license from the Technion Research and Development Foundation Ltd. Professor Timor Baasov, the inventor of our compounds, who has served as our senior consultant since our inception.

Eloxx recently announced a new program studying intravitreal administration of ERSG compounds for rare inherited retinal disorders with a focus on Usher Syndrome. Eloxx's preclinical candidate pool consists of a library of 170 novel ERSG drug candidates identified based on read-through potential and cytoplasmic ribosomal selectivity. The ESRG selected for Usher syndrome is currently in IND enabling studies. The intravitreal compounds show retinal tolerability at doses 10-fold greater than anticipated efficacy range in sensitive species.

In a rational design approach, Eloxx' synthetic aminoglycoside derivatives were developed through progressive steps in medicinal chemistry to eliminate structural components that mediated antibacterial effects, while at the same time retaining the features that promoted readthrough on eukaryotic ribosomes.

3.3 Selected Technology Profile

3.3.1 Translation Control Therapeutics Platform

Anima Biotech's proprietary technology enables visualization and monitoring of target protein translation via pulses of light emitted by ribosomes. The fully automated high-throughput screening system discovers small molecules that modulate the light, as they decrease or increase the target protein's production. The platform integrates proprietary technologies in biology, bioinformatics, image analysis, big data analysis and artificial intelligence algorithms in a cloud computing software architecture.

Overview

Anima's platform combines breakthrough novel biology with proprietary analysis software that runs in a big-data cloud architecture. Compound libraries are screened in a purpose-built, high performance Translation Control Lab. Using protein synthesis monitoring assays, tjhe company generates millions of images that show the impact of the different compounds on the translation of the target protein. Images are uploaded in real time into the project's private cloud where they are analyzed by proprietary bio-informatics, imaging and big data analysis algorithms to identify hit molecules that selectively control the translation of the target protein.

The confirmatory Part 2 portions of the SUNFISH and FIREFISH studies have completed enrollment and will conduct their primary efficacy analyses in Q4 2019 and Q1 2020, respectively.

The small molecule SMN2 splicing modulator **branaplam** originated from a high-throughput phenotypic screening hit, pyridazine, and evolved via multiparameter lead optimization at Novartis. In April 2015, a phase I/II study of oral branaplam administration to infants with type 1 SMA was initiated. However, enrolment was discontinued in mid 2016 because in animal studies conducted in parallel with the trial showed unexpected toxicities to the peripheral nerves and spinal cord, testes and blood vessels in the kidney. In September 2017, enrollment for the trial was resumed in Europe with different doses and modification of the trial design, giving participants a choice of a **weekly oral** treatment or delivery through a feeding tube. Interim results from part of the study demonstrate that branaplam has good safety and tolerability in SMA type 1 patients. Continued survival of 62% of treated patients and improvements in CHOP INTEND scores support continued evaluation of branaplam in SMA. Study results also underscore the importance of early intervention and sustained treatment in SMA type 1.

Eisai was developing **E7107**, a semisynthetic, urethane derivative of the natural product pladienolide B. E7107 is the first compound in a new class of anti-cancer agents targeting the spliceosome. Specifically, E7107 interacts with the Splicing factor 3B subunit 1 (SF3b1) to block the normal splicing of oncogenes. Development of E7107 was suspended after Phase I clinical trials due to an unacceptable profile of adverse events. E7107 has been studied in two separate phase I dose-escalation studies in patients with solid tumors. Pharmacodynamic analysis of the effects of E7107 on splicing of target mRNAs in peripheral blood mononuclear cells taken from the patients revealed that splicing inhibition was achieved *in vivo* and was commensurate to E7107 dose. While the drug was generally well tolerated and a maximal tolerated dose was established, an unexpected toxicity of bilateral **optic neuritis** was identified and resulted in suspension of both trials. It was unclear if this toxicity, which was not encountered in preclinical animal studies, is an on-target effect of SF3B1 inhibition or a specific toxicity associated with E7107.

