Target Product Selection - Where Can Molecular Pharming Make the Difference?

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Abstract: Four major developments have taken place in the world of Molecular Pharming recently. In the USA, the DARPA initiative challenged plant biotechnology companies to develop strategies for the large-scale manufacture of influenza vaccines, resulting in a successful Phase I clinical trial; in Europe the Pharma-Planta academic consortium gained regulatory approval for a plant-derived monoclonal antibody and completed a first-in-human phase I clinical trial; the Dutch pharmaceutical company Synthon acquired the assets of Biolex Therapeutics, an established Molecular Pharming company with several clinical candidates produced in their proprietary LEX system based on aquatic plants; and finally, the Israeli biotechnology company Protalix Biotherapeutics won FDA approval for the commercial release of a recombinant form of the enzyme glucocerebrosidase produced in carrot cells, the first plant biotechnology-derived biopharmaceutical in the world approved for the market. Commercial momentum is gathering pace with additional candidates now undergoing or awaiting approval for phase III clinical trials. Filling the product pipeline is vital to establish commercial sustainability, and the selection of appropriate target products for Molecular Pharming will be a critical factor. An interesting feature of the four stories outlined above is that they span the use of very different platform technologies addressing different types of molecules which aim to satisfy distinct market demands. In each case, Molecular Pharming was an economically and technically suitable approach, but this decision-making process is not necessarily straightforward. Although the various technologies available to Molecular Pharming are broad ranging and flexible, competing technologies are better established, so there needs to be a compelling reason to move into plants. It is most unlikely that plant biotechnology will be the answer for the whole biologics field. In this article, we discuss the current plant biotechnology approaches that appear to hold the greatest promise and in doing so attempt to define the product areas that are most likely to benefit from different Molecular Pharming technologies.

Keywords: Plant biotechnology, monoclonal antibodies, vaccines, plants.

1. KEY MOLECULAR PHARMING PLATFORMS

Molecular Pharming is the production of recombinant pharmaceutical proteins using plant biotechnology [1]. The potential benefits of Molecular Pharming in terms of economy, scalability and safety have been extensively discussed for the last 20 years, backed up by a large number of studies showing that pharmaceutical proteins can be expressed efficiently in many different plant species using diverse expression strategies [1]. Many biotech start-ups have been launched to capitalize on Molecular Pharming, but commercial uptake has been disappointingly limited [2]. Contributory factors include the diversity of the available platform technologies leading to uncertainty about their comparative technical and commercial benefits for the production of different target proteins, and the difficulty inherent in developing a regulatory framework without a standardized platform.

The biopharmaceutical industry has concentrated on a small number of platforms, including the bacterium Escherichia coli, yeasts such as Pichia pastoris, and a small number of well-characterized mammalian cell lines, such as Chinese hamster ovary (CHO) cells. Despite their different origins, all the cells are grown in fermenters and the principles governing the creation, growth and storage of producer cell lines, and the extraction and purification of the pharmaceutical products, are conceptually similar. In contrast, Molecular Pharming embraces a number of overlapping technologies with different advantages and limitations, which can be selected to match the characteristics of the intended protein product. Transformed plant cells and unicellular plants are cultivated in fermentation vessels in much the same way as bacteria, yeasts and mammalian cells. Plant tissues such as root cultures derived by infecting plants with the root pathogen Agrobacterium rhizogenes can be maintained in a similar way (see Schillberg et al., this issue). But Molecular Pharming can also be implemented using whole plants. As well as a wide choice of plant species, several different transformation approaches can be chosen, including the stable integration of DNA into the nuclear genome or the plastid genome, and transient expression by infiltrating leaves with expression vectors based on Agrobacterium tumefaciens, plant viruses or hybrids containing desirable properties of both [3]. For these platforms, the plants can be cultivated in soil or in hydroponic solutions, and they may be grown in containment facilities such as growth chambers and greenhouses, or they can be grown in the open.

The recent successes in Molecular Pharming have come about by copying the industry lead and focusing on a smaller number of key platform technologies. The three leading technologies are currently the production of recombinant proteins in plant cell cultures, in nuclear transgenic plants and by transient expression in leafy plants. These three approaches exploit different benefits of plant-based systems, i.e. plant cell cultures are beneficial because the cells are grown in containment, the product can be secreted and the cell culture system does not support human pathogens, transgenic plants are beneficial because they allow production on a massive scale suitable for high-demand/low-margin products, and transient expression is beneficial because production is rapid and can be scaled up to produce vaccines in response to emerging threats.

