Biotechnology Advances 33 (2015) 1572–1581

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Bringing plant-based veterinary vaccines to market: Managing regulatory and commercial hurdles☆

Jacqueline MacDonald [a], Ketan Doshi [b], Marike Dussault [c], J. Christopher Hall [d,e], Larry Holbrook [b], Ginny Jones [f], Angelo Kaldis [a], Cassidy L. Klima [g], Phil Macdonald [h], Tim McAllister [g], Michael D. McLean [d], Andrew Potter [i], Alex Richman [j], Heather Shearer [k], Oksana Yarosh [k], Han Sang Yoo [l], Edward Topp [a], Rima Menassa [a,*]

[a] Agriculture and Agri-Food Canada, 1391 Sandford Street, London, Ontario N5V 4T3, Canada
[b] Prairie Plant Systems Inc., 1 Plant Technology Road, Box 19A, RR#5, Saskatoon, Saskatchewan S7K 3J8, Canada
[c] Zoetis Inc., 333 Portage Street, Kalamazoo, MI 49007, USA
[d] PlantForm Corp., 120 Research Lane, Suite 200, Guelph, Ontario N1G 0B4, Canada
[e] School of Environmental Sciences, University of Guelph, Guelph, Ontario N1G 2 W1, Canada
[f] Elanco Canada Ltd., 797 Victoria Road, Route 116, Victoria, Prince Edward Island C0A 2G0, Canada
[g] Agriculture and Agri-Food Canada, 5403 1st Avenue South, Lethbridge, Alberta T1J 4B1, Canada
[h] Canadian Food Inspection Agency, 1400 Merivale Road, Ottawa, Ontario K1A 0Y9, Canada
[i] Vaccine and Infectious Disease Organization International Vaccine Centre (VIDO-InterVac), University of Saskatchewan, 120 Veterinary Road, Saskatoon, Saskatchewan S7N 5E3, Canada
[j] Agriculture and Agri-Food Canada, 174 Stone Road West, Guelph, Ontario N1G 4S9, Canada
[k] Canadian Food Inspection Agency, 59 Camelot Drive, Ottawa, Ontario K1A 0Y9, Canada
[l] College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form 20 July 2015
Accepted 27 July 2015
Available online 30 July 2015

Keywords:
Recombinant vaccine
Antibody
Immunotherapeutic
Molecular farming
Scale-up
Financing
Intellectual property
Freedom to operate

ABSTRACT

The production of recombinant vaccines in plants may help to reduce the burden of veterinary diseases, which cause major economic losses and in some cases can affect human health. While there is abundant research in this area, a knowledge gap exists between the ability to create and evaluate plant-based products in the laboratory, and the ability to take these products on a path to commercialization. The current report, arising from a workshop sponsored by an Organisation for Economic Co-operation and Development (OECD) Co-operative Research Programme, addresses this gap by providing guidance in planning for the commercialization of plant-made vaccines for animal use. It includes relevant information on developing business plans, assessing market opportunities, manufacturing scale-up, financing, protecting and using intellectual property, and regulatory approval with a focus on Canadian regulations.

Abbreviations: AFD, Animal Feed Division; ANVISA, National Health Surveillance Agency; APHIS, Animal and Plant Health Inspection Service; APVMA, Australian Pesticide and Veterinary Medicine Authority; BIS, Bureau of Indian Standards; CCVB, Canadian Centre for Veterinary Biologics; CEO, chief executive officer; CFIA, Canadian Food Inspection Agency; CFO, chief financial officer; CONABIA, National Agricultural Biotechnology Advisory Committee; COO, chief operating officer; CSO, chief scientific officer; CTNBio, National Technical Commission on Biosafety; DAFF, Department of Agriculture, Forestry and Fisheries; DBT, Department of Biotechnology; EC, European Commission; EFSA, European Food Safety Authority; EMA, European Medicines Agency; FDA, Food and Drug Administration; FSSAI, Food Safety and Standards Authority of India; FTO, freedom to operate; GAQSIQ, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; IP, intellectual property; MAPA, Ministry of Agriculture, Livestock, and Food Supply; MOA, Ministry of Agriculture; MOAC, Ministry of Agriculture and Cooperation; MOEF, Ministry of Environment and Forests; MOHFW, Ministry of Health and Family Welfare; MOST, Ministry of Science and Technology; NSS, National Surveillance System; OAGEBA, Office of Agricultural Genetic Engineering Biosafety Administration; OECD, Organisation for Economic Co-operation and Development; OGTR, Office of the Gene Technology Regulator; PCT, Patent Cooperation Treaty; PBO, Plant Biosafety Office; SAGPyA, Secretariat of Agriculture, Livestock, Fishery, and Food; SENASA, National Service of Health and Agrifood Quality; USDA, United States Department of Agriculture.

☆ The opinions expressed and arguments employed in this publication are the sole responsibility of the authors and do not necessarily reflect those of the OECD or of the governments of its Member countries.

* Corresponding author.
E-mail address: Rima.Menassa@agr.gc.ca (R. Menassa).

http://dx.doi.org/10.1016/j.biotechadv.2015.07.007

Contents

1. Introduction

The animal health field provides a unique opportunity for the application of plant-derived immunotherapeutics and vaccines. Infectious diseases have historically been a major cause of economic loss to the livestock industry worldwide, both directly as well as through disruptions in international trade. For example, bovine spongiform encephalopathy (mad cow disease) cost the Canadian beef and dairy industries over $5.3 billion in the two years following the identification of the first infected animal (Statistics Canada, 2006). Likewise, there are numerous other zoonotic pathogens, such as *Escherichia coli* O157, which do not affect the health of animals yet result in economic losses due to outbreaks of disease in human populations. A growing desire to control such pathogens is evident in public health initiatives such as One Health <www.onehealthinitiative.com/>, an international effort to expand collaboration across healthcare for humans, animals, and the environment. Of course, a multitude of economically important veterinary diseases exist that are not of significant risk to humans: notably foot-and-mouth disease, Newcastle disease, classical swine fever, porcine reproductive and respiratory syndrome, and porcine epidemic diarrhea.

