Chapter 7
The Production of Vaccines and Therapeutic Antibodies in Plants

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Abstract Biopharmaceuticals such as antibodies and recombinant subunit vaccines are generally produced on a commercial basis by process-scale fermentation in bacteria, yeast or animal cells. Plants and plant cells have joined the exclusive club of commercial production platforms comparatively recently, but they offer certain advantages over the more established systems particularly in terms of economy, scalability, response times and formulation options. After a promising start and then a rocky transition from early R&D to preclinical and clinical development, plants are now becoming more firmly established as an alternative to microbes and mammalian cells for the production of pharmaceutical proteins. Several plant-derived pharmaceuticals have undergone clinical trials and the first products for human use are approaching market authorization, with antibodies and vaccines strongly represented among these front runners. Although scientific advances have played an important role in the maturation of plant-based production technology, perhaps the most critical development has been the definition of a workable regulatory framework which has recently yielded the first processes for biopharmaceutical production in plants that comply with good manufacturing practice. In this chapter, we consider

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the state of the art in plant-based production systems for antibodies and vaccines and discus the development issues which remain to be addressed before plants become an acceptable mainstream production technology.

7.1 Introduction

Humans have relied on plants as a source of medicines since antiquity and it is estimated that up to 25% of drugs on the market today contain active pharmaceutical ingredients derived from plants (Raskin et al. 2002). More recently, plants have also been considered as heterologous production platforms for recombinant pharmaceutical proteins, which are usually produced by process-scale fermentation in bacteria, yeast or animal cells (Twyman et al. 2005; Desai et al. 2010). The first pharmaceutical proteins produced 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). These pioneering studies demonstrated that plants could produce stable and functional human proteins, leading to the concept of molecular farming, i.e. the commercial production of valuable recombinant proteins in plants on an agricultural scale (Schillberg et al. 2003). Since then, hundreds of pharmaceutical proteins have been produced in a variety of plants and plant-based systems, amassing a vast literature of proof-of-principle studies demonstrating the virtues of different plant species, cells/tissues and expression strategies (Ma et al. 2003; Twyman et al. 2003, 2005; De Muynck et al. 2010; Rybicki 2010).

These studies to a greater or lesser degree all emphasized the advantages of plants in three main areas: economy, scalability and safety. Plants are inexpensive compared to fermenter systems, they are also much more scalable, and in terms of safety they do not produce endotoxins like bacteria, nor do they support the proliferation of human viruses and prions like mammalian cells. Many companies were set up to exploit these advantages using an array of diverse plant systems, and the world looked forward to a new era of inexpensive medicines. Unfortunately, these early pioneers had not reckoned on the difficulties that would be encountered when translating their research into practical, industrial processes. There were technical limitations in that the yields from plants were not in the same league as those achieved using industry standards such as *Escherichia coli* and Chinese hamster ovary (CHO) cells, there were previously unrecognized differences between recombinant proteins produced in plants and animals because of different glycan structures, but most importantly there was a lack of regulations governing the use of plant systems which resulted in a huge roadblock in clinical development (Spok et al. 2008). The re-emergence of molecular farming as a viable production technology in the last few years has resulted from a decade of work to overcome technical limitations and to define and develop a suitable regulatory pathway for the production of pharmaceuticals in plants according to good manufacturing practice (Fischer et al. 2011).

7.2 Lead Products – Why Antibodies and Vaccines?

There are still no approved plant-derived pharmaceutical products for human use, although at the time of writing the FDA is on the brink of approving a recombinant form of glucocerebrosidase produced in carrot cells. A tobacco-derived subunit vaccine against Newcastle disease was approved by the USDA in February 2006 for use in poultry. Furthermore, a recombinant antibody that binds the *Hepatitis B virus* surface antigen was approved in Cuba by the Center for State Control of Medication Quality (CECMED) in June 2006. This is not used directly as a pharmaceutical product, but as an affinity reagent to purify the viral surface antigen (which is produced using conventional fermentation technology). However, it is important to include this product because the approval process is as rigorous as that for an active pharmaceutical ingredient.

