studying them in animals in the 20 years since it began studying antisense drugs. Even so, there is a push to identify new and better modifications, and many companies are converging on similar ones. This year, Isis plans to pick clinical candidates with bicyclic additions to the sugars in the nucleotides. With these modifications, nucleotides are positioned such that base-pairing becomes more stable, and potency is boosted, Crooke says. MDRNA of Bothell, Washington, acquired the patents for a similar technology, called bridged nucleic acids (BNAs), from Valeant Pharmaceuticals, headquartered in Aliso Viejo, California, this March. Both kinds of modification are similar to Locked Nucleic Acids, produced by Exiqon of Vedbaek, Denmark, and used by Santaris Pharma, which introduce a ring at the 2′ position of each sugar molecule. Such modifications increase the half-life by an order of magnitude compared with the types of oligonucleotide now in the clinic, says Santaris vice-president and chief scientific officer Henrik Ørum. "We inject these into animals systemically as naked molecules. Once they are in the tissues, they will have a half-life of weeks to many weeks."

Santaris also focuses on making its single-stranded molecules as small as possible, to aid their entry to cells. RXi has also used this strategy, designing 15-nucleotide double-stranded molecules.

Another approach that is in development is to conjugate antibodies or other specific targeting agents directly to the RNA molecules. Biotechnology company Dicerna Pharmaceuticals in Watertown, Massachusetts, is working with 27-nucleotide sequences, based on their proprietary Dicer Substrate Technology, which enter the RNAi-processing pathway earlier than other synthetic counterparts. It has partnered with Kyowa Hakko Kirin, the biopharmaceutical arm of which is based in Princeton, New Jersey, to provide the delivery technology. But like many companies, Dicerna is interested in multiple delivery strategies.

The rise of RNAi therapeutics companies has spurred the development of additional technology to track how oligonucleotides are processed by the body, but high-throughput purification and analysis technologies are not designed for such large molecules with a strong negative charge. Researchers who want to track nucleic-acid metabolites from humans and animals have a "huge problem", says Michael McGinley, bioseparations product manager for Phenomenex in Torrance, California. "You have polar [charged] oligonucleotides floating around in serum that like to bind to things. How are you going to pull them and their metabolites out and separate them from everything else that's there?" In September of last year, Phenomenex began selling kits and other products, such as its Clarity Oligo-MS and Clarity Oligo-RP chromatography columns, to researchers who are running preclinical and clinical studies. These kits provide high-throughput techniques to isolate synthetic oligonucleotides from biological fluids such as serum and urine, as well as from solid tissues, including liver and lung tissue. Although McGinley anticipates tweaking and improving the products over time, he says that they work well with all of the delivery devices, conjugates and modifications tested so far.

Potency potential

No matter what the approach, the delivery process is easier if researchers can achieve the same therapeutic effect with fewer molecules, so it is crucial to design small RNAs with high potencies. Most therapeutics companies are using a combination of open-access computer programs and their own algorithms to design siRNA sequences that will knock down a target mRNA. Indeed, some computer programs may generate thousands of sequences against a single mRNA. These are then assessed both by scientists and by bioinformatics programs. That's only the first step, says Khvorova of RXi. "All this computational screening is incredibly important, but then you need to do physical screening."

There are significant differences between small-molecule screening and RNA screening, says Caroline Shamu, director of the ICCB-Longwood Screening Facility at Harvard

MICRORNAS AS BIOMARKERS

In the 1990s, Carlo Croce, then director of the Kimmel Cancer Center in Philadelphia, Pennsylvania, was hunting for genes involved in chronic lymphocytic leukaemia. The disease was consistently associated with a lesion in chromosome 13, and so, back before the human genome was sequenced, his lab determined the identity of the nucleotides in an 800-kilobase stretch from the deleted region and began searching for protein-coding genes. "We failed, for six years," recalls Croce, now director of the Human Cancer Genetics Program at the Ohio State University in Columbus. His lead graduate student left science to go to business school.

After another false start and a lucky conversation, Croce obtained cells from a patient who had leukaemia involving a very small translocation, only about 30 kilobases. Croce examined this region thoroughly enough to convince himself that it contained no genes. Then he read about microRNAs, which had just been discovered in mice. Further experiments quickly revealed that the region encoded two microRNAs, which, in 2002, were the first to be implicated in disease[4].

ROSETTA GENOMICS

Rosetta Genomics is using microarrays and PCR to identify microRNA biomarkers and develop tests.

A few years later, researchers led by Todd Golub at the Broad Institute in Cambridge, Massachusetts, examined RNA molecules in tumours and reported that using just a small number of microRNAs, about 200, provided a better classification of tumours by type and source than using 16,000 messenger RNAs[5]. There's great potential, says Croce. "There is no doubt in my mind that microRNA can be used for diagnostic and prognostic purposes."

Indeed, although insurance companies will not yet pay for them, at least two companies offer services that involve testing for microRNAs in biopsies from patients with cancer: Rosetta Genomics in Rehovot, Israel, and Asuragen in Austin, Texas.

Such tests are possible because microRNAs are surprisingly stable both in the body and in paraffin blocks, says Muneesh Tewari, a researcher at the Fred Hutchinson Cancer Research Center in Seattle, Washington, and lead author on one of the first papers showing that microRNA can be extracted from plasma and serum[6]. "The hard part of this," he says, "is working with small quantities of starting RNAs and applying this to the technologies" that can identify them. Once extracted, known microRNAs can be identified by using microarrays and quantitative PCR; both known and unknown microRNAs can be identified by sequencing.

But finding microRNAs in samples from patients is only the first step to identifying which microRNAs carry information about disease, Tewari says. "Very little is known about the variation of microRNAs." The results of experiments can vary for reasons besides disease, says Jun Lu, a genetics researcher at Yale University in New Haven, Connecticut, and first author on the paper with Golub[5]. But, he says, "if it's a strong discriminator, then you should get the same answer no matter what platform you're using". M.B.