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PRODUCT R&D

EXPANDING SMALL MOLECULE HORIZONS

By Lauren Martz, Senior Writer

With a platform boasting more than a decade of preclinical rigor and $55.3 million in series A funding, Expansion Therapeutics Inc. is the latest company to step into the RNA-binding small molecule arena. The newco is setting its sights first on pathogenic RNA repeat expansions responsible for a range of rare genetic disorders.

As the most recent graduate of 5AM Ventures' 4:59 Initiative incubator, Expansion emerged from stealth mode yesterday, after twelve months' work with scientific founder Matthew Disney, a pioneer in the field who is professor of chemistry at The Scripps Research Institute.

5AM was joined in the series A by Sanofi Ventures, Novartis Venture Fund, Kleiner Perkins, RA Capital Management and Alexandria Venture Investments. Expansion President and CEO Kevin Forrest said that the involvement of corporate VCs reflects emerging interest in the field and that Sanofi Ventures, who joined in the seed round, makes "a great strategic fit" because it is one of the industry leaders in orphan diseases.

The announcement marks the third newco formed in as many years that aims to expand the scope of disease targets druggable by small molecules by developing compounds that bind RNA rather than protein.

Last March, Arrakis Therapeutics Inc. announced a $38 million series A round to use its bioinformatics and chemical biology platform to identify RNA-binding compounds for cancer, CNS diseases and rare genetic conditions. In 2015, Ribometrix Inc. was formed around a pair of high throughput platforms for screening RNA-binding molecules that were developed by Kevin Weeks, a professor at the University of North Carolina at Chapel Hill. Funding was not disclosed.

The budding field is part of the larger trend towards drug companies paying more attention to RNA.

In addition to the lengthening list of known types of RNA molecules that can serve as therapeutic agents or targets, such as siRNA, microRNA (miRNA), long non-coding RNA (lncRNA) and circular RNA, the field has identified epigenetic sites on RNA that form the platform of at least one newco, Storm Therapeutics Ltd., which launched in 2016.

Moreover, some companies and academic groups have stumbled upon small molecules that bind RNA or its translational machinery without a systematic screening method, such as an inhibitor of PCSK9 translation discovered last year by Pfizer Inc.

Traditionally, small molecules are designed to bind highly defined and stable pockets formed by the tertiary structure of protein targets. While the modality has several stability and PK advantages over biologics and nucleic acids, it is limited by the need for proteins with well-defined binding grooves at sites that modulate function.

Being able to drug RNA with small molecules opens up a vast number of additional targets, such as coding RNAs for proteins that can't be drugged directly, and non-coding RNAs — like miRNAs, lncRNAs and RNA repeat expansions — that contribute to disease pathology. The problem has been that the tertiary structure of RNA is more dynamic and less defined than that of proteins, making it hard to find binding molecules using standard protein-oriented screening platforms.

BIOCENTURY PRODUCT PROFILE

BIOCENTURY PRODUCT PROFILE	
INNOVATION STAGE	
Product	Small molecule targeting RNA repeat expansions
Concept	Small molecules that binds the CUG RNA repeat expansions in the DMPK gene responsible for myotonic dystrophy
Disease	Myotonic dystrophy type 1 (DM1)
Competition	Antisense oligonucleotides; gene editing therapies
Differentiation	Better tissue penetration and distribution than other modalities; more readily crosses the blood-brain barrier to address CNS manifestations of the disease
Administration	Oral
Risks	Potential for non-selective RNA binding
Development status	Discovery
Patents	Patented
Company; lead investigator	Expansion Therapeutics Inc.; Matthew Disney, The Scripps Research Institute

DISNEY WORLD

Disney and colleagues began creating a systematic method for developing small molecules that bind RNA targets about a dozen years ago, when much of the industry considered the feat impossible.

5AM managing partner Scott Rocklage told BioCentury Disney's work stood out for its high translational prospects. "We became really excited about the potential for Matt's long-term work to make an impact on challenging human diseases," he said.

Disney's goal was to create small molecule RNA-targeting probes analogous to those used to bind protein targets. The idea was to design the molecules based on the RNA secondary structure alone.

