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Plant-derived pharmaceuticals – the road forward

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Plant-derived pharmaceuticals are poised to become the next major commercial development in biotechnology. The advantages they offer in terms of production scale and economy, product safety, ease of storage and distribution cannot be matched by any current commercial system; they also provide the most promising opportunity to supply low-cost drugs and vaccines to the developing world. However, despite the promised benefits, the commercialization of plant-derived pharmaceutical products is overshadowed by the uncertain regulatory terrain, particularly with regard to the adaptation of good manufacturing practice regulations to field-grown plants. The success of such products also depends on careful negotiation of the intellectual property landscape, particularly the achievement of freedom-to-operate licenses for use in developing countries.

The roots of plants and pharmaceuticals

Plants and their products have been used for centuries to prevent and cure disease. More than a quarter of all the medicines used in the world today contain ingredients derived from plants [1]. However, it is only recently that biotechnology has been used to generate plants that produce specific therapeutic proteins, products that are traditionally synthesized using recombinant microbes or transformed mammalian cells [2]. The first of these plant-derived pharmaceutical proteins (PDPs) are now approaching commercial release [3,4]. This article considers the path ahead for PDPs and the challenges that must be addressed if these products are to achieve the commercial success of their counterparts produced using more traditional systems.

Current situation

The commercialization of PDPs will follow hot on the heels of research-grade plant-derived proteins. Three of these have been marketed to date: avidin, trypsin and β-glucuronidase, which are all produced in maize and developed by Prodigene Inc. (http://www.prodigene.com/) [5–7]. Many other companies with molecular farming portfolios are now initializing the large-scale production of technical proteins as a prelude to commercial release. Examples include the recent agreement between Large Scale Biology Corp. (http://www.lsbc.com/) and Sigma-Aldrich Co. (http://www.sigmaaldrich.com/) for the commercial-scale production of interferons α-2a and α-2b, and a similar agreement between Chlorogen Inc. (http://www.chlorogen.com/) and Sigma-Aldrich for the production of four undisclosed proteins.

In the case of proteins intended for medical or veterinary use, clinical trials form a necessary part of the product development pipeline. At the time of writing, at least ten unique PDPs have been submitted for clinical trials in humans (Table 1), although the total number is somewhat higher because several plant-derived recombinant subunit vaccines have been trialed independently by different groups. Large Scale Biology Corp. has submitted for phase I trials at least 12 scFv-type monoclonal antibodies produced using viral vectors in tobacco for the patient-specific treatment of non-Hodgkin's lymphoma. Other companies have products that have reached the end of pre-clinical testing and are poised for clinical trials. Such products include α-galactosidase for the treatment of Fabry disease (Large Scale Biology Corp.) and RhinoRx, a monoclonal antibody produced in tobacco for the treatment of respiratory syncytial virus infections in infants (Planet Biotechnology Inc., http://www.planetbiotechnology.com/). To date, only one PDP has been withdrawn from clinical trials. This was the monoclonal antibody Avicidin, developed jointly by the now disbanded Monsanto Protein Technology (http://www.mpt.monsanto.com/) and NeoRx Corp. (http://www.neorx.com/). Although the antibody showed promising results in patients with advanced colon and prostate cancer, several participants suffered side effects and the product was withdrawn. Similar side effects caused by the same antibody produced in mammalian cells indicated that this was not a problem unique to the plant-based production system.

Several PDPs for human use are approaching the market but it is likely that the first commercial PDP will

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Table 1. Plant-derived pharmaceutical proteins for potential medical use that have reached clinical development

Product	Medical condition treated by the drug	Source	Status	Refs
Vaccines				
E. coli heat labile toxin	Diarrhea	Maize, potato	Two independent phase I trials	[28,29]
HBsAg	Hepatitis B	Lettuce, potato	Two independent phase I trials	[30,31]
Norwalk virus capsid protein	Sickness and diarrhea	Potato	Phase I trials	[32]
Rabies glycoprotein	Rabies	Viral vectors in spinach	Phase I trials	[33]
Antibodies				
LSBC scFVs	Non-Hodgkin's lymphoma	Viral vectors in tobacco	At least 12 personalized antibodies submitted for phase I trials	Original study [34][a]
Avicidin	Colorectal cancer	Transgenic maize	Withdrawn from phase II trials in 1998	
CaroRX	Dental caries	Transgenic tobacco	Phase II trials	[35]
Other products				
Gastric lipase	Cystic fibrosis, pancreatitis	Transgenic maize	Phase II trials	[b]
Human intrinsic factor	Vitamin B12 deficiency	Transgenic *Arabidopsis*	Phase II trials	[c]
Lactoferrin	Gastrointestinal infections	Transgenic maize	Phase I trials	[b]

