



PROVACS

## Blueprint for the Development of Plant-derived Vaccines for the Poor in Developing Countries

Prepared by PROVACS-Production of Vaccines from Applied Crop Sciences

A Program of The Center for Infectious Diseases and Vaccinology  
The BioDesign Institute at Arizona State University, Tempe, Arizona

2005

Frontispiece: Aescapulus, son of Apollo.  
He was exceptionally skilled at medicine and became the symbol of medicine.  
(Designed by Charles Kazilek, ASU)

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## Executive Summary

This Blueprint is the result of a series of international consultations and demonstrates that the development of plant-derived vaccines is both straightforward and achievable. A particular benefit of plant-derived vaccines is that their production could easily and economically be established in developing countries. The production in plants of large quantities of vaccines for oral administration is poised to revolutionize the world of vaccination and help bring to the poor the promise of existing and new vaccines for controlling infectious diseases. Due to economic and market realities, this promise will be achieved only through the collaboration of the public and private sectors with significant investments by both parties. These investments however will yield rich dividends in the form of widely available, inexpensive, orally administered, heat stable vaccines. The document specifically discusses pathways to produce vaccines against hepatitis B virus (HBV) and

human papilloma virus (HPV) but also apply to other pathogens.

This Blueprint is based largely on four consultations. The first was an international meeting in Tempe, Arizona in November 2002 that brought together a group of experts in a wide range of disciplines to consider the issues in plant-derived vaccine development. This Blueprint contains much of the information presented at the November meeting. The second was a meeting in Annecy, France at the Fondation Mérieux in May 2003. The papers of that meeting have been published as a special issue of *Vaccine*[1]. The third was a meeting of a Blue Ribbon Panel in Sedona, Arizona in October 2004 (Members of the Panel are shown in the Appendix). An earlier draft of this Blueprint was reviewed by the members of the Panel and substantial changes were made based on their comments. The last meeting was held at WHO in Geneva in January 2005. This meeting

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Acknowledgement: Funding from the Rockefeller Foundation is gratefully acknowledged. WHO kindly organized a meeting on regulatory issues for which we are grateful. We also note with gratitude the contributions of the Fondation Mérieux and the Flinn Foundation.

Biodesign Institute. 2005. Blueprint for the Development of Plant-derived Vaccines for the Poor in Developing Countries: Intellectual Property Considerations. The Biodesign Institute, Arizona State University: Tempe.

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considered regulatory issues and a report will be forthcoming.

New and improved vaccines are urgently needed to address the global scourge of infectious diseases. Two primary goals for these new and improved vaccines, especially for developing countries, as evidenced by the priorities of the Gates Grand Challenge Program, are heat stability and oral needle-free delivery. Plant-derived vaccines may be a particularly attractive tactic to address these priorities and to stimulate manufacturing in developing countries. Using plants to produce vaccines was first proposed in the 1980s. Substantial progress has been achieved in the laboratory, and there have been a small number of promising clinical trials, but the promise of plant-derived vaccines remains unfulfilled. The envisioned vaccines would be highly purified products meeting national regulatory standards for uniformity, safety and efficacy.

Several potential characteristics of plant-derived vaccines could make them particularly attractive for controlling infectious diseases in developing countries.

- Plant-derived vaccines could be produced on a very large scale and at very low cost, perhaps as little as a few cents per dose.
- The vaccines would be orally active, thus eliminating the need for injection and the associated cost and safety concerns.
- Oral activity is associated with the ability of plant-derived vaccines to evoke mucosal immunity, which is valuable for a number of infections that are transmitted through the mucosa.
- Plant-derived oral vaccines should be heat stable, thus largely eliminating the need for a cold chain for these vaccines.
- It might be possible to make multi-antigen vaccines either by multiple gene splicing or by mixing various plant-derived vaccines.
- A very important potential aspect of plant-derived vaccines is that developing countries could launch and carry forward their development and ultimately their production.

Because production of vaccines in plants would represent a significant departure for the established vaccine manufacturers in terms of technology and associated skill sets, there has been reluctance on the part of these companies to enter this field. Thus, if these vaccines are to reach the poor in developing countries, the public sector will have to provide significant support.

Plants can be used to manufacture only subunit vaccines. Thus, a strategy to prove the feasibility of this production approach would be to focus on one or more subunit vaccines that are already made by conventional methods.

Vaccines, and particularly the new vaccines, made by traditional production methods suffer from several limitations including cost, requirement for injection, and the lack of heat stability, mucosal effectiveness and ability to be easily prepared as combination vaccines. Plant-derived vaccines can potentially address each of the limitations thereby greatly facilitating the delivery of vaccines in developing countries.

The first goal with respect to plant-derived vaccines is to prove them as viable and comparatively superior to one or more existing vaccine production methods. We propose the following criteria for candidate selection:

- A vaccine produced by a traditional method already exists—the basis for making quantifiable comparisons
- The candidate is a subunit vaccine—a requirement for plant-derived vaccines
- The target disease is important for the poor in developing countries—in keeping with the public sector priorities
- The vaccine could have important markets in both developed and developing countries and among both the poor and the non-poor—to facilitate collaboration with the private sector
- The number of candidates should be small to ensure a highly focused program

Given these five factors, we will describe the situation for two vaccines: hepatitis B virus (HBV) and human papilloma virus (HPV). Many other vaccines may be possible, but HBV and HPV cover the field well and represent attractive first targets. Other targets could include cholera, ETEC, and measles. Cholera and ETEC do not have significant developed country markets. Also, for ETEC and cholera, one probably needs immune responses to multiple fimbrial antigens as well as to toxins. (One attractive feature of developing plant-derived vaccines against enteric infections is the possibility of developing a single vaccine against multiple pathogens, which may be very important for this form of infection.) A Respiratory Syncytial Virus (RSV) vaccine would be highly desired, but the needed immunology of subunit vaccine candidates that would give safe, protective immunity is still to be deter-

mined. There is inadequate flu production capacity in the world, and so plant-derived flu vaccines could also be of interest. The research could focus on haemagglutinin produced in plants, but again the detailed immunology of an effective flu subunit vaccine is limited, and concerns about plant *vs.* mammalian cell glycosylation of the protein would need to be resolved.