Vaccines have the potential to reduce the burden of animal infections, but in many cases vaccines have not been produced that are both effective and cost-saving for the livestock industry. For example, a vaccine against *E. coli* O157 (Canadian license issued in 2008, now inactive) for use in cattle has a projected capacity to reduce human cases by nearly 85%, yet adoption of this vaccine by farmers was low (Matthews et al., 2013). Low adoption rates were due both to the cost of the vaccine and the need to handle animals three times for vaccine administration. Production of lower-cost vaccines in plants combined with oral administration by incorporating the product into livestock feed may be an avenue to increased adoption.

Although the concept of plant-derived veterinary vaccines dates back to 1993 (Usha et al., 1993), such vaccines are yet to be available on the market. Interest in the use of transgenic plants for pharmaceutical production has been growing over the past five years (Fig. 1); and interest in veterinary vaccines, in particular, has been increasing because regulatory approval can be significantly less onerous than that for human pharmaceuticals (Phan et al., 2013). The motivation for production of vaccines and other biologics from plants arises from an array of potential advantages over other production systems (Kolotilin et al., 2014). Depending on the plant system used, these advantages can include relatively high expression levels; effective post-translational modifications including proper folding and glycosylation; lower risk of contamination with animal pathogens or bacterial toxins; cost-of-production efficiencies; speed of development in the case of transient expression; stable, room temperature storage within seeds and oral administration of the product (Everett et al., 2012; Kolotilin et al., 2012; Tremblay et al., 2010).

Plant production platforms are diverse, and may involve the use of whole plants in a greenhouse or field, or plant cell culture; stable or transient expression; targeted or constitutive expression; expression from nuclear or organelle genomes; and expression of protein monomers, multimers, or virus-like particles. In addition, the unmodified version of the engineered plant may be a food or feed crop, or neither. The product may be intended for purification, or administration as a crude extract or whole plant tissue, and the planned route of administration may be oral, nasal, topical, or through injection. These factors all influence the advantages of the plant-based system, and can affect the steps in the commercialization process of a potential plant-made product. For example, while oral immunization is likely to be more convenient for the end-user, it often necessitates a very large dose to elicit the desired response, requiring milligram or gram quantities as opposed to the micrograms needed for injectable delivery (Rybicki, 2010).

Several excellent references for varied topics of interest for the commercialization of plant-made pharmaceuticals have recently been published. For products and platforms, a large array of plant species and

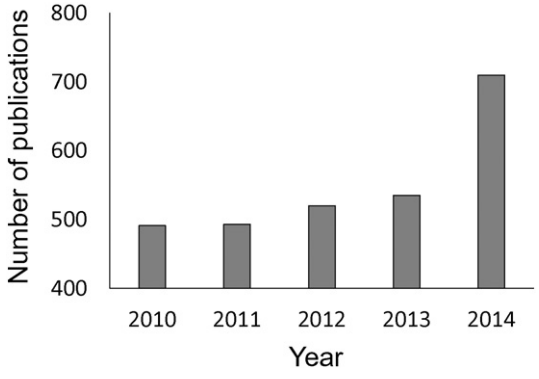

Fig. 1. Number of published articles on plant-made vaccines over the last five years (2010–2014). Articles were retrieved for each year using the search term "transgenic AND plant AND vaccine" in SpringerLink, ScienceDirect, Wiley, and Web of Science, and combined from all sources.

products is summarized in Paul and Ma (2011). The use of tobacco to make antibodies, vaccines and cytokines is presented by Tremblay et al. (2010); while recombinant plant-made cytokines are the focus of Sirko et al. (2011); and the plant-based production of antibodies for passive immunization is reviewed by Virdi and Depicker (2013). Plant cell suspension cultures from tobacco, rice, barley through to moss and the mechanics of bioreactor production are reviewed by Huang and McDonald (2012); and seed, leaf and cell bioreactor systems plus protein purification concerns are reviewed by Wilken and Nikolov (2012). Production of plant made veterinary vaccines with an example of purification using elastin like polypeptide fusions (ELPylation) is reviewed by Phan et al. (2013), and a unique purification method using hydrophobins from filamentous fungi is presented in Joensuu et al. (2010). Multiple plant transformation methods other than *Agrobacterium* transfer are effectively surveyed by Rivera et al. (2012), and the potential for higher yielding recombinant protein expression using chloroplast transformation is outlined in Bock and Warzecha (2010) and Scotti et al. (2012). An important potential strength of plant-based production may be the utility in oral delivery of edible vaccines with protein body-like encapsulation for gastrointestinal presentation, as previously reviewed (Jacob et al. 2013; Khan et al. 2012; Kwon et al. 2013). Mucosal, oral provision is being sought for low cost and ease of use without the need for refrigerated storage and transportation and has gained considerable international interest (Pelosi et al. 2012; Rybicki et al. 2012).

Although motivation is great, a knowledge gap exists between the ability to create and evaluate plant-based products in the laboratory, and the ability to take these products on a path to commercialization, including business planning, financing, and regulatory approval. This report provides guidance relevant to this process as an aid in planning for the commercialization of plant-made vaccines and biologics for veterinary use. Some of the content, especially that dealing with regulatory issues, has a Canadian focus consistent with the authors' expertise. However, information is provided that is relevant to multiple countries. The hope is that the information here will help more scientists get the products of innovation from the bench, to the field and to the animal.

2. Business planning

2.1. Collaborations

Since most scientists do not have sufficient ability or time to dedicate to financing and commercialization of technologies, an important step toward success is partnering with entrepreneurs or business associates to successfully finance and commercialize ideas.

