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PI3K Inhibitors As Potential Therapeutics for Autoimmune Disease

Jennifer Ball*, Sophie Archer* and Stephen Ward

*Department of Pharmacy & Pharmacology, University of Bath, Claverton
Down, BA2 7AY, UK*

Corresponding Author:

Stephen Ward

Department of Pharmacy & Pharmacology,

University of Bath,

Claverton Down,

BA2 7AY, UK

S.G.Ward @bath.ac.uk

Tel: 01225 383641

* These authors contributed equally to this work

Abstract:

Aberrant over-activation of the immune system can give rise to chronic and persistent self-attack, culminating in autoimmune disease. This is currently managed therapeutically using potent immunosuppression and anti-inflammatories. Phosphoinositide 3-kinase (PI3K) has been identified as an ideal therapeutic target for autoimmune diseases given its wide ranging roles in immunological processes. Recent studies into the function of selective PI3K inhibitors both *in vitro* and *in vivo* have yielded encouraging results, allowing progression into the clinic. Here we review their recent progress across a range of autoimmune diseases.

Introduction:

Autoimmune diseases arise through aberrant activation of T and B lymphocytes towards self-derived antigen, a response normally prevented by immunological tolerance [1,2]. Drug therapy for autoimmune disease was revolutionised with the introduction of biological agents targeting $\text{TNF}\alpha$ (e.g. infliximab) and more recently T lymphocytes (e.g. abatacept) and B lymphocytes (e.g. rituximab). These provide powerful immunosuppression and have surpassed the clinical efficacy of classic disease modifying anti-rheumatic drugs (DMARDs) [3]. However, the concern over adverse effects and high cost often limits the early prescription of biological agent. Furthermore, not all patients are responsive to biological therapy and effectiveness is unpredictable. Finally, biological treatment has not been shown to induce a drug-free remission so continuous treatment is required for therapeutic benefit [4]. Together with the expanding emergence of autoimmune disease in an increasing worldwide population, these factors underline the need for simpler, cost effective small molecule therapeutics. Here, we discuss the evidence that targeting of phosphoinositide 3-kinase (PI3K) with small molecule inhibitors could provide an effective, safe treatment for the control of autoimmune diseases including rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE) and autoimmune diabetes.

Phosphoinositide 3-Kinase Signalling:

Class I PI3K enzymes phosphorylate the D3 position on the inositol ring of phosphatidylinositol 4,5-bisphosphate $[\text{PI}(4,5)\text{P}_2]$ to generate $\text{PI}(3,4,5)\text{P}_3$ which controls a crucial signaling cascade implicated in a plethora of cellular responses (**Figure 1**). The class I PI3K isoforms are composed of a p110 kDa catalytic subunit and a tightly associated regulatory subunit (**Figure 1**). The three class IA p110 catalytic isoforms $\text{PI3K}\alpha$, $\text{PI3K}\beta$ and $\text{PI3K}\delta$ are activated downstream of a variety of receptors that are phosphorylated by tyrosine kinases upon cognate stimulus. The class IB catalytic isoform $\text{PI3K}\gamma$ is activated by G-protein $\beta\gamma$ subunits and signals downstream of G protein-coupled receptors (GPCRs) [5,6]. However recent evidence has suggested there may be greater diversity in receptor coupling to PI3K isoforms than originally thought, with $\text{PI3K}\beta$ also being coupled to GPCRs and $\text{PI3K}\gamma$ being coupled to tyrosine kinase-coupled receptors [7,8]. $\text{PI3K}\alpha$ and $\text{PI3K}\beta$ have a broad tissue distribution, while $\text{PI3K}\delta$ and $\text{PI3K}\gamma$ are predominantly expressed in leukocytes, but can be found in some cancer cells of non-leukocyte origin [5].

