Pharmaceutical BIOPROCESSING

Molecular pharming in plants and plant cell cultures: a great future ahead?

Keywords: biobetter • biopharmaceutical • bioreactor • emergency vaccine • molecular farming • plant biotechnology • plant cell culture • recombinant protein • transgenic • transient expression

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Plant biotechnology may not be a familiar concept to the general public, but it is a rapidly developing field of research that involves the use of plants, plant tissues and plant cell cultures to make or modify products and processes. The versatility of plants and plant cells can be harnessed to produce diverse products, including valuable proteins. This is often described as 'molecular farming' and it requires the introduction of foreign DNA into plants or plant cells, turning them into factories for the production of specific recombinant protein products. The term 'molecular pharming' is often used instead to highlight the production of protein-based biopharmaceuticals, which contributes to the sustainable production of drugs that promote human and animal wellbeing. Both terms also apply to the production of valuable secondary metabolites such as the anticancer drugs paclitaxel, vincristine and vinblastine, but we will focus on recombinant proteins and their use as biopharmaceuticals in this article.

The biopharmaceutical markets have expanded rapidly over the last 20 years, and are projected to more than double in volume over the next decade from US$200 billion in 2013 to at least US$500 billion in 2020. The two major biopharmaceutical production systems are microbes (mainly *Escherichia coli* and yeast) and mammalian cells such as the Chinese hamster ovary platform. In both cases, productivity has increased substantially over the last decade due to process optimization, platform standardization and genetic improvements. Both the US FDA and European Medicines Agency are familiar with these systems, and standard protocols can be followed to ensure the approval of new products. However, equivalent protocols are only just emerging for plant-based production systems, and only one plant-derived biopharmaceutical protein is currently on the market. With their established production infrastructure and regulatory framework, microbial and mammalian production systems have raced far ahead of their plant-based counterparts. No company will change their production host without a clear economic benefit, nor will they consider plants and plant cells for new products if there is no advantage over their incumbent technology. Furthermore, new companies will not base their manufacturing on a second-best option. Therefore, plant-based systems must begin to compete head-to-head with the established systems and, on a technological basis, we can already identify the areas where plant-based systems have the advantage, namely in terms of speed, improved product quality and scalability.

The international success story of molecular pharming began in 2006 with the US Department of Agriculture approval of a poultry vaccine against Newcastle disease developed by Dow AgroSciences (IN, USA) [1,2]. The vaccine was manufactured in transgenic tobacco cell suspension cultures and was a benchmark for the regulatory acceptance of plants as a manufacturing platform,

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although ultimately, the product was not marketed because the company withdrew from animal vaccine research. Currently, the brightest star in the molecular pharming sky is Elelyso™ (taliglucerase alfa) produced in carrot cells by the Israeli company Protalix Biotherapeutics (Carmiel, Israel) [2] and licensed to Pfizer Inc. (NY, USA). This is a recombinant form of the human enzyme glucocerebrosidase, which is used for the treatment of the lysosomal storage disorder Gaucher disease. The recombinant product met the primary end points in successful Phase III clinical trials in September 2009 and gained FDA approval in 2012. The product is currently on the US and Israeli markets, but the European Medicines Agency granted 10-year European marketing exclusivity to another product in 2010 and thus Elelyso cannot receive approval for Gaucher disease until 2020. Elelyso has a longer serum half-life than its Chinese hamster ovary-derived counterpart Cerezyme® (Genzyme, MA, USA) [3,4] and is produced by targeting the protein to the plant vacuole, which exposes terminal mannose residues on the glycan chains that are required for receptor binding. This avoids the need to trim the terminal sugars *in vitro*, which is part of the production process for Cerezyme. The long serum half-life has a strong impact on patient compliance because fewer doses are required. In addition, the disposable bioreactor production platform ProCellEx® (Protalix Biotherapeutics) can easily be scaled up to address market needs, and thousands of liters of cell suspension culture can be harvested weekly. From the traceability and cross-contamination point of view, disposable bioreactors are the best option for biopharmaceutical production.

Several plant-derived biopharmaceutical products are currently undergoing clinical trials. The EU FP6-funded academic consortium Pharma-Planta [5] pioneered the regulatory process for the entire European molecular pharming community by taking a tobacco-derived HIV-neutralizing monoclonal antibody from initial vector construction and gene transfer through all phases of development and manufacturing to launch a Phase I clinical trials, which concluded in 2011. The consortium worked closely with EU and national regulatory authorities to ensure the safety of the antibody and promote the acceptance of plant-based production platforms. In January 2014, a collaboration between Icon Genetics GmbH (Halle, Germany) [6] and Bayer Innovation GmbH (Düsseldorf, Germany) [7] resulted in the completion of a Phase I clinical trial for a personalized plant-derived vaccine for the treatment of non-Hodgkin's lymphoma. These examples represent important milestones in the history of plant molecular pharming and open the floodgates for the clinical development of additional products in the future.

There are many different plant-based production systems in development and one can evaluate and select the most suitable system for a given target product to achieve the optimal characteristics. However, this has diluted efforts to establish a standardized regulatory process and may delay overall progress in the field and fulfilment of industrial standards. Nevertheless, none of the plant-based systems under development are ideal for all target molecules, so the parallel development of different platforms is advantageous for the progress of molecular pharming. The potential of different plant-based production platforms is discussed in more detail below.

