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Review

Plant molecular farming for the production of valuable proteins – Critical evaluation of achievements and future challenges

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ABSTRACT

Recombinant proteins play an important role in many areas of our lives. For example, recombinant enzymes are used in the food and chemical industries and as high-quality proteins for research, diagnostic and therapeutic applications. The production of recombinant proteins is still dominated by expression systems based on microbes and mammalian cells, although the manufacturing of recombinant proteins in plants – known as molecular farming – has been promoted as an alternative, cost-efficient strategy for three decades. Several molecular farming products have reached the market, but the number of success stories has been limited by industrial inertia driven by perceptions of low productivity, the high cost of downstream processing, and regulatory hurdles that create barriers to translation. Here, we discuss the technical and economic factors required for the successful commercialization of molecular farming, and consider potential future directions to enable the broader application of production platforms based on plants.

1. The beginning of molecular farming in plants

In the early 1980s, a presentation on plant viruses at the Max-Plank Institute in Cologne inspired Fritz Kreuzaler to develop the concept of antibody-mediated disease resistance via the expression of pathogen-specific antibodies in plants. Although his contemporaries thought it unlikely that functional full-length antibodies would assemble from two heavy chains and two light chains without mammalian chaperones, Kreuzaler and his team nevertheless confirmed the presence of an assembled full-size antibody following the injection of cDNAs encoding the heavy and light chains of a murine IgM into the nuclei of *Acetabularia mediterranea*, an algal species with large cells used as model organism in cell biology. The sequences coding for the N-terminal signal peptide that allows co-translational translocation into the endoplasmic reticulum were removed from the antibody genes to allow the recombinant proteins to accumulate in the cytosol. The presence of the assembled antibody was confirmed using an anti-idiotope antibody that binds specifically to the assembled immunoglobulin and not to the individual antibody heavy and light chains. This groundbreaking work, presented initially as a doctoral thesis (Stieger, 1987) before publication in a journal article (Stieger et al., 1991), demonstrated for the first time that plant cells have the capacity to produce complex, multimeric mammalian proteins. Subsequently, a functional murine full-size IgG1 antibody was produced in transgenic tobacco plants (Hiatt et al., 1989) and recombinant human serum albumin was expressed in transgenic potato and tobacco plants as well as tobacco cell cultures derived from the transgenic line (Sijmons et al., 1990). In both studies recombinant proteins contained N-terminal signal peptides enabling their targeting to the secretory pathway favoring protein folding and stability resulting in higher accumulation levels (Schillberg et al., 1999).

These pioneering studies marked the beginning of molecular farming, which describes the utilization of plants as host platforms to produce valuable recombinant proteins. Many additional publications followed, often focusing on pharmaceutical proteins. This was based on assumptions that the production of such high-value proteins in plants would reduce costs compared to microbes and mammalian cells, which

Abbreviations: CHO, Chinese hamster ovary; EMA, European Medicines Agency; ETEC, enterotoxigenic *Escherichia coli*; FDA, Food and Drug Administration; Gb3, globotriaosylceramide; GLP, good laboratory practice; GMP, good manufacturing practice; GRAS, generally recognized as safe; hEGF, human epidermal growth factor; HEK, human embryogenic kidney; HN, hemagglutinin-neuraminidase; IND, investigational new drug; PK/PD, pharmacokinetic/pharmacodynamics; QVLP, quadrivalent virus-like particle; USDA, United States Department of Agriculture.

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require expensive fermenter infrastructure and clean rooms to ensure compliance with pharmaceutical good manufacturing practice (GMP). The cultivation of plants in greenhouses or the open field is indeed significantly less expensive and more scalable than fermenter systems (Twyman et al., 2003; Buyel et al., 2017). However, it was soon recognized that open-field cultivation makes it more difficult to control product quality and safety, and that downstream processing (the extraction and purification of target proteins from the plant matrix) is a major cost driver requiring process optimization, as discussed in more detail below.