[a]See also http://www.lsbc.com
[b]http://www.meristem.com
[c]http://www.cobento.com

be a veterinary vaccine. At least 30 such products have been expressed in plants, some providing protection against challenges with disease-causing agents [4]; this group of PDPs is likely to attract a smaller regulatory burden compared with pharmaceuticals for humans. Among the diseases that have been targeted in trials, notable examples include canine parvovirus, foot and mouth disease virus, porcine epidemic diarrhea virus, rabies virus and porcine transmissible gastroenteritis virus. In the case of porcine transmissible gastroenteritis virus, the trial carried out by ProdiGene Inc. showed for the first time that an oral vaccine produced in plants could protect livestock against virulent challenge [8]. The first product to reach the market could be a poultry vaccine developed by Dow AgroSciences (http://www.dowagro.com/), which has been proposed for market release sometime in 2006.

The extent of these activities indicates that the concerns held initially by many in the pharmaceutical industry, regarding the feasibility of producing a regulated product in plants, can be overcome. Even so, there are still no commercialized pharmaceutical products derived from plant biotechnology, and this represents the next major milestone. There are still some hurdles to overcome, many of which are not technical. In this review, we shall discuss some of these major issues. We will overview the regulatory status of PDPs and discuss the manufacturing issues surrounding the production of pharmaceuticals in plants. We shall also consider how plant biotechnology innovation and human health improvement can be achieved through intellectual property (IP) management. Finally, it has long been recognized that the poor in developing countries stand to benefit the most from PDPs. We will explore these issues from a developing-country perspective, focusing on priority health areas, the transfer of technology, and the co-development of plant biotechnology and its products with developing-country partners.

Regulation of pharmaceutical crops

The regulation of pharmaceutical crops needs to be addressed on a case-by-case basis, and involves several regulatory authorities. As well as adhering to the same strict regulations that apply to food and feed GM crops, such as those laid out by the 2001/18 EU regulations for Europe and the USDA/APHIS permit application policies for the USA, PDPs also need to satisfy the regulations set out by the agencies that oversee the production of pharmaceuticals. Draft documents addressing quality aspects in the production of medicinal products made in GM plants were published by the EMEA (The European Agency for the Evaluation of Medicinal Products, http://www.emea.eu.int/pdfs/human/bwp/076402en.pdf) and the FDA (US Food and Drug Administration, http://www.fda.gov/cber/gdlns/bioplant.pdf) in 2002. These guidelines address the scientific and technological hurdles that will have to be overcome before plants can be considered as alternatives to existing biopharmaceutical production systems up to the point of marketing [9].

One major concern over PDPs is the use of food and feed crops as production hosts. It is important to ensure that adequate segregation measures are in place, both in the field and post-harvest, which includes transport and processing. This is particularly important where there is a risk of admixture with commodities intended for the food and feed chains. Past actions by regulatory agencies and biotechnology companies reflect the importance and emphasis placed on this issue. A classic example is the case of Prodigene Inc., who accepted a civil penalty of US$250 000 after volunteer transgenic maize was found growing in a soybean crop one year after the pharmaceutical maize was harvested. In this case, the company also covered the cost of destroying the soybean crop and the clean up steps that followed. Thus, it is not only the production crop itself but also the likely impact it has on surrounding crops and crops planted later in the rotation cycle that must be taken into account.

Preventing the adulteration of food and feed crops with pharmaceutical products is not just about using hi-tech measures but mostly involves using common sense and diligence to follow adopted procedures. The isolation of the pharmaceutical crop from genetically compatible breeding materials and visually similar non-PDP products will go a long way towards the prevention of genetic and mechanical mixing. Parent seed for commercial PDP production as well as the commercial crops themselves need to be isolated from other plants of the same species and from wild relatives to avoid cross-pollination.