This Blueprint uses Innovation Systems Theory (IST). IST reveals that health product innovation takes place in a framework consisting of three major steps and six determinants[2].

The three steps, in simplified form, are:

- Candidate identification and animal studies
- Phase 1-3 clinical trial and production scale up
- Introduction and post-marketing surveillance.

The six determinants are:

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

The Blueprint lays out desired activities for each determinant at each step of development for both the public and private sectors.

New and improved vaccines are urgently needed to address the global scourge of infectious diseases. Two primary goals for these new and improved vaccines, especially for developing countries, as evidenced by the priorities of the Gates Grand Challenge Program, are heat stability and oral needle-free delivery. Plant-derived vaccines may be a particularly attractive tactic to address these priorities and to stimulate manufacturing in developing countries. Using plants to produce vaccines was first proposed in the 1980s. Substantial progress has been achieved in the laboratory, and there have been a small number of promising clinical trials, but the promise of plant-derived vaccines remains unfulfilled.

Over the last decade, the concept of plant-derived vaccines has grown more sophisticated. In the early years of research, investigators proposed that antigen-bearing fruits or vegetables could be consumed directly. While “immunization-by-eating” is a fascinating concept, detailed considerations have refined this view of plant-derived vaccines. Regulatory requirements will call for lot-to-lot consistency, uniformity of dosage, and purity, none of which are achievable through immunization-by-eating strategies. Thus the future of plant-derived vaccines lies in the development of either orally administered or injected vaccines. Orally administered vaccines may be developed in two forms. First, they may consist of purified antigen-bearing plant tissue, with or without excipients, delivered in a capsule. Or, they may be soluble dry powders of highly purified antigen and excipient protein prepared through a combination of membrane recovery, purification, concentration, and spray drying. This latter form would require the antigen to be delivered inside some form of encapsulation. The final product would look like dried milk and would be administered in a similar way except in very small quantities (a few milliliters).

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- Plant-derived oral vaccines should be heat stable, thus largely eliminating the need for a cold chain for these vaccines.
- It might be possible to make multi-antigen vaccines either by multiple gene splicing or by mixing various plant-derived vaccines.
- A very important potential aspect of plant-derived vaccines is that developing countries could launch and carry forward their development and ultimately their production.

The development of plant-derived vaccines will not be accomplished by market forces alone. Today’s research-based international vaccine companies are not interested in plant-derived vaccines because of concerns about investment returns and the lack of detailed regulatory frameworks, precedents, and skilled plant biologists in their existing research corps. For plant-derived vaccine development to move forward, the public sector will have to take the lead. However, if plant-derived vaccines are to become a major component of international immunization efforts, the private sector will eventually need to embrace this technology. Quite rightly, companies will want answers to tough questions, such as:

1. What is the unmet medical need, i.e. why would these products be used?
2. What is the product(s) that will emerge? If they are new versions of existing products, what are their comparative advantages? And will these advantages be important in a decade or so when the products emerge from development?
3. Can the product be made at the right scale and cost (i.e. manufacturing feasibility)?
4. Are there world-class people who can lead and support the effort?

This Blueprint is aimed at providing answers to these and related questions.

The first challenge with respect to plant-derived vaccines is to demonstrate that plants are a viable and attractive way to produce vaccines. There are many methods to produce vaccines, including attenuation of live pathogens (e.g., the Sabin polio vaccine), inactivation of live pathogens (e.g., rabies vaccine), isolation of pathogen subunits (e.g., hepatitis B vaccine from human plasma), and production of subunit vaccines using recombinant DNA technologies. Each of these methods has been used successfully on a commercial scale.

Plants have yet to be used on a commercial scale to produce a human vaccine or protein pharmaceuticals. The most advanced work for human use with plants involves monoclonal antibodies, but this work has yet to lead to a widely available commercial product. However, both animal vaccines and a patient-specific anticancer monoclonal antibodies have been approved by US regulatory authorities for clinical testing through Phase 3. These stand as important proofs

of principle. Plants can be used for only one type of vaccine—subunit vaccines—thereby limiting their use to a subset of all vaccines. When choosing vaccines as candidates for development, preference could be accorded to those for which the effective subunit is already known.

A preferred strategy to validate plant-derived vaccines, therefore, should be to produce a vaccine that is already made successfully by some other method. The work should be conducted to make it possible to compare the two methods of production and demonstrate conclusively that plants represent a significant improvement, by one or more measures, over proven methods for making vaccines. It is essential, then, to define clearly the criteria by which a plant-derived vaccine would be considered superior to a vaccine produced by conventional means. Having successfully met these criteria, there would be a firm basis for seeking to develop other vaccines in plants and concurrently encourage private investments.

### *Criteria for evaluating plants as a method to produce vaccines*

We propose that a plant-derived vaccine would be deemed superior to other methods of production, if it were not inferior in any significant way and met the following criteria:

- Its unit cost of production at a commercial scale was < 50% of the cost of production by the currently used method.
- It could be formulated and delivered in a readily acceptable orally administered form that achieved acceptable correlates of protection.
- It was demonstrated to be safe and, in particular, did not cause immune tolerance.
- It was shown to be heat stable, e.g., had a shelf life of at least 12 months at 27 degrees Celsius.
- It provided equal or enhanced levels of mucosal effectiveness compared to the existing vaccine.
- It could be produced readily in developing countries.

