High throughput remyelination

By Kai-Jye Lou and Lev Osherovich, Senior Writers

Remyelinating compounds are highly sought after but have been difficult to identify in part because there is a dearth of high throughput screening platforms to detect remyelination. Researchers at the **University of California, San Francisco** have now developed an assay to visualize the process in a microtiter plate and used it to identify compounds that can promote the process.¹

The screening platform has been licensed by **Inception 5 Inc.**, a built-to-buy incubator company partnered with **Roche**.²

Oligodendrocytes are glial cells that produce the myelin coating around the axons of CNS neurons. This insulating, lipid-rich coating allows the neuron to transmit action potential signals in an efficient manner.

In multiple sclerosis (MS), inflammatory immune cells attack the myelin coating. Loss of this coating results in less efficient action potential transmission in neurons and leads to the progressive neurological and neuromuscular deficits seen in MS. Although there are multiple drugs on the market that can diminish relapses associated with MS, none has been shown to reverse the course of the disease or promote remyelination. And only a handful of remyelinating compounds are in clinical development.

Jonah Chan, an associate professor in the Department of Neurology at the **University of California, San Francisco School of Medicine**, told *SciBX* that screens for compounds that promote remyelination have been designed to look for expression of myelin proteins in cells, but there was no way to know if these cells were making functional myelin.

Last October, researchers at **The Scripps Research Institute** reported on the use of such a system to screen a library of about 100,000 small molecules.^{3,4}

Chan noted that although cocultures of neurons and oligodendrocytes can recapitulate functional remyelination, current coculture systems are challenging to use for screening applications. He said that the cells can form overlapping processes and that the presence of cell bodies makes it difficult to quickly quantify the amount of myelin produced.

To address these limitations, Chan's team developed an assay that

could be used in high throughput screens to identify compounds that promote functional remyelination. The assay uses a standard 96-well plate in which each well contains an array of vertical axon-mimicking micropillars constructed from fused silica. Oligodendrocyte precursor cells (OPCs) cultured in these wells differentiate into oligodendrocytes that deposit myelin around the axon-mimicking micropillars.

"We found serendipitously that when you coculture oligodendrocytes with micropillars that resemble axons in size and shape, they form a ring of myelin around the pillars that can be quickly identified and measured," said Chan. "This was a big discovery for us because it allowed us to take the neurons out of the picture."

The team used its micropillar arrays to screen a library of about 1,000 drugs for molecules that enhanced OPC differentiation and myelination. The screen identified a cluster of antimuscarinic drugs, with the top two hits being clemastine and benztropine. In a mouse model of chemical-induced demyelination, clemastine increased recovery of myelin compared with vehicle.

Clemastine is an OTC antihistamine that also has antimuscarinic activity, whereas benztropine is a generic drug marketed to treat the symptoms of Parkinson's disease (PD).

Data were published in *Nature Medicine*.

"This assay takes oligodendrocyte differentiation to the next step of asking whether the cell is undergoing the necessary shape changes. It allows them to determine whether the cell is doing the right sort of morphological rearrangement," said Tassie Collins, VP of translational medicine at the **Myelin Repair Foundation (MRF)**.

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Progress toward therapies

Inception 5 is the fifth single-asset company spun out by the **Versant Ventures**-backed incubator **Inception Sciences Inc.** Roche will provide Inception 5 with undisclosed milestone-based funding and has an option to acquire the startup upon submission of its first IND.

The company is evaluating a portfolio of additional undisclosed preclinical compounds identified by Chan's group that could promote remyelination in MS. The company is not disclosing when it expects to select its lead compound.

Brian Stearns, senior director and head of chemistry at Inception Sciences, said that the UCSF screening platform has already been used to identify additional classes of compounds that could promote remyelination.

"In some cases, the targets have already been identified, and in other cases the targets must still be identified," added Daniel Lorrain, executive director and head of biology at Inception Sciences.

Inception 5 is scaling up its screening platform to discover proprietary compounds that promote remyelination.

However, competition could be on the horizon. Earlier this week, **Kadimastem Ltd.** announced a deal with the Merck Serono S.A. unit of **Merck KGaA** to use the biotech's screening platform to identify therapeutic candidates for MS and neurodegenerative diseases.

Box 1. Specific BETs on remyelination.

A team at the **Icahn School of Medicine at Mount Sinai** has linked BET bromodomain protein-regulated gene transcription to oligodendrocyte precursor cell (OPC) differentiation and identified a series of selective BET inhibitors that could enhance this process.⁵

BET bromodomain proteins contain two bromodomains—BRD1 and BRD2—and are epigenetic regulators of transcriptional activity involved in many signaling pathways.

In vitro, structure-guided studies identified a class of compounds selective for BRD1 over BRD2. In cultured mouse OPCs, the lead BRD1-selective inhibitor from the series—called olinone—increased OPC differentiation compared with baseline. In contrast, vehicle or a BRD2-selective inhibitor had no significant effect on this measure, whereas nonselective inhibitors targeting BRD1 and BRD2 decreased OPC differentiation.

“Our study describes olinone, a bromodomain inhibitor with high selectivity, as an inducer of the early stages of differentiation,” said Patrizia Casaccia, the co-corresponding author and chief of the Center of Excellence for Myelin Repair at the Friedman Brain Institute at the Icahn School of Medicine at Mount Sinai.

