TRANSLATIONAL REVIEW

Phosphoinositide 3-Kinase in Asthma: Novel Roles and Therapeutic Approaches

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Abstract

Asthma manifests as airway hyperresponsiveness and inflammation, including coughing, wheezing, and shortness of breath. Immune cells and airway structural cells orchestrate asthma pathophysiology, leading to mucus secretion, airway narrowing, and obstruction. Phosphoinositide 3-kinase, a lipid kinase, plays a crucial role in many of the cellular and molecular mechanisms driving asthma pathophysiology and represents an attractive therapeutic target. Here, we summarize the diverse roles of phosphoinositide 3-kinase in the pathogenesis of asthma and discuss novel therapeutic approaches to treatment.

Keywords: phosphoinositide 3-kinase; asthma; bronchodilator; inflammation

Asthma, characterized by airway hyperresponsiveness (AHR) and inflammation, manifests as wheezing, coughing, and shortness of breath (1, 2). Exposure to allergen, in part, drives the release of inflammatory mediators from immune cells, inducing mucus secretion, airway narrowing, and obstruction (3). These pathological responses are mediated by airway structural cells, including airway epithelial cells and airway smooth muscle (ASM) cells (4–6). Phosphoinositide 3-kinase (PI3K), a multifunctional lipid kinase, is central to the development of AHR and inflammation, and plays crucial roles in nearly all aspects of asthma pathogenesis. The centrality of PI3K to asthma pathogenesis makes PI3K an attractive therapeutic target. In this article, we review the diverse roles of PI3K in the pathogenesis of asthma, and discuss novel therapeutic approaches to treatment.

PI3K Activation and Function

Initially discovered in studies of carcinogenesis, PI3K mediates cell functions, including proliferation, metabolism, and motility—all of which are important to cancer progression (7). Other studies determined that PI3K plays a role in a myriad of diseases.

PI3K phosphorylates the D-3 position of the plasma membrane lipid phosphatidylinositol-4-5-bisphosphate, generating phosphatidylinositol-3-4-5-trisphosphate (PI[3,4,5]P_3) (8, 9). Cytosolic signaling proteins with pleckstrin homology domains accumulate at sites of PI3K activation by direct binding to PI(3,4,5)P_3. Proteins with pleckstrin homology domains include protein kinases, scaffolding proteins, and guanine exchange factors (GEFs), all of which will become activated and initiate signaling cascades (10–13). Notable effectors of PI3K signaling include protein serine/threonine kinases, Akt and phosphoinositide-dependent kinase (PDK) 1, as shown in Figure 1. Binding of Akt and PDK1 to PI(3,4,5)P_3 brings these proteins into proximity, inducing phosphorylation of Akt by PDK1. Activation of Akt by PDK1 activates mammalian target of rapamycin (mTOR) complex 1 and a host of other proteins, affecting cell growth and proliferation (14).

Signaling of the PI3K pathway terminates by dephosphorylation of PI(3,4,5)P_3 by the enzyme, phosphatase and tensin homolog (PTEN), the main endogenous PI3K inhibitor (15). PTEN is a tumor suppressor and is important in many cancers. The PI3K pathway is also

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Clinical Relevance

Ground-breaking studies that demonstrate the potential of phosphoinositide 3-kinase (PI3K) inhibitors for asthma treatment have recently emerged, namely, that PI3K inhibitors can bronchodilate. With the recent U.S. Food and Drug Administration approval of PI3K inhibitors for certain malignancies, the potential for repositioning PI3K inhibitors for asthma is more compelling than ever. This review illuminates the multifaceted roles of PI3K in asthma pathogenesis and the clinical potential of isoform-selective inhibitors in asthma.

inactivated by Src-homology domain 2–containing phosphatases SH2 domain-containing inositol phosphatase 1 and 2, found primarily in blood cells (16).