PI3K can be activated by 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. Mice in which the genes encoding PI3K γ or PI3K δ have been either ablated or altered to encode kinase-inactive mutants are viable, fertile and apparently healthy. However, when their immune system is challenged, they exhibit severely altered phenotypes demonstrating that PI3K γ and PI3K δ have non-redundant functions in leukocytes, and that the activities of these isoforms in immune cells are crucial during chronic inflammatory diseases [5]. Often these roles are quite distinct, requiring coordinated function of both isoforms at discrete steps of immune cell activation. Although PI3K dependent signalling has been identified as critical for immune function, aberrant overactive PI3K signalling is known to result in immune-related pathologies [9, 10, 11] providing substantial evidence that the inhibition of PI3K could provide a novel method of treating autoimmune disease.

PI3K as a Pharmacological Target:

The first compounds identified to block PI3K, the natural product wortmannin and Eli Lilly's LY294002, have served as useful experimental tools with which to explore the PI3K pathway [10]. X-ray crystallography data of PI3K γ bound to wortmannin and LY294002 helped to understand how these compounds fit into the ATP binding pocket [12] which in turn influenced attempts to design better compounds with increased potency and required selectivity. Furthermore mTOR shares high sequence homology within the ATP-binding pocket with PI3K and compounds originally developed as PI3K inhibitors were later shown to also target mTOR. Pan-PI3K and mTOR inhibitors are under current investigation for the treatment of cancers where the PI3K/Akt/mTOR pathway is central to the transformed phenotype of most cancer cells [10].

Due to their largely restricted expression to hematopoietic cells, the impact of pharmacological targeting of PI3K γ and PI3K δ should be largely restricted to the immune system. 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 difference in potency would be difficult to obtain. However, the discovery of the quinazolinone purine series, exemplified by the ICOS compound IC-87114 indicated that this task was possible [10]. IC-87114 demonstrated an IC₅₀ value of approximately 100 nM for PI3K δ lipid kinase activity while having negligible potency against the PI3K α and PI3K β isoforms. In 2006 several members of ICOS Corporation formed a spin-out company, Calistoga Pharmaceuticals. Calistoga developed CAL-101, a PI3K δ specific inhibitor, which has experienced successful proof of concept in clinical trials for treatment of B cell malignancies (13-16). This compound (recently renamed GS-1101) exhibits 40-300 fold selectivity over other PI3K isoforms (Figure 2). In addition, patents describing PI3K δ inhibitors have been filed by several other companies and the majorities are based on the same basic pharmacophore identified by ICOS [10].

Selective inhibition of PI3K γ has been accomplished in a series of compounds designed by Merck Serono SA based on the thiazolidinedione scaffold. One of these, AS-605240, has demonstrated superior potency for PI3K γ compared to related compounds. In general terms, the level of selectivity of compounds against PI3K γ versus other PI3K isoforms has been largely disappointing. However, using a chemoproteomics-based drug discovery platform, researchers at Cellzome have designed CZC24832 which exhibits superior selectivity for PI3K γ than previously reported compounds [10, 17].

Given the evidence from gene-targeted mice that PI3K γ and PI3K δ often work in concert and can have overlapping roles, dual inhibition of both targets with a single compound has been considered. TargeGen described two diaminopteridine-diphenol-based compounds (TG-101-110, TG-100-115) with good selectivity for both PI3K γ and δ . Infinity and Intellikine have an ongoing phase I trial with IPI-145 (**Figure 2**) and Rhizen Pharmaceuticals have recently announced the initiation of a phase I trial with novel compound RP6530 [18] which represent the only PI3K δ/γ inhibitors currently in clinical development [10].

