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GMP issues for recombinant plant-derived pharmaceutical proteins

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ABSTRACT

Recombinant proteins can be produced in a diverse array of plant-based systems, ranging from whole plants growing in the soil to plant suspension cells growing in a fully-defined synthetic medium in a bioreactor. When the recombinant proteins are intended for medical use (plant-derived pharmaceutical proteins, PDPs) they fall under the same regulatory guidelines for manufacturing that cover drugs from all other sources, and when such proteins enter clinical development this includes the requirement for production according to good manufacturing practice (GMP). In principle, the well-characterized GMP regulations that apply to pharmaceutical proteins produced in bacteria and mammalian cells are directly transferrable to plants. In practice, the cell-specific terminology and the requirement for a contained, sterile environment mean that only plant cells in a bioreactor fully meet the original GMP criteria. Significant changes are required to adapt these regulations for proteins produced in whole-plant systems and it is only recently that the first GMP-compliant production processes using plants have been delivered.

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1. Introduction

Plants produce a vast array of pharmacologically active products that have been used for thousands of years to prevent and cure diseases. However, in the last 15 years it has also become possible to use plants as heterologous expression platforms for recombinant proteins, including native and modified therapeutic proteins from humans (Ma et al., 2003; Twyman et al., 2005). The ability of plants to express human genes was established when Barta et al. (1986) showed that undifferentiated tobacco and sunflower callus tissue could produce transcripts of a human growth hormone fusion gene, although in this case no protein was detected. The first potentially therapeutic proteins expressed in plants were human serum albumin expressed in tobacco and potato leaves and suspension cells (Sijmons et al., 1990), and a monoclonal antibody that was expressed in tobacco leaves (Hiatt et al., 1989). From these pioneering studies emerged the concept of molecular farming, the production of valuable recombinant proteins in plants and plant cells (Schillberg et al., 2003).

Molecular farming gained support and interest within the plant biotechnology community because plants have several advantages over traditional platforms for recombinant protein production. Plants are inexpensive, they are highly scalable and they do not support

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human pathogens. Crops producing pharmaceutical proteins can be established with minimal upfront investment in infrastructure, unlike the major fermentation-based platforms. Researchers also embraced the immense diversity offered by different plant species and platforms (Twyman et al., 2003). There was a flurry of commercial activity in the 1990s based on these potential advantages, leading to unrealistic assumptions that plants might challenge the industry gold standards such as CHO cells and persuade drug developers and manufacturers to switch platforms. This did not happen for several reasons. As well as the huge existing investment in fermentation infrastructure, the main explanations for the lack of industrial interest were the technical limitations of plants (they did not perform anywhere near as well as the best mammalian cell lines) and the lack of regulations governing the use of plant systems for the manufacture of biopharmaceutical products (Spok et al., 2008).

2. Overcoming technical limitations

Research in the last decade has eliminated many of the technical hurdles that burst the initial molecular farming bubble, particularly the challenges of low yields, low recovery during processing and the potential impact of plant-specific glycans. In overcoming these limitations, researchers came up against an additional hurdle, which was the lack of support for translational research, clinical development and manufacturing according to GMP (Whaley et al., 2011; Yusibov et al., 2011).

