TARGETS & MECHANISMS

EXPANSION INTO TNBC

By Lauren Martz, Associate Editor

With an RNA-binding small molecule that sensitizes HER2-negative cancers to Herceptin, Matt Disney's latest study adds to the growing arsenal of preclinical therapies against triple-negative breast cancer. The only problem is the molecule may run the risk of worsening a patient's cancer before making it better.

Disney has been a pioneer in the growing field of RNA-binding small molecules, a technology that takes on the long-standing dogma that RNA is poorly druggable by conventional modalities. Disney spun out Expansion Therapeutics Inc. from The Scripps Research Institute, where he is a professor of chemistry and neuroscience, to develop treatments for RNA-expansion diseases. Last year, Expansion raised $55.3 million in a series A round co-led by 5AM Ventures, Kleiner Perkins, Novartis Venture Fund and Sanofi Ventures.

Disney's Scripps lab focuses on designing selective therapeutic RNA-binding molecules based on gene sequences. In a study published in the Journal of the American Chemical Society on Feb. 6, Disney's team designed a molecule that converts HER2-negative cancers to HER2-positive, sensitizing them to Roche's anti-HER2 mAb Herceptin trastuzumab, but driving up invasiveness in the process.

The study throws a new strategy into an indication that, by definition, is hard to treat.

Triple-negative breast cancer (TNBC) is characterized by the absence of the estrogen receptor, progesterone receptor and HER2. The indication affects about 20% of breast cancer patients, but there are no targeted therapies specifically approved for the indication. AstraZeneca plc and Merck & Co. Inc's PARP inhibitor Lynparza olaparib was approved in 2018 to treat HER2-negative cancers, which includes TNBC.

However, in a Phase III trial, it showed only a modest effect on progression-free survival and no effect on overall survival in the subset of HER2-negative patients with TNBC. Standard of care for TNBC is a combination of taxanes and anthracyclines in the neoadjuvant setting for first-line patients.

In January, Phoenix Molecular Designs described a biomarker, RSK2, that is activated in about 80% of TNBC tumors, based on unpublished preclinical studies on 65 TNBC tumors. The company is developing the RSK2 inhibitor PMD-026, and has partnered with Roche to develop a companion diagnostic (see “Reducing the R(isk in TNBC”).

TNBC has also been a large focus among studies presented at the American Association for Cancer Research (AACR) meeting over the last three years. In 2018, a study from the University of Tennessee identified the deubiquitinating enzyme OTULIN as a new target for the indication, linking OTULIN overexpression to TNBC and chemotherapy resistance (see “Hello RNA”).

Disney’s lab discovered the HER2 switch molecule, Targaprimir-515, through a screen for target sites in the transcriptome. The team optimized the molecule for specificity and selectivity using its computational model Inforna, and presented cell-based evidence of target engagement.
The paper also answers the question whether it will be possible to routinely achieve small molecule selectivity for an RNA target site. “This example, and another that we will disclose soon, suggest that prevailing view in the field that you can’t get molecules that are selective for RNA should be reconsidered,” said Disney (see Box: “Selective Focus”).

Still, switching on HER2 expression brings risks, since the treated cells exhibited greater potential to migrate.

### TARGETS & MECHANISMS

#### SELECTIVE FOCUS

Against a background of skepticism that small molecules can be designed to bind RNA with sufficient selectivity for drug development, Matt Disney’s team at The Scripps Research Institute tested the power of its computational model Inforna to optimize selectivity.

Several newcos working on RNA-binding small molecules told BioCentury that many people still question whether small molecules can be designed with high specificity for RNA targets — a perception that prevented companies from targeting the modality for years.

A major barrier is the fact that similar RNA motifs exist at different sites throughout the transcriptome.

Using Inforna, Disney’s team previously discovered a small molecule that selectively binds two targets, the precursor hairpins of miR-515 and miR-885, which share a common target motif. Inforna uses information about binding pairs of small molecules and RNA motifs to identify molecules against disease-relevant RNA targets (see “Selectivity Suite”).

In the new study, the group optimized the dual-selective molecule to bind only one of the targets. By using secondary structures of the two binding sites, the model identified an additional, adjacent binding site only present on miR-515, and used fragment-based assembly to create a molecule with over 3200-fold selectivity over the parent compound for the new site. The new molecule, Targaprimir-515, only bound RNA containing the original target site and the adjacent site.

Disney’s team confirmed Targaprimir-515 was selective for miR-515 using ChemCLIP (Chemical Cross-Linking and Isolation by Pull-Down), a cell-based screening method that attaches biotin plus a reactive molecule to the small molecule ligand to isolate and analyze its bound targets.