A vaccine meeting these criteria would represent a major advance in vaccine technology and would be especially valuable in developing countries.

### *Challenges with vaccines made by traditional methods*

The sustained use of vaccines for the poor in developing countries faces many challenges. These challenges include cost, requirement for injection, lack of heat stability, and lack of mucosal effectiveness. Also, as the number of vaccines increases, there is a need for combination vaccines.

**Costs:** The cost of vaccines remains a significant burden for developing countries, and the costs of future vaccines are projected to be considerably higher than current vaccines[3]. Several factors drive the projections for new vaccines:

- Regulatory requirements in developed countries, particularly for constructing production facilities and for final quality control, have increased dramatically in recent years. Almost all new vaccines are first produced in these developed countries, which requires the vaccine candidates to be tested in the countries of origin according to their high regulatory standards. (Developing a candidate vaccine first in a developing country is a new strategy being pursued in a few cases. The

recent licensure of a rotavirus vaccine in Mexico by GlaxoSmithKline Biologicals reflects this strategy.)

- Largely because of the dramatically increased costs of meeting regulatory requirements, intellectual property (IP) has taken on crucial importance. Many new vaccines are produced with proprietary methodologies, and patent holders vigorously prosecute their rights or protect their trade-secrets/know-how because of its inherent value.
- Some new vaccines may require multiple doses and booster immunizations. The concomitant requirement to buy these vaccines in continuous large quantities means that even small reductions in price could result in large absolute savings.
- In large multinational pharmaceutical companies, vaccines must compete for R&D resources against other products with high profit potential, such as those against heart disease and cancer. Thus, budget decisions must identify vaccines whose market values will generate comparable returns on investment.
- Production of vaccines with mammalian cells, such as Chinese Hamster Ovary (CHO) cells, which is an alternative subunit vaccine production system, does not enjoy economies of scale.

This combination of factors and the resulting higher costs of new vaccines have caused great concern about the potential availability of these vaccines to the poor. Traditionally, governments and multilateral- and bilateral agencies have had to pay only pennies per dose for traditional Expanded Program on Immunization (EPI) vaccines of the World Health Organization (WHO). A new vaccine (e.g., against *Haemophilus influenzae* type b) costs \$2 or more per dose or about 10- to 20-fold greater than the traditional vaccines.

**Requirement for injection:** The procurement, distribution, use, and disposal of syringes and needles continue to impede vaccine delivery. Of great concern is the high risk of unsafe injection caused by re-use, poor sterilization, improper disposal, and misuse of “sharps.” Orally active vaccines eliminate the need for injection and can be delivered by a wider range of service providers. High-quality, orally active vaccines derived from concentrated, dry plant tissues would also require much less sophisticated manufacturing technology than injected products.

**Lack of heat stability:** Maintenance of the cold chain and extending its reach to remote areas are proving to be daunting challenges. Ensuring continued high levels of coverage for existing and new vaccines is difficult, as are attempts to reach those who have not yet received the services of national immunization programs.

**Mucosal effectiveness:** Most current vaccines are injected and achieve effectiveness by stimulating cellular or humoral immunity. Mucosal effectiveness is important because it is seen as the most powerful means to prevent initial infection by pathogens at mucosal membranes, such as Human Immunodeficiency Virus (HIV), diarrheal agents (such as rotavirus, Norwalk virus, cholera, and Enterotoxigenic *Escherichia coli* (ETEC), and respiratory diseases such as pneumonia).

**Combination vaccines:** Combinations are highly valued because they reduce the need for multiple injections or administrations. The experience of the Vaccine Fund through the Global Alliance for Vaccines and Immunization (GAVI) has shown that developing countries accord very high priority to combination vaccines.

With the exception of combination vaccines, there has been little progress in addressing the challenges listed above. Cost of production continues to increase. Little research is underway to prepare orally active vaccines. GAVI and the Grand Challenges in Global Health program of the National Institutes of Health (NIH) have identified the use of sugar-glass technologies to improve heat stability in existing vaccines, but this technology can only increase the cost of the vaccine. Numerous combination vaccines are under development but they represent no savings; indeed, in some cases, the cost of the combined vaccine is more than the sum of the cost of the separate vaccines [e.g., Hepatitis B and Diphtheria-Tetanus-Pertussis (HBV-DTP) and similar combinations].

### *The Potential of Plant-Derived Vaccines*

Plant-derived vaccines could potentially address each of the challenges posed by existing methods of vaccine production.

**Low Cost:** Plant-derived vaccines may be very inexpensive. As described below (see page 17), the costs of producing plant-derived vaccines to meet regulatory requirements is being rigorously evaluated.

