Gene Therapy Briefs

The U.S. Patent and Trademark Office (www.uspto.gov) issued the first patent for an engineered CRISPR (clustered, regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) gene-editing system designed to enable scientists to modify genes and better understand the biology of living cells and organisms.

The Broad Institute applied for the patent in concert with the Jan. 3, 2013, publication in *Science* that described the use of the CRISPR enzyme Cas9 for genome editing (Cong *et al.*, 2013).

U.S. patent no. 8,697,359 is assigned to the **Broad Institute** (www.broadinstitute.org) and the **Massachusetts Institute of Technology** (MIT; www.mit.edu) and includes claims to the engineered CRISPR-Cas9 system and methods of using the system. The inventor is Feng Zhang, PhD, a Broad core member who is also the senior author of the *Science* article.

Dr. Zhang is also the W. M. Keck career development professor in biomedical engineering at MIT, an investigator at the McGovern Institute for Brain Research, and a cofounder of **Editas Medicine** (www.editasmedicine.com, Cambridge, MA). He formed Editas with four other gene-editing pioneers to translate their research into a new class of therapeutics designed to enable precise and corrective molecular modification to treat underlying causes of diseases at the genetic level.

CRISPR relies on cellular machinery that bacteria use to defend themselves from viral infection. Researchers have copied this cellular system to create gene-editing complexes that include a DNA-cutting enzyme, called Cas9, bound to a short RNA guide strand that is programmed to bind to a specific genome sequence, telling Cas9 where to make its cut.

At the same time, the researchers also deliver a DNA template strand. When the cell repairs the damage produced by Cas9, it copies from the template, introducing new genetic material into the genome. Scientists envision that this kind of genome editing could one day help treat diseases such as hemophilia, Huntington's disease, and others that are caused by single mutations.

"The CRISPR-Cas9 system is an extraordinary, powerful tool. The ability to edit a genome makes it possible to discover the biological mechanisms underlying human biology and, potentially, to treat certain human diseases," said Eric Lander, PhD, director of the Broad Institute (Editas Medicine, 2014).

Regeneron Pharmaceuticals (www.regeneron.com; Tarrytown, NY) will use the adeno-associated virus (AAV)-based drug-delivery platform of Avalanche Biotechnologies (www.avalanchebiotech.com; Menlo Park, CA) in a collaboration to commercialize its lead drug and new gene therapy products for ophthalmologic diseases, the compa-

nies said today. The collaboration could net Avalanche more than \$640 million.

Under the collaboration, Regeneron will apply the Avalanche Ocular BioFactory™ to discover and develop gene therapy vectors for ophthalmology. BioFactory is designed to use the body's own cells to produce therapeutic protein after only a single injection. That, in turn, blocks vascular endothelial growth factor (VEGF) signaling, treating the disease.

Avalanche says its platform reaches peak expression after 4–6 weeks, at which point the treatment can be maintained on an ongoing basis with a continuous, steady-state level of therapeutic protein versus a half-dozen or so reinjections per year with current treatments. Based on preclinical studies, Avalanche says the therapeutic effect from its lead product AVA-101 can be maintained for at least 18 months, with the potential to last for several years following treatment from a single injection.

Regeneron agreed to pay Avalanche an undisclosed, upfront cash payment—contingent payments of up to \$640 million tied to development and regulatory milestones—plus a royalty on worldwide net sales of collaboration products.

The collaboration covers up to eight distinct therapeutic targets, with Regeneron holding exclusive worldwide rights for each product it moves forward in clinical development. Avalanche has the option to share in development costs and profits for products directed toward two collaboration therapeutic targets it will select.

Regeneron has a time-limited right of first negotiation for certain rights to AVA-101 upon completion of an ongoing phase IIa trial. AVA-101 is Avalanche's gene therapy product targeting vascular endothelial growth factor (VEGF), now under development for the treatment of wet age-related macular degeneration (AMD).

"The collaboration will bring together Avalanche's novel platform technology with Regeneron's proprietary molecules and research capabilities, with the goal of creating a new class of next-generation biologics in ophthalmology," Thomas W. Chalberg, PhD, Avalanche's cofounder and CEO, said in a statement (Regeneron Pharmaceuticals and Avalanche Biotechnologies, 2014).

Avalanche's collaboration with Regeneron comes almost 2 weeks after Avalanche completed a \$55 million series B financing intended to advance AVA-101 and other clinical programs in retinal disorders. Avalanche said it will also use proceeds from the financing to invest in manufacturing and clinical infrastructure for AVA-101, as well as accelerate the development of pipeline programs based on Avalanche's proprietary BioFactory platform. (Avalanche Biotechnologies, 2014).

Existing investors were joined by new investors led by Venrock, including Deerfield, Adage Capital Management, Redmile Group, Rock Springs Capital, Sabby Capital, as well as an affiliate of Cowen & Co. and two undisclosed blue chip health-care funds.

