PKD: cease and de-cyst

By Michael J. Haas, Senior Writer

Marketed treatments for polycystic kidney disease manage symptoms but do not slow disease progression. A research team led by **Genzyme Corp.** now has shown that blocking biosynthesis of the glycosphingolipid glucosylceramide could help treat the underlying causes of PKD.[1] The findings could represent a new indication for Genzyme's eliglustat tartrate, a glucosylceramide synthase inhibitor that is in Phase III testing for Gaucher's disease.

The most common causes of PKD are mutations in *polycystic kidney disease 1* (*PKD1*) or *PKD2*, both of which code for proteins that are expressed in the cilia of renal epithelial cells. Although different *PKD* mutations can produce a variety of symptoms, all forms of the disease are characterized by the development of cysts in the kidneys that compromise the organ's function and can lead to renal failure.

Studies in the 1990s showed that levels of two glycosphingolipids—glucosylceramide and lactosylceramide—were higher in the kidneys of PKD patients[2] and in mouse models of PKD than in those of healthy controls.[3] Glycosphingolipids regulate cell surface receptors and other functions in cell membranes that are essential to processes such as the cell cycle.

In prior studies in animal models of PKD, the Genzyme team had found that direct inhibition of the cell cycle prevented cystogenesis. For the new study, the team wanted to know whether indirect inhibition of the cell cycle—through blockade of glycosphingolipids—would have a similar effect on PKD models, team leader Oxana Ibraghimov-Beskrovnaya told *SciBX*.

The company has done previous work on glycosphingolipid metabolism and has already developed inhibitors of glucosylceramide synthase (GCS)—which catalyzes a key step in glucosylceramide biosynthesis—to treat Gaucher's disease. This gave the team access to a specific GCS inhibitor for its PKD studies.

First, the team analyzed kidney tissue samples from PKD patients and three mouse models of PKD to confirm that high levels of glucosylceramide are a hallmark of PKD.

The researchers then treated the three mouse models of PKD with the GCS inhibitor Genz-123346 and saw decreased kidney levels of glucosylceramide and reduced formation of kidney cysts compared with no treatment.

Analysis of kidney tissue from treated mice showed that inhibiting GCS blocked signaling between protein kinase B (PKB; Akt) and mammalian target of rapamycin (mTOR; FRAP; RAFT1). Previous studies by several other teams[4–6] had already suggested mTOR as a potential target to treat PKD.

"Our ultimate goal is to develop a therapy for PKD," Ibraghimov-Beskrovnaya said. The proof-of-concept study reported in *Nature Medicine* "demonstrates that there is a molecular link between cystogenesis and abnormal glycosphingolipid metabolism."

Ibraghimov-Beskrovnaya is VP of cell biology and distinguished scientific fellow at Genzyme. The team included a researcher from the **University of Michigan**.

"GCS inhibition seems to have effects on cell cycle progression, which may act together with reduction of Akt/mTOR activity to slow cyst growth," Ibraghimov-Beskrovnaya said. "Thus, with a single drug we may affect multiple pathways of cystogenesis, which might be of benefit" in the treatment of PKD.

Indeed, mixed results from two clinical trials reported last month in *The New England Journal of Medicine* suggest that direct mTOR inhibition may not be sufficient to treat PKD. In separate Phase III trials, a German-Austrian team found that **Novartis AG**'s everolimus mTOR inhibitor slowed cytogenesis but not the decline of renal function in patients with advanced PKD,[7] whereas a Swiss-Belgian team found that **Pfizer Inc.**'s sirolimus mTOR inhibitor had no effect on cystogenesis in patients with early-stage PKD.[8]

> **"GCS inhibition seems to have effects on cell cycle progression, which may act together with reduction of Akt/mTOR activity to slow cyst growth. Thus, with a single drug we may affect multiple pathways of cystogenesis."**
> *—Oxana Ibraghimov-Beskrovnaya, Genzyme Corp.*

Novartis' everolimus (RAD001) is approved to prevent renal and heart transplant failure and graft rejection. Pfizer's sirolimus is approved to treat renal transplant and to prevent renal transplant rejection.

Stefan Somlo, professor of internal medicine and genetics and chief of nephrology at **Yale School of Medicine**, noted that GCS inhibition had no apparent side effects in the kidneys of normal mice, suggesting the treatment was safe.