2.1. Upstream production — intrinsic yield improvement

The yield problem has been addressed by developing new technical approaches to increase the expression of genes introduced into plants and to increase the stability of recombinant proteins accumulating in or secreted from plant tissues. The commercial and economic success of any plant-based production platform for recombinant proteins depends on the product yield, since the costs of upstream production relate to the amount of biomass produced, and the greater the amount of product per unit of biomass the better. The recombinant protein itself plays an important role in determining the yield. Some proteins will always be easier to produce commercially than others because of their intrinsic stability. In general terms, yields can be improved by designing optimal expression constructs to maximize transcription, mRNA stability and protein synthesis, increasing the transgene copy number and introducing transgenes into germplasm that is best suited for high-level expression (reviewed by Desai et al., 2010). In each case, however, an additional regulatory burden is introduced. In clinical development, the origins, purpose and safety of all components of the expression vector must be documented and explained, and there are also requirements to demonstrate the safety of the plant species and the impact of the transgene insertion event. This is a general regulatory issue affecting genetically modified plants, but there seems to be much less scrutiny of the same factors in, for example, CHO cells. The focus on transgene insertion as a regulatory issue has driven researchers to look for less contentious approaches for yield improvement which do not involve nuclear transformation. The most successful approaches thus far include the use of transient expression platforms based on *Agrobacterium tumefaciens* and/or plant viruses (Giritch et al., 2006; Huang et al., 2010; Pogue et al., 2010; Sainsbury and Lomonossoff, 2008; Vézina et al., 2009), and the development of plastid transformation systems although the latter is only applicable in a small number of species (Bock, 2007).

The subcellular localization of a protein contributes to its stability, so further yield improvements have been achieved by adding targeting sequences, such as a signal peptide to allow secretion (into the medium for plant cells, or to the apoplast in whole plants), a signal peptide and a KDEL/HDEL tetrapeptide so that secreted proteins are retrieved to the endoplasmic reticulum (ER), or a signal peptide and a transmembrane domain so the recombinant protein is concentrated in the membrane fraction (Sharma and Sharma, 2009). Another strategy involves the expression of recombinant proteins as fusions with stabilizing sequences, such as the elastin-like peptide repeat, which not only increases yields but also provides a convenient extraction method known as reverse transition cycling (reversible temperature-dependent precipitation) (Conley et al., 2009, 2011). A similar approach involves the use of oleosin fusions in oilcrops such as safflower, since oleosin is targeted to oil bodies and can be separated easily from bulk seed biomass, an approach pioneered by the Canadian biotechnology company SemBioSys, which recently developed a GMP process for the extraction of safflower-derived insulin. Another related approach involves fusion with the seed storage protein γ-zein, which results in the assembly of new storage organelles and increases yields by up to 300% (Torrent et al., 2009). This is being developed as Zera® technology by the Spanish biotechnology company ERA Biotech. Fungal hydrophobins can also be used to purify proteins by surfactant-based aqueous two-phase partitioning (Joensuu et al., 2010). Once again, however, these technical solutions introduce an additional regulatory burden. Not only must the developer explain the origin and purpose of these additional sequences, but the fact that they alter the primary structure of the protein itself means that much greater regulatory scrutiny focuses on the safety and comparability of the product. It is still 'safer' to express native versions of heterologous proteins in plants.

2.2. Downstream processing — yield of purified protein

In the late 1990s and early 2000s, many researchers in the field of molecular farming were more concerned to demonstrate that plants could produce adequate quantities of functional human proteins than how to get the product out. Downstream processing (DSP), the isolation and purification of the recombinant protein, is an integral part of every biomanufacturing process, but this was largely overlooked in the initial molecular farming boom, with the exception of the early pioneers at Prodigene Inc. who were launching plant-derived technical reagents onto the market (Menkhaus et al., 2004; Nikolov and Woodard, 2004). DSP is economically critical (since it represents up to 80% of overall production costs) and also a key component of the regulatory process for evaluating the safety of pharmaceutical products.

For all clinical products, DSP must conform to GMP and must be able to produce a sufficiently pure and homogeneous product with contaminants removed to acceptable levels. In the case of plants, one initial problem with GMP processes is to define the nature of those contaminants and satisfy the regulators that they have been reduced to safe levels. In bacteria and animal cells, the nature of the contaminants is well-known and the safety levels well established. Robust processes have been developed to remove endotoxins from bacterial cultures and viruses from mammalian cell cultures. Neither of these is relevant in plants, but instead plants might contain potentially harmful secondary metabolites and, of course, plant viruses. In our experience, the issue of plant viruses was a sticking point in the development of a GMP process for plants even though humans are exposed to large numbers of plant viruses daily in food. The problem was not that the regulators thought that plant viruses were dangerous, but that the inflexibility of the rules meant that an unquantified risk was sufficient to hold up development. To our knowledge there are no reports that plant viruses induce diseases in humans or other mammals.

