RNA Interference Advances to Early-Stage Clinical Trials

By Vicki Brower

Since the discovery of RNA interference (RNAi) in 1998, researchers have hoped to use the gene-silencing technique to shut down cancer-causing genes. Now, with a first phase I study in Nature and more trials reported at this year’s annual meeting of the American Society of Clinical Oncology (ASCO), the field seems closer to that goal.

In the Nature article, Mark Davis, Ph.D., of the California Institute of Technology and colleagues presented the results of an ongoing 15-person trial of a small-interfering RNA (siRNA) called CALAA-01. The siRNA, delivered systemically by means of a nanoparticle targeting transferrin, a protein that many cancer cells overexpress, accumulated in a dose-dependent manner in tumors. Patients have had side effects, but there has so far been no dose-limiting toxicity.

RNAi is an active area of research, and CALAA-01, which Calando Pharmaceuticals developed, is just one of several siRNAs in company pipelines. But which, if any, of these synthetic molecules will make it to later-phase trials remains unclear.

“This trial is an important first step for RNAi in cancer,” said Esther Chang, Ph.D., a professor of oncology at Georgetown University in Washington, D.C., and an RNAi researcher who was not involved in the study. But big questions still exist, she said, such as how much of the agent is hitting the target and causing apoptosis.

“It is not clear from Calando’s data that a sufficient amount of siRNA is reaching the cancer cells,” she said. “Also, the percentage of patients having side effects, about one-third, raises a red flag, and may indicate that the treatment is having significant off-target [immune activation] effects.”

RNAi drugs harness a natural biological pathway that interrupts the flow of genetic information from RNA and shuts down gene expression. Small, synthesized interfering RNAs mimic this process. The synthesized, double-stranded RNA molecules are designed to interfere with the function of a specific gene(s) by binding to and destroying mRNA, which carries protein-building instructions from the DNA.

RNAi’s goal is to prevent the production of cancer- or other disease-causing proteins in the first place, rather than preventing their damage or neutralizing them once they are produced, said John Maraganore, Ph.D., CEO of Alnylam.

“Instead of mopping up water from the floor, RNAi works by turning off the spigot,” he said.

Delivery Challenges

Targeting most cancers requires systemic delivery, which is more complex than delivering “naked” RNAi locally, according to David Evans, Ph.D., a vice president at Sirnaomics. Until relatively recently, most first-generation RNAi drugs were locally administered without a delivery vehicle, through injection or nebulizer, which is effective only with localized disease. Systemic delivery has met with many confounding problems: getting enough RNAi to the target cells, bypassing nontarget tissues, and preventing degradation of RNAi in the bloodstream and excretion before absorption. Researchers widely debate the optimal size of siRNAs for safety and efficacy. Treatments for primary brain, skin, and pancreatic cancers are experimentally using local delivery.

“There are three major issues in RNAi for cancer: delivery, delivery, delivery,” said David Corey, Ph.D., professor of pharmacology at the University of Texas Southwestern Medical School in Dallas. Experts agree that delivery systems, which include organic and nonorganic polymers, lipids and lipid variations – all nanoparticles – are currently the make-or-break factor for RNAi therapeutics.

Each system has advantages and drawbacks, with no one vehicle appropriate for all tissues, said Clive Jackson, Ph.D., senior principal scientist at AstraZeneca, which is collaborating with Silence Therapeutics on RNAi drugs. RNAi molecules have a short half-life and degrade rapidly without chemical modification to increase stability. Another delivery issue is that reaching target cells often requires large quantities of siRNA, Chang said.

Off-target effects from siRNA’s non-specific stimulation of the innate immune system are another serious challenge for...
RNAi therapies, which require chemical modification for this reason, said Philip Sharp, Ph.D., an institute professor at MIT and a founder of Alnylam.