With one plant-derived pharmaceutical product perched on the brink of approval, it is important to consider the clinical pipeline and what products are likely to follow. This reveals that there are two major classes of product in development – plant-derived antibodies and plant-derived subunit vaccines. The popularity of these products as development targets reflects their regulatory status and the strategic advantages of using plants to produce them. Antibodies are popular targets because they currently dominate the biopharmaceutical pipeline, representing up to 30% of biopharmaceuticals in development (Sheridan 2010). There is a large body of literature confirming that antibodies in a variety of formats can be expressed successfully in plants and remain functional after isolation from plant tissue (De Muynck et al. 2010). Perhaps most importantly, antibodies tend to have a stoichiometric mechanism of action and are therefore required in large doses. This means that for some antibodies, particularly those envisaged as topical reagents (e.g. as microbicides) production on the 100–1,000 kg scale would be necessary to cope with demand (Gottschalk 2009). Currently, only plants have the scalability to meet this challenge. Antibodies as topical reagents also attract less regulatory scrutiny than injectables, so it is no coincidence that topical antibodies envisaged as microbicides were chosen as fast-track candidates during the development of regulatory guidelines for plant-derived pharmaceuticals (Ma et al. 2005a, b).

Much the same reasoning applies to the development of subunit vaccines expressed in plants because many of the pioneers were developed as oral vaccines, to be administered in partly processed plant food (e.g. mashed potato or tomato paste), therefore sidestepping much of the regulatory scrutiny that would apply to vaccines administered by injection. Where oral vaccination is possible, antigens presented as part of a food matrix tend to remain more stable and are therefore more efficacious, although dosing can be difficult to control (Nochi et al. 2007). The development of human vaccines produced in plants has also been boosted by the large number of studies showing the efficacy of plant-derived vaccines for the prevention of diseases in other animals, culminating with the USDA approval of the Newcastle disease vaccine discussed above (Rybicki 2010). More recently, the development of transient expression platforms based on *Agrobacterium tumefaciens*,

plant viruses, or hybrids combining the advantages of both, has led to a resurgence of interest in plant-derived vaccines because transient expression in plants can be scaled up much more quickly than fermentation or egg-based platforms, therefore offering a rapid response to emerging pandemics or bioterrorism (D'Aoust et al. 2010; Rybicki 2010).

7.2.1 Antibodies Produced in Plants

Many antibodies have been expressed in a variety of different plant species and tissues, but it is important to distinguish between those produced as pharmaceutical proteins with the intention of at least partial extraction and purification, and those intended to function *in planta* as a strategy to tackle plant diseases (Schillberg et al. 2001). Pharmaceutical antibodies in plants often represent proof-of-principle studies with no subsequent development, but others are heading towards the clinic, and several plant-derived antibodies have already been evaluated in clinical trials or are poised to enter clinical trials. The ones we consider are listed below:

- Avicidin from maize (NeoRx/Moinsanto). Indicated for colorectal cancer, but withdrawn from phase II trials in 1998 because of adverse effects.
- CaroRx from tobacco (Planet Biotechnology). Indicated for dental caries, the antibody binds specifically to *Streptococcus mutans*, the bacterium that causes dental caries. Phase II trials completed and product is licensed in the EU as a medical device.
- BLX-301, an anti-CD20 optimized antibody for the treatment of non-Hodgkin B-cell lymphoma expressed in the aquatic plant *Lemna minor* by Biolex Inc.
- MAPP66, a combination of antibodies envisaged as a HSV/HIV microbicide and expressed in *N. benthamiana* using Bayer's MagnICON (magnifection) technology.
- 2G12, an HIV-neutralizing antibody expressed in tobacco (Fraunhofer IME/Pharma-Planta).

The two plant-derived antibody products that have completed phase II trials are Avicidin and CaroRx. Avicidin is a full length IgG specific for EpCAM (a marker of colorectal cancer) produced in maize and developed jointly by NeoRx and Monsanto. Although Avicidin demonstrated therapeutic efficacy in patients with advanced colon and prostate cancers, it was withdrawn from phase II trials in 1998 because treatment resulted in a high incidence of diarrhea (Gavilondo and Larrick 2000). The same issue arose with the equivalent antibody produced in mammalian cells and in all other respects the two antibodies were comparable (physicochemical properties, serum clearance, urine clearance and dosimetry). Therefore, the adverse effects were not linked in any way to the use of maize as the production platform.

