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REVIEW-THEMED ISSUE

Recombinant biologic products versus nutraceuticals from plants – a regulatory choice?

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Biotechnology has transformed the potential for plants to be a manufacturing source of pharmaceutical compounds. Now, with transgenic and transient expression techniques, virtually any biologic, including vaccines and therapeutics, could be manufactured in plants. However, uncertainty over the regulatory path for such new pharmaceuticals has been a deterrent. Consideration has been given to using alternative regulatory paths, including those for nutraceuticals or cosmetic agents. This review will consider these possibilities, and discuss the difficulties in establishing regulatory guidelines for new pharmaceutical manufacturing technologies.

Plants have always been a rich source of compounds to maintain or improve human health [1]. Historically these have been compounds that occur naturally in plants, but with the introduction of new plant biotechnology at the end of the last century, the possibility emerged to engineer plants to manufacture new compounds, including small molecules and biologics that originate from non-plant sources [2]. Very rapidly, the technology to genetically modify almost any plant species was developed, including all of the world's major food and feed crops, and with that arrived the prospect of delivering recombinant compounds of potential medical benefit, by the oral route [3].

This boom in plant biotechnology occurred at the same time as the explosion in university enterprise activities. A number of new companies including spin-offs were established to take advantage of growing interest in the field of 'molecular pharming' [4]. Although most of these ventures were clearly developing pharmaceutical drug targets, for some the regulatory path was not so clear and alternative routes for commercial development became of interest. For example, it was considered that some products could be developed as nutraceuticals (or food supplements), cosmetic ingredients or medical devices, the regulatory path for which are different (and less onerous) than for medicines.

In this article, we shall consider the circumstances under which a plant biotechnology product might be regarded as a nutraceutical or food supplement. We shall contrast this with how new medicines are regulated with specific reference to plant derived products and how this was applied to a monoclonal antibody produced in genetically modified plants [5]. We also consider the difficulties in establishing a new regulatory path for a novel biotechnology.

Nutraceuticals and related products

The populist term 'nutraceutical' was coined in 1989 [6, 7], but actually has no definition in US or European law. Nutraceuticals are sometimes also described as dietary

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supplements, functional foods, natural health products and 'foods for special health use' and as such, the term tends to blur the distinction between food and medicines. Dietary supplements for example, are recognized in the US as a separate regulatory category of food and are neither food nor drug (Dietary Supplement, Health and Education Act, 1994). They are defined as 'a product (other than tobacco) intended to supplement the diet that contains one or more of the following dietary ingredients; vitamins, minerals, amino acids, herbs or other botanicals; a concentrate, metabolite, constituent, extract or combination of the ingredients listed above'. They must also conform to other criteria:

- be intended for ingestion in pill, capsule, tablet, powder or liquid form;
- not be represented for use as a conventional food or as sole item of a meal/diet; and
- be labelled as a 'dietary supplement'.

This definition is quite distinct from a drug, which according to the US Food and Drug Administration (FDA) is 'an article intended to diagnose, cure, mitigate, treat or prevent disease', although clearly the marketing objectives of dietary supplements often cross into this spectrum.

In fact, dietary supplements do not fall under the FDA, whose remit is restricted to foods, additives, drugs and cosmetics. So whereas for new food additives and drugs, the manufacturer must conduct safety studies and submit the results to the FDA for review and pre-market approval, dietary supplements can be marketed without satisfying these criteria and need no pre-market testing.

In the Europe an Union, products are either regulated as foods or medicines, and on a European-wide basis, allowing each member state to apply its own regulatory framework. In the UK, for example, the Medicines and Healthcare Products Regulatory Authority has indicated that there are no plans to alter legislation to make specific provision for nutraceuticals (www.gov.uk/government/uploads/system/uploads/attachment_data/file/358665/Appendix6.pdf).

In Europe, a food is defined as 'any substance or product whether processed, partially processed or unprocessed intended to be, or reasonably expected to be, ingested by humans' (Regulation (EC) No. 178/2002). Nutraceutical products can be regulated as food, but there can be no implication of medical benefit, that is the suggestion that the product can treat or prevent disease. However, beneficial effects of nutraceuticals can be made as 'health claims' rather than 'medical claims'. For instance, claims must not state that a nutraceutical will prevent or cure a disease, only that it may help to improve health, possibly assisting in the avoidance of the onset of illness.

