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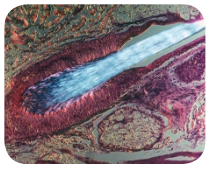

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FDA approves 'farmaceutical' drug from transgenic chickens

The US Food and Drug Administration (FDA) approval on 8 December of Alexion Pharmaceuticals' Kanuma (sebelipase alfa) is only the fourth for a recombinant protein drug produced in an unconventional expression system. The drug, which in the US will cost about $310,000 annually, is purified from the egg white of transgenic hens (*Gallus gallus*), a production method chosen because of the glycosylation pattern of the resulting protein. It is approved for treating two forms of lysosomal acid lipase (LAL) deficiency: a fatal, early-onset form called Wolman disease and cholesteryl ester storage disease, a less severe form that can cause liver fibrosis, cirrhosis and eventually liver failure. Despite its undoubted potential from a cost and performance perspective, molecular 'farming' has remained a minority pursuit in the decade following the first such approval, that of ATryn, a recombinant antithrombin produced in the milk of transgenic goats (**Table 1**). In volume terms, biologics produced in unconventional systems represent a tiny fraction of the industry's total output.

Herb Bendicks / Alamy Stock Photo

Eggs from transgenic chickens contain a human enzyme to treat a rare inherited disease.

Kanuma could mark a turning point in the development of the sector, given the blockbuster ambitions that Alexion, of Cheshire, Connecticut, has attached to the product. Last June, Alexion paid $8.4 billion in cash and shares to acquire its developer, Lexington, Massachusetts-based Synageva BioPharma (formerly AviGenics).

For the immediate future, however, the vast majority of biotech firms will stick to conventional bacterial, yeast and mammalian expression systems, such as Chinese hamster ovary (CHO). The latter has now become the standard production system for complex mammalian proteins, in both research and production environments. "Now there's a huge amount of inertia [against going] to another system," says Rob Etches, president and CEO of Crystal Bioscience, of Emeryville, California. "Moving away from CHO represents a risk that, at this point in time, nobody needs to take." Sijmen de Vries, CEO of Pharming, a company synonymous with the breeding of transgenic animals, concurs: "Let's be realistic, billions have been invested in the CHO standard, and tens of thousands of careers have been built on it."

It was not always thus. Back in the early 1990s, CHO and other cell-based systems were not as robust or efficient as they are now, and the future use of transgenic animals or plants as 'bioreactors' for the production of recombinant proteins seemed quite a realistic prospect. It was, for a time, a mainstream research topic within the biotech industry. Before Dolly the sheep—the first mammal to be cloned by somatic cell nuclear transfer (SCNT), all of twenty years ago—there was Herman the bull, the first transgenic bovine, who, like Dolly, also achieved a certain emblematic notoriety. Born in 1990, in the Netherlands, the animal was engineered via microinjection of a fertilized embryo to carry the gene encoding human lactoferrin, as well as regulatory sequences to induce the expression and secretion of the antimicrobial protein in the milk of female offspring.

The original project was developed by the European arm of GenPharm International, of Mountain View, California, now Pharming, of Leiden, the Netherlands. But its success was limited by the inefficiency of the gene transfer process and by the low yields obtained from those animals that were successfully modified. Despite subsequent improvements in both, the project never yielded a commercial product (*Nat. Biotechnol.* **20**, 484–487, 2002). PPL Therapeutics, the company spun out of the Roslin Institute in Edinburgh, Scotland on the back of Ian Wilmut's and Keith Campbell's pioneering work in mammalian SCNT, also failed to make headway in its efforts to produce α1-antitrypsin (AAT) in the milk of transgenic sheep, despite the backing of an alliance with Bayer AG, of Leverkusen, Germany. It went out of business in 2004.

A couple of years earlier, Pharming also came perilously close to going out of business when Genzyme (now part of Paris-based Sanofi) exited an alliance to develop α-glucosidase as an enzyme replacement therapy (ERT) for Pompe disease. After testing investors' patience for several years, it finally completed a long-drawn-out European approval process in 2010 for its hereditary angioedema (HAE) drug Ruconest (recombinant C1 esterase inhibitor), which is secreted in the milk of transgenic rabbits. An FDA approval followed in 2014, but sales remain low. Pharming lost its early

Table 1 Selected products using unconventional expression systems on the market or under development

