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# Getting selective for γ

*By Tim Fulmer, Senior Writer*

**Cellzome AG** and **Exelixis Inc.** have independently designed the first highly selective inhibitors of the γ-isoform of phosphoinositide 3-kinase.[1,2] Exelixis plans to out-license its inhibitors, whereas last week's acquisition of Cellzome by **GlaxoSmithKline plc** gives the pharma a new class of compounds for inflammatory and autoimmune diseases.

Phosphoinositide 3-kinase (PI3K) plays a central role in signaling pathways that contribute to cell growth, proliferation, motility and survival. The kinase occurs in four isoforms. The α- and β-isoforms are expressed in many tissues and cell types, and the γ- and δ-isoforms are expressed in immune cells.

Aberrant activation of PI3Kα and PI3Kβ is a key driver of many solid cancers, whereas activation of PI3Kγ and PI3Kδ can lead to hematological malignancies and inflammatory diseases.

For the past decade, the challenge has been to design PI3K inhibitors that selectively hit the isoform implicated in a given disease while sparing the function of the other isoforms. That has generally required chemists to optimize inhibitors that are 10–1,000 times more selective for one isoform over the others.[3]

Selective inhibitors of PI3Kα and PI3Kδ have moved into the clinic, and inhibitors of PI3Kβ are in preclinical testing (*see* **Table 1, "PI3Kγ and PI3Kδ inhibitor pipeline"**). Inhibiting PI3Kγ has been more difficult.

Genetic data from a variety of animal models suggest selectively inhibiting PI3Kγ could have a broad anti-inflammatory effect with potential utility in a range of diseases,[4] including rheumatoid arthritis (RA),[5] atherosclerosis[6] and diabetes.[7,8] Highly selective inhibition of PI3Kγ also should avoid the cardiotoxicity that has been associated with some PI3K inhibitors in mice that antagonize the PI3Kα isoform (*see* **Box 1, "Heartless PI3K inhibition"**).

Chemical scaffolds that work for the other three isoforms "often fail to potently inhibit PI3Kγ," Christian Rommel told *SciBX*. "The ATP-binding pocket of PI3Kγ is structurally distinct [from the other isoforms] and is more tight and narrow as well as less flexible."

Rommel is the former CSO of Intellikine Inc., which was acquired by **Takeda Pharmaceutical Co. Ltd.** this year for $190 million up front and up to $120 million in milestones. While at Intellikine, Rommel oversaw the development of INK1117, a PI3Kα-selective PI3K inhibitor that is in Phase I testing to treat solid tumors, and IPI-145, a dual inhibitor of PI3Kγ and PI3Kδ that is in Phase I testing to treat hematological malignancies.

Compared with dual inhibition of PI3Kγ and PI3Kδ, inhibiting only PI3Kγ "is thought to be more suitable for myeloid-triggered inflammatory processes implicated in atherosclerosis and certain forms of metabolic disorders," said Rommel.

## Going native

Cellzome researchers reasoned they might have a better chance of identifying a PI3Kγ-selective PI3K inhibitor by using initial compound screens in whole-cell extracts rather than in panels of purified recombinant kinases.

The goal was to test the activity of inhibitors in a setting that mimics physiology, VP of Research Operations Gitte Neubauer told *SciBX*. "The targets are full length, post-translationally modified, and their interactions with other proteins are largely preserved," she said.

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> ***—Matthew Patricelli, ActivX Biosciences Inc.***

"There has been a growing realization that recombinant protein kinases, which are often truncated and/or fusion proteins, do not exhibit the same activity and drug-binding properties as kinases in native cells and tissues," said Matthew Patricelli, director of technology at **ActivX Biosciences Inc.**

Proteome-wide analysis of compound selectivity against native kinases in the presence of cellular cofactors "is particularly useful to inform decisions, for example, in the lead optimization phase or in the clinical candidate selection process," said Henrik Daub, SVP of science and technology at **Evotec AG**.

ActivX's KiNativ platform and Evotec's Kinaxo platform both allow for proteome-wide kinase inhibitor profiling in cell and tissue extracts.[9,10]

To identify molecules that could serve as starting points for optimization of highly selective inhibitors of PI3Kγ, Cellzome used its Kinobeads chemical proteomics platform.

