Moving upstream in Huntington's

By Lauren Martz, Staff Writer

Researchers at **Harvard Medical School** and **The Scripps Research Institute** each have published new strategies to treat Huntington's disease, a condition for which there is only one approved drug (*see Table 1, Attacking Huntington's disease*).^{1,2} The Harvard group showed that inhibiting cytochrome C release from the mitochondria might effectively combat the disease. The Scripps team described a more upstream approach—using histone deacetylase (HDAC) inhibitors to curb transcriptional dysregulation.

Companies and academics interviewed by *SciBX* had safety concerns about both approaches, but they think that if these issues are addressed, the strategies could be used together.

Huntington's disease (HD) is a neurodegenerative disorder caused by CAG repeats in the *huntingtin* (*HTT*) gene that manifest as a range

of motor and cognitive defects. *HTT* is a 3,144 amino acid-long protein involved in many protein-protein interaction networks.³

Although HD is a monogenic disorder, the therapeutic challenge is posed by the myriad phenotypic effects that can culminate in neurodegeneration. Moreover, the exact route of pathogenesis is still ill defined. Known contributors—and thus potential points of therapeutic intervention—include transcriptional dysregulation, mitochondrial dysfunction, aberrant protein aggregation, excitotoxicity and disruptions in cellular trafficking.⁴

Stop the apoptosis

In a paper published in the *Journal of Neuroscience*, Robert Friedlander and colleagues at Harvard showed that intervention at the stage of mitochondrial dysregulation might provide neuroprotection. Friedlander is vice chairman of the Department of Neurosurgery and associate director of cerebrovascular surgery at **Brigham and Women's Hospital**.

Friedlander's team screened 1,040 neurological compounds on the market and in development by testing them on purified mitochondria. They identified 21 compounds that potently inhibited cytochrome C release. Of those, 16 also lowered cytochrome C release from mitochondria and lowered cell death in ST14A cells with mutated *HTT*.

The researchers chose one of the most potent compounds, methazolamide, for further analysis. Methazolamide is a generic approved to treat glaucoma.

Table 1. Attacking Huntington's disease. A number of companies are attempting to treat Huntington's disease (HD) by using different mechanisms. Some are targeting the disease by repairing the mutated protein responsible for the disease or eliminating its aggregation, whereas others are working further downstream at the points of mitochondria dysfunction, oxidative stress or excessive apoptosis, which all cause neuronal damage. Still other companies have compounds that protect or repair the neurons from the effects of the mutated protein. Selected compounds in development and on the market for HD are listed below.

Category	Company	Product	Description	Status
Aberrant apoptosis	Neurologix Inc. (OTCBB:NRGX)	dXIAP	Mutated form of the gene <i>X-linked inhibitor of apoptosis</i> (<i>XIAP</i>) delivered by adeno-associated virus (AAV) vector	Preclinical
Neuroprotection	Ovation Pharmaceuticals Inc./ Biovail Corp. (NYSE:BVF; TSX:BVF)/ Cambridge Laboratories Ltd.	Xenazine tetraabenazine	Selective inhibitor of vesicular monoamine transporter 2 (VMAT2)	Marketed
	NeuroSearch A/S (CSE:NEUR)	ACR16	Dopamine stabilizer	Phase III
	ReNeuron Group plc (LSE:RENE)	ReN005	Neuronal stem cell transplantation	Preclinical
Mitochondrial dysfunction	Amarin Corp. plc (NASDAQ:AMRN)/ Scil Group	Miraxion (AMR-101)	Eicosapentaenoic acid derivative that inhibits phospholipase A ₂ (PLA ₂) and caspases	Phase III
	Medivation Inc. (NASDAQ:MDVN)/ Pfizer Inc. (NYSE:PFE)	Dimebon	Small molecule that blocks an undisclosed target involving mitochondrial pores	Phase II
Mutated protein	Alnylam Pharmaceuticals Inc. (NASDAQ:ALNY)/Medtronic Inc. (NYSE:MDT)	ALN-HTT	Small interfering RNA (siRNA) against the <i>huntingtin</i> (<i>HTT</i>) gene delivered by Medtronic's implantable infusion pump	Preclinical
	Targeted Genetics Corp. (NASDAQ:TGEN)/ Merck & Co. Inc. (NYSE:MRK)	siRNA/AAV1	AAV vector delivering siRNA targeting <i>HTT</i>	Preclinical
Neuronal repair	Ceregene Inc.	CERE-120	AAV Type 2 vector encoding <i>neurturin</i> (<i>NTN</i>) gene	Preclinical
Oxidative stress	Avicena Group Inc. (Pink:AVCE)	HD-02	Therapeutic that incorporates a clinical form of creatine	Phase II
Protein aggregation	Prana Biotechnology Ltd. (ASX:PBT; NASDAQ:PRAN)	PBT2	Metal protein-attenuating compound (MPAC)	Phase I

