# Chapter 12
# Pharmaceuticals

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## 12.1 Introduction

Plants and their extracts have long been used as remedies for a variety of health conditions, and many modern pharmaceuticals are still derived from plants. With recombinant DNA technology and the advent of efficient transformation technologies for plant cells it is now possible to extend the use of plants for pharmaceutical purposes by using them as production platforms for biopharmaceuticals. As of 2006, more than 150 biopharmaceuticals were approved for use in human medicine (Walsh 2006). The vast majority of biopharmaceuticals are proteins of human or animal origin and include enzymes, blood factors, thrombolytics, monoclonal antibodies, cytokines, hormones, and growth factors. These proteins are currently produced mainly in bacterial, yeast, or animal cell cultures. As the demand for and diversity of biopharmaceuticals increase, we need additional production capacities to fulfill the market requirements. Therefore, plants and plant cells have been investigated as alternative production hosts.

Since the first report of the successful production of a monoclonal antibody in transgenic tobacco plants two decades ago (Hiatt et al. 1989) a great variety of proteins with potential pharmaceutical applications have been produced in plants (Basaran and Rodríguez-Cerezo 2008; Fischer et al. 2004; Schiermeyer et al. 2004; Twyman et al. 2005). These proteins are collectively referred to as plant-made pharmaceuticals (PMPs) and a large number of plant species have been evaluated as production platforms for these molecules (Sparrow et al. 2007). These include food crops such as maize, barley, potato, and tomato, non-food crops such as tobacco and others such as duckweed and moss. As well as intact plants, certain types of plant tissue cultures (e.g. hairy roots) and cell suspension cultures from various species

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have been investigated (Hellwig et al. 2004). In the following section we discuss the basic principles required to produce PMPs, and then we consider four case studies of plant-derived biopharmaceuticals that have moved from laboratory proof-of-principle studies into clinical development.

## 12.2 Expression Systems

The properties of a pharmaceutical recombinant protein often vary according to the expression platform used to produce it, which takes into account the plant species, the tissue from which the product is extracted, and the subcellular localization of the recombinant protein. The selection of an expression system therefore depends mostly on the required properties of the recombinant protein, but also on considerations such as downstream processing and regulatory issues.

### *12.2.1 Transient Expression Systems*

Transient expression systems do not involve transgenic plants or cells – the transgene encoding the pharmaceutical protein remains episomal rather than integrating into the host genome. Such systems are based either on the transient expression of episomal DNA following standard transformation (*Agrobacterium* or direct transfer by particle bombardment, see Chap. 1) or on the use of plant viruses as vectors. Although transient expression following standard transformation is mostly used as a rapid testing system to confirm gene transfer and expression, agroinfiltration (in which recombinant *Agrobacterium tumefaciens* are infiltrated into plant tissue) can be used to produce milligram amounts of recombinant pharmaceutical proteins within a short time-frame. This procedure is described in more detail by Fischer et al. (1999) and Sheludko (2008).

To date four types of expression systems have been developed using plant viruses as vectors (Lico et al. 2008). *Gene insertion vectors* are defined as those in which the pharmaceutical transgene resides within a complete viral genome and are usually based on tobacco mosaic virus (TMV) or potato virus X (PVX). In *gene substitution vectors*, an endogenous virus gene such as the coat protein gene is replaced by the pharmaceutical transgene. Both the insertion and substitution vector systems have limitations with respect to transgene size, genetic stability, and expression level. Furthermore, the elimination of the virus coat protein may impair its ability to spread systemically within a plant. *Peptide display vectors* have been employed particularly for the expression of small peptide epitopes for use as vaccines, although occasionally they have been used to display larger proteins. They are often based on cowpea mosaic virus (CPMV). When these peptides are fused with the coat protein of a virus, the resulting chimeric viral coat will display the epitope on its surface. Such viral particles have proven to be very effective for

vaccination because they are highly immunogenic, so no additional adjuvants are needed to induce an immune response in the host. The versatility of these vectors has been demonstrated with an experimental rabies vaccine that induced a humoral immune response in laboratory animals and human volunteers (Yusibov et al. 2002) and a cancer vaccine that induced a cellular immune response in mice (McCormick et al. 2006). However, while such vectors are useful for the production of chimeric viral particles, they cannot be used to synthesize intact recombinant pharmaceutical proteins.

