1 Recombinant biologic products versus nutraceuticals from plants -

- 2 a regulatory choice?
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16 Abstract

- Biotechnology has transformed the potential for plants to be a manufacturing source
- of pharmaceutical compounds. Now, with transgenic and transient expression
- 19 techniques, virtually any biologic, including vaccines and therapeutics, could be
- 20 manufactured in plants. But uncertainty over the regulatory path for such new
- 21 pharmaceuticals has been a deterrent. Consideration has been given to using
- alternative regulatory paths, including those for nutraceuticals or cosmetic agents.
- 23 This review will consider these possibilities, and discuss the difficulties in
- establishing regulatory guidelines for new pharmaceutical manufacturing
- 25 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

- health [1]. Historically these have been compounds that occur naturally in plants, b
 with the introduction of new plant biotechnology at the end of the last century, the
- 29 possibility emerged to engineer plants to manufacture new compounds, including
- 30 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
- ³⁴ oral route [3].
- 35 This boom in plant biotechnology occurred at the same time as the explosion in

36 university enterprise activities. A number of new companies including spin-outs were

- 37 established to take advantage of growing interest in the field of "molecular pharming"
- ³⁸ [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
- 40 commercial development became of interest. For example, it was considered that
- some products could be developed as nutraceuticals (or food supplements),
- 42 cosmetic ingredients or medical devices, the regulatory path for which are different
- 43 (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

45 this with how new medicines are regulated with specific reference to plant derived

47 products and how this was applied to a monoclonal antibody produced in genetically

- 48 modified plants [5]. We also consider the difficulties in establishing a new regulatory
- 49 path for a novel biotechnology.

50 Nutraceuticals and related products

The populist term "nutraceutical" was coined in 1989 [6, 7], but actually has no 51 definition in US or European law. Nutraceuticals are sometimes also described as 52 dietary supplements, functional foods, natural health products and "foods for special 53 health use" and as such, the term tends to blur the distinction between food and 54 medicines. Dietary supplements for example, are recognised in the USA as a 55 separate regulatory category of food and are neither food nor drug (Dietary 56 Supplement, Health and Education Act, 1994). They are defined as "a product (other 57 than tobacco) intended to supplement the diet that contains one or more of the 58 following dietary ingredients; vitamins, minerals, amino acids, herbs or other 59 botanicals; a concentrate, metabolite, constituent, extract or combination of the 60 ingredients listed above". They must also conform to other criteria: 61

- 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

- 68 prevent disease", although clearly the marketing objectives of dietary supplements69 often crosses into this spectrum.
- ⁷⁰ In fact, dietary supplements do not fall under the remit of the US 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 FDA for review and pre-market approval, dietary supplements can be
- marketed without satisfying these criteria and need no pre-market testing.
- In Europe, products are either regulated as foods or medicines, and on a European-
- vide basis, allowing each member state to apply its own regulatory framework. In the
- 77 UK for example, the Medicines and Healthcare Products Regulatory Authority has
- indicated that there are no plans to alter legislation to make specific provision fornutraceuticals
- 80 (www.gov.uk/government/uploads/system/uploads/attachment_data/file/358665/App 81 endix6.pdf).
- In Europe, a food is defined as "any substance or product whether processed,
- 83 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, ie 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,
- 89 only that it may help to improve health, possibly assisting in the avoidance of the
- 90 onset of illness.

91 Pharmaceutical regulation of plant derived drugs

- 92 Pharmaceutical manufacture by plant biotechnology is complicated by the fact that it 93 is an emerging technology. As such the regulatory framework was slow to become 94 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 95 "Guideline on the quality of biological active substances produced by stable 96 transgene expression in higher plants" [8]. Previous to that, a "Points to Consider" 97 document had been available from 2002, which had been drafted by the agency's 98 Biologics Working Party. This document had not been challenged by any emerging 99 product candidate, and was an immature document relating to how Good 100 Manufacturing Practice might be applied to plants. The uncertainty relating to 101 regulatory requirements for plant biotechnology products, and the prospect of "being 102 the first" to engage with the regulatory authority on a new technology was a major 103 disincentive for industry to develop this area in Europe. 104
- 105

106 Edible vaccines

- 107 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 supplement, and given the
- differences between regulatory oversight of drugs, foods and dietary supplements, it
- is perhaps not surprising that some SMEs become interested in the possibility of
- negotiating an alternative, less complicated and time-consuming regulatory path.
- Although the initial idea of vaccination through consumption of raw plant material (eg fruits) has been largely replaced by the concept of oral antigen delivery in processed plant material.
- A small number of human clinical trials involving oral delivery of antigen have been 117 undertaken. In all cases no major safety concerns were detected, and formulations 118 were well tolerated by individuals. The first trials in humans were conducted with the 119 LT-B antigen of enterotoxigenic strains of *E.coli* delivered in transgenic potato [12]. 120 After consumption of transgenic potato, both serological and mucosal responses 121 were detected: 91% of volunteers developed anti LT-B specific serum IgG, and 50% 122 123 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 124 results were observed. The authors noted that maize offers substantial benefits 125 compared to potato for delivery of edible vaccines, including the availability of raw 126 maize preparations, or processed options that require only minimal heat or pressure 127 treatments that would not denature antigens. 128
- 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].
- 134 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
- 138 antibody to NVCP.
- 139 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
- 143 vaccinated, showed detectable levels of rabies virus-neutralising antibodies, when
- 144 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 USA, where the regulatory burden
- 151 for early phase clinical trials has been easier to negotiate. In Europe, a Good
- 152 Manufacturing Practice (GMP) compliant manufacturing process has to be in place

153 with a GMP manufacturing licence awarded before any candidate product can be

- 154 tested in human volunteers.
- 155

156 **Creating a regulatory path for an emerging biotechnology for pharmaceuticals**

157 The manufacture of pharmaceuticals is regulated by law, and a code of practice

termed Good Manufacturing Practice (GMP) represents the minimum standard that a

159 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:

- Identify the key regulatory issues relating to the GMP-compliant production of plant-derived antibodies, following discussions and negotiations with European regulatory authorities.
- Develop a suitable transgenic plant line producing anti-HIV mAb 2G12 (known as P2G12).
- 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 neutralising 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].