A key feature is that one of the final steps in the process confirms whether the potency and selectivity recorded in isolated RNA screens translates to cells.

According to Disney, the biggest challenge has been finding potent and selective compounds given the "dearth of tools to assess target engagement in cells."

His solution — now part of the suite of technologies — is a cell-based method termed ChemCLIP (Chemical Cross-Linking and Isolation by Pull-Down). ChemCLIP attaches biotin plus a reactive module to the small molecule ligands, so that when added to cells, the compounds and their bound targets can be isolated, purified and analyzed.

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In 2008, while still at the University at Buffalo, his lab described a technology, dubbed Two-Dimensional Combinatorial Screening (2DCS), which pairs specific RNA motifs with small molecules capable of binding them to create a library of such interactions. The method involves hybridizing libraries of small molecules with RNA motifs to create a database of preferred binding pairs.

By pairing 2DCS with another technology his group developed two years later that ranks the selectivities and affinities of the interactions, Structure-Activity Relationships Through Sequencing (StARTS), the team developed a system to identify molecules that alter function of the isolated RNA motifs.

In 2014, after moving to Scripps, Disney created Inforna, a computational model that uses the binding partner information to identify small molecules that hit disease-relevant RNA targets. Inforna pairs the data collected using 2DCS and StARTS with structural information about RNA targets and the individual motifs they contain to identify lead compounds.

Combined, the technologies have generated small molecules for targets ranging from oncogenic miRNAs to mutant tau, although RNA repeat expansion targets have drawn much of Disney's time and resources.

In particular, the platform has identified allele-selective molecules that bind the toxic repeats responsible for myotonic dystrophy type 1 (DM1) and correct downstream pathologic effects, including abnormal mRNA splicing, *in vitro*.

Disney told BioCentury the platform can identify candidates that inhibit or enhance protein expression for any RNA target, or that can eliminate the targeted RNA entirely.

He declined to comment on the differences between his platform and those of Arrakis or Ribometrix, citing a lack of available information from the competitors.

The first-generation technology Disney developed at Buffalo was licensed to SMaRT Therapeutics Inc. According to Forrest, the IP "reverted back to the UB" after the company discontinued operations, and Expansion now has exclusive rights to both the

EXPANDING OPPORTUNITIES IN DM1

Expansion Therapeutics Inc. is developing small molecules that bind RNA targets, including the RNA repeat expansions responsible for myotonic dystrophy type 1 (DM1), an inherited adult-onset muscular dystrophy caused by a defect in the DMPK gene.

