REPRINT FROM APRIL 5, 2010

BioCentury

THE BERNSTEIN REPORT ON BIOBUSINESS™



*Product Discovery & Development*

# Breaking the cell barrier

**By Aaron Bouchie**
**Senior Writer**

The publication of data from a Phase I trial of CALAA-01 provided the first proof of concept in man that systemically delivered short interfering RNA can be targeted to specific cells. So far, RNAi therapeutics in the clinic have been limited to either local or non-targeted systemic delivery.

On the heels of the publication, two companies announced deals for targeted RNAi therapies.

On March 21, *Nature* published data on its website from the Calando Pharmaceuticals Inc. subsidiary of **Arrowhead Research Corp.** on three metastatic melanoma patients who received CALAA-01 in an escalating single-dose Phase I trial. Analysis of tumor biopsies after treatment showed CALAA-01 accumulated in the tumors in a dose-dependant fashion and was not detected in the adjacent epidermis.

> ***"Right now we're focusing on cancer. However, the basic RONDEL platform could be used to deliver siRNA to non-cancer tissues."***
>
> **Christopher Anzalone, Arrowhead Research**

Additional analysis showed the compound knocked down the target mRNA and protein, providing the first demonstration of the gene-silencing mechanism of action in man.

CALAA-01 is a short interfering RNA duplex targeting the M2 subunit of ribonucleotide reductase (RRM2) delivered via the RONDEL delivery system. RONDEL (RNAi/Oligonucleotide Nanoparticle Delivery) is composed of a cyclodextrin-containing polymer, polyethylene glycol (PEG), to promote stability, and a targeting ligand displayed on the exterior of the nanoparticle.

At least two marketed cancer drugs target ribonucleotide reductase: Gemzar gemcitabine from **Eli Lilly and Co.** and Clolar clofarabine from **Genzyme Corp.**

The targeting ligand used in CALAA-01 is a human transferrin, which engages transferrin receptors on cancer cells. These receptors are up-regulated in malignant cells.

Calando makes the RONDEL delivery vehicles and the siRNA payloads separately. The two vials are mixed at dosing, and the particles self-assemble. This makes it more cost-effective for the company to manufacture and ship, according to Arrowhead President and CEO Christopher Anzalone, because it can make the delivery vehicle in bulk and ship different siRNAs separately.

"Right now we're focusing on cancer. However, the basic RONDEL platform could be used to deliver siRNA to non-cancer tissues," he said. "We're studying only transferrin right now, but in the future we could use different targeting moieties."

Calando plans to continue the Phase I trial until it reaches the maximum tolerated dose, and will now approach pharmas for partnering opportunities. "We wanted to wait until this paper came out, so now we're set to have discussions," Anzalone said.

The Phase I data are the first for a targeted siRNA. Most siRNA therapeutics in development are delivered locally, for example, via inhalation to the lungs or injected into the eye or brain. Systemic delivery has been the ultimate goal, but the problem has been that naked siRNA is quickly broken down by

*See next page*

BioCentury™
THE BERNSTEIN REPORT ON BIOBUSINESS

PO Box 1246
San Carlos CA 94070-1246
Voice: 650-595-5333
Fax: 650-595-5589
www.biocentury.com

DAVID FLORES
President & CEO

KAREN BERNSTEIN, Ph.D.
Chairman & Editor-in-Chief

***Product Discovery & Development,***
*from previous page*

serum endonucleases.

At least four companies have systemically delivered siRNA therapies in the clinic, three of which use non-targeted lipid-based technologies to protect the siRNA from degradation: **Alnylam Pharmaceuticals Inc.**, **Silence Therapeutics plc** and **Tekmira Pharmaceuticals Corp.**

Because lipids naturally collect in the liver, Tekmira's and Alnylam's first indications are liver-related.

Tekmira plans to begin a Phase I trial of its second-generation ApoB SNALP product to treat hypercholesterolemia next half. The compound is an siRNA against apolipoprotein B-100 (APOB-100) mRNA encapsulated in liposomal stable nucleic acid lipid particles (SNALPs).

Phase I data from the first-generation product showed that two patients who received the highest dose had average reductions of ApoB protein and LDL cholesterol of 21.1% and 16.3%, respectively. The company stopped the trial when it saw what might have been a dose-limiting immune stimulation, a problem the second-generation product has been designed to avoid.

Alnylam's ALN-VSP, a lipid nanoparticle formulation of two siRNAs that target the kinesin spindle protein (KSP) and VEGF genes, is in Phase I testing to treat liver cancer, with interim data expected in mid-2010. ALN-VSP uses Tekmira's SNALP technology.