PI3Ks consist of heterodimers composed of a p110 catalytic subunit and a regulatory subunit. The PI3K family is divided into three classes (classes I, II, and III) based on structural and functional characteristics, as shown in Figure 1. Further divided into subclasses based on their p110 catalytic subunit, class IA PI3Ks are composed of p110α, p110β, and p110δ isoforms, whereas class IB contains solely the p110γ isoform. Class IA PI3Ks contain p85, p55, or p50 phosphotyrosine-binding regulatory subunits. Class IB PI3Ks contain p101 or p84/p87 regulatory subunits, which allow activation by the βγ subunits of G protein–coupled receptors (GPCRs) (17). Class II PI3Ks consist of three isoforms (C2α, C2β, and C2γ) that are ubiquitously expressed (18, 19). Class III PI3K includes only one member, vacuolar protein-sorting mutant 34, that regulates endocytosis, Toll-like receptor (TLR) signaling, and vesicular trafficking (20). Classes II and III are not well studied, due to the lack of highly selective pharmacological inhibitors. Class I is the most thoroughly studied PI3K family, and is the focus of this review.

Functions of Class I PI3K

Due to the limitations of selective inhibition strategies, the study of class I PI3K family members remains challenging (21). Unlike the p110δ and p110γ subunits, the p110α and p110β subunits are required for cell proliferation and embryonic development (22). Consequently, *in vivo* experiments requiring genetic knockdown of p110α and p110β pose obstacles.

The p110δ and p110γ isoforms are predominantly, but not exclusively, expressed in leukocytes, and play important roles in innate and adaptive immune responses (23). Activated TLRs in leukocytes recruit p110δ and p110γ and initiate signaling (24). In T cells, p110δ and p110γ negatively regulate TLR-induced IL-12 and IFN-γ production, facilitating Th1 responses and diminishing Th2 responses (25). p110δ knockout mice and mice expressing inactive p110δ catalytic subunit demonstrate impaired CD28-costimulated clonal expansion and differentiation, highlighting the necessity of p110δ in T cell activation (26, 27). Interestingly, patients with activated PI3K Δ syndrome manifest increased susceptibility to airway infections, bronchiectasis, and lymphoproliferation (28). p110γ-deficient mice display reduced thymocyte survival and T cell maturation, with a significant decrease in $CD4^+$ cells (29). PI3K also mediates B cell function, as p110δ knockout mice display impaired B cell receptor–mediated antigen presentation (30). In neutrophils, PI3K regulates nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase and is important for reactive oxygen species generation. Stimulation of numerous neutrophil receptors, including GPCRs, cytokine receptors, integrin receptors, and Fc receptors, activates GEFs. GEFs, such as phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 1 protein and cytohesin4, mediate chemotaxis, vesicle trafficking, degranulation, and NADPH oxidase activation (31).

PI3K serves to promote immune cell survival by modulating antiapoptosis signaling. The PI3K/AKT pathway inhibits proapoptotic proteins, including B cell lymphoma 2 and associated proteins. PI3K signaling also facilitates activation of prosurvival proteins, including B-cell lymphoma-extra large, induced myeloid leukemia cell differentiation protein, and NF-κB (32).

Collectively, these studies demonstrate that p110δ and p110γ are integral to the orchestration of both the innate and adaptive immune responses, including leukocyte migration, activation, B cell and T cell maturation, neutrophil NADPH oxidase activation, and antigen response.

PI3K and Asthma

Atopic asthma manifests when T cells mature into a Th2 subtype upon allergen exposure and release mediators that activate other immune cells, such as mast cells, granulocytes, and B cells. Activated immune cells then elicit responses from structural cells, such as ASM and airway epithelial cells, which culminate in AHR, inflammation, and remodeling. PI3Ks play important roles in the responses of airway immune cells and structural cells that mediate these pathophysiological processes.

The importance of PI3K in asthma is demonstrated by *in vivo* experiments that show that PI3K inhibitors prevent pathogenesis of allergen-induced AHR and inflammation (25, 33). IC87114, a p110δ-selective inhibitor, attenuated allergic airway inflammation and AHR in a murine model (34). p110δ also mediates lung inflammation induced by *Aspergillus fumigatus* via a mechanism involving endoplasmic reticulum stress (35). In addition, allergen-induced AHR does not develop in p110γ-deficient mice (36). Taken together, these experiments suggest that PI3K is necessary for the development of asthma.