203 The application and difficulties of precedent.

In drawing up a new set of rules (in this case, GMP for medicinal products of plant

205 biotechnology) it is always easiest to draw upon precedent from related areas. But

this brings its own challenges, particularly in trying to accommodate new

207 manufacturing within existing guidelines [21, 22].

208 Banking systems

209 One example of a challenge is the establishment of a banking system for the starting

210 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

216 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
- 219 preservation of the identity of the master bank. Master and working bank systems for 220 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.

- 227 <u>Transformation events</u>
- 228 The transformation event refers to the specific genetic alteration that occurred in the

cells used for production. In the case of mammalian cells (eg CHO) for mAb

- 230 production, a detailed characterisation 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
- 233 precedent of GM foods. Under GM food legislation in Europe, a precise
- characterisation 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 characterisation in genetically modified
- 237 plants for mAb production being much more onerous than those required from CHO
- 238 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].

241 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 245 246 food for consumers or further processing that is safe and wholesome. Some 247 organisations like the World Health Organisation had established GAP guidelines for medicinal plants [26]. Early expectations were that this precedent could be applied to 248 GM plants for pharmaceutical production. However, it rapidly became clear that the 249 established GAP systems were inadequate for this purpose, and a major part of 250 251 Pharma-Planta's effort was directed towards the establishment of revised SOPs for GAP for monoclonal antibody production. 252
- 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 (eg 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 (eg genetic characterisation of the transformation event) is applied, even though the same requirements are not applied to other technologies used for the same application.

260 Outcome of the Pharma-Planta project

- The most important outcomes from the Pharma-Planta project was the granting of a 261 GMP manufacturing license to Fraunhofer IME for plant derived monoclonal 262 antibodies by the national German regulatory authority, and the approval of the 263 clinical trial application by the national UK regulatory authority [5]. These two 264 achievements demonstrated that a GMP compliant process for transgenic plants 265 could be developed and was acceptable to pharmaceutical regulators. They 266 established a regulatory approach and path in Europe that could be adopted or 267 adapted by other parties. 268
- 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.

275 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.

281 <u>Elelyso</u>

Protalix, an Israeli enterprise established in 1993, had considerable success in 282 producing glucocerebrosidase (prGCD / ELELYSO[™]) in a carrot cell fermentation 283 system. Protalix advanced ELELYSO through clinical trials and subsequent new 284 drug approval regulation by the FDA, and it remains the only molecular pharming 285 product currently licensed for human use. Human glucocerebrosidase is an enzyme 286 287 involved in glycolipid metabolism, and deficiency of this enzyme leads to Gaucher's disease, an incapacitating condition for which the only treatment is continuous 288 289 enzyme replacement therapy. Gaucher's disease is generally considered an 'orphan 290 disease', based on the relatively low incidence and distribution of the condition 291 worldwide [27].

292 Recombinant human glucocerebrosidase had previously been marketed by

Genzyme (Cerezyme[™]) and Shire (VpriV[®]) using a mammalian cell production 293 294 platform. The uptake of human glucocerebrosidase into target cells (primarily 295 macrophages) requires the correct processing of four typically occupied glycosylation sites [27]. Paucimannosidic glycans are ligands for mannose receptors 296 297 expressed by macrophages, whereas the heterologous complex or high mannose glycans formed in mammalian cell cultures do not display correctly linked mannose 298 moieties required for binding. In order to expose these residues, downstream 299 enzymatic reactions are required, which adds to process cost and complexity. In 300 contrast, Protalix took advantage of the well-characterised plant secretory pathway 301 by modifying the protein to alter its accumulation pattern within the cells, leading to a 302 homogenous population of paucimannosidic glycans. 303

In 2009, the US 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.

312 Interberry-alpha

313 Interberry-alpha is recombinant canine interferon-alpha produced by the Hokusan

314 Co. Ltd in the National Institute of Advanced Industrial Science and Technology

315 (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.
- 318 Manufacturing and marketing approval for the product was granted by the Japanese
- 319 Ministry of Agriculture, Forestry and Fisheries, and processed strawberries were
- marketed from 2014 for the treatment of periodontal disease in dogs.

321 **Conclusions**

322 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.
- 324 Similarly all the edible vaccines tested so far have adopted a more complicated
- 325 pharmaceutical regulatory route. So, despite much discussion and conjecture within
- the field, it seems that most are choosing the conventional regulatory approach,
- presumably to realise 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.

331 The Pharma-Planta consortium project overcame a major roadblock by taking on the 332 challenge of being the first organisation in Europe to engage with the regulatory body and establish an accepted manufacturing process for transgenic plant derived 333 biologics. In so doing, it encountered many obstacles and difficulties which led to 334 considerable delay. Fortunately, this delay could be absorbed because of the public 335 nature of the project, whereas similar delay could spell disaster for a commercial 336 entity. There is thus a line of thought that suggests this type of "ice breaker" activity 337 should be a role of academia, given the commercial uncertainties that are ever 338 present. It is hoped that now this barrier has been overcome, that the decision to 339 adopt a pharmaceutical regulatory approach over other apparently simpler routes to 340 commercialisation will have become more straightforward. 341

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