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Ascidian closes $50M financing to rewrite the rules of RNA splicing

By Cormac Sheridan, Staff Writer

Ascidian Therapeutics Inc. secured $50 million in series A funding from its founder Apple Tree Partners (ATP) to take a therapy based on its RNA exon editing technology into a first-in-human trial in ABCA4 retinopathy and to advance additional programs in neuromuscular, neurological, and rare disease indications.

The company's name points to the molecular underpinnings of its platform. Ascidians – also known as tunicates or sea squirts – are marine invertebrates that perform trans-splicing, a type of pre-mRNA processing that is absent from mammalian cells. It involves the end-to-end ligation of exons generated from different RNA transcripts and it serves to increase the diversity of organisms' proteomes. In contrast, cis-splicing, which does occur in mammalian cells, involves the knitting together of all the exons from one particular transcript, after the non-coding introns have been removed.

Ascidian Therapeutics has developed a platform that enables it to introduce trans-splicing to human cells without employing any external enzymes. The spliceosome, an RNA-protein complex responsible for cis-splicing, can also support trans-splicing, once the appropriate molecular cues are provided. The Boston-based company delivers its RNA exon editors as DNA constructs using adeno-associated virus (AAV) vectors or other transduction technologies. These encode the exons of interest, along with additional components, including splice donor and acceptor sequences and other structural features.

Michael Ehlers, chief scientific officer and venture partner, ATP, and chairman, Ascidian

"The molecule itself is a sort of multi-modular RNA," Michael Ehlers, chief scientific officer and venture partner at ATP, told *BioWorld*. The company can screen for and optimize the individual components independently before knitting them into a single DNA sequence. When transferred to the nucleus of patients' cells, it is transcribed into an RNA molecule, which can be spliced into an endogenous pre-mRNA to produce an mRNA transcript that lacks the disease-causing sequences that are present in transcripts generated from patients' own DNA. Export to the cytoplasm and translation then follows in the usual way.

The trans-splicing phenomenon has been recognized for several decades, and preliminary work on exploiting it for therapeutic purposes was undertaken several years ago. Some of this activity was incubated in an earlier ATP-backed venture, Limelight Bio. But "it kind of got abandoned, because all the components of the technology weren't there," Ehlers said. Subsequent developments in high-fidelity RNA sequencing, next-generation DNA sequencing, barcoding technologies, and computational biology, have all enabled the development of the current platform.

Romesh Subramanian, CEO, Ascidian

Ehlers was the founding CEO at Ascidian but has now become chairman, following the recent appointment of RNA veteran Romesh Subramanian as CEO. Subramanian was previously founder and CEO at Waltham, Mass.-based Dyne Therapeutics Inc., which is developing oligonucleotide-based drugs for patients with muscle diseases, and before that he was co-founder of Translate Bio Inc. (formerly Rana Therapeutics Inc.), which Paris-based Sanofi SA acquired in 2021 for $3.2 billion.

Development of the platform has been "challenging", Subramanian told *BioWorld*. "The team here at Ascidian, under Michael's leadership, have really reduced it to practice." The approach enables the wholesale insertion into an mRNA transcript of large stretches of code, which opens up the possibility of developing a single therapy that can address large genes or disease-causing genes with high mutational variance. "We have a lot of optionality within the platform," he said. "The target space is quite large."

The company's lead program offers an illustration of this. The ABCA4 gene encodes the ABCA4 (ATP-binding cassette, sub-family A, member 4) protein, a membrane transporter expressed in photosensory rods and cones, which is responsible for exporting toxic vitamin A derivatives that are generated during the visual cycle. Its absence leads to retinal dystrophy and progressive vision loss, although the phenotype is highly variable given the diversity of the underlying mutations.

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More than 2,000 disease-associated variants have been identified so far, according to one recent review, including more than 900 that cause Stargardt disease. The wild-type gene exceeds the packaging limit of AAV vectors, so gene replacement is not a feasible therapeutic strategy. Neither, for the same reason, is single-base gene editing. Ascidian's therapy, which involves the rewriting of over 20 contiguous exons in the mRNA transcript – it contains 50 exons in total – does not eliminate all mutations but it does address about 60% of them.

The company reported in vivo nonhuman primate data at the 2022 American Society for Gene and Cell Therapy annual meeting in May. It proved to be safe and well-tolerated. Take-up and expression of the introduced exons were confirmed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis of the chimeric mRNA molecules (they can be distinguished from endogenous mRNAs as the exon junctions are dissimilar) and by immunoblot analysis of the resulting protein, which contained N-terminal epitope tags. Immunohistochemical staining confirmed transduction of the target photoreceptor cells. An accompanying in vitro study demonstrated up to 38% editing efficiency of the ABCA4 mRNA. "We know that 50% is phenotypically normal," Ehlers said.

Trans-splicing does not completely eliminate the cis-splicing that gives rise to mutated mRNA molecules, but delivering an excess of synthetic RNA molecules will ensure that they out-compete the endogenous mRNA for access to the spliceosome. At the same time, that will not lead to excessive and potentially dangerous protein production, as translation of the introduced RNA exons is limited by the availability of pre-mRNA molecules produced by the cell's endogenous transcription machinery. "It's pegged to the expression levels of the pre-mRNA that you're splicing," Ehlers said.

A first-in-human trial of this technology will constitute yet another milestone in the emergence of gene-based medicines. "We're not sharing the timeline yet," Subramanian said. IND-enabling studies are already underway. Given the program's current rate of progress, a clinical trial start may not be too far away.