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Two Birds with One Stone: Dual p110δ and p110γ Inhibition

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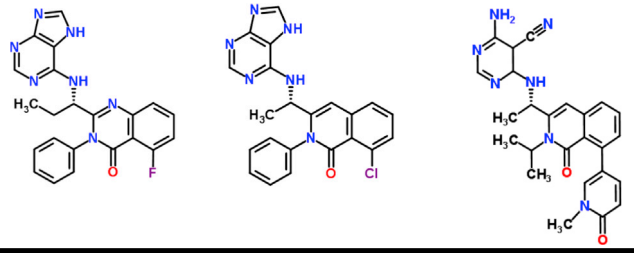
In this issue of *Chemistry & Biology*, Winkler and colleagues describe the discovery and preclinical development of IPI-145, a new inhibitor of the phosphoinositide 3-kinase (PI3K) isoforms p110δ and p110γ that have entered clinical trials.

The phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases of which there are eight distinct catalytic subunits expressed in mammalian cells. Most efforts to date have been focused on developing inhibitors against the class I PI3Ks p110α, p110β, p110γ, and/or p110δ, which use $PI(4,5)P_2$ as their preferred substrate to produce PIP_3 at the plasma membrane. PIP_3 serves as a membrane tether for many proteins, including Akt, and, as such, connects receptor tyrosine kinases and G protein coupled receptors to intracellular signaling networks (Okkenhaug, 2013). A number of drugs target all the class I PI3K isoforms with similar efficacy, and such compounds are being progressed through the clinic, primarily to treat nonhematological malignancies (Engelman, 2009). Significant progress has also been made in the design of inhibitors that discriminate among the different class I PI3K isoforms. Both p110γ and p110δ are expressed at much higher levels in cells of the immune system than in other cell types. Therefore, by targeting one or both of these isoforms, one can selectively block PI3K signaling in leukocytes with minimal effect on other tissues and organs (Banham-Hall et al., 2012).

Previously, the p110δ-selective inhibitor IC87114 and/or the p110γ selective inhibitor AS605240, often in combination with PI3K knockout and knockin mice, have been evaluated in mouse models of asthma, arthritis, and/or lupus (Banham-Hall et al., 2012). Broadly speaking, p110δ inhibitors block lymphocyte function, whereas p110γ inhibitors block innate immune cell migration and function. This is best demonstrated in mouse models of asthma, where genetic or pharmacological inhibition of p110δ reduced type 2 T cell responses, whereas inhibition of p110γ prevented eosinophil recruitment (Nashed et al., 2007; Takeda et al., 2009). However, dual inhibition of p110δ and p110γ was found to be more effective in an antibody-induced arthritis model where pathology is thought to mainly be contributed by innate immune cells (Randis et al., 2008).

Given the often nonoverlapping functions of p110δ and p110γ in immune cells, the rationale for inhibiting both is clear. Kevan Shokat and colleagues first described a dual p110δ-p110γ selective compound SW14 and found this to be more potent than a p110δ-selective compound at blocking TNF-α in a cell-based screen (Williams et al., 2010). However, dual p110γ-p110δ inhibition also presents potential risks, because p110δ and p110γ dual-deficient mice show severe pathology, probably linked to inappropriate T cell activation, which is presumed to result from profound T cell lymphopenia caused by a block in T cell development (Ji et al., 2007). A profound depletion of thymocytes was also observed after administration of the p110δ-p110γ dual-specific inhibitor CAL-130 (Subramaniam et al., 2012).