2. PLANT CELL AND WHOLE PLANT CULTURES

The majority of commercial biologics are currently produced using mammalian cells because they can produce active and correctly-folded complex glycoproteins. Fermentation in contained vessels using a uniform cell culture can be controlled precisely, which makes it suitable for the manufacture of pharmaceutical proteins in compliance with good manufacturing practice (GMP). This has served as an attractive paradigm in plant biotechnology, and approaches have been developed that combine the advantages of...
plant cells (e.g. the absence of pathogens that infect humans) with the benefits of existing bioreactor technologies [4]. Plant cell cultures have been used for more than a decade for the commercial production of small molecule drugs such as paclitaxel, but the commercial use of plant cells to produce biologics is in its infancy. The small number of current case studies nevertheless provides important examples to attract future investors.

Plant cell bioreactors are typically less expensive to operate and scale-up than their mammalian counterparts due to the simple growth medium requirements of plant cells, but the same manufacturing practices used with mammalian cells (such as cryopreservation and the establishment of master and working cell banks) have now been adapted for use with plant cells [4]. As it is possible to harvest secreted product, there are advantages in terms of product homogeneity and downstream purification costs. However, there are several factors that need to be taken into consideration when matching a target product to this platform. The first is the relatively long pre-manufacturing phase compared to transient expression (see below), which involves the establishment and characterization of highly-productive cell lines. Although recent advances have streamlined the selection of the most productive cells in a heterogeneous population [5], this nevertheless limits the capacity of cell culture platforms to deliver products for dynamic and unpredictable markets, such as patient-specific therapies and pandemic vaccines. The second drawback is the relatively high costs compared to other plant-based platforms in terms of initial capital expenditure and process development. The most appropriate target proteins for plant cell cultures are therefore high-value products with a predictable and constant market demand.

Protalix Biotherapeutics, an Israeli company established in 1993, has developed a recombinant form of the enzyme glucocerebrosidase (pGCD / ELELYSO™) which has completed clinical trials and is the first plant-derived biologic to gain FDA approval as a new drug. Human glucocerebrosidase is a neutral 60 kDa enzyme involved in glycolipid metabolism, whose absence leads to the lysosomal storage disorder Gaucher’s disease, a frequently incapacitating condition for which the only treatment is continuous enzyme replacement therapy. Gaucher’s disease is generally considered an ‘orphan disease’, based on the relatively low frequency of the condition worldwide.

Human glucocerebrosidase has previously been marketed by Genzyme (Cerezyme™) and Shire (Vupri®). Both manufacturers use mammalian cell cultures and the recombinant enzyme requires post-extraction modification in vitro before formulation. This is because the uptake of human glucocerebrosidase into target cells (primarily macrophages) is dependent on the correct processing of glycans on four typically-occupied acceptor sites [6]. Pauclamannosidic glycans are substrates for the mannose receptors expressed by macrophages, whereas the heterologous complex or high-mannose glycans formed in mammalian cell cultures do not display the correctly-linked mannose moieties required for binding, hence the need for enzymatic trimming in vitro. In contrast, Protalix have taken advantage of the well-characterized protein trafficking pathways in plants and targeted their version of the enzyme to the vacuole, producing a homogenous population of pauclamannosidic glycans. The resulting product does not require trimming before use which reduces the cost of downstream processing, but is as safe and efficacious as its (processed) mammalian counterpart [7,8].

Biolex Therapeutics (Pittsboro, NC, USA) has also focused on delivering products with superior clinical efficacy compared to existing treatments on the market using a production system based on the aquatic plant Lemna minor, which is commercially known as the LEX platform. The lead product candidate is locteron, a recombinant form of the human cytokine interferon-02b. This has proven difficult to produce in an active form using other production systems. Yields in bacteria are low because interferons tend not to fold correctly in the cytoplasmic environment, whereas the biological activity of interferons interferes with their production in mammalian cells [9]. Plants represent an ideal alternative because they fold and assemble mammalian proteins efficiently and interferons have no biological impact. In the LEX system, Biolex claim a minimum yield of 7% of total soluble protein when the protein is directed to the apoplast [10]. In addition to high yields, Biolex have coupled the active pharmaceutical ingredient with a proprietary sustained release formulation and have recently completed Phase II clinical trials demonstrating improved tolerability with reduced treatment cycles compared with equivalent drugs already on the market. Other Biolex product candidates include antibodies that are ‘glyco-optimized’ through the use of specialized Lemna strains that lack the ability to synthesize complex glycans. A human CD20-specific antibody produced in these engineered plants carries high-mannose glycans that are common to all eukaryotic glycoproteins before processing in the Golgi body, and these antibodies bind the human Fc receptor with ten times the affinity of a comparable antibody produced in mammalian cells and also display enhanced cell-mediated cytotoxicity [11]. Recently, the LEX platform developed by Biolex has been acquired by Synthon (Nijmegen, NL), a development which represents a substantial vote of confidence in plant cell manufacturing by the pharmaceutical generics industry.

