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Pretzel: modulating the mitochondrial genome

BY DANIELLE GOLOVIN, STAFF WRITER

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Pretzel launched Monday with three platforms to restore mitochondrial function in a variety of indications, including both primary and secondary mitochondrial diseases, using DNA editing and small molecules.

The company debuted with a $72.5 million series A financing led by Arch Venture Partners and Mubadala Capital. HealthCap, Cambridge Innovation Capital, Cambridge Enterprise, Angelini Ventures, GV, Invus, Eir Ventures, GU Ventures and Karolinska Institute Holding also participated.

Pretzel Therapeutics Inc., which is named after the preztel-like shape of the interior of mitochondria, aims to use its genome editing platform to address primary mitochondrial diseases with a known genetic origin. Though the company isn't disclosing the indications it's working on, such diseases often affect children and predominantly affect organs with the highest energy requirements, such as the heart, brain and skeletal muscles.

Human mitochondrial DNA is small and double-stranded, with each mitochondrion containing 2-10 copies of the genome and each cell containing 100–10,000 copies. Mitochondria with mutated DNA often co-exist with wild-type mitochondria in a cell, in a state known as heteroplasmy. The mutant-to-wild-type ratio of mitochondrial genomes has to exceed a threshold, thought to be about 60%, to impact cellular function and cause a phenotype.

Pretzel uses zinc finger nucleases to cut and destroy mutated mitochondrial DNA to reduce the mutant-to-wild-type DNA ratio in cells. "Once the cut is produced, there is no mechanism of repair inside the mitochondrial genome," co-founder and CSO Gabriel Martinez told BioCentury. The lesioned DNA then gets degraded.

Elimination of mutant mitochondrial DNA stimulates replication of the remaining wild-type mitochondrial DNA pool to maintain a predetermined total mitochondrial DNA copy number, shifting the heteroplasmic ratio in favor of wild-type.

"Through a process that is not completely understood, there is a preset number of copies of mitochondrial DNA that we need to have in our mitochondria to produce enough energy," said Martinez.

Pretzel co-founder Michal Minczuk revealed in Nature Medicine that elimination of mutant mitochondrial DNA in the heart using targeted zinc finger nucleases delivered via an adeno-associated viral (AAV) vector shifted mitochondrial DNA heteroplasmy and rescued molecular and physiological disease phenotypes in mice.

Minczuk is program leader and MRC investigator at the MRC Mitochondrial Biology Unit at the University of Cambridge.

Pretzel chairman and CEO Jay Parrish told BioCentury the company is still exploring which method it will use to deliver its zinc finger nucleases.

The company thinks zinc finger nucleases are ideal for editing the mitochondrial genome, as they are small enough to package in delivery vectors and after their production in the cytoplasm they efficiently cross the mitochondrial double membrane. CRISPR systems, by contrast, require guide RNAs that do not readily cross the mitochondrial membranes and are therefore restricted to nuclear gene editing.

Parrish cited Precision BioSciences Inc. (NASDAQ:DTIL) among Pretzel's competition; Precision uses I-CreI as its nuclease rather than zinc fingers, and showed in a 2021 Nature Communications publication that it can also cross the mitochondrial membrane to eliminate mutant mitochondrial DNA in mice. Precision's most advanced therapeutic candidates are allogeneic CAR T cells; the company's website does not yet list a mitochondrial gene editing program.

Pretzel's other two technology platforms are small-molecule based: one to modulate mitochondrial gene expression and one to modulate mitochondrial function.

The former involves small molecules that act on the enzymes involved in mitochondrial DNA replication, transcription and translation. "This could look like stabilization of transcription factors that leads to overall increased mitochondrial DNA copy number," said Parrish.

Pretzel co-founder Nils-Göran Larsson demonstrated in a 2019 paper that absolute levels of wild-type mitochondrial DNA may be more important than the ratio of mutant-to-wild-type DNA. In the Science Advances publication, increasing total mitochondrial DNA levels ameliorated pathology in multiple mouse tissues, even while the heteroplasmic ratio remained constant.

Larsson is professor of mitochondrial genetics at the Department of Medical Biochemistry and Biophysics at Karolinska Institute.

The company's third platform involves modulating mitochondrial quality control systems, using small molecules to target things such as mitochondrial proteases, which regulate trafficking, processing and activation of mitochondrial proteins, mitochondrial dynamics, mitophagy and apoptosis.

Parrish said this platform will mostly be used to treat secondary mitochondrial diseases where dysregulated energetics is a component. "In states like metabolic disorders where there's too much energy, or oncology where there's a rapid proliferation of cells, these types of approaches may be very effective."

For its small molecule programs, the company started with undisclosed targets of interest and performed high-throughput small molecule screens using assays developed by its co-founders to identify lead compounds.

Across the three platforms, Pretzel has two programs in the lead optimization stage, with four behind them, and expects to be in the clinic in the next couple of years. "Our goal with the financing is to get as many programs as far along as we can," said Parrish. "The other big component is to continue to build the team."

Parrish is a Venture Partner at Arch and co-founder of Rome Therapeutics Inc. He most recently co-founded and served as CBO of Vir Biotechnology Inc. (NASDAQ:VIR).

Rather than eliminate mutated mitochondrial DNA or modulate mitochondrial function, companies such as cellvie Inc. and LUCA Science Inc. are delivering healthy mitochondria via transplantation to revive cell energy production in dysfunctional or damaged cells, with an initial focus on ischemia-reperfusion injury. Both are preclinical.

COMPANY PROFILE
PRETZEL THERAPEUTICS INC.
Waltham, MA and Gothenburg, Sweden

Technology: Small molecules and zinc finger nucleases to modulate mitochondrial function

Origin of technology: University of Gothenburg, University of Cambridge and Karolinska Institute

Disease focus: Cancer, endocrine/metabolic, neurology

Clinical status: Preclinical

Founded: 2020 by Claes Gustafsson, Michal Minczuk, Nils-Göran Larsson, Gabriel Martinez and Paul Thurk

Academic collaborators: Undisclosed

Corporate partners: Undisclosed

Number of employees: 35

Funds raised: $72.5 million

Investors: Arch Venture Partners, Mubadala Capital, HealthCap, Cambridge Innovation Capital, Cambridge Enterprise, Angelini Ventures, GV, Invus, Eir Ventures, GU Ventures, Karolinska Institute Holding

CEO: Jay Parrish

Patents: Undisclosed

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