Evidence for a Role of PI3K in Autoimmune Pathology

Rheumatoid arthritis

RA is a chronic autoimmune disease, which predominantly causes inflammation in small joints of the hands and feet causing immobilization and disability. In RA, the structure of the synovium is transformed into a pannus-like tissue, which invades cartilage and erodes bone. Strong granulocyte and lymphocyte recruitment causes the inflamed synovium to consist of multiple inflammatory cells, such as macrophages, neutrophils, and T and B cells. Hyperplasia of synovial lining cells occurs, which are predominantly fibroblast-like synoviocytes [19] and damage to tissue is driven by aggressive inflammatory cytokine signaling in these joints. Given that PI3K γ has a pivotal role in mediating leukocyte migration and activation as well as mast cell degranulation [10], it was predicted that blocking PI3K γ may be an effective strategy to fight RA. The collagen induced arthritis (CIA) model reflects the immunological components of the disease and constitutes an acute T-cell-mediated autoimmune arthritis that focuses on the effector phase of arthritis. In this model, PI3K γ null mice exhibited reduced paw swelling, synovial inflammation and cartilage erosion, while inhibition of PI3K γ decreased neutrophil infiltration as well as Th17 differentiation, a pro-inflammatory helper T cell type characterized by expression of the cytokine IL-17 [17,20].

The transgenic overexpression of human tumor necrosis factor (hTNF) α leads to a chronic inflammatory destructive polyarthritis similar to human RA and is sensitive to neutralizing anti-TNF α antibodies. Loss of PI3K γ in human TNF α mice led to reduced arthritis compared to controls [21]. Interestingly, PI3K γ deficiency does not alter the recruitment of inflammatory cells as observed in the CIA model of RA, but it significantly reduces cartilage damage through reduced expression of matrix metalloproteinases in fibroblasts and chondrocytes. PI3K γ expression is significantly higher in the synovium of RA patients compared to synovium from osteoarthritis patients. Furthermore,

inhibition of PI3K γ reduced TNF α -induced MMP production in fibroblasts isolated from human RA patients [21]. This study therefore, provides further mechanistic insights into PI3K γ in RA that extends beyond its established role in immune cell migration.

PI3K δ mRNA and protein expression is also higher in RA than osteoarthritis synovium [22] and PI3K δ mRNA can be induced in cultured synoviocytes by inflammatory cytokines. In a K/BxN-serum transfer model of arthritis, in which neutrophils and LTB $_4$ participate in the effector phase of the inflammatory arthritis, selective inhibition of PI3K δ diminishes joint erosion to a level comparable to inhibition of its PI3K γ 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 LTB $_4$ -mediated neutrophil chemotaxis as described earlier [21]

Important differences between PI3K γ and PI3K δ have been noted in the mechanisms underpinning joint destruction. For example, in a model of osteoclastogenesis, the PI3K δ selective inhibitor IC-87114 significantly inhibited the generation of osteoclasts whereas selective inhibition of PI3K γ with AS-605240 had no effect [23]. Taken together, these lines of evidence suggest that dual inhibition of PI3K γ and PI3K δ would be more therapeutically beneficial than targeting one isoform alone.

Systemic Lupus Erythematosus (SLE)

SLE is a complex multisystem autoimmune disease characterised by the presence of autoreactive antibodies and chronic activation of the immune system. Typically, initial overproduction of memory CD4 $^+$ T lymphocytes leads to hyperactivity of polyclonal B lymphocytes and induces their rapid expansion and production of autoantibodies. The presence of anti-nuclear autoantibodies can lead to the formation of complexes that are retained in the kidney in half of all patients and lead to leukocyte infiltration, ultimately causing renal failure and glomerulonephritis [24]. Current therapies poorly control disease indicators, necessitating potent immunosuppression to achieve remission.

Several lines of evidence suggest dysregulated PI3K-dependent signalling in mouse models of SLE. For example, deletion of PI3K γ in a mouse model reduced the survival of pathogenic memory CD4 $^+$ lymphocytes, which ultimately led to inhibition of glomerulonephritis and enhanced survival rate. Furthermore, treatment of these mice with PI3K γ inhibitor AS-605240 also reduced autoantibody production and increased survival [25]. In addition, there is evidence that the PI3K/Akt/mTOR pathway is up-regulated in murine lupus nephritis [26]. Mice deficient in the src family kinase Lyn, show clinical, pathological and biochemical features of SLE and hyper-activation of PI3K signaling. Inactivation of PI3K δ was enough to halt disease in Lyn-deficient mice [27]. In this regard, increased PI3K δ but not γ activity was observed in SLE patients which correlated with resistance of activated and memory T cells to activation induced cell death and increased number of memory T cells. This highlights an important role for PI3K δ in SLE [28].