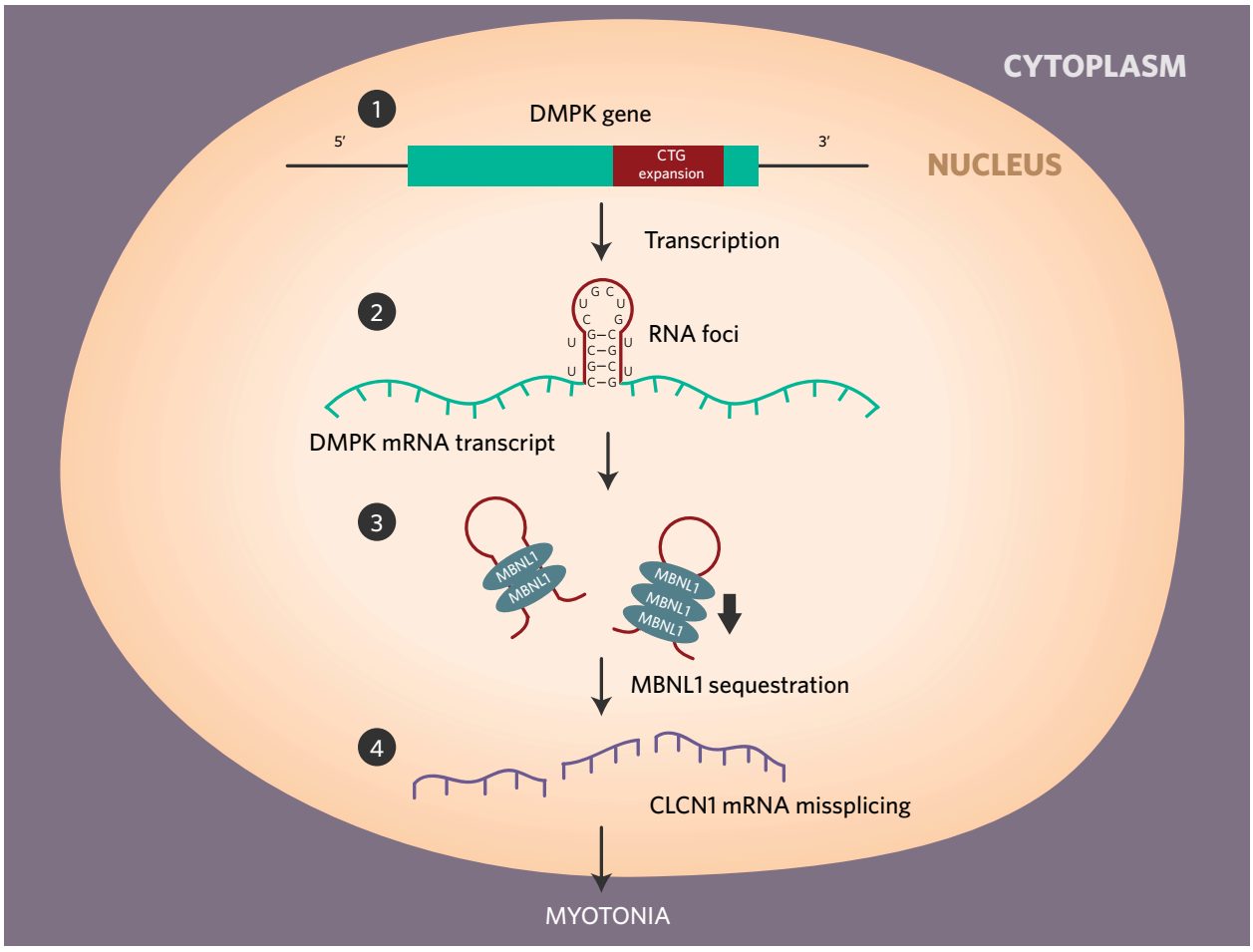
(1) DM1 is caused by an RNA triplet CTG expansion in the 3′ untranslated end of the DMPK gene.

(2) Some disorders caused by RNA repeat expansions result from disruption of the relevant gene, but in DM1, the pathology occurs due to effects on other proteins. When the DMPK gene is transcribed into an mRNA transcript, the repeated CUG RNA segments fold into RNA foci, or hairpin loops, that result from the hydrogen bonding between the C and G base pairs in the repeated segments.

(3) The RNA foci bind and sequester MBNL1, which normally regulates alternative splicing of pre-mRNAs, resulting in low levels of MBNL1 in the nucleus.

(4) The reduction in free MBNL1 leads to missplicing of mRNAs, including CLCN1, a chloride channel required for electrical excitability in muscle. CLCN1 mRNA missplicing contributes to the myotonia that occurs in DM1 pathology.

CLCN1 - chloride voltage-gated channel 1; DMPK - dystrophia myotonica-protein kinase; MBNL1 - muscleblind-like splicing regulator 1



first generation IP from Buffalo and the second generation IP from Scripps.

That IP includes about a dozen issued patents and a suite of patent applications covering key methods of compounds for targeting various repeats, identification of lead compounds using the platform, and methods for validation of the small molecules in cells including ChemCLIP.

EXPANSION'S REPEATS

Forrest said the company's focus is on diseases caused by RNA repeat expansions because it is clear that the toxic RNA species drive disease and there are no cures or effective treatments for them.

Disney's group has published data showing the platform can yield small molecules that engage target RNA "in an allele-selective and precise manner," said Forrest, preserving the healthy copy of the gene while disrupting the copy with pathogenic repeats.

gene, which create CUG repeat-containing RNA foci that bind and sequester MBNL1 — a protein required for gene splicing in muscle cells (see "Expanding Opportunities in DM1").