- Production of vaccine antigens in plants is highly efficient. For example, with the current state of technical development, enough hepatitis B antigen to vaccinate all babies in the world each year could be grown on roughly 200 acres of land, and all the HBV vaccine required annually for China could be produced on a 40-acre plot. The implications of batch-failure are also ameliorated. It may be possible to have 80 percent wastage with plant-derived vaccines and still achieve low costs. Thus, plant-derived vaccines may be of considerable value for vaccines that require multiple injections and booster doses. For example, plant-derived tuberculosis (TB) subunit vaccine to boost the immunity of adolescents who are at higher risk to infection (due to waning immunity from childhood vaccination) could result in substantial savings, and a plant-derived malaria vaccine could be of great value.
- Plant-produced antigens for oral delivery may be much less expensive because production does not require capital-intensive pharmaceutical manufacturing facilities and the associated high staff expenses. For example, enough antigen for one dose of hepatitis B vaccine could be produced in unprocessed plant material at a cost of \$0.005. This advantage of plant-derived vaccines is important because it can lead to a much lower go/no-go vaccine development decision making process. This is because it will not be necessary to make a large capital investment in a production facility for a vaccine that is under development.
- Plant-derived vaccines may also be cost efficient because development can proceed immediately in developing countries. One of the reasons that new vaccines are not introduced into developing countries soon after they first become available in developed countries is because of the high initial cost. This initial cost is set in order to recoup the high cost of R&D and the expense of the production facility, market development, etc. Developed country producers can afford to offer

“marginal cost of production” vaccines only after a large market is established for the vaccine. Since, with plant-derived vaccines, the need to recoup capital investments should be much lower, it will take considerably less time before the vaccine can be sold at a price close to the marginal cost of production. Additionally, the lower entry costs will allow manufacturers in the developing world to participate in new vaccine production.

**Needle free administration:** Vaccines produced through plant biotechnology methodology could be orally active, whereas most other new vaccines entering the market (produced in animal or yeast cell fermentation systems) must be injected.

**Lack of heat stability:** Vaccines produced *via* plant biotechnology to yield dried plant extracts should not require a sophisticated cold chain. They should be stable at room temperature to the same extent any dried food powder is stable.

**Mucosal effectiveness:** These vaccines stimulate the immune response at the mucosal level and thus would be especially effective against diseases—TB, pneumonia, flu, diarrheal diseases, sexually transmitted diseases (STDs), HIV, et al.—that infect through the mucosal system. In situations where a satisfactory traditional vaccine exists for primary immunization, a plant-derived vaccine could be of great value as a boost to ensure or enhance mucosal immunity. These vaccines may also have to evoke cellular mediated immunity.

**Combination vaccines:** Plant-derived vaccine technology could be applied to the development of vaccines combining numerous antigens. For example, it is theoretically possible to make a plant that produces antigens to stimulate effective immune response to cholera, ETEC, and rotavirus. Alternatively, several plant-derived vaccines could be blended prior to packaging for delivery.

As illustrated in Table 1, different plants have various characteristics that need to be taken into account.

**Table 1. Pros and cons of different plant types.**

Plant Type	Pros	Cons
<b>Tobacco</b>	Mature transformation and expression technology High biomass yields Potential for easy scale up	Alkaloid content Need for immediate processing of green leaves
<b>Cereals and legumes</b>	High protein yields Convenient seed storage for later processing Efficient downstream processing Known agronomics	Potential for gene escape into seed/food crops via pollen Cost of confinement production
<b>Fruit, leafy, and root vegetables</b>	Known agronomics in greenhouses Harvest and processing technologies available	Need for crop stewardship (to avoid contamination of foods) Need for immediate processing

## Which Plant-derived Vaccines?

### *Criteria for selecting lead candidates*

As noted earlier, the first goal with respect to plant-derived vaccines is to prove them as viable and comparatively superior to one or more existing vaccine production methods. We propose the following criteria for candidate selection:

- A vaccine produced by a traditional method already exists—the basis for making quantifiable comparisons
- The candidate is a subunit vaccine—a requirement for plant-derived vaccines
- The target disease is important for the poor in developing countries—in keeping with the public sector priorities
- The vaccine could have important markets in both developed and developing countries and among both the poor and the non-poor – to facilitate collaboration with the private sector
- The number of candidates should be small to ensure a highly focused program

Given these five factors, we will describe the situation for two vaccines: HBV and HPV. Many other vaccines may be possible, but HBV and HPV cover the field well and represent attractive first targets. Other targets could include cholera, ETEC, and measles. Cholera and ETEC do not have significant developed country markets. Also, for ETEC and cholera, one probably needs immune responses to multiple fimbrial antigens as well as to toxins. (One attractive feature of developing plant-derived vaccines against enteric infections is the possibility of developing a single vaccine against multiple pathogens, which may be very important for this form of infection.) A Respiratory Syncytial Virus (RSV) vaccine would be highly desired, but the needed immunology of subunit vaccine candidates that would give safe, protective immunity is still to be determined. There is inadequate flu production capacity in the world, and so plant-derived flu vaccines could also be of interest. The research could focus on haemagglutinin produced in

plants, but again the detailed immunology of an effective flu subunit vaccine is limited, and concerns about plant *vs.* mammalian cell glycosylation of the protein would need to be resolved.

### *Hepatitis B Virus (HBV)*

HBV infection causes acute and chronic serum hepatitis, liver cirrhosis, and, most important, liver cancer. Transmission routes include blood, sexual intercourse, and perinatal transfer. In the poorest countries (sub-Saharan Africa, most of Asia, and the Pacific), most people become infected during childhood and 8-10% of the general population becomes chronically infected in the absence of immunization. Of the 2 billion people who have been infected with HBV, over 350 million have chronic infections, leaving them at high risk of death from cirrhosis of the liver and liver cancer. These sequelae kill more than half a million people each year. Childhood infection commonly leads to the carrier state and increases the chance of HBV-related cirrhosis or liver cancer to 25%. The hepatitis B surface antigen (HBsAg) is the viral envelope protein, which is found in high concentration in the serum of HBV infected individuals[4]. In 1982, human serum-derived HBsAg began to be used as a vaccine. The current vaccines are recombinant HBsAg from yeast cell culture [5]. The existing HBV vaccine doesn't cure existing infections, but is 95% effective in preventing the carrier state; it is the first vaccine against a major human cancer.

