

Carrots produce drugs

Farming for Pharma

Carrots are known to be healthy. But they can do much more than produce beta-carotene if manipulated by genetic tricks. The Israeli biotech company Protalix Biotherapeutics forced carrot cells to produce drugs – the first therapeutic made by transgenic plants to be approved for human use.

Until recently, Protalix Biotherapeutics was one among many. The Israeli company develops plant expression systems to produce human therapeutics, as do many others. The use of crops as biological factories is often called “molecular pharming” or “biopharming”.

Since May 1st, however, Protalix Biotherapeutics has been unique. They are the first company worldwide to put a plant-derived “pharming drug” for commercial use on the market. The drug that received FDA approval is called taliglucerase alfa, a recombinant glucocerebrosidase to treat the rare hereditary Gaucher disease. It is produced in genetically engineered carrot cell suspension cultures and will be sold as “Elelyso”.

A not-so-novel technology

The possibility of producing pharmaceutical proteins in plants was hinted at in 1989 with the expression of monoclonal antibodies from genetically modified tobacco plants (Hiatt *et al.*, *Nature* 1989, 342:76-78) and – one year later – human serum albumin from tobacco and potato plants (Sijmons *et al.*, *Biotechnology* 1990, 8:217-221). A number of start-up companies were launched to commercialise these discoveries but not a single development appeared on the market. The persistent scepticism of the major pharmaceutical companies about this new technology was a deep blow to those who never gave up on the development of plant-made drugs.

Against all odds, the world’s first plant-made pharmaceutical was approved in the US in 2006 by the United States Department of Agriculture. It wasn’t for humans, but for the vaccination of poultry against the Newcastle disease virus. The US company Dow Agrosciences developed the production of the drug in tobacco, not grown on open land but in bioreactors.

Despite regulatory approval, the poultry vaccine remained a proof-of-concept and was never sold. But it paved the way for future plant-made therapeutics.

Approved but never sold

“Dow Agrosciences used the animal vaccine as an example to completely run through the process. A new platform needs to be approved, which can be difficult when authorities get in contact with it for the first time”, explains the plant physiologist Stefan Schillberg, head of the Molecular Biology Division at the Fraunhofer Institute for Molecular Biology and Applied Ecology Aachen. From 2004 to 2011, Schillberg contributed to Pharma-Planta, a consortium founded by the EU that developed a plant-made HIV drug and got its clinical testing under way (www.pharma-planta.net).

Protalix followed on Dow Agrosciences’ heels with its human drug Elelyso. Elelyso is an enzyme analogue that is similar to human lysosomal glucocerebrosidase, an enzyme that is dysfunctional in patients with Gaucher disease. There are more than 280 known mutations in the gene encoding glucocerebrosidase that lead to different forms of the autosomal recessive lysosomal storage disease.

The most common form of Gaucher disease (type I) does not usually affect the central nervous system whereas type II and type III are neuropathic forms. The dysfunctional lipid metabolism of Gaucher patients is caused by accumulation of glucocerebroside inside characteristic storage cells. When not cleaved by glucocerebrosidase the glycoprotein accumulates in the spleen, liver, lungs, bone marrow and even in the brain causing organ damage, low red blood cell counts and platelet and bone problems. A functional glucocerebrosidase can be restored by enzyme substitution therapy, which is the most common treatment of Gaucher disease and requires lifelong intravenous infusions.

Giants as competitors ...

The plant biochemist Yoseph Shaaltiel founded Protalix in 1993. Today the company employs 235 people but competes with much bigger rivals. The enzyme replacement therapy for Gaucher disease is a niche that was occupied for many years

by a single product called Cerezyme (imiglucerase). Cerezyme was developed in the eighties by the biotechnology pioneers of Genzyme (Boston, USA). The company employs over 12,000 people and is a giant in comparison to Protalix. Genzyme was acquired by Sanofi-Aventis in 2011. The recombinant glucocerebrosidase Cerezyme is produced in Chinese hamster ovary cells. It was approved by the FDA in 1994 and became one of the most expensive drugs ever. Treatment of a patient for one year costs more than €200,000.

Although Gaucher disease is very rare, affecting about 10,000 patients worldwide, of whom 6,000 are in treatment, Genzyme’s manufacturing capacity has had its limits in the past. Fifteen years after the approval of Cerezyme, Genzyme was forced to temporarily close a production plant in Allston Landing, USA because of viral contamination. The result was a worldwide shortage of Cerezyme and recommended dose-reductions in adult patients.

... with some problems

However, one man’s meat is another man’s poison. In 2010, one year after the incident, a second player, quicker than Protalix, appeared on the field. The English biopharmaceutical company Shire – with 5,250 employees huge in comparison to Protalix – got FDA-approval for Vpriv (Velaglucerase alfa). Thus the second beta-glucocerebrosidase analogue available for Gaucher patients was born. After sixteen years of market dominance the Cerezyme regime had a competitor.

Vpriv, like Cerezyme, is produced in mammalian cells but uses a human fibrosarcoma cell line. The end product costs about 15 percent less than Cerezyme. The approval of this competitor was followed by another issue in 2011 that caused the Genzyme management quite a headache. The global availability of Cerezyme was again limited by, “a temporary decrease in Cerezyme yields”, as stated by Genzyme in a letter to US health care providers. ▶▶



Are plant-based expression systems as powerful as this giant carrot, found by Ryan in a local supermarket?