PI3K and Asthma: Structural Cells

Structural cells, including ASM and epithelial cells, are the main effector cells of inflammatory mediators released during asthma. ASM cells proliferate and shorten upon exposure to inflammatory mediators, inducing airway remodeling and obstruction (6). Epithelial cells recruit eosinophils by releasing eotaxin. Eosinophils subsequently release major basic protein, inducing epithelial damage (4). PI3Ks play an important role in mediating both ASM and epithelial cell responses.

ASM, the pivotal cell type mediating AHR, is the primary target for bronchodilation, a major therapeutic strategy. In asthma, ASM maintains airway tone, secretes inflammatory mediators, and undergoes hypertrophy and hyperplasia. ASM shortening occurs upon agonist binding to a GPCR, resulting in an elevation of intracellular calcium, myosin light-chain (MLC)

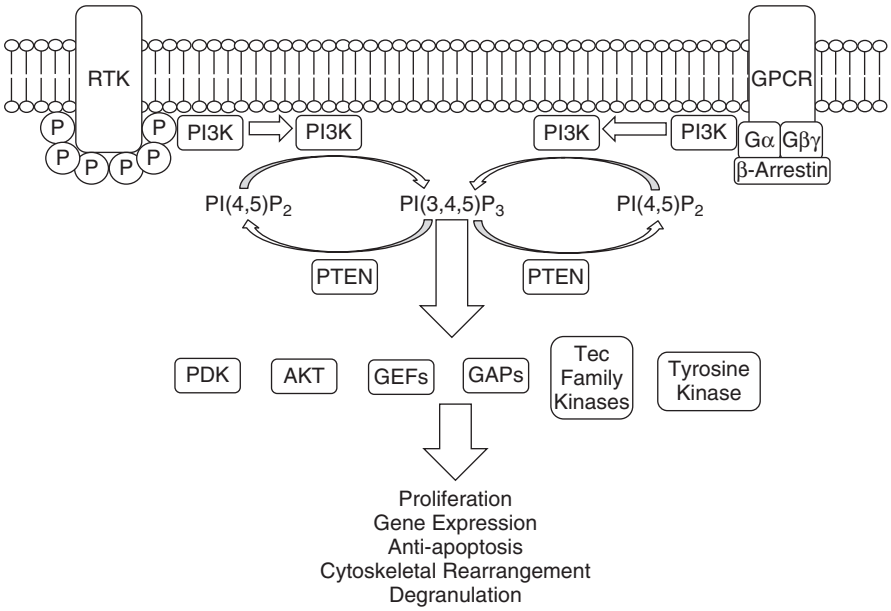

Figure 1. Class I phosphoinositide 3-kinase (PI3K) signaling. Class I PI3Ks are activated upon agonist binding to receptor tyrosine kinases (RTK) or G protein–coupled receptors (GPCRs). RTKs will recruit PI3Ks to phosphorylated tyrosine residues, resulting in activation of PI3Ks. GPCRs can activate PI3Ks via G proteins, such as Gα or Gβγ, and through β-arrestins. PI3K phosphorylates the D-3 position of the plasma membrane lipid phosphatidylinositol-4-5-bisphosphate (PI[4,5]P_2), generating PI(3,4,5)P_3. PI(3,4,5)P_3 will recruit proteins with pleckstrin homology domains, which include phosphoinositide-dependent kinase (PDK) 1, AKT kinase (AKT), guanine exchange factors (GEFs), GTPase accelerating proteins (GAPs), Tec family kinases, and nonreceptor tyrosine kinases. These signaling proteins will subsequently affect cellular functions, including proliferation, gene expression, antiapoptosis, cytoskeletal rearrangement, and degranulation. PTEN, phosphatase and tensin homolog.

phosphorylation, and actin–myosin cross-bridge cycling, via the canonical inositol trisphosphate and calmodulin–mediated pathway. In parallel, inhibition of MLC phosphatase by Rho kinase sustains MLC phosphorylation and maintains ASM tone.