To overcome the limitations with respect to transgene size, transgene stability and impaired virus spreading, *deconstructed vectors* have been developed that preserve beneficial virus replication functions but replace the reliance on systemic spreading with the ability to transfer DNA into many more cells (Marillonnet et al. 2004). The deconstructed vectors are based on TMV gene substitution vectors (with the transgene replacing the coat protein gene) so the virus can move to adjacent cells with the help of the movement protein but cannot spread systemically. Most virus vectors rely on systemic spreading to achieve high protein expression levels, but the deconstructed vectors take advantage of the efficiency of *Agrobacterium*-mediated gene delivery by enclosing the entire virus vector within a T-DNA, which is introduced into host cells by agroinfiltration. Under normal circumstances, episomal T-DNA is short-lived and expression is only possible for a limited time unless selection is used to propagate cells with integrated T-DNA. However, because TMV is an RNA virus that naturally replicates in the cytosol, the introduction of a virus DNA construct into the nucleus allows replication-competent virus genomes to be produced by transcription. The deconstructed vector system required several optimizations to achieve high-level expression. Efficient processing of the transcribed RNA was found when several intron sequences were added to the viral constructs to ensure correct processing and export from the nucleus. When those vectors were delivered by vacuum infiltration of recombinant *Agrobacterium tumefaciens* into whole *Nicotiana benthamiana* plants, a process termed Magnifection by its inventors, yields of up to 4 g $kg^{-1}$ fresh weight of leaf biomass could be achieved (Marillonnet et al. 2005). Even the production of hetero-oligomeric proteins such as antibodies is possible when multiple viral vectors are co-infiltrated into the plant host. Giritch et al. (2006) achieved yields of up to 0.5 g $kg^{-1}$ fresh weight of a human monoclonal IgG1 antibody when they expressed the heavy and light chains of the antibody on two separate non-interfering viral vectors based on TMV or PVX sequences, respectively (Giritch et al. 2006).

## *12.2.2 Stable Expression Systems*

### 12.2.2.1 Transplastomic Plants

The introduction of transgenes into the circular genome of plastids can be achieved by biolistic transformation methods (Verma et al. 2008), which is discussed in

detail in Chap. 2. The transgene is designed to contain flanking sequences homologous to endogenous plastid genes so that the transgene is inserted into predefined regions in the plastid genome by homologous recombination. Each plastid contains several hundred genome copies. Therefore the primary transformants must undergo multiple rounds of regeneration to achieve the homoplasmic state (in which all plastid genomes contain the transgene). This is routinely achieved by the introduction of an aminoglycoside 3″-adenylyltransferase gene that confers resistance to spectinomycin (Svab and Maliga 1993). Due to the presence of multiple genomes per plastid it is possible to achieve transgene copy numbers of up to 10 000 per cell (Bendich 1987). A further increase in the copy number can be achieved by targeting the transgene to duplicated regions of the plastid genome (Zoubenko et al. 1994). The high transgene copy number allows recombinant proteins to accumulate to high concentrations in transplastomic plants, often reaching 10% of the total soluble protein (TSP) or even higher (Daniell 2006). Since plastids are inherited maternally in most crop species, gene transfer via pollen is unlikely making this technique an important biosafety solution to outcrossing (Hagemann 2002).

Plastids are equipped with the enzymes required to assemble multisubunit proteins like the pentameric cholera toxin B subunit (Daniell et al. 2001) and to create disulfide bridges in molecules like the human growth hormone somatotropin (Staub et al. 2000). Native somatotropin has a phenylalanine residue at the N-terminus but recombinant somatotropin can only be produced in plastids with methionine at the N-terminus. To meet this challenge, the recombinant molecule was produced as a N-terminal fusion with ubiquitin, the latter being cleaved by an endogenous ubiquitin protease to yield the native N-terminal phenylalanine. Some recombinant proteins expressed in plastids are subject to N-terminal processing by the endogenous methionine aminopeptidase depending on the amino acid composition following the initiating N-formylmethionine (Fernandez-San Millan et al. 2007; McCabe et al. 2008). This has to be taken into account in transgene design when a defined N-terminus is critical for the functionality of the final protein product.