Pharmaceutical regulation of plant-derived drugs

Pharmaceutical manufacture by plant biotechnology is complicated by the fact that it is an emerging technology. As such the regulatory framework was slow to become established and still has not been thoroughly tested in any part of the world. Indeed, it was not until 2009 that the European Medicines Agency (EMA) published a 'Guideline on the quality of biological active substances produced by stable transgene expression in higher plants' [8]. Previous to that, a 'Points to Consider' document had been available from 2002, which had been drafted by the agency's Biologics Working Party. This document had not been challenged by any emerging product candidate, and was an immature document relating to how Good Manufacturing Practice (GMP) might be applied to plants. The uncertainty relating to regulatory requirements for plant biotechnology products, and the prospect of 'being the first' to engage with the regulatory authority on a new technology was a major disincentive for industry to develop this area in Europe.

Edible vaccines

The prospect of manufacturing medically important recombinant proteins in plants rapidly gave rise to the possibility of delivering recombinant vaccines and therapeutics in edible plant material as 'edible vaccines' [9]. This potentially obscures the lines between pharmaceutical and dietary supplements, and given the differences between regulatory oversight of drugs, foods and dietary supplements, it is perhaps not surprising that some small and medium-sized enterprises (SMEs) have become interested in the possibility of negotiating an alternative, less complicated and time-consuming regulatory path.

However, the initial idea of vaccination through consumption of raw plant material (e.g. fruits) has been largely replaced by the concept of oral antigen delivery in processed plant material [10, 11].

A small number of human clinical trials involving oral delivery of antigen have been undertaken. In all cases no major safety concerns were detected, and formulations were well tolerated by individuals. The first trials in humans were conducted with the LT-B antigen of enterotoxigenic strains of *E. coli* delivered in transgenic potato [12]. After consumption of transgenic potato, both serological and mucosal responses were detected: 91% of volunteers developed anti LT-B specific serum IgG, and 50% also developed anti-LT-B specific secretory IgA antibody (SIgA) in stool samples. In a later study in which volunteers were fed the same antigen in maize [13], similar results were observed. The authors noted that maize offers substantial benefits compared to potato for delivery of edible vaccines, including the availability of raw maize preparations, or processed options that require only minimal heat or pressure treatments that would not denature antigens.

Antigen-specific serum antibody responses were also detected in a trial in which volunteers were fed lettuce expressing hepatitis B surface [14]. When volunteers previously vaccinated conventionally against hepatitis B were fed the same antigen in potato, antigen-specific serum antibody responses increased up to 56-fold after three doses [15].

Tacket and co-workers expressed the Norwalk virus capsid protein (NVCP) in transgenic potatoes and conducted feeding trials in 24 volunteers [16]. Nineteen of the individuals

developed an immune response of some kind, although the level of serum antibody increases were modest, possibly because of pre-existing serum antibody to NVCP.

Finally, human trials have been conducted with rabies glycoprotein and nucleoprotein antigen peptides [17]. These antigens were fused to the alfalfa mosaic virus (AIMV) coat protein and this chimaera was expressed in spinach using a tobacco mosaic virus. Three out of nine volunteers, who had not previously been vaccinated, showed detectable levels of rabies virus-neutralizing antibodies, when fed spinach infected with the recombinant virus.

Overall, these studies have indicated that an immune response can be mounted in individuals fed transgenic plant material expressing a disease antigen. The approach so far for edible vaccines has been to adopt the pharmaceutical regulatory route, which may not be surprising given the nature of the target products and that they are being developed to address important medical needs.

All of these studies have been performed in the US, where the regulatory burden for early phase clinical trials has been easier to negotiate. In Europe, a GMP-compliant manufacturing process has to be in place with a GMP manufacturing licence awarded before any candidate product can be tested in human volunteers.

Creating a regulatory path for an emerging biotechnology for pharmaceuticals

The manufacture of pharmaceuticals is regulated by law, and a code of practice, termed'Good Manufacturing Practice' (GMP), represents the minimum standard that a medicines manufacturer must meet in their production processes. It was the absence of GMP guidelines for medicinal products of plant biotechnology that was a major disincentive for commercial development in this area.

Ultimately, it was an academic consortium, The Pharma-Planta project, funded by public research money in the European Union Framework 6 programme, that engaged first with the regulators and led to the maturation of the 'Points to Consider' document into a 'Guideline'. As expected, the process was slow and complicated by precedent in other regulatory areas. It does, however, provide a valuable insight into how new regulatory pathways are developed.

The Pharma-Planta project was an Integrated Project in the area of 'Plant platforms for immunotherapeutic biomolecule production'. The research consortium comprised 33 academic and industry partners in Europe and South Africa. The specific objectives of the project were to:

1 Identify the key regulatory issues relating to the GMP-compliant production of plant-derived antibodies, following discussions and negotiations with European regulatory authorities.
2 Develop a suitable transgenic plant line producing anti-HIV mAb 2G12 (known as P2G12).
3 Develop procedures for plant cultivation and downstream processing to address the key regulatory issues identified above.
4 Establish specifications for plant-derived mAbs acceptable for human use.
5 Design and perform a clinical trial to establish the safety of a plant-derived mAb.

