# Getting fat off PI3Kγ

*By Tim Fulmer, Senior Writer*

A team of Swiss researchers has shown that knocking out an isoform of the cancer target phosphoinositide 3-kinase led to weight loss and increased insulin sensitivity in mice.[1] The challenge will be designing compounds that are selective for the γ-isoform because its structural features have thus far made it much more intractable to drug design than the other isoforms.

Phosphoinositide 3-kinase-γ (PI3Kγ) is one of four isoforms (α, β, γ and δ) in the PI3K family. The kinases play a central role in signaling pathways that contribute to cell growth, proliferation, motility and survival.

There are at least 10 PI3K-targeting compounds in preclinical and clinical development for cancer and inflammation[2] (*see* **Table 1, "PI3K inhibitors"**). Depending on the indication, the goal ranges from designing compounds that hit a single isoform to creating compounds that target up to all four.

Unlike the other isoforms, which are expressed ubiquitously, PI3Kγ is primarily expressed in the bone marrow, and because the bone marrow gives rise to immune cells, selectively inhibiting PI3Kγ could have anti-inflammatory effects. Indeed, knocking out PI3Kγ in a variety of mouse models of inflammatory and autoimmune diseases decreased inflammation.[3,4]

Guided by prior work from several labs that showed chronic inflammation may contribute to obesity and insulin resistance,[5–7] the Swiss team hypothesized that blocking PI3Kγ could cause weight loss and improve glucose homeostasis.

The team was led by Giovanni Solinas and Matthias Wymann. Solinas is a professor of physiology and medicine at the **University of Fribourg**. Wymann is a professor of biomedicine at the **University of Basel**.

**Table 1. PI3K inhibitors.** Selected phosphoinositide 3-kinase (PI3K) inhibitors in or about to enter the clinic. There are four distinct isoforms—PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ.

| Company | Product | Isoform selectivity | Indication | Status |
|---|---|---|---|---|
| **Exelixis Inc.** (NASDAQ:EXEL)/ **Sanofi** (Euronext:SAN; NYSE:SNY) | XL147 (SAR245408) | All isoforms | Solid cancers | Phase II |
| **Gilead Sciences Inc.** (NASDAQ:GILD) | GS 1101 (CAL-101) | PI3Kδ | Hematological cancers | Phase II |
| **Novartis AG** (NYSE:NVS; SIX:NOVN) | BKM120 | All isoforms | Advanced solid tumors | Phase II |
| **Oncothyreon Inc.** (NASDAQ:ONTY) | PX-866 | All isoforms | Solid cancers | Phase II |
| **Novartis** | BYL719 | PI3Kα | Advanced solid tumors | Phase I |
| **Amgen Inc.** (NASDAQ:AMGN) | AMG319 | PI3Kδ | Hematological cancers | Phase I |
| **Intellikine Inc.** | INK1117 | PI3Kα | Solid tumors | Phase I |
| **Roche** (SIX:ROG; OTCQX:RHHBY)/ **Genentech Inc.** | GDC-0941 | All isoforms | Advanced solid tumors | Phase I |
| Roche/Genentech | GDC-0032 | PI3Kα | Solid tumors | Phase I |
| **Avila Therapeutics Inc.** | CNX-1351 | PI3Kα | Cancer | Preclinical |
| **Karus Therapeutics Ltd.** | KAR4141 | PI3Kδ | Inflammation | Preclinical |
| Karus Therapeutics | KAR4139 | PI3Kδ and PI3Kβ | Inflammation and cancer | Preclinical |
| **Pathway Therapeutics Inc.** | PI3Kδ inhibitors | PI3Kδ | Inflammation and hematological cancers | Preclinical |
| Pathway Therapeutics | PI3Kδ and PI3Kβ inhibitors | PI3Kδ and PI3Kβ | Inflammation and cancer | Preclinical |

To test its hypothesis, the team knocked out PI3Kγ in mice and placed them on a high-fat diet. The knockout mice had less weight gain and fatty liver, greater glucose control and lower adipose tissue inflammation than wild-type controls on an identical diet.

The PI3Kγ knockouts also showed greater thermogenesis than their wild-type littermates, suggesting that at least part of the weight loss resulted from increased heat production and energy expenditure by non–bone marrow cells, not from exercise.

Indeed, differences in physical activity between the knockout and wild-type mice did not correlate with differences in energy expenditure. Thus, weight loss in the knockouts was likely independent of their level of activity.

Data were published in the *Proceedings of the National Academy of Sciences*.

The Swiss team's findings are supported by an earlier paper from Japanese researchers that also looked at PI3Kγ knockout mice on a high-fat diet.[8]

However, because the two groups looked at different readouts from the animals, their observations differed.

Whereas the Swiss researchers observed increased thermogenesis and weight loss as a result of knocking out PI3Kγ, a group led by Kohjiro Ueki and Takashi Kadowaki saw somewhat different effects: improved insulin sensitivity coupled with decreased infiltration

of proinflammatory macrophages into adipose tissue.

