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REPRINT FROM OCTOBER 20, 2016

PRODUCT R&D

CHAPERONING THE DJ

By Karen Tkach, Staff Writer

In the latest example of post-dopamine thinking in Parkinson's disease, Cantabio Pharmaceuticals Inc. has moved upstream of the neurotransmitter with a strategy to block the protein misfolding and oxidative damage that drive neurodegeneration in the disease. Rather than the common approach of boosting dopamine signaling, Cantabio's strategy centers on enhancing the function of DJ-1, a target that is both a chaperone and an oxidative stress sensor, to rein in both pathways with a single compound.

"These two processes actually result in killing brain cells and causing the symptoms of the disease, and eventually the death of the patients," said CEO Gergely Toth.

The mainstay of PD regimens still involves therapies that compensate for the loss of dopaminergic neurons by augmenting levels of the neurotransmitter or sensitizing cells to its effects. However, because that approach only treats the disease's symptoms — not its cause — and is fraught with side effects, many researchers have shifted focus to disease modifying therapies that can slow cell death, in part by targeting proteins genetically linked to the disease, such as α-synuclein and LRRK2.

At least seven clinical-stage compounds target α-synuclein for PD, including three mAbs, two small molecule inhibitors, and two vaccines. And while no LRRK2-targeting agents have yet reached the clinic, at least five companies are investigating small molecule inhibitors that block the target's kinase activity.

DJ-1 mutations have also been linked to PD, but unlike α-synuclein and LRRK2 which contribute to PD pathology through aggregation and toxic gain-of-function, respectively, DJ-1 normally has a protective role that is lost in the disease, according to Cantabio.

The goal in targeting DJ-1, therefore, is to promote its function by developing pharmacological chaperones that can stabilize it and prevent misfolding.

Last month at the World Parkinson Congress, the company presented data for the first time on its DJ-1 chaperone program (see "Chaperoned Pairing").

Toth told BioCentury the chaperones are the "backbone of Cantabio's scientific approach." Because the small molecules can cross the blood-brain barrier, bind disease targets and either prevent misfolding or block aggregation, the approach can be applied to a variety of neurodegenerative diseases, he said.

At the front of the pipeline are CB101 and CB102, which each enhance DJ-1 function by stabilizing its dimeric conformation.

The company is also developing pharmacological chaperones for two Alzheimer's disease targets, β-amyloid and tau, where the idea is to stabilize each molecule's monomeric form to prevent aggregation.

BIOCENTURY PRODUCT PROFILE	
INNOVATION STAGE	
Product	A compound that stabilizes the dimeric form of DJ-1, a chaperone protein and oxidative stress sensor
Concept	Promoting the functional form of DJ-1 can block two of the pathways that lead to cell death in PD: the misfolding and aggregation of DJ-1 client proteins, including α-synuclein; and oxidative damage
Disease	Parkinson's disease
Competition	Agents that increase dopamine levels or sensitivity; compounds that directly block α-synuclein aggregation; LRRK2 inhibitors; stem cell therapies
Differentiation	Blocks the neuronal death that leads to dopamine loss; targets multiple disease pathways with a single compound
Administration	Oral
Risks	Off-target effects of enhancing DJ-1 function
Development status	Preclinical
Patents	Patent application filed
Company	Cantabio Pharmaceuticals Inc.

DJ MASTERMIND

DJ-1 is itself a chaperone that binds and prevents aggregation of α-synuclein, a major component of Lewy bodies, and prevents misfolding of other proteins.

In addition, DJ-1 senses oxidative stress and acts as a transcriptional activator. "It initiates overexpression of a number of proteins that counteract oxidative stress," said Toth. "It also initiates the overexpression of chaperones such as heat shock proteins, which protect against protein misfolding."

He added: "We think the advantage of targeting DJ-1 is that it's a sensor and a switch. If you can modulate that in the right way, you get a systematic response which we call redox protein homeostasis."