Hundreds of different proteins have been produced successfully in plants, including pharmaceutical proteins such as antibodies, vaccines, hormones and enzymes, as well as proteins for diagnostic, research and cosmetic applications. A unique feature of plant-based protein expression is the diversity of plant species and systems used for production. Whereas conventional biopharmaceutical manufacturing involves a small number of well-established platforms, such as the bacterium *Escherichia coli*, the yeast *Saccharomyces cerevisiae*, Chinese hamster ovary (CHO) cells, murine NS0 and Sp2/0 cells, and human embryonic kidney (HEK293) cells, many different plant species have been proposed as production platforms, including tobacco, potato, tomato, alfalfa, safflower, carrot, lettuce, strawberry, moss, duckweed, maize, wheat and rice. Furthermore, the nature of the system ranges from whole plants to organ cultures (such as hairy roots) and cell suspension cultures. Different expression strategies can also be exploited, such as (i) stable transgenic or transplastomic lines that are highly scalable vs transient expression in non-transgenic plants for rapid manufacturing; (ii) constitutive vs tissue-specific vs inducible expression; and (iv) different protein targeting strategies to control product accumulation or post-translational modification and to facilitate downstream processing (Nölke et al., 2003; Twyman et al., 2005; Schillberg et al., 2013). The diversity of molecular farming platforms and corresponding product categories has been reviewed (e.g. by Spiegel et al., 2018).

The variety of plant species, systems, expression methods and protein targeting strategies means that a suitable platform is likely to be available for any conceivable protein product. However, the systematic comparison of different platforms to identify the most suitable on a case-by-case basis is laborious and time-consuming, and the absence of a standard platform has obstructed efforts to optimize productivity and downstream processing. Accordingly, the molecular farming community has begun to consolidate around a few platforms, with tobacco (*Nicotiana tabacum*) and its close relative *N. benthamiana* as the most widely used species (Spiegel et al., 2018).

Ultimately, Kreuzaler's vision of antibody-mediated disease resistance in crops was also realized. Plants engineered to produce pathogen-specific full-size antibodies or fragments thereof have been generated, conferring tolerance or even complete resistance against viral, bacterial, and fungal pathogens (Tavladoraki et al., 1993; Voss et al., 1995; Safarnejad et al., 2011).

2. Success stories

Many of the early molecular farming studies demonstrated the feasibility of recombinant protein production in a given plant species and confirmed the functionality of the protein product using diagnostic and/or cell-based assays. In the case of pharmaceutical proteins, additional studies were often carried out in animals. For example, the hepatitis C virus E1E2 heterodimer produced transiently in lettuce triggered immune responses in mice (Clarke et al., 2017), and domain III of the West Nile virus envelope protein produced in *N. benthamiana* protected mice against lethal West Nile virus infections (Lai et al., 2018). The transient coexpression of Dengue virus structural proteins and truncated non-structural RNA-dependent RNA polymerase in *N. benthamiana* resulted in the formation of virus-like particles (VLPs), which induced a strong antibody response in mice (Ponndorf et al., 2020). These and similar studies have shown that plants can be used to produce vaccines and other proteins for biopharmaceutical applications.

Plants are particularly suitable for the production of animal vaccines because crude plant extracts can be fed to or injected into animals, avoiding protein purification and therefore significantly reducing production costs. This is essential for the manufacture of animal vaccines because the market will only tolerate products costing a few cents per dose (Topp et al., 2016). For example, piglets were protected against enterotoxigenic *E. coli* (ETEC) when fed on diets supplemented with *Arabidopsis thaliana* seeds expressing anti-ETEC antibodies (Virdi et al., 2013), and crude extracts of *N. benthamiana* leaves transiently expressing the influenza virus H5 trimer prevented the spread of avian influenza (H5N1) when injected into chickens (Phan et al., 2020).

Oral vaccines have also been tested in humans (Kurup and Thomas, 2020). Edible plants such as tomato, lettuce and banana have been selected because they have been granted GRAS (generally recognized as safe) status, and bananas also offer an additional advantage because the tough skin protects the vaccine-containing fleshy fruit from damage and desiccation. Human volunteers fed on transgenic raw potato tubers expressing the Norwalk virus capsid protein as VLPs showed a strong immune response to the antigen (Tacket et al., 2000). However, oral vaccines in plant tissues can be destroyed in the stomach before they make contact with the gut-associated lymphoid tissue, so high accumulation levels are required to ensure efficacy or the product must be encapsulated within storage compartments to offer a degree of protection. Furthermore, batch-to-batch consistency is difficult to achieve when using fruits or tubers. This can be addressed by producing oral vaccines in plant cell suspension cultures, allowing the preparation of homogenous mixtures with a uniform vaccine concentration (Schillberg et al., 2013; Arévalo-Villalobos et al., 2020).