The project was originally funded to run from 2004 to 2009, but as the development of a new regulatory pathway for plant-derived pharmaceuticals was time-consuming, it was extended until 2011.

In the case of monoclonal antibodies (mAbs), the 'gold standard' production platform is based on mammalian cell cultures that are well established in the industry and compliant with GMP. The differences between platforms based on sterile cell cultures and non-sterile whole organisms such as plants was one of the major concerns that led to doubts about the potential quality and consistency of mAbs produced in plants [18, 19].

An HIV neutralizing mAb (2G12) was selected that had previously been expressed in CHO cells at GMP, and tested in Phase I clinical trials in human volunteers. This provided an important advantage that a target specification had already been agreed with regulatory bodies and there was a considerable amount of safety data already available for the mAb.

The production of P2G12 in tobacco for clinical trials required the development of an entire production process from first principles, including transformation, the selection of lead events, the establishment of working practices for tobacco cultivation that satisfied the regulatory bodies in Europe, the definition of Master Seed Banks and Working Seed Banks, the development of a unique GMP-compliant downstream processing infrastructure and finally the completion of a first-in-human clinical trial to test the product for safety [5, 20].

The application and difficulties of precedent

In drawing up a new set of rules (in this case, GMP for medicinal products of plant biotechnology), it is always easiest to draw upon precedent from related areas. But this brings its own challenges, particularly in trying to accommodate new manufacturing within existing guidelines [21, 22].

Banking systems

One example of a challenge is the establishment of a banking system for the starting point of product manufacture. Systems for banking crop seeds have been well established in the agricultural industry for many years [23]. They generally involve a 'master' seed bank which is used to establish 'working banks' that are used for distribution to the agricultural industry. The master bank is relatively small, and as it diminishes, it can be replenished, thereby ensuring long-term continuity of supply.

Although similar terminology is used in the pharmaceutical sector, the principles underlying master and working banks are fundamentally different. A key issue is that the master bank may not be replenished, and that sufficient master bank supplies need to be established from the start for the

lifetime of the product. This ensures preservation of the identity of the master bank. Master and working bank systems for pharmaceuticals were developed with cell culture systems in mind, rather than whole organisms. The logistics of banking vials of cells for periods of up to 20 years differ significantly from those for banking plants or seeds and results in important consequences for the choice of banking system for plant production, and possibly for the plant species used for manufacture.

Following regulatory discussion, existing GMP rules were applied and replenishment of plant master seed banks for pharmaceutical production was not permitted.

Transformation events

The transformation event refers to the specific genetic alteration that occurred in the cells used for production. In the case of mammalian cells (e.g. CHO) for mAb production, a detailed characterization of the transformation event is not usually required by the regulators.

However, in the case of plants, a different approach was taken, due to the existing precedent of GM foods. Under GM food legislation in Europe, a precise characterization of the transformation event is necessary, including flanking DNA sequences, and single copy insertion events are significantly favoured [24]. This led to the requirements for transformation event characterization in GM plants for mAb production being much more onerous than those required from CHO manufacture. It was a significant deterrent to the use of plants with multiple transgene copies and insertion sites, which in turn restricted the product expression yields that were achievable [25].

Plant cultivation

A key component of the acceptance of plant manufacturing being GMP compliant was the establishment of standard operating procedures (SOPs) describing the cultivation of the plants [25].

'Good agricultural practice' (GAP) had previously been developed for production of food for consumers or further processing that is safe and wholesome. Some organizations like the World Health Organization had established GAP guidelines for medicinal plants [26]. Early expectations were that this precedent could be applied to GM plants for pharmaceutical production. However, it rapidly became clear that the established GAP systems were inadequate for this purpose, and a major part of Pharma-Planta's effort was directed towards the establishment of revised SOPs for GAP for monoclonal antibody production.

The three examples outlined above illustrate some of the difficulties in developing new regulatory paths. In some cases, systems that have been well established in other areas (e.g. food crop seed banking or good agricultural practice) are not deemed appropriate for a new manufacturing platform's compliance. In other cases, a precedent that was created for a completely different reasons (e.g. genetic characterization of the transformation event) is applied, even though the same requirements are not applied to other technologies used for the same application.

Outcome of the Pharma-Planta project

The most important outcomes from the Pharma-Planta project was the granting of a GMP manufacturing license to Fraunhofer IME for plant-derived monoclonal antibodies by the national German regulatory authority, and the approval of the clinical trial application by the national UK regulatory authority [5]. These two achievements demonstrated that a GMP-compliant process for transgenic plants could be developed and was acceptable to pharmaceutical regulators. They established a regulatory approach and path in Europe that could be adopted or adapted by other parties.

