Illumina sues Oxford Nanopore

In February, Illumina filed two lawsuits against sequencing company Oxford Nanopore for patent infringement. At the heart of the lawsuit is the use of *Mycobacterium smegmatis* porinA (MspA). Illumina is alleging that Oxford Nanopore's MinION and PromethION devices for single-molecule sequencing infringe US patent nos. 8,673,550 and 9,170,230, which use Msp porins as the basis for a protein nanopore sequencing system. The San Diego–based sequencing giant says it signed exclusive rights to license and develop the MspA technology from the University of Alabama Birmingham Research Foundation and the University of Washington. Illumina is now seeking damages as well as an injunction against further infringement. The Oxford-based biotech has not disclosed whether the hand-held MinION sequencer currently on sale and the larger benchtop version, PromethION, use Msp porins. In March, Oxford Nanopore's chief technology officer Clive Brown announced a collaboration with the laboratory of Han Remaut from VIB, a Ghent, Belgium–based research institute to develop a new DNA sequencing nanopore. The R9 pore is based on a *Escherichia coli* CsgG pore that resulted from screening over 700 mutations. The company intends to soon make flow cells containing the new R9 pore available for MinION and PromethION systems, and will discontinue its existing R7 flow cells by academic researchers in some early access programs. Oxford Nanopore has never discussed the type of pore it had used in its devices. It did rely on the alpha-hemolysin pore in its early development phase.

"**"'You know, Mosley, it's called the life sciences because it takes a lifetime to get your money back',"** Stephen Mosley, head of the Alaska Permanent Fund, recalls a conversation in which he was forewarned about investment timelines in biotech. The fund, which has billions of dollars from the state's massive oil revenues, invested more than $280 million in just three biotech startups, Juno Therapeutics (Seattle), Denali Therapeutics (S. San Francisco) and Codiak Biosciences (Woburn, MA, USA). Their $129-million investment in Juno alone is now worth $1 billion. (*Bloomberg News*, 3 March 2016)

Little known fact: the original name for Juno Therapeutics was **"FC Therapeutics, 'f' being a four-letter Anglo-Saxon word and 'c' being cancer,"** according to Hans Bishop, Juno's CEO. After receiving a large investment from the Alaska Permanent Fund, Juno was chosen, as a nod to the state (whose capital is Juneau). (*Bloomberg News*, 3 March 2016)

"By my last count [July, 2015], there are three new peer-reviewed publications that mention using CRISPR per day. And that number is only increasing." Jacob Corn, of UC Berkeley, opines that the true value of CRISPR will not be in genetic engineering, but in the biological insights gained from experimental use. (*The Washington Post*, 23 February 2016)"

can differentiate, with 100% accuracy and sensitivity, early- and late-pancreatic cancer from benign disease (*Nature* **523**, 177–182, 2015). That level of precision, which is unheard of in molecular diagnostics, sounds too good to be true. "Sure—that was my first reaction," says Williams, adding, however, that the data make for a "very convincing story," even if the findings remain preliminary at this point, and that the data need to be reproduced by others.

The broad liquid-biopsy category also encompasses the analysis of circulating tumor cells and cell-free, circulating tumor DNA (ctDNA) derived from dying cancer cells, in addition to exosomes. The area has engendered multi-billion-dollar market forecasts and some recent startup activity. Illumina, of San Diego, recently spun out Grail, with $100 million in venture capital funding, to apply what it calls 'ultra-deep sequencing' to the detection of ctDNA. Molecular Stethoscope, also of San Diego, is focusing on circulating cell-free RNA for early detection and monitoring of neurodegenerative disease, coronary artery disease and autoimmune disease.

Exosome's ExoDx *Lung(ALK)* test detects both exosomal RNA and ctDNA, in a single-step analysis. "No one has been able to harness the dual fraction before," Skog says. It can boost sensitivity in detecting rare cancer mutations that are not easily picked up in other liquid biopsies that rely on circulating tumor cells or ctDNA only. In one blinded study in m0/m1a NSCLC patients, whose mutation status is considered particularly difficult to assess by liquid biopsy, analyzing the exosomal RNA and ctDNA fractions improved sensitivity almost threefold (74% vs. 26%) over what could be obtained by evaluating ctDNA only. The test is the first of four that the company plans to make available this year from its Cambridge, Massachusetts, facility, which is certified under the Clinical Laboratory Improvement Amendments (CLIA) quality program run by the Centers for Medicare & Medicaid Services.

Given the immaturity of the field, scientists have yet to standardize methods for isolating and analyzing exosomes. Most academic researchers rely on ultracentrifugation for isolating exosomes, a technology that is unwieldy and inappropriate for most clinical settings. Exosome Diagnostics is pursuing two alternative approaches. One is a research protocol involving spin-column-based nucleic acid purification and the other, a current good manufacturing process (cGMP), involving quantitative PCR. Throughput is not an issue at this point, Skog says. "We have a medium-throughput assay now," he says. "We can easily do a few hundred samples in a day."

Ymir Genomics, an early-stage firm currently based at the LabCentral facility in Cambridge, Massachusetts, has developed two high-yield methods for isolating extracellular vesicles and extracellular nucleic acids from urine. One is based on a novel proprietary molecule that causes them to precipitate; the other is based on a widely available capture resin. "Most of the work done on methods development has been done on blood plasma and serum—very little has been done on urine," co-founder and CSO P. Shannon Pendergrast says. "It's much easier to work in blood serum and plasma, because the concentration of extracellular vesicles is much higher—1,000-fold higher has been reported." Urine has a couple of advantages: obtaining samples is even less invasive than taking blood samples; moreover, exosomes are stable in urine at room temperature for up to a week. Weissleder's group is focused on analyzing the protein rather than the nucleic acid content of exosomes. It is testing in clinical trials novel approaches for isolating and analyzing exosomes that can be easily deployed at the point of care. One is based on a magneto-electrochemical sensor (*ACS Nano.* **10**, 1802–1809, 2016). Another involves a surface-plasmon-resonance (SPR) chip, called the nano-plasmonic exosome (nPLEX) sensor, which uses metal-film-based arrays of 'nanoholes' with bound affinity ligands for detecting prespecified exosomal proteins (*Nat. Biotechnol.* **32**, 490–495, 2014).

Exosomes as therapeutics are at an even earlier stage, but the most logical path forward, Williams says, is to use exosomes to deliver therapeutic payloads. MD Anderson plans to launch a phase 1 study by the end of the year, building on Kalluri's work in animal models. Williams also holds out the possibility that exosomes may be naturally 'addressable' to particular tissues—and that their selectivity can be reprogrammed by modifying their producer cell lines. One emerging line of evidence—from David Lyden's laboratory at Weill Cornell Medical College, in New York—suggests that exosomes possess integrin profiles that are distinct from those of their respective parent cells and which, in the case of cancer, may contribute to or even dictate the formation of a premetastatic niche in specific organs (*Nature* **527**, 329–335, 2015). The study serves as an *in vivo* proof of the concept that using specific exosomal integrin blockers can combat cancer metastasis.

Even if exosome biology remains immature, it is attracting interest and cash that will accelerate its maturation. The early movers had an additional motivation, says Weissleder: "A wide open IP space." Whether they will reap the rewards remains an open question for now.

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