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PRODUCT DEVELOPMENT

NeuBase takes antisense oligos to more targets in more places

BY ALLISON JOHNSON, SENIOR WRITER

Newcomer NeuBase Therapeutics is re-engineering antisense oligos to access a broader range of targets, while simultaneously solving the modality's dosing and delivery issues.

With three approvals since the start of 2016, antisense oligonucleotides (ASOs) have started to hit their stride, yet first-generation technologies are limited on multiple fronts.

Most ASOs only target linear RNA, which means they fail to bind the more complex structures often assumed by mutant, disease-causing RNAs. And ASOs don't cross the blood-brain barrier, which has meant that Biogen Inc.'s spinal muscular atrophy therapy Spinraza nusinersen must be delivered intrathecally.

NeuBase aims to address those issues, plus several others, with its Peptide-nucleic acid AnTisense OLigonucleotide, or PATrOL, platform.

Founded by serial entrepreneur and CEO Dietrich Stephan, NeuBase Therapeutics Inc. made its trading debut on NASDAQ through a reverse merger on July 18, just 11 months after launching. Stephan told BioCentury the biotech took advantage of an RNA-hungry market to eschew the more traditional VC financing route,

enabling it to hand pick its board without restrictions. It attracted former Global Head of Innovation at Johnson & Johnson and current Vividion Therapeutics Inc. CEO Diego Miralles, as well as Franklyn Prendergast, who served on the board of Eli Lilly and Co. from 1995-2017 (see SideBar: "NeuBase Takes Control").

On July 30, the company added more big names to its roster when former National Cancer Institute Director Samuel Broder and serial entrepreneur George Church, a professor of genetics at Harvard Medical School, joined its SAB.

A key differentiator of NeuBase's PATrOL platform is its collection of double-sided nucleic acid bases, which are responsible for the oligos' ability to bind new target types. These "bifacial" bases can bridge two strands of RNA, allowing them intercalate into a variety of folded RNA structures, not just bind to linear strands.

The bases can also bind DNA, offering an entirely different mechanism for regulating protein expression.

The biotech's platform also improves delivery and dosing via a novel trafficking ligand and streamlines manufacturing via tweaks to the molecules' peptide backbones.

The trafficking ligand renders the oligos blood-brain barrier penetrant, solving the CNS delivery problem, and transports the molecules directly into the cytoplasm. The latter avoids the loss of therapy that normally occurs after endosomal uptake, and could enable lower dosing.

The components of PATrOL springboard off work by NeuBase's scientific founder and CSO Danith Ly at Carnegie Mellon University, where he remains a professor of chemistry.

NeuBase is applying PATrOL first to Huntington disease (HD) and myotonic dystrophy, and plans to take a program into the clinic in at least one of those indications in 1Q21. It also has a handful of earlier stage oncology programs.

The biotech has maintained about a \$66 million market cap since it began trading, and Stephan said it has 15-18 months of runway.

Doubling down on ASOs

NeuBase's Ly spent more than a decade perfecting the compatibility of bifacial nucleic acids with any double-stranded RNA or DNA structure.

Standard nucleic acids incorporated into ASOs bind to one base, a complementary A, C, U, or G in the single-stranded RNA target, but NeuBase's bases can bind an A, C, U/T, or G on either end.

"These are bases that can go into a hairpin, open it up, and then hydrogen bond to both the ascending and descending strands," Stephan said.

Last year, Ly published two studies in *Biochemistry* applying the concept to hairpins that form in mutant versions of mRNAs encoding *HTT* and *DMPK*, which cause HD and myotonic dystrophy, respectively.

Unlike previous versions of bifacial bases that have been reported in the literature since the 1990s, Stephan said Ly's key innovation was engineering each base to be the same size, which enables strong hydrogen bonding along the entire length of the ASO.