Eisai's affiliate H3 Biomedicine discovered the second generation orally available modulator of the SF3b complex, H3B-8800. Since mid 2016, a Phase 1 trial of H3B-8800 is underway in patients with hematologic malignancies. Results have not yet been reported, but the study is

distribution and central nervous system penetration. The RNA chemical probing technology SHAPE helps identify structured regions of RNA potentially amenable to drug binding (Mustoe, 2018).

Ribometrix's discovery pipeline focuses on targeting RNA molecules that fold into complex 3D structures yielding pockets amenable to small molecules with high selectivity and favorable drug-like characteristics. The Ribometrix platform provides a unique and comprehensive analysis of small molecule-RNA interactions, including 3D structural information, based on the inventions of the company's scientific co-founder, Kevin Weeks, Ph.D., at the University of North Carolina at Chapel Hill. Ribometrix is leveraging this platform to identify and optimize compounds to progress a broad pipeline of small molecule drug candidates.

Ribometrix technology also allows to figure out not just if a small molecule is binding to an RNA, but also where. That is key to knowing whether the small molecule is disrupting the RNA's function in the right way. Ribometrix is going after drug targets in cancer and neurology. Solomon, Ribometrix's CEO, says his company has been doing drug development for about a year since it got its seed funding. It has also picked targets, including c-myc, a famous cancer gene that has long been thought undruggable. The company is also pursuing a target in Huntington's disease (Xconomoy, Nov 13, 2018). Ribometrix is pursuing Huntington's disease and is targeting c-Myc mRNA for cancer, confirmed Katie Warner, co-founder and VP of RNA Biology (BioCentury Feb 21, 2019).

5.2.6 Saverna Therapeutics

Saverna Therapeutics is a Swiss biopharmaceutical start-up company founded with the vision to develop small molecule drugs that target non-coding RNA for the treatment of diseases with high unmet need. Saverna Therapeutics was founded in November 2017 by four former lab heads at Novartis, Switzerland, who have come together to leverage their expertise in a specialized and validated drug discovery platform tailored for identifying RNA-targeting small molecule compounds.

Non-coding RNA, despite being reported to be dysregulated in over 1000 diseases, are notoriously difficult targets for drug discovery. As such, Saverna Therapeutics' drug discovery activities have the potential to impact the development of a new field in drug discovery by

throughput screening and structural biology to identify hits against two of Storm's RNA modulating targets. The agreement has been expanded to support STORM's chemistry efforts on additional novel targets. Evotec will use its broad drug discovery platform to develop the compounds against these novel RNA targets.

Evotec is a drug discovery alliance and development partnership company which operates worldwide providing the highest quality stand-alone and integrated drug discovery solutions, covering all activities from target-to-clinic to meet the industry's need for innovation and efficiency in drug discovery.

Technology, Targets & Pipeline

According to the company's CEO, STORM Therapeutics is focusing on two classes of RNA-modifying enzymes, RNA methyltransferases and terminal uridylyltransferases (TUTases), and has already advanced two undisclosed targets in drug discovery (BioCentury Oct 26, 2017). „The Kouzarides and collaborators have performed multiple CRISPR drop-out screens that have led to numerous (10-20) candidate targets, of which METTL3 is one" (Hodgson, 2018). Methyltransferase-like 3 (METTL3) is an epigenetic writer that – together with METTL4 in a complex – methylates adenosine at the N^6 position, yielding 6-methyladenosine (m^6A). METTL3 levels are often elevated in cancer cells in vitro and in mouse models of human cancer.

STORM's CEO said that „one of the strongest cases for a drug target is one which methylates particular messenger RNAs. Our three main programs target three types of RNA" " (Global University Venturing, March 5, 2019).

STORM Therapeutics has further developped mass spectrometry and applies it to quantitative analysis of site-specific modifications in RNA species, such as tRNA, mRNA and microRNA.