With the advent of the Global Alliance for Vaccines and Immunization and the Vaccine Fund, prices for hepatitis B vaccine have fallen to less than \$0.30 per dose, but at least three injection doses are needed for effective immunization. The total immunization cost thus represents at least five times the cost of the next most expensive EPI vaccine – measles – and thus is an inhibitor to sustained use in developing countries. Furthermore, the current HBV vaccine does not yet reach about 60 percent of the world's children, including many in countries with high disease prevalence, largely due to combined costs of vaccine and delivery.

There are several considerations that make developing a plant-derived hepatitis B vaccine attractive:

- A Phase 1 clinical trial with a plant-derived hepatitis B vaccine has boosted antigen-specific serum antibody titers.
- The biology of hepatitis B vaccines is well understood, and the presence of serum antibodies specific to the surface antigen represents an accepted correlate of protection.
- Hepatitis B infection continues to be a major problem leading to significant disease burdens in developing countries.
- Existing vaccines have dropped dramatically in price but are still at levels beyond the resources of many developing countries.
- Hepatitis B vaccine is needed in both developed and developing countries; a development program can seek to meet the rigorous requirements of developed countries, thus helping to ensure that a high quality product emerges.

The pathway for development, therefore, is well defined. Such an inexpensive vaccine can be produced readily in developing countries and could significantly contribute to global health.

### *Human Papilloma Virus (HPV)*

At least 300-600 million women worldwide are HPV carriers; 30 million have low-grade cervical dysplasia and 10 million have high-grade dysplasia. About 42 million women in Africa are carriers of HPV. In South Africa alone, asymptomatic HPV infection occurs in 16-20% of women in all ethnic groups. Males are also carriers at similar rates. Currently there are about 100,000 cases of HPV-related cancer in men globally. An HPV vaccine would aim to control infection, decrease financial burdens associated with screening and treating HPV infections, and reduce the incidence of cervical cancer worldwide.

The main cause of cancer of the cervix is infection with certain high-risk types of HPV [6]. Cancer of the cervix is the second most common cancer in South African women [7]. Although cervical screening programs have reduced the number of cervical cancer cases in the developed world, this is not the case in Africa, where screening programs are either inadequate or non-existent. Therefore, in most parts of Africa the only hope of reducing disease is a successful HPV vaccination campaign. It is likely that

one or more HPV vaccines will be licensed in the near future.

**HPV vaccines.** The HPV vaccines presently undergoing human trials are peptide subunit vaccines composed of virus-like particles (VLPs) made in yeast, insect tissue cultures via baculovirus expression, or recombinant vaccinia virus [8]. The commercial vaccines being tested by Merck and Glaxo-SmithKline are comprised of 3 or 4 different VLPs, each derived from genes of different strains of HPV; this will add to the cost of production and formulation of what is likely to be a highly effective vaccine. This cost may pose daunting constraints for health-care systems in Africa and other parts of the developing world that need a safe, effective vaccine that is cheap to produce. But none of the present trial vaccines satisfy all these criteria. Peptide and subunit vaccines are expensive to produce. Vaccinia virus recombinants are cheap to produce, but there are concerns about safety, especially in immunosuppressed people. In general, recombinant live-vector vaccines, even when attenuated, are not suitable for the immunocompromised. This is of particular concern for vaccines going to developing countries, as it is most often not feasible to determine an individual's HIV status prior to immunization. Additional factors, such as malnutrition, may also depress the immune system. The current HPV vaccines in Phase 3 clinical trials evoke strong serum antibodies that seem essential for protection. Although it is not clear that plant-derived oral vaccines could achieve the needed priming of such responses, they may be useful in a prime-boost strategy.

**Oral vaccination against HPV.** HPV infects stratified epithelial tissues and does not spread systemically, suggesting strong mucosal immunity would provide the best protection against HPV infection. Although mucosal immunity is not as well understood as systemic immunity, there is evidence that antigen exposure at one mucosal surface—such as the gut—elicits an immune response at a distant mucosal site, such as the vagina or cervix [9]. Preliminary studies (unpublished) have tested oral immunogenicity of HPV16-L1 produced and delivered in transgenic plant tissue: ingestion of dried tissue ef-

fectively boosted responses to parenteral boosting. Therefore, oral immunization is potentially effective at inducing cervical and vaginal antibody responses. Issues remaining to be addressed in mucosal immunity include the possible short duration of the immune response, the requirement for boosting, and the possibility of inducing immune tolerance. The availability of plant-derived vaccines would allow these issues to be addressed. If frequent boosting is important, a low-cost and easily distributed oral vaccine from plants would be a potential solution.

A PATH report[10] provides a detailed set of recommendations for HPV vaccine needs. The report concludes that in addition to safety and efficacy, in developing countries it is necessary to consider cost of production and delivery. The report emphasizes that new vaccines must be available to the populations that need them most. It lists four major ways to reduce costs and increase vaccine coverage (*italics, our addition*):

- According priority to a vaccine that can be produced in developing countries rather than relying on imports (e.g., a recombinant bacillus Calmette-Guérin (BCG) or *edible vaccine*);
- Simplifying distribution by creating a stable vaccine with a long shelf life that does not require an expensive and logistically complex cold chain (e.g., DNA vaccines or *dried plant tissues or extracts*);
- Formulating an oral vaccine, since it is easier to administer, more acceptable to recipients, and can be less pure than a vaccine formulated for injection (*such as unit-dose dried plant tissues*);
- Developing a vaccine that creates long-lasting immunity with a single dose (e.g., recombinant live vector vaccines).

