EDITORS' pick

RaNA Therapeutics

This Cambridge, Massachusetts-based startup aims to use oligonucleotides to downregulate long noncoding RNAs in epigenetic disorders and to modulate crop traits.

RaNA Therapeutics co-founders Art Krieg and Jeannie Lee

now. RaNA is currently in the process of finalizing the specific oligonucleotide chemistry it will employ and is several years from starting a clinical trial. Because it will use single-stranded antisense oligonucleotides, the company hopes to avoid some of the delivery problems that have bedeviled the development of drugs based on double-stranded short interfering RNA (siRNA). It plans to administer its lncRNA inhibitors subcutaneously as a saline solution. Even so, as for other antisense approaches, endosomal escape of oligonucleotides, once they have been transported across the cell membrane, remains a challenge.

Several other startups are targeting epigenetic mechanisms, such as Copenhagen-based EpiTherapeutics, Cambridge, UK-based CellCentric, and Cambridge, Massachusetts-based Epizyme and Constellation Pharmaceuticals, all of which are working on enzymatic targets, such as histone methyltransferases, histone demethylases, histone trimethyl demethylases and ubiquitin modifiers. RaNA's closest competitor is Opko-Curna, which is also using oligonucleotides to target a noncoding RNA species called natural antisense transcripts (*Nat. Biotechnol.* **30**, 453–459, 2012). The company also aims selectively to boost the expression of target genes, such as sodium channel, voltage-gated, type I, alpha subunit (SC1NA), a brain sodium channel which, owing to haploinsufficiency, is poorly expressed in Dravet's disease, a severe form of childhood epilepsy. Both approaches promise greater specificity than that offered by targeting epigenetic mechanisms at the enzymatic level, but the downside is that they rely on an experimental therapeutic platform with, as yet, a limited number of target organs. In short, RaNA is working with unvalidated technology, applied to an emerging branch of biology. Its risk profile is as distinct as its technological approach.

Cormac Sheridan Dublin

The recent slew of publications from the Encyclopedia of DNA Elements (ENCODE) consortium has bolstered information on an emerging class of regulatory RNAs—the long noncoding RNAs (lncRNAs). Still poorly understood, lncRNAs play important roles in the epigenetic regulation of gene expression, and ENCODE has now described the general characteristics of 9,640 manually curated lncRNA loci (see <http://www.nature.com/encode/threads/non-coding-rna-characterization>). With information on this RNA class burgeoning, the potential for therapeutic modulation of lncRNAs has sparked investor excitement in RaNA Therapeutics, which closed a \$20.7-million Series A venture funding round back in January. The investor syndicate notably included St. Louis, Missouri-based agbiotech firm Monsanto.

Several commentators have aptly referred to lncRNA as the 'dark matter' of the cell. It requires a complete reassessment of the conceptual frameworks underpinning molecular biology, according to John Mattick, executive director of the Garvan Institute of Medical Research, in Sydney. Unlike dark matter, however, lncRNA molecules have been positively identified and are abundant. As a consequence, initial skepticism about their importance is on the wane, even if uncertainty remains about how exactly they regulate gene expression (*Nature* **482**, 339–346, 2012; doi:10.1038/nature10887).

Cambridge, Massachusetts-based Atlas Venture is a co-founder of RaNA, as is CEO Art Krieg, the founder and chief scientific officer of Coley Pharmaceutical, a pioneer in Toll-like receptor modulation that New York-based Pfizer acquired in 2007. Krieg had initially approached Atlas with a completely different proposition. After Coley's acquisition, he assumed responsibility for Pfizer's oligonucleotide drug research. When the big pharma firm later decided to cease internal research in this area, he sought to take on some of its projects with external support.

Atlas, however, was looking to build a company around the research of scientific founder Jeannie Lee, of Massachusetts General Hospital, in Boston, who is involved in unpicking the molecular mechanisms underlying the epigenetic phenomenon of female X-chromosome

inactivation in mammals. Developing female embryos inactivate expression of one of their two x-chromosomes, through a process that starts with the expression of a 17-kilobase (kb) noncoding RNA called Xist. Lee and co-workers identified a 1.6-kb ncRNA sequence within Xist, called RepA, that acts as the target for Polycomb repressive complex 2 (PRC2), a highly conserved regulator of chromatin structure (*Science* **322**, 750–756, 2008). PRC2, once recruited by RepA, silences gene expression by catalyzing the trimethylation of the lysine 27 residue within the H3 histone protein.

Lee's group subsequently developed an RNA immunoprecipitation and RNA sequencing (RIP-seq) method to identify over 9,000 noncoding RNAs that interact with PRC2 in embryonic stem cells (*Mol. Cell.* **40**, 939–953, 2010). This work, together with the demonstration that locked nucleic acids can be used selectively to disrupt PRC2-lncRNA interactions (*Proc. Natl. Acad. Sci. USA* **107**, 22196–22201, 2010), forms the basis of RaNA's intellectual property platform and its target and drug discovery activities.

The company aims to apply the same principle to treating human disease by de-repressing the expression of a relevant gene, using oligonucleotides that block the interactions between PRC2 and targeted lncRNAs and thereby prevent PRC2 recruitment to specific chromosomal loci. The company believes the approach may be applicable against a wide range of genetic conditions, ranging from haploinsufficiency conditions, in which a single healthy allele cannot produce enough protein, to complex immunological conditions and cancer. Krieg was initially doubtful about the potential of the strategy, given the complexity of the biology involved. However, pilot studies on 15 genes were all positive, resulting in a median fourfold increase in mRNA expression.

As lncRNAs are expressed at low levels, selective silencing of a target lncRNA may require only a small drug dose to obtain a strong increase in gene expression, according to Claes Wahlestedt, a researcher at the University of Miami, in Florida, and scientific founder of cuRNA, now the Opko-Curna subsidiary of Miami-based Opko Health. Whether such increases will result in durable, clinically meaningful effects remains an open question for