Natural Attraction to Liver

Early on, scientists discovered that siRNAs travel to and are absorbed by the liver. “That is why a lot of companies have put their resources into addressing liver cancer or metastasis of other cancers to the liver, as a low-hanging fruit,” said Babak Alizadeh, Ph.D., senior vice president for business development at Napajen in Burlingame, Calif., which is developing technology for antigen-presenting cells and does not work on drugs for the liver. “Several groups are just starting to see success with cell-specific targeting advances outside the liver,” Alizadeh said.

Alnylam is developing ALN-VSP02 for solid cancers with liver involvement. Composed of siRNAs that target vascular endothelial growth factor (VEGF)-A and kinesin spindle protein, the drug is in an open-label, ongoing phase I trial in 15 patients, primarily those with colorectal cancer with liver involvement. In June, Alnylam reported preliminary results at ASCO. Patients were infused every 2 weeks with the drug, which was mostly well tolerated with limited liver toxicity. One patient experienced an adverse effect, and another died of liver failure; both events were most likely related to the drug. Magnetic resonance images indicate early evidence of delivery, delivery, delivery. “There are three major issues in RNAi for cancer: delivery, delivery, delivery.”

“Another drug in early-stage testing, by Silence Therapeutics, targets the angiogenic and lymphangiogenic processes. Called Atu027, it is in a phase I trial primarily in patients with colorectal cancer metastasizing to the liver. This liposomal nanoparticle targets the PKN3 gene, which is necessary for metastasis. “It interferes with the endothelial lining of tumor blood vessels, [interferes with] cell migration, and reduces the oxygen supply of the tumor,” said the company’s chief executive, Phil Haworth, Ph.D. The open-label, dose-finding study is currently dosing its fifth of 11 cohorts, is on schedule, and is expected to be completed in the second half of 2011. Dose escalation is continuing, Haworth said. In preclinical studies, Atu027 demonstrated antitumor activity against several tumor types, including pancreatic and other gastrointestinal tumors, and non-small cell lung, prostate, and other cancers. These animal studies also showed no indication of genotoxicity in rodents and non-human primates, and demonstrated no indications of cardiovascular or pulmonary function impairment in primates, Haworth said.

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Beyond the Liver

Some siRNA researchers are looking at siRNA in other cancers. Jan Barciszewski, Ph.D., of the Institute of Bioorganic Chemistry of the Polish Academy of Science in Poznan, recently described a randomized trial with 46 patients with brain tumors with siRNA targeted to tenascin C. These tumors overexpress this protein, which contributes to tumor cell adhesion, invasion, proliferation, and migration. He and his colleagues from the Neurosurgery Clinic of the University of Medical Sciences in Poznan reported in March’s Cancer Biology and Therapy that they administered naked double-stranded RNA directly into the brain after surgery for patients with grade III glioma and glioblastoma multiforme. The median overall survival for the glioma patients was a statistically significant 72.3 weeks ($p=0.0001$), compared with 59.1 weeks for those on brachytherapy. Among those receiving the RNAi treatment, 83% had functional improvement scores, compared with 33% of those on brachytherapy. Median length of survival of all RNAi-treated patients in this study was 106.6 weeks, compared with 48.2 weeks in previous studies.
Barciszewski noted that primary brain tumors may be an ideal setting for RNAi therapy because administration is local, with no delivery issues, and because the drug can be placed in many locales where surgery may not be possible.

Sirnaomics is developing siRNA drugs for breast cancer and glioma. Both drugs target multiple genes—another new direction in siRNA research. Earlier this year, Alnylam and its collaborators at MIT reported developing a lipid delivery method that can carry five siRNAs at once in mice, instead of only one or two (Proceedings of the National Academy of Sciences online, Jan. 11, 2010).

For breast cancer, the company’s targets include epithelial growth factor receptor, VEGF, and COX-2. In glioma, the siRNAs are aimed at the same growth factors and angiotensin. The focus on receptor-mediated and angiogenic pathways is important, according to Napajen’s Alizadeh. “This will be the hallmark of a new era in RNA-targeting drugs,” he said.