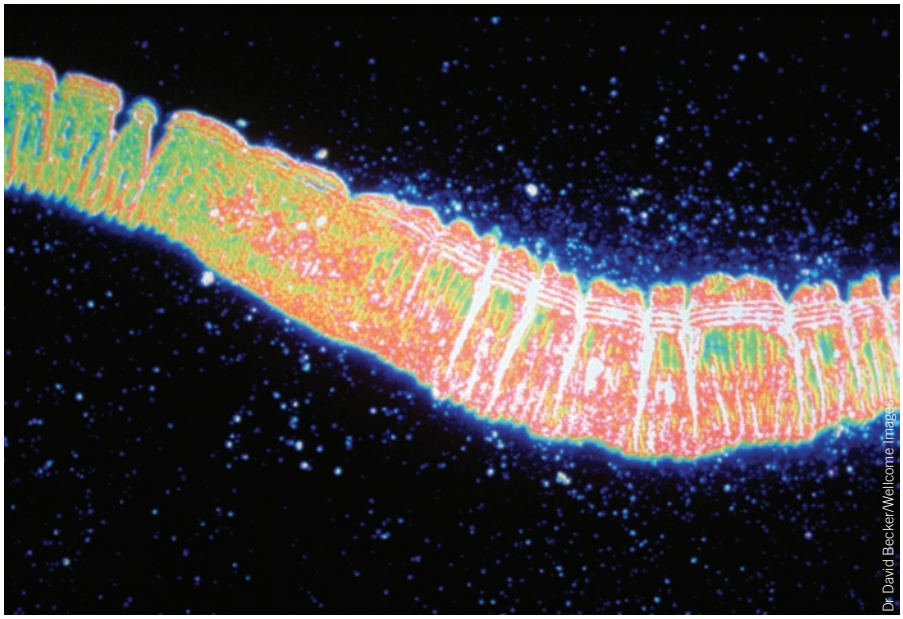
GSK/Sirtris compounds dogged by assay artifacts

In January, scientists at Pfizer Global Research and Development, in Groton, Connecticut, reported that small molecules developed by Cambridge, Massachusetts–based Sirtris Pharmaceuticals do not activate the sirtuin pathway that has been linked to longevity. The disconcerting discovery, published in *The Journal of Biological Chemistry* (published online, doi:10.1074/jbc.M109.088682, 8 January 2010), is not the first to cast doubt on compounds that target sirtuins. In a paper published last November (*Chem. Biol. Drug Des.* **74**, 619–624, 2009), researchers at Amgen of Thousand Oaks, California, also showed that a purported anti-aging compound in red wine, resveratrol, doesn't act on the pathway either. These findings are fueling skepticism not just concerning Sirtris and its resveratrol-like compounds but also about the due diligence process at London-based GlaxoSmithKline, which purchased the Cambridge, Massachusetts–based biotech in April 2008 for an eye-popping $720 million (*Nat. Biotechnol.* **26**, 595, 2008).

The controversy over Sirtris drugs reached a tipping point in January with a publication by Pfizer researchers led by Kay Ahn showing that resveratrol activates SIRT1 only when linked to a fluorophore. Although Ahn declined to be interviewed by *Nature Biotechnology*, a statement issued by Pfizer says the group's findings "call into question the mechanism of action of resveratrol and other reported activators of the SIRT1 enzyme."

Most experts, however, say it's too soon to write off Sirtris' compounds altogether, assuming they're clinically useful by mechanisms that don't involve sirtuin binding. And for its part, GSK won't concede that Sirtris' small molecules don't bind the targets. In an e-mailed statement, Ad Rawcliffe, head of GSK's WorldWide Business Development group, says, "There is nothing that has happened to date, including the publication [by Pfizer,] that suggests otherwise."

The evidence Sirtris brought to the table came with a complicated history, which GSK claims to have been well aware of. Scientists have for years expressed skepticism about the company's core premise: that sirtuins emulate the anti-aging benefits of calorie restriction, and that by activating sirtuins with drugs, it's possible to treat age-related diseases (*Nat. Biotechnol.* **26**, 371–374, 2008). In 2005, Matt Kaeberlein, of the University of Washington, Seattle, published the first data showing that calorie restriction doesn't activate sirtuins in yeast (*Science* **310**, 1193–1196, 2005). Those data have since been replicated in othera



In yeast and worms as the *C. elegans* pictured above, sirtuins can extend lifespan by up to 70 percent. Sirtris hopes to develop a pill that might do the same for humans, or at least ward off the diseases of aging.

laboratories, he says. In mice, calorie restriction triggers the SIRT1 enzyme, but the company's small molecules target SIRT1 in only some tissues and not all, Kaeberlein adds. Also in 2005, Kaeberlein and John Denu, from the University of Wisconsin, Madison, independently showed that resveratrol activates SIRT1 only when the sirtuin is bound to a fluorescent label. Pfizer scientists also report that Sirtris' small molecules, including SRT501—currently in phase 2 clinical trials to treat type 2 diabetes—bind SIRT1 only in the presence of this fluorophore. These drugs do, however, bind something: unlabeled native proteins, which presumably may influence sirtuin pathways in the cell. "We still don't know what resveratrol or the Sirtris compounds actually bind to in cells," says Kaeberlein.

In an e-mailed response, GSK's Rawcliffe states that the company knew SIRT1 modulation *in vitro* is complicated and was aware of the controversies surrounding the fluorescence-based assay and the precise mechanism of action of the published compound(s). Nonetheless, Rawcliffe says GSK remains confident that the activity seen in cell-based and animal studies are acting through an SIRT1-dependent mechanism.