Researchers from **Massachusetts Institute of Technology** (MIT; www.mit.edu) have used the CRISPR/Cas9 gene-editing methodology to cure mice of a rare liver disorder caused by a single genetic mutation.

The MIT investigators said their study (“Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype”), published March 30 in *Nature Biotechnology*, offered the first evidence that the technique can reverse disease symptoms in living animals. CRISPR, which provides a way to snip out mutated DNA and replace it with the correct sequence, holds potential for treating many genetic disorders (Yin *et al.*, 2014).

“What’s exciting about this approach is that we can actually correct a defective gene in a living adult animal,” says Daniel Anderson, PhD, the Samuel A. Goldblith associate professor of chemical engineering at MIT, a member of the Koch Institute for Integrative Cancer Research, and the senior author of the article (MIT, 2014).

For this study, the researchers designed three guide RNA strands that target different DNA sequences, near the mutation that causes type I tyrosinemia, in a gene that codes for an enzyme called FAH. Patients with this disease, which affects about 1 in 100,000 people, cannot break down the amino acid tyrosine, which accumulates and can lead to liver failure. Current treatments include a low-protein diet and a drug called NTCB, which disrupts tyrosine production.

In experiments with adult mice carrying the mutated form of the FAH enzyme, the researchers delivered RNA guide strands along with the gene for Cas9 and a 199-nucleotide DNA template that includes the correct sequence of the mutated FAH gene. “Delivery of components of the CRISPR-Cas9 system by hydrodynamic injection resulted in initial expression of the wild-type Fah protein in ~1/250 liver cells,” the investigators reported. “Expansion of Fah-positive hepatocytes rescued the body weight loss phenotype” (Yin, *et al.*, 2014).

While the team used a high-pressure injection to deliver the CRISPR components, Dr. Anderson said he envisions that better delivery approaches are possible. His lab is now working on methods that may be safer and more efficient, including targeted nanoparticles.

ReGenX Biosciences (<http://regenxbio.com>; Washington, DC) will apply its NAV technology platform in a collaboration launched with **AAVLife** (<http://www.aavlife.com>; Paris, France) to develop and commercialize new therapies for Friedreich’s Ataxia (FA). ReGenX granted AAVLife an exclusive worldwide license, with rights to sublicense—to deliver NAV rAAVrh10 vector via non-central nervous system (CNS) routes to treat FA in humans. AAVLife was also granted an option for a nonexclusive global license for CNS delivery of the vector for the treatment of FA in humans.

In return, AAVLife agreed to pay ReGenX undisclosed upfront and ongoing fees, plus fee payments tied to milestones and royalties on net sales of products incorporating NAV vectors. ReGenX would also receive a share of any sublicensing revenue.

NAV vector technology includes new AAV vectors such as rAAV7, rAAV8, rAAV9, and rAAVrh10. ReGenX has

treatment programs in development for hypercholesterolemia, mucopolysaccharidoses, and retinitis pigmentosa.

AAVLife joins several companies that have established collaborations with ReGenX. The companies include Chatham Therapeutics, Fondazione Telethon, Audentes Therapeutics, Lysogene, Esteve, and AveXis.

“REGENX has been engaged with the team at AAVLife, including its stakeholders like the Friedreich’s Ataxia Research Alliance (FARA), since first becoming aware of their gene therapy research results and during the company’s process of formation,” Ken Mills, ReGenX’s president and CEO, said in a statement. “We believe this license agreement will be a key component to the successful development of treatments for patients suffering with FA” (ReGenX Biosciences and AAVLife, 2014).

AAVLife entered into the collaboration with ReGenX on April 29, exactly two weeks after announcing that it had completed a \$12 million series A financing round intended to advance its FA gene therapy into clinical studies. Versant Ventures led the financing round, with participation from Inserm Transfert Initiative, a French seed-investment firm linked to the French National Institute of Health and Medical Research (Inserm) (AAVLife, 2014).

AAVLife was recently founded by a team that includes three of the nine researchers that published results in April showing that gene-replacement therapy prevented and corrected cardiac damage in an FA mouse model. The company aims to build on the work of Hélène Puccio, PhD, a research director at the **French Institute of Health and Medical Research** (INSERM; <http://english.inserm.fr/>). Dr. Puccio and two colleagues have been testing a gene-therapy approach in the cardiac FA mouse model she developed in her lab at the **Institut de Génétique Moléculaire et Cellulaire** (www.igbmc.fr/).

In a study published April 6 in *Nature Medicine*, Dr. Puccio and colleagues showed that a single intravenous injection of AAVrh10 expressing frataxin was not only capable of preventing the development of heart disease in the mouse, but also fully and rapidly treated the mice with advanced stages of heart disease, returning the heart to normal function. The study established the preclinical proof-of-concept for the potential of gene therapy in treating FA cardiomyopathy (Perdomini *et al.*, 2014).

