Genentech's new parADigm

By Lev Osherovich, Senior Writer

A study by a team at **Genentech Inc.** proposes a new mechanism for Alzheimer's disease pathogenesis by outlining a process that could be an early step in the neurodegenerative disorder.¹ The work also identifies a trio of new AD targets, which the company is pursuing with preclinical programs.

The study suggests that the previously ignored amino-terminal portion of amyloid precursor protein (APP), called N-APP, might be the main culprit behind AD. APP is a transmembrane protein that gives rise to N-APP and the much more commonly known β -amyloid ($A\beta$), a fragment that forms the amyloid fibers and plaques that are hallmarks of AD, although the precise role of those fibers and plaques in disease pathology is unclear.²

Whereas $A\beta$ has been the subject of a great deal of work because of its clear role in plaque formation, N-APP has been ignored because it was thought to be trimmed off and thus not play any role in AD.

The majority of disease-modifying candidate therapeutics—including the most advanced, a Phase III antibody called bapineuzumab from **Wyeth** and **Elan Corp. plc**—have focused on blocking the formation of $A\beta$ plaques (see **Box 1, "Galapagos goes gamma"**). Results on that front have been mixed, however.

An earlier Elan vaccine against $A\beta$ failed.³ And a Phase II trial of bapineuzumab missed its endpoint last fall. Wyeth and Elan are now running the Phase III trial of bapineuzumab in a subset of AD patients that the companies believe are genetically compatible with the therapeutic.

If Genentech's murine cell culture findings hold up in human cells and in mouse models of AD, the result could usher in a shift of therapeutic focus away from $A\beta$ and toward N-APP or its downstream effectors—a pair of proteins that control apoptosis.

The *Nature* study was authored by Anatoly Nikolaev and Marc Tessier-Lavigne, Genentech's EVP of research drug discovery, and collaborators at the **University of California, San Diego**. Nikolaev, previously Tessier-Lavigne's postdoc, is a scientist at Genentech.

The discovery

Tessier-Lavigne told *SciBX* that the initial focus of the study was not AD but rather embryonic nerve development and regeneration. In fact, the connection between nerve development and AD was a lucky outgrowth of Nikolaev's postdoctoral project on apoptosis in developing neurons.

During normal embryonic development, the axons of neurons grow extensively at first and are later pruned back through apoptosis

triggered by changes in neurotrophic growth factor levels. Previous work suggested that axon pruning requires one or more members of the tumor necrosis factor receptor (TNFR) family, according to Tessier-Lavigne.

"Most neurons express a receptor that mediates their destiny," he said. "They're poised to commit suicide but are prevented from doing so by trophic support."

The Genentech team analyzed the expression of all known TNFRs in the brains of embryonic mice and found that one protein, called tumor necrosis factor receptor superfamily, member 21 (TNFRSF21; DR6), was present in many cells undergoing apoptosis. DR6 was thus a likely suspect in conveying the cue for neurons to die.

In vitro, serum-starved neurons receiving an antibody against DR6 showed better axon survival than mock-treated controls.

The connection of these findings to AD became apparent when the team identified APP as the natural ligand for DR6.

Ordinarily, APP is bound to the cell surface. But the researchers discovered that when the neurons are deprived of growth factors, APP is cleaved by two AD-associated proteases, β -site APP-cleaving enzyme 1 (BACE1) and γ -secretase, plus an additional, as yet unidentified, protease.

Cleavage of APP by these enzymes frees two fragments to accumulate near the neuron's surface— $A\beta$ and N-APP. The team found that N-APP was responsible for DR6's proapoptotic effects.

In cultured neurons, recombinant N-APP triggered DR6 activation and axon degeneration, whereas immunodepletion of N-APP prevented degeneration caused by withdrawal of growth factors.

The Genentech team went on to identify an intracellular protease called caspase-6 apoptosis-related cysteine peptidase (CASP6; MCH2) as the downstream effector of the N-APP and DR6 signal. Inhibition of CASP6 prevented axonal degeneration upon growth factor withdrawal compared with what was seen in untreated controls.