PI3K activation is necessary for the modulation of ASM contraction and the accumulation of contractile proteins (37, 38). Importantly, PI3K contributes to airway tone via its regulation of Rho kinase. In human *ex vivo* small airways contracted to agonist, PI3K inhibitors evoke bronchodilation (39). p110δ and p110γ subunits are required for the development of AHR in mice (34, 40, 41). Furthermore, cytokine-mediated induction of CD38, a calcium signaling protein important to the development of AHR, was impaired after treatment with PI3K inhibitors (42). P110γ activity was found to be elevated in ASM derived from subjects with asthma, and was also important for β-2 adrenergic receptor resensitization (43). These studies highlight the importance of PI3K in ASM contraction and AHR development.

ASM also secretes chemokines and cytokines, such as IL-6, vascular endothelial growth factor (VEGF), and CXCL-8, which contribute to the recruitment of immune cells in asthma. PI3K is necessary for IL-6 secretion induced by transforming growth factor-β, a cytokine important to airway remodeling and AHR development (44). Under mechanical strain, ASM signals through PI3K/Akt/mTOR and extracellular signal-regulated kinase pathways, inducing hypoxia-inducible factor, a transcription factor required for VEGF expression (45). VEGF release by mechanical strain of human airway smooth muscle may account for the angiogenesis seen after repeated asthma exacerbations (45). In addition, PI3K and mitogen-activated protein kinase pathways regulate the synergy of IL-17 and IL-1β to enhance CXCL-8 expression (46). Taken together, these studies highlight the importance of PI3K to ASM chemokine and cytokine secretion.

In addition to ASM contraction and mediator secretion, PI3K plays an important role in airway remodeling. Airway remodeling refers to the structural changes that occur during asthma. ASM undergoes hyperplasia and hypertrophy to increase ASM mass. Th2 cytokines modulate airway contraction by secreting matrix metallopeptidase-1 from the ASM cells via PI3K activation (47). PI3K is also required for growth factor–induced cell migration (48–51). Furthermore, activation of class IA PI3K is sufficient to stimulate DNA synthesis and growth, which promote airway remodeling (52).

ASM derived from subjects with asthma display increased proliferation, responsiveness to contractile agonist, and mediator release, suggesting an epigenetic alteration. Small RNAs are essential to the establishment of an epigenetic signature, and profiling of small RNAs suggests that the PI3K pathway is enhanced in bronchial smooth muscle cells from patients with asthma (53).

The airway epithelium, considered an essential modulator of inflammation, lies at the interface between the host and the environment. The epithelium represents the first line of defense against microorganisms, toxicants, and allergens, and expresses many pattern recognition receptors to rapidly detect and respond to pathogen-associated molecular patterns found in microbes or to damage-associated molecular patterns released upon tissue damage, cell death, or cellular stress. Activation of epithelial pattern recognition receptors releases cytokines, chemokines, and antimicrobial peptides, which attracts and activates innate and adaptive immune cells. Studies show that endothelial cell activation is a key triggering event in the recognition of inhaled allergens that activates the local network of dendritic cells that coordinate the subsequent immune response. PI3K mediates epithelial responses to environmental stimuli. PI3K is important during viral-induced asthma exacerbations, due to its importance in virus internalization (54, 55). In an allergen-induced model of asthma, PI3K activation is increased and PTEN activation is decreased in the airway epithelium. PTEN protein expression and PTEN activity were also decreased in epithelial cells of mice exposed to allergen. Immunoreactive PTEN localized in epithelial layers around the bronchioles of control mice; PTEN rapidly disappeared in allergen-exposed lungs of mice, suggesting that the PI3K/PTEN

pathway modulates epithelial cell function in asthma (56).

PI3K also facilitates mediator release from epithelial cells (57, 58). PI3K inhibition prevents expression of IFN-γ–induced protein 10, a mediator released during virus-induced asthma exacerbations (59). PI3K also modulates inducible nitric oxide synthase and nitric oxide signaling in the airway epithelium, promoting the development of airway inflammation (59, 60). P110δ inhibition attenuates antigen-induced airway inflammation and hyperresponsiveness through the modulation VEGF-induced vascular leakage (61). PI3K inhibition also reduces the mucus hypersecretory phenotype and goblet cell metaplasia induced by IL-13, an important cytokine that is associated with asthma (62).