According to Toth, Cantabio is the first company to develop compounds targeting DJ-1, despite the target's established link to PD. He said that's because DJ-1 is not a typical drug target. "It's activated by oxidation, and as far as I know there are no drugs out there that target proteins like that. It's different; it's not a protease, it's not a kinase, it's not a GPCR," said Toth.

Consequently, Cantabio is hedging its bets by developing an alternative approach to increase the levels of functional DJ-1 in the brain. The company's CB201 is an engineered version of the target fused with a cell-penetrating peptide for delivery into the CNS.

KEEP IT TOGETHER

In the poster presentation, Cantabio detailed how it identified and characterized its DJ-1 pharmacological chaperones.

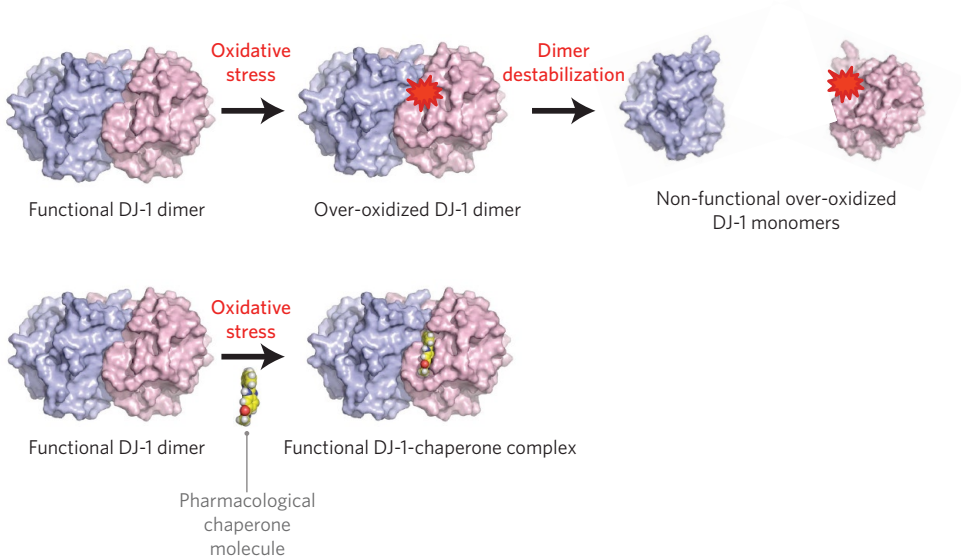
CHAPERONED PAIRING

Cantabio Pharmaceuticals Inc. (OTCQB:CTBO) is developing pharmacological chaperones to prevent protein misfolding in neurological diseases. The company's lead program for Parkinson's disease comprises chaperone molecules that stabilize the dimeric conformation of DJ-1, a protein that senses and coordinates cellular responses to oxidative stress and blocks α-synuclein aggregation.

When a **functional DJ-1 dimer** is subjected to pathological **oxidative stress**, such as that caused by the PD-associated herbicide rotenone, the protein can become **over-oxidized**, leading to **dimer destabilization**, in which DJ-1 loses its dimeric conformation to produce **non-functional over-oxidized DJ-1 monomers**. Loss of functional DJ-1 dimers results in cellular accumulation of oxidative damage and α-synuclein aggregates, which can drive cell death that contributes to PD pathology.

In the presence of a **pharmacological chaperone molecule**, DJ-1 subjected to oxidative stress can maintain its function through the formation of a **functional DJ-1-chaperone complex**, which stabilizes the dimeric conformation and restores DJ-1 oxidation to baseline levels.

α-synuclein (SNCA); DJ-1 (PARK7). *Source: Cantabio Pharmaceuticals Inc.*



Through a partnership with NovAliX S.A.S., Cantabio used a high throughput, surface plasmon resonance (SPR)-based chemical microarray to screen over 110,000 compounds for DJ-1 binding. The screen identified over one hundred hits that fell into eleven different molecular scaffold classes.

Toth noted the tightest binders did not make the best candidates, since they tended to inhibit the protein. "Our goal is not to interfere with the target's native function," he said.