In total, the findings paint a picture of a neurodegenerative pathway in early brain development that prunes excess axon growth. But because APP is a central player in both this process and AD, Tessier-Lavigne proposed that inappropriate activation of the pathway later in life could lead to AD (see **Figure 1, "New targets in Alzheimer's disease"**).

"When the neuron is healthy, this mechanism is kept in check, but when the neuron is committed to self-destruction, the process becomes activated," said Tessier-Lavigne. "The components of this system are present in the adult brain, suggesting that this process goes awry" in AD.

Beyond its link to AD, the normal purpose of APP had been poorly understood, thus complicating efforts to target the protein. The Genentech study "provides the best data regarding the natural role of APP," said Stephen Strittmatter, professor of neurology at **Yale School of Medicine**. "The data show clearly that a fragment of APP, N-APP, functions with DR6 in caspase-mediated axonal degeneration after growth factor withdrawal."

Backseat for $A\beta$?

At least 10 companies are developing therapies to prevent $A\beta$ accumulation or stimulate the removal of $A\beta$ deposits from AD-stricken

Box 1. Galapagos goes gamma

A Belgian team has identified a new Alzheimer's disease target—a G protein-coupled receptor that controls the production of γ -secretase, an enzyme involved in the processing of Alzheimer's amyloid precursor protein.⁶ The target is in the preclinical pipeline of **Galapagos N.V.**

The discovery, reported in *Science*, was the result of a collaboration between Galapagos and the laboratory of Bart De Strooper, a professor of human genetics at the **Catholic University Leuven** who is also a consultant to the company.

The team screened a cDNA library of druggable receptors to identify genes that influence the production of β -amyloid (A β), the toxic fragment of amyloid precursor protein (APP) that contributes to AD.

"We looked in neuronal cell lines for clones that processed APP into the wrong form of A β ," said Galapagos CEO Onno van de Stolpe.

The strongest hit was an orphan G protein-coupled receptor called GPR3. Overexpression of GPR3 increased

production of A β by more than eightfold compared with that seen in untreated controls. Likewise, transgenic mice with GPR3 had higher A β levels than controls, whereas GPR3 knockouts had lower A β levels.

De Strooper's academic team took on the *in vivo* characterization of GPR3 after Galapagos' discovery of the target. They found that mouse neurons transfected with extra GPR3 had higher levels of the γ -secretase complex on their surfaces than untreated controls. Moreover, blocking γ -secretase with a small molecule prevented the rise in A β levels caused by GPR3 overexpression. They also found that in mice and humans, GPR3 is expressed in the hippocampus and other brain areas involved in AD.

As a result, De Strooper suspects that GPR3 modulates the activity of γ -secretase, one of several proteases involved in A β production. However, he told *SciBX* that it's not yet clear if GPR3 plays a role in causing AD as it is only upregulated in a subset of AD patients.

GPR3 knockouts appear healthy, meaning the receptor is not essential for

viability, nor does GPR3 appear to affect the processing of other γ -secretase substrates such as Notch, the cleavage of which is needed for normal immune functions. Thus, if the usual challenges of blood brain barrier permeability can be met, GPR3 is a solid AD target, De Strooper told *SciBX*.

John Lin, senior director of neurodegeneration at **Pfizer Inc.**'s Rinat subsidiary, said the discovery of GPR3 "should come as welcome news to the traditional pharma industry, since GPCRs are most amenable to small molecule drug discovery."

De Strooper next plans to further characterize the expression and activity of GPR3 in AD patients and to figure out how the receptor controls γ -secretase. Meanwhile, van de Stolpe said Galapagos is moving ahead with small molecule screening to identify candidates for preclinical development.

The discovery has been patented and Galapagos is exploring partnering opportunities for its GPR3 program, according to van de Stolpe.

—LZO

brains (see **Table 1, "Alzheimer's targets"**). However, the discovery of N-APP's activation of apoptosis raises doubts about whether A β is really the central player.

"Overall this work represents a boost to AD research, as the field had been focused on the A β hypothesis almost to the exclusion of other ideas or alternative interpretations," said John Lin, senior director of neurodegeneration at **Pfizer Inc.**'s Rinat subsidiary.

Pfizer acquired Rinat in 2006, and the main driver of the deal was the biotech's RN1219, an antibody against A β that is now in Phase II trials for AD.