The company's top management team may include a chief executive officer (CEO), chief operating officer (COO), chief financial officer (CFO), and chief scientific officer (SCO), among others. Ideally, the team will have affiliations with prominent downstream organizations and with a diverse range of organizations (Higgins and Gulati, 2006). The CEO is head of this top management team, and deals with strategic, long-term challenges of the company. The COO is an optional, second-in command position, usually defined in relation to the CEO. He/she may act as co-leader; lead the implementation of specific strategies, such as change or expansion; or provide advice to a more inexperienced CEO (Bennett and Miles, 2006). The CFO creates budgets, evaluates business unit performance, and acts as a primary ambassador to investors (Zorn, 2004); while the CSO is responsible for developing research capabilities and evidence of the viability and utility of products, and his/her role and experience, in particular, can affect investor decisions (Higgins and Gulati, 2006).

A proper company will also require a board of directors or governors, to show the public that the company has sound governance and will manage its investments in a highly responsible, productive and professional manner. The board is a small group of individuals often consisting of one or two members of a company's executive, but it is mandatory that outside members are included, and these should be established professionals easily recognized by the public as individuals who are honest, capable of delivering results, and who have impeccable integrity.

2.2. Creating a business plan

A business plan is essential for commercialization. It is a management tool that outlines the company's activities and plans for success, and becomes the foundation on which business officers rely to seek financing. Such a document usually takes a large number of iterations before being fit to present to potential investors.

A typical plan for a biotechnology company will include a description of the commercial opportunity that the company is developing; a thorough but easy-to-understand description of the technology that will be used; a sound business model (how the company will make money); a description of the market or markets that products will target; a complete description of the competition that is already in place, or the likely competitors who will also be working in the same business space; strategies for investors, including a description of entry and exit points, as well as corporate strategies; the company's intellectual property (IP) plan, including a description of all patents currently held, those under development and the company's policy on freedom to operate (FTO); a financial review with projected profit and loss considerations as well as the company's plan for immediate and long-term cash flow; and a description of the regulatory requirements for products and waste materials (which is of particular importance in the area of therapeutic bioproducts).

2.3. Financing

Financing must be considered at all stages, from concept development to final sales (Fig. 2), and it is an increasingly challenging and ongoing process. Financing requirements expand tremendously as a company grows from its beginnings toward product sales. Many of the members of a company's executive are usually involved in fundraising, including most or all of the chief officers.

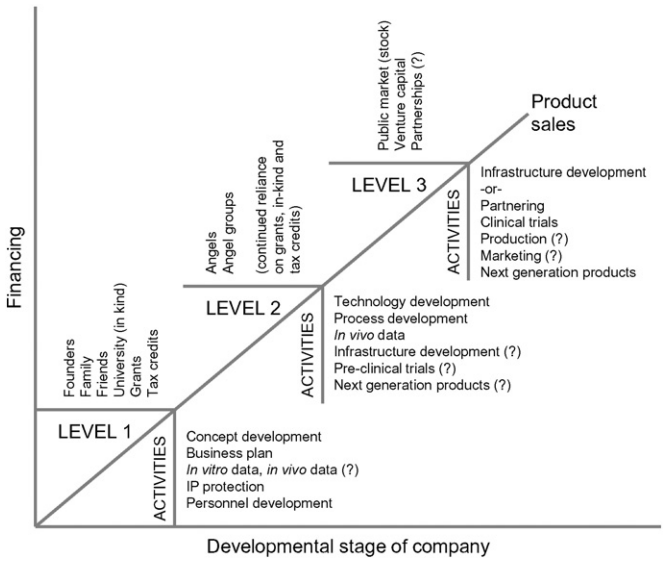

Fig. 2. Approximate chronology for company activities and investments. Divisions among levels are arbitrary. Question marks (?) indicate activities that may be undertaken at the subsequent level (*i.e.* level x + 1), or may not be undertaken by the company.

Early financing most often includes money from each of the founders, as well as investment from family and friends, research grants, and in-kind donations from an affiliated university or research institution. Investment from founders provides them with control of the company and gives subsequent investors faith that the founders are serious about the new company. Investors will be offered shares of company stock at fixed prices as outlined in the business plan, and there can be several series of preferred stock that are offered to investors before a company becomes a public entity and starts to sell or trade shares on an open market. Tax credits from local, provincial or federal sources are another source of revenue which should be sought. Additional opportunities could include income from contract research or production, or being sponsored by a larger and established company or a government agency. Initial activities supported by early financing should include concept development, development of a strong business plan, provision of supporting research data, development and protection of intellectual property (IP), and the hiring and training of technical personnel.

Invariably, fund-raising requires going beyond first-stage investors to seek further capital from "angel" investors. Angel investors are wealthy individuals or groups who are looking for start-up investment opportunities that allow for tremendous financial gain should the new enterprise become successful. Local communities often have investor meetings or clubs, such as those which might be hosted by a chamber of commerce. There are also many angel groups who advertise on the internet. Angel investors can provide substantial funding for technology refinement and process development, as well as for *in vivo* experimentation to demonstrate the efficacy of company technology. Other opportunities to find investment can come from networking at trade shows, or presenting at forums held by local, state, or provincial governments. These are often associated with ministries or departments that serve to promote technology transfer. Federal governments can also promote new companies through consulate- or embassy-associated investment meetings in foreign countries.

A third level of investment can include venture capital, partnerships, and entering into the stock market. Entry into a public stock market is a major step for a new company; however, this process usually occurs after all sources of venture capital investment are exhausted. Partnering with established companies for production, marketing and sales is often required. Activities include, but are not restricted to, establishment of company infrastructure, safety and efficacy trials, production and marketing. Next-product developments are also key to company growth.

3. Market viability

3.1. Target market & competitors

In order to attract collaboration and investment, and to make sure that a proposed product has a place in the market, an early step is to assess the value of the product and the potential for competition. Investment in a vaccine candidate depends on financial return on research and development investment, and this return depends to a large extent on the novelty of and need for the product. As average timelines for research, development, and registration of a novel product range from five to seven years (International Federation for Animal Health [IFAH] Europe, 2008), the target markets should be predicted at least this far in advance. Eventual and long-term consumer acceptance must also be gauged five to ten years in advance.