Oxford BioMedica (www.oxfordbiomedica.co.uk) said it regained worldwide rights to the RetinoStat[®] treatment for wet age-related macular degeneration (Wet AMD) it had been codeveloping with **Sanofi** (www.sanofi.com; Paris, France), after being told by the pharma giant it would not continue their partnership beyond a current ongoing phase I study. Sanofi said its decision was a matter of prioritizing other drug development projects and was not linked to unexpected results from an analysis of study data.

Oxford BioMedica said April 7 it successfully completed recruitment and dosing for the phase I trial, designed to assess the safety and aspects of biological activity of RetinoStat in the eye. Interim analysis of patient data has demonstrated safety and signs of efficacy, the company said, with study results expected toward year’s end (Oxford BioMedica, 2014a).

Given what Oxford BioMedica called expressions of interest from “both large pharmaceutical/biotech companies and financial investors” (Oxford BioMedica, 2014b) in the

event of a Sanofi pullout, plus the success of currently marketed Wet AMD treatments, “the Company is therefore confident that once the indicative results from the study are available there will be significant demand for what will then be a phase II-ready product for a major ocular indication. Those treatments include Lucentis® by **Novartis** (www.novartis.com; Basel, Switzerland) and Eylea (afibergcept) by **Regeneron Pharmaceuticals** (www.regeneron.com; Tarrytown, NY) and **Bayer HealthCare** (<http://healthcare.bayer.com>; Leverkusen, Germany).

“Whilst I am disappointed that Sanofi, who has been a good partner during the collaboration and paid for the phase I development to date, has decided to take forward only StarGen™ and UshStat® and not RetinoStat, I am excited to regain control over a product which will soon be phase II-ready,” John Dawson, Oxford BioMedica CEO, said in a statement (Oxford BioMedica, 2014b). “We are confident that once we have the indicative results from the full study, we will be in a position to repartner the product with terms that enhance shareholder value given the phase II-ready nature of this asset.”

Oxford BioMedica disclosed the end of the partnership with Sanofi on April 29—the same day that it also announced it had been awarded a grant of £2.2 million (\$3.7 million) by the U.K. Technology Strategy Board under its Biomedical Catalyst funding program. The award will help fund a phase I/II clinical trial of OXB-102 in Parkinson’s disease patients. OXB-102—an enhanced version of ProSavin®—uses the company’s LentiVector® gene delivery technology to deliver the genes for three enzymes that are critical to the biosynthesis of dopamine.

OXB-102 completed a phase I/II clinical trial in 2012, results of which were published in January 2014 in *The Lancet* (Palfi *et al.*, 2014). The drug demonstrated a favorable safety profile and a statistically significant improvement over baseline in motor function at 6 and 12 months after dosage. This substantial second award will support our plans to further develop OXB-102 through the next stage of the clinical process and to its next value inflection point,” Dawson said (Oxford BioMedica, 2014c).

Celladon (www.celladon.com; San Diego, CA) said April 10 that its lead product candidate Mydicar® (AAV1/SERCA2a) has been granted breakthrough therapy designation by the U.S. Food and Drug Administration (FDA) for reducing hospitalizations for heart failure in NYHA class III or IV chronic heart failure patients who are NAb negative. Celladon is the first drug developer to win the designation for a developmental gene therapy treatment.

Mydicar is the first in a new first-in-class therapy designed for patients with chronic heart failure due to systolic dysfunction. Mydicar uses genetic enzyme replacement therapy to correct the deficiency in SERCA2a, an enzyme that becomes deficient in heart failure patients and results in inadequate pumping of the heart. Celladon has developed a companion diagnostic to identify the patients who are AAV1 NAb negative and therefore eligible for Mydicar treatment.

FDA’s breakthrough therapy designation is intended to streamline drug development and review of innovative new medicines that address unmet medical needs for serious or life-threatening diseases or conditions. Drug developers obtain the designation by showing FDA preliminary clinical evidence indicating that a treatment may demonstrate a

substantial improvement over existing therapies on at least one clinically significant endpoint.

“Mydicar has the potential to provide transformative disease-modifying effects with long-term benefits in heart failure patients with a single administration,” Krisztina Zsebo, PhD, Celladon’s president and CEO, said in a statement. “Our goal is to bring Mydicar to market as quickly as possible in the United States, where we estimate approximately 350,000 heart failure patients with currently limited remaining treatment options could be eligible for therapy” (Celladon, 2014). The FDA based its designation on results from the phase I Calcium Up-Regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID 1) trial.

Celladon is currently evaluating Mydicar in the phase IIb CUPID 2 trial to determine its efficacy in reducing the frequency of and/or delaying heart failure-related hospitalizations. The trial is evaluating a single intracoronary infusion of Mydicar versus placebo added to a maximal, optimized heart failure regimen in patients with NYHA class III or IV symptoms of chronic heart failure due to systolic dysfunction. Patient enrollment has been completed and 250 patients have been randomized in this trial, the company said. Celladon expects to report results from CUPID 2 in April 2015.

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—Alex Philippidis