Multiple sclerosis (MS)

MS is an autoimmune disease whereby immune activation leads to destruction of the myelin sheath surrounding nerve fibres which impairs signals to and from the brain affecting muscle control, vision, balance and causing fatigue, loss of sensation or numbness. MS gets its name from the build-up of scar tissue (sclerosis) in the brain and/or spinal cord. 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. Considerable evidence now indicates that the T cell lineage most likely to be driving EAE pathogenesis are Th17 cells [29]. In a Th17-driven EAE model, the absence of PI3K γ delayed progression of motor dysfunction and reduced pro-inflammatory chemokines as well as numbers of infiltrating immune cells in the meninges of PI3K γ deficient mice [30]. Both genetic and pharmacological targeting of PI3K γ indicated that it plays an important role in mediating leukocyte survival rather than leukocyte adhesion in this experimental model of MS.

Another group has reported signaling through PI3K δ is required for full and sustained pathology of EAE. Thus, in PI3K δ -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 PI3K γ deficient mice, there were significant increases in the proportion of T cells undergoing apoptosis at early stages of EAE in the absence of PI3K δ activity. Furthermore, a profound defect in Th17 cellular responses during EAE was apparent in the absence of PI3K δ activity. The PI3K δ inhibitor, IC-87114, also had greater inhibitory effects on Th17 cell generation *in vitro* than it did on Th1 cell generation. Taken together, these data indicate that both PI3K γ and PI3K δ can contribute to the pathogenesis of EAE, influencing cell survival, differentiation and migration mechanisms [31]. Thus, dual targeting of PI3K γ and δ might be a therapeutic option for the treatment of EAE.

Type 1 Diabetes

Type 1 diabetes (T1D) is an autoimmune disease characterised by expansion and activation of T lymphocytes specific for components of pancreatic beta cells. Activated T lymphocytes infiltrate the islets of Langerhans and either kill beta cells or promote local inflammation of the pancreas, ultimately causing beta cell dysfunction and death [32]. Following promising early results in a number of animal disease models, the specific PI3K γ inhibitor AS605240 has recently been tested in NOD mice to investigate its effect on T1D. This revealed that PI3K γ is important in regulating the balance of T lymphocyte subsets during the pathogenesis of T1D. AS605240 suppressed autoreactive T lymphocytes and T lymphocyte infiltration into pancreatic islets whilst promoting regulatory T lymphocyte (Treg) expansion. Furthermore, PI3K γ inhibition prevented the development of diabetes in prediabetic NOD mice and reversed hyperglycemia in newly hyperglycemic NOD mice, possibly through expansion of the suppressive Treg lymphocyte subset [32]. This preclinical study offers an important indication of the potential successes of T lymphocyte targeted PI3K γ inhibition therapy for patients with T1D.

The PI3K δ isoform of PI3K has prominent roles in B lymphocyte activation, proliferation and antigen presentation which are associated with initiating and maintaining destructive inflammatory processes. For example, in a model of T1D, non-obese diabetic (NOD) mice that lack B cells do not develop spontaneous autoimmune diabetes seen in wild-type NOD mice [33]. In the NOD mouse model, the PI3K δ inhibitor IC87114 impaired infiltration of inflammatory cells into the pancreatic islets. IC87114 also prevented the *in vitro* activation of diabetogenic T lymphocytes by NOD B cells. Furthermore, IC87114 delayed the onset, reduced severity and prevented progression of autoimmune diabetes in this mouse model [34]. Thus, for the roles of both T- and B-lymphocytes in T1D, the PI3K pathway is a key driver of lymphocyte activation and expansion, making it an attractive target for therapeutic intervention.