Silence's lead is Atu027, an siRNA lipoplex targeting protein kinase N3 (PKN3). The compound does not use a targeting ligand but does use the company's AtuPLEX technology, which creates chemically modified liposomes that are taken up primarily by endothelial cells and tumor vasculature. Data from a Phase I trial to treat solid tumors are expected in December.

**Quark Pharmaceuticals Inc.**, the fourth company with a systemically delivered, non-targeted siRNA therapeutic in the clinic, uses chemical modifications instead of encapsulating particles to protect its siRNAs from degradation. Because naked siRNAs accumulate in the kidney, the company is starting with diseases of that organ.

Quark plans to start Phase II trials of QPI-1002 in June to prevent acute kidney injury (AKI) in patients undergoing major cardiovascular surgery and to treat and prevent delayed graft function (DGF) after renal transplantation. QPI-1002 inhibits the expression of p53.

To systemically deliver siRNA to tissues outside the liver and kidneys, companies are looking at a variety of targeting technologies, such as conjugating their delivery vehicles to aptamers, peptides, small molecules, antibodies and antibody fragments. Aside from Calando's CALAA-01, all these programs are in research or preclinical development.

"Alnylam has long held the belief that there is no magic bullet for delivery, and that many things would work depending on the target and indication," said CEO John Maraganore.

For example, Maraganore believes that a targeting ligand isn't needed to target tumors because their leaky vasculature allows molecules to enter the cells. "Cancer cells take things up easily. I'm not saying that targeting tumors is a bad thing, just that it's not clear to us that it's a required thing," he said.

The company has a program using systemic, non-targeted delivery in preclinical development to treat solid tumors.

Xioa-Dong Yang, VP of research and preclinical development at Silence, believes a targeting ligand will make a cancer siRNA even more efficacious because the interstitial pressure in leaky tumors tends to push out particles other than oxygen and nutrients.

Silence plans to combine its PolyTran biodegradable peptide-based polymers for targeted siRNA delivery with its AtuPLEX technology to further enhance delivery of siRNA to targeted tissues. Silence gained PolyTran when it acquired Intradigm Corp. in January.

"With a ligand, you have a greater chance of molecules staying in the tumor," Yang said. "The particle has to get into the tumor first, and a ligand helps that via endocytosis. Without a ligand, everything is by chance, and efficiency is much, much lower," he said.

Yang also noted that non-targeted delivery vehicles would be more likely to cause liver-related side effects, especially with lipid-based systems. "If 90% of the drug accumulates in the liver and only 10% in the tumor, then that is not very efficient," he said.

For this reason, he added, targeting also can bring down cost of goods.

Last Tuesday, **Dicerna Pharmaceuticals Inc.** and **Ipsen Group** announced a deal to develop conjugates of Dicerna's dicer substrate siRNA (DsiRNA) molecules and Ipsen's peptide targeting vectors for oncology and endocrinology indications.

The same day, Silence and partner **Dainippon Sumitomo Co. Ltd.** added new, undisclosed targets to a 2009 deal to evaluate Silence's siRNA molecules, lipid delivery and targeting technologies. The deal includes AtuPLEX but not PolyTran.

**Merck & Co. Inc.**, which bought Sirna Therapeutics Inc. for $1.1 billion in cash in 2006, would not disclose the status of its siRNA programs.

COMPANIES AND INSTITUTIONS MENTIONED

**Alnylam Pharmaceuticals Inc.** (NASDAQ: ALNY), Cambridge, Mass.
**Arrowhead Research Corp.** (NASDAQ: ARWR), Pasadena, Calif.
**Dainippon Sumitomo Co. Ltd.** (Tokyo:4506; Osaka:4506), Osaka, Japan
**Dicerna Pharmaceuticals Inc.**, Watertown, Mass.
**Eli Lilly and Co.** (NYSE:LLY), Indianapolis, Ind.
**Genzyme Corp.** (NASDAQ:GENZ), Cambridge, Mass.
**Ipsen Group** (Euronext:IPN), Paris, France
**Merck & Co. Inc.** (NYSE:MRK), Whitehouse Station, N.J.
**Quark Pharmaceuticals Inc.**, Fremont, Calif.
**Silence Therapeutics plc** (LSE:SLN), London, U.K.
**Tekmira Pharmaceuticals Corp.** (TSX: TKM), Burnaby, B.C.