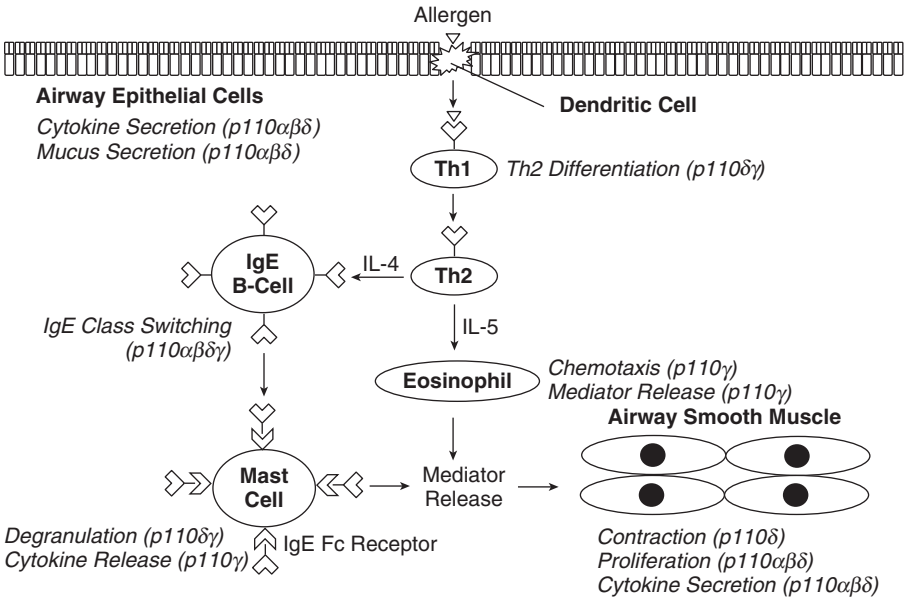

Figure 2. Functions of class I PI3K isoforms in cells mediating asthma pathophysiology. A schematic representation of the roles of class I PI3K isoforms in various cell types involved in asthma pathophysiology. Th1, T-helper cell type 1; Th2, T-helper cell type 2.

PI3K and Asthma: Immune Cells

Allergen exposure induces Th2 differentiation of T cells that, in turn, secrete cytokines that promote allergic inflammation. Th2 cytokines stimulate B cells to produce IgE and other antibodies. These cytokines include IL-4, which stimulates the production of IgE, IL-5, which activates locally recruited eosinophils, and IL-13, which stimulates mucus secretion from bronchial submucosal glands and promotes IgE production by B cells. As in other hypersensitivity reactions, IgE coats submucosal mast cells, and repeated exposure to the allergen triggers the mast cells to release granule contents and produce cytokines and other mediators, which collectively induce an asthma phenotype.

T cells, in part, promote the development of asthma, secreting cytokines that are sufficient to induce AHR and B cell class switching to IgE. Class I PI3Ks play roles in T cell activation, differentiation, and proliferation, with p110δ and p110γ being the main contributors, as shown in Figure 2. Upon activation by antigen-presenting cells, naive $CD4^+$ cells will proliferate and differentiate to various T helper effector subsets, including the Th2 subtype. Class I PI3Ks have important roles in T cell functions. T cell receptor engagement by antigen activates p110δ through tyrosine kinase signaling cascades (63). p110γ is recruited by G protein activation through GPCRs, which include chemokine receptors (64). Mice lacking both p110δ and p110γ are markedly impaired in T cell dFB CELLSevelopment, and are unable to rearrange the T cell receptor α and β chains (65). In naive $CD4^+$ T cells, evidence suggests that class I PI3Ks are activated upon engagement by antigen-presenting cells (66). PI3Ks are required for the differentiation of Th effector subsets, with Th2 differentiation being necessary for the development of atopic asthma (27).

