, PI3Kβ, and PI3Kδ, pairs with one of these regulatory subunits, which are responsible for the recruitment of the complex to the plasma membrane upon receptor ligation. Class IA isoforms are activated downstream of a variety of receptors that are phosphorylated by tyrosine kinases upon cognate stimulus. The class IB catalytic isoform PI3Kγ

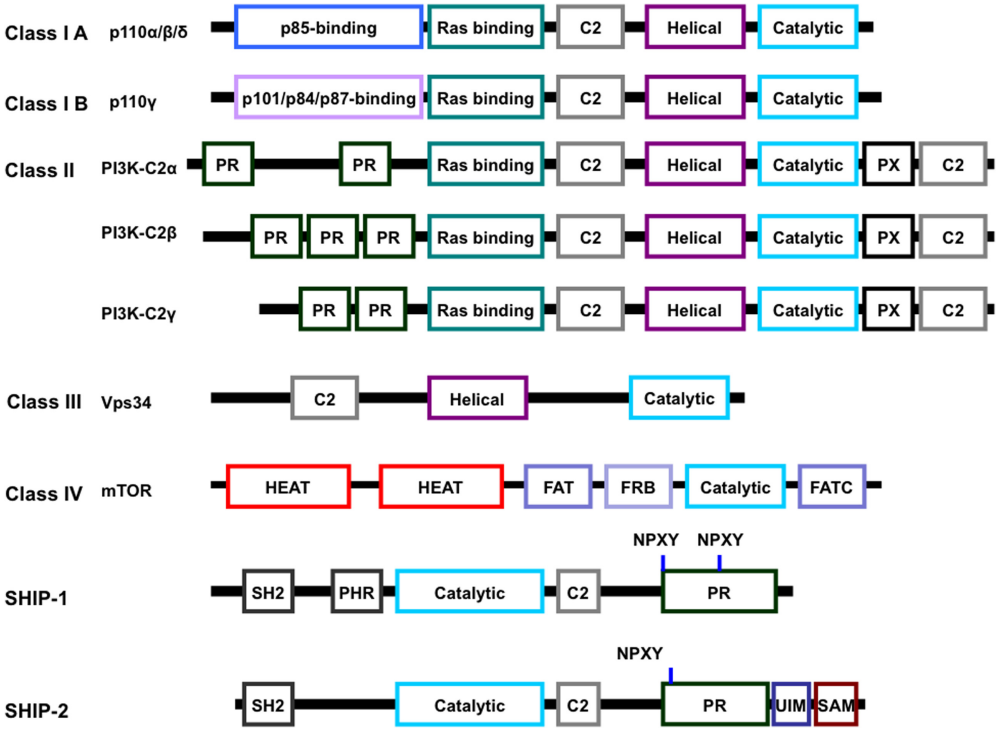

FIG. 1. Molecular structure of proteins in the PI3K signaling pathway. The class I, II, III, and IV PI3K protein families and the lipid phosphatases SHIP-1 and SHIP-2 possess a number of important protein domains within their molecular structures. The class I and II PI3K enzymes share a similar core domain structure of single catalytic, helical, C2, and Ras binding domains. In addition, class I PI3Ks have p85 regulatory subunit-binding (p110 α/β/δ) or p101/p84/p87-binding (p110γ) domains, whereas class II PI3K have proline rich (PR) and phox (PX) domains. The single class III PI3K, Vps34, has only catalytic, helical, and C2 domains. The class IV PI3K-related kinase mTOR has a catalytic domain surrounded by a FAT (FRAP, ATM, and TRRAP) and C-terminal FAT domain (FATC) as well as an FKBP12/Rapamycin (FRB) binding domain. At the N-terminal region, mTOR also has two HEAT (Huntington elongation factor 3, PR65/A, TOR) repeats composed of antiparallel α-helices. SHIP-1 and SHIP-2 possess a catalytic domain with N-terminal SH2 and C-terminal PR domains containing NPXY motifs. SHIP-1 also contains a PH related (PH-R) domain (Ming-Lum et al., 2012) and SHIP2 has a ubiquitin-interacting motif (UIM) and a sterile α motif (SAM).

pairs with either of the regulatory subunits p84/p87 or p101 and is activated by G-protein $\beta\gamma$ subunits and signals downstream of G protein-coupled receptors (GPCRs) (Bohnacker et al., 2009). Remarkably, PI3Kγ complexed with either p101 or p84 can produce phosphatidylinositol 3,4,5-trisphosphate [$PI(3,4,5)P_3$], only the p84-p110γ complex supports mast-cell degranulation, whereas either complex can activate Akt or mediate cell migration. However, some GPCRs seem to activate class IA PI3Ks, most notably PI3Kβ (Guillermet-Guibert et al., 2008). Furthermore, recent evidence has revealed that receptor tyrosine kinases and Toll-like/IL-1 receptors unexpectedly activate myeloid cell PI3Kγ (Schmid et al., 2011). This study also reported that treatment of myeloid cells with vascular endothelial growth factor-A (VEGF-A) causes cognate receptor tyrosine kinase (VEGF receptor 1) to physically associate with PI3Kγ. TLR/IL-1 receptors were reported to directly activate PI3Kγ in a Ras/p87-dependent manner, a mechanism distinct from the GPCR-stimulated PI3Kγ activation that occurs in a Ras/p101-dependent manner. Together with previous reports that PI3Kγ is involved in T-cell receptor (TCR) signaling (Alcázar et al., 2007), these observations have challenged the paradigm that PI3Kγ functions exclusively in GPCR signaling.

The tissue distribution of PI3K isoforms varies; PI3Kα and PI3Kβ seem to have a broad tissue distribution, whereas PI3Kδ and PI3Kγ are predominantly expressed in leukocytes. However, PI3Kγ is also expressed in the heart and the endothelium (Crackower et al., 2002; Patrucco et al., 2004) as well as in some tumors, most notably pancreatic and breast cancers (Edling et al., 2010; Brazzatti et al., 2012; Dituri et al., 2012). Likewise, PI3Kδ is found in some cancer cells of nonleukocyte origin, such as melanoma and breast cancer cells, as well as in neurons (Sawyer et al., 2003; Veerasingham et al., 2005).

Class I PI3K enzymes phosphorylate the D3 position on the inositol ring of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] to generate $PI(3,4,5)P_3$, which is located in the plasma membrane and acts as a docking site to recruit and activate pleckstrin homology (PH) domain containing proteins. Numerous PH-domain-containing proteins are activated by PI3K signaling, including PKB/Akt and PDK1, a range of adaptor/scaffolding proteins, and guanine nucleotide exchange factors, which regulate GTPases and hence cell motility and intracellular trafficking (Fig. 2).

PI3K signaling activity is tightly regulated by at least two lipid phosphatases: SH2-domain containing inositol-

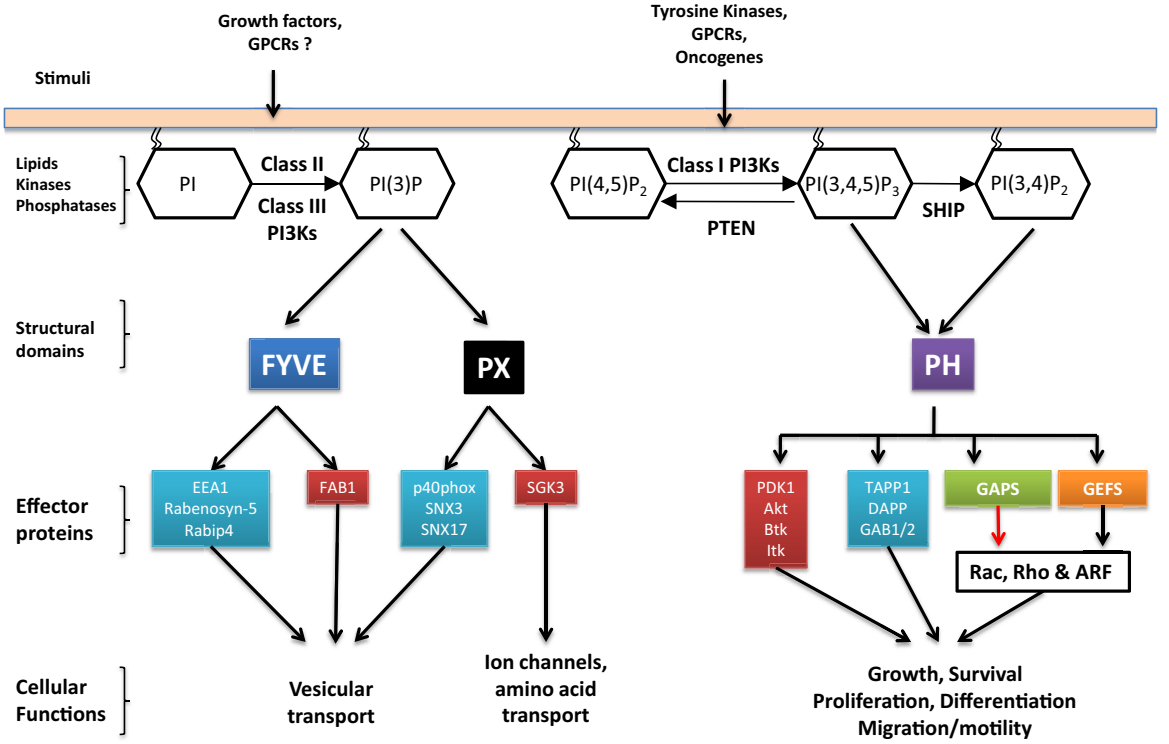

FIG. 2. PI3K signaling cascade. Lipid second messengers recruit components of the PI3K signaling pathway through specific domains. Activation of class I PI3K causes increased levels of the second messenger $PI(3,4,5)P_3$, which recruits and leads to the activation of a number of PH domain-containing proteins. These include kinases such as Akt, binding proteins such as Gab1/2, as well as guanine nucleotide exchange factors (P-REX, cytohesins) and GTPase activating protein (GAPs; e.g., ARAP3). The lipid phosphatase SHIP-1 dephosphorylates $PI(3,4,5)P_3$ to $PI(3,4)P_2$, which, unlike $PI(4,5)P_2$, can also act to recruit PH domain-containing proteins. Some PH domain-containing proteins are exclusively recruited to $PI(3,4)P_2$ and not $PI(3,4,5)P_3$, and some can bind either lipid. The class II and III PI3K family members catalyze the formation of PI3P from PI; this lipid acts to recruit proteins containing either Fab1, YGL023, Vps27, and EEA1 (FYVE) or PX domains. The recruitment and activation of PH, FYVE, and PX domain-containing proteins leads to initiation of signaling cascades that are important for a number of key cellular events.

5-phosphatase (SHIP) and phosphatase and tensin homolog (PTEN) (Harris et al., 2008). SHIP dephosphorylates $PI(3,4,5)P_3$ at the D5 position of the inositol ring to create phosphatidylinositol 3,4-bisphosphate [$PI(3,4)P_2$], whereas PTEN dephosphorylates the D3 position to create $PI(4,5)P_2$ (Fig. 2). Multiple forms of SHIP have been reported, expression of 145-kDa SHIP-1 (Fig. 1) being restricted to differentiated cells of the hematopoietic system, endothelial cells, hematopoietic stem cells, and embryonic stem cells (Kerr, 2008). In contrast, SHIP-2 (a 142-kDa protein that is highly homologous with SHIP-1 but is encoded by a different gene) and PTEN are expressed in both hematopoietic and nonhematopoietic tissues. SHIP-2 also has a broader phospholipid substrate specificity than SHIP-1, because it also hydrolyzes $PI(4,5)P_2$ in vitro (Ooms et al., 2009).

III. Role of Phosphoinositide 3-Kinases γ and δ in the Immune System

A diverse array of receptors expressed on leukocytes responsible for both innate (neutrophils, macrophages) and adaptive (T and B lymphocytes) immune responses as well as those that constitute a link (mast cells, eosinophils) between these two arms of the immune response are able to stimulate PI3K activation. Mice in which the genes encoding PI3Kγ or PI3Kδ have been either ablated or altered to encode kinase-inactive mutants (e.g., $PI3K\delta^{D910A}$ mice) are viable, fertile, and apparently healthy (Okkenhaug et al., 2002). However, as detailed in Table 1, when the immune system is challenged, the mice exhibit severely altered phenotypes demonstrating that PI3Kγ and PI3Kδ have nonredundant functions in neutrophils, B cells, T cells, mast cells, and dendritic cells and that the activities of these isoforms in immune cells are crucial during the onset, progression, and maintenance of chronic inflammatory diseases. Often these roles are quite distinct, requiring coordinated function of both isoforms at discrete steps of immune cell activation. This has been reviewed extensively elsewhere (Okkenhaug and Vanhaesebroeck, 2003; Vanhaesebroeck et al., 2005, 2010; Crabbe et al., 2007).

IV. Cooperation between Phosphoinositide 3-Kinase Isoforms in the Immune Response

A. Phosphoinositide 3-Kinase γ and δ Coordinate Leukocyte Migration

There is now strong evidence that PI3Kγ and PI3Kδ act in partnership to regulate immune cell signaling and function. The directed movement of leukocytes from the circulation into tissues or secondary lymphoid organs is essential for routine immunosurveillance and normal host defense during infection or injury. Cell migration was initially predicted to require input from PI3Kγ, given that many of the homeostatic and inflammatory mediators that regulate leukocyte trafficking function via GPCRs. Several studies demonstrated that PI3K inhibitors or genetic loss of PI3Kγ causes reduction in chemotactic responses of lymphocytes, neutrophils, macrophages, and eosinophils in a variety of in vitro and in vivo migration assays (Hirsch, 2000; Sasaki et al., 2000b; Reif et al., 2004; Smith et al., 2007; Lim et al., 2009; Thomas et al., 2009). The catalytic function of PI3Kγ is crucial for morphological changes associated with cell polarization by generating $PI(3,4,5)P_3$ at the leading edge and regulating Rac activity and the cytoskeleton reorganization (Costa et al., 2007; Ferguson et al., 2007; Barberis et al., 2009). An additional route by which class IA PI3Ks can influence cell migration has recently been uncovered and involves the regulation of transcription factors that regulate cell quiescence and expression of homing receptors on T cells. Hence, genetic and pharmacological studies have revealed that PI3Kδ and PDK1/Akt play an essential role in the events that lead to proteolytic shedding and reduced transcription of CD62L, CCR7 and S1P receptor-1 (Finlay et al., 2009; Waugh et al., 2009). However, PI3Kδ also regulates neutrophil migration as demonstrated by the ability of a PI3Kδ-specific inhibitor to reduce directional neutrophil movement in response to chemotactic agents (Sadhu et al., 2003; Puri et al., 2004). Moreover, several studies have also revealed the importance of endothelial activity of both PI3Kγ and PI3Kδ in regulating neutrophil interactions with the inflamed vessel wall (Puri et al., 2005, 2004).

The evidence discussed above points to partially overlapping roles of both PI3Kγ and PI3Kδ isoforms in regulating the complex interplay between leukocytes and the inflamed endothelium, as well as their activation responses. Indeed, analysis of neutrophil migration in vivo revealed that, in fact, although PI3Kγ is important in early chemokine-induced emigration, PI3Kδ replaces and maintains the delayed chemokine-induced neutrophil recruitment into inflamed tissues (Liu et al., 2007), suggesting a coordinated but temporally distinct role for these isoforms. This is consistent with observations that in tumor necrosis factor-α (TNFα)-primed human neutrophils, fMLP induces a biphasic increase in $PI(3,4,5)P_3$ accumulation, in which the first phase depends on the PI3Kγ isoform. The second phase of $PI(3,4,5)P_3$ accumulation depends on prior exposure to TNFα and is driven predominantly by PI3Kδ (Condliffe et al., 2005). There is also evidence for distinct yet complimentary roles for PI3Kγ and PI3Kδ at different stages of transendothelial migration of effector T cells (Jarmin et al., 2008; Ward and Marelli-Berg, 2009) as well as during mast cell degranulation in response to antigen receptor and chemokine signals (Laffargue et al., 2002; Ali et al., 2004, 2008; Willox et al., 2010; Kitaura et al., 2005). Indeed, mice that are both deficient in PI3Kγ and express the kinase-inactive $PI3K\delta^{D910A}$ mutant (unlike mice in which PI3K genes have been targeted individually) display severe impairment of thymocyte development, profound T-cell lymphopenia, and T-cell and eosinophil infiltration of mucosal organs, elevated IgE levels, and a skewing

TABLE 1

Immune cell-specific functions of class I PI3K isoforms and the phosphatase SHIP-1

Data were delineated by analysis of mice in which PI3K or phosphatases were genetically ablated, conditionally knocked down, or the kinase domain removed in the whole animal or specific cell types. A role for an isoform in a particular cellular process is indicated by alteration from wild type when that isoform is targeted; these roles are given for each specific cell type, although this is not intended to be an exhaustive list.

Cell Type	Phenotype of β KO/KD	Phenotype of δ KO/KD	Phenotype of γ KO	Phenotype of SHIP1 KO	References
Neutrophils	Dereased FcγR-induced ROS	Decreased migration	Decreased GPCR signaling, ROS production, migration	Spontaneous lung infiltration	Helgason et al., 1998; Sasaki et al., 2000b; Ji et al., 2007; Nishio et al., 2007; Randis et al., 2008; Kulkarni et al., 2011
Eosinophils	N.A.	N.A.	Decreased migration and infiltration	Increased lung infiltration	Sasaki et al., 2000b; Ji et al., 2007; Oh et al., 2007; Thomas et al., 2009
Basophils	N.A.	N.A.	N.A.	Increased FcεR1 signaling, histamine and IL4 release	Vonakis et al., 2007; Kuroda et al., 2011
Mast cells	N.A.	No degranulation or hypersensitivity, protection from passive anaphylaxis	Reduced degranulation, protection from passive anaphylaxis	Increased lung infiltration, degranulation, hyperplasia, cytokine production, proliferation	Huber et al., 1998; Laffargue et al., 2002; Ali et al., 2004, 2008; Kitaura et al., 2005; Haddon et al., 2009; Hochdörfer et al., 2011; Ma et al., 2011
Macrophages/ Monocytes	N.A.	Decreased migration, proliferation	Decreased migration	Increased lung infiltration, circulating cell numbers, M2 skewing, phagocytosis, myeloid suppressors; decreased NADPH oxidase activity	Helgason et al., 1998; Nakamura et al., 2002; Jones et al., 2003; Ghansah et al., 2004; Camps et al., 2005; Bishop et al., 2008; Kamen et al., 2008; Papakonstanti et al., 2008; Leung et al., 2009
Dendritic cells	N.A.	Decreased IL6 production, GPCR signaling, migration, cell survival	Decreased migration and cell numbers	Increased maturation and function; decreased development	Del Prete et al., 2004; Neill et al., 2007; Krishnamoorthy et al., 2008; Antignano et al., 2010a, 2010b, 2011; Delgado-Martín et al., 2011; Kuroda et al., 2011
NK cells	N.A.	Reduced migration	Reduced migration and development	Increased inhibitory receptor expression, cell numbers	Wang et al., 2002; Tassi et al., 2007; Saudemont et al., 2009
T cells	N.A.	Decreased pre-TCR and TCR signaling, proliferation, migration and trafficking, differentiation, Treg function,	Decreased TCR signaling, migration, $CD8^+$ cytotoxicity, $CD4^+$:$CD8^+$ ratio; increased apoptosis	Decreased Th17 differentiation; increased Th1 differentiation, Th2 cytokines, Treg numbers, lung infiltration, $CD8^+$ cytotoxicity	Helgason et al., 1998; Sasaki et al., 2000b; Okkenhaug et al., 2002, 2006; Rodríguez-Borlado et al., 2003; Barber et al., 2006; Kashiwada et al., 2006; Patton et al., 2006, 2011; Ji et al., 2007; Nombela-Arrieta et al., 2007; Oh et al., 2007; Tarasenko et al., 2007; Jarmin et al., 2008; Thomas et al., 2008; Collazo et al., 2009; Liu et al., 2009a; Rolf et al., 2010; Wei et al., 2010

TABLE 1—*Continued.*

Cell Type	Phenotype of β KO/KD	Phenotype of δ KO/KD	Phenotype of γ KO	Phenotype of SHIP1 KO	References
B cells	N.A.	Decreased BCR signaling, proliferation, maturation; increased IgE production, apoptosis	N.A.	Enhanced signaling and function, aberrant development, and positive selection.	Liu et al., 1998; Helgason et al., 2000; Sasaki et al., 2000b; Okkenhaug et al., 2002; Bilancio et al., 2006; Al-Alwan et al., 2007; Ji et al., 2007; Zhang et al., 2008; Leung et al., 2009

Treg, regulatory T cell.

toward Th2 immune responses (Ji et al., 2007). However, the serious defects in immune development observed in the PI3Kγδ-null mice prevent detailed dissection of the selective roles of these PI3K subunits in post-thymic responses.

Despite evidence for cooperation between these isoforms, it is important to highlight that genetic targeting of both PI3Kγ and -δ has revealed distinct contributions of these isoforms dependent on receptor and/or cell type and activation state. Thus, although both PI3Kγ and -δ are required for migration of neutrophils toward leukotriene B4 (LTB4), only the activity of PI3Kγ is responsible for the migration of cells in response to fMLP (Randis et al., 2008). Likewise, both PI3Kγ and PI3Kδ are necessary for natural killer (NK) cell migration to inflamed tissues and the uterus during early pregnancy in vivo and chemotaxis to CXCL12 and CCL3 in vitro. In contrast, PI3Kδ alone was required for NK cell distribution in steady state as well as for trafficking to lymphomas and for chemotaxis to S1P and CXCL10 in vitro (Saudemont et al., 2009). Other studies in murine models have shown that in vitro, both PI3Kδ and PI3Kγ are required for FcγRI-driven mast cell degranulation; in vivo, PI3Kγ (but not PI3Kδ) is dispensable for allergic responsiveness (Laffargue et al., 2002; Ali et al., 2004, 2008; Kitaura et al., 2005). The changing dependence on either or both of these PI3K isoforms is important to bear in mind when designing drugs to interfere with these isoforms in the immune system.

