PI3K Inhibitors As Potential Therapeutics for Autoimmune Disease

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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 TNFα (*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 modify 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 erythematous (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 [PI(4,5)P_2] to generate PI(3,4,5)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 PI3Kα, PI3Kβ and PI3Kδ are activated downstream of a variety of receptors that are phosphorylated by tyrosine kinases upon cognate stimulus. The class IB catalytic isoform PI3Kγ is activated by G-protein βγ 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 PI3Kβ also being coupled to GPCRs and PI3Kγ being coupled to tyrosine kinase-coupled receptors [7,8]. PI3Kα and PI3Kβ have a broad tissue distribution, while PI3Kδ and PI3Kγ 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 PI3Kγ or PI3Kδ 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 PI3Kγ and PI3Kδ 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 PI3Kγ 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 the their largely restricted expression to hematopoietic cells, the impact of pharmacological targeting of PI3Kγ and PI3Kδ 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 IC_{50} value of approximately 100 nM for PI3Kδ lipid kinase activity while having negligible potency against the PI3Kα and PI3Kβ isoforms. In 2006 several members of ICOS Corporation formed a spin-out company, Calistoga Pharmaceuticals. Calistoga developed CAL-101, a PI3Kδ 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 PI3Kδ 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 PI3Kγ 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 PI3Kγ compared to related compounds. In general terms, the level of selectivity of compounds against PI3Kγ 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 PI3Kγ than previously reported compounds [10, 17].

Given the evidence 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 considered. TargeGen described two diaminopteridine-diphenol-based compounds (TG-101-110, TG-100-115) with good selectivity for both PI3Kγ 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 PI3Kδ/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 PI3Kγ has a pivotal role in mediating leukocyte migration and activation as well as mast cell degranulation [10], it was predicted that blocking PI3Kγ 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, PI3Kγ null mice exhibited reduced paw swelling, synovial inflammation and cartilage erosion, while inhibition of PI3Kγ 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-TNFα antibodies. Loss of PI3Kγ in human TNFα mice led to reduced arthritis compared to controls [21]. Interestingly, PI3Kγ 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. PI3Kγ expression is significantly higher in the synovium of RA patients compared to synovium from osteoarthritis patients. Furthermore, inhibition of PI3Kγ reduced TNFα-induced MMP production in fibroblasts isolated from human RA patients [21]. This study therefore, provides further mechanistic insights into PI3Kγ in RA that extends beyond its established role in immune cell migration.

PI3Kδ mRNA and protein expression is also higher in RA than osteoarthritis synovium [22] and PI3Kδ mRNA can be induced in cultured synoviocytes by inflammatory cytokines. In a K/BxN-serum transfer model of arthritis, in which neutrophils and LTB4 participate in the effector phase of the inflammatory arthritis, selective inhibition of PI3Kδ diminishes joint erosion to a level comparable to inhibition of 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 earlier [21]

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 [23]. Taken together, these lines of evidence suggest that dual inhibition of PI3Kγ and PI3Kδ would be more therapeutically beneficial than targeting one isoform alone.

Systemic Lupus Erythematous (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 PI3Kγ 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 PI3Kγ 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 PI3Kδ was enough to halt disease in Lyn-deficient mice [27]. In this regard, increased PI3Kδ 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 PI3Kδ 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 PI3Kγ delayed progression of motor dysfunction and reduced pro-inflammatory chemokines as well as numbers of infiltrating immune cells in the meninges of PI3Kγ deficient mice [30]. Both genetic and pharmacological targeting of PI3Kγ 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 PI3Kδ is required for full and sustained pathology of EAE. Thus, 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 it did 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 [31]. Thus, dual targeting of PI3Kγ 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 PI3Kγ inhibitor AS605240 has recently been tested in NOD mice to investigate its effect on T1D. This revealed that PI3Kγ 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, PI3Kγ 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 PI3Kγ inhibition therapy for patients with T1D.

The PI3Kδ 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 PI3Kδ 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 nor 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 PI3Kγ and PI3Kδ 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)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 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)P_3 at the D5 position of the inositol ring to create phosphatidylinositol 3,4-bisphosphate [PI(3,4)P_2], whereas PTEN de-phosphorylates the D3 position to create PI(4,5)P_2 . **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?

