A RIPping target in Gaucher's disease

By C. Simone Fishburn, Senior Editor

Enzyme-replacement therapies are highly effective for most patients with Gaucher's disease but do little for those with the neurological childhood forms of the disease. Now, an Israeli and U.K. team has unlocked the mechanism of nerve destruction in neuronopathic Gaucher's disease and identified receptor-interacting serine-threonine kinase 3 as a new target.¹

The team is investigating possibilities for generating inhibitors of the protein kinase that cross the blood-brain barrier (BBB).

Gaucher's disease is caused by glucocerebrosidase (GBA; GCase) deficiency, which leads to accumulation of sphingolipids in lysosomes. In normal cells, GCase breaks down the lipid glucosylceramide (GlcCer). Mutations in *GCase* produce catalytically inactive or unstable enzyme, which results in accumulation of unmetabolized glucosylceramide.

Type I Gaucher's disease is the most common form and does not have a neurological component. Neuronopathic Gaucher's disease is divided into Types II and III.

Type II (infantile) Gaucher's disease affects newborns and is usually fatal within one to three years of birth.

Type III (juvenile) has a slower onset and often appears in early childhood with symptoms of abnormal eye movement followed later by seizures. The disease progresses more slowly than Type II Gaucher's disease, with patients generally living into their teens or twenties and sometimes longer.

Together, Types II and III affect about 5% of patients with Gaucher's disease in Western countries but are substantially more prevalent in Asian countries including the Indian subcontinent, China, Japan and Korea.

Companies have taken two main approaches to treating Gaucher's disease—enzyme replacement therapy (ERT) and substrate

reduction therapy (SRT)—with the common goal of preventing the buildup of lipids (see Table 1, “Gaucher's disease pipeline”).

ERT involves recombinant enzymes that replace the defective GCase. These enzymes do not cross the BBB and thus are not used in Types II and III disease.

SRT compounds prevent accumulation of the sphingolipids by inhibiting the synthesis of GCase substrates. These therapies can cross the BBB but have not shown efficacy in neuronopathic Gaucher's disease.

Although the pathway from defective GCase to lysosomal accumulation of lipids is well understood, little is known about the downstream part of the process, in which the lipid accumulation causes the cellular toxicity that underlies the disease.

A group headed by Tony Futerman at the **Weizmann Institute of Science** decided to look at how the buildup of lipids in lysosomes causes nerve cell destruction in Types II and III Gaucher's disease.

Futerman is a professor of biochemistry and director of The Nella and Leon Benoziyo Center for Neurological Diseases at Weizmann. His lab collaborated on

the study with researchers at the **University of Cambridge** and **University of Oxford**.

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—Neil Weinreb,
National Gaucher Foundation Inc.

Table 1. Gaucher's disease pipeline. Selected compounds on the market or in clinical development for Type I Gaucher's disease.

Source: BCIQ; BioCentury Online Intelligence

Company	Product	Description	Type of therapy	Status
Genzyme Corp.; Sanofi (Euronext:SAN; NYSE:SNY)	Cerezyme imiglucerase	Recombinant glucocerebrosidase (GBA; GCase) (rGCase)	Enzyme replacement therapy (ERT)	Marketed
Genzyme; Sanofi	Ceredase alglucerase	Alglucerase enzyme	ERT	Marketed
Protalix BioTherapeutics Inc. (NYSE-M:PLX; Tel Aviv:PLX); Pfizer Inc. (NYSE:PFE)	Elelyso alfataliglycerase	Plant cell-expressed rGCase	ERT	Marketed
Shire plc	Vpriv velaglucerase alfa	rGCase	ERT	Marketed
Actelion Ltd. (SIX:ATLN); UCB Group (Euronext:UCB)	Zavesca miglustat	Glucosyltransferase inhibitor	Substrate reduction therapy (SRT)	Marketed
Genzyme; Sanofi	Cerdelga eliglustat tartrate	Glucosylceramide synthase inhibitor	SRT	Phase III
Isu Abxis Co. Ltd. (KOSDAQ:086890)	ISU302	Biosimilar Cerezyme	ERT	Phase III
Protalix BioTherapeutics	PRX-112	Plant cell-expressed rGCase (oral)	ERT	Phase I
ExSAR Corporation	EXR-202 ^A	GCase-binding chaperone	Not applicable	Phase I

^AThe indication for EXR-202 may include neuronopathic Gaucher's disease.

Ripping ahead

In two mouse models of neuronopathic Gaucher's disease, the researchers found that the disease was not caused by apoptosis of neurons but by a different, less-extensively studied process called necroptosis.

Two of the most important proteins in necroptosis, receptor-interacting serine-threonine kinase 1 (Ripk1; Rip1) and Ripk3 (Rip3), were upregulated in symptomatic Gaucher's disease mice compared with controls.

The team also found higher RIPK1 levels in a postmortem human brain from a patient with Type II disease than in an age-matched control. The researchers were unable to test RIPK3 levels as they did not have a specific RIPK3 antibody.

Increased Ripk1 and Ripk3 expression also occurred in a mouse model of another neuronal lysosomal storage disease—Krabbe disease. However, levels were not altered in animal models of other lysosomal storage diseases including Niemann-Pick disease type C1, GM1 gangliosidosis and Sandhoff disease, despite the fact that some of these involve neuronal pathways.