Tobacco was the first domesticated crop in which plastid transformation was achieved, and only recently has that success been replicated in other crops, such as tomato (Ruf et al. 2001) and lettuce (Lelivelt et al. 2005). This means that most reports of transplastomic plants producing pharmaceutical proteins involve the use of tobacco, yet the accumulation of recombinant subunit vaccines in the chloroplasts or chromoplasts of edible plant tissues would offer additional opportunities for vaccine production and delivery (Kamarajugadda and Daniell 2006).

Despite the high transgene copy numbers in homoplasmic plants and the absence of position effects and post-transcriptional silencing, not all recombinant proteins can be expressed in plastids at high levels. The rotavirus coat protein VP6, a potential subunit vaccine for enteric infections, accumulated to 3% TSP in the young leaves of transplastomic tobacco plants, but could not be detected in older leaves due to proteolytic degradation (Birch-Machin et al. 2004). Similarly the HIV p24 antigen could be detected in the youngest leaves of transplastomic tobacco plants but not in mature leaves (McCabe et al. 2008). With a codon-optimized

construct, homogenous expression was achieved in leaves of all ages but the transplastomic leaves exhibited a yellow phenotype and rearrangements were detected within the plastid genome. Proteolysis is a general concern irrespective of the expression system. Foreign proteins are exposed to proteolysis in planta during biomass growth phase and upon cell disruption and downstream processing, and research is ongoing to identify and hopefully inhibit the proteases involved (Doran 2006; Schiermeyer et al. 2005).

#### 12.2.2.2 Nuclear Transgenic Plants

Many therapeutic proteins expressed in plants are glycoproteins, which means they must be targeted to the endomembrane system where glycan chains are added. Since plastids are unable to modify proteins by glycosylation, such proteins need to be expressed from transgenes integrated into the nuclear genome. Many plant species have been transformed successfully by either co-cultivation with *Agrobacterium tumefaciens* (Twyman et al. 2003) or by biolistic methods (Altpeter et al. 2005); see Chap. 1 for details. Gene stacking for the expression of hetero-oligomeric proteins can be achieved by crossing plants that express individual subunits, or by simultaneous transformation with multiple genes. The former approach has been used to assemble IgG and IgA class antibodies (Ma et al. 1994) as well as secreted antibodies comprising four different polypeptide chains: the heavy and light chains, the joining chain, and the secretory component (Ma et al. 1995).

Breeding programs (see Chap. 6) have been used to increase the yield of recombinant avidin in transgenic maize plants by a factor of 70 after six generations (Hood et al. 2002). Also, it is possible to introgress transgene(s) from laboratory model varieties into elite germplasm that is not readily accessible for transformation (Rademacher et al. 2008).

In contrast to plastid transgenes, nuclear transgenes can be subject to epigenetic phenomena such as position effects, transcriptional silencing (TGS) and post-transcriptional gene silencing (PTGS), the latter of which can be triggered by aberrant transcripts produced from truncated and rearranged transgenes, inverted repeats and by readthrough of tandem repeats generating mRNAs without poly-adenylate tails. This is discussed in more detail in Chap. 5. Although position effects can often be ameliorated by the use of matrix attachment regions (Abranches et al. 2005), these do not protect plants from PTGS triggered by complex transgene structures, so it is usually necessary to screen populations of independent primary transformants to identify plants with high-level expression. The targeted integration of transgenes into the nuclear genome by homologous recombination could circumvent problems caused by epigenetic phenomena, but this is very inefficient in most plant species with the notable exception of the moss *Physcomitrella patens* (Decker and Reski 2004). Very recently it was shown that homologous recombination in higher plant species can be facilitated by the use of engineered zinc finger nucleases (ZFN) that introduce DNA double-strand breaks at

specific sites in the genome, thus stimulating homologous recombination events (Kumar et al. 2006). This technology could facilitate very precise engineering of transgenic plants in the future.