The platform uses a resin matrix coated with immobilized analogs of small molecules that broadly bind kinases. The resin is incubated in a fresh cell extract along with a test compound or vehicle.

The arrangement sets up a competitive binding assay in which the test compound competes with the resin to bind PI3Kγ in the cell extract. Hits are then identified by reduced binding of PI3Kγ to the resin, which indicates the test compound binds PI3Kγ more strongly than the resin and thus could be the starting point for inhibitor optimization.

Previously, the researchers had validated the platform by confirming its ability to predict the known targets of a panel of multikinase inhibitors, including the cancer drugs Gleevec imatinib from **Novartis AG** and Sprycel dasatinib from **Bristol-Myers Squibb Co.**[11]

In the new work, the team modified the resin to bind PI3Kγ as well as potential off-target kinases PI3Kδ, mammalian target of rapamycin (mTOR; FRAP; RAFT1) and DNA-dependent protein kinase (DNA-PK), and then they screened a library of 16,146 small molecules for selective inhibition of PI3Kγ.

**Table 1. PI3Kγ and PI3Kδ inhibitor pipeline.** There are at least six companies developing inhibitors that are selective for phosphoinositide 3-kinase-δ (PI3Kδ) or selective for both PI3Kγ and PI3Kδ. Hematological malignancies and inflammation are the main indications being pursued. The $IC_{50}$ value indicates the concentration of compound needed to inhibit PI3K signaling by 50% and serves as a rough measure of the relative selectivity of an inhibitor for one isoform over another. Lower $IC_{50}$ values generally indicate greater potency.

*Source: BCIQ: BioCentury Online Intelligence. Shuttleworth, S.J.* et al. Curr. Med. Chem. ***18****, 2686–2714 (2011)*

| Company | Compound | Intended target(s) | Isoform selectivity $IC_{50}$ value (nM) | Lead indication(s) | Status |
|---|---|---|---|---|---|
| **Gilead Sciences Inc.** (NASDAQ:GILD) | GS-1101 | PI3Kδ | PI3Kα: >100,000<br>PI3Kβ: 1,820<br>PI3Kγ: 1,240<br>PI3Kδ: 70 | Chronic lymphocytic leukemia (CLL); non-Hodgkin's lymphoma (NHL) | Phase III |
| **Amgen Inc.** (NASDAQ:AMGN) | AMG 319 | PI3Kδ | Not available | Hematologic malignancies | Phase I |
| **Takeda Pharmaceutical Co. Ltd.** (Tokyo:4502)/ **Infinity Pharmaceuticals Inc.** (NASDAQ:INFI)/ **Mundipharma International Ltd.** | IPI-145 | PI3Kδ and PI3Kγ | Not available | Hematologic malignancies; autoimmune disorders | Phase I |
| **Exelixis Inc.** (NASDAQ:EXEL)/ **Merck & Co. Inc.** (NYSE:MRK) | XL499 | PI3Kδ | Not available | Allergic asthma; inflammation | Preclinical |
| **Karus Therapeutics Ltd.** | KAR4139 | PI3Kδ and PI3Kβ | Not available | Cancer; inflammation | Preclinical |
| | KAR4141 | PI3Kδ | Not available | Inflammation | Preclinical |
| **Pathway Therapeutics Inc.** | PI3Kδ | PI3Kδ | ≥100 times more selective over PI3Kα, PI3Kβ and PI3Kγ | Hematologic malignancies; inflammation; respiratory disorders | Preclinical |

The lead hit of that screen, CZC19091, was potent and at least 20 times more selective for PI3Kγ over the off-target kinases. However, the compound was poorly active in cellular assays and showed poor exposure in rats.

The researchers then made three chemical modifications to CZC19091, which led to the highly selective PI3Kγ inhibitor CZC24832. The compound was more potent than the parent molecule. CZC24832 also was 100 times more selective for PI3Kγ over PI3Kα and PI3Kδ and 30 times more selective over PI3Kβ. The compound showed strong activity in cellular assays and good exposure in rats.

In a mouse model of collagen-induced arthritis, CZC24832 decreased bone and cartilage destruction by 53% compared with vehicle control, confirming the compound's anti-inflammatory activity.