> "It's activated by oxidation, and as far as I know there're no drugs out there that target proteins like that. It's different; it's not a protease, it's not a kinase, it's not a GPCR."
>
> Gergely Toth, Cantabio

The company selected a subset of hits spanning a broad range of structural classes for evaluation in cellular models, in collaboration with researchers at Purdue University and the University of Antioquia. The authors described results from a representative compound that contained a molecular scaffold that differed from those in CB101 and CB102.

The researchers showed the compound had protective properties in two sources of neuronal cells treated with PD-linked herbicides that cause oxidative damage. One was a human neuroblastoma cell line treated with paraquat, in which the compound dose-dependently prevented cell death. The other involved neurons derived from human mesenchymal stem cells treated with rotenone, in which the compound blocked accumulation of reactive oxygen species (ROS), mitochondrial damage, and activation of the pro-apoptotic regulator caspase-3.

The compound also increased survival in a *Drosophila* model of paraquat poisoning.

Using two-dimensional nuclear magnetic resonance (NMR), along with computational docking and molecular dynamics simulations, the company identified the compound's likely binding interface on DJ-1, and predicted that it boosts resilience to oxidative stress by stabilizing the dimeric structure of DJ-1.

Toth said Cantabio uses computational methods to dissect the structural and biophysical effects of each of its small molecule chaperone candidates.

"We put in effort toward understanding the mechanism of action — where these compounds bind, what they do to the biophysics of our protein targets, and what do they do to their function," he said. "We have a very strong biophysical focus, because protein misfolding is a biophysical process."

The company also presented data showing the compound prevented excessive oxidation of DJ-1 in the stem cell-derived neurons. "We have a lot of evidence that the pharmacological chaperones do more than just stabilize," said Toth.

REDOX JOCKEY

Toth told BioCentury DJ-1-stabilizing chaperones might be useful for treating a variety of diseases involving oxidative stress, including both familial and sporadic forms of PD, and other neurodegenerative disorders such as AD.

"There are mutations that cause DJ-1 to lose its function via structural instability. As a result, DJ-1 is not able to fold fully, and so its not able to perform its native function," he said.

But Toth said wild-type DJ-1 is also susceptible to misfolding, which may underlie some cases of sporadic PD.

"There's a lot of evidence showing that when it becomes overoxidated, DJ-1 loses its function because it loses its structural integrity," he said. "There is a key cysteine residue which is highly reactive, and as oxidative stress rises, it gets oxidated very quickly."

Toth told BioCentury that Cantabio plans to pursue DJ-1 in the context of AD in the future, but is prioritizing PD due to its genetic association with the target.

He noted DJ-1's multifaceted function in cells, and likelihood of playing different roles in distinct cellular contexts and disease states, means the company will be on the lookout for off-target effects as it moves from flies into mammalian models.

"Models suggest overexpression of DJ-1 doesn't cause any toxicity," Toth said. "But it's possible enhancing DJ-1 could do some damage, so getting the pharmacology of our therapeutic candidates right will be very important."

Cantabio has filed patent applications on various DJ-1 pharmacological chaperones, and expects to submit INDs for its final PD clinical candidates in 2018.

COMPANIES AND INSTITUTIONS MENTIONED

Cantabio Pharmaceuticals Inc. (OTCQB:CTBO), Sunnyvale, Calif.
NovAliX S.A.S., Illkirch, France
Purdue University, West Lafayette, Ind.
University of Antioquia, Medellin, Colombia

TARGETS AND COMPOUNDS

α-synuclein (SNCA)
Caspase-3 (CASP3; CPP32)
DJ-1 (PARK7)
LRRK2 - Leucine-rich repeat kinase 2
tau (MAPT; FTDP-17) - microtubule-associated protein τ

REFERENCES

Toth, G., et al. "Identification of novel biologically active DJ-1 small molecule modulators with activity in cellular and in vivo models of oxidative stress relevant to Parkinson's disease." *Presented at the World Parkinson's Congress* **(2016).**

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