The greatest advantage of intact plants that are stably transformed to produce a target protein is their unparalleled scalability. For biopharmaceutical products, manufacturing will probably be restricted to greenhouses and other closed environments to ensure product safety and batch-to-batch consistency when production is carried out under controlled conditions. For example, ORF Genetics (Kopavogur, Iceland) [8] uses barley plants grown in greenhouses to produce recombinant growth factors, cytokines and interleukins in the cereal seeds for research purposes. Similarly, the Canadian company SemBioSys (AB, Canada) developed a safflower-based production system for insulin and completed Phase I/II clinical trials in 2009 before filing for bankruptcy in 2012. The SemBioSys platform was so efficient that theoretically 16 mid-sized Canadian farms could have produced enough insulin to meet the entire global demand. Although the current *E. coli* platform also meets this capacity, it is more expensive to establish in developing countries, which would therefore benefit most from the production of inexpensive medicines in plants.

The use of plant cell suspension cultures for molecular pharming is advantageous owing to the high product quality and scalability. Since the withdrawal of Dow AgroSciences from the animal vaccine market, Fraunhofer Institute for Molecular Biology and Applied Ecology [9] has continued to develop the tobacco BY-2 cell platform in the context of the EU-FP7-funded project CoMoFarm [10] combined with orbitally shaken bioreactor technology from Kühner (Basel, Switzerland) [11]. The 200 l OrbShake device was used for the large-scale cultivation of 100-liter BY-2 cell suspension cultures, resulting in cell growth and target protein yield comparable to standard cultivation using shake flasks, thus achieving a several 100-fold scale up without loss of productivity [12]. VTT Technical Research Centre of Finland used

traditional microbial bioreactors to cultivate the same BY-2 cells at the 600 l scale [13]. The growth kinetics of BY-2 cells in bioreactors was again strikingly similar to shake flasks, although the productivity was variable. This must be addressed by generating monoclonal cultures to achieve batch-to-batch consistency sufficient for industrial standards [14].

Transient expression systems have been developed to complement stably-transformed transgenic plants, and they benefit from the rapid onset of recombinant protein production. This is particularly useful for rapid response situations, such as the production of vaccines against epidemic diseases or bioterrorist threats. The plant material is propagated before the introduction of foreign DNA, allowing plants to be grown in the open if necessary (although greenhouse conditions are preferred from the perspective of GMP). The plants are moved into contained, GMP-compliant facilities for protein production. The Defense Advanced Research Projects Agency program in the USA has invested substantially into the development of this manufacturing technology and, for example, Medicago [15] has the capacity to manufacture 10 million vaccine doses per month and their pandemic and seasonal influenza vaccines are currently undergoing Phase I/II clinical development, and Kentucky Bioprocessing [16] has successfully produced vaccine-grade recombinant proteins with their manufacturing capacity of several hundred kilograms per day.

In the future, perhaps the greatest promise of molecular pharming is the use of edible host tissues as a vehicle for recombinant proteins such as oral vaccines and prophylactic antibodies. For example, Protalix Biotherapeutics has completed the Phase I clinical testing of a carrot cell line expressing recombinant glucocerebrosidase, which is orally administered rather than injected, and thus preferable in terms of patient compliance. Other companies are developing products expressed in cereal seeds, thus benefiting from natural forms of bioencapsulation. As stated above, transient expression is emerging as the ideal strategy to deploy emergency vaccines, particularly for zoonotic diseases such as influenza, but molecular pharming in general appears to well suited for the deployment of veterinary vaccines, for example, by adding plants or plant tissues expressing vaccine antigens to animal feed. In large-scale industries such as fish and poultry farming, oral vaccination saves the labor and stress involved in isolating and manually injecting thousands of animals. The plant-based production of vaccines is also ideal for companion animals, offering a robust and cost-effective platform for this sector.

One perceived drawback that is often discussed in the context of molecular pharming is the presence of plant-specific glycans on recombinant human proteins. Although it is now accepted that plant glycans pose little risk of immunogenicity (e.g., Elelyso, discussed above, contains typical plant glycans but no adverse effects have been reported even after multiple injections) a raft of technologies has been developed to remove plant glycans and add human-like counterparts, which have facilitated the development of 'biobetter' products with customized glycan profiles that enhance their activities. A recent breakthrough is the use of mutant plants lacking plant-specific N-glycan residues but expressing 11 human proteins acting in different subcellular compartments at different stages of the glycosylation pathway, resulting in the synthesis of a highly complex mammalian oligosaccharide structure [17].

Conclusion

Molecular pharming provides a safe and sustainable platform for the production of valuable recombinant proteins. However, the economic prospects of this technology depend heavily on the selection of suitable target proteins that can be produced more rapidly, less expensively or at a higher quality in plants or plant cell cultures compared with established microbial and mammalian systems. If the targets and platforms are chosen with care, the forecast for molecular pharming is indeed very promising.

Information resources

The International Society for Plant Molecular Farming (ISPMF) was established in February 2014. Membership is open to everybody and the idea is to promote and support excellent research, scholarship and practice in the field of plant molecular pharming. We are happy to invite all readers of *Pharmaceutical Bioprocessing* to join us at the frontier of plant molecular pharming. The ISPMF has regular meetings; for more information, please visit the ISPMF website [18].

Financial & competing interest disclosure

A Ritala and ST Häkkinen are employees of VTT Technical Research Centre of Finland, and S Schillberg is an employee of Fraunhofer Institute for Molecular Biology and Applied Ecology and Honorary Professor at the Justus-Liebig University of Gießen (Germany). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

The authors wish to thank Richard M Twyman for manuscript editing which was funded by the European Union Seventh Framework Programme under the grant agreement no. 227420 (CoMoFarm).

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