Finally, the team used the inhibitor to uncover a new role for PI3Kγ in the regulation of proinflammatory T cells. In a panel of human primary cell co-cultures exposed to inflammatory stimuli, CZC24832 inhibited RAR-related orphan receptor C thymus-specific isoform (RORγ2; RORγT) expression, blocking differentiation of naïve T cells into T helper type 17 (Th17) cells and reducing the number of IL-17–producing cells.

"Our results revealed a previously undescribed role of PI3Kγ in the regulation of $T_H17$ differentiation, supporting the involvement of PI3Kγ in the control of both innate and adaptive immune mechanisms," the authors wrote in their paper in *Nature Chemical Biology*.

Although the lead PI3Kγ inhibitor identified in the *Nature Chemical Biology* paper "is a very good and selective *in vivo* probe compound, it does not have ideal physical-chemical properties. Further optimization would be needed before it might be considered a clinical candidate," Neubauer said. She declined to provide additional details, and GSK did not respond to requests for comment.

For obesity-related diseases, "it will be important to evaluate the toxicity of CZC24832 following chronic, *in vivo* administration in the relevant animal models," said Giovanni Solinas, professor of physiology and medicine at the **University of Fribourg**.

Solinas and colleagues have shown that PI3Kγ promotes obesity and insulin resistance in mice on a high-fat diet.[7]

Cellzome and GSK have been working together since 2008 to discover and develop kinase-targeted therapeutics using the Kinobeads platform, and GSK had an exclusive option to license any product candidates.

Neubauer declined to disclose if any compounds that have been discovered under that deal target PI3Kγ or other PI3K isoforms.

**"Based on the data we've gathered so far, we believe PI3Kγ inhibitors have broad-spectrum anti-inflammatory activity and could find therapeutic uses in autoimmune indications like rheumatoid arthritis, multiple sclerosis and lupus, as well as in airway inflammation diseases such as asthma and COPD [chronic obstructive pulmonary disease]."**

***—Peter Lamb, Exelixis Inc.***

## Back to basics

Exelixis opted for a different approach than Cellzome and stuck with the standard structure-based design methods Exelixis used to discover and optimize selective inhibitors of PI3Kα, PI3Kβ and PI3Kδ.

"We had two key things working in our favor that made us believe we could use a traditional structure-based design approach" to discover PI3Kγ inhibitors, Exelixis CSO and EVP of discovery research Peter Lamb told *SciBX*. "First, we have a very large in-house screening library of about 4.5 million compounds that includes a broad range of scaffolds and chemotypes. Second, we have X-ray crystal structures of PI3Kγ bound to some of those chemotypes, which, we believed, could inform and guide our optimization of inhibitors that would be at least 10 times

### Box 1. Heartless PI3K inhibition.

A study published in *Science Translational Medicine* by researchers from the **State University of New York at Stony Brook** showed that blocking phosphoinositide 3-kinase-α (PI3Kα) signaling in the heart triggered prolongation of the QT interval in mice and in cultured canine ventricular myocytes.[12]

In mice, cardiac-specific knockout of Pi3kα led to increased action potential duration (APD) compared with that seen in wild-type animals, whereas cardiac-specific knockout of Pi3kβ had minimal effects on APD. When the Pi3kβ knockout mice were treated with a PI3Kα inhibitor, the animals showed APD length comparable to that in the Pi3kα knockout mice.

Moreover, echocardiogram (ECG) readings of hearts isolated from the Pi3kα knockout mice showed the QT interval was almost twice as long as that in wild-type controls. In cultured canine myocytes, **Novartis AG**'s PI3Kα and mammalian target of rapamycin (mTOR; FRAP; RAFT1) dual inhibitor, BEZ235, increased APD compared with vehicle.

Those results suggest that in the mouse heart, reduced signaling by the Pi3kα isoform solely mediates APD prolongation.

The authors concluded that patients treated with PI3K inhibitors and other drugs targeting PI3K signaling in the heart "should be closely monitored for QT prolongation and cardiac arrhythmias."

Corresponding author Richard Lin told *SciBX* that he and corresponding author Ira Cohen "are actively investigating how to counter the off-target proarrhythmic effect caused by PI3K inhibitors and other drugs that inhibit PI3K signaling." He declined to provide additional details.

Lin and Cohen are professors of physiology and biophysics at SUNY Stony Brook. Cohen also is director of the university's Institute of Molecular Cardiology.