"Polycystic kidney disease is an important unmet need, and the development of novel therapeutic candidates is vital," said Gideon Bollag, SVP of research at **Plexxikon Inc.** The *Nature Medicine* paper "provides a novel approach to identify such a candidate."

Plexxikon and partner **Roche** are developing PLX5568 (R7376), a Raf kinase inhibitor in Phase I testing to treat PKD.

Bollag said the *Nature Medicine* paper did not fully address several key questions, such as when GCS becomes active during human disease, whether the enzyme is expressed primarily in cystic kidney cells and whether the biosynthetic products of GCS primarily target kidney cells during disease progression.

Ibraghimov-Beskrovnaya said the team is examining these questions in its mouse models. But "we would also be interested in verifying any findings in human tissues," she said.

Somlo also noted that "much of the data in the *Nature Medicine* paper are derived from mouse models that are not orthologous to human PKD."

An orthologous disease model has a defect in the same gene that causes the human form of disease, whereas a nonorthologous model has a defect in a different gene that gives rise to a disease that resembles the human condition. Ibraghimov-Beskrovnaya's team used an orthologous *Pkd1* knockout neonatal mouse model and two nonorthologous, adult mouse models with mutations in other genes that cause kidney cystogenesis.

"The confirmatory work in the orthologous early-onset mouse PKD model is reassuring, but additional studies in that model" and orthologous adult mouse models would strengthen the validation of GCS as a target to treat the human disease that results from *PKD* defects, Somlo said.

Bollag noted that testing GCS inhibition in an orthologous adult model "would parallel the sequence of validation studies for the vasopressin 2 receptor antagonists, which were first tested in nonorthologous models and subsequently showed efficacy in an adult orthologous PKD model."

The most advanced vasopressin 2 receptor antagonist for PKD is **Otsuka Pharmaceutical Co. Ltd.**'s tolvaptan (OPC-41061), which is in Phase III testing.

De-cysting persistence

Ibraghimov-Beskrovnaya pointed out that the orthologous and nonorthologous models used by her team each had its advantages and disadvantages. Thus she said the team would "continue to use whatever models seem most appropriate for the research questions we are trying to address."

Among those questions is what role other glycosphingolipids and related molecules, such as lactosylceramide and the ganglioside GM3, might play in PKD progression. "This follow-on work will be important to understanding which target within the glycosphingolipid pathway is the most relevant for the treatment of PKD," she said.

She said Genzyme is evaluating whether to advance undisclosed compounds that target the glycosphingolipid pathway into clinical development for the indication.

The company has not disclosed the patent status of the findings reported in *Nature Medicine*. "Although we have not planned to partner this program, we remain open to discussions which would accelerate patients' access to this therapy, particularly in emerging markets," said Ibraghimov-Beskrovnaya.

Haas, M.J. *SciBX* 3(26); doi:10.1038/scibx.2010.785
Published online July 1, 2010

REFERENCES

1. Natoli, T. *et al. Nat Med.*; published online June 20, 2010; doi:10.1038/nm.2171
   Contact: Oxana Ibraghimov-Beskrovnaya, Genzyme Corp., Framingham, Mass.
   e-mail: oxana.beskrovnaya@genzyme.com
2. Chatterjee, S. *et al. J. Lipid Res.* **37**, 1344–1344 (1996)
3. Deshmukh, G. *et al. J. Lipid Res.* **35**, 1611–1618 (1994)
4. Tao, Y. *et al. J. Am. Soc. Nephrol.* **16**, 46–51 (2005)
5. Wahl, P. *et al. Nephrol. Dial. Transplant.* **21**, 598–604 (2006)
6. Shillingford, J. *et al. J. Am. Soc. Nephrol.* **21**, 315–327 (2010)
7. Walz, G. *et al. N. Engl. J. Med.*; published online June 26, 2010; doi:10.1056/NEJMoa1003491
8. Serra, A. *et al. N. Engl. J. Med.*; published online June 26, 2010; doi:10.1056/NEJMoa0907419

COMPANIES AND INSTITUTIONS MENTIONED

Genzyme Corp. (NASDAQ:GENZ), Cambridge, Mass.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Otsuka Pharmaceutical Co. Ltd. (Tokyo:4768), Tokyo, Japan
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Plexxikon Inc., Berkeley, Calif.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
University of Michigan, Ann Arbor, Mich.
Yale School of Medicine, New Haven, Conn.