B. Phosphoinositide 3-Kinase β and δ Coordinate the Respiratory Burst

Once neutrophils encounter bacteria, they generate reactive oxygen species (ROS) as part of the respiratory burst to destroy bacteria and resolve infection. Neutrophils isolated from PI3Kγ-null mice show reduced superoxide generation, but in a model of invasive fungal infection of the lung, neutrophils from PI3Kγ-null mice were similar to wild-type cells in their ability to produce ROS in response to *Aspergillus fumigates* hyphae (Boyle et al., 2011). In this model, loss of both PI3Kγ and PI3Kδ isoforms partially reduced ROS generation. However, neutrophils from mice lacking PI3Kβ and expressing kinase-dead PI3Kδ^{D910A} displayed a marked reduction in neutrophil ROS generation. The importance of PI3Kβ and its cooperation with PI3Kδ was further underlined by a study from the same group that demonstrated PI3Kβ plays an essential, nonredundant role in the efficient activation of mouse neutrophils by IgG-containing immune complexes (Kulkarni et al., 2011). Neutrophils detect these immune complexes through Fc receptors for IgG (FcγRs). PI3Kβ was shown to act downstream of FcγR phosphorylation, and PI3Kβ-deficient neutrophils produced lower concentrations of ROS in response to immune complexes than control neutrophils. This effect of PI3Kβ deficiency was most pronounced at low immune-complex densities, but at high densities, PI3Kδ could partly compensate for the loss of PI3Kβ in ROS generation. The study also suggests that LTB4 is produced in response to FcγR ligation and signals in an autocrine or paracrine manner through its GPCR BLT1 to enhance immune complex-induced ROS production. Remarkably, this signaling loop in response to FcγR ligation was not abolished in PI3Kγ-deficient neutrophils, despite the known role for PI3Kγ downstream of BLT1. Rather, although the response to LTB4 alone required PI3Kγ, the response to costimulation with LTB4 and immune complexes was largely dependent on PI3Kβ. Hence, PI3Kβ can integrate signals from FcγRs and BLT1 and plays a crucial role in the response to immune complexes (Kulkarni et al., 2011). This was further illustrated in mice lacking PI3Kβ, which were markedly protected in a model of autoantibody-induced skin blistering. Given that the loss of PI3Kβ does not affect humoral responses or neutrophil-mediated killing of *Staphylococcus aureus* (Kulkarni et al., 2011), inhibition of PI3Kβ might be an effective option to treat certain inflammatory conditions but avoid general adverse effects on protective immunity.

V. Emergence of Chemical Tools to Target Phosphoinositide 3-Kinase Signaling

A. The Pathfinder Inhibitors: The Influence of Crystal Structure Elucidation and Cancer

The first compounds to block PI3K, the natural product wortmannin and Eli Lilly's 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (LY294002), have served as useful experimental tools with which to explore the

PI3K pathway. Wortmannin was first identified nearly 40 years ago (Wiesinger et al., 1974), although its inhibitory effects on PI3K were not identified until the early 1990s (Arcaro and Wymann, 1993), when it was found to covalently interact with the ATP binding pocket of PI3K. LY294002, developed by Lilly Research Laboratories (Indianapolis, IN), was an important first step in the synthesis of compounds to target PI3K (Vlahos et al., 1994). A reversible competitive inhibitor of the ATP binding pocket of PI3K, LY294002 is less potent than wortmannin and has selectivity and toxicity issues (Gharbi et al., 2007). The value of these crude but effective inhibitors in advancing the PI3K field should not be underestimated and is evidenced (at the time of writing) by just over 6000 PubMed hits for LY294002 alone. Indeed, these compounds allowed the initial identification or hint of a role for PI3K in various biological processes.

The usefulness of wortmannin and LY294002, as both research tools and potential drugs, was tempered by their broad targeting of all PI3K isoforms and off-target effects (Gharbi et al., 2007). Despite this, the simpler planar structure of LY294002 has helped the design of many more selective pan-PI3K and isoform-selective inhibitors. In particular, X-ray crystallography data of PI3Kγ bound to wortmannin and LY294002 helped the understanding of the manner by which these compounds fit into the ATP binding pocket (Walker et al., 2000), which in turn influenced attempts to design better compounds with increased potency and required selectivity. Although this was in part fuelled by the desire for better anti-inflammatory drugs, the single most important catalyst for PI3K inhibitor design was arguably the potential application of PI3K inhibitors as anticancer drugs. The PI3K/Akt/mTOR pathway is one of the most commonly activated pathways in human cancer and is central to the transformed phenotype of most cancer cells (Manning and Cantley, 2007; Engelman, 2009). It can be activated by amplification or activating mutation of either PI3Kα or upstream receptor tyrosine kinases, and by mutations or deletions downstream in the pathway or of regulatory elements such as the phosphatase PTEN (Yuan and Cantley, 2008; Liu et al., 2009b). Increased PI3Kα activity or activity-enhancing mutations in the *PI3KCA* gene are common in a number of cancers, including breast, endometrial, and glioblastoma (Samuels et al., 2004; Kok et al., 2009).

Crystal structures of PI3Ks have revealed six regions within the ATP binding pocket: hydrophobic regions I (the affinity pocket) and II, the hinge region, the P loop, the start of the activation loop (DFG motif), and the specificity pocket (Walker et al., 2000). Designing compounds that explore or create these pockets has improved potency and selectivity of PI3K inhibitors (Knight et al., 2006; Folkes et al., 2008; Berndt et al., 2010). These have influenced subsequent efforts to develop PI3K inhibitors with improved selectivity and reduced toxicity. In addition, mTOR shares high sequence homology in the hinge region with PI3K, and therefore some compounds originally developed as PI3K inhibitors were later shown to also target mTOR. Furthermore, pan-PI3K isoform and mTOR inhibitors often exert synergistic effects in functional measures. Approximately 18 drugs now in clinical development target the PI3K signaling cascade; of these, half target both mTOR and PI3K, most target one or more PI3K isoforms, and some inhibit DNA protein kinase (Table 2).

B. Where Is the Best Site to Block the Phosphoinositide 3-Kinase Signaling Cascade?

The targeting of PI3K in cancer has prompted much debate regarding exactly the optimal point to inhibit in this cascade. The most popular targets to date have been PI3K catalytic isoforms, Akt, and mTOR (Fig. 3). Inhibitors of class I PI3K would prevent accumulation of PI(3,4,5)P_3, which is necessary for full activation of Akt/PKB. However, Akt can be phosphorylated and activated by inhibitor of nuclear factor κB kinase subunit ε (IKBKE) independently of the recognized PI3K/PDK1/mTORC2/PH domain-mediated mechanism and thus, PI3K inhibition may not fully silence Akt/PKB and hence mTORC1 activation (Guo et al., 2011). Direct targeting of Akt has been explored with some success, particularly with respect to cancer (Hers et al., 2011; Tan et al., 2011). This would avoid disrupting effects of PI3K that are mediated by other downstream effectors of PI3K signaling, which may or may not be desirable depending on the disease context. Paradoxically, inhibition of Akt could actually increase PI3K-dependent activation of those effectors, because Akt activation leads to increased mTORC1 activity, which operates a well known negative feedback mechanism that inhibits insulin-stimulated PI3K activation (Guertin and Sabatini, 2009). Because of this negative feedback, when mTORC1 is active, the PI3K-Akt pathway is suppressed, whereas if mTORC1 is inhibited (for instance, with rapamycin), PI3K-Akt signaling can actually be enhanced. Indeed, success in developing effective mTOR inhibitors was tempered by the recognition that currently approved inhibitors of mTORC1 fail to block the activation of Akt via mTORC2 at Ser-473 (Moore et al., 2011). In cancer cells, losing this feedback inhibition may actually promote survival and counter the potential therapeutic benefits of mTORC1. Moreover, success in developing effective mTOR inhibitors was tempered by the recognition that currently approved inhibitors of mTORC1 fail to block the activation of Akt via mTORC2 at Ser-473 (Moore et al., 2011). Thus, inhibition of both mTORC1 and mTORC2 would therefore be predicted to give a better potential for efficacy by removing the mTORC2-mediated phosphorylation of Akt. In this regard, both ATP competitive [e.g., AZD8055 ([5-[2,4-bis-[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl]-2-methoxyphenyl]methanol) from AstraZeneca] and allosteric (from Pathway Therapeutics, San Francisco, CA) inhibitors of mTORC1 and mTORC2 have been

TABLE 2

TABLE of compounds targeting the PI3K pathway currently in clinical trials. List of PI3K pathway inhibitors currently in clinical trials or approved for treating inflammatory disease and/or cancer. Stages of development are given and indications for treatment are based upon clinical trials information at clinicaltrials.gov. Where available, references are given for the chemical structures of these compounds.

Compound	Company	Target	Status	Indications	Structure Reference
PI3K inhibitors					
XL147	Exelixis/Sanofi	PI3K	Phase I/II	Solid tumors/lymphoma	N.A.
PX-866	Oncothyreon	PI3K	Phase I/II	Solid tumors	Ihle et al., 2004
GDC0941	Genentech/Roche	PI3K	Phase I	Solid tumors/non-Hodgkin lymphoma	Folkes et al., 2008
BKM120	Novartis	PI3K	Phase I/II	Solid tumors/leukemia	Koul et al., 2012
ZSTK474	Zenyaku Kogyo	PI3K	Phase I	Neoplasms	Yaguchi et al., 2006
GS-1101 (CAL-101)	Gilead	PI3Kδ	Phase II	Non-Hodgkin lymphoma/ leukemia/allergic rhinitis	Ikeda et al., 2010
BYL719	Novartis	PI3Kα	Phase I	Solid tumors	N.A.
INK1117	Intellikine	PI3Kα	Phase I	Solid tumors	N.A.
IPI-145	Infinity	PI3Kα/γ	Phase I	Advanced hematologic malignancies	N.A.
Akt inhibitors					
Perifosine	Keryx	Akt	Phase III	Colorectal cancer/multiple myeloma	Yap et al., 2008
MK2206	Merck	Akt	Phase I/II	Solid tumors	Hers et al., 2011
PI3K/mTOR inhibitors					
BEZ235	Novartis	PI3K/mTOR	Phase I/II	Solid tumors	Maira et al., 2008
BAY80-6946	Bayer	PI3K/mTOR	Phase I	Neoplasms	N.A.
BGT226	Novartis	PI3K/mTOR	Phase I	Solid tumors	Chang et al., 2011
XL765	Exelixis/Sanofi	PI3K/mTOR	Phase I	Solid tumors	N.A.
SF1126	Semafore Pharma	PI3K/mTOR	Phase I	Solid tumors/multiple myeloma	Garlich et al., 2008
GSK2126458	GSK	PI3K/mTOR	Phase I	Solid tumors	Knight et al., 2010
PF-04691502	Pfizer	PI3K/mTOR	Phase I/II	Solid tumors	
GDC-0980	Genentech/Roche	PI3K/mTOR	Phase I/II	Solid tumors/non-Hodgkin's lymphoma/renal carcinoma	Sutherlin et al., 2011
PKI-587	Pfizer	PI3K/mTOR	Phase I	Neoplasms	Venkatesan et al., 2010
mTOR inhibitors					
OSI-027	OSI Pharma	mTOR/catalytic site	Phase I	Solid tumors/lymphoma	Bhagwat et al., 2011
AZD8055	AstraZeneca	mTOR/catalytic site	Phase I/II	Solid tumors/lymphoma	Chresta et al., 2010
INK128	Intellikine	mTOR/catalytic site	Phase I	Solid tumors/multiple myeloma	N.A.
Ridaforolimus	Ariad/Merck	mTORC1	Phase III	Metastatic soft-tissue and bone sarcomas	Yap et al., 2008
Everolimus	Novartis	mTORC1	Approved	Renal cell carcinoma/neuroendocrine tumors/subependymal giant cell astrocytoma	Yap et al., 2008
Temsirolimus	Wyeth	mTORC1	Approved	Renal cell carcinoma	Yap et al., 2008
SHIP-1 activator					
AQX-1125	Aquinox	SHIP-1	Phase II	Airway inflammation	N.A.

N.A., not available; AZD8055, (5-(2,4-bis((3*S*)-3-methylmorpholin-4-yl)pyrido(2,3-*d*)pyrimidin-7-yl)-2-methoxyphenyl)methanol; BAY80-6946, 2-amino-*N*-(7-methoxy-8-(3-morpholinopropoxy)-2,3-dihydroimidazo[1,2-*c*]quinazolin-5-yl)pyrimidine-5-carboxamide; BEZ235 (2-methyl-2-[4-(3-methyl-2-oxo-8-quinolin-3-ylimidazo[4,5-*c*]quinolin-1-yl)phenyl]propanenitrile) BGT226, 8-(6-methoxypyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethylphenyl)-1,3-dihydroimidazo(4,5-*c*)quinolin-2-one; GDC-0980, 1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno(3,2-*d*)pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxypropan-1-one; GSK2126458, 2,4-difluoro-*N*-(2-(methyloxy)-5-(4-(4-pyridazinyl)-6-quinolinyl)-3-pyridinyl)benzenesulfonamide; OSI-027, 4-[(5*Z*)-4-amino-5-(7-methoxyindol-2-ylidene)-1*H*-imidazo[5,1-*f*][1,2,4]triazin-7-yl]cyclohexane-1-carboxylic acid; PF-04691502, 2-amino-8-(4-(2-hydroxyethoxy)cyclohexyl)-6-(6-methoxypyridin-3-yl)-4-methylpyrido(2,3-d)pyrimidin-7(8*H*)-one; PKI-587, 1-(4-((4-(dimethylamino)piperidin-1-yl)carbonyl)phenyl)-3-(4-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)phenyl)urea 1,3,4,7,10,11,12,13,14,15,16,17-dodecahydro-2-oxa-cyclopenta(*a*)phenanthren-11-yl)ester; XL765, *N*-[4-[[3-(3,5-dimethoxyanilino)quinoxalin-2-yl]sulfamoyl]phenyl]-3-methoxy-4-methylbenzamide.

reported, although no structures have been published for the latter compound. However, the best inhibition of PI3K signaling (at least in the context of anticancer drugs) would be predicted to be achieved by dual targeting PI3K and mTORC1/2, because this would effectively shut down signaling at both proximal and intermediate sites of the cascade (Fig. 3). This would be the case with SF1126 [(8*S*,14*S*,17*S*)-14-(carboxymethyl)-8-(3-guanidinopropyl)-17-(hydroxymethyl)-3,6,9,12,15-pentaoxo-1-(4-(4-oxo-8-phenyl-4*H*-chromen-2-yl)morpholino-4-ium)-2-oxa-7,10,13,16-tetraazaoctadecan-18-oate; Semaphore Pharmaceuticals, Indianapolis, IN], a conjugate of LY294002 with an RGD-containing peptide. The purpose of the RGD peptide was to increase solubility and binding to specific integrins within the tumor compartment. This results in enhanced delivery of the active compound to the tumor vasculature and tumor while limiting its damaging systemic effects. SF1126 inhibits not only PI3K and mTOR but also a select number other cancer targets such as DNA protein kinase, PIM1, and PLK1 (Gharbi et al., 2007). Hence, SF1126 therefore effectively shuts down PI3K-Akt-mTOR signaling. It has shown promising preclinical results alone or in combination with the anticancer drug doxorubicin and not only decreases proliferation but also has antiangiogenic and pro-apoptotic actions (Garlich et al., 2008; Ozbay et al., 2010; Peirce et al., 2011).

The optimal site for therapeutic intervention in the PI3K signaling cascade may require targeting of one or

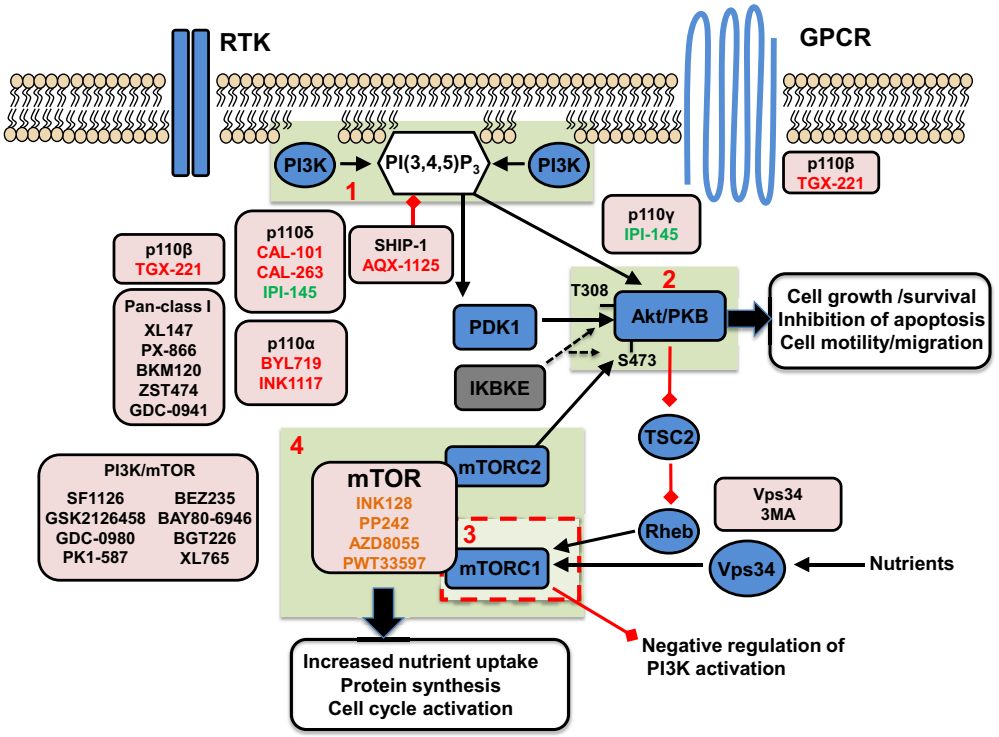

FIG. 3. Sites of intervention of PI3K/mTOR-targeting inhibitors. Heterodimeric PI3Kα, PI3Kβ, and PI3Kδ complexes are activated downstream of growth factors, antigen and cytokine receptors, whereas PI3Kγ activation is triggered downstream of GPCRs. PI3K phosphorylates $PI(4,5)P_2$ to generate $PI(3,4,5)P_3$. This reaction can be reversed by the action of PTEN (not shown). Alternatively, the 5′ phosphatase SHIP-1 can convert $PI(3,4,5)P_3$ to $PI(3,4)P_2$. Akt and PDK1 directly bind to $PI(3,4,5)P_3$ and are thereby recruited to the plasma membrane. The phosphorylation of Akt at Thr308 (which is in the activation loop of Akt) by PDK1 and at Ser-473 (which is in a hydrophobic motif of Akt) by mTORC2 results in full activation of this protein kinase. In turn, Akt phosphorylates several cellular proteins (not shown) to facilitate cell survival and cell cycle entry. In addition, Akt phosphorylates and inactivates tuberous sclerosis 2 (TSC2), a GTPase-activating protein for Ras homolog enriched in brain (RHEB). Inactivation of TSC2 allows RHEB to accumulate in the GTP-bound state and thereby activate mTORC1. Additional activation of mTORC1 can occur via input from other pathways upon nutrient activation. This figure shows potential sites for pharmacological intervention in the PI3K/Akt/mTOR signaling cascade (green shaded areas). 1, inhibitors of class I PI3K would prevent accumulation of $PI(3,4,5)P_3$, which is necessary for full activation of Akt/PKB. This approach would not prevent activation of mTORC1 via PKB-independent avenues or mTORC2 activation. 2, Akt can be phosphorylated and activated by IKBKE, independently of the recognized PI3K/PDK1/mTORC2/PH domain-mediated mechanism; thus, PI3K inhibition may not fully silence Akt/PKB and hence mTORC1 activation. 3, mTORC1 inhibitors will alleviate a negative feedback mechanism that targets the PI3K pathway and potentially enhance PI3K-Akt signaling. 4, inhibition of both mTORC1 and mTORC2 would therefore be predicted to give a better potential for efficacy by removing the mTORC2-mediated phosphorylation of Akt. However, the best inhibition of PI3K signaling would be predicted to be achieved by dual targeting PI3K and mTORC1/2, because this would effectively shut down signaling at both proximal and intermediate sites of the cascade. Direct targeting of Akt in particular has been explored with some success but is not depicted here. Targets of inhibitors currently in clinical trial are depicted: those in red represent compounds that are selective for a single PI3K isoform; inhibitors in green exhibit PI3Kγ/δ dual selectivity; inhibitors in orange are mTOR catalytic site inhibitors. Compounds targeting SHIP-1 are allosteric activators. Negative regulatory events are depicted by red lines.

more components of the signaling cascade and will likely depend on the particular molecular pathology driving a given disease. From an immunological perspective, targeting of mTOR in the immune system, particularly in T cells, has long been the focus for the development of immunosuppressive drugs such as rapamycin and its analogs, which suppress mTOR activity via an allosteric mechanism (Guertin and Sabatini, 2009). We now appreciate that mTOR provides a critical link between metabolic demands and cellular function and plays a role in regulating diverse immune cells, including neutrophils, mast cells, NK cells, $\gamma\delta$ T cells, macrophages, dendritic cells, T cells, and B cells (Delgoffe and Powell, 2009; Mills and Jameson, 2009; Thomson et al., 2009; Weichhart and Säemann, 2009). Some of the new mTORC1/mTORC2 inhibitors developed primarily as anticancer agents have been characterized in the context of immune diseases. The mTORC1/2 inhibitor PP242 [(2*Z*)-2-(4-amino-1-propan-2-yl-2*H*-pyrazolo[3,4-*d*]pyrimidin-3-ylidene)indol-5-ol] has potent antileukemic effects in vitro and in vivo but leaves lymphocyte function largely intact (Janes et al., 2010). PP242 has also shown promise in treating multiple myeloma (Hoang et al., 2010), whereas another mTORC1/2 inhibitor, INK128 [3-(2-amino-5-benzoxazolyl)-1-(1-methylethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine], developed by Intellikine (La Jolla, CA), inhibits cell proliferation both in vitro and in vivo. INK128 has entered phase I clinical trials as a therapeutic agent for the treatment of breast cancer (ClinicalTrials.gov identifier NCT01351350), lymphoma (ClinicalTrials.gov identifier NCT01058707), and multiple myeloma (ClinicalTrials.gov identifier NCT01118689). The dual targeting of PI3K/mTOR in inflammatory/autoimmune disease has not been extensively investigated. Nevertheless, dual pan-PI3K/mTORC1/2 inhibitors seems to cause greater immune suppression than mTORC1/2 alone, with greater reduction of hematopoietic

colony formation and B- and T-cell proliferation, a lowered fraction of B cells with germinal center phenotype, as well as percentages of total splenic B and T lymphocytes. Pan-PI3K-TORC1/2 inhibitors also suppress immune rejection of melanoma xenografts (López-Fauqued et al., 2010).

One concern of the dual PI3K/mTOR inhibitor approach is that it is so robust that it might lead to general immunosuppression. This may be acceptable in transplantation settings but possibly not for other diseases, such as inflammatory and autoimmune diseases, where a lighter touch might be more appropriate to dampen the deleterious effects of an overactive immune system rather than silence it completely. The long appreciated important role of PI3Kδ and -γ (as well as a growing understanding of the contribution of PI3Kβ), in both innate and adaptive immunity has therefore led to the search for selective pharmacological tools with which to target these enzymes in inflammation and autoimmune disorders.