Ming-Ming Zhou added that results showing the variable effects from targeting different bromodomains suggest that highly selective modulation of gene transcription, rather than broad

modulation, is going to be important for the development of new therapeutics for multiple sclerosis (MS) and related human disorders. Zhou is the other co-corresponding author, a professor and chair of the Department of Structural and Chemical Biology and co-director of the Experimental Therapeutics Institute at the Icahn School of Medicine.

“They pretty convincingly demonstrate that [olinone] is selective for one bromodomain within the BET-family proteins,” said Jose Lora, executive director of preclinical sciences at **Constellation Pharmaceuticals Inc.** “We didn’t have any indication that these proteins regulated pathways involved in myelin regeneration.”

Constellation’s BET bromodomain inhibitor CPI-0610 targets both BRD1 and BRD2 and is in Phase I testing to treat various hematologic malignancies. Last year, Lora’s group published work showing that a BET bromodomain inhibitor reduced symptoms in a mouse model of experimental autoimmune encephalomyelitis (EAE).⁶

“Assuming the biology is relevant *in vivo*, the compound could be a very interesting tool. One could imagine combining a BRD1-selective bromodomain inhibitor with agents that control inflammation in the context of MS,” Lora said.

Both Daniel Lorrain, executive director and head of biology at **Inception Sciences Inc.**, and Jonah Chan, an associate professor in the Department

of Neurology at the **University of California, San Francisco School of Medicine**, had concerns about the safety of targeting BET bromodomain proteins in MS as these proteins regulate multiple signaling pathways across many cell types.

“Bromodomain inhibitors can have dramatic effects on gene transcription, so safety studies in animals will be essential,” Chan told *SciBX*.

Although increasing the selectivity of compounds like bromodomain inhibitors could improve safety, the potential trade-off could be loss of efficacy if such compounds lose their ability to target related proteins that contribute to disease pathology.

Lora suggested that a next step could be to carry out *in vitro* knock-down experiments on individual BET bromodomain proteins and then attempt to rescue the effects with mutant variants of those proteins. He added that mutants with functional Brd2 but not Brd1 should recapitulate the effect of treatment with a BRD1-selective inhibitor such as olinone.

The Mount Sinai researchers are pursuing development of olinone and related compounds in MS and are evaluating the compounds in rodent models of remyelination. Mount Sinai has filed a patent application covering olinone and related compounds and their use for treating human diseases including MS. The technology is available for licensing. —K-JL & LZO

The platform includes assays to detect OPC differentiation into oligodendrocytes and cocultures to measure myelination of neuronal axons by oligodendrocytes.

Collins said that the MRF is also working on a scalable coculture system with live axons and oligodendrocytes. She said that such a system is important to have because there is evidence suggesting that axons and oligodendrocytes have dynamic interactions that affect the process of myelination.

The myelination-promoting compounds that are already in the clinic arose from targeted efforts to understand biological processes controlling remyelination. These include **Biogen Idec Inc.**’s BIIB033, a mAb against leucine-rich repeat neuronal protein 1 (LINGO-

1), and **Acorda Therapeutics Inc.**’s remyelinating antibody rHlgM22, which binds to lipid rafts on oligodendrocytes. BIIB033 is in Phase II

testing to treat relapsing-remitting MS (RRMS), and rHlgM22 is in Phase I trials to treat MS.

MRF is also evaluating multiple preclinical compounds and cell therapies that could protect neurons against demyelination and promote remyelination. The MRF’s most advanced candidate is MRF-008, an undisclosed generic antihypertensive drug in preclinical development for MS. The foundation plans to start a Phase I trial of MRF-008 in 4Q14 or 1Q15.

In addition, a team from the **Icahn School of Medicine at Mount Sinai** has published data in *Chemistry & Biology* suggesting that selective BET bromodomain protein inhibitors could

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accelerate OPC differentiation and potentially promote myelination (see Box 1, “Specific BETs on remyelination”).

Chan said that his group is now evaluating various screening hits in *in vivo* models of local demyelination. His group also is trying to develop a new animal model in which neurons remain more intact than those found in the standard experimental autoimmune encephalomyelitis (EAE) mouse model.

Lorrain said that such a model would be important because in the current EAE model there might be too much axonal damage to assess the efficacy of remyelinating therapies.

Moreover, a separate UCSF School of Medicine group led by Ari Green, assistant clinical director of the UCSF Multiple Sclerosis Center, initiated the investigator-led Phase II ReBUILD trial earlier this year to evaluate clemastine in patients with RRMS. Green collaborates with Chan's group.

UCSF has a pending patent covering the micropillar array screening platform.

Lou, K.-J. & Osherovich, L. *SciBX* 7(30); doi:10.1038/scibx.2014.885
Published online Aug. 7, 2014

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COMPANIES AND INSTITUTIONS MENTIONED

Acorda Therapeutics Inc. (NASDAQ:ACOR), Ardsley, N.Y.
Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Constellation Pharmaceuticals Inc., Cambridge, Mass.
Icahn School of Medicine at Mount Sinai, New York, N.Y.
Inception 5 Inc., San Diego, Calif.
Inception Sciences Inc., San Diego, Calif.
Kadimastem Ltd. (Tel Aviv:KDST), Ness Ziona, Israel
Merck KGaA (Xetra:MRK), Darmstadt, Germany
Myelin Repair Foundation, Saratoga, Calif.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
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