## 12.3 Post-Translational Modifications

The most important post-translational modification for biopharmaceuticals is N-glycosylation, since ~30% of all approved biopharmaceuticals are glycoproteins. Glycan chains affect half-life, stability, and functionality. The glycosylation machinery in plants is similar but not identical to its mammalian counterpart. In both cases, the N-glycosylation of a peptide chain starts with the co-translational transfer of an oligosaccharide precursor to asparagine residues within the consensus sequence N-X-S/T (X is any amino acid but proline) in the endoplasmic reticulum (ER). As the protein matures, the oligosaccharide precursor is trimmed to finally yield a glycoform known as the high mannose type, which is identical in plants and mammals. When the glycoprotein travels further down the secretory pathway the glycans are modified stepwise by enzyme activities located in the Golgi apparatus. The final complex-type N-glycans differ between plants and mammals. Plant glycoproteins contain β1,2-xylose and α1,3-fucose, which are absent in mammals, whereas mammalian glycoproteins contain β1,4 galactose and terminal neuraminic acid residues that are absent in plants. Proteins with plant-specific glycans may induce an immune response upon subcutaneous injection in mammals (Bardor et al. 2003). Although this response would be desirable in the case of plant-derived vaccines, it might limit the use of plant-derived therapeutics that have to be administered on a regular basis.

Therefore different strategies have been pursued to produce glycoproteins in plants with humanized glycans. By adding the H/KDEL sequence motif to the C-terminus of pharmaceutical proteins, they are effectively retained within the endoplasmic reticulum, which prevents the plant-specific modification in the Golgi from taking place. This strategy has been employed for the production of a mouse/human chimeric IgG1 antibody against the human chorionic gonadotropin (Sriraman et al. 2004). Other approaches aim to modify the endogenous plant glycosyltransferase system responsible for the transfer of β1,2-xylose and α1,3-fucose residues. In the moss *Physcomitrella patens*, the genes for β1,2-xylosetransferase and α1,3-fucosetransferase have been disrupted by gene targeting (Koprivova et al. 2004). This double knockout mutant has been used to produce secreted human erythropoietin that lacks the plant specific core β1,2-xylose and core α1,3-fucose (Weise et al. 2007). In plants that are less amenable for gene targeting, an RNA interference (RNAi) approach has been used to silence the endogenous β1,2-xylosetransferase and α1,3-fucosetransferase genes. By transforming the duckweed *Lemna minor* with a vector coding for the human anti-CD30 antibody MDX-060 and an inverted repeat construct homologous to β1,2-xylosetransferase and α1,3-fucosetransferase, a recombinant antibody was produced lacking

plant-specific N-glycans (Cox et al. 2006). The plant-derived MDX-060 antibody was compared to its counterpart produced in Chinese hamster ovary (CHO) cells in terms of its binding characteristics with respect to the human Fc receptor and its antibody-dependent cell-mediated cytotoxicity (ADCC) activity. The plant-derived antibody had >10-fold higher affinity for the human Fc receptor and 20-fold higher ADCC activity against a tumor cell line in vitro compared to the CHO-derived antibody. Similarly the HIV-1 neutralizing antibody 2G12 that was produced in a fucosyltransferase and xylosetransferase T-DNA *Arabidopsis* double-knockout line had a homogeneous mammalian-like N-glycosylation pattern (Schahs et al. 2007).

To further humanize the glycan structures of PMPs, the human β1,4-galactosyltransferase cDNA has been introduced into tobacco (Bakker et al. 2006; Fujiyama et al. 2007) and alfalfa (Sourrouille et al. 2008). Glycan analysis of a mouse IgG antibody that was expressed in the transgenic tobacco lines revealed the presence of terminal galactose residues in a subset of the analyzed glycopeptides. Surprisingly, the level of plant-specific core fucose and core xylose residues was also significantly reduced.