Another important aspect of DSP is that whereas some of the unit operations are developed based on the properties of the product (e.g. affinity purification for product capture), others have to be developed based on the properties of the expression host. Particularly the early steps, which involve the removal of host-cell contaminants, need to be tailored for plants because the contaminants in plants differ significantly from those in mammalian cells or bacteria. DSP for whole plant tissues must, for example, deal with the removal of fibers, oils and metabolic byproducts on a crop-by-crop basis, key examples including nicotine

from tobacco leaves and oxalic acid from alfalfa as well as cellulose more generally.

2.3. Plant glycans

Another important technical and regulatory aspect of molecular farming is the status of plant glycans. Many therapeutic proteins are in fact glycoproteins, and the glycans can be necessary for full biological activity, stability, immunogenicity, correct targeting and appropriate pharmacokinetic properties. N-glycosylation occurs in several stages, the first of which involves the attachment and modification of an oligosaccharide precursor in the ER. This is similar in all eukaryotes. The nascent N-glycan is then processed by enzymes resident in the Golgi apparatus, and these enzymes are distinct in plants, mammals, insects and yeast, resulting in different oligosaccharide structures. Plant-derived glycoproteins differ from those of mammals in that they carry specific β1,2-xylose and core α1,3-fucose residues that are not found in mammals, but lack β1,4-galactose and sialic acid residues that are present in mammals (Gomord et al., 2010).

There is no evidence that plant glycans are immunogenic in or harmful to humans and it should be noted that CHO cells originate from rodents and therefore also produce non-human glycans. Nevertheless, the potential impact of plant glycans on protein structure, activity and safety has given pause to the regulators and again has caused researchers to innovate around a regulatory bottleneck, e.g. by expressing aglycosylated derivatives of proteins lacking the glycan attachment sites, by targeting proteins to the ER and thus avoiding Golgi-specific modifications to ensure all glycans are of the universal 'high-mannose' type (Sriraman et al., 2004; Triguero et al., 2005), and by glycoengineering, where host production lines have been engineered to prevent the addition of plant-type glycans and/or to add human type glycans (Gomord et al., 2004).

This has been achieved using gene knockout and RNA interference techniques in Arabidopsis, tobacco, duckweed and moss to abolish plant-specific glycosylases (Decker and Reski, 2004; Schahs et al., 2007; Strasser et al., 2004, 2008) and through the expression of the entire mammalian pathway for sialic acid synthesis to allow protein galactosylation and sialylation (Castilho et al., 2010; Strasser et al., 2009). It should be noted, however, that the differences between plant and human glycans can be beneficial in some cases. The key example here is Uplyso (taliglucerase alfa), a recombinant form of glucocerebrosidase produced in carrot cells by the Israeli biotechnology company Protalix BioTherapeutics in concert with Pfizer. Glucocerebrosidase is a glycoprotein, and its function depends on terminal mannose residues that are preserved when it is produced in carrot cells. In contrast, the current market leader Cerezyme (imiglucerase) produced by Genzyme Corp. is manufactured in CHO cells and has terminal sialic acid residues that need to be enzymatically removed in vitro to expose the critical mannose residues. For this reason the plant-derived product is advantageous.