CaroRx is a chimeric secretory IgA/G produced in transgenic tobacco plants which has completed phase II trials sponsored by Planet Biotechnology Inc. (Ma et al. 1998). Secretory antibody production requires the expression of four separate polypeptides,

which in this case were initially expressed in four different plant lines that were crossed over two generations to stack all the transgenes in one line. The antibody binds to the major adhesin SA I/II of *Streptococcus mutans*, the bacterium that causes tooth decay in humans. Topical application following elimination of bacteria from the mouth helped to prevent recolonization by *S. mutans* and led to the replacement of this pathogenic organism with harmless endogenous flora. To circumvent the regulatory vacuum surrounding plant-derived pharmaceuticals in the late 1990s and early 2000s, CaroRx was registered as a medical device.

BLX-301 has completed phase I trials. BLX-301 is an anti-CD20 antibody indicated for non-Hodgkin lymphoma which produced in the Lex system developed by the US biotechnology company Biolex Inc. and based on duckweed (*Lemna minor*). This plant has a number of significant advantages for the production of recombinant pharmaceutical proteins as it can be grown in sealed, aseptic tanks under constant conditions with a chemically defined medium, which ensures batch-to-batch consistency and allows clones to be scaled up rapidly in the company's GMP facility. The Lex system also allows the glycan structures of the antibody to be controlled and optimized (Cox et al. 2006), thus Biolex claims that BLX-301 is more potent and efficacious than equivalent antibodies produced in mammalian cells and has fewer adverse effects.

MAPP66 is an antibody cocktail envisaged as a microbicide to prevent the transmission of HIV and HSV, and it has also completed phase I trials. MAP66 is produced by transient expression in *Nicotiana benthamiana* using the magnifection technology. The principle of magnifection, developed by Icon Genetics (now part of Bayer) is that genes are delivered as part of a recombinant plant virus (in this case *Tobacco mosaic virus*) but the systemic spreading of the virus is made unnecessary through the use of *A. tumefaciens* as a delivery vehicle (Marillonnet et al. 2005; Gleba et al. 2005). The bacterium delivers the viral genome to so many cells that local spreading is sufficient for the entire plant to be infected and this overcomes an often critical limitation of plant viruses which is that the host range, efficiency of infection and ultimately the speed at which recombinant proteins accumulate all depend on its natural ability to spread systemically through the plant. Taking the systemic spreading function away from the virus and relying instead on the bacterium to deliver the viral genome to a large number of cells allows the same viral vector to be used in a wide range of plants (Gleba et al. 2004).

Four monoclonal antibodies with potent HIV-neutralizing activity have been identified (b12, 2G12, 2F5 and 4E10) and have been shown to prevent virus transmission in animal models (Cardoso et al. 2005). Combinations of 2–4 of these antibodies would therefore make effective microbicides, and the demand for such products particularly in endemic regions such as sub-Saharan Africa would mean multi-ton scale production, which would only be possible in plants. Tobacco and maize plants have been used to produce 2G12 (Ramessar et al. 2008; Rademacher et al. 2008) and 2F5 (Sack et al. 2007), and tobacco plants producing 2G12 have been developed within the EU Framework Program 6 project Pharma-Planta as a pioneer to help define the regulatory pathway for plant-derived pharmaceuticals (Ma et al. 2005a). The objective of this project was to take candidate pharmaceutical

products from gene to clinic, defining the regulatory pathway in concert with the appropriate regulatory bodies along the way. This process has been largely successful and a phase I clinical study of the plant-derived 2G12 antibody commenced in July 2011.

7.2.2 Vaccine Candidates Produced in Plants

Plant-derived vaccines can be divided into two categories – those designed for veterinary use and those designed for medical use. The Newcastle disease vaccine for poultry was the first plant-derived pharmaceutical product to be approved, and there is a large body of both immunogenicity and challenge data to support the efficacy of such vaccines, including numerous clinical studies (Twyman et al. 2005).