Other clinical trials have been carried out to test plant-derived antibodies or replacement human proteins such as insulin for diabetes, gastric lipase for cystic fibrosis, and glucocerebrosidase for Gaucher's disease (Yao et al., 2015; Spiegel et al., 2018). The production process used to manufacture pharmaceutical proteins for clinical trials must comply with GMP, which is easier to achieve with cell suspension cultures and hairy roots in bioreactors because production can take place entirely within a cleanroom environment, analogous to systems based on microbes and mammalian cells. This is not possible for whole plants grown in soil, Rockwool blocks or hydroponic systems. Therefore, the regulatory authorities allow upstream manufacturing to take place in a non-GMP environment as long as the clarified extracts of plant tissue are aseptically transferred to the GMP environment for further processing (Fischer et al., 2012, 2013, Sack et al., 2015). Even so, the upstream cultivation step must be well controlled to ensure uniform plant growth and to avoid contact with insects, rodent excreta and pesticides, because inconsistent growth conditions and contaminants can affect product yield and quality. The development of these guidelines enabled the production of an HIV-specific monoclonal antibody in transgenic tobacco plants for use in a first-in-human phase I clinical trial, which evaluated a single vaginal administration of the antibody in healthy females. This confirmed the safety and tolerability of the plant-derived pharmaceutical protein (Ma et al., 2015).

3. Market maturity

The first molecular farming products, which were commercialized more than 20 years ago, were avidin and β-glucuronidase produced in transgenic maize plants (Hood et al., 1997, 1999). Both proteins are used as research reagents, and avidin is still sold by Merck via its subsidiary MilliporeSigma (catalog no. A8706). Since then, many diagnostic and technical proteins have been produced in plants, and some companies (e.g. Leaf Expression Systems, Agrenvec, and Diamante) have added this protein category to their portfolios. The advantages of these proteins produced in plants include the authentic presentation of plant allergens for the detection of allergies (Santoni et al., 2019) and the absence of contamination with bacterial endotoxins or animal-derived

components, which is a prerequisite when preparing growth factors and cytokines for animal cell culture or food applications. The latter is true for stably transformed plants, but most transient expression systems involve the infiltration of leaf tissues with bacteria, which produce endotoxins under stress. In this case, special care has to be taken to remove these toxins during downstream processing (Arfi et al., 2016). Another molecular farming product already on the market is human epidermal growth factor (hEGF) produced by ORF Genetics in transgenic barley plants, which are cultivated all year round in Icelandic greenhouses powered by geothermal energy (Magnusdottir et al., 2013). The purified protein is used as a bioactive cosmetic ingredient (Schouest et al., 2012).

Thus far, only one plant-derived biopharmaceutical product has received market authorization following the completion of phase I–III clinical trials in humans. This product is taliglucerase alfa, a recombinant form of human glucocerebrosidase produced in plant cell suspension cultures by the Israeli company Protalix Biotherapeutics (Zimran et al., 2018a). Taliglucerase alfa was approved by the US Food and Drug Administration (FDA) in May 2012 for injection as an enzyme replacement therapy for the treatment of adult patients with Gaucher's disease and was marketed as Elelyso. Another version of the recombinant enzyme (imiglucerase) is produced in CHO cells (Hollak et al., 2010). After purification from the culture medium, the glycan chains of imiglucerase must be processed enzymatically *in vitro* to expose terminal mannose residues that are required for the efficient uptake of the enzyme into macrophages. In contrast, taliglucerase alfa does not require these additional processing steps because it is targeted to the cell vacuole, where the complex type *N*-linked glycans are trimmed to the paucimannosidic form, exposing the mannose residues naturally (Shaaltiel et al., 2007). Trimming in the vacuole does not remove plant-specific α1,3-fucose and β1,2-xylose residues that have the potential to trigger immune responses, although the treatment of Gaucher's disease patients with taliglucerase alfa has been well tolerated in multiple clinical trials, with no reported anti-glycan effects during clinical development or post-authorization (Pastores et al., 2014, 2016; Zimran et al., 2018a, b).