References:

1. Arkwright, P.D, et al. (2002). Autoimmunity in human primary immunodeficiency diseases. Blood. 99(8):2694-2702.

2. Walsh, S.J. (2000). Autoimmune Diseases: A Leading Cause of Death among Young and Middle-Aged Women in the United States. American Journal of Public Health. 90:1463-1465.

3. Van der Woude, D. et al. (2009). Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. Arthritis Rheum. 60(8):2262-71

4. Emery, P. et al (2009). A cost-effectiveness of biological therapeutic sequences for rheumatoid arthritis in the UK. Value Health. 12:A439-A439

5. Vanhaesebroeck, B. et al. (2010). The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol. 11:329–341.

6. Bohnacker T et al. (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.

7. Schmid MC 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.
   >>This study refutes current dogma that PI3Kγ is activated only by GPCRs by demonstrating that myeloid cell PI3Kγ is unexpectedly activated by tyrosine kinases and Toll-like/IL1-receptors. Whereas GPCRs activate PI3Kγ in a Ras/p101-dependent manner, receptor tyrosine kinase and Toll-like/IL1 receptors directly activate PI3Kγ in a Ras/p87-dependent manner. These studies reveal that PI3Kγ is a single convergent point controlling tumor inflammation and progression.

8. Guillermet-Guibert J, et al. (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 U S A* **105**, 8292–8297.

9. Blunt, M.D. and Ward, S.G. (2012). Targeting PI3K isoforms and SHIP in the immune system: new therapeutics for inflammation and leukemia. Curr Opin Pharmacol. 2012 Aug;12(4):444-51.

10. Foster, J.G. et al. (2012). Inhibition of PI3K signaling spurs new therapeutic opportunities in inflammatory/autoimmune diseases and hematological malignancies. Pharmacol Review. 64(4):1026-54.

11. Yuan, T.L. and Cantley, L.C. (2008). PI3K pathway alterations in cancer: Variations on a theme. Oncogene. 27(41):5497-5510.

12. Walker EH, et al. (2000). Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol Cell* **6**, 909–919.

13. Berndt A, et al.: The p110delta structure: mechanisms for selectivity and potency of new PI(3)K inhibitors. Nat Chem Biol 2010, 6:117-124.
>> Describes the first crystal structures of the catalytic subunit of PI3Kδ. Revealing the structure of this enzyme provides exciting new insights into the behavior of the PI(3)K enzymes, and particularly into the reasons for isoform selectivity of small-molecule inhibitors. This study provides the first detailed structural insights into the active site of a class IA PI3K occupied by non-covalently 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.

14. Lannutti BJ, 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 2011, 117:591-594.
>>Using tumor cell lines and primary patient samples representing multiple B-cell malignancies, this study demonstrated that constitutive PI3K pathway activation is PI3Kδ-dependent. CAL-101 blocked constitutive PI3K signaling, resulting in decreased phosphorylation of Akt and other down-stream effectors, an increase in poly(ADP-ribose) polymerase and caspase cleavage and an induction of apoptosis. These effects were been observed across a broad range of immature and mature B-cell malig-nancies, thereby providing a impetus for subsequent clinical evaluation of CAL-101

15. Hoellenriegel J, et al. (2011) The phosphoinositide 3-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. Blood 2011, 118:3603-3612.
>>This study reveals that PI3K□ inhibition by CAL-101 disrupts crosstalk between the CLL and its microenvironment in several ways: (i) CLL chemokine receptor function and signaling are modulated by CAL-101, causing diminished leukemia cell chemotaxis and migration beneath marrow stromal cells; (ii) CAL-101 impairs CLL cell viability, both by disrupting BCR signaling but also by

antagonizing support from nurse- like cells and by interrupting paracrine secretion of chemokines by CLL cells; (iii) CAL-101 also reduced the exaggerated production of other chemokines and cytokines that occurred when CLL cells were co-cultured with nurse-like cells.

16. Fruman DA. (2012) PI3K signalling in B- and T-lymphocytes: new developments and therapeutic advances. Biochem J. 2012 Mar 15;442(3):465-81.