A key driver for a new product opportunity is the identification of end-user needs, including convenience of administration, confidence in positive safety and efficacy outcomes, and the cost or return on investment for the end-user's use of the product. A target profile which will meet end-user requirements must be identified, including the targeted label claim (prevention of disease X caused by organism Y); product attributes that can differentiate it in the market, like an easier route or method of administration (*e.g.* single vs multiple-dose; intranasal, oral, or parenteral administration); and the potential market value, including estimation of the total target market and the potential market share (IFAH Europe, 2008). The product profile should also align with existing management practices and consider the minimum vaccination age, the route and interval of administration, as well as the desired onset and duration of immunity. The effect of maternal antibodies on vaccine performance should also be considered. The targeted market may be a large, widespread need across multiple species (*e.g.* leptospirosis; influenza) or a small niche where no or few products are currently available. Emerging infectious diseases (*e.g.* porcine epidemic diarrhea syndrome) can also be a prime area of market development. Market opportunities may also arise from efficacy concerns with current disease management options, or safety concerns with existing products.

As part of the market assessment, potential competitors need to be identified and evaluated against the proposed product. The easiest and least expensive place to start is by internet-based searching for research focused on the same disease areas and targets, as well as those using similar technologies or platforms. Affiliations and acknowledgements for published peer-reviewed research can identify relevant academia-industry ties that may already exist. Searches of patent literature will provide information on the intellectual property landscape for areas of interests (see Section 3.3) as well as identify potential competitors. Additional information can be gathered from networking at relevant conferences and meetings.

3.2. Product development

For a new plant-based vaccine candidate at the research stage, an important milestone is proof-of-concept to show that the candidate is safe and efficacious using research batches produced at very small scale. For commercial-scale production, however, the manufacturing scale-up and quality control must demonstrate that the vaccine can be produced in a manner that minimizes batch-to-batch variability and the potential for batch rejection due to inadequate quality (USDA-APHIS, 2014a). This development phase is lengthy and expensive; therefore the feasibility of scale-up is a key factor that should be considered early in the vaccine development process. The cost of goods must be consistent and favorable to ensure continued profitability for the manufacturer and cost-effectiveness for the customer. These costs should be included in analyses along with the availability and cost of commercial production facilities. If production facilities are unavailable, the cost to build and certify production facilities must be included. Note that individual market differences in manufacturing site requirements may reduce economies of scale, thus increasing product costs (IFAH Europe, 2008).

While oral delivery of the vaccine without purification is expected to be most economically advantageous for product development and use, certain products may require purification. In such cases, downstream processing of plant-made immunotherapeutics can account for up to 80% of production expenses (Fischer et al., 2012) with hidden costs for processes such as buffer preparation and equipment cleaning, tests to validate the effectiveness of cleaning parameters, and activities relating to product quality control and assurance. Validation, quality control, and quality assurance can account for over 50% of labor costs (Wilken and Nikolov, 2012). An acceptable product shelf-life is also critical for logistics and inventory management, both for the manufacturer and for the end-user. Even so, it is estimated that with 1% protein expression and 50% protein recovery from purification, the cost of plant based protein is 10 and 1000 fold lower than microbial and mammalian based expression systems, respectively (Xu et al., 2012).

Post-licensing activities should also be considered. Reference requalification studies are needed to ensure the continued potency and efficacy of the product (USDA-APHIS, 2011). Vaccinovigilance programs are invaluable in monitoring and ensuring continued field safety of the product; data arising from these activities can determine if label statements or use recommendations need adjustment to ensure safety

and efficacy of the product. Market support studies may be needed to support the product's use in smaller markets or unique geographic or husbandry areas. Finally, further development programs may have to be initiated to expand the product's label claims in order to address evolving market needs or animal management practices (*e.g.* use in pregnant animals; decreased volume of vaccine doses).

Certain go/no-go points during product development should also be considered, with possible exit strategies for each, such as commercial licensing of products/services, or the sale of companies/technologies to strategic investors such as pharmaceutical companies or mature biotechnology companies. Commercial licensing of vaccines effectively enables the sale of each product as it arises, while preserving the core technologies associated with vaccine production for future discoveries. Given the highly technical nature and specific expertise required for production of plant-made vaccines, a licensing strategy may offer the highest probability of success. This is particularly true in today's business climate, where roughly 60% of new products are licensed from third parties. However, there is no standard template, with potential partners interested in anything that adds value to their pipelines or provides a market advantage. This includes know-how, technology or products. Ultimately, success can be measured by financial return, vaccine approval, vaccine sales and/or market share.

3.3. Intellectual property

Intellectual property (IP) considerations are an important component in bringing technologies to market and must be looked at from the planning stage all the way through commercialization, with advice from an IP professional. They play a key role in assessing the novelty of a proposed product, the potential competition, and the ability to produce and market the product without infringing on the rights of others, also known as "freedom to operate" (FTO). For very early stage research, a preliminary approach to identify the majority of relevant existing patents, using free online resources, may be beneficial (Miralpeix et al., 2014).

