UC's latest CRISPR patent

The University of California, Berkeley, the University of Vienna and Emmanuelle Charpentier have been granted a third US patent covering the use of CRISPR–Cas9 gene-editing technology.

The patent, US 10,113,167, covers the use of RNA to modify a target DNA, where the RNA can be delivered to the target either complexed with Cas9 protein or by genetically modifying cells such that they will express and produce Cas9 inside the cell to form a functional CRISPR–Cas9 complex. These CRISPR–Cas9 methods apply to eukaryotic and cell-free environments.

Despite having three issued patents, the University of California still lags behind its rival the Broad Institute of MIT and Harvard in terms of intellectual property rights for CRISPR–Cas9 technology in the United States. Patents assigned to the Broad were granted sooner. And, in a recent dispute, a US appeals court affirmed Broad's ownership of a key patent describing the use of CRISPR–Cas9 in eukaryotic cells (*Nat. Biotechnol.* **36**, 1026, 2018).

Despite Broad's dominant position, the University of California has several pending patent applications for CRISPR–Cas9 technology, including an application that was involved in the recent court case. Here, the court ruled that that the University of California's application, which described CRISPR–Cas9 in any cell type and cell-free systems, did not interfere with Broad's eukaryotic patents, meaning both methods could be patentable. The court did not rule on the validity of the patents.

Both the University of California and the Broad have licenced their intellectual property. Yet it remains unclear whether Broad, University of California or other parties "will ultimately have sufficiently strong or comprehensive patent protection to give licensees confidence in their rights to develop CRISPR technology," said patent attorney Kevin Noonan in his Patent Docs blog.

Charlotte Harrison

"These are like the most earnest people you have ever met. … The nefarious image just does not fit these folks. They're all very smart, they're all well-intentioned, they want to solve problems, and I think some of them are kind of like blown back by the tone of the coverage." Patrick O'Connor of the Alliance to Protect Medical Innovation, comes to the defense of the biotech industry as calls mount for greater transparency. (*STAT*, 16 November 2018)

"If it walks like a drug company interest group and talks like a drug company interest group and represents drug companies, then it's probably a drug company special interest group." Ben Wakana, executive director of Patients for Affordable Drugs, speaking of the Alliance to Protect Medical Innovation. (*STAT*, 16 November 2018)

serotypes," he says. It does offer cost benefits, he says, because the process dispenses with the need for bacterial plasmids to introduce the vector components to the producer cell line, but consistency can be a problem.

Yposkesi, located in Evry, France, claims a ten-fold improvement in vector production using the conventional HEK293 cell process. This yield resulted from replacing polyethylenimine with an as yet undisclosed transfection agent, which improves transfection efficiency by up to fivefold, in a highly efficient HEK293 cell line. "We have identified a subpopulation which are high producers," he says. The company, a spin-out from the not-for-profit gene therapy research organization Généthon, can achieve up to 70% vector purity without the need for cumbersome and expensive ultracentrifuges. "Ultracentrifugation is not an easily scalable manufacturing technique," he says.

For all their challenges, viral vectors remain the most effective way of delivering large quantities of DNA to target cells. The repertoire of available capsids, particularly AAV vectors, has expanded in the past two decades—an effort spearheaded by Wilson following the death of Jesse Gelsinger in a trial of an adenovirus-based therapy for ornithine transcarbamylase deficiency, a trial Wilson led. The 11 available AAV serotypes can be further extended by pseudotyping—mixing and matching different viral genomes and capsid proteins—so the resulting constructs allow selective, if not specific, targeting of particular organs or tissue types. But what remains largely unchanged are the DNA payloads the vectors carry—for the most part they involve a transgene under the control of a strong viral promoter, as well as the sequences encoding capsid assembly functions. "The capsid [only] gets you so far," says David Venables, CEO of Edinburgh-based Synpromics. An AAV9-based vector, for example, will efficiently deliver its payload to the central nervous system, but, he adds, it will not do so exclusively. "You've still got exposure elsewhere."

Synpromics is developing promoters that allow greater control of transgene expression in terms of both location and strength. It's not the first company to develop cell-selective promoters, nor is it the first to develop inducible switches that can dial up or down the level of gene expression required. But it is attempting to overcome the shortcomings of existing systems, which, says Venables, are "leaky" in terms of their expression profiles and which require the coexpression of additional factors, such as transcriptional activators or repressors, to work. They are neither able to shut down expression completely nor able to crank it up sufficiently when required. "The amplitude of the dial-up tends to be quite low." The company has not yet unveiled the workings of its inducible expression switches, but it has secured commercial agreements with six of the ten leading gene therapy firms, he says. One of them is UniQure, of Amsterdam. It recently reported preclinical data in a nonhuman primate model indicating that a liver-directed gene therapy, under the control of a Synpromics-developed liver-selective promoter, resulted in an eightfold increase in gene expression compared with current approaches.

Touchlight has developed a method to obtain viral vectors using *in vitro* DNA amplification, which eliminates the need for plasmid transfection and avoids the packaging of unwanted bacterial DNA into viral capsids. "Part of your drug product is contaminated by antibiotic resistance genes," says Linden. "In the traditional system, you can't get around it." Touchlight avoids the problem by using a dual-enzyme system, comprising a phage DNA polymerase and protelomerase. Via rolling-circle DNA replication, a circular template containing the sequence of interest is replicated into a concatemer, a long continuous sequence, which is then processed into individual 'doggybone' DNA (dbDNA) molecules. So-called because of their shape, these double-stranded, covalently closed DNA molecules can be introduced into producer cells lines for capsid assembly and packaging using the same triple transfection method currently used for bacterial plasmids. "You do exactly the same, except you don't package the crap," says Linden. What's more, the generation of dbDNA vectors is rapid. "You can make gram amounts of DNA within two weeks at scale in GMP [good manufacturing practice]," he says.

The technology can be used to produce any DNA-based medicines, including gene therapies, DNA vaccines and what Linden terms "DNA-launched" products, such as antibodies. Touchlight recently entered a collaboration with the Janssen Biotech arm of Johnson & Johnson, which is evaluating the technology for undisclosed genetic therapies in infectious disease and oncology. Linden, who, as Pfizer's former vice president of gene therapy, helped to develop the pharma firm's gene therapy strategy, says a dbDNA-based AAV gene therapy could reach in the clinic within one to two years.

Linden expects gene therapy to progress in a hybrid fashion in which new ideas and innovations are bolted onto the existing technologies. Because they are potentially curative, AAV and other viral vectors are here to stay, he says, "and what we have to do is address the limitations." Mark Levi, senior consultant with Parexel, regards this as being feasible, given the incentives involved, "I think you'll see manufacturing rise to the occasion and deliver," he says. "Where there's money to be made it'll get done."

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