Seven human clinical trials involving plant-derived subunit vaccines have also been reported. Tacket et al. (1998) performed the first such trial with transgenic potatoes expressing the enterotoxigenic *E. coli* (ETEC) labile toxin B-subunit (LTB), one of the most potent known oral immunogens. The LTB content of the tubers varied between 3.7 and 15.7 μg g^{-1} fresh weight. Fourteen volunteers were given either transgenic or non-transgenic potato on days 0, 7, and 21 of the trial. Almost all of those consuming the transgenic potatoes showed at least four-fold increases in serum IgG against LTB while no such increase was seen in those consuming the non-transformed potatoes. Five of these individuals also demonstrated at least a four-fold increase in anti-LTB IgA, detected in stool samples. There were few side effects, such as nausea and diarrhea. A more recent trial using LTB expressed in processed corn seed produced similar results to the potato study (Tacket et al. 2004). The same group also described the results of a clinical trial performed using transgenic potato tubers expressing the Norwalk virus capsid protein (NVCP) (Tacket et al. 2000). Twenty adult volunteers were given two or three 150-g doses each of raw transgenic potato tuber containing 215–750 μg NVCP. Although only 50% of the NVCP subunits assembled into virus-like particles in the potato cells, thus reducing the effective dose of the vaccine, nearly all of the volunteers showed significant increases in the numbers of IgA antibody-forming cells (AFCs), and six of these individuals also showed increases in IgG AFCs. There were also noticeable increases in serum antibodies against NVCP and stool IgA antibodies in a few of the participants.

A clinical trial has also been carried out using orally delivered HBV surface antigen produced in lettuce (Kapusta et al. 1999). Two of three volunteers who were given two 150-g doses of transgenic lettuce containing about 2 μg of the antigen per dose, produced protective serum antibodies after the second dose, although the titers declined in a few weeks. Even so, the study confirmed that naïve subjects could be seroconverted by the oral delivery of a plant-derived viral antigen. A similar trial in the United States involved the HBV surface antigen expressed in transgenic potatoes although participants in this trial had already been seroconverted with the standard, yeast-derived vaccine (Richter et al. 2000). Of 33 participants given either two or three 1-mg doses of the antigen, about half showed increased serum IgG titers against the virus.

Yusibov et al. (2002) have carried out a trial involving 14 volunteers given spinach infected with *Alfalfa mosaic virus* vectors expressing the rabies virus glycoprotein and nucleoprotein. Five of these individuals had previously received a conventional rabies vaccine. Three of those five and all nine of the initially naïve subjects produced antibodies against rabies virus while no such response was seen in those given normal spinach.

In all the above cases, the subunit vaccine was delivered orally as part of a matrix of plant material, and the advantage of using plants was convenience of the oral delivery route and the efficacy mediated by presentation of the antigen as a bioencapsulated formulation. In contrast, McCormick et al. (1999) produced a single chain antibody fragment designed to confer passive immunity against non-Hodgkin lymphoma using *Tobacco mosaic virus* as a vector in tobacco plants, but purified the vaccine for administration by injection. In preclinical development, a vaccine based on the well-characterized mouse lymphoma cell line 38C13 stimulated the production of anti-idiotype antibodies capable of recognizing 38C13 cells, providing immunity against lethal challenge with the lymphoma. Here the advantage of plants was not the potential for oral delivery, but the rapid production that can be achieved by transient expression, allowing the development of patient-specific vaccines for personalized B-cell lymphoma therapy. At least 12 such vaccines have been tested in phase II trials (McCormick et al. 2008).

The rapid production that is possible in plants is useful not only for personalized medicines but also for scaling up production as a rapid response to pandemic threats. Researchers at Medicago Inc. in Canada and at Fraunhofer CMB in Newark, Delaware, recently achieved the impressive feat of producing gram quantities of vaccine against an emerging influenzavirus strain within 1 month of isolating the hemagglutinin sequence (Rybicki et al. 2010). The Medicago vaccine was subsequently tested in a phase I clinical study to demonstrate safety (Landry et al. 2010).

7.3 Recent Advances in Production Technology

7.3.1 Optimizing Yields and Recovery

The yields of recombinant proteins produced in plants are generally much lower than those routinely achieved in fermenter systems and this has been a challenge both in terms of establishing the credibility of molecular farming and also in the development of economically viable processes and (in the case of oral vaccines) efficacious products. The recombinant protein itself plays an important role in determining the yield because some proteins are inherently less stable than others, and some proteins produce unanticipated negative effects on plant growth. In general terms, yields can be improved by optimizing 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). However, many of the problems with low expression levels are restricted to nuclear transgenic plants, and very high

expression levels can be achieved using transient expression platforms based on Agrobacterium and/or plant viruses (Giritch et al. 2006; Sainsbury and Lomonossoff 2008; Vézina et al. 2009; Huang et al. 2010; Pogue et al. 2010). For the time being, transient expression is likely to be the first choice for products required rapidly in large quantities. Even so, transgenic systems have a number of advantages despite their longer development times, slower scaling-up and the increased regulatory burden that comes with the GM label – these include the permanent genetic resource represented by an integrated transgene (no requirement for the reintroduction of bacteria or viruses in every production generation), the absence of genetically modified bacteria and viruses, and the batch-to-batch consistency. Transgenic plants are likely to remain the system of choice for products required in large quantities but not as an urgent response.

