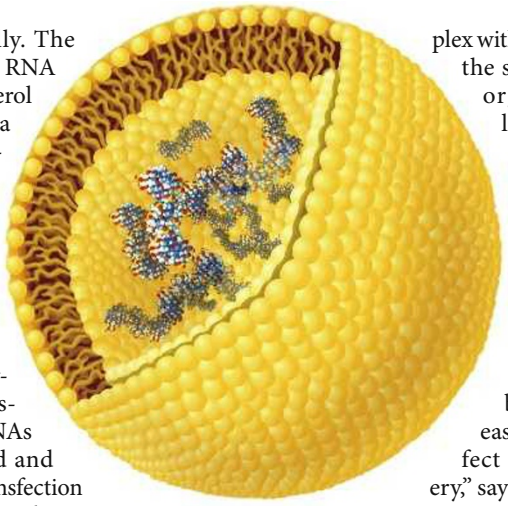
delivered systemically. The team used a synthetic RNA conjugated to cholesterol and stabilized with a partial phosphorothioate backbone and 2′-*O*-methyl sugar modifications on both the sense and antisense strands of the RNA.

Since this study, both lipid- and polymer-based vehicles for systemic delivery of siRNAs have been developed and tested. At Polyplus-transfection in Illkirch, France, researchers have taken advantage of the difference between cationic polymers and cationic lipids for systemic delivery to different organs. "We are interested in delivery to the lung and have used systemic administration of the cationic polymer polyethylenimine for delivery," says Patrick Erbacher, the company's chief scientific officer. "But for tumour injections, we use either a cationic polymer or a cationic lipid formulation."

With siRNAs conjugated to lipids or encapsulated in liposomes or lipid nanoparticles, several companies have achieved stable and efficient systemic delivery to organs including the liver, pancreas, kidneys and even to some types of tumour. And polymers that can complex with siRNA can deliver the short sequences to organs such as the lungs, spleen and kidneys.



Liposomes offer one way of achieving systemic delivery of siRNAs.

ENCAPSULA NANOSCIENCES

Researchers at Altogen Biosystems based in Las Vegas, Nevada, are exploring cationic lipids and biodegradable polymers for *in vivo* delivery, but have not found it easy. "There is no perfect method for delivery," says Andreas Kim, the company's vice-president of research and development.

"Nothing really works amazingly well. All methods have their advantages and disadvantages." In mice, he notes, lipid-based delivery of siRNA is very efficient but tends to induce an inflammatory response to the lipid formulation. Delivery vehicles based on biodegradable polymers, on the other hand, don't cause inflammatory responses but are not delivered as efficiently and the effects seem to be more transient than their lipid-based counterparts.

Alnylam is using liposomes to deliver siRNAs to the liver. "It is easier to target things in the liver with liposomes because about 95% of the injected dose for liposomal formulations goes to the liver," Maraganore says. Liposomes are synthetic analogues of the cell membrane and are made up of hydrophilic and hydrophobic regions that form spherical 'packages' in aqueous conditions. Alnylam is using this approach to target two genes in the liver — one involved in regulating levels of low-density lipoprotein in the blood and the other involved in liver cancer (targeting both vascular endothelial growth factor and kinesin spindle protein).

The ability to use lipid-based delivery vehicles to target the liver has made the organ a popular starting point for many companies. Merck, for example, is using lipid nanoparticles that, like liposomes, take advantage of endogenous mechanisms for uptake, but have no specific ligand attached for targeting.

John Maraganore is confident systemic delivery of siRNA can be achieved.

ALNYLAM PHARMACEUTICALS

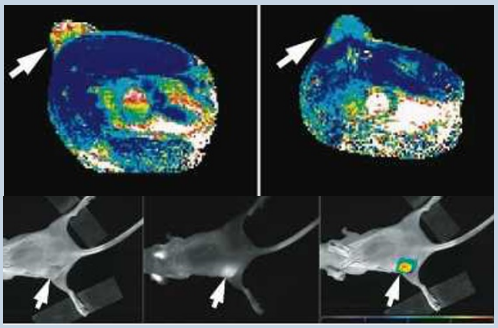
Although lipid nanoparticles are its main focus, Merck is also exploring other delivery vehicles — in some cases through external collaborations. "We are working aggressively in the licensing arena — inviting people to work with us in an evaluation phase," says Sachs. In October, the company finalized a licensing agreement with

THE INSIDE TRACK

How to deliver short-interfering RNAs (siRNAs) to specific tissues is only part of the problem facing researchers. They also need to find out whether the RNA has reached its intended target. Anna Moore, a radiologist at Harvard Medical School in Boston, Massachusetts, is well aware of the issue. "There was no way to use a clinical imaging modality to see the delivery of siRNA," she says.

When researchers want to see whether an siRNA has been reached a particular tissue, they usually perform histological analysis followed by reverse transcription PCR to see whether the target gene was silenced. "You can do this with mice, but when you move on to humans it becomes impractical," Moore says.

Researchers can track siRNAs *in vivo* using bioluminescence imaging or by tracking green fluorescent proteins. But bioluminescence imaging is not a clinical modality. So Moore and her colleagues decided to try magnetic resonance imaging (MRI).



Now you see it: the nanoparticle system devised by Anna Moore's team allows siRNA delivery to be seen in MRI scans (top) and optical scans (bottom).

NATURE MED. (REF. 5)

The first step was to design an siRNA delivery vehicle that could be imaged by MRI. Moore and her team used a nanoparticle containing an iron oxide core. They coated it with dextran, which could have various targeting features added to it relatively easily.

Although iron oxide can be imaged using MRI, the group also attached a fluorescent dye, Cy 5.5, to the dextran coat for optical imaging. "We wanted to correlate the imaging data with microscopic findings," says Moore.

The iron oxide nanoparticle generates a bright spot on the MRI image. The exact target of the nanoparticle can then be confirmed by the fluorescent dye and by doing microscopy for histological analysis.

Two further attachments were then made to the nanoparticle via the dextran coating: a membrane translocation peptide that can cross cell membranes and an siRNA. With this, Moore and her colleagues thought they had a particle that could target and image delivery to tumour cells *in vivo*. But the imaging showed that the nanoparticle went to the liver and kidneys, and was present in other organs as well[5].

Moore and her team plan to continue with the nanoparticles, trying to make them more efficient in terms of delivery and target uptake. But the real value of these nanoparticles might be their versatility. As different siRNAs or targeting peptides can be attached to the dextran coat, a large range of therapeutic siRNAs and peptides can be tested.

"My lab is really interested in imaging other pathologies such as diabetes, which is far from cancer but the imaging approaches are very similar," says Moore. "And that is the beauty of this technology — you can apply it to different pathologies." N.B.