VI. Development of Phosphoinositide 3-Kinase Inhibitors with Which to Target the Immune System

A. Phosphoinositide 3-Kinase δ Inhibitors

Given the high degree of similarity that exists between the amino acids forming the ATP-binding pockets of the four class I PI3Ks, it was expected that isoform-selective inhibitors with a reasonable (at least 50-fold) difference in potency would be difficult to obtain. However, the discovery of the quinazolinone purine series, exemplified by the ICOS Corporation (Bothell WA) compound IC-87114 (2-[(6-aminopurin-9-yl)methyl]-5-methyl-3-(2-methylphenyl)quinazolin-4-one), indicated that this task was possible (Sadhu et al., 2003). IC-87114 demonstrated an IC_{50} value of approximately 100 nM for PI3Kδ lipid kinase activity and was also potent in cell assays that are dependent on the catalytic activity of this isoform but had negligible potency against the PI3Kα and PI3Kβ isoforms (Table 3).

In 2006, several members of ICOS Corporation formed a spin-off company, Calistoga Pharmaceuticals (Seattle, WA). Calistoga developed CAL-101 (5-fluoro-3-phenyl-2-[(1*S*)-1-(7*H*-purin-6-ylamino)propyl]quinazolin-4-one), a PI3Kδ-specific inhibitor, which has experienced successful proof of concept in clinical trials for treatment of B-cell malignancies (in section VII.A). This compound (which was acquired by Gilead in February 2011 and recently renamed GS-1101) exhibits 40- to 300-fold selectivity over other PI3K isoforms (Table 3). Calistoga Pharmaceuticals is also exploring inhibition of PI3Kδ in inflammatory conditions using CAL-101 and CAL-263 (Norman, 2011), another PI3Kδ-selective inhibitor (ClinicalTrials.gov identifiers NCT00836914 and NCT01066611). In addition, patents have been filed by several other companies [Amgen (Thousand Oaks, CA), Intellikine, and Incyte Systems (Pal Alto, CA)] describing PI3Kδ inhibitors, and the majority are based on the same basic pharmacophore identified by ICOS (Norman, 2011). However, additional scaffolds have now been reported by several companies; almost all of these are with intended indications against B-cell lymphomas (Norman, 2011).

TABLE 3
Compounds targeting the PI3K isoforms

Individual IC_{50} values for each PI3K inhibitor are derived from in vitro assays of inhibitor activity against purified protein activity. Values for CZC24832 are derived from chemoproteomic competition binding assays using cell extracts (K_i) or cell-based assays (IC_{50}) that are either PI3Kγ-dependent (C5a-induced Akt phosphorylation or fMLP-induced neutrophil migration), PI3Kα-dependent (insulin-induced Akt phosphorylation), or class 1A-dependent (CSF-induced monocytic cell migration).

Compound	IC_{50} Values [K_i]					Reference
	PI3K Isoform				mTOR	
	p110α	p110β	p110δ	p110γ		
	nM					
XL147[a]	39	383	36	23	>15,000	Shapiro et al., 2009
PX-866[a]	5.5	>300	2.7	9		Ihle et al., 2004, 2005
GDC0941[a]	3	33	3	75	580[b]	Folkes et al., 2008
BKM120[a]	Not available					
ZSTK474	16	44	4.6	49	>100,000	Kong and Yamori, 2007
BYL719[a]	Not available					
INK1117[a]	15	>100–1000-fold higher	>100–1000-fold higher	>100–1000-fold higher		Rommel, 2011
TGX-221	~5000	~5–10	~100	>10,000		Jackson et al., 2005
IC-87114	>100,000	~2000–16,000	70–130	1240–61,000	>100,000	Bilancio et al., 2006; Knight et al., 2006
GS-1101 (CAL-101)[a]	820	565	2.5	89	>1000	Lannutti et al., 2011
AS-605240	60	270	300	8		Camps et al., 2005
CZC24832						
K_i		~6000	35,000	~2		Bergamini et al., 2012
IC_{50}	>30,000	>30,000	>30,000	~1000		
TG-100-115	1300	1200	235	83		Doukas et al., 2006
TG-101-110	1200	107	64	85		Doukas et al., 2006
IPI-145 (INK-055)[a]	>60	>60	32	5.7		Boyle et al., 2009

[a] Related compounds in clinical development.
[b] Binding affinity.

A better understanding of the molecular mechanisms of isoform selectivity of PI3Kδ inhibitors has been made possible by a recent report describing the crystal structure of the PI3Kδ catalytic core, both free and complexed with a broad panel of new and mostly PI3Kδ-selective PI3K inhibitors. Comparison of the PI3Kδ structure with those of other isoforms reveals several important features for inhibitor design: PI3Kδ is more flexible than PI3Kγ, and most of the PI3Kδ-selective inhibitors adopt a propeller shape that allows them to exploit this conformational plasticity by opening up and then binding into a "specificity pocket." In contrast, pan-specific PI3K inhibitors adopt a flat structure rather than the propeller shape and do not access the specificity pocket. This new understanding was used to design novel inhibitors that retained very high selectivity toward PI3Kδ by exploiting the selectivity pocket, combined with much increased potency by targeting the affinity pocket. This study provides the first detailed structural insights into the active site of a class IA PI3K occupied by noncovalently bound inhibitors and suggests mechanisms to increase the potency of inhibitors without sacrificing isoform selectivity and also how to optimize solubility, pharmacokinetics/metabolism, and pharmacodynamic behavior (Berndt et al., 2010).

B. Phosphoinositide 3-Kinase γ Inhibitors

Selective inhibition of PI3Kγ has been accomplished in a series of compounds designed by Merck Serono SA based on the thiazolidinedione scaffold. One of these, AS-605240 [5-(quinoxalin-6-ylmethylidene)-1,3-thiazolidine-2,4-dione] has demonstrated superior potency for PI3Kγ compared with related compounds (Table 3), can be administered orally and has high membrane permeability (Barber et al., 2005; Camps et al., 2005). Despite the promising preclinical data using these compounds (described in section VII), PI3Kγ inhibitors have yet to undergo further development or clinical proof of concept. In general terms, the level of selectivity of compounds against PI3Kγ versus other PI3K isoforms has been largely disappointing. Possible reasons for the relatively slow progress in developing PI3Kγ inhibitors include the close structural conservation of class I PI3Ks and other lipid kinases in the ATP-binding pocket and the limited ability of the commonly used in vitro assays based on recombinant enzymes, to predict cellular and in vivo kinase selectivity. Researchers at Cellzome (Heidelberg, Germany) made a recent advance in this area. They developed a chemoproteomics-based drug-discovery platform that enables multiplexed high-throughput screening of native proteins in cell extracts, thus preserving their post-translational modifications and protein interactions (Bergamini et al., 2012). Using affinity enrichment of target kinases afforded by immobilized ATP-competitive lipid kinase inhibitors, the potency of small-molecule test compounds was evaluated in competition binding assays. This approach allows targeting of proteins with low expression and measurements in human primary cells. The chemoproteomic strategy led to the design of CZC24832 [5-(2-amino-8-fluoro-[1,2,4]triazolo[1,5-*a*]pyridin-6-yl)-N-*tert*-butylpyridine-3-sulfonamide], which exhibits superior selectivity for PI3Kγ than compounds reported previously (Camps et al., 2005; Bergamini et al., 2012). Despite this recent success, the lack of highly selective inhibitors of PI3Kγ has hampered detailed mechanistic studies of PI3Kγ in human primary cells and has hitherto precluded human clinical studies in inflammation, because adverse effects caused by the simultaneous inhibition of off-target kinases, in particular class IA PI3Ks, are intolerable for treatment of chronic non–life-threatening diseases.

C. Dual Phosphoinositide 3-Kinase γ/δ Inhibitors

Given the evidence outlined earlier from gene-targeted mice that PI3Kγ and PI3Kδ often work in concert and can have overlapping roles, dual inhibition of both targets with a single compound has been investigated. TargeGen (San Diego) described two diaminopteridine-diphenol-based compounds with good selectivity for both PI3Kγ and δ (Table 3). TG-101-110 [6- (1*H*-indol-4-yl)-pteridin-2,4-diamine::6-(1*H*-indol-4-yl)pteridine-2,4-diamine] inhibits PI3Kγ and -δ but also displays PI3Kβ inhibition close to the IC_{50} values for PI3Kγ and -δ (Doukas et al., 2006). TG-100-115 (3-[2,4-diamino-7-(3-hydroxyphenyl)pteridin-6-yl]phenol) has a better selectivity profile for PI3Kγ and -δ over PI3Kα and -β and shows minimal activity on a range of other protein kinases (Doukas et al., 2006). TG-100-115 was initially tested for safety and efficacy in a phase I/II clinical trial for patients with myocardial infarct. Although this compound does not seem to have been developed further, Infinity Pharmaceuticals (Cambridge, MA) and Intellikine have ongoing phase I trials with IPI-145 [*N*-(6-(4-amino-1-((8-methyl-1-oxo-2-o-tolyl-1,2-dihydroisoquinolin-3-yl)methyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[*d*]thiazol-2-yl)acetamide] (Tables 2 and 3). This compound represents the only PI3Kδ/-γ inhibitor currently in clinical development (Norman, 2011).

D. Phosphoinositide 3-Kinase β Inhibitors

TGX-221 [9-(1-anilinoethyl)-7-methyl-2-morpholin-4-ylpyrido[1,2-a]pyrimidin-4-one] is a PI3Kβ isoform-specific inhibitor developed initially as a novel therapeutic agent for the treatment of thrombosis (Jackson et al., 2005; Straub et al., 2008). The series of PI3Kβ inhibitors exemplified by TGX-221 was designed around substitutions of chemical groups of LY294002 that convey selectivity for PI3Kβ over other PI3K isoforms (Table 3) (Jackson et al., 2005). TGX-221 is effective in treating thrombus formation by platelets in vivo in both rats and mice. There are species differences in the effect of TGX-221 treatment on homeostatic bleeding times, which are unaffected in rats (Jackson et al., 2005) but increased in mice (Bird et al., 2011). PI3Kβ inhibition should there-

fore be approached with caution. However, the increasing recognition of a role for PI3Kβ in the immune system would suggest therapeutic applications beyond those initially envisaged for thrombosis. Evidence of cooperation between the β and δ isoforms suggests that development of dual PI3Kβ/δ inhibitors may have therapeutic promise; indeed, compounds with such selectivity have been reported, suggesting that this approach is feasible (Knight et al., 2006).

VII. Therapeutic Potential of Inhibitors Targeting Phosphoinositide 3-Kinase γ and δ For Immune Disorders

Given that PI3Kγ and -δ as well as the β isoform have multiple nonredundant roles in immune cells (Table 1), it is not surprising that PI3K is implicated in many inflammatory and autoimmune disease states as well as hematological malignancies (Fig. 4). The etiology of these diseases is often complex, multifactorial, and poorly understood. So, the discovery and development of an arsenal of inhibitors selectively targeting one or more PI3K isoforms not only will provide tools with which to better understand disease mechanisms but also will hopefully provide more efficacious therapeutic tools. We now describe the preclinical and early clinical trial data that assess the therapeutic potential of inhibitors targeting the β, γ, and δ isoforms of PI3K.

A. B-Cell Malignancies

PI3Kδ is a signaling hub for diverse stimuli in B lymphocytes, including the B-cell antigen receptor, the CD19 coreceptor, cytokines, chemokines, and Toll-like receptors (Fruman and Rommel, 2011). Mouse models of PI3Kδ deficiency indicated a key role in multiple aspects of B-lymphocyte biology and function (Table 1), including development, proliferation, and cell survival (Fruman and Rommel, 2011). These models also indicated that acute inhibition of PI3Kδ could be prove efficacious against various pathological B-cell conditions, including lymphoma. CAL-101 has been studied extensively in patients with relapsed or refractory B-cell malignancies. There are at least 15 types of mature B-cell lymphoma that can have distinct pathological and clinical features and hence may often require diverse treatment strategies (Küppers, 2005). In preclinical studies using chronic lymphocytic leukemia (CLL) cell lines and primary pa-

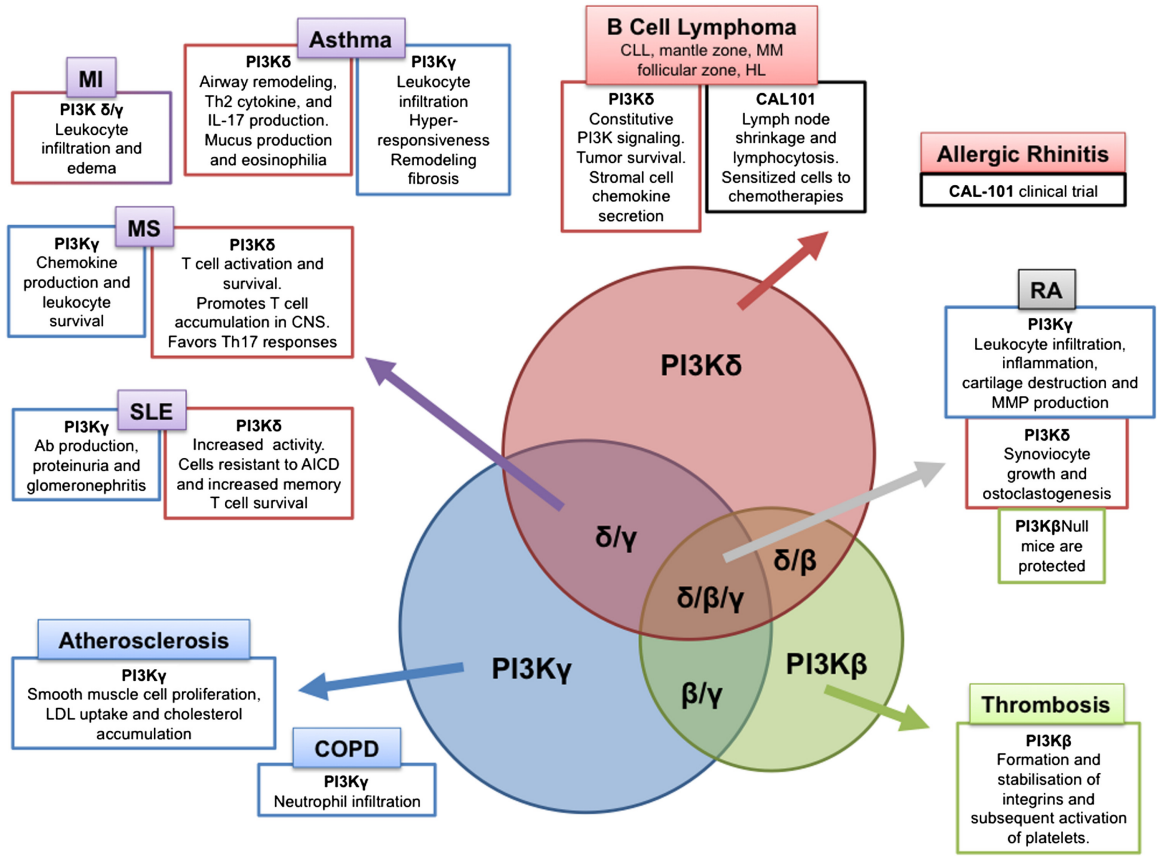

FIG. 4. Individual and combined roles of class I PI3K β, δ, and γ isoforms in inflammatory and autoimmune disease as well as hematological malignancies and thrombosis. Genetic targeting of specific and multiple class I PI3K isoforms in mice, as well as the use of isoform-selective and pan-PI3K inhibitors in rodents and humans, have revealed multiple roles for these enzymes in immune cell function (Table 1). Here, the relative involvement of single or multiple isoforms in diseases is summarized. These include: thrombosis (PI3Kβ); B-cell lymphomas (PI3Kδ); atherosclerosis and COPD (PI3Kγ); MS, MI, SLE, asthma, and allergic rhinitis (PI3Kδ and -γ); and RA (PI3Kβ, -δ, and -γ). The PI3Kδ inhibitor CAL-101 has been investigated in clinical trials for two of these diseases, allergic rhinitis and B-cell lymphomas.

tient CLL samples, CAL-101 blocked constitutive PI3K signaling resulting in decreased phosphorylation of Akt and decreased cell viability of cell lines. In patient samples of CLL, there is increased PI3Kδ expression and activity that is reduced by CAL-101. These effects have been observed across a broad range of other immature and mature B cell malignancies, including $CD5^+$ mantle zone B-cell lymphomas, follicular lymphomas and multiple myeloma (Herman et al., 2010; Ikeda et al., 2010; Fruman and Rommel, 2011; Hoellenriegel et al., 2011; Lannutti et al., 2011). Ongoing clinical evaluation of CAL-101 has revealed that it causes rapid lymph node shrinkage and lymphocytosis (Fruman and Rommel, 2011; Hoellenriegel et al., 2011; Lannutti et al., 2011). This inhibitor has therefore demonstrated an essential role for PI3Kδ in constitutive PI3K signaling that is required for the survival of several different types of malignant B cells. Oncogenic mutations of components of the PI3K signaling pathway are infrequent in B-cell malignancies. A potential mechanism for PI3K activation in this setting is tonic antigen-independent B-cell receptor (BCR) signaling that requires PI3Kδ for the transduction of proliferation and survival signals.

The malignant B-cell microenvironment is important for disease progression and CAL-101 was tested in assays that model CLL microenvironment interactions in vitro. CAL-101 inhibited CLL cell chemotaxis toward CXCL12 and CXCL13 in Boyden chamber type assays and migration beneath stromal cells (pseudoemperipolesis) (Hoellenriegel et al., 2011). This is consistent with data from mouse models indicating that PI3Kδ is the dominant isoform required for B-cell migration (Reif et al., 2004). CAL-101 also down-regulated secretion of chemokines in stromal cocultures and after BCR triggering. CAL-101 reduced survival signals derived from the BCR or from nurse-like cells and inhibited BCR- and chemokine receptor-induced Akt and extracellular signal-regulated kinase activation in CLL cells. In stromal cocultures, CAL-101 sensitized CLL cells toward chemotherapies such as bendamustine (Hoellenriegel et al., 2011). These results are consistent with clinical data showing marked reductions in circulating CCL3, CCL4, and CXCL13 levels and a surge in lymphocytosis during CAL-101 treatment. Hence, CAL-101 displays a dual mechanism of action in which it both decreases cell survival and reduces interactions that retain CLL cells in protective tissue microenvironments.

Hodgkin lymphoma (HL) is a malignant lymphoma of B-cell origin (Thomas et al., 2004). The malignant cells, known as Reed-Sternberg (RS) cells, represent less than 2% of the tumor mass, the remainder being composed of a mix of reactive inflammatory cells attracted by the RS cells. HL cell lines and primary samples from patients with HL have been reported to express high levels of PI3Kδ and constitutive PI3K pathway activation (Meadows et al., 2012). As with CLL, CAL-101 was able to reduce the positive interaction between stromal cells and malignant RS cells; this may be due, in part, to reduced release of the chemokine CCL5 by RS cells. Cell-cycle arrest and apoptosis was also induced in HL cell lines, and dual inhibition of PI3Kδ and mTOR may be of benefit in the treatment of HL.

B. Rheumatoid Arthritis

RA is a chronic autoimmune disease that predominantly affects joints of the hands and feet, causing immobilization and disability. In RA, the structure of the synovium is transformed into a pannus-like tissue that invades cartilage. The inflamed synovium consists of inflammatory cells, such as macrophages, neutrophils, and T and B cells, as well as hyperplasia of synovial lining cells, which are predominantly fibroblast-like synoviocytes. Strong granulocyte and lymphocyte recruitment into these joints is one of the major causes of the onset of RA (Firestein, 2003). Damage to the synovial tissue is driven by aggressive inflammatory cytokine signaling in these joints. Given that PI3Kγ has a pivotal role in mediating leukocyte migration (Hirsch, 2000; Sasaki et al., 2000a; Reif et al., 2004; Smith et al., 2007; Lim et al., 2009; Thomas et al., 2009) and activation as well as mast-cell degranulation (Ali et al., 2008; Willox et al., 2010), it was predicted that blocking PI3Kγ might be an effective strategy to fight RA. This was explored with both genetic and pharmacological approaches (using the PI3Kγ-selective inhibitor AS-605240) in distinct animal models of arthritis. When arthritis was induced by injection of monoclonal antibodies to type II collagen (a model that focuses on the effector phase of arthritis), PI3Kγ-null mice exhibited reduced paw swelling, synovial inflammation, and cartilage erosion. In wild-type mice, oral administration of AS-605240 effectively inhibited antibody-induced inflammation and cartilage destruction to a degree similar to that observed in the PI3Kγ-null mice (Camps et al., 2005). When arthritis was induced by injection of bovine type II collagen along with adjuvant, (a model in which an adaptive immune response contributes to disease development similarly to the human disease), AS-605240 reduced symptoms correlating with decreased neutrophil infiltration (Camps et al., 2005). CZC24832 also shows anti-inflammatory effects in a collagen-induced arthritis model that correlated with reduced Th17 differentiation, a pro-inflammatory helper T-cell type characterized by expression of the cytokine IL-17 (Weaver and Murphy, 2007). Indeed, CZC24832 treatment also led to reduced IL-17 production (Bergamini et al., 2012). This confirms the long-held belief that pharmacological inactivation of PI3Kγ alone can lead to amelioration of inflammatory disease.

Although widely used as a disease model for RA, murine collagen-induced arthritis in mice reflects mainly the immunological components of the disease and constitutes an acute T-cell-mediated autoimmune arthritis. Therefore, alternative animal models of RA have been developed that

resemble the chronic, destructive phase of RA. For example, transgenic overexpression of human tumor necrosis factor α leads to a chronic inflammatory destructive polyarthritis that is similar to human RA and is sensitive to blockade of TNFα by treatment with neutralizing anti-TNFα antibodies. Loss of PI3Kγ in human TNFα transgenic mice led to reduced arthritis compared with control mice (Hayer et al., 2009). It is noteworthy that PI3Kγ deficiency does not alter the recruitment of inflammatory cells as observed in the collagen-induced arthritis model of RA, but it significantly reduces cartilage damage through reduced expression of matrix metalloproteinases in fibroblasts and chondrocytes (Hayer et al., 2009). In vitro analyses demonstrate that the decreased invasiveness of fibroblasts is mediated by reduced phosphorylation of Akt and extracellular signal-regulated kinase. PI3Kγ expression is significantly higher in the synovia of patients with RA compared with that from patients with osteoarthritis. Furthermore, inhibition of PI3Kγ using AS-252424 (5-[[5-(4-fluoro-2-hydroxyphenyl)-2-furanyl]methylene]-2,4-thiazolidinedione) reduced TNFα-induced matrix metalloproteinase production in fibroblasts isolated from patients with RA (Hayer et al., 2009). This study, therefore, provides further mechanistic insights into PI3Kγ in RA that extends beyond its established role in immune cell migration.