The Pharma-Planta clinical trial was completed in November 2011. It represented the first ever administration of a plant-derived mAb by the vaginal route in humans and the first use of a GMP-compliant transgenic plant-derived mAb in humans. No major safety issues were identified, the plant-derived antibody was safe and well tolerated in healthy women when administered intravaginally in single doses of up to 28 mg.

The first commercial products of molecular pharming

In parallel with these developments in Europe, the first two products of molecular pharming have been brought to the market in recent years. The first, Elelyso, is an enzyme replacement therapy for humans, and the second, Interberry-alpha, also a biologic, is targeted at the veterinary market. In both cases, the products were developed and licensed as pharmaceuticals by the appropriate regulatory authority.

Elelyso

Protalix, an Israeli enterprise established in 1993, had considerable success in producing glucocerebrosidase (prGCD/ELELYSO™) in a carrot cell fermentation system. Protalix advanced ELELYSO through clinical trials and subsequent new drug approval regulation by the FDA, and it remains the only molecular pharming product currently licensed for human use. Human glucocerebrosidase is an enzyme involved in glycolipid metabolism, and deficiency of this enzyme leads to Gaucher's disease, an incapacitating condition for which the only treatment is continuous enzyme replacement therapy. Gaucher's disease is generally considered an 'orphan disease', based on the relatively low incidence and distribution of the condition worldwide [27].

Recombinant human glucocerebrosidase had previously been marketed by Genzyme (Cerezyme™) and Shire (VpriV®) using a mammalian cell production platform. The uptake of human glucocerebrosidase into target cells (primarily macrophages) requires the correct processing of four typically occupied glycosylation sites [27]. Paucimannosidic glycans are ligands for mannose receptors expressed by macrophages, whereas the heterologous complex or high mannose glycans formed in mammalian cell cultures do not display correctly linked mannose moieties required for binding. In order to expose these residues, downstream enzymatic reactions are required, which adds to process cost and complexity. In contrast, Protalix took advantage of the well-characterized

plant secretory pathway by modifying the protein to alter its accumulation pattern within the cells, leading to a homogeneous population of paucimannosidic glycans.

In 2009, the FDA and Genzyme issued a notification to healthcare professionals about the potential for foreign particle contamination of several Genzyme products including Cerezyme™ (FDA Safety Alert, 2009). This event is believed to have triggered awareness of the lack of FDA-approved therapeutic alternatives and interest in identifying manufacturing alternatives.

The subsequent commercial approval for Protalix's ELELYSO resulted almost immediately in the signing of a collaboration agreement with Pfizer for further development and commercialization.

Interberry-alpha

Interberry-alpha is recombinant canine interferon-alpha produced by Hokusan Co. Ltd in the National Institute of Advanced Industrial Science and Technology (AIST), Hokkaido, Japan. Interberry-alpha is manufactured in genetically modified strawberries in a hermetically sealed 'Type 2' facility specifically designed for transgenic plants and the avoidance of gene release into the environment. Manufacturing and marketing approval for the product was granted by the Japanese Ministry of Agriculture, Forestry and Fisheries, and processed strawberries were marketed from 2014 for the treatment of periodontal disease in dogs.

Conclusions

It is perhaps interesting that both ELELYSO and Interberry-alpha were produced in edible plant species and could have adopted a food supplement regulatory path. Similarly all the edible vaccines tested so far have adopted a more complicated pharmaceutical regulatory route. So, despite much discussion and conjecture within the field, it seems that most are choosing the conventional regulatory approach, presumably to realize the advantages of medical claims, and possibly because ultimately, this is considered to be the 'right' path to take. It is likely however, that all future decisions will be taken case-by-case, and on the basis of commercial considerations and regulatory approaches taken at national level.

The Pharma-Planta consortium project overcame a major roadblock by taking on the challenge of being the first organization in Europe to engage with the regulatory body and establish an accepted manufacturing process for transgenic plant-derived biologics. In so doing, it encountered many obstacles and difficulties which led to considerable delay. Fortunately, this delay could be absorbed because of the public nature of the project, whereas similar delay could spell disaster for a commercial entity. There is thus a line of thought that suggests this type of 'ice breaker' activity should be a role of academia, given the commercial uncertainties that are ever present. It is hoped that now this barrier has been overcome, the decision to adopt a pharmaceutical regulatory approach over other apparently simpler routes to commercialization will have become more straightforward.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare support from The Hotung Foundation and the Ettore Majorana Foundation and Centre for Scientific Culture (JM) for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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