Many N-glycan structures of human origin contain terminal sialic acid residues. The presence or absence of this moiety strongly affects the plasma half-life of the corresponding glycoprotein. In the case of human erythropoietin, enzymatic removal of the terminal sialic acid residues reduces the serum half-life of this protein from >5 h to <2 min when injected intravenously in rats (Erbayraktar et al. 2003). Therefore efforts are ongoing to engineer the pathway that leads to the formation of CMP-sialic acid and the transfer of sialic acid to recombinant proteins in plants. Some of the key mammalian enzymes in this pathway – α2,6-sialyltransferase, UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase, N-acetylneuraminic acid phosphate synthase, and CMP-N-acetylneuraminic acid synthetase – have already been expressed successfully in *Arabidopsis* (Castilho et al. 2008; Wee et al. 1998).

In contrast to the numerous reports on the significance of N-glycosylation much less is known about the O-glycosylation of PMPs. This type of glycosylation occurs on hydroxyproline residues that are formed by the action of prolyl hydroxylases. This reaction typically occurs on clustered proline residues that are found within the extensin family of hydroxyproline-rich glycoproteins (HRGPs). A similar proline-rich sequence motif is found in the hinge region of human IgA1 antibodies, and consequently it became a substrate for O-glycosylation when a recombinant antibody of this subclass was produced in maize kernels (Karnoup et al. 2005). Whether O-glycosylation limits the use of such PMPs remains to be determined. In a recent study, a synthetic O-glycosylation motif consisting of a tandem repeat of the dipeptide serine/proline (SP) was fused to the C-terminus of human interferon α2b. As expected the chimeric proteins had higher molecular masses (up to 75 kDa for IFNα2-$(SO)_{20}$) due to the presence of O-glycans. When injected intravenously into mice, this engineered chimeric protein had a 13-fold longer serum half-life compared to standard IFNα2. The increased serum half-life was explained by slower renal clearance and greater resistance towards proteolytic degradation (Xu et al. 2007).

## 12.4 Downstream Processing

Downstream processing includes the post-harvesting steps needed to extract, purify, and formulate the active pharmaceutical ingredient. The first steps of this process are largely dictated by the expression system (Menkhaus et al. 2004). Although a secreted protein might be purified directly from the growth medium, possibly after a concentration step, an intracellular protein needs to be liberated from the surrounding cell matrix before purification. The crude extract will be subjected to clarification procedures consisting of centrifugation or filtration steps to remove cell debris and particles. During these initial steps, measures must be taken to limit the potential degradation of the target protein by proteolysis and to prevent unintended modification of the product by oxidation or the addition of phenolic groups (Pierpoint 2004). Additional adjustments of the feedstream with respect to the pH or salt concentration might be necessary to meet the needs for the subsequent chromatography steps, with two or more orthogonal separation methods typically used to maximize purity and contaminant removal (Drossard 2004). Potential contaminants include endogenous plant proteins, metabolites, and nucleic acids, and also environmental contaminants such as agrochemicals or microbes and insects associated with the plant. The actual chromatographic methods used depend on the physicochemical properties of the target protein. Common techniques include affinity chromatography with Protein A or lectins, ion exchange chromatography, hydrophobic interaction chromatography, and size exclusion chromatography. The design of the downstream process largely depends on the level of purity required for the protein's intended use, e.g. proteins for injection must be purer than proteins intended for topical administration.

When PMPs enter clinical development, the production process has to meet certain quality criteria that are defined by current good manufacturing practices (cGMP). The regulations for biopharmaceuticals (as defined by the Food and Drug Administration (FDA) in the USA and by the European Medicines Agency (EMEA) in the EU) apply for all biopharmaceuticals irrespective of the production platform. Whereas the production of biopharmaceuticals in plant suspension cells cultivated in fermenters is very similar to microbial systems and mammalian cells, the production process is quite different when whole plants are used as the expression hosts. To address these differences, guidance for the production of PMPs has been issued by the FDA and the EMEA, and consultations are underway to refine the regulations (Spok et al. 2008).