In addition, HPV vaccines would be delivered mainly to young women, and immunization systems for them are yet to be established. Thus any strategy that can reduce costs and simplify administration will be valuable if not essential.

A plant-derived vaccine could directly address the first three PATH-identified targets, and further research could provide material to address the fourth.



# The Blueprint



## A Framework for the Blueprint

Having reviewed the potential benefits of plant-derived vaccines and having identified two lead candidates for initial focus, we now turn to the Blueprint for development of the lead candidates.

This Blueprint uses innovation systems theory (IST). IST derives from research begun in the early 1970s that initially sought to understand Japan's rapid technical and economic success [11]. The studies then spread to the Asian Tigers. Since the mid-1990s, IST scholars have increasingly turned their attention to developing countries. However, little attention has been devoted to biomedical research and almost none to developing technologies for diseases of the poor in developing countries.

The power of IST is that it takes a systems approach to understanding innovation. It seeks to identify the major factors that influence success and the ways that policy makers and managers can help accelerate innovation.

IST reveals that health product innovation takes place in a framework consisting of three major steps and six determinants[2].

The three steps, in simplified form, are:

- Candidate identification and animal studies
- Phase 1-3 clinical trial and production scale up
- Introduction and post-marketing surveillance

The six determinants are:

- R&D in the public and private sectors
- Ability to manufacture new health technology products to high standards
- National distribution systems in both the public and private sectors
- International distribution systems including supply through international organizations such as UNICEF, the operation of global funds, and

trade among countries (National Distribution Systems and Domestic Markets include both public and private sector sales and distribution of new health technologies. International Distribution Systems and Export Markets include both international commercial sales and public sector procurement and distribution through agencies such as the Vaccine Fund and UNICEF)

- Systems to manage IP
- Systems for drug and vaccine regulation to achieve safety and efficacy.

Progress requires attention to these determinants, which are exhaustive—there are no others. A comprehensive Blueprint for product innovation, therefore, must attend to each of the steps and determinants. But there is an additional factor. Health product innovation for diseases of the poor requires the establishment and operation of public-private partnerships. The public sector generally lacks certain capabilities of the private sector, which is better at manufacturing, reaching markets, and dealing with regulatory challenges. Thus, for each step and for each determinant, it is necessary to identify the role of the public and the private sectors. Finally, because this innovation system is dynamic, the Framework is constantly updated as the work proceeds.

A fundamental aspect of IST is the *dynamic linkage* of determinants. Progress in one is facilitated by progress in the others; conversely, lack of progress in one can inhibit progress in the others. It is common in the public sector to carefully plan each R&D step while paying much less attention to the other determinants. Because the determinants are dynamically linked, however, success can be achieved more efficiently through consistent attention to each of the six determinants. This Blueprint seeks to achieve this.



## *The Determinants of the Framework*

Because the Framework includes work in a wide range of disciplines for which no one organization has all the requisite skills, strategic partnerships—particularly with developing country organizations—are essential for success.

This Blueprint describes the required product development efforts for oral vaccines against hepatitis B and HPV. First, we describe some general research challenges for plant-derived vaccines.

### *Research and Development*

Successfully developing plant-derived vaccines means expressing relevant antigens in appropriate plant tissues, formulating the active ingredient into homogeneous, temperature-stable batches, and ultimately preparing concentrated dosages that are appropriate for easy administration in unit doses. To this end, the research and development plan should address the following aspects that apply to all plant-derived vaccines. Important progress is being achieved in each of these areas.

#### **Technical issues**

- Selection of a potent candidate antigen/or antigen combination for the subunit vaccine.
- Determination of appropriate crop/plant to produce a vaccine product of maximal yield and stability with the lowest manufacturing costs, while also considering its adaptation to the country in question, technical feasibility, genetic containment, and regulatory requirements.
- Refinement/optimization of antigen expression in plants
- Protein engineering for optimal mucosal targeting of antigens
- Assessment of the importance of glycosylation in immunogenicity in plant-derived vaccines and identification of ways to improve antigenic properties through modified glycosylation.
- Determination of the optimal means for preventing proteolytic breakdown.
- Assessment and discovery of adjuvants, as well as their means of delivery, to enhance oral immunogenicity and to overcome/prevent tolerance. Adjuvants pose particular problems with plant-derived vaccines that are delivered with some of the plant material remaining. The adju-

vant may “adjuvantize” other bystander plant proteins. It will very likely be better to enhance the antigen content of plants to improve its immunogenicity whenever possible.

- Evaluation of different plant propagation methods for optimal harvesting of plant materials for vaccine preparation, while also establishing appropriate protocols for genetic containment of the genetically engineered crop
- Evaluation of both humoral and cellular immunity with plant-derived vaccines (e.g., to both HBV and HPV).

#### **Process technology**

- Optimization of plant tissue drying for antigen recovery and heat-stable storage
- Good Manufacturing Practice (GMP) protocols and methodologies for purified (injectable) or oral formulations
- Development of quality assurance (QA) and quality control (QC) processes
- Formulation of product and design of appropriate packaging

#### **Clinical phase:**

- Preclinical animal studies. It may be reasonable to proceed without conducting animal toxicology tests. Protocols do not exist; they would take a great deal of time; and it would be difficult to interpret the data.
- Control studies can be started easily as there is no requirement for an Investigational New Drug (IND) approval from the US FDA to “feed” individuals beyond normal institutional animal welfare requirements. However, ethical review will be necessary.
- Measure amplitude and type of immune response.
- Details of administration. If given orally, should the individual be fasted or should there be a protective carrier? What is the impact of stomach acid on antigen integrity and immunogenicity? What is the optimal timing of immunization dose delivery?
- Safety, especially tolerance studies. The intestinal flora of children in developing countries appear to be significantly different than in developed countries, and there are associated major differences in the intestinal mucosa (shorter villi, flat-

tening, thicker and less absorptive). The prevalence of diarrheal disease and parasite infections may also influence the effectiveness of plant-derived vaccines.