Although taliglucerase alfa is the only plant-made recombinant protein approved for pharmaceutical use in humans following standard clinical approval, some further product candidates have now reached late-stage clinical studies. For example, pegunigalsidase alfa is a recombinant form of human globotriaosylceramide (Gb3) produced in tobacco cells by Protalix Biotherapeutics as an enzyme replacement therapy for Fabry disease, and is currently undergoing phase III testing (van der Veen et al., 2020). A plant-derived vaccine for seasonal influenza has also been tested in two phase III studies by the Canadian company Medicago, which has large-scale production facilities in Canada and the US. Influenza hemagglutinin proteins were transiently expressed in *N. benthamiana* to form enveloped VLPs that resemble wild-type influenza virions but do not contain viral RNA. Monovalent VLPs composed of hemagglutinins from the strains recommended for 2017/2018 or 2018/2019 conventional vaccines were combined to produce each season's quadrivalent virus-like particle (QVLP) formulation. Both clinical studies showed that the plant-derived QVLP vaccine provided substantial protection against respiratory infections caused by influenza virus (Ward et al., 2020). Although more studies over several seasons are required to demonstrate the full potential of the plant-derived seasonal influenza vaccine, it is clear that transient expression platforms in particular could facilitate the rapid stockpiling of emergency vaccines (e.g., for new strains of pandemic influenza, and other emerging and re-emerging diseases).

Accordingly, transient expression in *N. benthamiana* was used to produce ZMapp, an experimental cocktail of three monoclonal antibodies for the treatment of Ebola patients (Park and Wi, 2016). The three monoclonal antibodies (c13C6, c2G4, and c4G7) were produced separately by the US company Mapp Biopharmaceutical and combined into a clinical-grade product within 4–6 months. ZMapp was first used on humans during the 2014 West Africa Ebola virus outbreak, and was granted temporary authorization under the animal efficacy rule before clinical trials. Five of seven patients receiving the experimental drug survived, but ~9 g of the cocktail was required per patient, which in turn requires 30–50 kg of leaf tissue. Unsurprisingly, a consortium of non-profit, governmental, and industrial partners was subsequently formed to produce Ebola-specific antibodies in CHO cells to meet the demand generated by an Ebola epidemic (Pettit et al., 2016). The NIH ran a clinical trial of plant-produced ZMapp in 2015 with subjects from Sierra Leone, Guinea and Liberia. However, the epidemic waned and the trial closed early, leaving it statistically underpowered. Detailed structural analysis of ZMapp subsequently revealed that c2G4 and c4G7 recognize overlapping epitopes and compete to bind the Ebolavirus envelope glycoprotein. The more recent chimeric MIL77E cocktail containing only c13C6 and c2G4 was produced in CHO cells and achieved full protection in non-human primates challenged with a lethal Ebolavirus strain (Qiu et al., 2016).

In 2006, the United States Department of Agriculture (USDA) approved an animal health vaccine developed by Dow AgroSciences LLC produced in stably transformed transgenic tobacco NT-1 suspension cells (Fox, 2006). The vaccine protects poultry against the Newcastle disease virus, and was the first biopharmaceutical product derived from plant cell cultures to receive regulatory approval (Schillberg et al., 2013). The vaccine is the recombinant hemagglutinin-neuraminidase (HN) glycoprotein, one of two viral surface glycoproteins and the major surface antigen that induces neutralizing antibodies and a protective immune response in domestic poultry and other avian species. The HN protein was extracted from the tobacco cells and the crude extract was injected into chickens, thus reducing the production costs enough for commercial viability. The unpurified HN protein conferred full protection in virus challenge studies, but was not commercialized by Dow AgroSciences following a strategic business decision.

4. Barriers to wider market penetration

The small number of molecular farming products reaching the market compared to the large number of research studies indicates the presence of a commercial development bottleneck, particularly in the pharmaceutical industry. The most widely perceived limitations of molecular farming include (i) the low productivity of plants, (ii) the high costs of downstream processing, and (iii) the slow translation to applications.