Recent studies have revealed that PI3Kδ mRNA and protein expression is higher in RA than in osteoarthritis synovium, and PI3Kδ mRNA can be selectively induced in cultured synoviocytes by inflammatory cytokines. Indeed, inhibition of PI3Kδ diminished PDGF-mediated synoviocyte growth (Bartok et al., 2012). In a K/BxN-serum transfer model of arthritis, in which neutrophils and LTB4 participate in the effector phase of the inflammatory arthritis, genetic deletion, or selective inhibition of PI3Kδ diminishes joint erosion to a level comparable with its PI3Kγ counterpart. Induction and progression of joint destruction was profoundly reduced in the absence of both PI3K isoforms and is consistent with both isoforms being required for LTB4-mediated neutrophil chemotaxis, as described previously (Randis et al., 2008).

Important differences between PI3Kγ and PI3Kδ have been noted in the mechanisms underpinning joint destruction. For example, in a model of osteoclastogenesis, the PI3Kδ-selective inhibitor IC-87114 significantly inhibited the generation of osteoclasts, whereas selective inhibition of PI3Kγ with AS-605240 had no effect (Toyama et al., 2010). Taken together, these lines of evidence suggest that dual inhibition of PI3Kγ and PI3Kδ would be more therapeutically beneficial than targeting one isoform alone. However, in the same K/BxN model, PI3Kβ-null mice were also partially protected, whereas mice that were also deficient in PI3Kδ activity were highly resistant to disease (Kulkarni et al., 2011). Hence, inhibition of PI3Kβ (alone or in combination with targeting of PI3Kδ) may also offer potential in the treatment of RA in certain situations.

C. Systemic Lupus Erythematosus

SLE is a chronic inflammatory disease, characterized at early stages by an increase in long-lasting, autoreactive memory CD4$^+$ T lymphocytes (Kuroiwa and Lee, 1998; Wakeland et al., 1999). Dysregulated T cells lead to polyclonal B cell activation, generalized B-cell expansion, hypergammaglobulinemia, and increased autoantibody production. Circulating anti-DNA antibodies form complexes that are retained in kidneys and locally activate the complement cascade. As the disease progresses, T cells and macrophages infiltrate the kidney and amplify the local inflammatory response culminating eventually in glomerulonephritis and renal failure (Kuroiwa and Lee, 1998; Wakeland et al., 1999).

Mouse SLE involves abnormal activation of CD4$^+$ T cells that accumulate as activated memory cells and contribute to disease pathogenesis (Jevnikar et al., 1994; Borlado et al., 2000; Lang et al., 2003). Deletion of PI3Kγ in this model reduced the survival of pathogenic memory CD4+ T lymphocytes, which ultimately led to a reduction in disease (Barber et al., 2005). In the MRL$^{lrp/lpr}$ multigenic SLE-prone mouse model (in which SLE susceptibility correlates with mutations at several loci, as for human SLE5), AS-605240 and related compounds increased survival and reduced autoantibody production, proteinuria, and glomerulonephritis. Mice treated with AS-605240 showed no adverse side effects after 3 months of treatment (Barber et al., 2006). Mice expressing an activating mutation of the p85 regulatory subunit p65(PI3K) in T cells develop an SLE-like disease because of the increased survival of mature CD4$^+$ T lymphocytes (Borlado et al., 2000). Deletion of PI3Kγ in this model reduced the survival of pathogenic memory CD4$^+$ T lymphocytes. Together, these results validate the PI3Kγ isoform as a target for SLE treatment (Barber et al., 2005, 2006). More recently however, increased PI3Kδ but not -γ activity was observed in patients with SLE. This increased activity made activated and memory T cells more resistant to activation induced cell death. This resulted in an increased number of memory T cells and highlights an important role for PI3Kδ in SLE (Suárez-Fueyo et al., 2011).

D. Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is an induced method of autoimmune inflammation of the central nervous system (CNS) commonly used to model diseases such as MS in rodents (Seil, 1972; Finsen et al., 2002). EAE is characterized by infiltration of inflammatory cells into the CNS that drive destruction of neurons, causing MS-like symptoms, including ataxia, paralysis of limbs, and loss of weight.

Until recently, MS and EAE were thought to be driven by autoreactive Th1 cells. However, considerable evi-

dence now indicates that the T-cell lineage most likely to be driving EAE pathogenesis are Th17 cells (Kleinschek et al., 2007). In a Th17-driven EAE model, the absence of PI3Kγ delayed progression of motor dysfunction (Rodrigues et al., 2010). Lower levels of the proinflammatory chemokines CCL2 and CCL5 were observed in the meninges of PI3Kγ-deficient mice after EAE induction. Consequently, there were markedly reduced numbers of infiltrating immune cells. Both genetic and pharmacological targeting of PI3Kγ indicated that it plays a more important role in mediating leukocyte survival than in mediating leukocyte adhesion in this experimental model of MS. However, using the same EAE model, another group (Haylock-Jacobs et al. 2011) reported that signaling through PI3Kδ is required for full and sustained pathologic features of EAE. In PI3Kδ-inactivated mice, T-cell activation and function during EAE was markedly reduced, and fewer T cells were observed in the CNS. Reminiscent of observations made in the PI3Kγ deficient mice, there were significant increases in the proportion of T cells undergoing apoptosis at early stages of EAE in the absence of PI3Kδ activity. Furthermore, a profound defect in Th17 cellular responses during EAE was apparent in the absence of PI3Kδ activity. The PI3Kδ inhibitor IC-87114 also had greater inhibitory effects on Th17-cell generation in vitro than on Th1-cell generation. Taken together, these data indicate that both PI3Kγ and PI3Kδ can contribute to the pathogenesis of EAE, influencing cell survival, differentiation, and migration mechanisms (Haylock-Jacobs et al., 2011). Thus, dual targeting of PI3Kγ and -δ might be a therapeutic option for the treatment of EAE.

E. Airway Disorders

Asthma is a chronic disorder in which the airway occasionally constricts, becomes inflamed, and is lined with excessive amounts of mucus, often in response to one or more triggers, such as exposure to an allergen. Airway eosinophilia, mucus accumulation, elevated serum IgE levels, and airway hyper-responsiveness are fundamental characteristics of allergic asthma. Th2 cells, together with other inflammatory cells such as mast cells, neutrophils, B cells, and eosinophils, play an essential role in the pathophysiology of this disease (Medina-Tato et al., 2006, 2007). With regard to asthma, inhibition of PI3Kδ may be of primary importance. For example, the early-phase response in asthma is largely driven by mast-cell degranulation (Bloemen et al., 2007), and although both PI3Kδ and -γ contribute to mast-cell activation (Laffargue et al., 2002; Ali et al., 2008), the former seems to predominate (Ali et al., 2008). Subsequent events during the late-phase response, such as IgE hyperproduction, may also depend primarily on PI3Kδ (Okkenhaug et al., 2002; Al-Alwan et al., 2007). In contrast, lipopolysaccharide (LPS) and smoke-induced pulmonary inflammations are probably driven by chemokine-induced neutrophil recruitment, and PI3Kγ is the isoform primarily responsible for this response (Thomas et al., 2005).

In an ovalbumin (OVA) model of asthma, PI3Kδ^{D910A} mice or treatment with the PI3Kδ inhibitor IC-87114 leads to decreased Th2 cytokine and mucus production as well as reduced eosinophil recruitment, airway remodeling, and hyper-responsiveness (Lee et al., 2006; Nashed et al., 2007; Farghaly et al., 2008). More recently, it has become apparent that pharmacological inhibition of PI3Kδ with IC-87114 also reduces the levels of IL-17 in the OVA-induced mouse model (Park et al., 2010). GlaxoSmithKline has developed an inhaled PI3Kδ inhibitor that, in the OVA model, reduced IL-13 levels and eosinophilia in bronchoalveolar lavage fluid, and effects were comparable with treatment with corticosteroids. It is noteworthy that in an in vitro steroid-insensitive assay of asthma, this PI3Kδ inhibitor was still active (Amour et al., 2011). In a model of allergic airway inflammation induced by cockroach antigen, IC-87114 significantly inhibited airway eosinophil recruitment, resulting in attenuation of airway hyper-responsiveness, reduced mucus secretion, and expression of various pro-inflammatory molecules (Kang et al., 2012). In vitro observations, suggested that the reduced eosinophil recruitment observed in IC-87114-treated cockroach antigen-challenged mice, may be caused by a more direct effect of the inhibitor on eosinophil trafficking (rolling, adhesion, and migration) rather than Th2 cytokines and eosinophil-active chemokines (Kang et al., 2012). This is in contrast to observations in the OVA model, in which IC-87114 treatment resulted in a significant reduction in Th2 cytokines (Lee et al., 2006).

There is also strong evidence that PI3Kγ contributes to the immune processes that underpin allergen-induced airway inflammation. In the OVA-induced model of asthma, challenge of PI3Kγ-null mice with allergen resulted in a greatly reduced number of infiltrating leukocytes in their bronchoalveolar lavage fluid compared with wild type, as well as decreased hyper-responsiveness, airway remodeling, and fibrosis (Lim et al., 2009; Takeda et al., 2009; Thomas et al., 2009). In addition, PI3Kγ seems crucial for eosinophil-mediated inflammation in a mouse model of pleurisy involving intrapleural administration of OVA (Pinho et al., 2005). Given evidence implying a role for both PI3Kγ and -δ in allergic airway inflammation in mice, it is interesting to note that the dual PI3Kγ/δ inhibitor TG-100-115 reduced airway inflammation in the OVA model (Doukas et al., 2009).

Other airway disorders may also benefit from the targeting of PI3Kγ and -δ. For example, PI3Kδ-selective inhibitor CAL-101 has been tested in a clinical trial for allergic rhinitis (ClinicalTrials.gov identifier NCT00836914). Furthermore, other non–allergen-based mouse models of airway inflammation exploiting knockout mice have revealed that PI3Kγ is required for neutrophil recruitment in both chemokine airway instillation and intraperitoneal LPS models of lung injury in PI3Kγ-null mice

(Yum et al., 2001; Thomas et al., 2005). Likewise, the dual PI3Kγ/δ inhibitor TG-100-115 reduced neutrophilia and TNFα production in response to LPS and was effective as an intervention after cigarette smoke exposure (Doukas et al., 2009). However, it remains unclear whether the intrapulmonary TG100-115 levels reported in this study led to inhibition of PI3K isoforms other than PI3Kγ, thereby making the task of assigning biological outcomes to inhibition of specific isoforms difficult. This work supports the value of a dual-specificity inhibitor such as TG100-115 in treating distinct respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). The smoke model is of particular interest because, like human COPD, it is steroid-resistant. Steroid resistance is the result of several alterations within the cell, including dysregulation of the glucocorticoid receptor, attenuated histone deacetylase activity, and increased proinflammatory gene transcription (Adcock et al., 2008). The demonstration that aerosolized TG100-115 provides efficacy as an interventional therapy in a smoke-induced neutrophilia model therefore suggests the potential use of TG100-115, not only for patients with COPD but also for steroid-resistant patients with severe asthma (Adcock et al., 2008).

Despite the encouraging preclinical and clinical data relating to use of inhibitors targeting the δ and γ isoforms of PI3K, there are some potential caveats. For example, PI3Kδ negatively regulates B cell antibody class switching (Zhang et al., 2008). One potential cause for concern is that increased IgE levels were recorded when PI3Kδ is inactivated or inhibited with IC-87114, because IgE drives hyper-responsiveness. In contrast, loss of PI3Kγ did not have this effect (Takeda et al., 2009).

PI3K has also been proposed as a respiratory disease target on the basis of its role in mediating smooth muscle and endothelial cell proliferation and, by extension, airway remodeling (Goncharova et al., 2002; Krymskaya, 2007); this represents another area worthy of future research. Indeed, a role for the α and β isoforms has recently been proposed in remodeling of airway smooth muscle cells, a key process involved in asthma. PIK75 (*N*-[(*E*)-(6-bromoimidazo[1,2-*a*]pyridin-3-yl)methylideneamino]-*N*,2-dimethyl-5-nitrobenzenesulfonamide hydrochloride), a PI3Kα-selective inhibitor, demonstrated a role for this isoform in fibronectin deposition and survival of asthmatic cells in response to TGFβ treatment. In addition, PI3Kα and, in part, PI3Kβ also facilitated secretion of VEGF and IL-6 from these cells (Moir et al., 2011).

F. Myocardial Infarction

When blood flow to the heart is reduced as a result of cardiovascular disease, this causes myocardial ischemia, which can lead to apoptosis of cardiac myocytes. A reperfusion event restores blood flow, but oxidative damage results in an increase in inflammatory immune cell infiltration and tissue damage. This wave of ischemia/reperfusion events eventually leads to myocardial infarction (MI) (Frangogiannis et al., 2002). Given evidence for inflammatory activation as an important pathway in disease progression in chronic heart failure, the PI3Kγ and PI3Kδ isoforms have been considered potential therapeutic targets. Indeed, in both rat and porcine occlusion models of MI, dual inhibition of PI3Kγ and -δ using TG-100-115 showed promising therapeutic benefit (Doukas et al., 2006). Inhibition of both isoforms causes a decrease in inflammatory cell infiltration in vivo and a reduction in edema and disease severity.

Atherosclerosis is a chronic disease of large arteries and is the primary cause of MI and stroke (Hansson and Libby, 2006). PI3Kγ levels are reported to be increased in atherosclerotic lesions, and a lack of PI3Kγ was found to protect against atherosclerosis (Fougerat et al., 2008). This was due in part to a reduced ability of PI3Kγ-null T cells and macrophages to infiltrate into lesions. In addition, loss of PI3Kγ also caused the lesions to have an increase in collagen and smooth muscle. This resulted in more stable plaques in PI3Kγ knockout mice, which were less likely to form a thrombus (Fougerat et al., 2008). PI3K, along with Erk1/2, integrates monocyte chemotactic protein-3 signaling to promote smooth-muscle cell proliferation, a process important for thickening of artery walls in atherosclerosis (Maddaluno et al., 2011). Accordingly, the PI3Kγ-selective inhibitor AS-605240 was effective in treating mouse models of established atherosclerosis (Chang et al., 2007; Fougerat et al., 2008). PI3K also has important roles in macrophage function in atherosclerotic legions. Use of AS-605240 or PI3Kγ-null mice demonstrated that PI3Kγ is partially required for low-density lipoprotein uptake and cholesterol accumulation in macrophage foam cells (Anzinger et al., 2012). However, PI3K signaling is also required for the action of liver X receptor ligands, which cause the efflux of cholesterol from foam cells and prevent development of atherosclerotic legions (Huwait et al., 2011).

VIII. Beyond the Class I Phosphoinositide 3-Kinase Isoforms: Increased Understanding of a Role for Class II and III Phosphoinositide 3-Kinases in the Immune System

There is increasing interest in developing inhibitors for class II and class III PI3Ks, primarily as anticancer agents. However, as the role of class II and III PI3Ks in the immune system is further explored, inhibitors of these PI3Ks may have additional applications in immune cell settings as outlined below.

A. Class II Phosphoinositide 3-Kinases

Class II PI3Ks (comprising the α, β, and γ isoforms) are structurally distinct members of the PI3K family that have received relatively little attention compared with their class I cousins (Fig. 1). The primary product of class II PI3Ks is generally considered to be phosphatidylinositol 3-phosphate [PI(3)P], although this is largely

based on in vitro evidence (Falasca and Maffucci, 2012). Although PI3KC2α and PI3KC2β have a wide tissue distribution and are both expressed in leukocytes, PI3KC2γ has a more restricted pattern of expression and is absent from most leukocytes.

Unlike the class I PI3Ks, the class II PI3Ks are activated by agonist-induced relocalization of constitutively active class II enzyme to the plasma membrane via interaction of their N and C termini with adaptor signaling molecules, such as Grb2 in epidermal growth factor receptor signaling (Wheeler and Domin, 2001; Mazza and Maffucci, 2011). Agonists of other receptor tyrosine kinases and GPCRs as well as membrane-associated complexes have also been described as activators of class II PI3Ks as shown in Fig. 5 (Turner et al., 1998; Gaidarov et al., 2001; Arcaro et al., 2000, 2002; Maffucci et al., 2005; Das et al., 2007).

One of the hallmark features of class II PI3Ks is their relatively low sensitivity to the classic PI3K inhibitors wortmannin and LY294002 (Domin et al., 1997). Consequently, the involvement of class II PI3Ks in biological processes may well have been underestimated. Conversely, some class I PI3K inhibitors, such as the PI3Kα/mTOR inhibitor PI-103 [3-(4-morpholin-4-ylpyrido[2,3]furo[2,4-*b*]pyrimidin-2-yl)phenol], actually exhibit comparable activity toward PI3KC2β but, crucially, are much less potent toward PI3KC2α (Knight et al., 2006). Progress in understanding the biological role of class II PI3Ks has been relatively slow compared with that for the four class I isoforms. This is due in part to the paucity of class II PI3K-selective pharmacological tools and the lack of a knockout mouse. However, the recent report of a PI3KC2α knockout mouse will hopefully shed light on the biological role of this enzyme (Harris et al., 2011a).

Despite the lack of selective pharmacological tools with which to target class II PI3Ks, molecular approaches have implicated the class II PI3KC2β and its primary product PI(3)P in the regulation of cell adhesion, actin reorganization, and migration in nonimmune cell systems (Domin et al., 2005; Maffucci et al., 2005). Certainly, class II PI3K isoforms are activated by CCR2 chemokine agonists in monocytic cell lines (Turner et al., 1998). The mechanism of interaction of class II PI3K with GPCRs is unclear, but the protein FROUNT, which is structurally similar to clathrin and facilitates leukocyte infiltration in response to CCR2 and CCR5, may be one possible mediator (Gaidarov et al., 2001; Terashima et al., 2005; Toda et al., 2009). Class II PI3KC2β, but not PI3KC2α, has been demonstrated to play an important

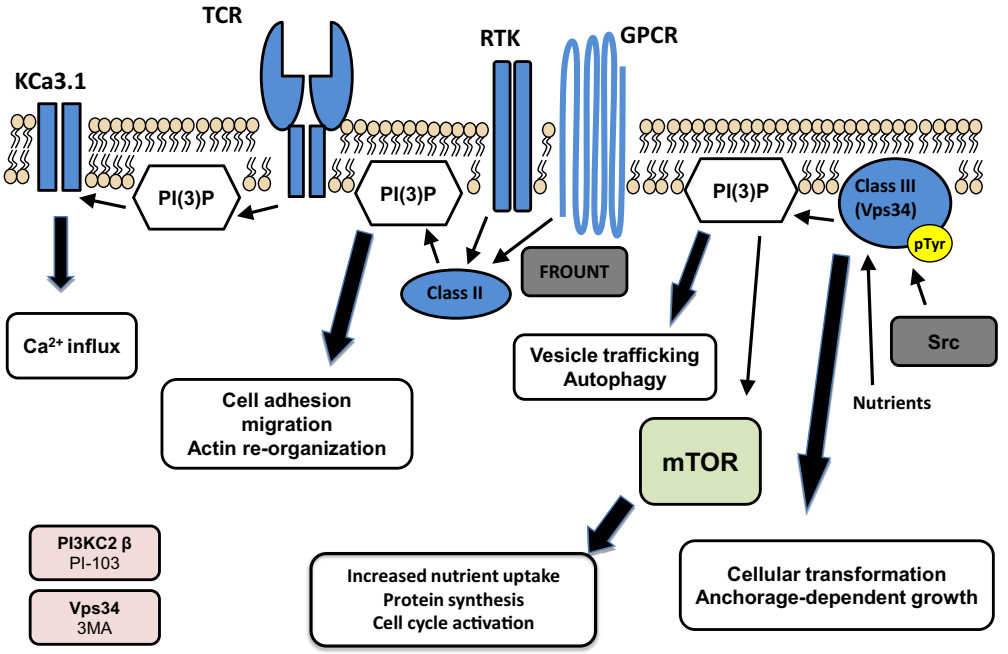

FIG. 5. Activation and function of class II and III PI3Ks. PI(3)P is a product of both class II and III PI3K enzymes, although the structure and the mechanisms of activation of these enzymes are different. Stimulation of cells with agonists for receptor tyrosine kinases including EGF, stem cell factor, and insulin or activation of chemokine GPCRs causes relocation of constitutively active class II PI3Ks to the plasma membrane (Wheeler and Domin, 2001; Mazza and Maffucci, 2011). Membrane-associated complexes, such as clathrin, intersectin (not depicted), and FROUNT are also involved in class II relocalization (Gaidarov et al., 2001; Terashima et al., 2005; Das et al., 2007; Toda et al., 2009). Nutrients such as amino acids can activate the class III enzyme Vps34, which, through PLD, promotes mTORC1 signaling (Byfield et al., 2005; Nobukuni et al., 2005; Yoon et al., 2011). Vps34 can also be tyrosine-phosphorylated in a Src-dependent manner. Little is known about the function of both enzyme families; however, the class II PI3KC2β has roles in migratory process downstream of CCR2 and CCR5 chemokine GPCRs (Domin et al., 2005; Maffucci et al., 2005; Cai et al., 2011) as well as the positive regulation of KCa3.1 downstream of TCR activation. The latter is critical for maintenance of negative membrane potential that facilitates sustained calcium entry into T cells via calcium release-activated Ca^{2+} channels. Increased cytosolic Ca^{2+} then mediates the transcriptional activation of a number of genes critical for T-cell activation. PI(3)P generated by Vps34 is required for vesicle and IL-7RA trafficking, autophagy, as well as cell transformation and growth (McLeod et al., 2011). Although selective class II inhibitors have yet to be described, the dual class I/mTOR inhibitor PI-103 has been shown to inhibit PI3KC2β. Inhibitors that display limited (3MA) or more selectivity (PIK-93) for Vps34 have also been described (Miller et al., 2010).

and unexpected role in $CD4^+$ T-cell activation downstream of the TCR (Srivastava et al., 2009; Cai et al., 2011).

B. Class III Phosphoinositide 3-Kinases

The single class III PI3K Vps34 exhibits considerable homology with the catalytic subunits of other PI3Ks, particularly at the level of domain organization. Vps34 has a structurally uncharacterized N-terminal region of approximately 50 amino acids, followed by C2, helical, and kinase domains that are approximately 30% homologous with PI3Kγ, but it has no Ras binding domain (Vanhaesebroeck et al., 2010). Vps34 enzymes are unique among PI3Ks in that they will only use phosphatidylinositol as a substrate. Vps34 could therefore share protein effectors with the class II PI3Ks, but it is not clear whether the functions of class II and class III PI3Ks overlap.