## 12.5 PMPs in Advanced Development

### *12.5.1 Glucocerebrosidase*

Recombinant glucocerebrosidase is needed to replace the nonfunctional enzyme present in patients with the monogenic disorder Gaucher disease, which is characterized by the inability to degrade glucosylceramides, which therefore accumulate

in the lysosomes of phagocytes. Clinical symptoms of the disease include hepatosplenomegalia, anemia, and thrombocytopenia. Patients are currently treated with a recombinant version of the enzyme (imiglucerase, Cerezyme) that is currently produced in CHO cells. The purified recombinant enzyme needs additional in vitro enzymatic treatments to expose the mannose residues of its N-glycan chains. These terminal mannose residues facilitate uptake of the enzyme into macrophages. The complex production process of imiglucerase makes it one of the most expensive biologicals to date with an annual treatment cost of ca. US $200 000 per patient (Kaiser 2008).

The Israeli biopharmaceutical company Protalix has developed an alternative production process using transgenic suspension cells derived from carrot roots (Shaaltiel et al. 2007). The 497-amino-acid enzyme is genetically fused to the N-terminal signal peptide from *Arabidopsis thaliana* basic endochitinase and to a C-terminal vacuolar targeting sequence from tobacco chitinase A. The purified plant-derived glucocerebrosidase (prGCD) contains two additional amino acids at the N-terminus and seven additional amino acids on the C-terminus compared to the mature human enzyme. Because prGCD is targeted to the vacuole, the complex type N-glycans are trimmed to expose mannose residues, a glycan structure known as the paucimannose type. This eliminates the need for artificial trimming of the glycans during downstream processing. At present, prGCD is undergoing a clinical phase III study to assess its safety and efficacy in Gaucher patients.

### *12.5.2 Insulin*

Millions of people suffer from insulin-dependent diabetes mellitus (type I diabetes), which is now among the most common causes of death in industrialized countries. The total demand for insulin exceeds 8000 kg year$^{-1}$. The mature insulin molecule is a small (5.8 kDa) non-glycosylated protein consisting of a 21-amino-acid A chain and a 30-amino-acid B chain connected by two disulfide bonds. The two chains are derived from a single precursor polypeptide (proinsulin) in which they are connected by a linking C-chain. The C-chain is cleaved off by limited proteolysis upon secretion from the Langerhans cells in the pancreas. Recombinant insulin is produced either by the separate expression of recombinant A and B chain mini-genes or by mimicking the natural route from proinsulin. Insulin was the first biopharmaceutical produced by recombinant DNA technology. Recombinant human insulin achieved market approval in 1982 and current demands are met by production processes using *Escherichia coli* (Chance and Frank 1993) and *Saccharomyces cerevisiae* (Kjeldsen 2000). Plant-derived human insulin has been produced by the genetic fusion of a mini-proinsulin polypeptide with a shortened C-chain to the C-terminus of oleosin, which allows expression in oilseed crops and accumulation in oil bodies. The chimeric protein is targeted to the ER-derived

oleosomes in the seeds, and because of the unique properties of these organelles, they can be purified easily by floating centrifugation and the recombinant insulin can be proteolytically cleaved off and further purified by chromatography. Human recombinant insulin ($DesB_{30}$ insulin) produced with this method in *Arabidopsis* seeds is as effective in an insulin tolerance test as human standard insulin (Nykiforuk et al. 2006). The Canadian company SemBioSys Genetics has commercialized the oleosin fusion system and uses safflower (*Carthamus tinctorius*) for the production of insulin. The company recently announced the launch of a clinical phase I/II trial in the UK with healthy volunteers to demonstrate the equivalence of the plant-derived insulin (SBS-1000) to currently marketed recombinant human insulins (Moloney et al. 2008).

### *12.5.3 Idiotype Vaccines*

Idiotype vaccines are patient-specific vaccines developed for patients suffering from clonal diseases such as B cell lymphoma (non-Hodgkin lymphoma, NHL). The malignant cells carry individual immunoglobulins (idiotypes) on their surface which can be used to trigger a specific immune response. Currently, the vaccines are produced from patient B-cell tumor cells that are expanded as human/mouse heteromyelomas. The monoclonal idiotype antibody is subsequently fused to an immunogenic carrier protein such as keyhole limpet hemocyanin (KLH) and injected, usually together with granulocyte-macrophage colony stimulating factor (GM-CSF) as an adjuvant. These vaccines are currently in clinical development (Sinha et al. 2008).