Because antibodies are complex multimeric glycoproteins, the stability (and therefore the yield) depends greatly on their ability to fold and assemble correctly, which in turn depends on their subcellular localization (this is not such a critical factor with vaccine candidates, which tend to be smaller and simpler proteins). The yield of an antibody is enhanced by adding a signal peptide to allow secretion to the apoplast, or even better a signal peptide and a KDEL/HDEL tetrapeptide so that secreted proteins are retrieved to the endoplasmic reticulum (ER), reflecting the favorable environment for protein folding, the presence of chaperones and the absence of significant protease activity (Sharma and Sharma 2009). In some cases, antibody stability can be increased by coexpressing the corresponding antigen although subsequent removal of the antigen after the complex has been extracted increases the complexity of downstream processing. Alternatively, stability can be increased by fusion to a stabilizing protein partner. For example, Floss et al. (2008) showed that the fusion of elastin-like peptides (ELPs) to the C-terminus of antibody 2F5 enhanced its stability without affecting its binding activity. The ELP also provides a convenient extraction method known as reverse transition cycling, which is based on reversible temperature-dependent precipitation (Conley et al. 2009, 2011). A similar 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).

7.3.2 Dealing with Plant Glycans

The other major technological challenge facing the pioneers of molecular farming was the impact of differences between plants and mammals in terms of protein glycosylation (Twyman et al. 2005). Plant-derived recombinant human glycoproteins tend to contain the carbohydrate groups β(1,2)xylose and α(1,3)fucose, which are not found in mammals, but they do not contain terminal galactose and sialic acid residues that are found in native human glycoproteins because the corresponding enzymes are not present in plants (Gomord et al. 2010). The impact of glycan differences is more important for antibodies than vaccine candidates because

antibodies are expected to bind antigens and carry out their effector functions, whereas antigens are expected solely to generate an immune response. Glycans are necessary for the biological activity of some proteins, and incorrect glycans may influence the solubility, stability or activity of a protein and thus its pharmacokinetic properties. Plant glycans may be immunogenic in some mammals but there is no evidence for this in humans, and it should be noted that CHO cells, which are rodent in origin, also produce non-human glycans. Nevertheless, the potential impact of plant glycans on protein structure, activity and safety has resulted in regulatory pressure on researchers to either remove or 'humanize' plant glycans, e.g. by expressing aglycosylated derivates 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 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 (Strasser et al. 2004, 2008; Decker and Reski 2004; Schahs et al. 2007) and through the expression of the entire mammalian pathway for sialic acid synthesis to allow protein galactosylation and sialylation (Strasser et al. 2009; Castilho et al. 2010).

It is important to state that non-authentic glycans are not necessarily always a disadvantage, and can affect the solubility, stability and biological activity of a protein positively as well as negatively, e.g. by extending its half life or increasing its affinity for a target. BLX-301 has already been cited as one example of beneficial glycan modification in plants, where the optimized glycan structures increase the potency of the antibody. In a different sense, Uplyso (taliglucerase alfa), the recombinant glucocerebrosidase produced in carrot cells which is poised for FDA approval, provides another example of beneficial plant glycans. Here the protein lacks sialic acid residues which are present in the equivalent protein produced in CHO cells (Cerezyme, imiglucerase). However, because sialic residues reduce the potency and longevity of the protein, they are removed from Cerezyme after purification by in vitro treatment with an exoglycosidase. It is ironic that plant glycans were once regarded as a serious bottleneck in the commercial development of molecular farming, but the extensive technology for glycan modification that has developed in response now makes plants the most versatile platform for the production of pharmaceutical proteins whose properties can be augmented or improved by glycan engineering.