(i) Low productivity. Compared to industrial microbial and mammalian production platforms, the protein yields of many plant-based systems are rather low. Recombinant protein levels rarely exceed 100 μg/kg fresh weight of plant tissue or per liter in suspension cell cultures, despite extensive research to optimize protein expression and stability. This research has focused not only on intrinsic factors such as expression strategies, protein fusion and targeting strategies (Hauptmann et al., 2015; Schwestka et al., 2020), and the co-expression of protease inhibitors (Mandal et al., 2016), but also on external factors such as nutritional and physical cultivation parameters affecting plant growth and fitness (Twyman et al. 2013). However, some plant-based systems can achieve extraordinary expression levels: for example, up to 4 g/kg fresh weight by transient expression (Marillonnet et al., 2005; Yamamoto et al., 2018) and 46 % of total soluble protein in transgenic plants (De Cosa et al., 2001). But it has to be considered that high levels in transient expression studies were achieved when using the model proteins such as the green fluorescent protein (GFP), a particularly simple and stable protein. The exceptional high productivity in transgenic plants was realized via transplastomic expression, but expression in the chloroplast may not be suited for all secretory proteins, which represent the vast majority of pharmaceutical proteins. For balance, it is notable that not all proteins are produced in high quantities by microbes and mammalian cells, although expression levels of 5–10 g/L are not uncommon in industrial processes and exceptional titers of 20 g/L have been achieved for some antibodies (Pujar et al., 2017; Schillberg et al.,

2019). The intrinsic protein biosynthesis capacity of plant cells is impressive, reaching a cell-specific productivity (qP) of 8 pg per cell per day (Havenith et al., 2014), only one order of magnitude below elite CHO lines (Hansen et al., 2017). But in terms of volume, a typical plant cell is ~1000 times larger than a CHO cell and ~10,000 times larger than a bacterial cell, resulting in a comparatively much smaller number of protein-producing cell units per kilogram of tissue or liter of medium.

The large size of the plant cell relative to animal cells and microbes is mainly conferred by the vacuole, which stores water, ions, and various metabolites. The size of plant cells in suspension cultures can therefore be reduced by increasing the osmolality of the culture medium, causing the vacuole to shrink and allowing more cells to fit in the same packed cell volume (unpublished data). This approach has been shown to increase productivity (Ullisch et al., 2012; Vasilev et al., 2013). Even so, a substantial vacuole is still present in the cells and the change in volume is not sufficient to close the productivity gap with mammalian cells and microbes entirely. Interestingly, the creation of cell-free lysates by removing the vacuole to concentrate the protein biosynthesis apparatus can achieve higher protein yields than intact plant cells (Buntru et al., 2014, 2015). Tobacco BY-2 cell lysates can synthesize recombinant proteins with yields of up to 3 mg/mL lysate by *in vitro* transcription-translation in batch reactions, which is ~15 times higher than other eukaryotic cell-free systems (Schillberg et al., 2019, unpublished data). The tobacco BY-2 lysate has been commercialized by the German company LenioBio and is currently used for screening in small reaction volumes of 50–100 μL, and for the production of recombinant proteins that are difficult to express in living plant cells due to their toxicity (Havenith et al., 2017; Huck et al., 2017). Cell-free batch reactions have been successful at volumes of up to 6 mL, and the process is now being scaled up to 1 L and 10 L, which will allow the production of tens of grams of protein per batch.