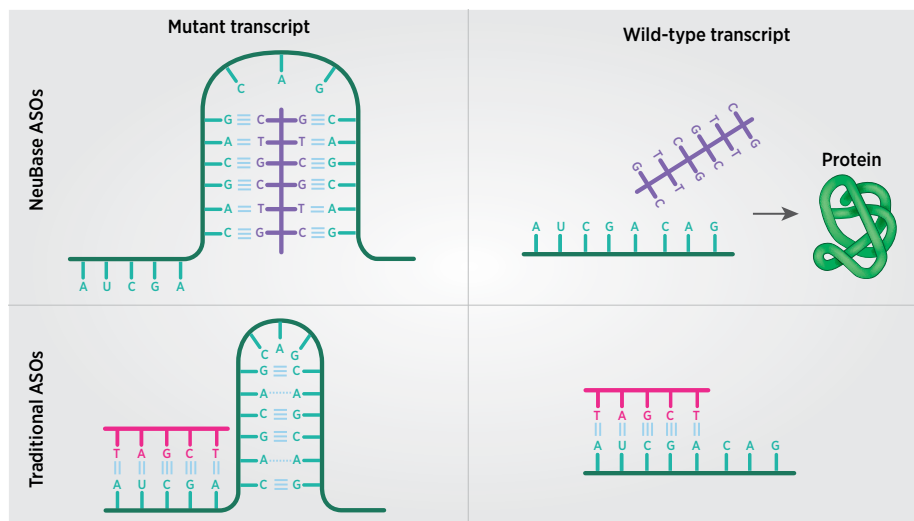
"In order to get the hydrogen bonding even down the hairpin, these bases have to be the same size. You can imagine if you had a really big base and a small one, you wouldn't get stable intercalation of the drug," he said.

NeuBase's selectivity advantage

By incorporating double-sided bases into its antisense oligonucleotides (ASOs), NeuBase Therapeutics Inc. can target hairpin structures unique to mutant mRNA transcripts, leaving wild-type transcripts intact. The company is using the feature to treat autosomal dominant diseases that arise when one copy of a gene is mutated.

Whereas traditional ASOs bind linear segments of RNA, NeuBase's ASOs work by inserting themselves between the two strands in an RNA hairpin and forming hydrogen bonds with both.

This means that in autosomal conditions like Huntington disease, NeuBase's ASOs can achieve selective degradation of the mutant, disease-causing transcript, allowing the wild-type version to carry on its cellular functions. The company believes this will give it a safety advantage over traditional ASOs, which don't discriminate between the two types of transcripts.



NeuBase is taking advantage of this ability to bind double-stranded regions of RNA to treat genetic disorders that are autosomal dominant, meaning they arise when one copy of a gene is mutated.

Because mutant transcripts often take on secondary structures that wild-type transcripts don't, targeting these structures enables allele-specific treatment that spares the wild-type gene (see Figure: "NeuBase's Selectivity Advantage").

"In Huntington disease, humans need that wild-type protein to function correctly," Stephan said, so selectively modulating the mutant transcript while leaving the wild-type one intact could provide a safety advantage.

NeuBase's first two preclinical programs target unique hairpins in mutant *HTT* mRNA and in mutant *DMPK* mRNA.

Wave Life Sciences Ltd. is the only ASO company testing an allele-specific *HTT* therapy in the clinic, according to BioCentury's BCIQ database.

Wave's approach to avoiding wild-type *HTT* is to target SNPs unique to mutant *HTT*. Wave and partner Takeda Pharmaceutical Co. Ltd. are developing two ASOs that each bind a different SNP. WVE-120101 and WVE-120102 are being tested in the Phase Ib/IIa PRECISION-HD1 and PRECISION-HD2 trials, respectively. Topline data expected from both by year end.

Wave estimates that together, the SNPs occur in up to 70% of HD patients. NeuBase expects its approach to treat 100% of HD patients.

No companies have allele-specific ASOs for DMPK in clinical testing, but two have small molecules that aim to treat the DM1 form of myotonic dystrophy.

Expansion Therapeutics Inc. began a Phase I trial in June testing ERX-963 to treat DM1. The company's platform identifies small molecules that can bind to folds in RNA, such as those formed by CUG repeats in mutant DMPK.