Vps34 is thought to be a potential anticancer target as a result of its roles in autophagy (Burda et al., 2002; Backer, 2008; Simonsen and Tooze, 2009) and its involvement in regulating nutrient input into the mTORC/S6 kinase 1 signaling pathway (Byfield et al., 2005; Nobukuni et al., 2005; Yoon et al., 2011). Furthermore, Vps34 is tyrosine-phosphorylated by Src, and this phosphorylation is essential for Src-mediated cellular transformation and anchorage-dependent growth. Protein levels of Vps34 also correlate with tumorigenic activity of human breast cancer cells (Hirsch et al., 2010). Information relating to the role of Vps34 specifically in immune cells is limited, but class III (along with class II) PI3Ks regulate various aspects of vesicle trafficking, so there is obvious potential for their involvement in activities such as antigen processing and cytotoxic responses. Indeed, Vps34 has recently been shown to be required for the trafficking of IL-7Rα to the surface of naive T lymphocytes, a process key for their survival (McLeod et al., 2011). Vps34's role in autophagy suggests it may prove important for immune recognition of tumor antigens, regulation of T-cell homeostasis, and immune tolerance (Li et al., 2008; Nedjic et al., 2008; Walsh and Edinger, 2010). There is considerable evidence that class III PI3K is important for phagocytosis because PI(3)P accumulates on phagosomal membranes (Vieira et al., 2001) and contributes to phagosomal maturation and pathogen destruction (Fratti et al., 2001; Vieira et al., 2001; Ellson et al., 2006b). Indeed, some virulent strains of bacteria (e.g., *Mycobacterium tuberculosis*) are thought to evade host defenses by interfering with PI(3)P metabolism (Deretic et al., 2006). The binding of PI(3)P to the PX domain of p40*phox* plays an important role in phagosomal oxidase activation during engulfment of serum-opsonized *S. aureus* and IgG-opsonized particles (Ellson et al., 2006a; Ellson et al., 2006b; Suh et al., 2006). Although the source of phagosomal PI(3)P accumulation may not necessarily be restricted to Vps34, small interfering RNA targeting of Vps34 has revealed that this enzyme is responsible for the synthesis of PI(3)P on phagosomes containing *S. aureus* and *Escherichia coli* (Anderson et al., 2008). Thus, there may be opportunities to target Vps34 in destructive inflammatory/autoimmune diseases in which there is dysregulated phagosomal activity and antigen presentation of self-molecules, for example.

As with the class II PI3Ks, the lack of Vps34-specific inhibitors has restricted our understanding of the biological/pathogenic importance of Vps34. It is noteworthy that both yeast and human Vps34 share a lysine residue that, in human class I PI3Ks, is the site of covalent modification by the pan-PI3K inhibitor wortmannin (Wymann et al., 1996; Walker et al., 2000). Indeed, Vps34 is inhibited by both wortmannin and LY294002 (Backer, 2008), and although 3-methyladenine (3MA) has been suggested to be a specific inhibitor of hVps34, in fact it inhibits class I and II PI3Ks as well (Ito et al., 2007). However, the crystal structure of Vps34 in complex with 3MA has been solved (Miller et al. 2010). 3MA is often used as an inhibitor of autophagy, and the IC_{50} values for Vps34 and PI3Kγ are 25 and 60 mM, respectively (Miller et al., 2010). 3MA has a slight preference for the hydrophobic ring comprising Phe673, Tyr746, and Leu812 that encircles the 3-methyl group of 3MA and that is unique and specific to Vps34. Hence, 3MA may be a useful lead for development of compounds with improved selectivity for Vps34 over other PI3K isoforms (Miller et al., 2010).

IX. SH2-Domain Containing Inositol-5-phosphatase-1: An Alternative Target for Selective Modulation of Phosphoinositide 3-Kinase in the Immune System

Although there has been considerable success in designing PI3Kδ-selective inhibitors with promise against lymphoid malignancies, the progress in designing PI3K inhibitors for anti-inflammatory/autoimmune applications has been disappointing. Until recently (Bergamini et al., 2012), it has proven particularly difficult to design inhibitors of PI3Kγ with sufficient windows of inhibitor selectivity over other PI3K isoforms to achieve selective action on the immune system. The design of small molecules with appropriate selectivity for class II and III PI3Ks over other classes of PI3Ks remains a challenge (Knight et al., 2006; Miller et al., 2010), and their largely ubiquitous expression makes selective targeting of the immune system problematic. As such, alternative targets for pharmacological intervention of PI3K signaling have been sought, with a view to obtain selective effects on the immune response. One such target, the lipid phosphatase SHIP-1, due to its expression in cells of hematopoietic linage, has shown particular promise and is considered below.

A. Role of SH2-Domain Containing Inositol-5-phosphatase-1 in the Immune System

SHIP-1 translocates to the plasma membrane after surface receptor stimulation and hydrolyzes the PI3K-generated second messenger PI(3,4,5)P_3 to PI(3,4)P_2. As a result, SHIP-1 is able to modulate PI(3,4,5)P_3-mediated signaling and hence the proliferation, differentiation, survival, activation, and migration of hematopoietic cells (Harris et al., 2008). SHIP-1 has been implicated in signaling pathways triggered by cytokine, chemokine, antigen, and IgG engagement in a variety of immune cells (Lioubin et al., 1994; Liu et al., 1994; Harris et al., 2008). Genetic analysis of SHIP-1 mutant mice (Table 1), has revealed a pivotal role for SHIP-1 in regulating the receptor repertoire and cytolytic function of NK cells, B lymphocyte development and antibody production, the myeloid cell response to bacterial mitogens, development of marginal zone macrophages, lymph node recruitment of dendritic cells, and mast cell degranulation (Leung et al., 2009). SHIP-1 also plays a critical role in homeostasis of myeloid and lymphoid effector and regulatory cells (Ghansah et al., 2004; Rauh et al., 2005; Locke et al., 2009; Kuroda et al., 2011) and plays an important role in establishing endotoxin tolerance in macrophages (Sly et al., 2004) as well as regulating leukocyte polarization during migratory responses (Harris et al., 2011b).

The key regulatory role of SHIP-1 has been exploited by several opportunistic pathogens that target these phosphatases to evade immune detection. Thus, lymphocytes are particularly sensitive to the cytolethal distending toxin subunit B (CdtB), an immunotoxin produced by *Actinobacillus actinomycetemcomitans,* that can hydrolyze PI(3,4,5)P_3 to PI(3,4)P_2. Exposure to CdtB leads to cell-cycle arrest and death by apoptosis. The lipid phosphatase activity of CdtB may therefore result in reduced immune function, facilitating chronic infection with *A. actinomycetemcomitans* and other enteropathogens that express Cdt proteins (Shenker et al., 2007). The measles virus evades destruction by the immune system, at least in part, by targeting negative regulation of PI3K/Akt signaling. It induces expression of the SHIP-1 homolog SIP-110, which depletes cellular PI(3,4,5)P_3 pools, suggesting that the threshold for activation signals leading to induction of T-cell proliferation is raised (Avota et al., 2006). The predominant expression in hematopoietic cells coupled with targeting of this protein by pathogens to avoid immune recognition suggests that SHIP-1 might offer opportunities for the design of new drugs targeting PI3K-dependent signaling.

B. SH2-Domain Containing Inositol-5-phosphatase-1 Can Act as a Tumor Suppressor in Hematological Malignancies and Is Crucial to Antitumor Immune Responses

The PI3K-dependent signaling pathway has a well established role in regulating cell survival, proliferation, and differentiation (Manning and Cantley, 2007). As we have seen, the PI3K/Akt/mTOR pathway is one of the most commonly activated pathways in human cancer (Engelman et al., 2006; Manning and Cantley, 2007). Indeed, there has been a long appreciation that one of the negative regulators of PI3K signaling, the 3′ lipid phosphatase PTEN, is frequently lost in many cancers (Hollander et al., 2011). Likewise, SHIP-1 expression is frequently lost, down-regulated, or mutated in many hematological malignancies, including acute myeloid leukemia (Luo et al., 2003). Scanning of the SHIP-1 3′ untranslated region has revealed perfect sequence complementarity with the seed sequence of miR-155. Elevated levels of miR-155 and consequent diminished SHIP-1 expression have been linked to B-cell lymphomas (Rodriguez et al., 2007; Costinean et al., 2009; O'Connell et al., 2009). In addition, it has been reported that oncogenic proteins, including BCR/Abl (implicated in chronic myelogenous leukemia), induce SHIP-1 down-regulation by a variety of mechanisms (Ruschmann et al., 2010). Consistent with its role as a tumor suppressor, SHIP-1 restricts development of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Ghansah et al., 2004; Locke et al., 2009). Thus, SHIP-1 deficiency leads to an expansion of MDSCs and regulatory T cells and, hence, suppression of antitumor immune responses. This may be another mechanism for increased tumorigenesis if SHIP-1 expression is reduced. However, the role of SHIP-1 in leukemia seems more complex than initially thought. In this regard, there is evidence that SHIP-1 can actually support cancer cell survival as a small-molecule inhibitor of SHIP-1 induces apoptosis of multiple myeloma cells (Brooks et al., 2010). This is consistent with its production of PI(3,4)P_2, which is known to facilitate Akt activation and thereby cell proliferation, survival, and tumorigenesis (Manning and Cantley, 2007). Others have shown that SHIP-1 inhibits CD95/APO-1/Fas-induced apoptosis in T cells by promoting CD95 glycosylation independently of its phosphatase activity (Charlier et al., 2010).

X. Manipulation of SH2-Domain Containing Inositol-5-phosphatase-1 Catalytic Activity with Small Molecules

A. Allosteric SH2-Domain Containing Inositol-5-phosphatase-1 Activators

SHIP-1 is a particularly ideal target for development of potential therapeutics for treating immune and hematopoietic disorders because its hematopoietic-restricted expression would limit the effects of a specific SHIP-1 agonist to target cells, and hence would probably minimize off-target tissue effect. One would predict, for example, that activators of SHIP-1 would lead to a reduction of cellular PI(3,4,5)P_3 and hence mimic the effect of PI3K inhibitors. In 2005, the natural product pelorol [methyl(4*aS*,6*aR*,11*aR*,11*bS*)-9,10-dihydroxy-4,4,6*a*,11*b*-tetramethyl-1,2,3,4*a*,5, 6,11,11*a*-octahydrobenzo[*a*]fluorene-7-carboxylate] extracted from the tropical marine sponge *Dactylospongia elegan,* was identified

as the first SHIP-1 activator (Yang et al. 2005). Pelorol and subsequent synthetic derivatives were found to bind allosterically to a newly identified C2 domain of SHIP-1, leading to enzyme activation. Two of these compounds based on the structure of pelorol, AQX-016A [(6*aR*,11*aR*,11*bS*)-4,4,6*a*,7,11*b*-pentamethyl-1,2,3,4*a*,5, 6,11,11*a*-octahydrobenzo[*a*]fluorene-9,10-diol] and AQX-MN100 were effective in the inhibition of immune cell activation and reducing inflammation in vivo using a model of endotoxin shock in mice (Ong et al., 2007). Moreover, these molecules have been successfully used to kill multiple myeloma cells in vitro indicating that SHIP-1 agonists could be effective anticancer agents (Kennah et al., 2009).

Aquinox Pharmaceutical Inc. (Richmond, BC, Canada) is currently developing a small-molecule SHIP-1 activator to exert negative effects on the PI3K pathway and that is intended for clinical use. A lead compound termed AQX-1125 with an undisclosed structure, has been shown to reduce Akt phosphorylation in the leukemic T cell line MOLT-4 and murine splenic B cells (Stenton et al., 2011). As expected, AQX-1125 had no effect on Jurkat cells, another leukemic T cell line that has previously been demonstrated to be deficient in SHIP-1 expression (Astoul et al., 2001). AQX-1125 was able to inhibit the chemotactic response of numerous human leukocyte populations, including activated T cells and neutrophils. It is noteworthy that AQX-1125 exhibited potent anti-inflammatory effects, significantly reducing OVA-induced leukocyte lung infiltration in Brown Norway rats (Stenton et al., 2011). Hence, these results indicate promising potential for SHIP-1 activators in the treatment of inflammatory disorders. AQX-1125 has passed phase I clinical trials in 2011, with phase IIa clinical studies initiated in late 2011 for the treatment of mild and moderate asthma (Aquinox Pharmaceuticals, 2011). With regard to the latter, the recent finding that TLR stimulation augments IgE plus Ag-induced TNFα and IL-6 production from mucosal mast cells (Ruschmann et al., 2012) might explain the exacerbation of IgE-mediated allergic episodes by infectious agents (Qiao et al., 2006). Because IgE synergizes with TLR ligands to trigger cytokine production from SHIP-1-null mucosal mast cells, activating SHIP-1 specifically in these cells might be useful for treating chronic inflammatory diseases such as asthma.

B. SH2-Domain Containing Inositol-5-phosphatase-1 Inhibitors

A small-molecule inhibitor of SHIP-1 has also been reported, although its site of action is unclear (Brooks et al., 2010). Using high-throughput screening, 3α-aminocholestane (3AC) was identified as a potent SHIP-1 inhibitor. Treatment of mice with 3AC led to increased numbers of MDSCs that repress allogeneic T-cell responses. Indeed, 3AC reduced ability of peripheral lymphoid tissues to prime myeloid-associated responses and protected against graft-versus-host disease, which involves priming of allogeneic T cells and is a common complication arising after bone marrow transplantation. The results with 3AC are consistent with observations from SHIP-1-deficient mice, which express more myeloid suppressor cells than their WT counterparts and accept allogeneic bone marrow grafts with a reduced incidence of graft-versus-host disease (Ghansah et al., 2004; Kerr, 2008). In addition SHIP-1-null mice are better able to accept bone marrow transplants compared with control mice (Wang et al., 2002), and SHIP-1 deficient mice have shown reduced cardiac graft rejection compared with control mice (Paraiso et al., 2007). The inhibition of SHIP-1 using pharmacological compounds may therefore offer the potential to aid transplant acceptance in patients undergoing transplant surgery. SHIP-1 inhibitors increased levels of granulocytes, red blood cells, neutrophils, and platelets in mice and could therefore have potential to improve blood cell number in patients with myelodysplastic syndrome or myelosuppressive infection. 3AC also triggered the apoptosis of human acute myeloid leukemia cell lines, consistent with the antiapoptotic nature of SHIP-1 under some circumstances.

These SHIP-1-targeting small molecules offer interesting therapeutic opportunities. However, SHIP-1 can have either inhibitory or activating roles in cell signaling that are determined by whether signaling pathways distal to PI3K are promoted by the SHIP-1 substrate $PI(3,4,5)P_3$ or product $PI(3,4)P_2$ (Harris et al., 2008; Kerr, 2011). Moreover, SHIP-1 product and substrate can both influence Akt activation and cell survival. This may explain in part why both activators and inhibitors of SHIP-1 have shown efficacy against leukemic cells (Kerr, 2011). As with PI3K, the targeting of SHIP-1 with activators or inhibitors is not without its risks. SHIP-1 deficiency leads to Crohn's disease-like ileitis in mice, most likely as a result of defects in mucosal T-cell immunity (Kerr et al., 2011). Moreover, in addition to a core catalytic domain responsible for the hydrolysis of the 5′-phosphate on $PI(3,4,5)P_3$, SHIP-1 encodes multiple structural domains (SH2 and proline-rich regions as well as NPXY motifs that become tyrosine-phosphorylated and bind the phosphotyrosine-binding domain motif) that facilitate interaction with partner proteins (Harris et al., 2008). Targeting the catalytic activity of SHIP-1 may be beneficial because it will avoid any scaffolding roles of SHIP-1. Conversely, if the scaffolding role of SHIP-1 contributes to the pathological outcomes, such small molecules would be predicted to be ineffective.

Inhibition of SHIP-2, which shares protein domains and 35% homology with SHIP-1 (Fig. 1) has also been explored, because this lipid phosphatase has been implicated in diabetes and obesity (Ooms et al., 2009). Owing to its ubiquitous expression, SHIP-2 is expressed along with SHIP-1 in leukocytes and in diseases in which these two isoforms perform nearly or completely redundant functions, so pharmacological targeting of both isoforms may be preferential. Indeed, targeting of multiple

myeloma cells by compounds that target both SHIP-1 and -2 can cause cell death (Fuhler et al., 2012). Compounds that selectively target SHIP-2 have also been reported, including inhibitors of SHIP-2 catalytic activity (Suwa et al., 2009) and a novel cell-impermeant biphenyl 2,3′4,5′,6-pentakisphosphate (Vandeput et al., 2007). Despite the lack of cell penetration by this latter compound, it has provided an invaluable tool for the study of SHIP-2. Indeed, the solving of the crystal structure of biphenyl 2,3′4,5′,6-pentakisphosphate revealed that upon binding of this compound to the phosphatase domain of SHIP-2, a flexible loop folds over and encloses the ligand (Vandeput et al., 2007; Mills et al., 2012). Targeting of this conformational change in the structure of SHIP-2 could present a novel feature for selectively targeting this lipid phosphatase. To fully exploit the potential of SHIP-1 and SHIP-2 inhibition for the treatment of inflammatory, autoimmune disorders and malignancies, much more needs to be learned about the role of SHIP-2 in immune cell function.

XI. Conclusions

There has been an astounding evolution of PI3K inhibitors in the past 20 years, from the early chemical tool compounds wortmannin and LY294002 to drugs that are showing promise as anticancer agents in clinical trials. The availability of crystal structures of the catalytic domains has facilitated understanding of isoform selectivity profiles and, in turn, has influenced the design of new chemical entities engineered to fit the required selectivity profile. The most progress in targeting PI3K signaling has been made in the cancer field, where the requirement for PI3Kα/pan-class I isoform selectivity is perhaps less rigorous. The clinical trials of these compounds show evidence of single-agent therapeutic activity in patients with cancer. Crucially, they are well tolerated, and early concerns about potential effects on glucose metabolism seem unfounded so far. There has been reasonable success in producing selective inhibitors of PI3Kδ, and PI3Kγ/δ inhibitors can also be produced for potential use in inflammation. There is also emerging potential for PI3Kβ and dual PI3Kβ/δ inhibitors for the treatment of immune/inflammatory disease, and PI3Kγ-selective inhibitors have only very recently been described. The difficulties of developing PI3Kγ inhibitors with sufficient selectivity over PI3K isoforms has led, in part, to the exploitation of endogenous and leukocyte-restricted regulators of PI3K signaling: the lipid phosphatase SHIP-1. Small-molecule regulators of this protein have shown early promise in terms of selective potential anti-inflammatory actions and are currently in phase I clinical trials to evaluate the safety, tolerability, and pharmacokinetics.

The option of using PI3K isoform-specific inhibitors for cancer treatment must be considered with care, because the function of a single isoform can potentially be involved not only in promoting both tumor progression but may also be pivotal to antitumor immunity. A failure in NK cell-mediated clearance of cancerous cells has been reported in studies using PI3Kδ knockout mice. Although this isoform promotes the progression of leukemia, PI3Kδ depletion results in a defective ability of NK cells to de-granulate and kill a large variety of target cells (Zebedin et al., 2008). Nevertheless, CAL-101 induces apoptosis of malignant cells without affecting normal T cells or NK cells. However, the effect of CAL-101 on NK or $CD8^+$ and cell-mediated cytolytic functions of these cells has not yet been fully explored (Herman et al., 2010). Hence, the therapeutic benefits arising from targeting PI3K isoforms could depend on a balance between the benefit of purging cancer cells and the disadvantages of immunological impairment.

Despite the progress made in pharmacological targeting of PI3K signaling, some important issues need to be considered. There is considerable plasticity within the PI3K signaling pathway, making it unlikely that PI3K inhibitors will provide a universal therapy across the range of diseases in which PI3K has been implicated. For example, in IL-3-dependent hematopoietic progenitor cells (which express all four class I PI3K isoforms), persistent inhibition of selected PI3K isoforms can allow the remaining isoform(s) to couple to upstream signaling pathways in which they are not normally engaged. Such functional redundancy of class IA PI3K isoforms upon sustained PI3K inhibition has implications for the development and use of PI3K inhibitors in both cancer and inflammatory/autoimmune disease (Foukas et al., 2010). Second, targeting of PI3K catalytic isoforms does not necessarily silence PI3K signaling, because Akt can be phosphorylated and activated by IKBKE independently of the recognized PI3K/PDK1/mTORC2/PH domain-mediated mechanisms and sustain malignant transformation (Guo et al., 2011). Third, PI3K/Akt signaling requires spatial compartmentalization in plasma membrane microdomains, such that PDK1 is found in membrane lipid rafts in response to growth factors, whereas the negative regulator PTEN is primarily localized in nonraft regions. Dysregulation of this membrane compartmentalization (e.g., forced relocalization of PTEN to the lipid rafts) undermines PI3K/Akt signaling and may underlie pathological complications such as insulin resistance rather than overactivity of the signaling pathway per se (Gao et al., 2011). Fourth, there is a growing appreciation of the noncatalytic scaffolding roles of the class I catalytic isoforms. For example, PI3Kγ and the p85/87 participate in a macromolecular complex that includes protein kinase A. This complex regulates PDE3b, which in turn modulates cardiac contractility (Patrucco et al., 2004). Likewise, a kinase-independent role for PI3Kβ in the endocytic process has been proposed (Jia et al., 2008; Hirsch et al., 2009). Finally, resistance mechanisms to PI3K-targeted therapy have recently been reported in which the PI3K/mTOR inhibitor BEZ235 could be evaded by gene amplification of either of two proto-

oncogenes, c-*myc* and eukaryotic translation initiation factor 4E. These apparently bypass the inhibitors by acting downstream of the pharmacologically inhibited targets (Ilic et al., 2011). The issues outlined above are not necessarily restricted to targeting the immune system and can be applied to other settings.

Despite these caveats, it is clear that PI3K (particularly the β, γ, and δ isoforms) have important nonredundant roles in multiple cells of the immune system. Consequently, alterations of the PI3K signaling pathway can lead to inflammatory and autoimmune disorders as well as leukemia. This, together with growing appreciation of the crystal structure of the catalytic isoforms, which help define the structure-activity rules for obtaining selectivity, will spur the continued design and development of improved PI3K inhibitors that are more selective and potent and have negligible off-target effects. These offer opportunities to manipulate the PI3K signaling network in immune cells for inflammation and transplantation as well as cancer. The latter may include nonleukemic cancers, given the up-regulation of PI3Kγ and PI3Kδ in some forms of nonimmune cell cancers, although it might be difficult to avoid effects on the immune system that might impair the endogenous antitumor response. Other elements of the PI3K family (class II and III isoforms) and its regulatory networks (e.g., SHIP-1) may offer complementary or alternative strategies depending on the exact disease. The intense interest around PI3K isoforms shared by immunologists, biomedical researchers, and pharmacologists should ultimately yield badly needed therapies for major pathologic conditions.

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Wrote or contributed to the writing of the manuscript: Foster, Blunt, Carter, and Ward.

References

Adcock IM, Ford PA, Bhavsar P, Ahmad T, and Chung KF (2008) Steroid resistance in asthma: mechanisms and treatment options. *Curr Allergy Asthma Rep* **8:**171–178.

Al-Alwan MM, Okkenhaug K, Vanhaesebroeck B, Hayflick JS, and Marshall AJ (2007) Requirement for phosphoinositide 3-kinase p110delta signaling in B cell antigen receptor-mediated antigen presentation. *J Immunol* **178:**2328–2335.