Many of these considerations are equally relevant to the development of certain conventional crops where genetic admixtures can occur. For example, there is already experience in the EU and the USA in the production of High Erucic Acid Rape (HEAR), which is used to make oils for industrial processing. Erucic acid is toxic and so production protocols are tightly defined and controlled to prevent mixing between HEAR seeds and rapeseed intended for food and feed. Because any potential mixing might be difficult to detect, further precautionary measures are advisable for PDPs, including effective handling and labeling protocols. Even within GM food crops, several high-profile cases, such as the Aventis Starlink corn [10], Syngenta's Bt10/Bt11 corn [11] and the presence of unapproved GM rice for sale in China [12], demonstrate the need for tight regulation, strict agricultural practices, transparency and better labeling controls, along with high penalties for breaching such regulations.

As the number of PDPs gaining approval for field release and entering clinical trials rises, the current GM regulations will need to adapt to better accommodate these products. Food and feed industries, international regulatory activities, non-governmental organizations and public interest groups will play a major role in the evolving regulatory framework.

Producing plant-derived pharmaceutical proteins under current Good Manufacturing Practice

Good Manufacturing Practice (GMP) is a component of quality assurance that ensures that a pharmaceutical product is manufactured to a quality appropriate for its intended use on a consistent basis [13]. In practice, this means that the manufacturing process is completely defined, both in terms of materials and procedures, from start to finish. Appropriate certified facilities and equipment must be available, processes and analytical methods must be validated, and staff must be adequately trained. There must also be an unbroken information chain that allows the final product and all the equipment and materials used in its manufacture to be traced back to source, as well as a complete, consistent manufacturing protocol for every batch of product, to be reviewed and approved before its release.

There are several challenges that need to be addressed when applying the principles of GMP to plant-derived pharmaceuticals. The concept of pharmaceutical GMP was originally developed for drugs manufactured by chemical synthesis and later adapted for biopharmaceuticals produced by cell culture and fermentation technologies in closed, precisely monitored and controlled systems. In the case of terrestrial plants, natural variations and inconsistencies in growth, soil and weather conditions limit our ability to establish an equivalent level of control. Some of these limitations can be addressed by growing plants in glasshouses or other types of contained environment, or through the use of hydroponics, but such solutions limit the scale of production, a major advantage of plant-based production [2]. The use of plant cell cultures, which can be maintained in a precisely controlled environment, offers an alternative [14]. It eliminates some major disadvantages of pharmaceuticals produced in microbes or animal cells, such as the risk that pathogens or oncogenic DNA could be present in the cells or the media. Again, this reduces the scale of production and makes the entire production cycle dependent on fermenters and skilled staff to operate them, which eliminates another advantage of pharmaceutical production in terrestrial plants (i.e. that most of the production cycle can be managed by workers without specialized technological knowledge or skills).

With animal cells and microbes, a tiered banking system consisting of master and working cell banks is used to provide adequately characterized and documented starting material for the production process, thereby offering an effectively infinite source of the original production system throughout the life-cycle of the product. With transgenic plants, determining the equivalent of a master or working cell bank is less straightforward. A seed bank would seem to be the most logical but there are no clear guidelines as to what seeds should be stored (e.g. seeds from the primary hemizygous transformant, homozygous transgenic seeds from selfed plants or hybrid seeds representing the actual production plants), how much seed should be stored and under what conditions, and how long storage should be continued. These issues need to be addressed and covered by the appropriate legislation from the FDA and equivalent bodies before PDPs can become a mainstream technology.

Moving on from the cultivation and harvesting of plant material, GMP must also apply to the subsequent downstream processing stages of pharmaceutical production. This involves isolating and purifying the pharmaceutical product from harvested plant biomass. The initial stages of processing (harvesting, extraction, clarification) vary the most and have to be optimized in a system-specific manner. Even with non-clinical proteins, downstream processing can account for $>80\%$ of overall production costs, although the actual cost depends on the purity that is required. There is no reason for the downstream processing steps in PDP production to differ from the standards applied to other biopharmaceutical production systems [15] but, again, there has been little help from the regulatory agencies so far [16,17]. As already stated, regulatory guidance for biopharmaceutical production in plants under GMP conditions currently exists only as draft legislation published in 2002.