Major pharmaceutical companies have shown limited interest in Molecular Pharming using plant cell cultures so far, perhaps because they are satisfied with the much higher yields that can be achieved with microbes and mammalian cells. However, Dow AgroSciences (Indianapolis, USA) did enter the field by producing a subunit vaccine against Newcastle disease in poultry, manufactured using transgenic tobacco cells, specifically the cell line NT-1. These cells were cultivated in a traditional reusable bioreactor in what became known as the Concert platform [12]. No attempt has been made to commercialize this product despite regulatory approval being granted by the USDA in 2006 [13]. This early venture may have failed to achieve commercial success due to inadequate product yields (8 µg/ml culture) [12] but it was nevertheless an important breakthrough in terms of proof-of-principle and regulatory acceptance. Development of further products using the Concert platform were envisaged (including a vaccine for “shipping fever,” a common respiratory infection in cattle) but no further progress has been reported.

A common characteristic among many of the lead products produced by Molecular Pharming in plant cells is their exploitation of post-translational modification (usually glycosylation) as a differentiator and unique selling point. The capability to control glycan structures exists because plant glycans were initially regarded as a significant drawback that might affect the activity, stability and immunogenicity of plant-derived pharmaceuticals. This led to efforts in many different plant-based platforms to eliminate plant-type glycans and humanize the glycans on recombinant glycoproteins [15]. In hindsight, much of the anxiety over the impact of plant glycans was unwarranted and there is little evidence for significant detrimental effects, but one positive outcome was the innovative development of glycan control technology which now provides a platform to develop glyco-engineered protein variants with superior properties that cannot be produced in mammalian cells. For example, the German biotechnology company Grevenovation GmbH has developed a photosynthetically-active production platform based on the moss Physcomitrella patens that exploits the efficiency of homologous recombination in this species to introduce genetic modules that influence the glycosylation patterns of recombinant proteins [16]. Work by other groups in transient expression systems has demonstrated that it is possible to recapitulate certain features of mammalian glycosylation in the model tobacco species N. benthamiana. Such precise control of glycosylation potentially offers real advantages over mammalian bioreactors for target proteins that rely on specific glycosylation profiles, particularly the degree of sialylation because these highly-charged residues have a
significant impact on the serum half-life of many therapeutic proteins, including erythropoietin, follicle-stimulating hormone and thyroid-stimulating hormone [17-19].

The addition of glycans to the hydroxyl group of hydroxyproline residues (hyp-O-glycosylation) is a process unique to plant cells, which could also be exploited to develop superior versions of pharmaceutical proteins. For example, covalently attached tags that induce arabinogalactan-type hyp-O-glycosylation have recently been found to increase the yield of interferon-c2 in tobacco BY-2 cells, in addition to increasing the serum half-life of the purified protein [20].

The use of plant cells and whole plant cultures presents a number of unique opportunities for innovation to reduce process costs, including the potential elimination of certain steps in the manufacturing process chiefly due to the inability of plants to propagate mammalian-tropic viruses. Removing the need for virus clearance and inactivation would significantly reduce the operating costs compared to mammalian cell systems, and would increase the throughput of plant cell culture manufacturing, opening the technology to a range of lower-value biopharmaceuticals such as passive immunotherapeutic antibodies and microbial prophylactics. Costs can also be reduced by using larger-scale bioreactors. Phyton Biotech currently operate using conventional stirred-tank bioreactors with a capacity of up to 75,000 liters, whereas Protalix use disposable 400-liter bubble-column type bioreactors made from flexible plastic (see Schillberg et al., this issue). Wave reactor systems are particularly suitable for photosynthetic cultures such as algae or moss, which require consistent illumination. Although culture medium requirements vary between systems, particularly between de-differentiated cells and organized tissues, there is certainly room to improve media compositions and cultivation regimes. For example, high-nitrate medium has recently been found to boost the yield of recombinant proteins in BY-2 cultures between 10 and 20-fold [21].