(ii) High costs of downstream processing. The production of recombinant proteins by microbes and mammalian cells is facilitated by the secretion of those proteins to the medium, because most host proteins are retained within the cell. In principle, this also applies to plant cell suspension cultures, hairy roots and rhizosecretion systems based on the hydroponic cultivation of whole plants (Häkkinen et al., 2014; Raven et al., 2015; Madeira et al., 2016). However, plant cells also secrete a number of host proteins into the medium, including proteases that can degrade the target recombinant protein, which makes the purification process more challenging (Mandal et al., 2016; Schillberg et al., 2019). In most whole-plant systems, the recombinant protein is engineered to be retained within a storage compartment inside the cell or secreted to the apoplast (the space outside the plasma membrane including the cell wall). Purification can be achieved in some cases by centrifugation, drawing the protein from the apoplast into a buffer. Typically, however, the plant tissue and cells must be disrupted by homogenization to release the target protein, simultaneously releasing large quantities of host proteins as well as insoluble particles and fibers. The clarification of plant tissue homogenates therefore requires additional filtration and precipitation steps, which increase the costs of downstream processing. Nevertheless, efficient larger-scale processes have been established for recombinant protein extraction and purification from plant tissues (Ma et al., 2015). For example, a human antibody was produced in transgenic tobacco plants at yields of 400 mg/kg fresh leaf weight. From 200 kg of harvested leaf material, 77 *g* of antibody was isolated by mechanical extraction, sequential filtration with decreasing exclusion sizes, and purification by Protein A chromatography, CaptoAdhere chromatography, ultrafiltration, and a final diafiltration step (Schillberg et al., 2019). The purity of the antibody in the final eluate was >90 % and the cost per gram of purified antibody was €1137 including labor, consumables, infrastructure depreciation for plant cultivation and downstream processing, and all necessary analytics. The downstream process costs represented 84 % of the overall costs. Another report calculated the manufacturing costs for a lectin produced by transient expression in *N. benthamiana* (Alam et al., 2018). Techno-economic modeling assumed a yield of 520 mg/kg leaf biomass, a recovery efficiency of 70 %, and a purity of >99 %. The estimated total cost per gram of purified lectin was $106, an order of magnitude lower than the antibody described above. The difference mainly reflected the cost of the Protein A matrix for the first antibody purification step. Downstream processing costs for the plant-derived lectin therefore represented <50 % of the overall costs. This techno-economic modeling is promising because it suggests that the costs of manufacturing plant-derived proteins are low enough to compete with microbial and mammalian production processes, although additional larger-scale processes must be tested to confirm these theoretical projections.

(iii) Slow translation to applications. The third major barrier to the uptake of molecular farming is the uncertain IP and regulatory landscape compared to industrial microbial and mammalian cell expression systems, which have a long record particularly in the area of biopharmaceutical manufacturing. Industry therefore continues to perceive molecular farming as a risk and in most cases prefers to rely on its tried and tested platforms. Molecular farming companies tend to own IP portfolios for their own expression systems, which should in theory provide confidence to industrial partners. However, the restriction to individual proprietary technologies effectively causes industry partners to be locked in by the limitations of individual platforms, which restricts their freedom to operate.

To address this constraint, the EU-funded project Pharma-Factory aims to provide freedom to operate for transient expression in plants, including tools such as expression vectors, *Agrobacterium* strains, and plant varieties, as well as the corresponding procedures (https://pharmafactory.org). This could build a new strategy for the commercial exploitation of molecular farming, in which unique IP would be assigned to the product rather than the platform. More effort is required in this direction, especially to facilitate the translation of plant-made pharmaceutical proteins to the clinic. Many academic studies demonstrate the production and functionality of plant-made biopharmaceuticals as a lead-in for clinical development. However, preclinical and clinical studies are often blocked because the candidate is not suitable for further development, or there is insufficient clinical expertise or translational funding. Fig. 1 shows the development pipeline for pharmaceutical proteins, highlighting the extensive evaluation of pharmacokinetic/pharmacodynamic (PK/PD) properties, safety and efficacy, administration route, dosing and dosing schedule in animal models prior to investigational new drug/clinical trial authorization (IND/CTA) status. Furthermore, active pharmaceutical ingredients must be produced according to good laboratory practice (GLP) and GMP to enable toxicity studies. A target product profile (TPP) should be developed outlining (i) the disease indication(s) to be investigated, (ii) the patient population to be treated (e.g., patient selection and/or stratification based on biomarkers), (iii) clinical endpoints to be achieved using single or combination therapy, and the comparator, (iv) tolerable side effect profile, and (v) market access strategy. Most plant molecular farming studies do not consider any of these issues.

Clinical development optionally begins with Phase 0 for the assessment of PK/PD but this is often rolled together with Phase I (safety assessment). If safety is confirmed, Phase II trials assess preliminary efficacy in small groups, usually compared to a placebo, and also establish dosing requirements (Phase IIa) and therapeutic range (Phase IIb). In some cases, Phase I/II data may be gathered at the same time. Phase III trials confirm safety and efficacy in larger groups prior to market authorization, although this stage may be skipped or carried out with small groups under orphan drug rules where the patient population is small (taliglucerase alfa benefited from orphan drug designation). Following successful Phase III trials, marketing authorization for molecular farming products is sought under the rules set out by the appropriate regulatory authority, for example a biologics license application (BLA) under the FDA system or market authorization via the EMA's Committee for Medicinal Products for Human Use (CHMP). In addition, reimbursement by the health care systems in every country of