Alcázar I, Marqués M, Kumar A, Hirsch E, Wymann M, Carrera AC, and Barber DF (2007) Phosphoinositide 3-kinase gamma participates in T cell receptor-induced T cell activation. *J Exp Med* **204:**2977–2987.

Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, et al. (2004) Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. *Nature* **431:**1007–1011.

Ali K, Camps M, Pearce WP, Ji H, Rückle T, Kuehn N, Pasquali C, Chabert C, Rommel C, and Vanhaesebroeck B (2008) Isoform-specific functions of phosphoinositide 3-kinases: p110 delta but not p110 gamma promotes optimal allergic responses in vivo. *J Immunol* **180:**2538–2544.

Amour A, Barrett V, Carter P, Deakin A, Down K, Felton L, Harrison Z, Le J, Lucas F, Mitchell C, et al. (2011) PI3Kδ inhibition: a future paradigm for the inhaled therapy of asthma (Abstract 133). Keystone Symposia on Molecular and Cellular Biology: PI 3-Kinase Signaling Pathways; 2011 Feb 13–18; Keystone, CO. Keystone Symposia, Silverthorne, CO.

Anderson KE, Boyle KB, Davidson K, Chessa TA, Kulkarni S, Jarvis GE, Sindrilaru A, Scharffetter-Kochanek K, Rausch O, Stephens LR, et al. (2008) CD18-dependent activation of the neutrophil NADPH oxidase during phagocytosis of *Escherichia coli* or *Staphylococcus aureus* is regulated by class III but not class I or II PI3Ks. *Blood* **112:**5202–5211.

Antignano F, Hamilton M, Patterson S, Ho V, Cohen C, Levings MK, and Krystal G (2011) SHIP-deficient dendritic cells, unlike wild type dendritic cells, suppress T cell proliferation via a nitric oxide-independent mechanism. *PloS One* **6:**e21893.

Antignano F, Ibaraki M, Kim C, Ruschmann J, Zhang A, Helgason CD, and Krystal G (2010a) SHIP is required for dendritic cell maturation. *J Immunol* **184:**2805–2813.

Antignano F, Ibaraki M, Ruschmann J, Jagdeo J, and Krystal G (2010b) SHIP negatively regulates Flt3L-derived dendritic cell generation and positively regulates MyD88-independent TLR-induced maturation. *J Leukoc Biol* **88:**925–935.

Anzinger JJ, Chang J, Xu Q, Barthwal MK, Bohnacker T, Wymann MP, and Kruth HS (2012) Murine bone marrow-derived macrophages differentiated with GM-CSF become foam cells by PI3Kγ-dependent fluid-phase pinocytosis of native LDL. *J Lipid Res* **53:**34–42.

Aquinox Pharmaceuticals (2011) Aquinox pharmaceuticals receives regulatory clearance to initiate first clinical study of AQX-1125, a novel oral anti-inflammatory compound (press release). Available at: http://www.aqxpharma.com/content/aquinox-pharmaceuticals-initiates-two-phase-iia-clinical-studies-airway-inflammation.

Arcaro A, Khanzada UK, Vanhaesebroeck B, Tetley TD, Waterfield MD, and Seckl MJ (2002) Two distinct phosphoinositide 3-kinases mediate polypeptide growth factor-stimulated PKB activation. *EMBO J* **21:**5097–5108.

Arcaro A, Zvelebil MJ, Wallasch C, Ullrich A, Waterfield MD, and Domin J (2000) Class II phosphoinositide 3-kinases are downstream targets of activated polypeptide growth factor receptors. *Mol Cell Biol* **20:**3817–3830.

Arcaro A and Wymann MP (1993) Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: the role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. *Biochem J* **296:**297–301.

Astoul E, Edmunds C, Cantrell DA, and Ward SG (2001) PI 3-K and T-cell activation: limitations of T-leukemic cell lines as signaling models. *Trends Immunol* **22:**490–496.

Avota E, Harms H, and Schneider-Schaulies S (2006) Measles virus induces expression of SIP110, a constitutively membrane clustered lipid phosphatase, which inhibits T cell proliferation. *Cell Microbiol* **8:**1826–1839.

Backer JM (2008) The regulation and function of class III PI3Ks: novel roles for Vps34. *Biochem J* **410:**1–17.

Barber DF, Bartolomé A, Hernandez C, Flores JM, Fernandez-Arias C, Rodríguez-Borlado L, Hirsch E, Wymann M, Balomenos D, and Carrera AC (2006) Class IB-phosphatidylinositol 3-kinase (PI3K) deficiency ameliorates IA-PI3K-induced systemic lupus but not T cell invasion. *J Immunol* **176:**589–593.

Barber DF, Bartolomé A, Hernandez C, Flores JM, Redondo C, Fernandez-Arias C, Camps M, Rückle T, Schwarz MK, Rodríguez S, Martinez-A C, Balomenos D, Rommel C, and Carrera AC. Camps M, Rückle T, Schwarz MK, Rodríguez S, et al. (2005) PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nat Med* **11:**933–935.

Barberis L, Pasquali C, Bertschy-Meier D, Cuccurullo A, Costa C, Ambrogio C, Vilbois F, Chiarle R, Wymann M, Altruda F, et al. (2009) Leukocyte transmigration is modulated by chemokine-mediated PI3Kgamma-dependent phosphorylation of vimentin. *Eur J Immunol* **39:**1136–1146.

Bartok B, Boyle DL, Liu Y, Ren P, Ball ST, Bugbee WD, Rommel C, and Firestein GS (2012) PI3 kinase δ is a key regulator of synoviocyte function in rheumatoid arthritis. *Am J Pathol* **180:**1906–1916.

Bergamini G, Bell K, Shimamura S, Werner T, Cansfield A, Müller K, Perrin J, Rau C, Ellard K, Hopf C, et al. (2012) A selective inhibitor reveals PI3Kγ dependence of T(H)17 cell differentiation. *Nat Chem Biol* **8:**576–582.

Berndt A, Miller S, Williams O, Le DD, Houseman BT, Pacold JI, Gorrec F, Hon WC, Liu Y, Rommel C, et al. (2010) The p110delta structure: mechanisms for selectivity and potency of new PI(3)K inhibitors. *Nat Chem Biol* **6:**244.

Bhagwat SV, Gokhale PC, Crew AP, Cooke A, Yao Y, Mantis C, Kahler J, Workman J, Bittner M, Dudkin L, et al. (2011) Preclinical characterization of OSI-027, a potent and selective inhibitor of mTORC1 and mTORC2: distinct from rapamycin. *Mol Cancer Ther* **10:**1394–1406.

Bilancio A, Okkenhaug K, Camps M, Emery JL, Ruckle T, Rommel C, and Vanhaesebroeck B (2006) Key role of the p110delta isoform of PI3K in B-cell antigen and IL-4 receptor signaling: comparative analysis of genetic and pharmacologic interference with p110delta function in B cells. *Blood* **107:**642–650.

Bird JE, Smith PL, Bostwick JS, Shipkova P, and Schumacher WA (2011) Bleeding response induced by anti-thrombotic doses of a phosphoinositide 3-kinase (PI3K)-β inhibitor in mice. *Thromb Res* **127:**560–564.

Bishop JL, Sly LM, Krystal G, and Finlay BB (2008) The inositol phosphatase SHIP controls *Salmonella enterica* serovar typhimurium infection in vivo. *Infect Immun* **76:**2913–2922.

Bloemen K, Verstraelen S, Van Den Heuvel R, Witters H, Nelissen I, and Schoeters G (2007) The allergic cascade: review of the most important molecules in the asthmatic lung. *Immunol Lett* **113:**6–18.

Bohnacker T, Marone R, Collmann E, Calvez R, Hirsch E, and Wymann MP (2009) PI3Kgamma adaptor subunits define coupling to degranulation and cell motility by distinct PtdIns(3,4,5)P3 pools in mast cells. *Sci Signal* **2:**ra27.

Borlado LR, Redondo C, Alvarez B, Jimenez C, Criado LM, Flores J, Marcos MA, Martinez AC, Balomenos D, and Carrera AC (2000) Increased phosphoinositide 3-kinase activity induces a lymphoproliferative disorder and contributes to tumor generation in vivo. *FASEB J* **14:**895–903.

Boyle DL, Rommel C, Topolewski K, and Firestein GS (2009) A novel PI3 kinase/inhibitor suppresses collagen-induced arthritis (Abstract). *Arthritis Rheum* **60 (Suppl 10):**669.

Boyle KB, Gyori D, Sindrilaru A, Scharffetter-Kochanek K, Taylor PR, Mócsai A, Stephens LR, and Hawkins PT (2011) Class IA phosphoinositide 3-kinase β and δ regulate neutrophil oxidase activation in response to *Aspergillus fumigatus* hyphae. *J Immunol* **186:**2978–2989.

Brazzatti JA, Klingler-Hoffmann M, Haylock-Jacobs S, Harata-Lee Y, Niu M, Higgins MD, Kochetkova M, Hoffmann P, and McColl SR (2012) Differential roles for the p101 and p84 regulatory subunits of PI3Kγ in tumor growth and metastasis. *Oncogene* **31:**2350–2361.

Brooks R, Fuhler GM, Iyer S, Smith MJ, Park MY, Paraiso KH, Engelman RW, and Kerr WG (2010) SHIP1 inhibition increases immunoregulatory capacity and triggers apoptosis of hematopoietic cancer cells. *J Immunol* **184:**3582–3589.
Burda P, Padilla SM, Sarkar S, and Emr SD (2002) Retromer function in endosome-to-Golgi retrograde transport is regulated by the yeast Vps34 PtdIns 3-kinase. *J Cell Sci* **115:**3889–3900.
Byfield MP, Murray JT, and Backer JM (2005) hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. *J Biol Chem* **280:**33076–33082.
Cai X, Srivastava S, Sun Y, Li Z, Wu H, Zuvela-Jelaska L, Li J, Salamon RS, Backer JM, and Skolnik EY (2011) Tripartite motif containing protein 27 negatively regulates CD4 T cells by ubiquitinating and inhibiting the class II PI3K-C2β. *Proc Natl Acad Sci USA* **108:**20072–20077.
Camps M, Rückle T, Ji H, Ardissone V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C, Françon B, et al. (2005) Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat Med* **11:**936–943.
Chang JD, Sukhova GK, Libby P, Schvartz E, Lichtenstein AH, Field SJ, Kennedy C, Madhavarapu S, Luo J, Wu D, et al. (2007) Deletion of the phosphoinositide 3-kinase p110gamma gene attenuates murine atherosclerosis. *Proc Natl Acad Sci USA* **104:**8077–8082.
Chang KY, Tsai SY, Wu CM, Yen CJ, Chuang BF, and Chang JY (2011) Novel phosphoinositide 3-kinase/mTOR dual inhibitor, NVP-BGT226, displays potent growth-inhibitory activity against human head and neck cancer cells in vitro and in vivo. *Clin Cancer Res* **17:**7116–7126.
Charlier E, Condé C, Zhang J, Deneubourg L, Di Valentin E, Rahmouni S, Chariot A, Agostinis P, Pang PC, Haslam SM, et al. (2010) SHIP-1 inhibits CD95/APO-1/Fas-induced apoptosis in primary T lymphocytes and T leukemic cells by promoting CD95 glycosylation independently of its phosphatase activity. *Leukemia* **24:**821–832.
Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE, Vincent JP, Ellston R, Jones D, Sini P, et al. (2010) AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. *Cancer Res* **70:**288–298.
Collazo MM, Wood D, Paraiso KH, Lund E, Engelman RW, Le CT, Stauch D, Kotsch K, and Kerr WG (2009) SHIP limits immunoregulatory capacity in the T-cell compartment. *Blood* **113:**2934–2944.
Condliffe AM, Davidson K, Anderson KE, Ellson CD, Crabbe T, Okkenhaug K, Vanhaesebroeck B, Turner M, Webb L, Wymann MP, et al. (2005) Sequential activation of class IB and class IA PI3K is important for the primed respiratory burst of human but not murine neutrophils. *Blood* **106:**1432–1440.
Costa C, Barberis L, Ambrogio C, Manazza AD, Patrucco E, Azzolino O, Neilsen PO, Ciraolo E, Altruda F, Prestwich GD, et al. (2007) Negative feedback regulation of Rac in leukocytes from mice expressing a constitutively active phosphatidylinositol 3-kinase gamma. *Proc Natl Acad Sci USA* **104:**14354–14359.
Costinean S, Sandhu SK, Pedersen IM, Tili E, Trotta R, Perrotti D, Ciarlariello D, Neviani P, Harb J, Kauffman LR, et al. (2009) Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-binding protein beta are targeted by miR-155 in B cells of Emicro-MiR-155 transgenic mice. *Blood* **114:**1374–1382.
Courtneidge SA and Heber A (1987) An 81 kd protein complexed with middle T antigen and pp60c-src: a possible phosphatidylinositol kinase. *Cell* **50:**1031–1037.
Crabbe T, Welham MJ, and Ward SG (2007) The PI3K inhibitor arsenal: choose your weapon!. *Trends Biochem Sci* **32:**450–456. *Cell* **110:**737–749.
Das M, Scappini E, Martin NP, Wong KA, Dunn S, Chen YJ, Miller SL, Domin J, and O'Bryan JP (2007) Regulation of neuron survival through an intersectin-phosphoinositide 3′-kinase C2beta-AKT pathway. *Mol Cell Biol* **27:**7906–7917.
Delgado-Martín C, Escribano C, Pablos JL, Riol-Blanco L, and Rodríguez-Fernández JL (2011) Chemokine CXCL12 uses CXCR4 and a signaling core formed by bifunctional Akt, extracellular signal-regulated kinase (ERK)1/2, and mammalian target of rapamycin complex 1 (mTORC1) proteins to control chemotaxis and survival simultaneously in mature dendritic cells. *J Biol Chem* **286:**37222–37236.
Delgoffe GM and Powell JD (2009) mTOR: taking cues from the immune microenvironment. *Immunology* **127:**459–465.
Del Prete A, Vermi W, Dander E, Otero K, Barberis L, Luini W, Bernasconi S, Sironi M, Santoro A, Garlanda C, et al. (2004) Defective dendritic cell migration and activation of adaptive immunity in PI3Kgamma-deficient mice. *EMBO J* **23:**3505–3515.
Deretic V, Singh S, Master S, Harris J, Roberts E, Kyei G, Davis A, de Haro S, Naylor J, Lee HH, et al. (2006) Mycobacterium tuberculosis inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. *Cell Microbiol* **8:**719–727.
Dituri F, Mazzocca A, Lupo L, Edling CE, Azzariti A, Antonaci S, Falasca M, and Giannelli G (2012) PI3K class 1B controls the cell cycle checkpoint promoting cell proliferation in hepatocellular carcinoma. *Int J Cancer* **130:**2505–2513.
Domin J, Harper L, Aubyn D, Wheeler M, Florey O, Haskard D, Yuan M, and Zicha D (2005) The class II phosphoinositide 3-kinase PI3K-C2beta regulates cell migration by a PtdIns3P dependent mechanism. *J Cell Physiol* **205:**452–462.
Domin J, Pages F, Volinia S, Rittenhouse SE, Zvelebil MJ, Stein RC, and Waterfield MD (1997) Cloning of a human phosphoinositide 3-kinase with a C2 domain that displays reduced sensitivity to the inhibitor wortmannin. *Biochem J* **326:**139–147.
Doukas J, Eide L, Stebbins K, Racanelli-Layton A, Dellamary L, Martin M, Dneprovskaia E, Noronha G, Soll R, Wrasidlo W, et al. (2009) Aerosolized phosphoinositide 3-kinase gamma/delta inhibitor TG100–115 [3-[2,4-diamino-6-(3-hydroxyphenyl)pteridin-7-yl]phenol] as a therapeutic candidate for asthma and chronic obstructive pulmonary disease. *J Pharmacol Exp Ther* **328:**758–765.
Doukas J, Wrasidlo W, Noronha G, Dneprovskaia E, Fine R, Weis S, Hood J, Demaria A, Soll R, and Cheresh D (2006) Phosphoinositide 3-kinase gamma/delta inhibition limits infarct size after myocardial ischemia/reperfusion injury. *Proc Natl Acad Sci USA* **103:**19866–19871.
Edling CE, Selvaggi F, Buus R, Maffucci T, Di Sebastiano P, Friess H, Innocenti P, Kocher HM, and Falasca M (2010) Key role of phosphoinositide 3-kinase class IB in pancreatic cancer. *Clin Cancer Res* **16:**4928–4937.
Ellson CD, Davidson K, Ferguson GJ, O'Connor R, Stephens LR, and Hawkins PT (2006a) Neutrophils from p40phox−/− mice exhibit severe defects in NADPH oxidase regulation and oxidant-dependent bacterial killing. *J Exp Med* **203:**1927–1937.
Ellson C, Davidson K, Anderson K, Stephens LR, and Hawkins PT (2006b) PtdIns3P binding to the PX domain of p40phox is a physiological signal in NADPH oxidase activation. *EMBO J* **25:**4468–4478.
Engelman JA (2009) Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* **9:**550–562.
Engelman JA, Luo J, and Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* **7:**606–619.
Falasca M and Maffucci T (2012) Regulation and cellular functions of class II phosphoinositide 3-kinases. *Biochem J* **443:**587–601.
Farghaly HS, Blagbrough IS, Medina-Tato DA, and Watson ML (2008) Interleukin 13 increases contractility of murine tracheal smooth muscle by a phosphoinositide 3-kinase p110delta-dependent mechanism. *Mol Pharmacol* **73:**1530–1537.
Ferguson GJ, Milne L, Kulkarni S, Sasaki T, Walker S, Andrews S, Crabbe T, Finan P, Jones G, Jackson S, et al. (2007) PI(3)Kgamma has an important context-dependent role in neutrophil chemokinesis. *Nat Cell Biol* **9:**86–91.
Finlay DK, Sinclair LV, Feijoo C, Waugh CM, Hagenbeek TJ, Spits H, and Cantrell DA (2009) Phosphoinositide-dependent kinase 1 controls migration and malignant transformation but not cell growth and proliferation in PTEN-null lymphocytes. *J Exp Med* **206:**2441–2454.
Finsen B, Antel J, and Owens T (2002) TNFalpha: kill or cure for demyelinating disease? *Mol Psychiatry* **7:**820–821.
Firestein GS (2003) Evolving concepts of rheumatoid arthritis. *Nature* **423:**356–361.
Folkes AJ, Ahmadi K, Alderton WK, Alix S, Baker SJ, Box G, Chuckowree IS, Clarke PA, Depledge P, Eccles SA, et al. (2008) The identification of 2-(1*H*-indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin-4-yl-thieno[3,2-*d*]pyrimidine (GDC-0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 kinase for the treatment of cancer. *J Med Chem* **51:**5522–5532.
Fougerat A, Gayral S, Gourdy P, Schambourg A, Rückle T, Schwarz MK, Rommel C, Hirsch E, Arnal JF, Salles JP, et al. (2008) Genetic and pharmacological targeting of phosphoinositide 3-kinase-gamma reduces atherosclerosis and favors plaque stability by modulating inflammatory processes. *Circulation* **117:**1310–1317.
Foukas LC, Berenjeno IM, Gray A, Khwaja A, and Vanhaesebroeck B (2010) Activity of any class IA PI3K isoform can sustain cell proliferation and survival. *Proc Natl Acad Sci USA* **107:**11381–11386.
Frangogiannis NG, Smith CW, and Entman ML (2002) The inflammatory response in myocardial infarction. *Cardiovasc Res* **53:**31–47.
Fratti RA, Backer JM, Gruenberg J, Corvera S, and Deretic V (2001) Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. *J Cell Biol* **154:**631–644.
Fruman DA and Rommel C (2011) PI3Kδ inhibitors in cancer: rationale and serendipity merge in the clinic. *Cancer Discov* **1:**562–572.
Fuhler GM, Brooks R, Toms B, Iyer S, Gengo EA, Park MY, Gumbleton M, Viernes DR, Chisholm JD, and Kerr WG (2012) Therapeutic potential of SH2 domain-containing inositol-5′-phosphatase 1 (SHIP1) and SHIP2 inhibition in cancer. *Mol Med* **18:**65–75.
Gaidarov I, Smith ME, Domin J, and Keen JH (2001) The class II phosphoinositide 3-kinase C2alpha is activated by clathrin and regulates clathrin-mediated membrane trafficking. *Mol Cell* **7:**443–449.
Gao X, Lowry PR, Zhou X, Depry C, Wei Z, Wong GW, and Zhang J (2011) PI3K/Akt signaling requires spatial compartmentalization in plasma membrane microdomains. *Proc Natl Acad Sci USA* **108:**14509–14514.
Garlich JR, De P, Dey N, Su JD, Peng X, Miller A, Murali R, Lu Y, Mills GB, Kundra V, et al. (2008) A vascular targeted pan phosphoinositide 3-kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. *Cancer Res* **68:**206–215.
Ghansah T, Paraiso KH, Highfill S, Desponts C, May S, McIntosh JK, Wang JW, Ninos J, Brayer J, Cheng F, et al. (2004) Expansion of myeloid suppressor cells in SHIP-deficient mice represses allogeneic T cell responses. *J Immunol* **173:**7324–7330.
Gharbi SI, Zvelebil MJ, Shuttleworth SJ, Hancox T, Saghir N, Timms JF, and Waterfield MD (2007) Exploring the specificity of the PI3K family inhibitor LY294002. *Biochem J* **404:**15–21.
Goncharova EA, Ammit AJ, Irani C, Carroll RG, Eszterhas AJ, Panettieri RA, and Krymskaya VP (2002) PI3K is required for proliferation and migration of human pulmonary vascular smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* **283:**L354–L363.
Guertin DA and Sabatini DM (2009) The pharmacology of mTOR inhibition. *Sci Signal* **2:**pe24.
Guillermet-Guibert J, Bjorklof K, Salpekar A, Gonella C, Ramadani F, Bilancio A, Meek S, Smith AJ, Okkenhaug K, and Vanhaesebroeck B (2008) The p110beta isoform of phosphoinositide 3-kinase signals downstream of G protein-coupled receptors and is functionally redundant with p110gamma. *Proc Natl Acad Sci USA* **105:**8292–8297.
Guo JP, Coppola D, and Cheng JQ (2011) IKBKE activates Akt independent of phosphatidylinositol 3-kinase/PDK1/mTORC2 and PH domain to sustain malignant transformation. *J Biol Chem* **286:**37389–37398.
Haddon DJ, Antignano F, Hughes MR, Blanchet MR, Zbytnuik L, Krystal G, and McNagny KM (2009) SHIP1 is a repressor of mast cell hyperplasia, cytokine production, and allergic inflammation in vivo. *J Immunol* **183:**228–236.
Hansson GK and Libby P (2006) The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* **6:**508–519.
Harris DP, Vogel P, Wims M, Moberg K, Humphries J, Jhaver KG, DaCosta CM, Shadoan MK, Xu N, Hansen GM, et al. (2011a) Requirement for class II phosphoinositide 3-kinase C2alpha in maintenance of glomerular structure and function. *Mol Cell Biol* **31:**63–80.
Harris SJ, Parry RV, Foster JG, Blunt MD, Wang A, Marelli-Berg F, Westwick J, and Ward SG (2011b) Evidence that the lipid phosphatase SHIP-1 regulates T lymphocyte morphology and motility. *J Immunol* **186:**4936–4945.