- Dose ranging and timing. It will be essential to show that progressively increasing the dosage leads eventually to a plateau in immune response. In a booster situation it is easier to find the right dose. The studies can start at low doses and move higher. The goal is the smallest dose that gives response. Since there is no animal model for guiding oral administration, one could begin in humans.
- Humoral vs. mucosal vs. other immune responses. Monitoring IgE responses may be important.

In the following paragraphs, we examine in greater depth three important research issues: understanding mucosal immunization, achieving adequate immunogenicity of orally-delivered vaccines, and immune tolerance.

### **Understanding mucosal immunization**

The mucosal immune system consists of molecules, cells, and organized lymphoid structures intended to provide immunity to pathogens that impinge upon mucosal surfaces. This includes both innate barriers, such as mucous, epithelium, and innate immune mechanisms, and adaptive host immunity, which at mucosal surfaces consists predominantly of cell-mediated immunity, as manifested by CD4+ T cells, secretory immunoglobulin A (S-IgA), and antigen-specific cytotoxic T-lymphocytes (CTLs). These responses are normally accompanied by the synthesis of secretory antibodies (especially S-IgA), the hallmark of mucosal immunity, which provide an important first line of defense against invasion of deeper tissues by pathogens. Design of non-replicating subunit immunogens to act at mucosal surfaces, in particular using virus like particles (VLPs), has been shown to require evaluation of the protein's recognition by immune effector sites on mucosal surfaces, particularly in the gut. The subunit mucosal vaccine candidates may lack some important immunostimulatory features of the original pathogens and thus sometimes may not elicit sufficiently strong immune responses; this suggests the need to include mucosal adjuvants in some mucosal vaccine formulations. Several candidate immunomodulatory adjuvants are known that may enhance mucosal immunization and

efficiently activate the immune system, leading to the development of strong, specific acquired immunity. Although exposure to antigens via the mucosal immune system may induce secretory IgA and mucosal CTL responses, it is not clear whether mucosal immunization induces long-lived memory responses. This subject requires further investigation to establish appropriate dosage, antigen/adjuvant combination, and prime and boost dosage regimens to ensure induction of immunological memory.

One important issue is the extent to which adjuvants of plant-derived vaccines will also provide adjuvanting effects to the other "bystander" plant components of the vaccine when it is delivered as an unpurified plant sample (such as dried plant tissue).

### **Achieving Adequate Immunogenicity of Orally Delivered Vaccines**

It is well known that mucosal and especially oral immunization usually requires higher amounts of subunit vaccine immunogens as compared to proteins formulated for injection, usually with adjuvants. An oral presentation may require 100 times higher antigen levels to achieve equivalent immune responses probably because of protein degradation in the gut and less efficient immune recognition sites in the gut. For plant-derived vaccines, the most significant potential advantage is the ability to achieve comparatively high levels of antigen in the plant samples in a cost-effective production system. What remains to be determined, however, is the absolute level of protein that will be needed in a unit dose to achieve the needed immune response, and whether it will be practical to deliver this in a dosage of acceptable volume/size. Two approaches have been developed to try to improve the subunit vaccine mucosal efficacy. These are still under analysis in plant expression systems.

First is the incorporation of candidate adjuvants into the plant-derived samples. It is important to determine if mucosal adjuvants will be needed to obtain efficacious immune responses to orally delivered subunit antigens of HBV [12] and HPV [13]. Researchers need to study the use of different adjuvants that show promise from previous studies, some of which can be produced in plant expression systems. Cholera toxin (CT) and *E. coli* heat-labile enterotoxin (LT) are potent mucosal adjuvants for which mutant attenuated-toxicity forms retain adjuvant effects [14].

A second approach involves saponins. Saponins are glycoalkaloid compounds that have immunomodulatory effects. They have been tested extensively in vaccine studies, including oral delivery[18]. The use of commercially available food-grade *Quillaja saponaria* saponin for evaluation of adjuvant activity in animal trials may increase the acceptability of this adjuvant addition to unprocessed plant samples.

It is recognized that regulatory considerations may slow progress on plant-derived vaccines that depend upon adjuvants for effectiveness. The optimal strategy for oral immunization would be to achieve levels of the antigen in the plant tissue that would, by themselves, generate a robust immune response.

### **Immune tolerance**

Many orally delivered antigens, especially food antigens, induce a state of immunologic unresponsiveness, known as oral tolerance, which occurs in numerous animal models and with a variety of antigens, as well as in humans. Tolerance may possibly be terminated or prevented by various manipulations, depending upon the cellular basis of the state of tolerance. Clements et al. have been able to prevent the induction of oral tolerance by delivering the antigen in the presence of specific compounds (certain mucosal adjuvants) that stimulate the immune response to foreign proteins. In the human studies with plant-derived vaccines conducted to-date, oral tolerance has not been observed [20, 21].

We now turn to an examination of the research and development issues specific to HBV and HPV.

### **HBV**

For the hepatitis B vaccine, proof of principle has been demonstrated in phase 1 human clinical trials using HBsAg in fresh plant tissue to boost serum antibody titers in volunteers previously immunized by the injectable vaccine. Since this work was done using fresh plant material (PNAS in press, 2005), subsequent research has focused on accumulation of pre-clinical and phase 1 data with freeze dried material, its stability, the development of GMP standards, and production of clinical lots for Phase 2 trials. Successful work in either priming or boosting studies would represent the achievement of a major milestone. The first studies should be without adjuvant.