Biotechnology and innovation

Biotechnology innovation requires more than research and development (R&D) and good health systems. Three

decades of innovation research have taught us a great deal that is directly applicable to the development of plant-derived products. The development of new products for the people who need them cannot be achieved without interactions between many players in institutions, governments and the private sector. Product innovation is a complex process involving numerous failures, feedback loops and changing collaborations. How are health research priorities set? What are the regulatory barriers? What is the impact of intellectual property regimes? Conversely, imaginative and potentially effective policy innovations cannot work without addressing a complex web of complementary and competing forces. How do governments allocate funds for health? How do civil service systems work? How will public opinion affect the acceptance of a new technology? How are the prices of urgently required drugs and vaccines determined? These are all issues that must be addressed in the development of plant-derived vaccines and therapies.

It is important to find a way to address this complexity. There is continuing dismay at the low level of resources for health compared with the documented needs even though resources have been increasing over the past few years. New funds for R&D, product procurement and program delivery have been made available. The EU and the US NIH have recently awarded new grants for the development of plant-derived vaccines and drugs. Thus, we are at an important crossroads in global health where rapid advances are possible.

Studies on innovation systems [18] show that there are certain essential elements for success, including the development of networks of institutions concerned with, and focusing on, various determinants of innovation. A second insight is that innovation is not a linear process that runs, in the case of health, from laboratory bench to bedside, but rather is a complex three-dimensional system where there are frequent switchbacks, failures and bursts of unexpected progress [19]. A third insight obtained from innovation studies is the centrality of private companies. In market economies, virtually all products, including most vaccines and drugs, are produced and sold by for-profit companies (see article by Eva Dantas on the Science and Development Network website: http://www.scidev.net/dossiers/index.cfm?fuseaction=policybrief&policy=61&section=362&dossier=13). For health, six determinants of innovation have been identified [20]:

- R&D in the public and private sectors.
- Ability to manufacture new health technology products to high standards.
- National distribution systems in the public and private sectors.
- International distribution systems including supply through international organizations such as UNICEF, the operation of global funds, and trade among countries.
- Systems to manage IP for countries and organizations.
- Systems for drug and vaccine regulation to achieve safety and efficacy.

These six determinants are inclusive, that is, they span all the determinants that are involved in health innovation; the literature shows that the determinants of innovation are dynamically linked [21]. Progress overall requires progress in each determinant. If a program effectively addresses all six determinants, it is likely to succeed. More importantly, if it fails to address one or more of the determinants it is likely to fail. This brings us to the importance of good IP management. Only gradually over the past couple of decades has the public sector come to understand the importance of IP. Generally this realization has come about as a result of being forced to address IP problems rather than through a pro-active effort to deal with the complex issues of IP in a constructive, forward-looking way. This situation is changing and there is increasingly serious public sector attention given to IP management. Although this is important for commercial interests in the West, an overall improvement in IP management for biotechnology innovation is necessary around the world if we are to achieve the aim of providing products for the improvement of health in developing countries. Moreover, there has to be a real pro-active commitment, and not just lip service, towards making knowledge and IP available to developing countries for humanitarian purposes. One innovative approach has been adopted by the Pharma-Planta consortium, an EU-funded project, to develop recombinant pharmaceuticals in plants (http://www.pharma-planta.org) – 76 scientists have signed a Statement of Intent for Humanitarian Use of all knowledge that is generated during the project.

Potential of plant-derived pharmaceutical proteins – a developing-country perspective

In the developing world, traditional herbal medicines have long been a key component of the health-care system. The concept of a plant-based pharmaceutical platform would bring agriculture to medicine in a way that would allow predominantly agricultural economies to improve their healthcare and other needs. Specifically, there is enormous potential for the cost-effective production of molecules that could help to control infectious diseases, particularly the overwhelming problem of HIV/AIDS.