Drug	Company	Mechanism	Production process	Indication	Status
ATryn (recombinant antithrombin)	Revo Biologics (formerly GTC Biotherapeutics)	Inhibits thrombin and factor Xa, the key serine proteases involved in blood coagulation	Purified from the milk of transgenic rabbits goats	Prevention of thromboembolic events (blood clots) in patients with hereditary antithrombin (AT) deficiency undergoing surgery or giving birth	EU approval 2006; FDA approval February 2009
Ruconest (conestat alfa)	Pharming	Human recombinant C1-esterase inhibitor	Purified from the milk of transgenic rabbits	Acute hereditary angioedema attacks	EU approval October 2010; FDA approval July 2014;
Elelyso (taliglucerase alfa)	Pfizer, Protalix Biotherapeutics	ERT	Produced in transgenic carrot-based plant cell expression system	Type 1 Gaucher disease	FDA approval May 2012; approval declined in EU due to market exclusivity rules
Kanuma (sebelipase alfa)	Alexion Pharmaceuticals	ERT	Purified from the egg white of transgenic hens (*Gallus gallus*)	Lysosomal acid lipase deficiency	EU approval September 2015; FDA approval December 2015
Pandemic flu vaccine	Medicago (Quebec, Canada)	Pandemic influenza vaccine	Virus-like particles transiently expressed in tobacco (*Nicotiana benthamiana*) leaves	Prevention of H5N1 influenza infection	Phase 2
VEN150 (recombinant lactoferrin)	Ventria Bioscience (Fort Collins, Colorado)	Iron-sequestering glycoprotein with antimicrobial and anti-inflammatory effects	Expressed in the grain endosperm of genetically modified rice	Inflammation in HIV	Phase 2
SBC-103 (recombinant α-N-acetylglucosaminidase)	Alexion	ERT	Purified from the egg white of transgenic hens (*Gallus gallus*)	Mucopolysaccharidoses IIIB	Phase 1/2
ZMapp	Mapp Biopharmaceutical,LeafBio (San Diego, Calif.); Defyrus (Toronto, Canada); Kentucky Bioprocessing (Owensboro, Kentucky)	Cocktail of three chimeric antibodies directed against Ebola virus surface glycoprotein	Produced in transgenic tobacco plants	Ebola virus infection	Phase 1/2
PRX-106	Protalix Biotherapeutics	Oral tumor necrosis factor-α inhibitor	Produced with carrot and tobacco cell culture techonology	Inflammatory bowel disease	Phase 1
Moss-aGal (agalsidase)	Greenovation Biotech	ERT	Produced in glycoengineered *Physcomitrella patens* moss cell line	Fabry disease	Phase 1

Sources: company websites; FDA, EMA, PubMed, clinicaltrials.gov. ERT, enzyme replacement therapy.

lead, and the market for HAE drugs is now dominated by Dublin, Ireland-based Shire. In hindsight, de Vries—who joined Pharming in 2008 and who led its turnaround—says many of the early movers in the transgenics field were poorly led. "You can also question the companies' choice of products," he adds.

In that context, much of the credit for the sale of Synageva and the approval of Kanuma must go to Sanj Patel, a former Genzyme executive who steered AviGenics away from being a platform company to become a product company with a focus on rare diseases. The original AviGenics organization was formed in Athens, Georgia, in 1996 to employ a retroviral approach to generating transgenic chickens for the production of recombinant proteins. The chick egg production system has certain advantages, notes Etches, who previously worked with Origen Therapeutics, a now defunct firm that sought to produce polyclonal antibodies in the eggs of transgenic chickens.

Chick egg whites lack proteases in the cell lysate that plague other production systems. These give rise to breakdown products that can cause immunogenicity problems. "In the chick egg, you don't have any proteases. In fact you have a bunch of protease inhibitors," he says. Even so, severe hypersensitivity reactions occurred in 21 of 106 patients who received Kanuma during clinical trials, three of whom developed anaphylaxis. The issue necessitates strict medical supervision during administration of the drug.

But the scaling flexibility and cost profile of the production system are both favorable, according to Alexion. "We have the ability to scale as needed by increasing the number of animals producing the egg whites," states Julie O'Neill, Alexion's executive vice president of global operations, via e-mail. "Costs and productivity associated with the egg white expression platform are comparable to standard biologics production processes." The Synageva acquisition brought Alexion a second program that also employs the same production system. SBC-103, a recombinant form of α-N-acetylglucosaminidase, is undergoing a phase 1/2 trial in patients with mucopolysaccharidosis IIIB. "We are also in the early stages of evaluating whether this platform could be used in the treatment of other lysosomal storage diseases that have central nervous system manifestations," O'Neill notes.