As to GSK's due diligence in the Sirtris deal, Rawcliffe remains confident that their process did an "excellent job in allowing us to understand the full view of the scientific field and to place Sirtris in perspective." Even so, unsubstantiated comments on Derek Lowe's 'In the Pipeline' blog (http://pipeline.corante.com/) allege that the deal went through against the advice of some internal GSK scientists and that similar due diligence processes on the Sirtris compounds at other companies, such as Basel, Switzerland–based Novartis and Amgen, had raised flags about artifacts in the assays.

According to Uwe Schoenbeck, Pfizer's chief science officer for external R&D and innovation (who did not comment directly on either GSK or Sirtris), due diligence is essentially a risk assessment. "You're looking to make the best educated guess about a company with the data available to you," he says. Pharmaceutical companies are generally looking for a strategic fit, he explains, and trying to identify opportunities that neither they nor the acquired firms can exploit in isolation. The first step is a nonconfidential exchange of data and information. Assuming both parties share mutual interest in a deal, the due diligence then moves on to an exchange of confidential information coordinated in part through a materials transfer agreement. This gives both parties access to what's known as a confidential data package containing sensitive information—raw data and chemical structures of lead compounds, for instance—that showcase a biotech's competitive advantage.

Due diligence investigators represent cross-sectional areas of expertise—discovery and development, toxicology and intellectual property, among others—assembled under a single lead, Pfizer's Schoenbeck says. According to another due diligence expert, who requested not to be identified, GSK's aim in buying Sirtris would have been to secure access to lead compounds, with proof of concept for therapeutic utility. That view was confirmed by Rawcliffe, who said the promise that sirtuin biology could yield "transformational medicines" was apparent to GSK during the acquisition. The fact that GSK paid $720 million for Sirtris ($22 a share, as compared with the market's valuation of $12 a share) also suggests that GSK was competing with other suitors, which drove up the offer price.

How each company assesses the promise and value of a biotech's assets, however, varies "philosophically" from pharma company to pharma company, Schoenbeck says. Pharma scientists engaged in due diligence may try to replicate a biotech firm's results in a pilot study. This is especially true when the data are uncertain, as would have been the case with Sirtris. Peter DiStefano, chief scientific officer with Cambridge, Massachusetts–based Elixir Pharmaceuticals, claims the commercial Fluor-de-Lys fluorometric detection assay kit from Enzo Lifesciences that Sirtris relies on for binding evidence can be unreliable, given that compounds often bind to the fluorophore itself. Results from that test should be confirmed with counterscreens, and more expensive and cumbersome radiolabeling assays that provide more definitive conclusions, he says. GSK's Rawcliffe would not say whether the company had used counterscreens in its due diligence. And when asked whether the company had done its own pilot study of the Sirtris compounds, he appeared to answer in the negative. "As part of this diligence, we investigated these controversies in a number of ways," he wrote. "[That included] speaking to many people on both sides of the argument. We are satisfied with the outcome of that process."

Sources interviewed for this article speculate that GSK and Sirtris might have more convincing data that they haven't yet shared with the public. And for his part, Rawcliffe claims GSK/Sirtris are planning to publish results that, he says, elucidate how their small molecules might activate SIRT1 and how that relates to disease processes.

But Brian Kennedy, also from the University of Wisconsin, counters that Sirtris has been threatening to publish these data for years. When Kennedy was a postdoctoral student at the Massachusetts Institute of Technology, he studied under Leonard Guarente, who is now an advisor to Sirtris. He's since published findings showing that resveratrol does not bind the yeast sirtuin SIR2 (*J. Biol. Chem.* **280**, 17038–17045, 2005). And together with Kaeberlein, he found that calorie restriction does a good job at extending yeast lifespan even in a *SIR2*-gene knockout strain, suggesting the two pathways are unrelated. Kennedy says he's perplexed when Sirtris scientists—notably the company's cofounder David Sinclair—claim possession of compounds that bind SIRT1 more effectively than resveratrol, when data suggest that resveratrol doesn't bind SIRT1 in the first place. "It's possible that Sirtris and GSK have information that resolves these issues," he concedes. "I haven't seen it, so at this time I remain skeptical. I agree their motivation isn't necessarily to enlighten the public, but you have to wonder by this point why they're holding it back."

At the same time, Kennedy acknowledges that resveratrol and other purported sirtuin activators do seem to confer metabolic benefits in rodents, even if they don't extend lifespan. "That's a conundrum," he says. These metabolic benefits were recently refuted in the new Pfizer paper, which found that the Sirtris compounds don't lower plasma glucose in obese mice fed high-fat diets, as reported earlier by Sirtris scientists. GSK spokesperson Janet Morgan attributes those contradictory findings in part to impurities in Pfizer's prepared versions of the Sirtris compounds. And Kaeberlein says that of all Pfizer's new findings, these appear to be the least robust. "[Pfizer] didn't seem to make a strong case for this," he says. "And if they had, that would have been a surprise, because of all the things resveratrol might do, its benefits in diabetes and obesity seem to be the most believable." Both Kennedy and Kaeberlein suggest resveratrol and resveratrol-like compounds might yield health benefits through other pathways.

Thomas Hughes, president and CEO of Cambridge, Massachusetts–based Zafgen, says he's not surprised that GSK went after Sirtris, despite the controversial nature of its research. "The whole field of drug discovery has been incredibly energized by this prospect of anti-aging biology and its influence on new pathways that could work in diseases like Alzheimer's and diabetes," he says. "And when you have something so potentially disruptive, it's rare to have a situation where you can't pull equal stacks of papers that support or refute the idea."

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