Once a potential IP asset is created, a patent or other form of protection may be needed in order to prevent others from using the ideas for their own gain. In the case of a plant platform in combination with a chosen antigen, the IP landscape is complex, and continually changes over time as technologies mature and patent office rules and guidelines change. For example, the first claim from a patent allowed in 1997 (resulting from a filing made in 1988) was very broad:

> A transgenic plant, comprising and expressing a DNA sequence coding for an antigen of a pathogenic microorganism or an antigenic determinant thereof, said antigen or antigenic determinant thereof eliciting a secretory immune response in a human or other animal upon oral administration of tissue of said plant (Curtiss and Cardineau, 1997);

A more recently issued patent is likely to be significantly more narrow, such as this first claim from a patent allowed in 2011 (resulting from a filing made in 2007):

> A method of inducing an immunoprotective response against a strain of Avian Influenza Virus (AIV) in an animal or human which comprises; a) expressing in a plant cell a DNA sequence encoding a hemagglutinin (HA1) variable region polypeptide comprising SEQ ID NO: 1 or 7; b) preparing a vaccine composition using the HA1 variable region polypeptide expressed in said plant cell; c) administering said vaccine composition to an animal or human such that a protective immune response is induced in said animal or human; and d) exposing said animal or human to a challenge strain of AIV having a HA1 variable region polypeptide that has at least 70% and less than 85.0%, sequence identity to SEQ ID NO: 1 or 7 (Webb and Henry, 2011).

The breadth of what one can obtain in a patent claim is much narrower than it used to be. While the reasons for such narrowing are many, the standards for obtaining a broad patent claim are now quite high. The principle that a patent specification must be sufficient to enable one skilled in the art to practice the invention is now strictly enforced. This was not the case several years ago and as a result broad claims were allowed without the inventors truly enabling the invention through the specification.

Complexity in both IP protection and FTO stems partly from the fact that the technology crosses multiple fields of innovation, such as genetic transformation and transient expression in plants including all the cloning, delivery, marker systems, *etc.*; protein engineering; antigen production; extraction and processing; adjuvant, formulation, and immunotherapy (Biodesign Institute, 2005). This means that there may be several IP hurdles to overcome when attempting to bring a product to market, as FTO across all of these fields must be considered. An FTO assessment for plant-based vaccines can therefore come with a high degree of uncertainty when large numbers of patents have to be considered (Krattiger and Mahoney, 2007). One must also consider patents that have just been recently applied for, not just those which have been issued. An FTO assessment should be continually revisited as there is generally an 18 month time period in which patent applications are not made public, and court decisions have impacted biotechnology patents significantly over the years. Protecting IP through a patent when there is already substantial prior art in the area means that claims will likely be narrow as in the example above.

While manageable, navigating the complexity takes time and can add substantial costs that offset the advantages of plant-based systems. There are several strategies that potentially can help keep costs down such as exploiting niche markets, working with off-patent technologies, cross licensing, partnerships, and others. If there is no opportunity to work around a technology one can look at acquiring, or licensing IP. An IP owner is not required to license their technology but many wish to recoup the costs they've incurred protecting their IP. There is a good chance a license will be needed at some point given the broad range of technologies involved in producing a plant-derived vaccine or therapeutic.

The Patent Cooperation Treaty (PCT) provides an internationally unified procedure for filing patent applications, but each country or region prosecutes applications and issues patents. Parties also accept patent applications directly without going through the PCT process. When choosing jurisdictions one needs to look at protection where the product will be made and sold. Global protection is not necessary if markets are geographically limited. In general, cost savings may be achieved by filing a PCT application if one requires patent coverage in many jurisdictions, while direct filings to state offices will cost less if fewer jurisdictions are needed. However, not all countries are party to the PCT, such as Argentina and some members of the European Union.

In protecting an IP asset one might want to consider keeping the IP as a trade secret instead of seeking patent protection. A trade secret by definition is something that is not disclosed publicly and can last indefinitely as long as the information does not become known. Unlike a patent, there is no protection once information related to a trade secret is disclosed. In the field of plant vaccines trade secrets might be in the form of know-how with respect to a processing facility or scaling up a technology. Protecting the trade secret would mean limiting access to a facility. The following link provides a good discussion on the advantages of trade secrets compared with patents: <http://www.wipo.int/sme/en/ip_business/trade_secrets/patent_trade.htm>

Further information on IP fundamentals is covered by patent offices and other online resources:

http://www.wipo.int/about-ip/en/
http://www.epo.org/learning-events.html
http://www.uspto.gov/
http://www.patentlens.net/

4. Regulations

4.1. Overview

As is the case with IP and FTO, regulations for plant-based vaccines can be complicated by the intricacy of technology, and requirements are changing to keep pace with technological advances. The development of transgenic crops has pushed countries to develop regulations that address public concerns about food, feed and environmental safety while still allowing access to these crops for growers. Ideally, the regulatory framework should be constructed to adequately address safety concerns while fostering the development and deployment of transgenic crops inside a country. Regulatory frameworks have emerged that are based either on the process of genetic engineering, or on the final product.

Regulations regarding transgenic plants in the European Union, which are possibly the most restrictive, use a strongly precautionary, process-based approach (Sparrow et al., 2013). In addition, once a transgenic plant is approved, Member States have the ability to opt out of allowing the plant to be cultivated or used as food or feed based on "legitimate reasons" other than risks to health or the environment, but including cultural traditions (European Commission, 2015; European Parliament and the Council of the European Union, 2015). In contrast, the regulatory framework for genetically engineered plants in the USA is product-based, arising from the premise that genetically engineered organisms are not inherently risky (Sparrow et al., 2013). Uniquely, the Canadian system regulates transgenic plants as "plants with novel traits"; a classification based entirely on the presence of a newly expressed trait, regardless of the method of introduction (CFIA, 2014a).

Regulatory responsibilities are generally divided between various government departments and sometimes non-governmental expert bodies. There are differences between countries in terms of the authorities responsible for implementing regulations (Table 1), the legal framework that is the basis of regulations, and the regulatory processes. However, the information guiding risk assessments that underlie the safe deployment of transgenic crops is very similar.

