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COMMENTARY

Targeting LDL Cholesterol With LNA

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Cardiovascular disease is the number one cause of death in the industrialized world. Much of the morbidity and mortality from heart disease can be linked to elevated low-density lipoprotein (LDL) cholesterol, and one of the key genes that increases LDL cholesterol is Proprotein convertase subtilisin/kexin type 9 (PCSK9). In a recent study in non-human primates, Lindholm *et al*. demonstrate that a 1 month course of weekly subcutaneous therapy with either of two locked nucleic acid (LNA) antisense oligonucleotides targeting PCSK9 rapidly decreases the expression of both PCSK9 and circulating LDL cholesterol without reported toxicity.[1] The reduction in serum LDL is sustained for more than a month after the last dose of the LNA antisense.

LDL is normally removed from the circulation on binding the LDL receptor, LDLR. PCSK9 functions by increasing the rate of degradation of the LDLR, secondarily preventing LDL clearance. PCSK9 thus increases circulating LDL, thereby also increasing the risk of atherosclerosis. Intriguingly, PCSK9 does not appear to be required for any other function, as humans completely lacking PCSK9 are perfectly healthy, and in fact have elevated expression of LDLR and much lower circulating LDL, with reduced risk of cardiovascular disease.[2] Based on these and other genetic data PCSK9 is widely considered to be a validated target, and inhibitors of PCSK9 are excellent therapeutic candidates. Given that nearly all patients with elevated LDL will be treated with statins, the gold standard for therapy of hyperlipidemias, it is extremely important that PCSK9 inhibitors have been shown in primate models to provide further reductions in LDL beyond what can be achieved with statins alone.[3] Several PCSK9 inhibitors are in preclinical and clinical development by biotech and pharmaceutical companies, using approaches including small molecules, monoclonal antibodies, antisense and RNA interference.[4]

Against this backdrop of clear need for PCSK9 antagonists, the results of Lindholm *et al*. reveal the great progress over recent years in the development of antisense oligonucleotides against targets expressed in the liver. Two different antisense oligonucleotides, SPC4061 and SPC5001, are compared to a control, SPC3088 (the control was used for the *in vitro* studies, but not *in vivo*). All three of these oligonucleotides had phosphorothioate backbones and were designed as LNA gapmers 13–16 bases long with two or three LNA modifications at the 5′ end, three LNA modifications at the 3′ end, and a DNA core (to facilitate RNAse H-mediated cleavage of the complementary PCSK9 mRNA target). Both of the antisense oligonucleotides showed similar activity *in vitro*, whether by transfection or unassisted uptake (also referred to as "gymnotic delivery") into HepG2 cells. There has been some speculation that perhaps unassisted uptake would better predict *in vivo* efficacy than transfection experiments, but such was not the case in the results of Lindhom *et al*.: despite similar *in vitro* activity of the two antisense oligonucleotides, SPC5001 proved to be significantly more potent than SPC4061 in the multiple dose monkey study, reducing circulating PCSK9 protein by 85% compared to 50% in the monkeys dosed with SPC4061. Both antisense gapmers were present within liver tissue at similar concentrations (~50 µg oligonucleotide/gram of tissue), excluding sequence-dependent differences in liver uptake. At present, there appears to be no *in vitro* correlate for *in vivo* efficacy with antisense oligonucleotides, and experimenters wishing to develop the most potent compound with the best therapeutic index may be well advised to proceed into primate testing with multiple candidate compounds before selecting a lead for clinical development.

The *in vivo* efficacy of the SPC5001 was both rapid and sustained. Serum PCSK9 was reduced by >50% as early as 24 hours after the first dose. The target protein concentration was ~85% reduced during the dosing period, with a gradual recovery during the subsequent 2 months. Even 1 month after the last dose of SPC5001, monkeys still showed approximately a 50% reduction from baseline PCSK9 concentration.[1]

In a fairly typical previous study using 20 base-long 2′-O-methoxyethyl antisense gapmers the oligonucleotide concentration in the liver required for 50% inhibition of the target ApoB mRNA was ~300 µg/g of liver tissue.[5] Although one cannot really compare potency across different targets, and of course the Lindholm *et al*. study is not a pharmacokinetic analysis allowing the derivation of a liver EC50, the fact that Lindholm *et al*. show a liver concentration of just 50 µg oligonucleotide SPC5001/gram of tissue is associated with a >80% reduction in target RNA suggests a much greater *in vivo* potency of the relatively short gapmer LNA designs compared to the earlier 20mer gapmer designs using 2′-O-methoxyethyl.

The safety profile is paramount for any compound under consideration for chronic administration, such as a PCSK9

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inhibitor. Some LNA oligonucleotides sequences are hepatotoxic,[6] but other LNA antisense compounds of the same length and pattern of modifications can be quite nontoxic: LNA antisense gapmers to survivin and hypoxia-inducible factor-1α have been dosed in human clinical trials above 4 mg/kg weekly for as long as over a year with an acceptable safety profile (http://webcast.aacr.org/portal/p/2011annual552). The results of Lindholm *et al*. are quite encouraging in providing further evidence for the fundamental safety of the LNA platform, showing no worrisome changes in serum chemistries or pathologic evidence of organ toxicity within the 30-day study, in which monkeys were dosed subcutaneously once at 20 mg/kg followed by four weekly doses at 5 mg/kg. Although not commented on in the present study, subcutaneous injection of gapmer oligonucleotides commonly induces injection site reactions, which are typically transient, mild, and reversible.

Translation of animal studies into the clinic is often challenging. The first human phase I safety and tolerability clinical trial of SPC5001 opened 18 May 2011, but was terminated 4 October 2011 (http://clinicaltrials.gov/show/NCT01350960). Henrik Ørum, the Santaris CSO, provided the following comment on the clinical trial termination: "we observed pharmacology as expected but weren't satisfied with the therapeutic window given that the drug is intended for chronic use. Still like the target, though, so we are looking for a fast follow-up with the appropriate profile." (H. Ørum, personal communication, Santaris CSO, 7 December 2011).

The LNA modification combines several desirable properties for the development of therapeutic oligonucleotides. The constrained sugar provides a substantial increase in Tm, the LNA is highly resistant to nucleases, and it greatly reduces immune stimulatory effects compared to unmodified oligonucleotides. The shorter length of the LNA gapmers in the current study compared to traditional 2′-O-methoxyethyl 20mer gapmers should reduce the level of serum protein binding, and may conceivably allow faster exit from endosomes due to the reduced charge, although that has yet to be demonstrated. It is possible that there may also be other properties of LNA modifications that provide further advantages for *in vivo* applications, and remain to be identified. Constrained sugars have become accepted as the next generation in development of antisense therapeutics to the point that in 2010 Isis adopted a constrained sugar, IScEt, for its upcoming antisense programs (http://www.isispharm.com/Antisense-Technology/Antisense-Drug-Discovery-Platform/Medicinal-Chemistry.htm).

Great scientific insights or breakthroughs seldom lead directly and smoothly into new therapeutic platforms. Instead, the initial innovation that launches a new field typically requires successive further innovations for clinical translation. Over the last two decades the field of antisense therapeutics has overcome many challenging barriers and built up a solid technical foundation. Recent studies such as Lindholm *et al*. leave little doubt that the development of short LNA gapmers for antisense therapy represents an important further innovation that will support the wider contributions of antisense technology to therapeutic pipelines.

Conflict of Interest

A.M.K. is employed by and holds stock in RaNA Therapeutics, which seeks to develop oligonucleotides for therapeutic applications, possibly including LNA or LNA-like modifications.

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