3. Testing the market with non-clinical products

After technical issues, one of the remaining hurdles preventing the pharmaceutical industry from embracing molecular farming was the absence of a commercial pedigree in other areas. This was addressed by using plants to produce non-medical proteins on a successful commercial basis. The key player here was the US biotechnology company Prodigene Inc., which developed maize as a commercial platform for the production of enzymes and technical reagents including avidin and β-glucuronidase (GUS), both of which are used as diagnostic agents in molecular biology (Hood, 2002). An important principle demonstrated by these case studies was that molecular farming can be economically viable even when the natural source of a protein is abundant (e.g. egg whites for avidin and *Escherichia coli* for GUS) and where a market is already established. Both products were marketed by Sigma-Aldrich Fine Chemicals (SAFC, St Louis, MI) for a successful trial period, and it is notable that SAFC is now one of the few companies to possess an approved GMP facility for the extraction and purification of biologics from plants. In the case of avidin, the best-performing transgenic lines produced the reagent at a level equivalent to 2% of the aqueous protein extracted from dry seeds (230 mg per kg of transgenic seed), and the product was nearly identical in structure and activity to native avidin (Hood et al., 1997). The protein remained stable in seeds stored at 10 °C and was not affected by commercial processing operations (dry milling, fractionation and hexane extraction). Similarly, the best GUS transgenic lines produced 0.7% of the product as a proportion of water-soluble seed protein, equivalent to about 80 mg per kg of dry seeds (Witcher et al., 1998). Purified recombinant GUS was similar in molecular mass, physical and kinetic properties to native GUS isolated from *E. coli*. Transgenic seed containing recombinant GUS could be stored at an ambient temperature for up to 2 weeks and for at least 3 months at 10 °C without significant loss of enzyme activity, and could be subjected to normal maize processing operations as discussed above without loss of GUS activity (Kusnadi et al., 1998). Prodigene Inc. had developed several further products such as laccase and trypsin, the latter still available from SAFC, and was beginning to focus on medically-relevant proteins when a breach of environmental regulations resulted in massive fines and clean-up costs which soon caused the company to cease trading (Naqvi et al., 2011).

Several other companies are producing medically relevant proteins in plants on a successful commercial basis although not for clinical use. For example, the US biotechnology company Ventria Bioscience uses field-grown rice for the production of human albumin, transferrin, lactoferrin and lysozyme, whereas ORF Genetics (based in Iceland) produces human growth hormone and cytokines in barley seeds. These products are currently commercialized for diagnostic use, academic and private research, and in the case of growth hormone as a cosmetic additive (distributed by Sif Cosmetics, Iceland). Another relevant example is the production of aprotinin in tobacco by Kentucky BioProcessing (Owensboro, KT), because this company also has a GMP facility approved for the manufacture of biological products in plants and acts as a contract manufacturing organization for plant-derived pharmaceuticals. Therefore, even though no clinical development is envisaged in the short term for the products discussed above, the companies nevertheless demonstrate high quality and batch-to-batch reproducibility which can lead to the development of GMP-compliant processes in the future for other products (Ma et al., 2005a, 2005b). A further GMP facility in Japan is focusing on veterinary medicine and has produced canine interferon-α purified from transgenic strawberries to treat canine periodontal disease.

4. The regulation of plant-derived pharmaceuticals

4.1. Historical perspective

At the turn of the millennium, regulatory guidance for the production of recombinant pharmaceutical proteins in plants existed only as draft legislation based strongly on the existing regulations for mammalian cells. This legislation was therefore inappropriate for applications involving whole plants. Drug development is a long and costly process and plants are newcomers squaring up against long-established and firmly-entrenched platforms based on mammalian cells and microbes. For complex human proteins such as glycoproteins, mammalian cells were the only viable platform for nearly 20 years, and naturally the regulations for drug manufacturing had developed alongside this platform and were tailored in its favor (Wurm, 2004). The regulatory framework for CHO cells, first used for the production of tissue plasminogen activator (r-tPA, Activase) in 1987, reflects the status of CHO cells as a founding technology in the biopharmaceutical industry. Over the last 20 years, extensive data

concerning cell behavior and safety have accumulated, and there have been vast improvements in performance with CHO cultures now producing recombinant proteins at a yield of 5–10 g/L in the best cases, an order of magnitude greater than in the 1980s and early 1990s (Wurm, 2004). Plants, with lower yields, no history of commercial success and no regulatory approval, could not possibly compete against this type of opponent and could only survive where all newcomers initially take hold — in niche markets.