► A big player dangles and the dwarf is new in its role. Although Protalix would certainly have preferred to enter the market before Shire did, it may not be too late to come up with a new Gaucher treatment. With its FDA approval in May 2012, Elelyso became the third available enzyme substitution therapy for Gaucher disease.

... and another as a partner

But Protalix does not have to stem the worldwide marketing of Elelyso alone. Another giant named Pfizer was interested in a piece of the cake. Protalix and Pfizer agreed on a commercial partnership in 2009. Pfizer already completed a nearly €50 million up-front payment to Protalix followed by a €20 million milestone payment in June. Revenue and expenses for the drug will be shared on a 60 percent (Pfizer) to 40 percent (Protalix) basis.

Protalix kept a putative ace up its sleeve, however. They did not licence the marketing rights of Elelyso in their home country to Pfizer but kept exclusive rights for Israel. Gaucher disease is of special interest in Israel because of the frequency the genetic disease occurs among the Jewish population. Worldwide it affects approximately one in every 75,000 births but in Ashkenazi Jews, which compose 80 percent of the Jewish population worldwide, the incidence of Gaucher disease type I is about one in every 950 people.

The approval of Elelyso had a history of three years. The FDA denied the application in the first instance asking the company for additional clinical data. Finally, the reapplication was successful. The crucial factor for the approval of Elelyso was a phase III trial in which 32 Gaucher patients were randomised to two groups each treated with a different dose of the drug. The primary efficacy end point – a reduction in spleen volume – was reached with both of the doses. Moreover, Elelyso was shown to be safe (Zimran *et al.*, *Blood* 2011, 118(22):5767-73). According to the company, interim results of a switchover trial, in which the medication of patients was switched from Cerezyme to Elelyso, would indicate the possibility of safely switching to the Protalix drug.

But do patients have the motivation to switch their existing treatment? From a financial point of view, probably not. Out-of-pocket drug-related expenses like co-payments or the cost of co-insurance

can be covered by assistant programmes offered by Genzyme and Shire to US patients. Although the new competitor Pfizer also provides such a programme, the number of patients that switch from a working Cerezyme or Vpriv therapy to Elelyso will likely be limited.

Competition for customers

Three drug makers have to share the cake and the smallest piece will probably go to Protalix, simply because they were late to the table. However, new pieces of cake can be generated by the recruitment of previously untreated patients through improvements in the diagnosis of Gaucher disease. Protalix and Pfizer have the approximately 600 Brazilian and 400 Australian patients in their sights. Regarding Brazil, Pfizer has

already entered into a supply agreement with the Brazilian government for Elelyso. Prior to that, Protalix and Pfizer have had to wait for marketing authorisation to sell Elelyso in Israel, Europe, Australia and Brazil.

Elelyso is a Protalix top product but not the only one. The small company has three compounds in preclinical development and one, the recombinant human acetylcholinesterase PRX-105, recently completed a phase I clinical trial. The drug

can be used as a biodefense agent, since it is designed to bind poisons like nerve gas before they cause neurological damage. Like all Protalix drug candidates, PRX-105 is produced in plant cells. Furthermore, an orally available glucocerebrosidase recently reached preclinical testing. The idea is to produce a juice of lyophilised transgenic plant cells containing the therapeutic enzyme. Within the intestine, the drug would be released from the plant cells to reach target cells via the bloodstream. “Here could be a big advantage of the production system of Protalix since carrot cells are already established as food”, noted Schillberg.

Benefits of pharming?

In an interview with *Bloomberg*, Protalix Chief Executive Officer David Aviezer called drugs made by plant cells a, “new generation of therapeutic proteins.” Really? What

are the advantages of the plant system in comparison to microorganisms, animal or human cells?

“First of all, it depends on the particular protein. In case of Elelyso, the glycosylation was a main factor” says Schillberg. Different groups of organisms add different sugar chains to their proteins. In order to be effective in patients, Elelyso’s oligosaccharide chains need to terminate with mannose residues. Mannose is recognised by receptors on macrophages determining a signal for subsequent drug uptake. Complex sugar chains of proteins made by mammalian cells do not usually terminate with mannose residues. Genzyme’s Cerezyme, which is produced in hamster cells, needs additional *in vitro* modification steps to remodel its sugar chains. Since carrot cells produce a glycosylation pattern as desired, Protalix can circumvent this expensive protein remodelling. That makes Elelyso 25 percent less expensive than its competitor Cerezyme.

“About 80 percent of the total costs of a protein drug typically do not result from the production *per se* but from additional steps like isolation, purification and modification”, explains Schillberg.

Which plant-made drugs might we expect in the near future? Schillberg believes that, “the plant-process may become particularly important for the production of vaccines. Here we need sufficient amounts in a relatively short time, which is possible in plants because of the rapid production process.”

Looking ahead

One day, insulin producing *E. coli* may go into retirement, leaving the field to genetically modified safflower plants. The system was developed by the Canadian company Sembiosys. Although their plant-made insulin has already been successfully tested in clinical trials, the company went bankrupt. New money came last year from the Chinese company Tasly Pharmaceuticals through a joint venture resulting in the transfer of the know how to China. A few months later, Sembiosys closed its doors in Canada and no longer operates as a company.

“Since China invests a lot of money into biotechnology, I can image that Chinese safflower insulin could become the second plant-made drug in the world”, says Schillberg. And so, Chinese insulin-producing *E. coli* might become the first transgenic bacteria to be retired.

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Frauenhofer researcher, Stefan Schillberg, worked for more than ten years with plant expression systems. He believes that plants might become important for vaccine production, too.