In a human breast cancer cell line, Targaprimir-515 decreased levels of miR-515 compared with an antisense oligonucleotide against the same target, with 20-fold greater potency (IC_{50}=0.3 μm vs. IC_{50}=6 μm). The small molecule was also more selective than the oligo, which decreased levels of other miRNAs.

“We do understand we’re taking a cancer that’s not invasive, and making it invasive, so there’s a balance we have to strike here but the output to render a tumor sensitive is very exciting,” Disney said.

Disney plans to advance Targaprimir-515 through preclinical development. Expansion’s pipeline has not been focused on cancer, but Disney told BioCentury the company will consider licensing the new molecule.

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“Some think we have to be able to identify every binding partner — RNA or protein — of every small molecule, and others think in the end, only the effects we see in models matter,” Warner said.

“If you think about every protein-targeted drug, how many have been profiled at all at the RNA level? A vanishingly small number if any, yet if you put something in a model and it works fine, it probably doesn’t matter which RNA motifs it’s binding,” she added.

— Lauren Martz
Disney's lab discovered the molecule's effect on HER2 through a series of cell-based experiments designed to elucidate its biological function. Earlier studies by other groups had established that miR-515 suppresses SK1 — an enzyme that phosphorylates sphingosine — and its downstream target S1P, thereby blocking a pathway that promotes cell proliferation and migration. By disrupting miR-515, Targaprimir-515 increased expression of both targets by up to four-fold in a human breast cancer cell line. On one hand, that makes the cells more cancerous, but on the other, further experiments showed it renders them more susceptible to HER2-mediated killing.

Using proteomics analysis, the group identified HER2 as a downstream target of the SK1 pathway, showing that HER2 expression increased when the SK1 pathway was activated. The team also demonstrated Targaprimir-515 flipped a normally HER2-deficient breast cancer cell line into a HER2-expressing cancer, driving up expression four-fold.

In an important finding, Disney's group showed that pre-treatment with the small molecule sensitized the previously HER2-deficient cells to Herceptin and Kadcyla, an antibody-drug conjugate (ADC) comprising Herceptin and an emtansine cytotoxin payload that triggers apoptosis. Kadcyla is marketed by the Genentech Inc. unit of Roche.

At a molecular level, Targaprimir-515 targets pri-miR-515, the primary transcript that contains a hairpin region from which the mature miRNA is cleaved. Once cleaved, the mature miRNA regulates translation by suppressing expression of target genes. In a breast cancer cell line expressing pri-miR-515 pre-treated with Targaprimir-515, Herceptin decreased viability in a dose-dependent manner. The mAb had no effect on cells lacking miR-515. Similarly, Kadcyla induced apoptosis of only the pri-miR-515-expressing cell line.

The molecule could be developed for breast cancer patients with HER2-negative tumors, including TNBC tumors most in need of new treatments. About 30-40% of TNBC patients respond to Herceptin, and this mechanism may be able to treat some of the remaining patients expressing pre-miR-515.

Disney's team is assessing what percentage of HER2-negative breast cancer cells express miR-515. In addition, he thinks the compound could expand the indications where Herceptin can be used to other cancers that are HER2-negative but express miR-515.

“About 30% of the time, HER2-positive breast cancers mount resistance to Herceptin. Perhaps the cascade these people are working on may re-sensitize them,” Dunn said, adding that resistance can arise for a variety of reasons, including loss of HER2 expression.

Disney agreed that overcoming acquired resistance is a possible application for the molecule. However, Targaprimir-515 also increased migration of breast cancer cell lines 2.5-fold, suggesting the researchers will need to weigh the risks of increased invasion potential with the benefits of inducing Herceptin sensitivity.

Dunn isn’t convinced the invasion risk is a big concern, and noted that if the therapy ultimately kills the cells, a “transient stimulation of invasion doesn’t matter.”
The increased invasion is a concern for two reasons: turning the cells more invasive before eliminating them could make the cancer worse before it gets better; and there's no guarantee that cells treated with Targaprimir-515 will respond to Herceptin, meaning the small molecule could make the cancer worse.

The Scripps study is not the first example of a compound that induces a target's expression to trigger drug sensitivity, but it isn't clear if earlier examples carried the same risk. A 2018 study from a group at the Netherlands Cancer Institute showed that treating melanoma cells with a MAP kinase

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**HER2 CONVERSION**

The Scripps Research Institute identified a new RNA-binding small molecule, Targaprimir-515 (TGP-515), that converts HER2-negative breast cancer cells into HER2-positive cells. TGP-515 could offer a new mechanism to treating triple-negative breast cancer (TNBC) by sensitizing TNBC tumors to HER2-targeted therapies.

Targaprimir-515 works by binding miR-515's hairpin precursor (pri-miR-515) to block production of the miRNA, resulting in upregulation of HER2. miR-515 is a suppressor of SK1 expression, which is required for production of S1P, and S1P induces HER2 expression.