IPI-145, which was originally developed by Intellikine as INK-1197, is a p110δ-selective inhibitor that, at higher concentration, is designed to also inhibit p110γ. IPI-145 is remarkably similar in structure to GS-1101 (now called Idelalisib), which Gilead acquired from Calistoga Pharmaceuticals in 2011. To help distinguish between the effect of dual inhibition of p110δ and p110γ versus inhibition of p110δ alone, another new compound IPI-3063, which is very potent and selective for p110δ, was used. In this issue of *Chemistry & Biology*, Winkler et al. (2013) compared the cellular IC_{50} values of GS-1101, IPI-145, and IPI-3063 in cell-based assays, using Akt phosphorylation as a readout for PI3K activity (summarized here in Figure 1). These results indicate that IPI-145 and GS-1101 are about 50- to 100-fold selective for p110δ over p110γ, respectively. However, IPI-145 is more potent than GS-1101, and p110γ inhibition can be achieved at relatively low IPI-145 concentrations, which are readily achievable in vivo. It is also worth noting that, at higher concentrations, both IPI-145 and GS-1101 show inhibitory activity toward p110β. The consequence, if any, of concomitant p110β inhibition is not considered further in the current study, but should not be ignored, especially as p110β can contribute to antibody dependent neutrophil activation (Kulkarni et al., 2011). Because IPI-145 is more potent against p110δ than against p110γ, Winkler et al. (2013) suggest that they can gradually include p110γ inhibition by increasing the dose of the drug. In order to demonstrate that IPI-145 can inhibit a p110γ-dependent response in vivo, a rat air pouch model was used. In this model, it has previously been demonstrated that p110γ is required for the recruitment of neutrophils to KC/GRO—an IL-8 family chemokine. IPI-145 administered at 10 mg/kg, resulting in plasma levels exceeding 100 nM, prevented neutrophil recruitment. This concentration should be sufficient to block p110γ as well as p110δ. By contrast, IPI-3063 administered at 50 mg/kg showed no inhibition of neutrophil recruitment, nor did a lower dose of 1 mg/kg of IPI-145. These results confirm that p110δ does not inhibit neutrophil recruitment and demonstrate that, at 10 mg/kg, IPI-145 effectively inhibits



	GS-1101	IPI-145	IPI-3063
p110α	2221 (8487)	1410 (1602)	1900
p110β	135 (838)	26.2 (85)	102
p110δ	4.9 (11)	0.36 (2.5)	0.1
p110γ	520 (546)	19.6 (27)	418
p110γ/p110δ	**106 (50)**	**54 (10.8)**	**>4000**

Figure 1. Chemical Structure and PI3K Isoform Selectivity of GS-1101, IPI-145, and IPI-3063
Structures and cellular IC_{50} values (nM) against the PI3K p110α, p110β, p110δ, and p110γ isoforms are shown as reported by Winkler et al. (2013) in this issue. PI3K activity, as determined by Akt phosphorylation, was measured in SCOV cells stimulated with serum (p110α), 786-0 cells stimulated with serum (p110β), RAJI cells stimulated with anti-IgM (p110δ), or RAW264.7 cells stimulated with C5a (p110γ). Enzymatic IC_{50} values (nM) measured in presence of 3 mM ATP are shown in brackets for GS-1101 and IPI-145.

p110γ as well as p110δ. Armed with this insight, Winkler et al. (2013) tested the effectiveness of IPI-145 in rat models of arthritis and asthma and a mouse model of lupus. In the arthritis model, therapeutic administration of IPI-145 caused a dose-dependent reduction of ankle swelling. The maximal response was observed with administration of 10 mg/kg of compound, but doses as low as 0.5 mg/kg also gave significant results. However, only the 10 mg/kg dose blocked cellular influx into the joints and eliminated ankle swelling completely, suggesting that p110γ inhibition was required to completely reverse ankle swelling. IPI-145 also effectively blocked eosinophil recruitment and cytokine production in the asthma model and reduced autoantibody production, proteinuria, and histopathology in the lupus model. In each case, maximal effect was achieved with 10 mg/kg doses, whereas partial responses were observed with 1 mg/kg doses predicted to only inhibit p110δ. The authors do not report whether they observed pathologies associated with dual p110γ-p110δ inhibition, but this could be a caveat, at least for long term administration of high doses of IPI-145.

Both IPI-145 and GS-1101 are showing promise as anti-leukemic agents and are moving rapidly through phase II/III clinical trials. In addition, IPI-145 is being evaluated for the treatment of allergic asthma and severe rheumatoid arthritis in phase II trials. The preclinical data presented here provide an important rationale for progressing IPI-145 through these trials, the results from which are keenly anticipated. In addition, IPI-145 may show a benefit in T cell acute lymphoblastic leukemia, where both p110δ and p110γ contribute to cell survival proliferation (Subramaniam et al., 2012). Finally, the recent discovery of primary immune deficient patients with activating mutations in the *PIK3CD* gene, which encodes p110δ, suggest that inhibitors such as IPI-3063, or indeed low doses of IPI-145, may help restore normal immune function in some patients (Angulo et al., 2013; Lucas et al., 2013).

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