7.3.3 Downstream Processing and Good Manufacturing Practice

The first decade of molecular farming research focused almost exclusively on upstream productivity, increasing yields and protein stability. Comparatively little attention was paid to downstream processing, and almost nothing was done to address the challenges of large-scale processing which would be an integral component of any commercial platform.

One company that did look into downstream processing in detail was Prodigene Inc., which developed maize as a commercial platform for the production of protein technical reagents used in research (Hood 2002). Much of the accepted wisdom about downstream processing in plant-based production systems came from their detailed studies of avidin and β-glucuronidase (GUS), which were commercially viable and marketed by Sigma-Aldrich Corp. alongside avidin from chicken eggs and GUS from *E. coli*. In both cases, the company demonstrated equivalence to the native product, stability during processing operations such as dry milling, fractionation and hexane extraction, and yields equivalent to 1–2% of soluble seed protein, which is the common benchmark cited for commercial viability in molecular farming (Hood et al. 1997; Witcher et al. 1998; Kusnadi et al. 1998). Before their demise, Prodigene Inc. was working on processing strategies for plant-derived antibodies and vaccines, and these processes provided foundations for the development of GMP processes that are now applied in other plants. Several aspects of downstream processing have to be customized specifically for plant systems, including the removal of fibers, oils and other by-products from certain crops, and process optimization for the treatment of different plant species and tissues (Menkhaus et al. 2004; Nikolov and Woodard 2004).

Two distinct strategies have arisen for creating a regulatory pathway for plant-derived pharmaceuticals, one based on the development of systems that are similar in concept to the industry standards, and the other based on the modification of existing regulations to deal with whole plants. In the first category, processes have been developed based on cultivated plant cells, analogous to processes for microbes and mammalian cells, e.g. the tobacco BY-2 system developed by Fraunhofer and Dow AgroSciences, and the carrot cell platform (ProCellEx) developed by Protalix BioTherapeutics (Aviezer et al. 2009). Further systems were developed based on algae, moss and aquatic plants, all linked by the ability to use closed vessels, defined synthetic media and controlled growth environments (Cox et al. 2006; Franklin and Mayfield 2005; Decker and Reski 2004). These systems provide containment, consistency and are similar to mammalian cells both conceptually and practically (Hellwig et al. 2004; Tiwari et al. 2009; Xu et al. 2011). In the second category, new concepts were developed such as the replacement of cell banking with seed banking, and new paradigms to embrace the biological differences between complex multicellular organisms and single cells (Ma et al. 2005a; Whaley et al. 2011; Fischer et al. 2011). After much preparatory work, this has resulted in the successful development and application of GMP processes involving contained systems such as carrot cells (Protalix Biotherapeutics), moss (greenovation, Frieberg) and Lemna (Biolex Therapeutics). Several organizations also now offer GMP manufacturing based on transient expression in tobacco or *Nicotiana benthamiana*, e.g. Kentucky BioProcessing (Owensboro, KY), Bayer/Icon Genetics (Halle, Germany), Fraunhofer CMB (Newark, DE), Medicago (Quebec, Canada) and Texas A&M University/G-Con LLC (College Station, TX). Finally, and uniquely, the Fraunhofer IME in Aachen, Germany, has a GMP facility for the production of recombinant antibodies in transgenic tobacco plants.

7.4 Conclusions and Outlook

A number of plant-derived pharmaceutical products are now very close to market authorization, thanks to the development of GMP-compliant production processes and the successful completion of clinical trials. The first product is likely to be Uplyso, the recombinant glucocerebrosidase produced in carrot cells by Protalix Biotherapeutics in concert with Pfizer, but many of the remaining products in the pipeline are either antibodies or vaccines, reflecting the key advantages of plants – economy, scalability and rapid response. 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 antibody as a medical device and focusing on oral vaccination and topical prophylaxis as primary administration routes. Now GMP processes are available for whole plants as well as contained plant-based systems, it is likely that the pipeline will fill rapidly with candidates falling into three main categories: (a) antibodies and vaccines required in 100–1,000 kg amounts, which exceed the capacity of traditional platforms; (b) vaccine candidates required as a rapid response to bioterrorism threats or emerging pandemics; and (c) pharmaceutical proteins whose efficacy or pharmacokinetic properties can be improved by glycan modification in vivo, which is now more advanced in plants that any other production platform.

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