Plant cell cultures are promising as an emerging platform because they share the benefits of other fermenter systems (containment, process control, recovery of secreted products to simplify purification) while also providing unique benefits (absence of endotoxins and human pathogens, simple medium requirements, propensity for glycan modification) and they now have a regulatory pedigree by yielding two approved products, one for animal use and one for human use. There is a significant opportunity for innovation with this platform, both in terms of biological diversity (suspension cells, root cultures, moss, algae, aquatic plants) and technological potential (strain improvement, medium optimization, novel bioreactor formats). The development of additional products and movement within the field to a small number of well-characterized production systems will increase the industrial value of this emerging technology.

3. TRANSIENT EXPRESSION PLATFORMS

Transient expression is a phenomenon that occurs when genes are introduced into plant tissues and are expressed for a short period without stable DNA integration into the genome [22,23]. All gene transfer processes lead to transient expression, and in each case the vector carrying the transgene (a plasmid in the case of particle bombardment, a T-DNA sequence in the case of Agrobacterium-mediated transformation and a chimeric virus genome in the case of viral transduction) remains episomal for a variable period, until it is degraded. The only requirement to achieve transient expression is that the transgene is housed in a functional expression construct, minimally consisting of a promoter and polyadenylation sequence flanking the coding region, but often containing additional sequences designed to boost transgene expression or target the protein to a specific subcellular compartment (see Twyman et al., this issue).

Many transient expression platforms are based on partial or complete plant virus genomes because these carry sequences that promote transgene expression, vector replication and/or systemic spreading, thus increasing yields. The potential of geminiviral vectors has been investigated [24] but extremely high-yielding platforms have been developed using RNA viruses [25,26]. Commercial development has focused on hybrid systems that incorporate components of the T-DNA transfer system and virus replication functions. For example, the iBioLaunch platform from iBio Inc. (Newark, USA) is a self-replicating vector based on Tobacco mosaic virus (TMV) which is integrated into the typical binary plasmid system utilized by A. tumefaciens. The bacterium achieves the initial infection of plant cells, and the viral components then promote cell-to-cell spreading and the amplification of the initial transcript [27]. The magnICON system developed by Icon Genetics (formerly owned by Bayer Innovation, Dusseldorf, Germany; now a subsidiary of Nomad Bioscience, Halle, Germany) also features a deconstructed TMV genome [28]. Separate pro-vectors recombine in planta with help from a bacterial integrase supplied in trans, and the viral RNA-dependent RNA polymerase then amplifies the initial mRNA.

One of the advantages of transient expression strategies is that they can be used to produce two or more different gene products at the same time. This is achieved using multi-expression cassettes in the iBioLaunch platform, and also the Proficia platform developed by Medicago Inc. (Quebec City, Canada) for transient expression in alfalfa [29]. These vectors can be used to produce different subunits of multimeric protein, to co-express a protein product along with an inhibitor of post-transcriptional gene silencing, or to co-express a protein with enzymes that modify the glycan structures in planta [30,31]. Multimeric proteins such as antibodies can also be produced by co-infecting plants with non-competing vectors derived from different viruses, such as TMV and Potato virus X (PVX) [32]. Multimeric proteins are exceptional, e.g. up to 4 g/kg fresh weight (30% total soluble protein) for green fluorescent protein using the magnICON system [20].

In planta with help from a bacterial integrase supplied in trans, the recombinant bacteria carrying vectors for transient expression can be introduced into plants using a needle-less syringe [33], by wounding [34,35] or by soil inoculation [36], but vacuum infiltration following the immersion of leaves into a bacterial suspension remains the preferred method because it can be scaled up effectively and the entire process can be carried out in a GMP environment [23]. Icon Genetics, Medicago and iBio Inc. all use automated vacuum infiltration in their production line [29,37,38]. In the case of Medicago, their pilot plant in Quebec City has the capacity to infiltrate 1200 plants per day using computer-controlled vacuum infiltration and the automated transport of plants to and from the infiltration unit, whereas their new facility in North Carolina will have the capacity to infiltrate 15,000 plants per day and manufacture 10 million doses of pandemic influenza vaccine per month [39]. Large-scale transient expression could also be implemented in open fields using a process called Spray N’ Trait developed by Nomad Bioscience, which involves spraying the aerial parts of field-grown plants with a bacterial suspension containing an abrasive and an agricultural spray adjuvant [40]. This could circumvent the technical and economic barriers currently limiting the use of vacuum infiltration and transient expression to the production of high-margin recombinant pharmaceutical products by removing the need for contained indoor facilities and growth rooms [41,42].