17. Bergamini G, et al. (2012). A selective inhibitor reveals PI3Kγ dependence of T(H)17 cell differentiation. *Nat Chem Biol*. Doi: 10.1038/nchembio.957
>>This paper describes chemoproteomics-based drug discovery platform that enables multiplexed high-throughput screening of native proteins in cell extracts, thus preserving their post-trans- lational modifications and protein interactions. 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

18. Rhizen Pharmaceuticals S.A. announces initiation of a “First in Human” Phase-1 trial of RP6530, a dual PI3K delta/gamma inhibitor, in patients with hematological malignancies

19. Firestein GS (2003). Evolving concepts of rheumatoid arthritis. *Nature* **423**, 356–361.

20. Camps M, et al. (2005). Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat Med* **11**, 936–943.

21. Hayer S, et al. (2009). PI3Kgamma regulates cartilage damage in chronic inflammatory arthritis. *FASEB J* **23**, 4288–4298.

22. Bartok, B et al., (2012). PI3 kinase δ is a key regulator of synoviocyte function in rheumatoid arthritis. Am J Pathol. 180:1906-1916.
>>Using novel selective PI3Kδ inhibitor INK007, the authors demonstrate PI3Kδ has a key role in fibroblast-like synoviocytes. This study is the first to document enhanced PI3Kδ expression in RA synovium and highlight the potential of targeting PI3Kδ in rheumatoid arthritis treatment.

23. Toyama S, (2010). Inhibitory effects of ZSTK474, a novel phosphoinositide 3-kinase inhibitor, on osteoclasts and collagen-

induced arthritis in mice. *Arthritis Res Ther* **12**, R92.

24. Wakeland EK et al., (1999). Genetic dissection of systemic lupus erythematosus. *Curr Opin Immunol* **11**, 701–707.

25. Barber, D.F. et al. (2005). PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. Nature Medicine. 11(9):933-5.

26. Stylianou, K. et al. (2011). The PI3K/Akt/mTOR pathway is activated in murine lupus nephritis and downregulated by rapamycin. Neprhol Dial Transplant. 26(2):498-50

27. Maxwell, M.J. et al. (2012). Attenuation of phosphoinositide 3-kinase δ signaling restrains autoimmune disease. J Autoimmun. 38(4):381-91

28. Suárez-Fueyo A et al., (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.

29. Kleinschek MA. et al. (2007). IL-25 regulates Th17 function in autoimmune inflammation. *J Exp Med* 204, 161–170

30. Rodrigues DH, Vilela MDC, Barcelos LDS, 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.

31. Haylock-Jacobs S et al., (2011). PI3Kδ drives the pathogenesis of experimental autoimmune encephalomyelitis by inhibiting effector T cell apoptosis and promoting Th17 differentiation. *J Autoimmun* **36**, 278–287.
> This study provides several novel insights into the role of PI3Kδ in CNS autoimmune disease and particularly, in regard to T helper cell function. PI3Kδ activity is required for efficient Th17 differentiation and function both in vitro and in vivo and protects $CD4^+$ T cells from undergoing apoptosis during an autoimmune response. These effects on T cells are apparently independent of DC function.

32. Durand, C.A. et al. (2013). Selective pharmacological inhibition of phosphoinositide 3-kinase p110delta opposes the progression of autoimmune diabetes in non-obese diabetic (NOD) mice. Autoimmunity. 46(1):62-73.

>Using a mouse model, this study investigates how targeting PI3K signaling in B-lymphocytes with p110δ isoform selective inhibitor IC87114 can limit autoimmune processes that contribute to type 1 diabetes. Crucially for the therapeutic potential of PI3K inhibitors, this

inhibitor proved beneficial in managing this autoimmune disease as both a preventative and therapeutic treatment.

33. Serreze, D.V. et al. (1996). B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new “speed congenic” stock of NOD.Ig mu null mice. J. Exp. Med. 184(5):2049–2053.

34. Azzi, J. et al. (2012). The novel therapeutic effect of phosphoinositide 3-kinase-y inhibitor AS605240 in autoimmune diabetes. Diabetes. 61(6):1509-18.

35. 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.

36. Ilic, N. et al. (2011). PI3K-targeted therapy can be avaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E(e11=4E) axis. PNAS. 108(37):E699-708.

>Identifies resistance mechanisms to PI3K-targeted therapy. Effects of the PI3K/mTOR inhibitor BEZ235 could be evaded by gene amplification of either of two proto- oncogenes, c-*myc* and eukaryotic translation initiation fac- tor 4E. These apparently bypass the inhibitors by acting downstream of the pharmacologically inhibited targets.