In HD cell lines, the drug inhibited release of both cytochrome C and the proapoptotic mitochondrial protein Smac/Diablo. In a mouse model of HD, methazolamide delayed disease onset, extended lifespan and lowered both cytochrome C release and neuronal death compared with the effects of saline controls.

“The release of cytochrome C occurs fairly early in the cell death process, so it is a good step to target,” Friedlander told *SciBX*.

He added that many of the compounds addressed by the group’s screening approach are already on the market for other indications. “These are FDA-approved drugs. We already know that methazolamide is safe and can cross the blood-brain barrier,” Friedlander said.

“In theory, this is a shorter pathway to an approved compound for Huntington’s disease treatment” compared with the discovery of new compounds, said Robert Pacifici, CSO of the **CHDI Foundation Inc.** “But it is important to look out for pitfalls. For example, minocycline, which is an approved antibiotic that was identified in the screen, was used in clinical testing for amyloid lateral sclerosis and the trials were halted because the compound was actually making people worse.”

CHDI is a not-for-profit organization that supports the discovery and development of HD therapeutics.

Pacifici was also concerned that the compounds in the *Journal of Neuroscience* article might have effects on processes other than cytochrome C release. He noted that methazolamide is primarily a carbonic anhydrase inhibitor, so it has at least one other function.

Other researchers were concerned about the long-term effects of shutting off apoptosis in HD patients.

“There is concern that apoptosis is necessary to prevent cancer. Therefore, an increased risk of cancer could be an adverse effect” of an apoptosis inhibitor, said Ashley Bush, Australian Research Council Federation Fellow at the **Mental Health Research Institute of Victoria** and a founding scientist of **Prana Biotechnology Ltd.**

Prana’s PBT2 is a metal protein-attenuating agent that blocks generation of reactive oxygen species and subsequent apoptosis. The compound has completed Phase I testing to treat HD.

David Rubinsztein, a professor of molecular neurogenetics at the **University of Cambridge**, agreed that cancer is a potential issue. “There is a chance that inhibiting apoptosis could increase the risk of cancer, and, with Huntington’s disease, a long-term treatment is necessary, which could increase that risk,” he said.

Potential safety issues aside, Pacifici questioned whether inhibiting apoptosis is the most efficacious way to treat HD. “You are preventing the neurons from dying, but it is not clear whether having a sick neuron is actually better than having a dead neuron,” he said.

Friedlander’s team showed that methazolamide delayed disease onset and extended lifespan by up to 27% and 20%, respectively, in mice. He said the most important next step for his group is testing their findings in the clinic.

Belinda Tsao-Nivaggioli, chairman and CEO at **Avicena Group Inc.**, agreed that clinical trials are the logical next step. “In Huntington’s disease, if a therapeutic shows activity of increasing life by about 17–20% in preclinical models, it is usually worth moving forward into clinical testing,” she said.

Avicena’s HD-02, a therapeutic that incorporates a clinical form of creatine, has completed Phase II testing in HD.

Brigham and Women’s Hospital of the Harvard Medical School has filed a patent application for the inhibitors of cytochrome C release. The compounds are available for licensing worldwide, said Friedlander.

Transcription prescription

In a paper in the *Proceedings of the National Academy of Sciences*, Elizabeth Thomas and colleagues at Scripps used a new HDAC inhibitor, HDACi 4b, to correct the transcriptional dysregulation associated with HD.

