Silencing KRAS

By Lauren Martz, Staff Writer

Oncogenic KRAS is notoriously difficult to target because of a lack of well-defined surface pockets for drug binding. Nucleic acid therapeutics can bypass that issue but have their own problems including tissue targeting, cellular uptake and degradation in the blood. **Silenseed Ltd.** thinks it has addressed the issues of the latter modality with a localized, sustained siRNA delivery technology dubbed LODER.¹

A paper in the *Proceedings of the National Academy of Sciences* provides the first detailed look at the technology and suggests that delivery of siRNA targeting KRAS (K-Ras) using LODER (local drug eluter) may cause pancreatic tumor regression.

Oncogenic KRAS mutations are found in several cancer types, including more than 90% of pancreatic cancers, and are associated with aggressive tumor growth.² The most common oncogenic KRAS mutation in pancreatic cancers involves a gain-of-function substitution of glycine to aspartate at codon 12, dubbed G12D.

Previous studies have shown that siRNA-mediated knockdown of KRAS G12D in pancreatic cancer cells inhibits cell growth *in vitro*,³ but translating the findings to the *in vivo* setting has been hampered by targeting, uptake and degradation issues.

In their *PNAS* paper, Eithan Galun and colleagues showed that LODER effectively targeted mutant KRAS *in vivo* and treated pancreatic cancer in mice.

LODER technology involves encapsulating siRNA therapeutics in a biodegradable polymeric matrix that protects the nucleic acid from degradation and allows long-term controlled release at the site of implantation.

Galun is a cofounder of Silenseed and director of the Goldyne Savad Institute of Gene Therapy at the **Hadassah Medical Center**. The paper included researchers from **Tel Aviv University**, **The Hebrew University of Jerusalem**, the **Chaim Sheba Medical Center** and the **Shaare Zedek Medical Center**.

The group incubated LODERs containing anti-KRAS G12D siRNA (siG12D LODER) in saline or mouse liver tissues. The siRNA remained stable and intact for at least 97 days in saline and 60 days in mouse liver tissue.

In human pancreatic cancer cells expressing KRAS G12D, siG12D LODER decreased mutant KRAS levels and induced cancer cell death comparably to free siRNA against the target. These findings suggest LODER delivery does not alter siRNA activity.

The next step was finding out whether the system could deliver active siRNA to tumors *in vivo*.

In mice with subcutaneous human pancreatic cancer tumors, intratumoral implantation of siG12D LODER decreased tumor volume and

cancer cell proliferation and increased survival compared with no treatment or implantation of empty LODER.

The team saw similar results in mice orthotopically transplanted with the same human cancer cells.

Implantation of siRNA-loaded LODERs into the pancreas did not affect body weight, liver weight or behavior.

"LODER is designed to deliver oligonucleotide-based drugs regionally over many months and potentially also a few years. It preserves RNA or DNA without degradation *in vivo* and in humans and can be programmed to optimize the release curve," said Silenseed CEO Amotz Shemi.

Rolf Brekken, an associate professor of surgical oncology and of surgery and pharmacology at **The University of Texas Southwestern Medical Center**, added, "The LODER technology appears to provide sustained delivery of therapy in a target organ. This is a significant improvement over the current standard for RNAi strategies, which is basically systemic injection of liposomal formulations loaded with the RNAi construct."

"One advantage of local delivery approaches such as LODER is that it reduces the need to stabilize the siRNA against nucleases in the blood," noted David Brown, director of discovery and *in vivo* research at **Mirna Therapeutics Inc.**

Mirna's lead candidate, MRX34, is a liposome-formulated mimetic of the tumor suppressor microRNA-34 (miR-34) that is in Phase I testing to treat liver cancer. The company also has miR-Rxlet7, a microRNA let-7 (MIRLET7; LET-7) mimetic that antagonizes KRAS, in preclinical testing for lung cancer.

LODER running

Silenseed has completed Phase I/II testing of siG12D LODER in patients with pancreatic cancer and is planning a Phase II/III trial.

The single-arm Phase I/II trial evaluated siG12D LODER plus chemotherapy and showed disease stability in all patients and a decrease in the level of pancreatic cancer biomarker CA 19-9 in 64% of patients. There was one serious adverse event associated with the treatment.

Shemi said that the upcoming trial will measure overall survival and quality of life. He said that the company hopes to launch siG12D LODER by 2018.

Shemi also said that the LODER platform can be extended to additional types of therapeutics including small molecules, plasmids and mAbs. In addition to expanding the potential therapeutic cargo, Silenseed thinks that LODER technology could be applied to oncogenes in brain, breast, prostate and thyroid, and regional cases of cervical, colorectal and esophageal tumors.

He added that the platform may even be applied to non-oncology indications such as localized chronic pain and degenerative diseases.

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Southwestern Medical Center**

But in cancer, Brown said, “The application will be restricted to localized tumors, which will limit its utility with the greater problem in cancer—metastases. The approach won’t successfully treat patients with advanced cancer.”

He also said that Silenseed will need to show that local delivery to tumors translates to actual entry into tumor cells. “Efficient delivery of siRNAs into cells typically requires that the molecules be encapsulated in liposomes or polymer nanoparticles which can interact with the plasma membrane and be taken up by the cell,” he said.

Brekken said that an additional challenge is that surgeons must be able to implant the biodegradable polymeric matrix in the target organ, which could be challenging in some cancers.

Brown did say that pancreatic cancer is probably the ideal candidate for Silenseed’s approach.

“Systemic drug delivery for pancreatic cancer is almost impossible due to the high tumor pressure, meaning pancreatic tumors are generally hypovascular with a lot of stroma that prevents blood flow through the tumors and therefore prevents the systemic delivery of drugs into the tumors,” he said. “Locally delivered implants overcome this high–tumor pressure problem.”

Shemi told *SciBX* that Silenseed holds a patent covering LODER and has filed for additional IP. He said that Silenseed is discussing LODER product partnerships with undisclosed companies.

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COMPANIES AND INSTITUTIONS MENTIONED

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