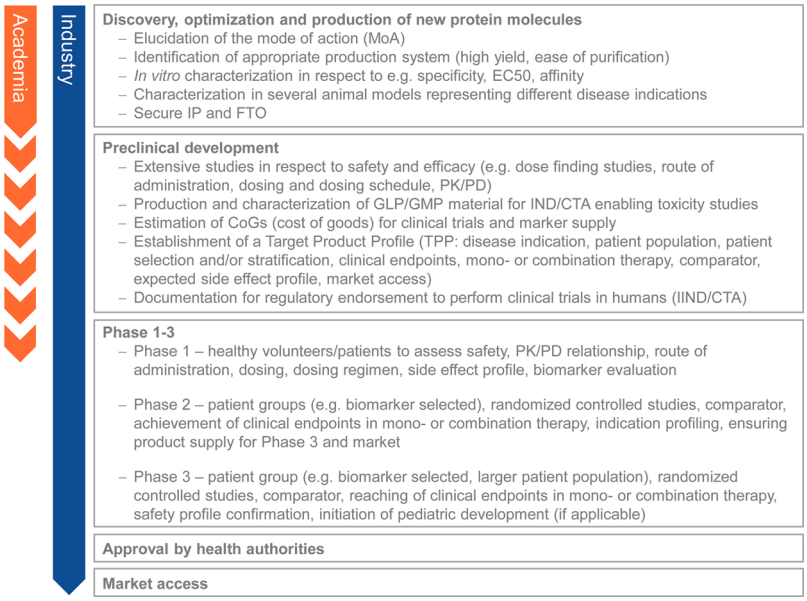

Fig. 1. From bench to market. Many academic molecular farming projects focus on the production of pharmaceutical proteins and demonstrate proof of concept by testing protein functionality in cell-based assays, animal models and in some cases human clinical trials. However, the successful marketing of pharmaceutical proteins requires additional steps that generally require industry resources or collaborations.

approval has to be negotiated. Considering this long and complex development pipeline, it is necessary to support joint translational projects involving academic groups and the pharmaceutical industry to provide more success stories for plant-made proteins.

5. Strategic advances and future perspectives

Plant molecular farming is still a niche platform in terms of the broad market introduction of protein products. The large size of plant cells and the resulting lower number of protein synthesis factories per unit volume compared to microbial and mammalian cells may be one of the greatest limitations. However, plant-based protein production has several unique benefits, including the advanced technology for glycan modification, animal component-free and endotoxin-free manufacturing of allergens and diagnostic proteins (also important for the production of therapeutics for some religious communities, vegans, and people with animal allergies), and the affordable production of mucosal vaccines and therapeutics, because topical and oral administration avoids the need for expensive downstream processing, reducing the manufacturing costs enough to make such products much more economically competitive. But the most important advantage is the production speed of transient expression systems, allowing the manufacturing of recombinant proteins within a few days. This platform is particularly suitable for the production of emergency vaccines and diagnostics, for example as countermeasures against novel influenza virus strains and SARS-CoV-2, which are needed within a few weeks or months after confirming the virus gene sequence (Capell et al., 2020; Rosales-Mendoza et al., 2020). The recent COVID-19 pandemic has once again shown that, in such emergency scenarios, protein production capacities become scarce very quickly, because the manufacturing of other drugs and diagnostics cannot be stopped or delayed in the face of a new disease. Transient expression in plants provides a strategy to close production gaps quickly: the plants can be grown while the pathogen's genome sequence is investigated, and are then ready for protein production as soon as antigen sequences are available. Many academic and industrial groups are therefore using transient expression in plants to produce diagnostics and therapeutics against SARS-CoV-2. The extent to which molecular farming can assert itself over other protein production systems remains to be seen, but the unique benefits of transient expression should allow plants to carve an important niche market for recombinant proteins as diagnostic and therapeutic agents against infectious diseases.

Credit author statement

Stefan Schillberg and Ricarda Finnern have written and revised the manuscript.

Declaration of Competing Interest

Stefan Schillberg is member of the Scientific Advisory Board of LenioBio GmbH distributing the tobacco BY-2 cell-free lysate developed by Fraunhofer IME and Dow AgroSciences.

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