"Most of the neuro world is focused on A β , but this paper suggests that APP is involved in another pathway," said David Hung, CEO of **Medivation Inc.**, which is also partnered with Pfizer in AD.

The companies are running a Phase III AD trial of Dimebon, a neuroprotective small molecule with a mechanism of action that is not fully understood.

Whether the neurodegeneration seen in AD is the consequence of A β , N-APP or a combination of the two should become apparent with further animal studies.

Tessier-Lavigne acknowledged that A β is toxic to neurons in a variety of preclinical models, including the cultured neurons used in the *Nature* study. He noted, however, that therapies to decrease A β plaques have not yet translated to improved outcomes in clinical trials.⁴

"The APP-death receptor model is not mutually exclusive with the β -amyloid hypothesis," said Mark Albers, assistant professor of neurology at **Massachusetts General Hospital** and **Harvard Medical School**.

Tessier-Lavigne agreed that both parts of APP may be in play in AD. "The two mechanisms could be operating together," he said. "The question is: what's their relative contribution" to pathology.

Regardless of the answer, Tessier-Lavigne believes that interfering with the N-APP/DR6/CASP6 pathway could block one of the earliest steps in AD pathogenesis because axon degeneration precedes full-blown neuron death in many AD models. "The obvious entry points would be targeting N-APP, DR6 and CASP6," he said.

"If, in fact, axon and synapse deficits are a major trigger of pathophysiology in AD and other neurodegenerative disorders, then targeting these as early as possible is an obvious and important therapeutic strategy," said Ronald Oppenheim, professor of neurobiology and anatomy at **Wake Forest University School of Medicine**.

"When you look at AD brains at autopsy, the single greatest correlate of brain cell death is synapse loss," which is a consequence of axon degeneration, said Medivation's Hung. He added that Dimebon has protected neurons from synaptic degeneration in preclinical studies.

If N-APP does contribute to AD pathogenesis, therapeutics that inhibit the cleavage of APP, such as inhibitors of BACE1 and γ -secretase, might work better than antibodies or small molecules aimed directly at

Figure 1. New targets in Alzheimer's disease. A trio of papers identifies new steps and targets in the pathogenesis of Alzheimer's disease. Nikolaev *et al.* show that, during development, withdrawal of neuronal survival factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) leads to abnormal processing of amyloid precursor protein (APP), followed by neuronal death. Nagahara *et al.* show that transgenic expression of BDNF prevents neurodegeneration in a variety of animal models of AD⁵. Thathiah *et al.* show that GPR3, a G protein-coupled receptor, regulates the activity of γ-secretase, an enzyme involved in APP processing.

Together, the studies suggest the following model:

NGF and BDNF signaling through neurotrophic tyrosine receptor kinases (NTRKs) [a] prevents the cleavage of APP by β-site APP-cleaving enzyme 1 (BACE1) [b]. BACE1 together with γ-secretase [c] converts APP into a toxic amyloid-forming β-amyloid (Aβ) fragment [d] and a soluble N-terminal fragment (N-APP) [e].

In neuronal development and possibly in AD, N-APP engages tumor necrosis factor receptor superfamily, member 21 (TNFRSF21; DR6) [f], which activates caspase-6 apoptosis-related cysteine peptidase (CASP6; MCH2) and a related enzyme, CASP3 (CPP32) [g] to promote neuronal apoptosis [h].

Targets marketed with * are being pursued by various companies for the development of AD treatments (see **Table 1, "Alzheimer's targets"**).

Aβ. BACE1 and γ-secretase are needed to produce both Aβ and N-APP. Inhibiting these enzymes would leave APP intact and unable to activate DR6 or form Aβ aggregates.

The most advanced γ-secretase inhibitor is **Eli Lilly and Co.**'s LY450139, which is in a Phase III trial for AD. The leader in the BACE1 race is **CoMentis Inc.**, whose CTS-21166 completed a Phase I AD trial last year. The compound is partnered with **Astellas Pharma Inc.**

The *Nature* paper also boosts the prospects for neuroprotective therapies such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). For example, the Genentech team found that N-APP was no longer toxic in the presence of NGF, suggesting that growth factors allow cells to bypass DR6's proapoptotic signal. (See **New home for BDNF, page 6.**)

Finally, the proapoptotic pathway proposed in the Genentech study

may be relevant to other neurodegenerative disorders, such as Huntington's disease (HD) and Parkinson's disease (PD), as well as traumatic brain injury and stroke.