B cells secrete IgE into circulation, which will then bind to high-affinity high-affinity IgE receptors on the surface of mast cells. PI3K negatively regulates IgE expression and IgE cell surface receptor expression levels. Blockade of PI3K signaling markedly enhanced B cell IgE class switching and increased IgE levels *in vivo*, despite reduced type 2 cytokine production (67, 68).

Mast cells, the central effector cell in allergic diseases, are present in increased numbers in the airways of patients with asthma. Binding of allergen to IgE on the cell surface induces a signal transduction cascade that releases mediators, such as histamine and prostaglandin D2. The release of histamine and prostaglandin D2 evokes bronchoconstriction. IgE-mediated mast cell degranulation is augmented by PI3K (69). In addition, PI3K was found to mediate VEGF release in murine models, which, in turn, promotes vascular leakage in asthma (70).

Increased numbers of eosinophils are also present in the airways of some patients with asthma. The recruitment, growth, and survival of eosinophils are promoted by factors released from airway epithelial cells, Th2 cells, and mast cells. Eosinophils express a variety of proinflammatory cytokines, Th2 cytokines, and chemokines that can activate mast cells and stimulate the epithelium. Eosinophils can also present antigen to T cells, and release growth factors, such as transforming growth factor-β, promoting inflammation in asthma. Inhibition of PI3K blunts eosinophil chemotaxis, a function important in asthma (71, 72). In addition, PI3K induces mediator release in allergic asthma, irrespective of allergen challenge model (73). Eosinophils derived from subjects with atopic asthma have elevated PI3K activation, suggesting that PI3K may be an important mediator of eosinophil function in asthma (74).

Neutrophils are present in some, but not all, patients with asthma. Neutrophilic migration and activation releases mediators that contribute to asthma exacerbations.

PI3K is necessary for neutrophil migration and degranulation in asthma (73, 75).

PI3K Inhibition as a Potential Therapeutic Strategy for Asthma

PI3K has gathered much attention as a potential therapeutic target in asthma, as outlined in Table 1 (76). Pan inhibitors of class I PI3K (wortmannin and LY294002) have toxicity profiles and unfavorable pharmacokinetic characteristics that preclude their clinical use (77). The focus, therefore, has shifted to isoform-selective PI3K inhibitors. PI3Kα inhibitors have been studied in the treatment of solid tumors, but have not been approved for clinical use, as shown in Table 2 (78). PI3Kδ and PI3Kγ, as well as dual inhibitors, are promising candidates. PI3Kγ inhibitors exhibit anti-inflammatory properties, but none have been approved for clinical use. Idelalisib, a PI3Kδ inhibitor, has been approved for the treatment of multiple hematologic malignancies.

Side effects, such as leukopenia, colitis, and skin rashes, have occurred upon treatment with idelalisib (79). Given the anti-inflammatory and bronchodilator effects, PI3Kδ inhibition may be a promising strategy for clinical development, especially with inhaled formulations to minimize side effects. Dual PI3Kδ/PI3Kγ inhibitors have also been effective in mitigating inflammatory disorders in animal models, and may be an alternate approach (77). IPI-145 (duvelisib) and RV-1729 are PI3Kδ/PI3Kγ combination inhibitors that are being developed for clinical use (75, 80). GSK2269557, an inhaled PI3Kδ inhibitor, is being developed for severe asthma (80). TG100-115, another PI3Kδ/PI3Kγ combination inhibitor, has been effective in allergen-induced asthma models (81).

AKT and mTOR complex 1 are activated downstream of PI3K, and oral inhibitors, including miltefosine and rapamycin, have been developed for clinical use. None of these drugs have been tested for use in airway diseases. Rapamycin has shown promise in inhibiting mTOR activation in chronic obstructive pulmonary disease (82), and inhibits allergic inflammation in allergen-challenged mice, while inhibiting eosinophil differentiation (83, 84).