Conclusions:

The γ and δ isoforms of PI3K have important non-redundant 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, potent and with negligible off-target effects. These offer opportunities to manipulate the PI3K signaling network in immune cells not only for autoimmune diseases but also for inflammatory conditions and transplantation as well as cancer. The latter could include targeting non-leukemic cancers, given the up-regulation of PI3K γ and PI3K δ in some forms of non-immune cell cancers, though it might be difficult to avoid effects on the immune system which might impair the endogenous anti-tumor response.

While there is optimism for targeting PI3K in autoimmune disease, there still remains key questions surrounding this therapeutic approach (Box 1). Furthermore, PI3K signalling comprises many areas of functional redundancy and plasticity, so targeted therapies are unlikely to have an effect across the range of conditions in which PI3K is involved. Second, targeting of PI3K catalytic isoforms does not necessarily silence PI3K signaling since Akt can be activated independently of the recognized PI3K/PDK1/mTORC2/PH domain-mediated mechanisms [35]. Furthermore, worryingly, the emergence of resistance mechanisms to PI3K targeted therapy has recently been reported [36]. Nevertheless, the emerging success of PI3K inhibitors in clinical trials for cancer and malignancies there remains hope that effective candidates will progress into trials for autoimmune diseases. The intense interest around PI3K isoforms, shared by immunologists, biomedical researchers, and pharmacologists, should ultimately yield badly needed therapies for major autoimmune conditions.

Figure Legends:

Figure 1: a) Schematic of PI3K signalling. Class I PI3K enzymes phosphorylate the D3 position on the inositol ring of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] to generate PI(3,4,5)P₃ which is located in the plasma membrane and acts as a docking site to recruit and activate pleckstrin homology (PH) domain containing proteins that in turn regulate downstream effector proteins. Numerous PH-domain containing proteins are activated by PI3K signaling, these include protein kinase B/Akt and 3'-phosphoinositide-dependent kinase-1 (PDK1), a range of adaptor/scaffolding proteins as well as guanine nucleotide exchange factors (GEFs) which regulate GTPases and hence cell motility and intracellular trafficking. PI3K signaling activity is tightly regulated by at least two lipid phosphatases: SH2-domain containing inositol-5-phosphatase (SHIP) and phosphatase and tensin homolog (PTEN). SHIP de-phosphorylates PI(3,4,5)P₃ at the D5 position of the inositol ring to create phosphatidylinositol 3,4-bisphosphate [PI(3,4)P₂], whereas PTEN de-phosphorylates the D3 position to create PI(4,5)P₂. **b) Structural domains of PI3K Class I enzymes.** Class IA and IB share core domain structure of single Ras binding, C2, helical and catalytic domains. Class IA have a p85 regulatory subunit-binding (p110α/β/δ) domains, whilst Class IB enzymes have p101/p84/p87-binding (p110γ) domains.

Figure 2: Individual and combined roles of Class I PI3K γ and δ isoforms in autoimmune disease. Here the relative involvement and selective inhibitors of PI3K isoforms in autoimmune disease are summarized.

Box 1. Current key questions regarding the use of PI3K inhibitors in autoimmune and inflammatory disease

1. How reflective are rodent models of the actual human disease with regard to the role of PI3Ks?
2. Will selective small molecule inhibitors of PI3Ks gain the same benefit as gene ablation strategies (either by gene deletion or knock-in mutation)?
3. Will isoform selective inhibitors avoid the toxicity problems associated with LY294002
4. Do isoform selective inhibitors retain sufficient efficacy to alter disease progression?
5. Are any side effects of PI3K inhibitors (isoform selective, isoform preference, pan-isoform and multi-kinase) commensurate with the disease being treated?

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