"Aberrant shedding of APP and activation of DR6 may be operative in many diseases of the nervous system," said Harvard's Albers.

"In HD, there's been a lot of focus on activation of CASP6," Tessier-Lavigne noted. "This begs the question of whether this process is activated in striatal neurons, which are affected in HD."

Back to the bench

The next test is whether interfering with N-APP, DR6 and CASP6 will have a disease-modifying effect in standard animal models of AD. Designing the right experiments is a challenge, as most models are biased toward overproduction of Aβ and may not necessarily have higher levels

Table 1. Alzheimer's targets. Selected therapeutics in development for Alzheimer's disease.

Target	Company	Product	Description	Status in AD
β -Amyloid ($A\beta$)	Elan Corp. plc (NYSE:ELN)/ Wyeth (NYSE:WYE)	Bapineuzumab (AAB-001)	Humanized mAb against $A\beta$	Phase III
	Cytos Biotechnology AG (SIX:CYTN)/ Novartis AG (NYSE:NVS; SIX:NOVN)	CAD106	Vaccine with a fragment of the $A\beta$ protein	Phase II
	Elan/Wyeth	ACC-001	$A\beta$ -related immunotherapeutic conjugate	Phase II
	Eli Lilly and Co. (NYSE:LLY)	LY2062430	Antibody against soluble $A\beta$	Phase II
	Pfizer Inc. (NYSE:PFE)	PF-4360365 (RN1219)	Humanized mAb against $A\beta$	Phase II
	Affiris GmbH/GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	Affitope vaccine	Vaccine against $A\beta$	Phase I
	Genentech Inc. (NYSE:DNA)/ AC Immune S.A.	Anti- $A\beta$	mAb against $A\beta$	Phase I
	MorphoSys AG (FSE:MOR)/ Roche (SIX:ROG)	R1450	HuCAL-derived human mAb against $A\beta$	Phase I
APP N-terminal (N-APP)	Genentech	N-APP antagonist	N-APP antagonist	Discovery
β -site APP-cleaving enzyme 1 (BACE1)	CoMentis Inc./Astellas Pharma Inc. (Tokyo:4503)	CTS-21166	BACE1 inhibitor	Phase I
Caspase-6 apoptosis-related cysteine peptidase (CASP6; MCH2)	Genentech	CASP6 inhibitor	CASP6 inhibitor	Discovery
γ -Secretase	Eli Lilly/Elan	LY450139	γ -Secretase inhibitor	Phase III
	Humanetics Corp.	NIC5-15	γ -Secretase inhibitor	Phase II
	Wyeth	Begacestat (GSI-953)	γ -Secretase inhibitor	Phase I
G protein-coupled receptor 3 (GPR3)	Galapagos N.V. (Euronext:GLPG; Pink:GLPYY)	GPR3 inhibitor	GPR3 inhibitor	Preclinical
Nerve growth factor (NGF)	Ceregene Inc.	CERE-110	Adeno-associated viral vector encoding NGF	Phase I
Tumor necrosis factor receptor superfamily, member 21 (TNFRSF21; DR6)	Genentech	DR6 antagonist	DR6 antagonist	Discovery

of N-APP than normal animals. Thus, a combination of further cell culture and animal studies may be the way forward.

The Genentech team's "elegant study has been done primarily in neurons derived from the spinal cord and retinal ganglion cells in the mouse," said Albers. "It remains to be demonstrated that these mechanisms are operative in neurons that are more classically associated with Alzheimer's disease, such as hippocampal neurons and cortical neurons."

Tessier-Lavigne said experiments to test the role of the new targets in AD models are underway and that therapeutics aimed at all three targets are in preclinical development at Genentech.

Genentech has filed multiple patents covering the research and its potential commercial applications, according to company spokesperson Robin Snyder.

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Published online Feb. 26, 2009

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Elan Corp. plc (NYSE:ELN), Dublin, Ireland
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