The evolution of the regulatory framework bifurcated at this point, with some developers striving to meet the existing regulations by putting forward plant-based platforms that behaved similarly to mammalian cells, e.g. the tobacco BY-2 system (CONCERT) developed by Fraunhofer and Dow AgroSciences, and the carrot cell platform (ProCellEx) developed by Protalix BioTherapeutics (Aviezer et al., 2009a,b). Here the emphasis was on containment, consistency and similarity to mammalian cells both conceptually and practically, and the products from these systems are therefore GMP-compliant and closest to the market (Hellwig et al., 2004; Tiwari et al., 2009; Xu et al., 2011; see also Table 1). Other developers stuck with whole plants and courted the regulators in order to fashion a new set of regulations. This required concepts such as cell banking to be replaced with seed banking, and new paradigms to be established to embrace the biological differences between complex multicellular organisms and single cells. Here the emphasis was on novelty, and borrowed much from the concepts already in place for genetically modified food crops (Ma et al., 2005a, 2005b; Whaley et al., 2011). Products developed from whole plant systems are trailing behind those from plant cells, but several are now in clinical development (Table 1).

4.2. General regulations governing pharmaceuticals derived from plants

The licensing of most drugs and diagnostics in the US is overseen by the FDA, whereas veterinary vaccines are separately regulated by USDA Center for Veterinary Biologics. The first draft guidelines for plant-derived pharmaceutical proteins were jointly developed by the USDA and FDA, and covered all imaginable platforms, including transgenic terrestrial plants, aquatic plants, moss and algae, as well as transient expression systems using plant viruses (FDA/USDA, 2002). These guidelines are flexible in terms of GMP considerations, requiring information about plant characteristics, the manufacturing process and pre-clinical testing regardless of which platform is chosen.

The situation in Europe is more complex. Recombinant pharmaceutical proteins derived from plants must adhere to Regulation 2309/93, which covers all medicinal products, but additional regulations apply specifically to plant-derived pharmaceuticals (Spok et al., 2008). These drugs are overseen by the relevant national authorities during the research and early clinical development phases but EMA takes precedence for commercialization. In 2002, EMA published draft guidance notes on 'the quality of biological active substances produced by stable transgene expression in higher plants' (EMEA, 2002) accompanying the FDA/USDA document discussed above. However, the EMA guidance was narrow in scope (only covering stable transgenic plants) and because it was drawn principally from the regulations covering cells and fermenters, certain regulations were difficult if not impossible to implement with whole-plant systems.

Some of the regulatory concepts developed and applied in cell culture systems that needed to be modified and redefined specifically for plants included master and working cell banks, which were replaced with master and working seed banks; batch-to-batch consistency, which had to take into account the natural variation between different plant organs; and standard operating procedures for different production systems and downstream processing requirements, a point we consider in more detail below. There still remains some overlap in authority between EFSA (the European Food Safety Authority) which deals with the practicalities of growing genetically modified plants, and EMA, which deals with the products from those plants. After an extensive period of consultation with researchers, updated guidelines addressing these issues were published in 2008 and came into effect in February 2009 (EMEA, 2008). Importantly, the development of new regulatory guidelines for the production of biopharmaceutical products in plants overlapped with the introduction of a new Clinical Trials Directive stipulating that all biopharmaceutical products intended for

Table 1
Plant-derived pharmaceutical products in clinical development manufactured using approved GMP-compliant processes.