In Canada, the regulation of plant-produced veterinary vaccines falls primarily under the authority of the Canadian Food Inspection Agency (CFIA) (Fig. 3). Within the CFIA, vaccines and their manufacturing facilities are regulated by the Canadian Centre for Veterinary Biologics (CCVB), feeds by the Animal Feed Division (AFD), and field-grown plants with novel traits by the Plant Biosafety Office (PBO). Distinctively,

Table 1
Regulators responsible for aspects of plant-made veterinary vaccines in various countries. AFD, Animal Feed Division; ANVISA, National Health Surveillance Agency; APHIS, Animal and Plant Health Inspection Service; APVMA, Australian Pesticide and Veterinary Medicine Authority; BIS, Bureau of Indian Standards; CCVB, Canadian Centre for Veterinary Biologics; CFIA, Canadian Food Inspection Agency; CONABIA, National Agricultural Biotechnology Advisory Committee; CTNBio, National Technical Commission on Biosafety; DAFF, Department of Agriculture, Forestry and Fisheries; DBT, Department of Biotechnology; EC, European Commission; EFSA, European Food Safety Authority; EMA, European Medicines Agency; FDA, Food and Drug Administration; FSSAI, Food Safety and Standards Authority of India; GAQSIQ, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; MAPA, Ministry of Agriculture, Livestock, and Food Supply; MOA, Ministry of Agriculture; MOAC, Ministry of Agriculture and Cooperation; MOEF, Ministry of Environment and Forests; MOHFW, Ministry of Health and Family Welfare; MOST, Ministry of Science and Technology; NSS, National Surveillance System; OAGEBA, Office of Agricultural Genetic Engineering Biosafety Administration; OGTR, Office of the Gene Technology Regulator; PBO, Plant Biosafety Office; SAGPyA, Secretariat of Agriculture, Livestock, Fishery, and Food; SENASA, National Service of Health and Agrifood Quality; USDA, United States Department of Agriculture.

Regulatory aspect	Regulators							
	Canada	USA	EU	Brazil	China	India	Australia	Argentina
Plants grown in containment	Environment Canada[a]	USDA-APHIS[b]	Member state authorities[c]	NSS[d]	OAGEBA[e]	MOEF, MOST-DBT[f]	OGTR[g]	SAGPyA-CONABIA[d]
plant field trials	CFIA-PBO[h]	USDA-APHIS[c]	Member state authorities[c]	NSS, CTNBio[d]	OAGEBA[e]	MOEF, MOST-DBT[f]	OGTR[g,i]	SAGPyA-CONABIA[d]
Commercial release of plants	CFIA-PBO[h]	USDA-APHIS[c]	Member state authorities, EC, EFSA[c]	NSS, CTNBio[d]	OAGEBA[e]	MOEF, MOST-DBT[f]	OGTR[g]	SAGPyA-CONABIA[d]
Vaccine safety & efficacy	CFIA-CCVB[j]	USDA-APHIS[k]	member state authorities, EMA[c]	MAPA[l]	MOA-Veterinary Bureau[m]	MOHFW[n]	APVMA[o]	SAGPyA-SENASA[p]
Use in livestock feed	CFIA-CCVB, CFIA-AFD[q]	USDA-APHIS[r] FDA[c,k]	member state authorities, EC, EFSA[c]	MAPA[l]	MOA- Veterinary Bureau[m]	BIS (not compulsory)[s]	DAFF, APVMA[t]	SAGPyA-SENASA[d,p]
Safety of meat, milk and eggs derived from treated livestock	Health Canada[u]	FDA[v]	member state authorities, EC, EFSA, EMA[w]	MAPA[l]	MOA- Veterinary Bureau[m], GAQSIQ[x]	MOHFW-FSSAI[y]	APVMA[z]	SAGPyA-SENASA[p]

[a] Canadian Environmental Protection Act, 1999.
[b] USDA-APHIS, 2007.
[c] Sparrow et al., 2013.
[d] Chenault Chamberlin, 2010.
[e] Chen et al., 2011.
[f] Choudhary et al., 2014.
[g] Tribe, 2012.
[h] CFIA, 2012a.
[i] OGTR, 2001.
[j] CFIA, 2014b.
[k] USDA-APHIS, 2013.
[l] MAPA Strategic Management Office, 2010.
[m] MOA, 2009.
[n] MOHFW, 2005.
[o] APVMA, 2008.
[p] SENASA, 2010.
[q] CFIA, 2014d.
[r] USDA-APHIS, 2014b.
[s] BIS, 2014.
[t] DAFF, 2013.
[u] Health Canada, 2013.
[v] FDA, 2014.
[w] European Parliament and the Council of the European Union, 2009.
[x] GAQSIQ, 2007.
[y] MOHFW, 2011.
[z] APVMA, 2014.