Because *Ripk1* knockout mice did not survive more than three days, the team focused on RIPK3.

The most direct evidence of a central role for RIPK3 came when the team treated *Ripk3*^{-/-} knockout mice with a chemical that induces Gaucher's disease and found that the animals did not develop the disease.

The mice showed none of the weight loss or decreased motor coordination seen in the wild-type mice that received the compound. Knockouts lived for more than 100 days on average compared with an upper limit of 40 days for the symptomatic mice.

In addition, whereas the neuronopathic Gaucher's disease mice had increased signs of systemic inflammation in the liver and spleen, the chemical caused no such effects in the Ripk3-deficient mice. In contrast to the *Ripk1*^{-/-} mice, the *Ripk3*^{-/-} knockouts showed no overt pathology in their development or overall health.

Results were published in *Nature Medicine*.

Futerman told *SciBX* that the data suggest RIPK3 has two pivotal effects in the disease. The first is to cause nerve cell death via necroptosis, and the second is to promote inflammation in both the CNS and systemic organs.

From target to clinic

According to Neil Weinreb, the paper represents a conceptual breakthrough for neuronopathic Gaucher's disease. "The key question for the field has been how the process of storing lipids in Gaucher's causes cell death. This paper gets to the heart of that," he told *SciBX*. "If you can block the storage of lipids in lysosomes and neutralize their activity, that would be very good. This is the first major step in that direction."

Weinreb, a clinical Gaucher's disease practitioner, is a member of the board of the **National Gaucher Foundation Inc.** and regional coordinator of the International Collaborative Gaucher Group,

which runs the Gaucher Registry.

Futerman told *SciBX* that the initial challenge will be to synthesize RIPK3 inhibitors with pharmaceutical properties. According to Futerman, commercially available RIPK inhibitors do not cross the BBB and have half-lives of under an hour.

"We are very intrigued with the findings and are interested in validating the target—and if it is a major contributor in Gaucher's, Krabbe and other diseases, then we'd be interested in looking for antagonists as therapeutics," said Seng Cheng, head of research and early development in the rare diseases division at the **Genzyme Corp.** unit of **Sanofi**.

Cheng said that a key experiment would be to develop an antagonist and test it in an animal model. But he added that antisense oligonucleotides or RNAi could also be used in Gaucher's disease models to bolster the case for RIPK3.

However, finding the best translational path might not be simple because the patient population is so small, and electing whether to test proof of concept in Type II or Type III disease is complex.

Weinreb thinks that RIPK3 inhibitors might be better suited for Type III Gaucher's disease than Type II because its slower onset could increase the chance of starting treatment before excessive neuronal damage has occurred.

However, Norman Barton, VP of R&D at **Shire plc**, told *SciBX* that it will be hard to find appropriate endpoints for a clinical trial in patients with Type III disease because it progresses much more slowly than Type II.

The endpoints used in Type I disease would not be relevant for Types II and III.

Cheng said that Genzyme has joined with physicians and others in the field to develop outcome measures for neuronopathic Gaucher's disease and said that finding a biomarker would be a real boost.

According to Barton, patients with Type II disease would be a better population for demonstrating proof of concept if they can be identified early enough. In that group, the progression of the disease could enable any positive effect of a RIPK3 inhibitor to be detected more definitively and more rapidly.

Barton said that any attempts to achieve proof-of-concept for either Type II or Type III Gaucher's disease would likely require a partnership with a global commercial organization.

"But a large organization would not want to go solo on developing a RIPK3 inhibitor. They would want it to be a risk-shared activity involving an alliance with an academic center and venture people," he told *SciBX*.

According to Barton, rare diseases generally require alliances to move programs forward. The small numbers of patients, complexity of clinical trials and significant financial investment all contribute to a high risk that is better shared between multiple parties.

Cheng agreed that a multiparty effort is needed to take the findings forward as designing the right trials and finding patients for

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rare diseases can be difficult for a single company to do successfully. He said that Genzyme traditionally operates by including patients, a foundation and a commercial partner.

Gregory Macres, founder of the **Children's Gaucher Research Fund**, which focuses on neuronopathic Gaucher's disease and supported the *Nature Medicine* study, said that his organization is seeking ways to participate in such a consortium.

A provisional patent application has been filed in the U.S. by **Yeda Research and Development Company Ltd.**, the technology transfer company of the Weizmann Institute. The IP is available for licensing.

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Contact: Anthony H. Futerman, Weizmann Institute of Science, Rehovot, Israel
e-mail: tony.futerman@weizmann.ac.il

COMPANIES AND INSTITUTIONS MENTIONED

Children's Gaucher Research Fund, Granite Bay, Calif.
Genzyme Corp., Cambridge, Mass.
National Gaucher Foundation Inc., Tucker, Ga.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
Shire plc (LSE:SHP; NASDAQ:SHPG), Dublin, Ireland
University of Cambridge, Cambridge, U.K.
University of Oxford, Oxford, U.K.
Weizmann Institute of Science, Rehovot, Israel
Yeda Research and Development Company Ltd., Rehovot, Israel