Transient expression platforms are commercially advantageous despite the absence of a permanent genetic resource because the production timelines are much shorter than cell cultures (measured in days rather than months from first gene transfer) and the yields are exceptional, e.g. up to 4 g/kg fresh weight (30% total soluble protein) for green fluorescent protein using the magnICON system [28]. The absence of human pathogens is an advantage shared by all plant-based platforms, but this is a particular strength in the case of transient expression systems because they may be called upon to supply emergency vaccines in response to a pandemic or terrorism threat, where the shutdown of a mammalian cell fermentation facil-
ity caused by contamination (e.g. [43,44]) would leave the target population extremely vulnerable.

The ability to manufacture large quantities of protein in a short time and to rapidly scale up the manufacturing process makes transient expression technologies ideally suited to meet the surge in capacity required to manufacture vaccines for emerging infectious diseases. During the 2009-2010 H1N1 pandemic, supply-chain analysis showed that vaccines would only become available in large amounts after the peak of viral infection and only in high-income countries [45] because of the lead time of 4-6 months required for traditional egg-based production and the dependence on a reliable supply of embryonated eggs [46]. Plant-based transient expression systems could reduce these lead times and produce large quantities of vaccines in the critical period before egg-based manufacturing reaches the necessary capacity. During the H1N1 pandemic, Medi- cago evaluated the capacity of their Proficia system to manufacture influenza vaccines, and found that the first batches of H1N1 virus-like particles (VLPs) could be produced three weeks after the Centers for Disease Control and Prevention released the new influenza hemagglutinin (HA) sequence [29]. Similar lead times were reported for the H5N1 VLP vaccine [47]. The yields of H1N1 and H5N1 VLP were up to 50 mg/kg fresh weight [29,47]. More recently, Medicago has announced positive results from the Canadian phase II clinical trial of this H5N1 vaccine [48] and the US phase I clinical trial of the H1N1 seasonal vaccine, with further plans to proceed to phase IIa clinical trials of their seasonal trivalent vaccine [30].

In collaboration with the Fraunhofer Centre for Molecular Biotechnology, iBio Inc. has investigated the potential of their iBioLaunch platform to produce pandemic and seasonal influenza vaccines, and have successfully produced the H3N2 strain HA with a yield of ~200 mg/kg fresh weight [50]. The subunit was also expressed as a fusion protein with lichenase B polypeptides (LicKM) which act as adjuvants (trade name iBioModulator) [51] with a yield of ~100 mg/kg fresh weight. This elicited HA-specific immune responses in ferret challenge studies [52]. A truncated, plant-codon optimized H5N1 HA subunit (HAI-05) was produced with a yield of ~60 mg/kg fresh at the 1-kg manufacturing scale and 50 mg/kg at the 5-kg and 50-kg manufacturing scales [53]. Phase I clinical trials of the H5N1 product began recruiting towards the end of 2010 [54]. The H1N1 HAC1 subunit was produced with a yield of ~90 mg/kg fresh weight at the 5-kg and 50-kg manufacturing scales [53] and a phase I clinical trial sponsored by DARPA showed that the product was safe and well tolerated at all dose levels [55].

In 2011, iBio Inc. announced the successful production of NaAPR1M-74, a hockworm-derived vaccine antigen, by transient expression in tobacco [56]. It has proven impossible to produce this protein efficiently using microbes or mammalian cells, but the partially purified plant-derived vaccine candidate is now undergoing final purification steps at the Walter Reed Army Institute for Research (WRAIR) to generate enough material for evaluation in phase I clinical trials. Furthermore, the company has also announced the successful expression of an anti-inflammatory neumair-midase monoclonal antibody with a yield of ~400 mg/kg fresh weight, which is broadly active against Tamiflu-resistant influenza virus strains [57]. Other reported vaccine candidates produced by transient expression include an antibody-based vaccine against malaria, fused to LicKM, which blocks the transmission of Plasmodium falciparum, produced with a yield of 800 mg/kg fresh weight [58]; a Human papillomavirus HPV16 E7 subunit vaccine, also fused to LicKM, produced with a yield of ~100 mg/kg fresh weight [59-61]; and a non-glycosylated monoclonal antibody against anthrax, produced with a yield of up to 250 mg/kg fresh weight, which provides better protection than glycosylated forms against respiratory anthrax in a non-human primate model [43, 44]. These examples demonstrate the flexibility of the transient production platform and provide an indication that complex recombinant subunit vaccines might be the most promising future direction for these technologies.