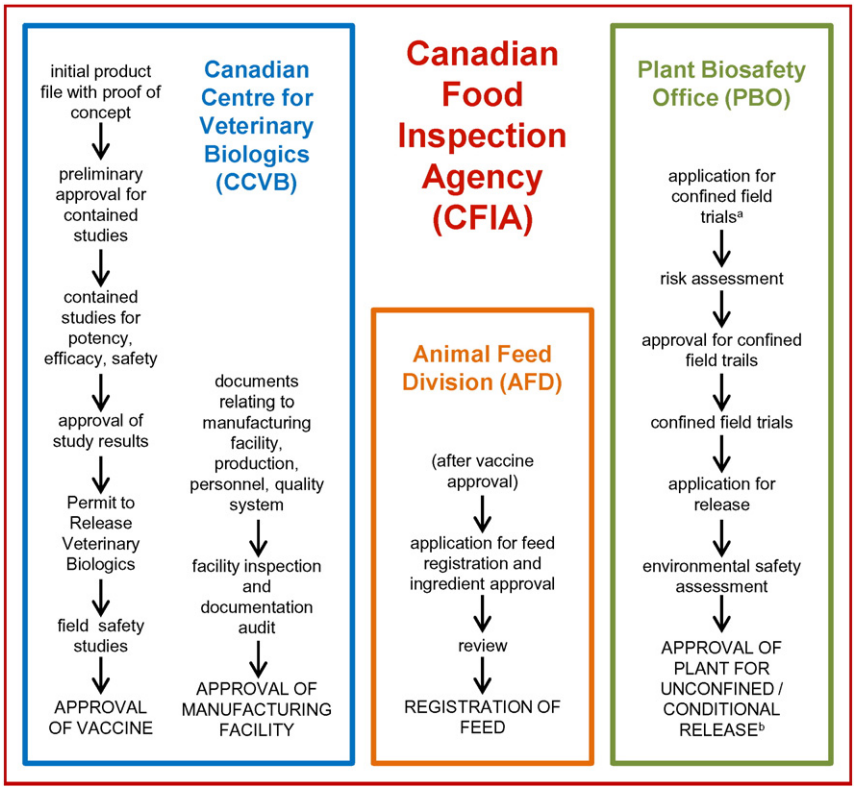

Fig. 3. Flowchart for regulatory approval of plant-made vaccines in Canada. Under the authority of the CFIA, vaccines and their manufacturing facilities are regulated by the CCVB, feeds by the AFD, and field-grown plants with novel traits by the PBO. Risk assessment for the environmental release of aquatic plants with novel traits is the responsibility of Fisheries and Oceans Canada (Fisheries and Oceans Canada, 2007, 2014). Approval is required from all relevant regulatory parties (*e.g.* if a plant that produces a vaccine is to be grown outdoors and administered to livestock by incorporating the product into feed, then the CCVB, AFD, and PBO must be consulted). While the CCVB, AFD, and PBO will share information, it is ultimately the responsibility of the applicant to contact, and obtain approval from, all relevant offices (CFIA, 2012a). [a]Applications to the PBO compare the product to a plant with a history of safe use (CFIA, 2014a). Aspects of the modified plant that are determined to be equivalent to the counterpart are accepted and the assessment then focuses on the differences (CFIA, 2014c). [b]Note that, due to the nature of the product, plants that produce vaccines or other pharmaceutical compounds may need to be grown under physical and reproductive confinement, even during commercialization (CFIA, 2012a).

risk assessment for the environmental release of aquatic plants with novel traits is the responsibility of Fisheries and Oceans Canada (Fisheries and Oceans Canada, 2007, 2014). Approval is required from all relevant regulatory parties depending on the intended use and release of the product. For example, if a plant produces a vaccine that is to be administered to livestock orally *via* feed, and the plant is to be grown in the field, then the CCVB, AFD, and PBO must all be consulted. Even if a vaccine-producing plant is not intended for use as feed or food, if its unmodified counterpart is a source of feed or food, assessment would require consultation with the AFD or the Novel Foods Section of Health Canada, respectively, to weigh potential risks associated with the release of the product into a commodity stream for which it is not intended (CFIA, 2014c). The CFIA, therefore, has historically discouraged molecular farming in major feed or food crops, as well as in crops pollinated by bees that contribute to commercial honey production (CFIA, 2015). While the CFIA allows field trials in food and feed crops, it imposes greater isolation distances for such crops (see Section 4.4) (CFIA, 2015).

4.2. Vaccine safety & efficacy

According to the CFIA, veterinary vaccines are categorized as veterinary biologics, a classification which also includes antibodies used to treat or prevent disease in animals. For approval in Canada, a veterinary biologic must meet the following general criteria:

- The product must be pure, *i.e.*, prepared to a final form and relatively free of extraneous micro-organisms and extraneous material, as determined by established test methods and approved in the production outline (CFIA, 2012b).
- It must be safe, potent and efficacious.
- Each biologically active component must be relevant to infectious animal disease conditions and animal genetics in Canada.
- The product must be manufactured in a facility acceptable to the Canadian Centre for Veterinary Biologics (CCVB)
- The product must be produced and tested in accordance with generally accepted good manufacturing practices and quality assurance standards (CFIA, 2014b).

Preliminary proof-of-concept data to support safety and efficacy must be submitted with an initial product file, which will trigger a phased review (Fig. 3). Preliminary approval from the CFIA–CCVB is needed prior to conducting further studies under contained conditions to support product potency, efficacy and safety. For use of experimental biologics in field studies outside of biocontainment facilities, a Permit to Release Veterinary Biologics (PRVB) is required (CFIA, 2014b). Approval of the PRVB is based on an environmental assessment, and would include consideration of the potential transfer of residues into the environment through excreta from the livestock consuming the product, and other mechanisms; and the potential for worker or bystander exposure (CFIA, 2013, 2014c). The CFIA-CCVB will also assign withdrawal periods to the vaccine based on the potential of vaccine, adjuvant or excipient residues to persist in livestock, including in the meat, milk, or eggs. Approval of any meat, milk, or eggs from vaccinated livestock is further subject to a food safety review from Health Canada.

Approval of a facility to manufacture veterinary biologics is also required. This involves a review of the facility, personnel, manufacturing

and quality control/quality assurance documents, and a pre-licensing inspection of all premises where manufacturing, testing, preservation, packaging, labeling, storage and distribution of the biologic are performed (CFIA, 2014b).

If the plant-made vaccine, whether in whole plant tissue such as leaves or seeds or as purified protein, is incorporated into feed for oral administration, the feed product will also need to be registered under the Feed Act and Regulations after approval of the vaccine component by the CCVB (CFIA, 2014d; Fig. 3). A product intended to be both a veterinary biologic and a feed would be classified and regulated primarily as a biologic by the CCVB, according to an established hierarchy for products with multiple claims (CFIA, 2014e); however, the CCVB may coordinate a joint review involving the AFD for approval (CFIA, 2014d, 2014e).