Although promising, PI3K inhibition as a therapeutic strategy is not without challenges. Problems include lack of specificity, adverse effects, and loss of efficacy. PI3K inhibitors, like most kinase inhibitors, target the ATP-binding pocket. Adequate inhibitor potency must be achieved to compete with ATP for binding. The structural similarity of the ATP-binding pocket in all kinases makes specific targeting difficult. Having an improved understanding of the ATP-binding pocket structure may target the development of inhibitors with improved specificity and potency. Adverse effects occur due to the myriad important functions of PI3K in other tissues. Inhaled delivery systems can promote topical deposition, while minimizing system effects. Development of inhaled PI3K inhibitors with adequate potency has been difficult, but the many preclinical studies and

Table 1. Characteristics of Phosphoinositide 3-Kinase in Asthma

Cell/Animal	Class I PI3K Subunits	Role in Asthma	References
Mice	p110δ	AHR	25, 33
	p110δ	Inflammation	35
	p110γ	Inflammation	36
	p110γ	Remodeling	36
Airway smooth muscle	p110δ, p110γ	Contraction	37–42
	p110α, p110β, p110δ	Proliferation	18
	p110α, p110δ	Gene Expression	42
	p110γ	β-2 Adrenergic Receptor resensitization	43
	p110δ	IL-6 secretion	44
	p110α, p110β, p110δ	VEGF expression	45
	p110α, p110β, p110δ	CXCL-8 expression	46
	p110α, p110β, p110δ	MMP-1 secretion	47
	p110α, p110β, p110δ	Proliferation	48–52
Airway epithelial cells	p110α, p110β, p110δ	IP-10 secretion	59
	p110α, p110β, p110δ	Nitric oxide signaling	59, 60
	p110α, p110β, p110δ	Mucus secretion	62
T cells	p110δ, p110γ	Th2 differentiation	27, 63–66
B cells	p110α, p110β, p110δ, p110γ	IgE class switching	67, 68
Mast cells	p110δ	Degranulation	69
	p110γ	Cytokine release	70
Eosinophils	p110γ	Chemotaxis	71, 72
	p110γ	Mediator release	73
Neutrophils	p110δ, p110γ	Migration	75
	p110α, p110β, p110δ, p110γ	Degranulation	73

Definition of abbreviations: AHR, airway hyperresponsiveness; CXCL-8, C-X-C motif chemokine ligand 8; IP-10, IFN-γ–induced protein 10; MMP-1, matrix metallopeptidase 1; P110α, phosphatidylinositol 3-kinase catalytic subunit α isoform; P110β, phosphatidylinositol 3-kinase catalytic subunit β isoform; P110δ, phosphatidylinositol 3-kinase catalytic subunit δ isoform; P110γ, phosphatidylinositol 3-kinase catalytic subunit γ isoform; PI3K, phosphoinositide 3-kinase; Th2, T-helper cell type 2; VEGF, vascular endothelial growth factor.

Table 2. Phosphoinositide 3-Kinase Inhibitors in Clinical Trials for Asthma and Chronic Obstructive Pulmonary Disease

Compound	Target	Indication	Clinical Trial Identifier
Idelalisib (CAL-101)	p110δ	Chronic lymphocytic leukemia	FDA and EMA approved, 2014
GSK2269557	p110δ	Asthma	NCT01462617, Phase 1
IPI-145	p110δ/p110γ	Asthma	NCT01653756, Phase 2
RV-1729	p110δ/p110γ	Asthma/COPD	NCT01813084, Phase 1
AQX-1125	SHIP1 activator	COPD	NCT01954628, Phase 2

Definition of abbreviations: COPD, chronic obstructive pulmonary disease; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; P110δ, phosphatidylinositol 3-kinase catalytic subunit δ isoform; P110γ, phosphatidylinositol 3-kinase catalytic subunit γ isoform; SHIP, SH2 domain-containing inositol phosphatase.

early clinical trials provide hope for future success of PI3K inhibition in airway diseases.

Conclusions

Asthma represents a syndrome that manifests as immune cell activation, inflammatory mediator release, and development of airway obstruction. PI3K, a crucial signaling molecule, plays a role in nearly all aspects of asthma pathophysiology. Inhibition of PI3K blunts mucus production, prevents mast cell degranulation, deters immune cell recruitment, and facilitates bronchodilation, all of which are therapeutically beneficial. Accordingly, PI3K represents an attractive target for the treatment of asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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