Both PI3Kα-selective inhibitors and PI3Kα and mTOR dual inhibitors are in the clinic.

PI3Kα-selective inhibitors include GDC-0941 from **Roche**, which is in Phase II testing for breast cancer, and BYL719 from Novartis and INK1117 from **Takeda Pharmaceutical Co. Ltd.**, both of which are in Phase I testing for solid tumors.

PI3Kα and mTOR dual inhibitors include BEZ235 from Novartis, which is in Phase I/II testing for solid cancers; XL765 from **Exelixis Inc.** and **Sanofi**, which is in Phase Ib/II testing for gliomas and solid tumors; and PWT33597 from **Pathway Therapeutics Inc.**, which is in Phase I testing for solid tumors.

None of the companies responded to requests for comment.

However, data presented at the 2009 and 2010 American Society of Clinical Oncology and American Association of Cancer Research meetings showed that BEZ235 had no dose-limiting toxicities and had mild adverse events including nausea, diarrhea and anemia. XL765 also showed good tolerability with adverse events similar to those of BEZ235.[13]

*—TF*

more selective for PI3Kγ over the other isoforms."

An *in vitro* high throughput screen of the company's entire compound library revealed two hits that, with subsequent optimization, generated a series of about 30 small molecules that bound PI3Kγ with varying levels of potency and selectivity.

The four most potent and selective inhibitors had PI3Kγ $IC_{50}$ values of 5, 8, 18 and 34 nM and were all 10–500 times more selective for PI3Kγ over the other isoforms. The compounds also had good plasma exposure in mice and rats.

In a mouse model of inflammation-associated mast cell degranulation, all four compounds decreased degranulation compared with vehicle and did so at levels comparable to those seen in Pi3kγ knockout mice.

Finally, in a mouse model of chemokine-induced proinflammatory neutrophil recruitment, the 34 nM inhibitor lowered recruitment by 50% compared with vehicle ($p<0.05$).

Results were published in the *Journal of Medicinal Chemistry*.

"Based on the data we've gathered so far, we believe PI3Kγ inhibitors have broad-spectrum anti-inflammatory activity and could find therapeutic uses in autoimmune indications like rheumatoid arthritis, multiple sclerosis and lupus, as well as in airway inflammation diseases such as asthma and COPD [chronic obstructive pulmonary disease]," said Lamb.

Exelixis wants to partner its PI3Kγ inhibitor program. "We are not actively working on this program now, as almost all of the company's resources are focused on our lead cancer compound, cabozantinib," he said.

Cabozantinib (XL184), an inhibitor of c-Met receptor tyrosine kinase (MET; HGFR) and VEGF signaling, is in a Phase III trial to treat medullary thyroid cancer and in two Phase II trials to treat a broad range of solid cancers. Next month the company will present data on the trials at the annual American Society of Clinical Oncology meeting in Chicago.

Exelixis also is interested in licensing its preclinical PI3Kα inhibitor program, said Lamb. Earlier this year, the company exclusively licensed its preclinical PI3Kδ program to **Merck & Co. Inc.**

The PI3Kγ inhibitors in both papers are covered by patents.

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## COMPANIES AND INSTITUTIONS MENTIONED

**ActivX Biosciences Inc.**, La Jolla, Calif.
**Bristol-Myers Squibb Co.** (NYSE:BMY), New York, N.Y.
**Cellzome AG**, Heidelberg, Germany
**Evotec AG** (Xetra:EVT), Hamburg, Germany
**Exelixis Inc.** (NASDAQ:EXEL), South San Francisco, Calif.
**GlaxoSmithKline plc** (LSE:GSK; NYSE:GSK), London, U.K.
**Merck & Co. Inc.** (NYSE:MRK), Whitehouse Station, N.J.
**Novartis AG** (NYSE:NVS; SIX:NOVN), Basel, Switzerland
**Pathway Therapeutics Inc.**, San Francisco, Calif.
**Roche** (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
**Sanofi** (Euronext:SAN; NYSE:SNY), Paris, France
**State University of New York at Stony Brook**, Stony Brook, N.Y.
**Takeda Pharmaceutical Co. Ltd.** (Tokyo:4502), Osaka, Japan
**University of Fribourg**, Fribourg, Switzerland