The speed and scalability of transient expression platforms are ideal not only for rapid response situations, but also for the development of personalized medicines. The now-defunct Large Scale Biology Corporation (Vacaville, USA) produced patient-specific idiotypic vaccines for the treatment of non-Hodgkins lymphoma based on single-chain variable antibody fragments produced at levels of up to 30 mg/kg fresh weight using their TMV-based vector Geneware [64]. The antibodies were shown to confer protection to vaccinated mice in tumor challenge studies [35] and were well-tolerated by patients immunized with personalized idiotypic vaccines derived from their own tumors in phase I clinical trials, which also revealed that the majority of patients developed cellular or humoral immune responses [64]. Icon Genetics, along with its former parent company Bayer Innovation, has continued the idiotype vaccine project by using non-competing virus-based vectors to produce full-length idiotype-containing antibody vaccines for the treatment of 20 patients. The full length antibodies were produced in tobacco plants at high yields (sufficient to provide the 225 mg of product required for quality control and the treatment of each patient) within two weeks of cloning of the antigenic sequence [65]. The FDA has approved these vaccines for phase I clinical trials [66].

Finally, transient expression platforms may also be ideal for another niche market, which is the production of orphan drugs for the treatment of rare diseases. For example, the iBioLaunch system has been used successfully to express the C1 esterase inhibitor, which is indicated for the treatment of hereditary angiodema, and α1-antitrypsin, which is indicated for the treatment of emphysema caused by α1-antitrypsin deficiency [67]. The company has also produced human α-galactosidase, which is indicated as an enzyme replacement therapy for Fabry’s disease, as part of a program to commercialize ‘biosimilar’ or ‘biobetter’ pharmaceuticals [68].

Transient expression platforms are exciting because they are extremely rapid and scalable, which is important at both ends of the market. On one hand, they are suitable for the rapid production of large amounts of vaccines to deal with an emerging pandemic or bioterrorism threat. Here, they are advantageous over established platforms because they have the capacity and response time to provide vaccines within weeks of a threat emerging, rather than the several months it would take for a conventional response. At the other end of the scale they are economical for the production of pharmaceuticals for very small markets, such as orphan diseases and individualized therapies.

4. PLATFORMS BASED ON TRANSGENIC PLANTS

In the early years of Molecular Pharming, the most widely used strategy for recombinant protein expression was the stable introduction of expression constructs into the nuclear genome to create stably transformed plant lines. This approach is advantageous because transformation is a straightforward procedure in many domesticated crops. It is usually carried out by one of two methods, namely Agrobacterium-mediat ed transformation or particle bombardment with DNA-coated gold beads. Once a transgenic plant line has been recovered it is a permanent genetic resource, in contrast to transient expression platforms where each round of production requires a gene transfer step. Transgenic plants are ultimately the most scalable of all the Molecular Pharming platforms, because the line can be used to produce seeds, which bulk up the number of plants in each generation. The seeds can also be used to establish master and working seed banks, which serve the same purpose as master and working cell banks in cell culture platforms. The major disadvantages of transgenic plants are the long lead times for development and scale-up, the unreliable yields of recombinant protein and concerns about the spread of pharmaceutical crops in the environment and into the food chain by outcrossing and seed dispersal [1-3]. As
with the other major platforms discussed above, the strengths and weaknesses of transgenic plants should act as a guide when it comes to selecting a target product.