In a study published on Feb. 6 in the *Journal of the American Chemical Society*, the Scripps team showed that decreasing miR-515 expression with TGP-515 increased expression of SK1, S1P and HER2 in breast cancer cell lines and sensitized the cancer cells to HER2-targeting therapies Herceptin trasuzumab and Kadcyla aldo-trastuzumab emtansine.

HER2 (EGFR2; ErbB2; neu) - Epidermal growth factor receptor 2; miR-515 - MicroRNA-515; S1P- Sphingosine-1-phosphate; SK1 - Sphingosine kinase 1
pathway inhibitor increased expression of AXL, making the cells more susceptible to Genmab A/S' ADC against the target, HuMax-AXL-ADC (see “Unexpected Combinations”).

Disney’s lab is studying the molecule in animal models of HER2-negative cancer. “We have to figure out whether we can fine tune dosing to sensitize the cancer but without making a bad cancer even worse.”

Genentech declined to comment on the potential combination of Herceptin and Targaprimir-515.

FIELD OF INDICATIONS

The Scripps molecule could represent a new avenue for Expansion, which has programs in myotonic dystrophy types 1 and 2, and other undisclosed repeat disorders.

While the growing field of companies with RNA-binding small molecule technologies is likewise picking first from repeat disorders, the other two companies launched in the last four years have already disclosed cancer programs.

Repeat expansion disorders are caused by toxic fragments of repeated RNA sequences inserted into different genes. They are a good fit for the technology because the RNA structures are well documented, there are no effective therapies for most, and many affect the CNS, where small molecules can more easily penetrate than competing antisense oligonucleotides.

Ribometrix is pursuing Huntington’s disease (HD) and is targeting MYC mRNA for cancer, said Katie Warner, co-founder and senior director of research at Ribometrix.

Arrakis Therapeutics Inc. has programs on undisclosed repeat expansion disorders in neurology, as well as programs in oncology and rare genetic diseases.

“RNA repeat expansions are pretty straightforward choices for the molecules,” said Warner. “For HD, there’s nothing to treat it, the RNAs are among the few with publicly available structural information, and based on the oligo work, there’s a nice path forward.”

By interfering with a protein’s mRNA, RNA-binding small molecules create an opportunity for targets like MYC that lack druggable binding pockets on the protein.

Other ways to hit cancer with RNA-binding small molecules include targeting cancer-promoting non-coding RNAs, such as miRNA.

Warner noted that it still isn’t clear which types of RNA targets will be the best fit for the molecules. "In a few years when the field is farther along and we know more about the biology and the effects of modulating different types of targets, we may find out that we really shouldn't target messages, or up-regulate certain RNAs, for example, but we're doing the studies now to figure that out.”

According to Arrakis CEO Michael Gilman, cancer is a high priority area for small molecule RNA-binders because, in addition to the unmet need, “oligonucleotide drugs, for example, don’t penetrate solid tumors as well as small molecules can.”

Although RNA-binding small molecules can be applied to a wide variety of indications, Warner thinks the field is turning to cancer because the risk profile is a good fit for a new modality.

“Other indications are inherently more risky. Cancer is a good first step because other indications require higher doses for longer periods of time in a very safe way,” she said.

While all three companies are converging on the same two groups of indications, Warner thinks the field will open up as it matures. “If you bias yourself toward indications known to be less challenging, in a few years you can go back to the indications you passed over.”

COMPANIES AND INSTITUTIONS MENTIONED

Arrakis Therapeutics Inc., Waltham, Mass.
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Expansion Therapeutics Inc., San Diego, Calif.
Genentech Inc., South San Francisco, Calif.
Genmab A/S (CSE:GEN; Pink:GMXAY), Copenhagen, Denmark
Merck & Co Inc. (NASDAQ:MRK), Kenilworth, N.J.
Netherlands Cancer Institute, Amsterdam, Netherlands
Phoenix Molecular Designs, Vancouver, Canada
Ribometrix Inc., Durham, N.C.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
The Scripps Research Institute, La Jolla, Calif.
University of Tennessee, Knoxville, Tenn.
U.S. Food and Drug Administration (FDA), Silver Spring, Md.

TARGETS

- AXL (UFO) - AXL receptor tyrosine kinase
- HER2 (EGFR2; ErbB2; neu) - Epidermal growth factor receptor 2
- miR-515 - MicroRNA-515
- miR-885 - MicroRNA-885
- MYC (c-myc) - v-myc myelocytomatosis viral oncogene homolog
- OTULIN - OTU deubiquitinase with linear linkage specificity
- RSK2 (RPS6KA3) - Ribosomal protein S6 kinase 90kDa polypeptide 3
- S1P - Sphingosine 1-phosphate
- SIK1 - Sphingosine kinase 1