Major obstacles to the delivery of existing vaccines include the expense and logistics – how to deliver highly perishable preparations to large populations in remote areas without proper roads and storage systems. PDPs offer advantages that make them particularly suited for use in developing countries. They might be amenable to local or regional production in close proximity to those populations in need of them. Developing countries often have significant processing capacity for bulk plant material, for example, in the form of fruit juice extraction, sugarcane milling, wheat and maize milling. Thus, it would be feasible to site large primary extraction facilities in poor countries so that they could derive maximum benefit from the technology. Strategies such as recombinant protein targeting to seeds, or the use of high-volume economic food-processing technologies (such as freeze

drying) could limit the need for a cold chain between the point of production and the point of delivery.

The development of PDPs could be initiated in developing countries in parallel with developed countries. Moreover, in many cases, progress in human clinical trials might be more relevant in developing countries, particularly in determining efficacy, as is already being discovered in HIV studies.

Unfortunately, there is a general lack of awareness of PDP technology and its potential, a problem compounded by the absence of a commercial pharmaceutical product derived from plants. In addition, biotechnology acceptance and concerns about biosafety and bioethics are major issues in developing countries. Many of these countries are still grappling with establishing biosafety policies for first-generation transgenic plants, and even where such policies exist, the relevant infrastructure for their implementation is often inadequate. PDP crops present even greater challenges to ensure that they are controlled in the laboratory and in experimental lots. This is further complicated by the potential for contamination and environmental risk because, to date, some of the most suitable plants for novel protein expression also happen to be staple food crops in less developed countries. Although crop plants are proven expression systems for novel molecules [22,23], the absence of systematic regulations designed specifically for the PDP sector creates concern about contaminated food and environmental risks. Precedence and negative attitudes in developed countries, particularly in Europe, impact strongly on the current decision-making processes in developing countries. Strong trade ties exist, and agricultural food products constitute a significant component of goods exported from the developing countries. Consumers in Europe and other Western countries have expressed concerns over supermarket shelves stocked with any GM-contaminated food; they have demanded product labeling and are willing to pay a premium for products that are guaranteed to be GM-free. Europe is a crucial market for developing countries, and this has a strong negative impact on the adoption of these technologies.

Vaccines and microbicides are not profitable products. The poor return on investment is a disincentive to private sector participation. For this technology to move forward, it is likely that the public sector will have to take a lead. Historically, most developing-country governments have invested poorly in research, and often lack the skilled manpower and necessary infrastructure to develop such a technology. However, a few developing countries have enthusiastically adopted GM crops; the acreage of such crops in these countries has been increasing steadily and is projected to increase significantly in coming years [24]. Developing countries that have taken the lead in this field are already building their own capacity in biotechnology, and might well be leaders in the adoption of plant biopharmaceutical manufacturing platforms, particularly given that they possess the advanced infrastructure and legislative capacity needed to develop or adopt these technologies. These countries include China, India, Brazil, Argentina and South Africa. For example, South Africa has emerged as a keen adopter of GM technology, and has developed a national biotechnology strategy along with a substantial budget and a commitment to public investment in PDP initiatives. The rest of the continent is following suit – the New Partnership for Africa's Development (NEPAD) is aiming to build four bioscience centers of excellence in different parts of the continent. The first such centre opened in November 2004 in Nairobi, Kenya. There are now several reports in the literature from institutions in developing countries describing the development of PDPs, notably the production of a foot and mouth disease vaccine in Argentina [25], rabies antibodies in India [8], hepatitis B virus monoclonal antibodies in Cuba [26] and human papillomavirus antigens in South Africa [27]. It is these countries that can ultimately harness the technology and become regional centers of production and distribution for such molecules. An added advantage is the emphasis that could be placed on regionally important diseases. For example, rabies is endemic in Southeast Asia, yet barely registers in the West other than as a potential bioterrorism threat.

This is still a young technology but the absence of PDPs on the market makes it more difficult to convince governments and major pharmaceutical companies that this technology is a viable alternative to established systems. A tangible product is therefore urgently required. Using the early products as a basis to develop validated production methods and regulatory frameworks, PDPs could immensely improve the health delivery systems of many developing countries and thereby improve the lives of millions of the world's poorest people.

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