Product	Product type	Developer	Platform	Development stage	GMP process	Comments
Uplyso (taligucerase alfa)	Enzyme (glucocerebrocidase) indicated for Gaucher disease	Protalix BioTherapeutics	Transgenic carrot suspension cells (ProCellEx™)	Phase III complete, market authorization pending	In-house	Market approval imminent (Shaaltiel et al., 2007)
Insulin	Hormone	SemBioSys Inc.	Transgenic safflower seeds	Phase I/II complete, phase III in progress	In-house	Maurice Moloney, personal communication
Influenza virus VLP	Subunit vaccine (avian influenza)	Medicago Inc.	*N. benthamiana* transient expression by agroinfiltration	Phase I complete	In-house	Rapid-response vaccine (D'Aoust et al., 2008; 2010)
Influenza virus HA	Subunit vaccine (avian and swine influenza)	Fraunhofer CMB	*N. benthamiana* transient expression by agroviral launch vector	Phase I complete	In-house	Rapid-response vaccine (Shoji et al., 2009; 2011)
BLX-301	Antibody (indicated for non-Hodgkin's lymphoma)	Biolex Inc	Transgenic duckweed (LEX System)	Phase II	In-house	http://www.biolex.com
MAPP66	Antibodies (indicated as combination HSV/HIV microbicide)	Bayer/ICON	*N. benthamiana* transient expression with MagnICON virus-based vectors	Phase I	In-house	http://www.bayer-innovation.com
P2G12	Antibody (indicated as HIV microbicide)	Pharma-Planta	Transgenic tobacco	Phase I in progress	Fraunhofer IME, Aachen	http://www.pharma-planta.net
Single-chain scFv fragments (individualized)	Antibody (non-Hodgkin lymphoma)	Large Scale Biology Corp (no longer trading)	Tobacco leaves infected with tobacco mosaic virus vectors	Phase I complete	Produced using the Kentucky BioProcessing GMP facility	Personalized vaccines against individual B-cell tumors (McCormick et al., 2008)
NoroVAXX	Norwalk virus subunit vaccine	Arizona State University	Transgenic potato tuber	Phase I complete	Produced using the Kentucky BioProcessing GMP facility	Oral vaccine candidate (Tacket et al., 2000)

phase I trials must be manufactured according to GMP. The importance of GMP compliance thus became relevant from the very beginning of clinical development. Some plant-derived pharmaceutical products have been tested in phase I trials without the need for GMP manufacture but all products entering clinical development now and in the future must comply with GMP conditions before human trials can be authorized.

4.3. GMP strategies for pharmaceutical proteins in whole plants

The elements of a GMP strategy for plant-derived pharmaceutical proteins can be difficult to define, but we have identified four key points that we believe are essential for the development of a broadly effective, GMP-compliant regulatory framework.

First, and perhaps the most important, we need to find suitable crops and focus on their development as industry standards. The diversity of plants used for molecular farming is seen as a technological advantage, but in industry terms it is messy and divisive. Away from plants, industry has focused on a few well characterized platforms. Standardization, with all its potential economic and regulatory benefits, is currently impossible with different parts of the molecular farming community advocating the use of production candidates ranging from alfalfa to zucchini. We need to define the CHO of vegetables and the *E. coli* of greens and focus development resources on this small collection of plant species with very specific advantages. These decisions will be driven by many factors (both economic and technological) as well as by the complex IP landscapes that surround the different platforms. Many plant-based production systems are economical in development, but may be more difficult to deploy commercially when complex IP issues need to be addressed and the owners of background IP demand royalties. Commercial success may therefore depend as much on how these IP issues are negotiated as on the intrinsic merits of each platform.

Second, we need to determine the most suitable cultivation setting: greenhouse or open field, increased containment or more cost-efficient production? Any economic advantages provided by plants must be recovered in the upstream production phase because DSP technologies and costs focus on the product and therefore converge regardless of the production platform. Open field cultivation is hard to beat in terms of process economy and plants tend to be fittest in their natural environment. However, this is balanced against the additional risks posed by pests, parasites, anthropogenic pollution and all the unforeseeable variation in environmental conditions with downstream consequences in terms of batch-to-batch reproducibility and the presence of unwanted adventitious agents such as insect carcasses and bird droppings. Greenhouses increase the costs of the upstream phase but offset this with the benefits of controlled, reproducible and adjustable cultivation conditions using technology that is well established by the food industry. Furthermore, when the value of the product justifies the investment, standard greenhouses can be replaced with fully-automated, computer-controlled factories virtually shut off from the outside environment, with in-process monitoring of plant health, morphology and nutritional status in a soil-free cultivation system with frequency-optimized illumination, atmosphere and temperature, all under feedback control. In terms of GMP, this would be the best approach but the costs might be prohibitive for larger-volume, lower-margin products including generics.