Ueki believes the two sets of findings are complementary and provide a rationale for using PI3Kγ inhibition in metabolic disorders.

"Taken together, the two studies suggest that a PI3Kγ inhibitor could simultaneously reduce insulin resistance and suppress weight gain in humans. Thus, a single compound could potentially treat the harmful effects of high caloric intake and an overly sedentary lifestyle," Ueki told *SciBX*.

**"Despite the head start provided to medicinal chemists by those crystal structures, not a single γ-selective inhibitor has been taken through early phase clinical trials."**
***—Stephen Shuttleworth, Karus Therapeutics Ltd.***

Ueki and Kadowaki are professors of medicine at **The University of Tokyo Graduate School of Medicine**. Ueki is also director of the division of diabetes and metabolic diseases at **The University of Tokyo Hospital**.

## Finding an inhibitor

Both the Swiss and Japanese researchers want to test PI3Kγ inhibitors in preclinical models of metabolic disorders.

The Swiss team "has undisclosed partners who have PI3Kγ inhibitors," according to Wymann.

Designing selective inhibitors of PI3Kγ is more difficult than blocking other PI3K isoforms, said Christian Rommel, CSO of **Intellikine Inc.**

"Compared to other PI3K isoforms, balancing isoform selectivity with potency has been especially difficult with PI3Kγ inhibitors. Based on the protein's X-ray crystal structure, one reason for that difficulty might be how the structure-activity relationship of PI3Kγ differs significantly from that of the other isoforms," said Rommel. "As a result, it may be more challenging using inhibitors of the other isoforms to guide our design of inhibitors of PI3Kγ."

Rommel has first-hand experience with the challenges of inhibiting PI3Kγ. Before joining Intellikine in 2007, he was head of target research at **Merck KGaA**'s **Merck Serono S.A.** unit, where he designed AS-605240, a PI3Kγ inhibitor that he said lacked the proper therapeutic index for testing in humans.

Since the first X-ray crystal structures of PI3Kγ were disclosed in 2000, "there has been ample opportunity for scientists to generate and assess the therapeutic potential of a diverse range of γ-selective inhibitors," said Stephen Shuttleworth, CSO of **Karus Therapeutics Ltd.** "Yet, despite the head start provided to medicinal chemists by those crystal structures, not a single γ-selective inhibitor has been taken through early phase clinical trials," he said.

Karus has two PI3K inhibitors in preclinical development for inflammation and cancer: KAR4141, which is selective for PI3Kδ, and KAR4139, which is selective for PI3Kδ and PI3Kβ.

According to Shuttleworth, the challenges associated with developing those two PI3K inhibitors were "fairly generic and common to most small molecule drug programs: obtaining requisite potency and specificity, confirming a clean toxicity profile, establishing a strong IP position."

Given the important role PI3Kγ plays in the immune system, "it will also be necessary to carefully study the impact a γ-selective inhibitor might have on the host defense system, in particular, the potential for opportunistic infection when treating inflammation," said David Matthews, VP of drug discovery and exploratory development at **Pathway Therapeutics Inc.**

Pathway's lead compound is PWT33597, a dual inhibitor of PI3Kα and mammalian target of rapamycin (mTOR; FRAP; RAFT1) that is in a Phase I trial to treat advanced solid tumors.

Whatever challenges are unique to PI3Kγ, Intellikine is continuing to pursue inhibitors of the target. Last year the company partnered with **Infinity Pharmaceuticals Inc.** to develop isoform-selective inhibitors of PI3Kγ and PI3Kδ.

"We believe that PI3Kγ is preclinically an extremely well-validated target in inflammation and that it's time to test the target in the clinic. Moreover, the role it plays in inflammation potentially links it to a number of other key diseases, including atherosclerosis, pain and obesity. The key remaining challenge is to identify compounds that hit the target hard and cleanly," said Rommel.

Rommel declined to disclose any details of the discovery strategy the two companies plan to use as they move forward with their PI3Kγ program.

Intellikine's lead compound, INK128, is in Phase I testing to treat advanced solid tumors and multiple myeloma (MM). INK128 inhibits mTOR complex 1 (mTORC1) and mTORC2, which act downstream of the PI3Ks.

The Swiss team's findings are not patented, according to Solinas.

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## COMPANIES AND INSTITUTIONS MENTIONED

**Infinity Pharmaceuticals Inc.** (NASDAQ:INFI), Cambridge, Mass.
**Intellikine Inc.**, La Jolla, Calif.
**Karus Therapeutics Ltd.**, Chilworth, U.K.
**Merck KGaA** (Xetra:MRK), Darmstadt, Germany
**Merck Serono S.A.**, Geneva, Switzerland
**Pathway Therapeutics Inc.**, San Francisco, Calif.
**University of Basel**, Basel, Switzerland
**University of Fribourg**, Fribourg, Switzerland
**The University of Tokyo Graduate School of Medicine**, Tokyo, Japan
**The University of Tokyo Hospital**, Tokyo, Japan