The result is progressive muscle wasting and weakness affecting the skeletal muscles and several other body systems. Expansion's strategy is to block that process and restore proper function to the cells.

"When small molecules bind CUG repeats, they free MBNL1, which enhances expression of muscle-specific chloride ion channels, for example, to improve myotonia," said Disney.

According to Forrest, the decision to start with DM1 was based on the expertise and progress from Disney's lab, as well as the clinical trial design opportunities.

"The biology of these repeats is well advanced, we've already seen selectivity of small molecules for type 1 myotonic dystrophy repeats, and there is an opportunity for early biomarker-guided clinical efficacy studies that will produce rapid readouts in humans," Forrest told BioCentury.

> "Implicit in the platform is the opportunity to go after 'undruggable' targets like KRAS, and that's also something we're reviewing."
>
> Kevin Forrest, Expansion Therapeutics

About 30 rare diseases are characterized as repeat expansion disorders, including myotonic dystrophies, Huntington's disease (HD) and certain forms of familial amyotrophic lateral sclerosis (ALS).

In all cases, they are caused by pathogenic, short, repeated segments of RNA that disrupt the function of normal proteins. In some, the repeated segments occur within a coding region, disrupting translation and preventing the protein from carrying out its function. In others, the repeated segments, in either coding or non-coding regions, bind and sequester proteins, preventing them from carrying out their cellular functions.

Expansion's lead indication is DM1, which falls into the latter category, and is the most common adult-onset muscular dystrophy. It is caused by repeated CTG sequences in the DMPK

He added that Disney's lab has also developed a technology that can eliminate repeated segments by destroying the target RNA after the small molecule binds, although he would not disclose if that technology will be incorporated into the lead candidate.

At least two other companies are targeting DM1 RNA repeat expansions with other modalities. Ionis Pharmaceuticals Inc. has the antisense oligonucleotide BIIB065, which targets the CUG repeats, in Phase I/II testing to treat DM1. Locana Inc. has a preclinical RNA-targeted CRISPR-Cas9 platform targeting DM1 and other microsatellite repeat expansion disorders.

According to Disney, Expansion's small molecules offer better tissue penetration than alternative modalities, and other PK advantages that translate to efficacy.

Forrest added that DM1 is a "system-wide disease" with effects in the cardiovascular, gastrointestinal, endocrine and central nervous systems, in addition to the classical presentation in skeletal muscles. "Small molecules will be advantageous because the volume of distribution will allow them to address a number of disease-affected tissues," he said.

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The company is not disclosing timelines for clinical development.

After DM1, the company's intends to target DM2, which is caused by an expanded CCTG sequence in the CNBP gene. It also has sights on other undisclosed expansion repeat diseases.

Forrest told BioCentury the company is "actively evaluating" programs outside of expansion repeats, including ones where binding RNA could address historically intractable targets.

"Implicit in the platform is the opportunity to go after 'undruggable' targets like KRAS, and that's also something we're reviewing," he said. In addition, the company is open to partnerships.

COMPANIES AND INSTITUTIONS MENTIONED

Arrakis Therapeutics Inc., Waltham, Mass.
Expansion Therapeutics Inc., San Diego, Calif.
Ionis Pharmaceuticals Inc. (NASDAQ:IONS), Carlsbad, Calif.
Locana Inc., San Diego, Calif.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Ribometrix Inc., Chapel Hill, N.C.
The Scripps Research Institute, La Jolla, Calif.
Storm Therapeutics Ltd., Cambridge, U.K.
University at Buffalo, Buffalo, N.Y.
University of North Carolina at Chapel Hill, Chapel Hill, N.C.

TARGETS

Cas9 - CRISPR-associated protein 9
CNBP - CCHC-Type Zinc Finger Nucleic Acid Binding Protein
DMPK - Dystrophia myotonica-protein kinase
KRAS - K-Ras
MBNL1 - Muscleblind-like splicing regulator 1
PCSK9 - Proprotein convertase subtilisin/kexin type 9

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