AMO Pharma Ltd. is planning a pivotal trial testing small molecule GSK3B inhibitor tideglusib (AMO-02) to treat DM1. It completed a Phase II trial of the therapy last year showing "most patients" showed improvements in cognitive function, fatigue and the ability to perform daily tasks.

ASOs meet DNA

Another application of double-sided bases is targeting DNA, and according to Stephan, no other company has antisense therapies that can do that.

"This is compelling because now we can target regions of genes like a promoter with an ASO, have the ASO land on that promoter and sterically inhibit RNA polymerase from transcribing that gene," Stephan said.

NeuBase can do the same thing with other regulatory elements like enhancers and repressors, he added.

Beyond these conventional transcriptional regulators, NeuBase is applying the steric hindrance its ASOs can deliver to a white space just starting to gain a foothold in industry: controlling gene expression by modulating the 3-D structure of chromatin (see "[Exploring 3-D Genomic White Space](#)" & "[A Venture View of Biological White Space](#)").

The idea is to prevent modulators from accessing chromatin and changing its shape, which could help control the expression of many genes, not just one.

The indications NeuBase is pursuing in this area are undisclosed.

Breaching barriers

NeuBase plans to leverage a novel targeting peptide to send its ASOs to the brain with systemic delivery, avoiding the need for intrathecal administration.

While the details of NeuBase's targeting peptide are undisclosed, Stephan said it is based on a roughly 12 amino acid stretch of the HIV tat protein that was shown in the 1990s to traffic payloads into cells throughout the body.

"Based on that, we distilled down the active groups of that peptide and figured out how to decorate the [ASO] backbone with it," said Stephan.

The ability to systemically deliver ASOs to the CNS is one of the major reasons the company is leading with neurology indications, he noted.

All three therapies in clinical testing for HD are delivered intrathecally. In addition to Wave's WVE-120101 and WVE-120102, Roche and Ionis

NeuBase takes control

Having gone the VC-backed route for 14 other startups, NeuBase Therapeutics Inc. CEO Dietrich Stephan sought more control over the direction of his latest company and felt the market was hungry enough for its gene silencing antisense technology to make a public listing feasible. NeuBase completed a reverse merger on July 12 with shell company Ohr Pharmaceutical Inc.

"This blend of gene therapy and RNA-targeting therapeutics is probably the hottest space in life sciences right now," said Stephan, citing Ionis Pharmaceuticals Inc.'s \$9.2 billion market cap and the June IPO of preclinical antisense company Stoke Therapeutics Inc., which raised \$142 million through the listing, giving it a valuation of \$566.3 million.

Dietrich said avoiding the VC route gave him and the company three primary degrees of freedom.

The first was selecting NeuBase's board of directors.

"I selected the board, and it's comprised of people who are deeply experienced drug developers. Between the five of us, we've brought at least a dozen drugs to market and have all the scars to show for it," he said.

The board includes Vividion Therapeutics Inc. CEO Diego Miralles. Until 2016, Miralles was Global Head of Innovation at Johnson & Johnson where he founded and launched J&J's JLABS incubators.

Also on the board is Franklyn Prendergast, who served on the board of Eli Lilly and Co. from 1995-2017.

The second freedom, he said, "is that we can take capital when we want and on whatever terms we want based on the status of our data and the capital markets. We are not dependent on the whims of a handful of investors that undermine our valuation and take preferential positions in terms of their equities," he said.

The third is more business development opportunities.

As a public company, Stephan said NeuBase gains increased visibility to potential pharma partners for non-core assets, and conversely, "we have a liquid instrument, so we can also make acquisitions using our equities that are very difficult to do in a private company."

— Allison Johnson

Pharmaceuticals Inc. have IONIS-HTTRx (RG6042) in Phase III trials for HD.

Another benefit of NeuBase's targeting peptide is that it allows the ASOs to enter the cytoplasm directly, rather than take an indirect route via the endosome.

Naked ASOs don't have the proper chemistry to penetrate cell membranes so drug developers typically package them with targeting moieties such as antibodies or other ligands against cell surface receptors that can trigger entry to the cell via endocytosis.