Storm's CEO emphasizes that "To understand RNA modifications and their role in a given RNAs function, you must have the means to measure and quantitate the levels of that modification on your given RNA. There is currently no technology widely available to do this – some modifications can be detected by sequencing or immunoprecipitation methods, but these are not comprehensive or quantitative. However, STORM has resolved this challenge developing unbiased proprietary mass spectrometry methods to detect and quantitate RNA modifications in a sequence specific manner. This allows us to accurately determine the effects of our small

Naturally occurring antibiotics such as tetracylines, macrolides, and aminoglycosides, as well as synthetic oxazolidinones, act by disrupting the function of bacterial ribosomes—the sites of mRNA's translation into protein—which themselves are made of RNA. And **bacterial riboswitches,** noncoding structures in mRNA molecules that regulate gene expression by binding to metabolites and ions, have been successfully targeted using small molecules such as Merck's **ribocil**.

For the first time, Merck applied the Automated Ligand Detection System (ALIS), an indirect affinity-selection mass spectrometry (AS-MS) technique, for the selective detection of small molecule-ncRNA interactions, high-throughput screening against large unbiased small-molecule libraries, and identification and characterization of novel compounds (structurally distinct from both FMN and ribocil) that target the FMN riboswitch Rizvi, 2018). Crystal structures reveal that different compounds induce various conformations of the FMN riboswitch, leading to different activity profiles. Thus, ncRNA can be broadly targeted by chemically diverse yet selective small molecules as therapeutics.

Merck developed a cell-based riboswitch regulated gene reporter assay as well as an in vitro riboswitch RNA aptamer-binding assay to further validate the mechanism of action of ribocil and to facilitate the discovery of more potent analogues (Balibar, 2018).

Merck began with the idea of finding a compound that blocks the bacterial riboflavin synthesis pathway. Riboflavin is an essential nutrient for humans and bacteria alike, but while humans must consume it as part of their diet, bacteria can either scavenge riboflavin from the environment or, if supplies are lacking, make their own. Merck devised a simple phenotypic screen. The researchers tested roughly 57,000 antibacterial synthetic small molecules on cultures of *E. coli* bacteria looking for ones whose killing ability was neutralized by the presence of riboflavin (The Scientist Apr 01, 2019).

The team found one molecule that fit the criteria and called it ribocil. To investigate the molecule's mechanism of action, they applied it to cultures of E. coli cells until colonies emerged that were resistant to its effect. The researchers then sequenced the whole genomes of each of the resistant bacterial strains to find which genes were mutated.

While all of the 19 resistant strains did have mutations in a gene called *RibB* (which produces one of the riboflavin synthesis enzymes), the mutations did not affect the protein itself. They altered a

Rizvi NF, Howe JA, Nahvi A et al.
Discovery of Selective RNA-Binding Small Molecules by Affinity-Selection Mass Spectrometry
ACS Chem Biol 2018; 13: 820-831

Roy B, Friesen WJ, Tomizawa Y et al.
Ataluren stimulates ribosomal selection of near-cognate tRNAs to promote nonsense suppression
Proc Natl Acad Sci USA 2016; 113: 12508-12513

Rzuczek SG, Colgan LA, Nakai Y et al.
Precise small-molecule recognition of a toxic CUG RNA repeat expansion
Nat Chem Biol 2017; 13: 188-193

Salani M, Urbina F, Brenner A et al.
Development of a Screening Platform to Identify Small Molecules That Modify ELP1 Pre-mRNA Splicing in Familial Dysautonomia
SLAS Discov 2019; 24: 57-67

Santag S, Siegel F, Wegner AM et al.
BAY 1143269, a novel MNK1 inhibitor, targets oncogenic protein expression and shows potent anti-tumor activity
Cancer Lett 2017; 390: 21-29

Seiler M, Peng S, Agrawal A et al.
Survey of spliceosome gene mutations and associated splicing defects across 33 cancer types
Cancer Res 2017; 77 (13 Suppl): abstract nr 383 (online access to abstract)

Seiler M, Yoshimi A, Darman R et al.
H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers
Nat Med 2018; 24: 497-504

Teng T, Tsai J, Puyang X et al.
A chemogenomic approach reveals the action of splicing modulators at the branch point adenosine binding pocket defined by the PHF5A/SF3b complex
Cancer Res 2017; 77 (13 suppl): abstract nr 126 (online access to abstract)