Recently, a plant-based production platform has been established to shorten the time required to derive such a vaccine from the patient's biopsy. For this purpose, the variable domains of the idiotype are cloned as single-chain antibodies (scFv) and transiently expressed using a viral vector system in *Nicotiana benthamiana* plants (McCormick et al. 2003). Plant-derived idiotype vaccines have been tested in a phase I clinical trial on 16 NHL patients after an initial chemotherapy (McCormick et al. 2008). The safety and immunogenicity of the vaccines were analyzed at two different doses and in the presence or absence of GM-CSF. Most of the patients displayed a cellular immune response while only three patients mounted a vaccine-specific humoral immune response. There were no severe adverse reactions in these trials. The development of plant-derived idiotype vaccines was initiated by the US-based Large Scale Biology Company (which filed for Chapter 11 bankruptcy in 2006). Further research has been undertaken by the German company Icon Genetics, which is a subsidiary of Bayer. The Bayer group has announced its intention to conduct another phase I clinical trial in 2009, using the Icon Genetics Magnifection technology discussed above.

### *12.5.4 Interferon*

There are three classes of interferons (IFN-α, IFN-β, IFN-γ) with the α and β classes grouped together as 'type I interferons' because they share 30% sequence identity and recognize the same receptor, and IFN-γ representing the 'type II interferons' because it is more distantly related and recognizes a distinct receptor. All three interferons have been produced as recombinant proteins and are approved for the treatment of various conditions, including chronic viral infections (especially hepatitis B and C), multiple sclerosis and certain types of cancer. All the interferons have a low molecular mass (∼30 kDa) and are thus efficiently eliminated from the body by renal filtration. Unmodified interferons therefore have a plasma half-life of ∼4 h. The plasma half-life can be increased significantly by attaching the polymer polyethylene glycol (PEG), up to a maximum of ∼25 h.

IFN-α2b produced in duckweed (*Lemna minor*) by the United States company Biolex Therapeutics is currently being evaluated in clinical trials (De Leede et al. 2008). IFN-α2b is a 19-kDa single-chain non-glycosylated protein that is currently produced in *E. coli* for therapeutic use. In a clinical phase I dose escalation study, the plant-derived interferon (BLX-883) was administered in a controlled release formulation in poly(ether-ester) microspheres (Locteron) to healthy volunteers. The product was well tolerated at all tested doses and the most common adverse effects (influenza-like symptoms, injection site reactions, headache) were similar to those observed in test subjects receiving Peginterferon alpha-2b (PEGIntron). In a subsequent clinical phase I/II trial, the antiviral properties of Locteron are being tested in patients with chronic hepatitis C, in comparison with PEGIntron.

## 12.6 Conclusion

The large number of biopharmaceuticals now in clinical development demonstrates the need to increase current production capacities and to identify and develop alternative production systems. Plants are regarded as attractive production platforms because they can offer a virtually unlimited supply when cultivated on an agricultural basis. However the cultivation of transgenic pharmaceutical plants in the open field requires appropriate safety measures to exclude the contamination of food and feed supplies. From the above-mentioned case studies, only safflower is cultivated in the open field, whereas the others are propagated in closed systems such as greenhouses, basins, and fermenters.

As plant expression platforms continue to improve in terms of post-translational modifications and overall protein yield, more PMP processes are likely to become economically feasible in the future. The whole field will definitely profit if one of the PMPs currently undergoing clinical development receives market approval. A step in this direction has been made in the field of veterinary medicine when

Dow AgroSciences received approval in 2006 for their poultry vaccine against Newcastle disease, produced in cultivated tobacco suspension cells. It is also clear that the development and approval of PMPs will be encouraged by the establishment of solid production guidelines by the relevant regulatory bodies.

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