The problem with endocytosis, said Stephan, is that only a small fraction of the ASO molecules escape the endosome, which means that only a small percentage of the administered dose ever sees its target.

NeuBase's peptide has a positive charge, enabling it to interact with the negatively charged cell membrane and enter the cytoplasm directly, side-stepping the endosomal pathway.

"NOW WE CAN TARGET REGIONS OF GENES LIKE A PROMOTER WITH AN ASO."

DIETRICH STEPHAN, NEUBASE THERAPEUTICS INC.

"Being able to flip right into the cytoplasm achieves a higher effective dose at the site of the target," compared with ASOs that get stuck in the endosomal or lysosomal compartments, said Stephan.

According to NeuBase, it should be able to deliver doses that are about tenfold lower than those required for ASOs that enter cells via endosomes.

However, NeuBase's targeting peptide does not give the ASOs cell-type specificity, a property that most other ASO developers are aiming to achieve by attaching cell type-selective ligands to their compounds.

Delivery to select cells reduces the chance of side effects, while ensuring ASOs aren't diluted out in circulation by being taken up into non-target tissues.

Stephan said NeuBase's targets do not require cell type-selective delivery because "the specificity comes from where the mutant transcript is expressed. The therapy should only engage in cells that express a pathogenic mRNA."

He added that for multi-organ diseases, it's beneficial to deliver nucleic acid therapies broadly.

"People think of Huntington's as a brain disease, but there are pathologies outside of the brain such as in the pancreas," and that's where systemic administration of a non-cell type selective ASO can benefit.

Long-term solutions

Another departure from standard ASOs is NeuBase's decision to engineer its molecules with a peptide backbone instead of a sugar backbone, a move that could give its molecules greater durability than either other ASOs or the RNA-targeting small molecules gaining traction in the gene control space.

Stephan explained that whereas sugar backbones carry a negative charge, NeuBase's peptide backbone is non-polar. This means that when NeuBase's ASOs bind a target, there is no negative charge repelling the naturally negatively charged RNA and DNA backbones, he said.

Without the opposing forces, "the hydrogen bonding that occurs between bases will have a much higher strength and you basically have to boil them off," he said.

The company has also figured out how to lock its peptide backbones in specific conformations, which confers manufacturing advantages.

Early peptide-based ASOs were not produced with uniform shapes, because amino acids have chiral centers and take on multiple conformations. This diversity of shapes caused self-aggregation and required purification steps to isolate the conformation of interest.

By using amino acids with locked centers, NeuBase's ASOs don't self-aggregate and they can be designed to adopt the right symmetry from the start, a right-handed helical structure complimentary to that of an RNA hairpin or a DNA double-helix, said Stephan.

"Because it matches the conformation of our target, the [ASO] can bind more tightly when it does engage versus something that has different chiral centers across the molecule," he said.

The added binding strength translates into even longer durability, though it will depend on how frequently the cell uses the gene, Stephan said. For example, tumor cells may require more frequent dosing due to a higher level of active translation and transcription, while post-mitotic neurons will require less frequent dosing.

"We think that with oral or subcutaneous or even IV dosing, we're probably somewhere between once a week and once a month to target RNA. At the DNA level, I could imagine dosing once every six months or once every year," he said. ■

COMPANIES AND INSTITUTIONS MENTIONED

Amo Pharma Ltd., Surrey, U.K.

Biogen Inc. (NASDAQ:BIIB), Cambridge, Mass.

Expansion Therapeutics Inc., San Diego, Calif.

Carnegie Mellon University, Pittsburgh, Pa.

Ionis Pharmaceuticals Inc. (NASDAQ:IONS), Carlsbad, Calif.

NeuBase Therapeutics Inc. (NASDAQ:NBSE), Pittsburgh, Pa.

Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland

Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Tokyo, Japan

Wave Life Sciences Ltd. (NASDAQ:WVE), Cambridge, Mass.

TARGETS

DMPK - Dystrophin myotonia-protein kinase

GSK3B - Glycogen synthase kinase 3 beta

HTT - Huntingtin

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