4.3. Environmental risk assessment

The living plants that produce a vaccine must also be maintained in a way that is safe for the environment. For transgenic plants, the environmental risk assessment generally estimates the risks associated with their release and cultivation in comparison with wild type plants. Countries with established regulatory programs for environmental risk assessment of transgenic plants consider similar safety concerns to be important, and these are addressed on a case-by-case basis prior to commercialization. Documents such as the OECD consensus documents (OECD, 2014) provide guidance on the array of potential concerns that are considered and their use in environmental safety assessment. The common considerations for the safety assessment of any transgenic plant would also apply to the safety assessment of plants producing a vaccine. These concerns generally include: gene transfer to related species; changes in weediness potential; secondary (indirect) and non-target adverse effects; and enhanced capacity to harbor plant pests (Macdonald, 2012).

Hazards can potentially exist for non-target organisms including plants, beneficial organisms (pollinators, biological control organisms, and soil microbes), and terrestrial or aquatic animals, and these concerns can increase if there are consequences for endangered or culturally important species. Non-target organisms may be exposed to the recombinant product through direct ingestion of the transgenic plant, or ingestion or parasitism of organisms that have fed on the plant. The recombinant product may itself cause adverse effects, or it may lead directly or indirectly to the expression of another product that causes adverse effects. Such effects may include toxicity, allergenicity, or oral tolerance in wild or domesticated animals (Kirk et al., 2005; Macdonald, 2012). It is therefore important to identify the threatened, endangered, and beneficial species that occur in the area where the plant will be grown. The potential consequences of the transgenic plant affecting soil micro-flora or fauna must also be considered. Appropriate indicator organisms can be chosen based on the potential for exposure to the product under field conditions, which would be affected by tissue-specific expression (Macdonald, 2012).

Risks to non-target organisms may be studied using a tiered approach: should adverse effects be observed in the laboratory, field studies may be needed to evaluate the actual abundance of non-target species that would come in contact with the transgenic plant. For instance, compared to laboratory test doses, insects are normally exposed to smaller amounts of toxins in the field because of more varied food choices and other environmental factors (Macdonald, 2012).

Adverse effects to field workers could also arise through physical contact with the transgenic plant, or use of the plant, its parts, or products, and need to be evaluated (Macdonald, 2012).

4.4. Contained production & field trials

Transient production systems in contained facilities are subject to oversight by Environment Canada (Canadian Environmental Protection Act, 1999), but other Government of Canada departments and provincial authorities would have oversight of transport to production facilities, waste disposal and waste water handling.

Confined research field trials are regulated by the CFIA (land plants) or by Fisheries and Oceans Canada (aquatic plants). An essential aspect of conducting these trials, where potential hazards are incompletely characterized, is to minimize unintended exposure outside of the designated field trial area. This is achieved by preventing pollen, seed or propagable materials from escaping the trial area; imposing conditions of reproductive isolation; assuring that volunteer plants are removed during the post-harvest period; and requiring that all vegetative or propagable material is destroyed according to approved methods (*e.g.* deep burial, incineration *etc.*) or securely stored and labeled. Government inspectors will inspect during the growing season and in the post-harvest period to ensure compliance with these conditions. Additionally, field trials for molecular farming must be surrounded by a 50 m perimeter of land that cannot be used for food or feed production, including livestock grazing; restrictions may be imposed on the use of field trial land in subsequent years for growing food or feed, or for grazing; and disposal of plant residue after harvest must be witnessed by an inspector of CFIA. Molecular farming in food or feed crops will also be subject to greater isolation distances (CFIA, 2015).

4.5. Unconfined release

In Canada, once a plant is approved for unconfined environmental release, there are limited or no conditions imposed on its release. Consequently, the crop is handled and managed just like any other conventionally bred commodity. For vaccine-producing plants that are not food or feed crops, and therefore have a considerably reduced possibility of ending up in the food or feed supply, conditions for environmental release would be based on a risk assessment as described above. However, the approval of vaccine-producing food and feed crops for release is currently limited because existing regulations require that such crops also be approved for both food and feed use. Unlike plants that are intended for food or feed, crops that have been modified to produce vaccines or other therapeutic products may not be safe as food or feed and as a result it is unlikely that they would ever be granted unconfined environmental release. Consequently, such plants may be required to grow under conditions that provide for physical and reproductive confinement, even during commercialization (CFIA, 2012a). Oversight would be required throughout the life cycle of the product and conditions would need to be imposed on production (CFIA, 2012c).

To address the potential risks of producing therapeutic or industrial products in plants and their effect on the environment and the possibility of direct or indirect entry into the food and feed stream, the CFIA has convened an inter-departmental working group to examine these issues and explore the development of an appropriate program that ensures that there is regulatory oversight for these products throughout their life cycle. The work of this group is ongoing and the CFIA has embarked on some first steps to explore a potential regulatory approach for plants that produce industrial products. While therapeutic products are outside of the scope of the pilot stage of this program, their future inclusion will be considered if the program is more fully implemented.

5. Conclusions

Successful commercialization of plant-made veterinary vaccines will require cooperation with entrepreneurs or business associates, the creation of a suitable business plan, and multiple stages of financing. The target market should be predicted well in advance as it typically takes five to seven years to go from research to product registration; and the ability for economical scale-up must also be assessed to determine whether the product can be produced cost-effectively at consistent quality. In the field of plant-based vaccines, intellectual property (IP) protection and freedom to operate (FTO) are complicated by the

intricacies of the technology, and their requirements are continually changing. Similar complications may arise when seeking product approval, as regulatory responsibilities will be divided between various offices and agencies, and continually change to keep pace with technological advances. While there are variations between countries in terms of how plant-based vaccines are regulated, in general, they must be shown to be safe, efficacious, and environmentally benign in order to gain approval.

In addition to the proof-of-concept studies typically performed in laboratories, the commercialization of a plant-made vaccine involves numerous steps and collaborations across various groups. These items should be considered prior to significant investment in a potential product.

Acknowledgments

This collaborative manuscript is an output from a workshop held in London, Ontario on September 23–25, 2013 and sponsored by the OECD Co-operative Research Programme on Biological Resource Management for Sustainable Agricultural Systems, whose financial support made it possible for some of the invited speakers to attend.

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