In parallel with transgenic animals, plant-based production systems are emerging. Improving yields have eliminated the once controversial prospect of growing genetically modified crops to produce pharmaceuticals in open field systems. Contained greenhouse facilities, as well as newer bioreactor vessels for culturing transgenic plant cells, are now sufficient. Protracted manufacturing problems that emerged at a Genzyme CHO facility in Allston, Massachusetts, in 2009 created an opportunity for Protalix Biotherapeutics, of Carmiel, Israel, to win approval for its ERT for Gaucher disease, Elelyso (taliglucerase alfa),

which is produced in a closed plant cell–based system, contained within disposable plastic chambers. New York-based Pfizer's move to in-license—and subsequently acquire—rights to this product outside of Israel and Brazil (where Protalix has entered a technology transfer and supply agreement with the country's health ministry) shows big pharma is at least open to the technology, even if it not yet getting involved in early-stage development.

"That was an important step, I think," says Julian Ma, professor of molecular immunology at St. George's University of London, in the UK. Also significant, he adds, was the production last year of a cocktail of anti–Ebola virus antibodies from transgenic tobacco. "All of a sudden plant antibodies became known around the world, despite everyone calling them secret serum to begin with," says Ma. Criticism arising from the shortage of the drug was misplaced, he adds. "The miracle was there was enough to treat nine patients, based on the stage of development at that time." Ma led an EU-funded research program that conducted the first clinical trial of a plant-produced monoclonal antibody, a topically applied HIV drug called P2G12, produced by transgenic tobacco. His group is now further refining the production process to generate an intravenous version of the same drug.

Glycosylation patterns in plant-produced proteins can differ from those obtained in mammalian culture systems. They tend to be homogeneous however, whereas mammalian cell production systems generate a wider distribution of glycosylation variants. "Essentially we got a single glycoform," Ma says. "We don't see it with all antibodies." Greenovation Biotech, of Freiburg, Germany, has engineered the glycosylation machinery of its producing strain of moss, *Physcomitrella patens*, in order to eliminate plant specific α-1,3-fucose and β-1,2-xylose residues and maximize the numbers of proteins with N-terminal mannose residues, so as to favor uptake by kidney cells (*J. Inherit. Metab. Dis.* 27 August 2015, doi:10.1007/s10545-015-9886-9). The company recently obtained clearance to conduct the first trial of a therapeutic produced from this system, α-galactosidase A, in patients with Fabry disease (*Nat. Biotechnol.* **33**, 1122, 2015). "The dosing begins in April," says CEO Thomas Frischmuth. "We have to produce two new batches of the enzyme." The company, which, like Protalix, also uses a closed, disposable cell culture system, is at present scaling up production from 300 liters to 5,000 liters. That would be sufficient to cover the world's entire population of patients with Fabry disease. "Our production is now very close to what mammalian cell systems are."

Production systems based on mammalian cell culture continue to set the standard, and alternative systems have failed to keep pace with them over the past two decades. But they are filling niches that conventional systems have failed to address adequately—and the next two decades are unlikely to be a repeat of the last two. As the case of Synageva shows, the opportunity exists. "It's all about getting products out onto the market," Ma says.

Cormac Sheridan *Dublin*

Obama's cancer moonshot

President Barack Obama in his final year in office is endorsing a nationwide effort to accelerate the testing of immunotherapy drug combinations to fight cancer. The president announced the project—dubbed Cancer MoonShot 2020—at his last State of the Union address, delivered on January 16. The effort will assemble an Apollo-sized team, known as the National Immunotherapy Coalition (NIC). This will include industry players such as Thousand Oaks, California–based Amgen and Summit, New Jersey–based Celgene, big pharma, and small biotechs like Culver City, California–based NantWorks, as well as oncologists, academic institutions, insurers, and senior officials from the FDA and the National Cancer Institute. Patrick Soon-Shiong, founder and chairman of the group of companies, is the driving force behind the coalition, and Vice President Joe Biden, whose son died of cancer last year, will be leading the moonshot project. The NIC will test more than 60 molecules in different combinations in 20 tumor types. The multitrial initiative aims to enroll 20,000 patients, who will have their whole genomes sequenced and be tested with proteomic diagnostics to match to the appropriate immunotherapies. The coalition could be a boon for the biotech industry, though observers note that Bristol-Myers Squibb, Merck and Roche, those with the most advanced immunotherapies, are already running combination trials with anti-PD-1/PD-L1 therapies, and were absent from the proceedings. *LM*