Her team showed that HDACi 4b was not cytotoxic *in vitro*, and it caused a twofold increase in acetylation of H4, which opens the chromatin structure to facilitate transcription. In a mouse model of HD, the compound improved motor performance and coat appearance compared with the effects of vehicle controls.

Compared with the control group, mice receiving HDACi 4b also lost less body and brain weight.

Thomas, assistant professor in the Department of Molecular Biology at Scripps, told *SciBX* that transcriptional dysregulation is one of the earliest pathological mechanisms in HD. Even though the exact pathway of disease pathology has not been confirmed, she said it is thought that the defects in gene expression precede mitochondrial dysfunction and therefore inhibition

of HDAC might act upstream of inhibitors of cytochrome C release.

“Because transcriptional dysregulation is very likely a core pathogenic mechanism of disease, our compounds should also help relieve the downstream pathologies,” said Thomas.

“We’ve looked at the effects of the compounds at the beginning of symptoms and are now looking at the effects of administration before symptom onset. If we find that it works better before symptoms occur, genetic screening and early treatment could be more effective ways to treat the disease,” she noted.

Thomas also said her lab is working to resolve toxicity issues associated with HDAC inhibitors. The first marketed HDAC inhibitor, Zolinza vorinostat (suberoylanilide hydroxamic acid) from **Merck & Co. Inc.**, is associated with side effects including nausea, vomiting and diarrhea, tiredness, upper respiratory infection, deep vein thrombosis and pulmonary embolus. The pan-HDAC inhibitor is approved to treat cutaneous T cell lymphoma (CTCL).

“The toxicity profile would be unacceptable” in HD patients, said Pacifici. “Pan inhibitors are not the way to go.”

Thomas said that Scripps has filed a patent for HDACi 4b and other similar compounds. **Repligen Corp.** holds rights to the patent and is conducting preclinical work to determine ideal doses and metabolic effects of the compounds.

“More than a dozen HDAC enzymes have been identified, and we are targeting members of class 1,” said Repligen president and CEO Walter Herlihy. “The compounds have high inhibitory potential for HDAC3 specifically.”

He added that “we are analyzing toxicity and pharmacology, which includes bioavailability and the ability of the compounds to cross the blood-brain barrier.”

Herlihy told *SciBX* that Repligen hopes to identify a pharmaceutical

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**—Robert Pacifici,
CHDI Foundation Inc.**

candidate by the end of this year and to start Phase I testing in 2009, pursuing the initial indication of Friedreich's ataxia (FA).

Combinations

Although the treatment strategies presented by the Harvard and Scripps teams are different, they are not necessarily competing approaches. Indeed, companies and institutions contacted by *SciBX* hope both strategies could fit into the treatment space.

"I think a cocktail-type approach will be the best answer for this disease, as it is for cancer and HIV," said Friedlander.

Tsao-Nivaggioli agreed. "Probably in the end it will be combination treatment. We will still need to rely on some symptomatic treatments and some to slow disease progression," she said.

In addition to symptomatic treatments, the HDAC- and cytochrome C-inhibiting approaches could be coupled with therapies that aim to fix the source of the disease: the mutated protein.

Rubinsztein said exclusively targeting downstream effects such as neuron damage might not address all of the pathogenic mechanisms of the disease, and patients might see little efficacy. "It would be more appealing to remove the toxic protein itself," he said.

Ideally, an HD therapy would correct the mutant gene. However, said Rubinsztein, "one would have to chop out the extra repeats and would have to do it in a high enough proportion of relevant cells in the brain. There might be a way to do it in a limited number of cells, but this poses quite a challenge."

He concluded: "The next best thing would be to remove the toxic protein."

Rubinsztein's lab is currently trying to enhance degradation of the mutated protein.

The CHDI foundation and **Isis Pharmaceuticals Inc.** are trying to lower mutant HTT protein expression with antisense oligonucleotides under an agreement that was expanded last year.

"One important thing to consider is that rodent studies have proven that htt is an essential protein and its knockdown is embryonically lethal. We now need to determine how much can be knocked down for therapeutic benefit and how much would be detrimental," Pacifici said.

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