The diversity of transgenic platforms is limited only by the number of different plant species in the world, but the benefits of three major types of crops have been recognized. The first type of system consists of leafy crops that generate a large amount of biomass, the prime example being tobacco (Nicotiana tabacum). This species produces 1-100 tons of leaf biomass per hectare per year depending on the cultivar and cropping method, and since it is neither a food nor a feed crop there is a low risk that pharmaceutical crops will enter the food/feed chain. Tobacco is also a favorite laboratory model which means that transformation, regeneration and transgene expression are well-characterized and efficient. Because one of the principal reasons for choosing transgenic plants over the other platforms is their overall scalability, the combined advantages of high biomass yield and a long successful history expressing a vast catalog of recombinant pharmaceutical proteins makes tobacco one of the leading platforms for commercial Molecular Pharming [3].

One of the drawbacks of leafy crops is that leaf tissue is watery and proteins degrade rapidly, so cropped tobacco leaves must either be processed immediately or frozen to ensure the recombinant product remains stable. Where this is not possible, cereal and legume crops can be considered as an alternative because they have large, protein-rich seeds, and recombinant proteins accumulating in the storage tissues (e.g. the endosperm in cereal seeds) are maintained in a stable environment and can often survive without significant degradation for months or even years when stored at ambient temperatures [69,70]. Several companies have explored the commercial prospects of Molecular Pharming in seeds, with varying degrees of success. Maize was developed as a platform by the now-defunct US company Prodigene Inc., which produced a range of seed-derived recombinant technical reagents including avidin and β-glucuronidase, which were the first commercial products of Molecular Pharming [71,72]. In choosing these targets, the company set out to demonstrate that Molecular Pharming was competitive even where the proteins could be regarded as commodities and where a market was already in place. Prodigene launched a range of maize-derived proteins which were marketed in collaboration with Sigma-Aldrich Fine Chemicals (St Louis, USA) until a breach of environmental regulations resulted in a huge fine and the company ceasing to trade. They were also working on various pharmaceutical products including antibodies and oral vaccines. Importantly, Prodigene were the first company to seriously investigate the economic impact of downstream processing in the context of Molecular Pharming, and developed a number of successful approaches to recover intact and functional recombinant proteins from maize seeds [73,74]. They also studied the impact of germplasm and breeding strategies on protein accumulation in the seed [75].

Rice and barley seeds have also been developed as commercial platforms, because unlike maize they are self-pollinating which should reduce the risk of outcrossing during field-based production. Ventria Bioscience (Fort Collins, USA) uses its proprietary Express-Tec platform in rice for the production of a range of recombinant pharmaceutical proteins including human albumin, transferrin, lactoferrin and lysozyme, and vaccines against rabies and Lyme disease. Its lead product (VEN100, whose active ingredient is lactoferrin) was safe and well-tolerated in a phase II clinical trial, where it significantly reduced the incidence of antibiotic-associated diarrhea in high-risk patients [76]. ORF Genetics is based in Iceland, and uses its ORFeus system to produce a portfolio of about 50 products in the endosperm of barley seeds, which are safe to grow in fields because barley is not cultivated in Iceland and there are no compatible weed species. Their product portfolio includes human growth hormone and various cytokines, which are currently licensed for diagnostic use, academic and private research, and in the case of growth hormone as a cosmetic additive (distributed by Sif Cosmetics, Iceland).

The final main category of transgenic crops in Molecular Pharming are fruits and vegetables, which are generally chosen when the product is envisaged as an oral vaccine that is sensitive to heat and which therefore cannot be cooked before consumption [77,78]. The ability to use plants for the delivery of oral vaccines is another niche advantage over conventional production platforms, and a number of vaccine antigens have been expressed in tissues such as tomato fruits, potato tubers and lettuce leaves for this purpose. Even so, it is now recognized that cereal seeds provide additional advantages over fruit and vegetable crops, particularly in terms of the long-term stability of vaccines and the bioencapsulation offered by cereal storage compartments, which delay the degradation of protein antigens in the gut and increase the contact time between antigens and immune effector cells (see Holbauer and Stoger, this issue).

The benefits of commercial Molecular Pharming in transgenic plants were considered as part of the EU Pharma-Planta project, in which eight target products were initially selected indicated for a range of diseases (HIV/AIDS, tuberculosis, rabies and diabetes) all characterized by a large affected population and therefore the need to produce the corresponding proteins inexpensively and on a massive scale (100 kg to 1 ton). Two HIV-neutralizing antibodies (2G12 and 2F5) were eventually chosen as fast-track product candidates that would be prioritized for production development. Both tobacco and maize plants were developed as platforms, tobacco because of the larger biomass yield and maize because the stability of the product would be useful in a developing country context. High yields of both antibodies were achieved in these two platforms and in tobacco BY-2 cells [79-81]. One of the key objectives of the project was to take a candidate pharmaceutical product from gene to clinic, defining the regulatory pathway along the way by consulting the appropriate authorities. This process was ultimately successful, resulting in the establishment of a GMP-compliant process for the production of 2G12 in transgenic tobacco leaves and the completion of a successful phase I clinical trial to demonstrate safety [82].