Harris SJ, Parry RV, Westwick J, and Ward SG (2008) Phosphoinositide lipid phosphatases: natural regulators of phosphoinositide 3-kinase signaling in T lymphocytes. *J Biol Chem* **283:**2465–2469.
Hayer S, Pundt N, Peters MA, Wunrau C, Kühnel I, Neugebauer K, Strietholt S, Zwerina J, Korb A, Penninger J, et al. (2009) PI3Kgamma regulates cartilage damage in chronic inflammatory arthritis. *FASEB J* **23:**4288–4298.
Haylock-Jacobs S, Comerford I, Bunting M, Kara E, Townley S, Klingler-Hoffmann M, Vanhaesebroeck B, Puri KD, and McColl SR (2011) PI3Kδ drives the pathogenesis of experimental autoimmune encephalomyelitis by inhibiting effector T cell apoptosis and promoting Th17 differentiation. *J Autoimmun* **36:**278–287.
Helgason CD, Damen JE, Rosten P, Grewal R, Sorensen P, Chappel SM, Borowski A, Jirik F, Krystal G, and Humphries RK (1998) Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. *Genes Dev* **12:**1610–1620.
Helgason CD, Kalberer CP, Damen JE, Chappel SM, Pineault N, Krystal G, and Humphries RK (2000) A dual role for Src homology 2 domain-containing inositol-5-phosphatase (SHIP) in immunity: aberrant development and enhanced function of b lymphocytes in ship –/– mice. *J Exp Med* **191:**781–794.
Herman SE, Gordon AL, Wagner AJ, Heerema NA, Zhao W, Flynn JM, Jones J, Andritsos L, Puri KD, Lannutti BJ, et al. (2010) Phosphatidylinositol 3-kinase-δ inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. *Blood* **116:**2078–2088.
Hers I, Vincent EE, and Tavaré JM (2011) Akt signalling in health and disease. *Cell Signal* **23:**1515–1527.
Hirsch DS, Shen Y, Dokmanovic M, and Wu WJ (2010) pp60c-Src phosphorylates and activates vacuolar protein sorting 34 to mediate cellular transformation. *Cancer Res* **70:**5974–5983.
Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, Sozzani S, Mantovani A, Altruda F, and Wymann MP (2000) Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* **287:**1049–1053.
Hirsch E, Braccini L, Ciraolo E, Morello F, and Perino A (2009) Twice upon a time: PI3K's secret double life exposed. *Trends Biochem Sci* **34:**244–248.
Hoang B, Frost P, Shi Y, Belanger E, Benavides A, Pezeshkpour G, Cappia S, Guglielmelli T, Gera J, and Lichtenstein A (2010) Targeting TORC2 in multiple myeloma with a new mTOR kinase inhibitor. *Blood* **116:**4560–4568.
Hochdörfer T, Kuhny M, Zorn CN, Hendriks RW, Vanhaesebroeck B, Bohnacker T, Krystal G, and Huber M (2011) Activation of the PI3K pathway increases TLR-induced TNF-α and IL-6 but reduces IL-1β production in mast cells. *Cell Signal* **23:**866–875.
Hoellenriegel J, Meadows SA, Sivina M, Wierda WG, Kantarjian H, Keating MJ, Giese N, O'Brien S, Yu A, Miller LL, et al. (2011) The phosphoinositide 3′-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. *Blood* **118:**3603–3612.
Hollander MC, Blumenthal GM, and Dennis PA (2011) PTEN loss in the continuum of common cancers, rare syndromes and mouse models. *Nat Rev Cancer* **11:**289–301.
Huber M, Helgason CD, Damen JE, Liu L, Humphries RK, and Krystal G (1998) The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. *Proc Natl Acad Sci USA* **95:**11330–11335.
Huwait EA, Greenow KR, Singh NN, and Ramji DP (2011) A novel role for c-Jun N-terminal kinase and phosphoinositide 3-kinase in the liver X receptor-mediated induction of macrophage gene expression. *Cellular signalling* **23:**542–549.
Ihle NT, Paine-Murrieta G, Berggren MI, Baker A, Tate WR, Wipf P, Abraham RT, Kirkpatrick DL, and Powis G (2005) The phosphatidylinositol-3-kinase inhibitor PX-866 overcomes resistance to the epidermal growth factor receptor inhibitor gefitinib in A-549 human non-small cell lung cancer xenografts. *Mol Cancer Ther* **4:**1349–1357.
Ihle NT, Williams R, Chow S, Chew W, Berggren MI, Paine-Murrieta G, Minion DJ, Halter RJ, Wipf P, Abraham R, et al. (2004) Molecular pharmacology and antitumor activity of PX-866, a novel inhibitor of phosphoinositide-3-kinase signaling. *Mol Cancer Ther* **3:**763–772.
Ikeda H, Hideshima T, Fulciniti M, Perrone G, Miura N, Yasui H, Okawa Y, Kiziltepe T, Santo L, Vallet S, et al. (2010) PI3K/p110{delta} is a novel therapeutic target in multiple myeloma. *Blood* **116:**1460–1468.
Ilic N, Utermark T, Widlund HR, and Roberts TM (2011) PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. *Proc Natl Acad Sci USA* **108:**E699–E708.
Ito S, Koshikawa N, Mochizuki S, and Takenaga K (2007) 3-Methyladenine suppresses cell migration and invasion of HT1080 fibrosarcoma cells through inhibiting phosphoinositide 3-kinases independently of autophagy inhibition. *Int J Oncol* **31:**261–268.
Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, Wright CE, Kenche V, Anderson KE, Dopheide SM, Yuan Y, et al. (2005) PI 3-kinase p110beta: a new target for antithrombotic therapy. *Nat Med* **11:**507–514.
Janes MR, Limon JJ, So L, Chen J, Lim RJ, Chavez MA, Vu C, Lilly MB, Mallya S, Ong ST, et al. (2010) Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. *Nat Med* **16:**205–213.
Jarmin SJ, David R, Ma L, Chai JG, Dewchand H, Takesono A, Ridley AJ, Okkenhaug K, and Marelli-Berg FM (2008) T cell receptor-induced phosphoinositide-3-kinase p110delta activity is required for T cell localization to antigenic tissue in mice. *J Clin Invest* **118:**1154–1164.
Jevnikar AM, Grusby MJ, and Glimcher LH (1994) Prevention of nephritis in major histocompatibility complex class II-deficient MRL-lpr mice. *J Exp Med* **179:**1137–1143.
Ji H, Rintelen F, Waltzinger C, Bertschy Meier D, Bilancio A, Pearce W, Hirsch E, Wymann MP, Rückle T, Camps M, et al. (2007) Inactivation of PI3Kgamma and PI3Kdelta distorts T-cell development and causes multiple organ inflammation. *Blood* **110:**2940–2947.
Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, et al. (2008) Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. *Nature* **454:**776–779.
Jones GE, Prigmore E, Calvez R, Hogan C, Dunn GA, Hirsch E, Wymann MP, and Ridley AJ (2003) Requirement for PI 3-kinase gamma in macrophage migration to MCP-1 and CSF-1. *Exp Cell Res* **290:**120–131.
Kamen LA, Levinsohn J, Cadwallader A, Tridandapani S, and Swanson JA (2008) SHIP-1 increases early oxidative burst and regulates phagosome maturation in macrophages. *J Immunol* **180:**7497–7505.
Kang BN, Ha SG, Ge XN, Reza Hosseinkhani M, Bahaie NS, Greenberg Y, Blumenthal MN, Puri KD, Rao SP, and Sriramarao P (2012) The p110δ subunit of PI3K regulates bone marrow-derived eosinophil trafficking and airway eosinophilia in allergen-challenged mice. *Am J Physiol Lung Cell Mol Physiol* **302:**L1179–L1191.
Kashiwada M, Cattoretti G, McKeag L, Rouse T, Showalter BM, Al-Alem U, Niki M, Pandolfi PP, Field EH, and Rothman PB (2006) Downstream of tyrosine kinases-1 and Src homology 2-containing inositol 5′-phosphatase are required for regulation of CD4+CD25+ T cell development. *J Immunol* **176:**3958–3965.
Kennah M, Yau TY, Nodwell M, Krystal G, Andersen RJ, Ong CJ, and Mui AL (2009) Activation of SHIP via a small molecule agonist kills multiple myeloma cells. *Exp Hematol* **37:**1274–1283.
Kerr WG (2008) A role for SHIP in stem cell biology and transplantation. *Curr Stem Cell Res Ther* **3:**99–106.
Kerr WG (2011) Inhibitor and activator: dual functions for SHIP in immunity and cancer. *Ann NY Acad Sci* **1217:**1–17.
Kerr WG, Park MY, Maubert M, and Engelman RW (2011) SHIP deficiency causes Crohn's disease-like ileitis. *Gut* **60:**177–188.
Kitaura J, Kinoshita T, Matsumoto M, Chung S, Kawakami Y, Leitges M, Wu D, Lowell CA, and Kawakami T (2005) IgE- and IgE+Ag-mediated mast cell migration in an autocrine/paracrine fashion. *Blood* **105:**3222–3229.
Kleinschek MA, Owyang AM, Joyce-Shaikh B, Langrish CL, Chen Y, Gorman DM, Blumenschein WM, McClanahan T, Brombacher F, Hurst SD, et al. (2007) IL-25 regulates Th17 function in autoimmune inflammation. *J Exp Med* **204:**161–170.
Knight SD, Adams ND, Burgess JL, Chaudhari AM, Darcy MG, Donatelli CA, Luengo JI, Newlander KA, Parrish CA, Ridgers LH, et al. (2010) Discovery of GSK2126458, a highly potent inhibitor of PI3K and the mammalian target of rapamycin. *ACS Med Chem Lett* **1:**39–43.
Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, et al. (2006) A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell* **125:**733–747.
Kok K, Geering B, and Vanhaesebroeck B (2009) Regulation of phosphoinositide 3-kinase expression in health and disease. *Trends Biochem Sci* **34:**115–127.
Kong D and Yamori T (2007) ZSTK474 is an ATP-competitive inhibitor of class I phosphatidylinositol 3 kinase isoforms. *Cancer Sci* **98:**1638–1642.
Koul D, Fu J, Shen R, LaFortune TA, Wang S, Tiao N, Kim YW, Liu JL, Ramnarian D, Yuan Y, et al. (2012) Antitumor activity of NVP-BKM120—a selective pan class I PI3 kinase inhibitor showed differential forms of cell death based on p53 status of glioma cells. *Clin Cancer Res* **18:**184–195.
Krishnamoorthy N, Oriss TB, Paglia M, Fei M, Yarlagadda M, Vanhaesebroeck B, Ray A, and Ray P (2008) Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma. *Nat Med* **14:**565–573.
Krymskaya VP (2007) Targeting the phosphatidylinositol 3-kinase pathway in airway smooth muscle: rationale and promise. *BioDrugs* **21:**85–95.
Kulkarni S, Sitaru C, Jakus Z, Anderson KE, Damoulakis G, Davidson K, Hirose M, Juss J, Oxley D, Chessa TA, et al. (2011) PI3Kβ plays a critical role in neutrophil activation by immune complexes. *Sci Signal* **4:**ra23.
Kuroda E, Antignano F, Ho VW, Hughes MR, Ruschmann J, Lam V, Kawakami T, Kerr WG, McNagny KM, Sly LM, et al. (2011) SHIP represses Th2 skewing by inhibiting IL-4 production from basophils. *J Immunol* **186:**323–332.
Kuroiwa T and Lee EG (1998) Cellular interactions in the pathogenesis of lupus nephritis: the role of T cells and macrophages in the amplification of the inflammatory process in the kidney. *Lupus* **7:**597–603.
Küppers R (2005) Mechanisms of B-cell lymphoma pathogenesis. *Nat Rev Cancer* **5:**251–262.
Laffargue M, Calvez R, Finan P, Trifilieff A, Barbier M, Altruda F, Hirsch E, and Wymann MP (2002) Phosphoinositide 3-kinase gamma is an essential amplifier of mast cell function. *Immunity* **16:**441–451.
Lang TJ, Nguyen P, Papadimitriou JC, and Via CS (2003) Increased severity of murine lupus in female mice is due to enhanced expansion of pathogenic T cells. *J Immunol* **171:**5795–5801.
Lannutti BJ, Meadows SA, Herman SE, Kashishian A, Steiner B, Johnson AJ, Byrd JC, Tyner JW, Loriaux MM, Deininger M, et al. (2011) CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. *Blood* **117:**591–594.
Lee KS, Lee HK, Hayflick JS, Lee YC, and Puri KD (2006) Inhibition of phosphoinositide 3-kinase delta attenuates allergic airway inflammation and hyperresponsiveness in murine asthma model. *FASEB J* **20:**455–465.
Leung WH, Tarasenko T, and Bolland S (2009) Differential roles for the inositol phosphatase SHIP in the regulation of macrophages and lymphocytes. *Immunol Res* **43:**243–251.
Li Y, Wang LX, Yang G, Hao F, Urba WJ, and Hu HM (2008) Efficient cross-presentation depends on autophagy in tumor cells. *Cancer Res* **68:**6889–6895.
Lim DH, Cho JY, Song DJ, Lee SY, Miller M, and Broide DH (2009) PI3K gamma-deficient mice have reduced levels of allergen-induced eosinophilic inflammation and airway remodeling. *Am J Physiol Lung Cell Mol Physiol* **296:**L210–L219.
Lioubin MN, Myles GM, Carlberg K, Bowtell D, and Rohrschneider LR (1994) Shc, Grb2, Sos1, and a 150-kilodalton tyrosine-phosphorylated protein form complexes with Fms in hematopoietic cells. *Mol Cell Biol* **14:**5682–5691.
Liu D, Zhang T, Marshall AJ, Okkenhaug K, Vanhaesebroeck B, and Uzonna JE (2009a) The p110delta isoform of phosphatidylinositol 3-kinase controls susceptibility to *Leishmania major* by regulating expansion and tissue homing of regulatory T cells. *J Immunol* **183:**1921–1933.
Liu L, Damen JE, Cutler RL, and Krystal G (1994) Multiple cytokines stimulate the

binding of a common 145-kilodalton protein to Shc at the Grb2 recognition site of Shc. *Mol Cell Biol* **14:**6926–6935.
Liu L, Puri KD, Penninger JM, and Kubes P (2007) Leukocyte PI3Kgamma and PI3Kdelta have temporally distinct roles for leukocyte recruitment in vivo. *Blood* **110:**1191–1198.
Liu P, Cheng H, Roberts TM, and Zhao JJ (2009b) Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* **8:**627–644.
Liu Q, Oliveira-Dos-Santos AJ, Mariathasan S, Bouchard D, Jones J, Sarao R, Kozieradzki I, Ohashi PS, Penninger JM, and Dumont DJ (1998) The inositolpolyphosphate 5-phosphatase ship is a crucial negative regulator of B cell antigen receptor signaling. *J Exp Med* **188:**1333–1342.
Locke NR, Patterson SJ, Hamilton MJ, Sly LM, Krystal G, and Levings MK (2009) SHIP regulates the reciprocal development of T regulatory and Th17 cells. *J Immunol* **183:**975–983.
Luo JM, Yoshida H, Komura S, Ohishi N, Pan L, Shigeno K, Hanamura I, Miura K, Iida S, Ueda R, et al. (2003) Possible dominant-negative mutation of the SHIP gene in acute myeloid leukemia. *Leukemia* **17:**1–8.
López-Fauqued M, Gil R, Grueso J, Hernandez-Losa J, Pujol A, Moliné T, and Recio JA (2010) The dual PI3K/mTOR inhibitor PI-103 promotes immunosuppression, in vivo tumor growth and increases survival of sorafenib-treated melanoma cells. *Int J Cancer* **126:**1549–1561.
Ma P, Vemula S, Munugalavadla V, Chen J, Sims E, Borneo J, Kondo T, Ramdas B, Mali RS, Li S, et al. (2011) Balanced interactions between Lyn, the p85{alpha} regulatory subunit of class IA phosphatidylinositol-3-kinase, and SHIP are essential for mast cell growth and maturation. *Mol Cell Biol* **31:**4052–4062.
Maddaluno M, Di Lauro M, Di Pascale A, Santamaria R, Guglielmotti A, Grassia G, and Ialenti A (2011) Monocyte chemotactic protein-3 induces human coronary smooth muscle cell proliferation. *Atherosclerosis* **217:**113–119.
Maffucci T, Cooke FT, Foster FM, Traer CJ, Fry MJ, and Falasca M (2005) Class II phosphoinositide 3-kinase defines a novel signaling pathway in cell migration. *J Cell Biol* **169:**789–799.
Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chène P, De Pover A, Schoemaker K, et al. (2008) Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther* **7:**1851–1863.
Manning BD and Cantley LC (2007) AKT/PKB signaling: navigating downstream. *Cell* **129:**1261–1274.
Mazza S and Maffucci T (2011) Class II phosphoinositide 3-kinase C2alpha: what we learned so far. *Int J Biochem Mol Biol* **2:**168–182.
McLeod IX, Zhou X, Li QJ, Wang F, and He YW (2011) The class III kinase Vps34 promotes T lymphocyte survival through regulating IL-7Rα surface expression. *J Immunol* **187:**5051–5061.
Meadows SA, Vega F, Kashishian A, Johnson D, Diehl V, Miller LL, Younes A, and Lannutti BJ (2012) PI3Kδ inhibitor, GS-1101 (CAL-101), attenuates pathway signaling, induces apoptosis, and overcomes signals from the microenvironment in cellular models of Hodgkin lymphoma. *Blood* **119:**1897–1900.
Medina-Tato DA, Ward SG, and Watson ML (2007) Phosphoinositide 3-kinase signalling in lung disease: leucocytes and beyond. *Immunology* **121:**448–461.
Medina-Tato DA, Watson ML, and Ward SG (2006) Leukocyte navigation mechanisms as targets in airway diseases. *Drug Discov Today* **11:**866–879.
Miller S, Tavshanjian B, Oleksy A, Perisic O, Houseman BT, Shokat KM, and Williams RL (2010) Shaping development of autophagy inhibitors with the structure of the lipid kinase Vps34. *Science* **327:**1638–1642.
Mills RE and Jameson JM (2009) T cell dependence on mTOR signaling. *Cell Cycle* **8:**545–548.
Mills SJ, Persson C, Cozier G, Thomas MP, Trésaugues L, Erneux C, Riley AM, Nordlund P, and Potter BV (2012) A synthetic polyphosphoinositide headgroup surrogate in complex with SHIP2 provides a rationale for drug discovery. *ACS Chem Biol* **7:**822–888.
Ming-Lum A, Shojania S, So E, McCarrell E, Shaw E, Vu D, Wang I, McIntosh LP, and Mui AL (2012) A pleckstrin homology-related domain in SHIP1 mediates membrane localization during Fcγ receptor-induced phagocytosis. *FASEB J* **26:**3163–3177.
Moir LM, Trian T, Ge Q, Shepherd PR, Burgess JK, Oliver BG, and Black JL (2011) Phosphatidylinositol 3-kinase isoform-specific effects in airway mesenchymal cell function. *J Pharmacol Exp Ther* **337:**557–566.
Moore SF, Hunter RW, and Hers I (2011) mTORC2 protein complex-mediated Akt (protein kinase B) serine 473 phosphorylation is not required for Akt1 activity in human platelets [corrected]. *J Biol Chem* **286:**24553–24560.
Nakamura K, Malykhin A, and Coggeshall KM (2002) The Src homology 2 domain-containing inositol 5-phosphatase negatively regulates Fcgamma receptor-mediated phagocytosis through immunoreceptor tyrosine-based activation motif-bearing phagocytic receptors. *Blood* **100:**3374–3382.
Nashed BF, Zhang T, Al-Alwan M, Srinivasan G, Halayko AJ, Okkenhaug K, Vanhaesebroeck B, Hayglass KT, and Marshall AJ (2007) Role of the phosphoinositide 3-kinase p110delta in generation of type 2 cytokine responses and allergic airway inflammation. *Eur J Immunol* **37:**416–424.
Nedjic J, Aichinger M, Emmerich J, Mizushima N, and Klein L (2008) Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* **455:**396–400.
Neill L, Tien AH, Rey-Ladino J, and Helgason CD (2007) SHIP-deficient mice provide insights into the regulation of dendritic cell development and function. *Exp Hematol* **35:**627–639.
Nishio M, Watanabe K, Sasaki J, Taya C, Takasuga S, Iizuka R, Balla T, Yamazaki M, Watanabe H, Itoh R, et al. (2007) Control of cell polarity and motility by the PtdIns(3,4,5)P3 phosphatase SHIP1. *Nat Cell Biol* **9:**36–44.
Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, Gulati P, Byfield MP, Backer JM, Natt F, Bos JL, et al. (2005) Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. *Proc Natl Acad Sci USA* **102:**14238–14243.
Nombela-Arrieta C, Mempel TR, Soriano SF, Mazo I, Wymann MP, Hirsch E, Martínez-A C, Fukui Y, von Andrian UH, and Stein JV (2007) A central role for DOCK2 during interstitial lymphocyte motility and sphingosine-1-phosphate-mediated egress. *J Exp Med* **204:**497–510.
Norman P (2011) Selective PI3Kδ inhibitors, a review of the patent literature. *Expert Opin Ther Pat* **21:**1773–1790.
Oh SY, Zheng T, Bailey ML, Barber DL, Schroeder JT, Kim YK, and Zhu Z (2007) Src homology 2 domain-containing inositol 5-phosphatase 1 deficiency leads to a spontaneous allergic inflammation in the murine lung. *J Allergy Clin Immunol* **119:**123–131.
Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, et al. (2002) Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* **297:**1031–1034.
Okkenhaug K, Patton DT, Bilancio A, Garçon F, Rowan WC, and Vanhaesebroeck B (2006) The p110delta isoform of phosphoinositide 3-kinase controls clonal expansion and differentiation of Th cells. *J Immunol* **177:**5122–5128.
Okkenhaug K and Vanhaesebroeck B (2003) PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol* **3:**317–330.
Ong CJ, Ming-Lum A, Nodwell M, Ghanipour A, Yang L, Williams DE, Kim J, Demirjian L, Qasimi P, Ruschmann J, et al. (2007) Small-molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. *Blood* **110:**1942–1949.
Ooms LM, Horan KA, Rahman P, Seaton G, Gurung R, Kethesparan DS, and Mitchell CA (2009) The role of the inositol polyphosphate 5-phosphatases in cellular function and human disease. *Biochem J* **419:**29–49.
Ozbay T, Durden DL, Liu T, O'Regan RM, and Nahta R (2010) In vitro evaluation of pan-PI3-kinase inhibitor SF1126 in trastuzumab-sensitive and trastuzumab-resistant HER2-over-expressing breast cancer cells. *Cancer Chemother Pharmacol* **65:**697–706.
O'Connell RM, Chaudhuri AA, Rao DS, and Baltimore D (2009) Inositol phosphatase SHIP1 is a primary target of miR-155. *Proc Natl Acad Sci USA* **106:**7113–7118.
Papakonstanti EA, Zwaenepoel O, Bilancio A, Burns E, Nock GE, Houseman B, Shokat K, Ridley AJ, and Vanhaesebroeck B (2008) Distinct roles of class IA PI3K isoforms in primary and immortalised macrophages. *J Cell Sci* **121:**4124–4133.
Paraiso KH, Ghansah T, Costello A, Engelman RW, and Kerr WG (2007) Induced SHIP deficiency expands myeloid regulatory cells and abrogates graft-versus-host disease. *J Immunol* **178:**2893–2900.
Park SJ, Lee KS, Kim SR, Min KH, Moon H, Lee MH, Chung CR, Han HJ, Puri KD, and Lee YC (2010) Phosphoinositide 3-kinase δ inhibitor suppresses interleukin-17 expression in a murine asthma model. *Eur Respir J* **36:**1448–1459.
Patrucco E, Notte A, Barberis L, Selvetella G, Maffei A, Brancaccio M, Marengo S, Russo G, Azzolino O, Rybalkin SD, et al. (2004) PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. *Cell* **118:**375–387.
Patton DT, Garden OA, Pearce WP, Clough LE, Monk CR, Leung E, Rowan WC, Sancho S, Walker LS, Vanhaesebroeck B, et al. (2006) Cutting edge: the phosphoinositide 3-kinase p110 delta is critical for the function of CD4+CD25+Foxp3+ regulatory T cells. *J Immunol* **177:**6598–6602.
Patton DT, Wilson MD, Rowan WC, Soond DR, and Okkenhaug K (2011) The PI3K p110δ regulates expression of CD38 on regulatory T cells. *PloS one* **6:**e17359.
Peifer C and Alessi DR (2008) Small-molecule inhibitors of PDK1. *ChemMedChem* **3:**1810–1838.
Peirce SK, Findley HW, Prince C, Dasgupta A, Cooper T, and Durden DL (2011) The PI-3 kinase-Akt-MDM2-survivin signaling axis in high-risk neuroblastoma: a target for PI-3 kinase inhibitor intervention. *Cancer Chemother Pharmacol* **68:**325–335.
Pinho V, Souza DG, Barsante MM, Hamer FP, De Freitas MS, Rossi AG, and Teixeira MM (2005) Phosphoinositide-3 kinases critically regulate the recruitment and survival of eosinophils in vivo: importance for the resolution of allergic inflammation. *J Leukoc Biol* **77:**800–810.
Del Prete A, Vermi W, Dander E, Otero K, Barberis L, Luini W, Bernasconi S, Sironi M, Santoro A, Garlanda C, et al. (2004) Defective dendritic cell migration and activation of adaptive immunity in PI3Kgamma-deficient mice. *EMBO J* **23:**3505–3515.
Puri KD, Doggett TA, Douangpanya J, Hou Y, Tino WT, Wilson T, Graf T, Clayton E, Turner M, Hayflick JS, et al. (2004) Mechanisms and implications of phosphoinositide 3-kinase delta in promoting neutrophil trafficking into inflamed tissue. *Blood* **103:**3448–3456.
Puri KD, Doggett TA, Huang CY, Douangpanya J, Hayflick JS, Turner M, Penninger J, and Diacovo TG (2005) The role of endothelial PI3Kgamma activity in neutrophil trafficking. *Blood* **106:**150–157.
Qiao H, Andrade MV, Lisboa FA, Morgan K, and Beaven MA (2006) FcepsilonR1 and toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells. *Blood* **107:**610–618.
Randis TM, Puri KD, Zhou H, and Diacovo TG (2008) Role of PI3Kdelta and PI3Kgamma in inflammatory arthritis and tissue localization of neutrophils. *Eur J Immunol* **38:**1215–1224.
Rauh MJ, Ho V, Pereira C, Sham A, Sly LM, Lam V, Huxham L, Minchinton AI, Mui A, and Krystal G (2005) SHIP represses the generation of alternatively activated macrophages. *Immunity* **23:**361–374.
Reif K, Okkenhaug K, Sasaki T, Penninger JM, Vanhaesebroeck B, and Cyster JG (2004) Cutting edge: differential roles for phosphoinositide 3-kinases, p110gamma and p110delta, in lymphocyte chemotaxis and homing. *J Immunol* **173:**2236–2240.
Rodrigues DH, Vilela MC, Barcelos LS, Pinho V, Teixeira MM, and Teixeira AL (2010) Absence of PI3Kgamma leads to increased leukocyte apoptosis and diminished severity of experimental autoimmune encephalomyelitis. *J Neuroimmunol* **222:**90–94.
Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, et al. (2007) Requirement of bic/microRNA-155 for normal immune function. *Science* **316:**608–611.
Rodríguez-Borlado L, Barber DF, Hernández C, Rodríguez-Marcos MA, Sánchez A,