Third, we also need to evaluate the relative merits of stable and transient expression carefully. These systems currently compete head-to-head, with transient expression technology the more advanced because process development is not delayed by regeneration times and the need to bulk up production lines by breeding and seed banking. Several organizations offer GMP manufacturing based on transient expression in tobacco, or its close relative *Nicotiana benthamiana*, including Kentucky BioProcessing (Owensboro, KY), Icon Genetics (Bayer; Halle, Germany), Fraunhofer CMB (Newark, DE) and Medicago (Quebec, Canada). An additional facility is being constructed by Texas A&M University (College Station, TX) and G-Con, LCC. Fraunhofer IME in Aachen, Germany, has the only European GMP facility for biopharmaceutical production in transgenic plants, while the METI project has constructed such a facility in Japan, SAFC has a GMP facility for transgenics in the US and SemBioSys has a GMP facility for transgenic safflower in Canada. Transient systems are rapid and can already deliver impressive recombinant protein expression levels, whereas transgenic plants have long development timelines and their potential for controlled high-level expression still remains to be tested to its limits. But transient expression also has some disadvantages, such as the process complexity introduced by the infiltration procedure, and the regulatory burden inherent in the large-scale use of genetically modified bacteria and/or viruses leading to more stringent containment. Furthermore, the transformation, characterization, banking, fermentation and proper disposal of the bacteria under GMP conditions all add to the overall process costs. Stable transgenic plant lines are permanent genetic resources that do not require the reintroduction of bacteria in every production generation, and batch-to-batch consistency is therefore higher. The greater inherent variation in transient expression systems means that a controlled and contained environment is much more of a requirement. The principal advantage of transient systems is their scalability, which makes them ideal for rapid-response situation such as the production of vaccine candidates against emerging pandemic diseases. Transient and stable production platforms therefore appear suited to different niches in the biopharmaceutical industry.

Finally, we need to address the early stages of DSP, where the processing of plant material differs significantly from the processing of mammalian cells, yeast or bacteria. Even when working in the most technologically-advanced production greenhouses, it is not possible to achieve the level of containment offered by classic bioreactors and it makes no sense to try to emulate the containment offered in a fermentation platform. Therefore, there will always need to be a boundary between upstream production and primary processing in a non-GMP environment, and the meticulous hygiene in a GMP-compliant DSP cleanroom. The challenge is to facilitate handling of the process input (the plants) while minimizing quality risks for the product associated with working in a horticultural rather than a pharmaceutical environment in the early process steps. This requires the implementation of a smooth transfer for process intermediates into the cleanroom area without compromising its quality. In our experience, single-use components and closed systems should be used wherever possible in the early processing stages to fulfill these demands and was a key step in the development of our own GMP process for transgenic plants.

5. Outlook

A number of plant-derived pharmaceutical products are now very close to the market, with authorization for the Protalix/Pfizer drug Uplyso expected in late 2011. The first generation of molecular farming products fall into two categories from a regulatory point of view, the first comprising pharmaceutical proteins manufactured in whole plants for non-medical use (e.g. as technical reagents) and the second comprising pharmaceutical proteins manufactured in plant-based bioreactor systems (plant cells or sterile aquatic plants) which are targeted for clinical development. The absence of a GMP system for whole plants until recently has resulted in some inventive approaches to innovate around the regulations, including registering a plant-derived protein as a 'medical device' and the development of vaccine candidates for administration in plant tissue as oral vaccines. Now GMP processes are available for whole transgenic plants and transient expression systems, so the first products are entering clinical trials. We foresee a rapid increase in the number of plant-derived pharmaceutical proteins entering clinical development, particularly topical reagents (which attract a lower regulatory burden than injectables), rapid-response vaccines and niche products such as

Uplyso and BLX-301 which perform better than their counterparts produced in mammalian cells because of the unique characteristics of plant glycans.

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