Transgenic platforms are promising because they are stable resources that are enormously scalable. This is vital for the production of pharmaceutical products required in bulk, particularly low-margin products and those targeted to under-developed regions. In this context, large scale also means production at levels previously unachievable for biologics, e.g. monoclonal antibodies on the ton scale. The production of an antibody-based HIV microbicide is a good example because it would be impossible to achieve using conventional fermenters due to the cost and the limited scale. As a microbicide component, the antibodies (like 2G12) must be applied in milligram amounts on a daily basis (and an effective microbicide ideally requires an antibody cocktail targeting two or more HIV epitopes). The only viable solution at present would be to produce the antibody on an agricultural scale, which can only be achieved using transgenic plants. This scalability also opens up exciting new horizons in disease prevention and treatment. If biologics like monoclonal antibodies can be made available in a way similar to chemical pharmaceutical entities, then this would open enormous new possibilities in a number of important medical fields, such as anti-microbials.

A final important consideration is the issue of access to global health. Of all the production platforms available to Molecular Pharming, perhaps transgenic plants alone offer the possibility to bring about a sea-change in how medicines are made available to the poor in under-developed countries. Transgenic plants offer the most cost-efficient form of Molecular Pharming, reflecting the economy of scale and the low-tech and inexpensive infrastructure. An additional advantage is that agricultural expertise is common-
place across the globe and pharmaceutical plants could therefore be transferred or “farmed out” readily. The use of seed-based expression systems would provide product stability in the absence of a cold chain so that a stockpile of unprocessed crude product could be accumulated and stored. If expression levels were sufficient, depending on specific applications, there is also important potential for minimal processing approaches where crude extracts could be used instead of highly purified and reformulated drug products. These include topical formulations, where established food processing technologies could be employed to process harvested plant material like seeds into a final formulation.

5. SUMMARY AND OUTLOOK

Molecular Pharming is a diverse field with a range of alternative production platforms offering different advantages and disadvantages. Three types of platform stand out as commercial leaders, albeit for different reasons: cell and whole plant cultures, transient expression and transgenic plants. This ability to offer different platforms that complement the characteristics of different products and their market dynamics may be considered one of the major strengths of the field.

Cell/tissue culture platforms are less expensive to set up and operate than comparable mammalian systems, but can handle complex multimeric proteins that cannot be expressed in bacteria. These platforms share the benefits of other culture systems in terms of containment and control, but also their drawbacks in terms of scalability. They also offer the unique benefit of glycan modification, which can produce ‘biobetter’ counterparts of pharmaceuticals produced in mammalian cells. They are best suited to the production of proteins with limited and predictable markets where glyco-optimization might provide a competitive edge, such as orphan drugs.

Transient expression platforms provide two major benefits - a rapid onset of production and rapid scalability. This makes them suitable for broad classes of products, i.e. subunit vaccines required quickly in large amounts, specialized products with a small market (e.g. orphan drugs and personalized medicines), and as a competing technology with current fermentation systems for products like monoclonal antibodies. Because transient expression systems are based on the repetitive use of recombinant microbes for gene transfer, they must be housed in specialized contained facilities and are therefore more suitable for the provision of high-margin pharmaceutical products.

Finally, transgenic plants provide the benefits of a permanent genetic resource, high yields and virtually unlimited scalability, although with a longer run-up period than transient systems. The availability of diverse leafy crops, seeds, fruits and vegetables allows a wide choice of platforms tailored to the requirements of different products, markets and manufacturing regions. Transgenic plants are best suited for the production of very high-volume and low-margin products, and are still the best system for the simultaneous expression of multiple polypeptides (e.g. antibodies). The use of seeds has several benefits in terms of storage and transport stability, oral vaccination and minimal processing, making them the best choice for resource-poor areas. They are ideal for the production of antibodies, complex vaccines including multi-component products and other ‘commodity’ pharmaceuticals.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Target Product Selection - Where Can Molecular


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