Hirsch E, Wymann M, Martínez-A C, and Carrera AC (2003) Phosphatidylinositol 3-kinase regulates the CD4/CD8 T cell differentiation ratio. *J Immunol* **170:**4475–4482.
Rolf J, Bell SE, Kovesdi D, Janas ML, Soond DR, Webb LM, Santinelli S, Saunders T, Hebeis B, Killeen N, et al. (2010) Phosphoinositide 3-kinase activity in T cells regulates the magnitude of the germinal center reaction. *J Immunol* **185:**4042–4052.
Rommel C (2011) Targetting PI3Kα and TORC1/2 alone or in combination for the treatment of solid tumor (Abstract 013). Keystone Symposia on Molecular and Cellular Biology: PI 3-Kinase Signaling Pathways; 2011 Feb 13–18; Keystone, CO. Keystone Symposia, Silverthorne, CO.
Rommel C, Camps M, and Ji H (2007) PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Immunol* **7:**191–201.
Ruschmann J, Antignano F, Lam V, Snyder K, Kim C, Essak M, Zhang A, Lin AH, Mali RS, Kapur R, et al. (2012) The role of SHIP in the development and activation of mouse mucosal and connective tissue mast cells. *J Immunol* **188:**3839–3850.
Ruschmann J, Ho V, Antignano F, Kuroda E, Lam V, Ibaraki M, Snyder K, Kim C, Flavell RA, Kawakami T, et al. (2010) Tyrosine phosphorylation of SHIP promotes its proteasomal degradation. *Exp Hematol* **38:**392–402, 402.e1.
Sadhu C, Masinovsky B, Dick K, Sowell CG, and Staunton DE (2003) Essential role of phosphoinositide 3-kinase delta in neutrophil directional movement. *J Immunol* **170:**2647–2654.
Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, et al. (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* **304:**554.
Sasaki T, Irie-Sasaki J, Horie Y, Bachmaier K, Fata JE, Li M, Suzuki A, Bouchard D, Ho A, Redston M, et al. (2000a) Colorectal carcinomas in mice lacking the catalytic subunit of PI(3)Kgamma. *Nature* **406:**897–902.
Sasaki T, Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I, et al. (2000b) Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. *Science* **287:**1040–1046.
Saudemont A, Garçon F, Yadi H, Roche-Molina M, Kim N, Segonds-Pichon A, Martín-Fontecha A, Okkenhaug K, and Colucci F (2009) p110gamma and p110delta isoforms of phosphoinositide 3-kinase differentially regulate natural killer cell migration in health and disease. *Proc Natl Acad Sci USA* **106:**5795–5800.
Sawyer C, Sturge J, Bennett DC, O'Hare MJ, Allen WE, Bain J, Jones GE, and Vanhaesebroeck B (2003) Regulation of breast cancer cell chemotaxis by the phosphoinositide 3-kinase p110delta. *Cancer Res* **63:**1667–1675.
Schmid MC, Avraamides CJ, Dippold HC, Franco I, Foubert P, Ellies LG, Acevedo LM, Manglicmot JR, Song X, Wrasidlo W, et al. (2011) Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3Kγ, A single convergent point promoting tumor inflammation and progression. *Cancer Cell* **19:**715–727.
Seil FJ (1972) Multiple sclerosis. Current etiological concepts. *Calif Med* **116:**25–33.
Shapiro G, Kwak E, Baselga J, Rodon J, Scheffold C, Laird AD, Bedell C, and Edelman G (2009) Phase I dose-escalation study of XL147, a PI3K inhibitor administered orally to patients with solid tumors (Abstract 3500). *J Clin Oncol* **27 (Suppl):**15s.
Shenker BJ, Dlakic M, Walker LP, Besack D, Jaffe E, LaBelle E, and Boesze-Battaglia K (2007) A novel mode of action for a microbial-derived immunotoxin: the cytolethal distending toxin subunit B exhibits phosphatidylinositol 3,4,5-triphosphate phosphatase activity. *J Immunol* **178:**5099–5108.
Simonsen A and Tooze SA (2009) Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *J Cell Biol* **186:**773–782.
Sly LM, Rauh MJ, Kalesnikoff J, Song CH, and Krystal G (2004) LPS-induced upregulation of SHIP is essential for endotoxin tolerance. *Immunity* **21:**227–239.
Smith LD, Hickman ES, Parry RV, Westwick J, and Ward SG (2007) PI3Kgamma is the dominant isoform involved in migratory responses of human T lymphocytes: effects of ex vivo maintenance and limitations of non-viral delivery of siRNA. *Cell Signal* **19:**2528–2539.
Srivastava S, Di L, Zhdanova O, Li Z, Vardhana S, Wan Q, Yan Y, Varma R, Backer J, Wulff H, et al. (2009) The class II phosphatidylinositol 3 kinase C2beta is required for the activation of the K+ channel KCa3.1 and CD4 T-cells. *Mol Biol Cell* **20:**3783–3791.
Stenton GR, Ogden N, Roberts H, Cross JL, Mackenzie LF, and Szabo C (2011) AQX-1125, a modulator of the SHIP1/PI3K pathway, suppresses chemotaxis and inflammation (Abstract 149). Keystone Symposia on Molecular and Cellular Biology: PI 3-Kinase Signaling Pathways; 2011 Feb 13–18; Keystone, CO. Keystone Symposia, Silverthorne, CO.
Straub A, Wendel HP, Dietz K, Schiebold D, Peter K, Schoenwaelder SM, and Ziemer G (2008) Selective inhibition of the platelet phosphoinositide 3-kinase p110beta as promising new strategy for platelet protection during extracorporeal circulation. *Thromb Haemost* **99:**609–615.
Suh CI, Stull ND, Li XJ, Tian W, Price MO, Grinstein S, Yaffe MB, Atkinson S, and Dinauer MC (2006) The phosphoinositide-binding protein p40phox activates the NADPH oxidase during FcgammaIIA receptor-induced phagocytosis. *J Exp Med* **203:**1915–1925.
Sutherlin DP, Bao L, Berry M, Castanedo G, Chuckowree I, Dotson J, Folks A, Friedman L, Goldsmith R, Gunzner J, et al. (2011) Discovery of a potent, selective, and orally available class I phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) kinase inhibitor (GDC-0980) for the treatment of cancer. *J Med Chem* **54:**7579–7587.
Suwa A, Yamamoto T, Sawada A, Minoura K, Hosogai N, Tahara A, Kurama T, Shimokawa T, and Aramori I (2009) Discovery and functional characterization of a novel small molecule inhibitor of the intracellular phosphatase, SHIP2. *Br J Pharmacol* **158:**879–887.
Suárez-Fueyo A, Barber DF, Martínez-Ara J, Zea-Mendoza AC, and Carrera AC (2011) Enhanced phosphoinositide 3-kinase δ activity is a frequent event in systemic lupus erythematosus that confers resistance to activation-induced T cell death. *J Immunol* **187:**2376–2385.
Takeda M, Ito W, Tanabe M, Ueki S, Kato H, Kihara J, Tanigai T, Chiba T, Yamaguchi K, Kayaba H, et al. (2009) Allergic airway hyperresponsiveness, inflammation, and remodeling do not develop in phosphoinositide 3-kinase gamma-deficient mice. *J Allergy Clin Immunol* **123:**805–812.
Tan S, Ng Y, and James DE (2011) Next-generation Akt inhibitors provide greater specificity: effects on glucose metabolism in adipocytes. *Biochem J* **435:**539–544.
Tarasenko T, Kole HK, Chi AW, Mentink-Kane MM, Wynn TA, and Bolland S (2007) T cell-specific deletion of the inositol phosphatase SHIP reveals its role in regulating Th1/Th2 and cytotoxic responses. *Proc Natl Acad Sci USA* **104:**11382–11387.
Tassi I, Cella M, Gilfillan S, Turnbull I, Diacovo TG, Penninger JM, and Colonna M (2007) p110gamma and p110delta phosphoinositide 3-kinase signaling pathways synergize to control development and functions of murine NK cells. *Immunity* **27:**214–227.
Terashima Y, Onai N, Murai M, Enomoto M, Poonpiriya V, Hamada T, Motomura K, Suwa M, Ezaki T, Haga T, et al. (2005) Pivotal function for cytoplasmic protein FROUNT in CCR2-mediated monocyte chemotaxis. *Nat Immunol* **6:**827–835.
Thomas MJ, Smith A, Head DH, Milne L, Nicholls A, Pearce W, Vanhaesebroeck B, Wymann MP, Hirsch E, Trifilieff A, et al. (2005) Airway inflammation: chemokine-induced neutrophilia and the class I phosphoinositide 3-kinases. *Eur J Immunol* **35:**1283–1291.
Thomas MS, Mitchell JS, DeNucci CC, Martin AL, and Shimizu Y (2008) The p110gamma isoform of phosphatidylinositol 3-kinase regulates migration of effector CD4 T lymphocytes into peripheral inflammatory sites. *J Leukoc Biol* **84:**814–823.
Thomas M, Edwards MJ, Sawicka E, Duggan N, Hirsch E, Wymann MP, Owen C, Trifilieff A, Walker C, Westwick J, et al. (2009) Essential role of phosphoinositide 3-kinase gamma in eosinophil chemotaxis within acute pulmonary inflammation. *Immunology* **126:**413–422.
Thomas RK, Re D, Wolf J, and Diehl V (2004) Part I: Hodgkin's lymphoma–molecular biology of Hodgkin and Reed-Sternberg cells. *Lancet Oncol* **5:**11–18.
Thomson AW, Turnquist HR, and Raimondi G (2009) Immunoregulatory functions of mTOR inhibition. *Nat Rev Immunol* **9:**324–337.
Toda E, Terashima Y, Sato T, Hirose K, Kanegasaki S, and Matsushima K (2009) FROUNT is a common regulator of CCR2 and CCR5 signaling to control directional migration. *J Immunol* **183:**6387–6394.
Toyama S, Tamura N, Haruta K, Karakida T, Mori S, Watanabe T, Yamori T, and Takasaki Y (2010) Inhibitory effects of ZSTK474, a novel phosphoinositide 3-kinase inhibitor, on osteoclasts and collagen-induced arthritis in mice. *Arthritis Res Ther* **12:**R92.
Turner SJ, Domin J, Waterfield MD, Ward SG, and Westwick J (1998) The CC chemokine monocyte chemotactic peptide-1 activates both the class I p85/p110 phosphatidylinositol 3-kinase and the class II PI3K-C2alpha. *J Biol Chem* **273:**25987–25995.
Vandeput F, Combettes L, Mills SJ, Backers K, Wohlkönig A, Parys JB, De Smedt H, Missiaen L, Dupont G, Potter BV, et al. (2007) Biphenyl 2,3′,4,5′,6-pentakisphosphate, a novel inositol polyphosphate surrogate, modulates Ca^{2+} responses in rat hepatocytes. *FASEB J* **21:**1481–1491.
Vanhaesebroeck B, Ali K, Bilancio A, Geering B, and Foukas LC (2005) Signalling by PI3K isoforms: insights from gene-targeted mice. *Trends Biochem Sci* **30:**194–204.
Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, and Bilanges B (2010) The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol* **11:**329–341.
Veerasingham SJ, Yamazato M, Berecek KH, Wyss JM, and Raizada MK (2005) Increased PI3-kinase in presympathetic brain areas of the spontaneously hypertensive rat. *Circ Res* **96:**277–279.
Venkatesan AM, Dehnhardt CM, Delos Santos E, Chen Z, Dos Santos O, Ayral-Kaloustian S, Khafizova G, Brooijmans N, Mallon R, Hollander I, et al. (2010) Bis(morpholino-1,3,5-triazine) derivatives: potent adenosine 5′-triphosphate competitive phosphatidylinositol-3-kinase/mammalian target of rapamycin inhibitors: discovery of compound 26 (PKI-587), a highly efficacious dual inhibitor. *J Med Chem* **53:**2636–2645.
Vieira OV, Botelho RJ, Rameh L, Brachmann SM, Matsuo T, Davidson HW, Schreiber A, Backer JM, Cantley LC, and Grinstein S (2001) Distinct roles of class I and class III phosphatidylinositol 3-kinases in phagosome formation and maturation. *J Cell Biol* **155:**19–25.
Vlahos CJ, Matter WF, Hui KY, and Brown RF (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4- morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (LY294002). *J Biol Chem* **269:**5241–5248.
Vonakis BM, Vasagar K, Gibbons SP Jr, Gober L, Sterba PM, Chang H, and Saini SS (2007) Basophil FcepsilonRI histamine release parallels expression of Src-homology 2-containing inositol phosphatases in chronic idiopathic urticaria. *J Allergy Clin Immunol* **119:**441–448.
Wakeland EK, Wandstrat AE, Liu K, and Morel L (1999) Genetic dissection of systemic lupus erythematosus. *Curr Opin Immunol* **11:**701–707.
Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, and Williams RL (2000) Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol Cell* **6:**909–919.
Walsh CM and Edinger AL (2010) The complex interplay between autophagy, apoptosis, and necrotic signals promotes T-cell homeostasis. *Immunol Rev* **236:**95–109.
Wang JW, Howson JM, Ghansah T, Desponts C, Ninos JM, May SL, Nguyen KH, Toyama-Sorimachi N, and Kerr WG (2002) Influence of SHIP on the NK repertoire and allogeneic bone marrow transplantation. *Science* **295:**2094–2097.
Ward SG and Marelli-Berg FM (2009) Mechanisms of chemokine and antigen-dependent T-lymphocyte navigation. *Biochem J* **418:**13–27.
Waugh C, Sinclair L, Finlay D, Bayascas JR, and Cantrell D (2009) Phosphoinositide (3,4,5)-triphosphate binding to phosphoinositide-dependent kinase 1 regulates a

protein kinase B/Akt signaling threshold that dictates T-cell migration, not proliferation. *Mol Cell Biol* **29:**5952–5962.
Weaver CT and Murphy KM (2007) The central role of the Th17 lineage in regulating the inflammatory/autoimmune axis. *Semin Immunol* **19:**351–352.
Wei X, Han J, Chen ZZ, Qi BW, Wang GC, Ma YH, Zheng H, Luo YF, Wei YQ, and Chen LJ (2010) A phosphoinositide 3-kinase-gamma inhibitor, AS605240 prevents bleomycin-induced pulmonary fibrosis in rats. *Biochem Biophys Res Commun* **397:**311–317.
Weichhart T and Säemann MD (2009) The multiple facets of mTOR in immunity. *Trends Immunol* **30:**218–226.
Wheeler M and Domin J (2001) Recruitment of the class II phosphoinositide 3-kinase C2beta to the epidermal growth factor receptor: role of Grb2. *Mol Cell Biol* **21:**6660–6667.
Whitman M, Downes CP, Keeler M, Keller T, and Cantley L (1988) Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* **332:**644–646.
Wiesinger D, Gubler HU, Haefliger W, and Hauser D (1974) Antiinflammatory activity of the new mould metabolite 11-desacetoxy-wortmannin and of some of its derivatives. *Experientia* **30:**135–136.
Willox I, Mirkina I, Westwick J, and Ward SG (2010) Evidence for PI3K-dependent CXCR3 agonist-induced degranulation of human cord blood-derived mast cells. *Mol Immunol* **47:**2367–2377.
Wymann MP, Bulgarelli-Leva G, Zvelebil MJ, Pirola L, Vanhaesebroeck B, Waterfield MD, and Panayotou G (1996) Wortmannin inactivates phosphoinositide 3-kinase by covalent modification of Lys-802, a residue involved in the phosphate transfer reaction. *Mol Cell Biol* **16:**1722–1733.
Yaguchi S, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, Hirono S, Yamazaki K, and Yamori T (2006) Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. *Journal of the National Cancer Institute* **98:**545–556.
Yang L, Williams DE, Mui A, Ong C, Krystal G, van Soest R, and Andersen RJ (2005) Synthesis of pelorol and analogues: activators of the inositol 5-phosphatase SHIP. *Org Lett* **7:**1073–1076.
Yap TA, Garrett MD, Walton MI, Raynaud F, de Bono JS, and Workman P (2008) Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. *Curr Opin Pharmacol* **8:**393–412.
Yoon MS, Du G, Backer JM, Frohman MA, and Chen J (2011) Class III PI-3-kinase activates phospholipase D in an amino acid-sensing mTORC1 pathway. *J Cell Biol* **195:**435–447.
Yuan TL and Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. *Oncogene* **27:**5497–5510.
Yum HK, Arcaroli J, Kupfner J, Shenkar R, Penninger JM, Sasaki T, Yang KY, Park JS, and Abraham E (2001) Involvement of phosphoinositide 3-kinases in neutrophil activation and the development of acute lung injury. *J Immunol* **167:**6601–6608.
Zask A, Verheijen JC, and Richard DJ (2011) Recent advances in the discovery of small-molecule ATP competitive mTOR inhibitors: a patent review. *Expert Opin Ther Pat* **21:**1109–1127.
Zebedin E, Simma O, Schuster C, Putz EM, Fajmann S, Warsch W, Eckelhart E, Stoiber D, Weisz E, Schmid JA, et al. (2008) Leukemic challenge unmasks a requirement for PI3Kdelta in NK cell-mediated tumor surveillance. *Blood* **112:**4655–4664.
Zhang TT, Okkenhaug K, Nashed BF, Puri KD, Knight ZA, Shokat KM, Vanhaesebroeck B, and Marshall AJ (2008) Genetic or pharmaceutical blockade of p110delta phosphoinositide 3-kinase enhances IgE production. *J Allergy Clin Immunol* **122:**811–819.e2.