To meet FDA requirements for some prototype vaccines, and as discussed above, it is necessary to determine if plant-derived vaccines induce tolerance when administered orally to experimental animals (especially non-human primates). It will be necessary to determine whether test animals orally immunized with a plant-derived HBsAg are subject to oral tolerance. This can be easily determined by treating them with oral doses and subsequently injecting them with the existing purified HBsAg vaccine. The advantages of HBsAg as a test antigen for these studies are that serum IgG is the correlate of protection against HBV disease, it is easy to measure accurately, and it is also the immunological parameter most often influenced by oral tolerance.

Other work could include improving potency by co-expression with other hepatitis antigens. While the use of the HBsAg has proven effective in current vaccines, other hepatitis B antigens, such as the core antigen, may prove valuable for mucosal immunization[22]. Their use, either alone or in combination with HBsAg, is being investigated. Improving targeting to the mucosal system by fusion to other mucosal targeting proteins such as LTB is another area of investigation.

### **HPV**

It is likely that the first commercial vaccines will target HPV 16, 18, 31 and 45 because they are the most prevalent in human carcinomas. Research on a plant-derived HPV vaccine should probably focus on these strains.

Prophylactic studies have used L1 and L2 proteins as antigenic targets. L1 comprises 80% of the capsid protein; L2 is the remainder. These proteins can form VLPs, structurally intact viral capsid proteins without the oncogenic DNA or proteins, the inclusion of which is not desirable in making a vaccine. Research has shown that plant expressed L1 proteins assemble into VLPs [23]. Plants offer the opportunity to express chimeric VLPs with the L1 proteins from various HPV strains. It is not clear how such a strategy may impact epitope presentation and ultimately efficacy. Alternatively, VLPs produced separately could be blended in batch processing.

Such critical issues as the effectiveness and duration of immune response induced by oral vaccination

will need to be examined in animal studies, along with the use of adjuvants.

The next major milestone will be animal and human clinical trials to show the effectiveness of plant-derived vaccines in establishing protective immunity. If the goal is proof of principle, it may be best to limit a plant-derived vaccine to types 16 and 18 to reduce the number of variables in the study.

### Regulatory Affairs

The work with regulatory agencies should identify and address key regulatory issues to establish plants as a viable and cost-effective vaccine production platform technology.

Thanks to the efforts of the FDA, European regulatory agencies, and WHO, the development of new parenteral vaccines is a relatively standardized process. The United States Department of Agriculture (USDA) and FDA began addressing the issue with respect to plant derived vaccine by organizing a scientific information exchange conference on the topic in Ames, Iowa in April 2000. In addition, WHO convened a meeting in Geneva in January 2005 that considered the development of regulations for plant-derived vaccines. This meeting led to the preparation of a manuscript[24]. The Summary section of this manuscript states:

*The meeting concluded that existing guidelines for the development, evaluation, and use of vaccines made by traditional methods can be applied to plant-derived vaccines. For plant-derived vaccines some specific issues will have to be addressed. These include, but are not restricted to, containment of the plants including disposal of waste materials. It was noted that plant-derived monoclonal antibodies have been produced and approved for clinical testing by at least one country and all applicable regulatory and Good Manufacturing Practice (GMP) requirements are in place for this type of product. An innovator wishing to bring a plant-derived vaccine to market should consult closely with regulatory authorities to ensure that all appropriate studies are undertaken.*

The USDA issues permits for the importation, interstate movement, and field testing of “pharma

plants.” This includes inspections of laboratories, greenhouses, and field sites. Generally, the Animal and Plant Health Inspection Service (APHIS) of USDA is the first agency that applicants developing “pharma plants” must work with before initiating reviews with FDA.

The regulations to ensure ‘genetic containment concern the unintentional transfer of genes for pharmaceuticals to crops used for food production. The solutions for the necessary genetic containment are largely at hand (greenhouse production, use of male sterile production lines, etc.) but need to be fully implemented according to defined GMP.

Some issues could be addressed by using plants that are unsuitable for human consumption. For example, tobacco is used in some production systems, and tomato varieties can be selected that are colorless, have an unpalatable flavor, and yet are still adapted to contained greenhouse cultivation. Or the plants might be grown in a unique way, such as propagating only the roots in a culture medium and harvesting the vaccine from the roots.

Once created, plants expressing vaccines can be maintained as genetic stocks (seeds, or seedlings maintained in long-term tissue cultures by clonal propagation). This would allow a validated seed stock to be maintained for use by licensed manufacturing entities in the developing world. The technology for maintaining genetically validated transgenic plant lines is already in place in agricultural systems for current generation “GM crops.” These are being grown in the US and several developing countries.

Plant-derived vaccine programs should work closely with the FDA, USDA, and WHO to assure conformance with appropriate regulatory requirements for the production, processing, formulation, and delivery of plant-derived vaccines. The programs should, at least initially, utilize glasshouse-grown plants for vaccine production to ensure isolation from food crops production. Adapting existing agronomic practices for “manufacturing” of vaccines will therefore preclude the need for extensive crop production research. Field production of vaccines could be employed, if guidelines are developed that ensure public acceptance of such a practice.

An important issue is whether plant-derived vaccines need to be sterile. It would be best if they did

not have to meet this requirement because of the significant costs and problems of ensuring sterility at all appropriate stages. In particular, the oral HBV vaccine should, if possible, be a non-sterile product. Sterilization adds only modestly to the cost of high-cost pharmaceuticals, but would add a much greater fractional cost to a low-cost pharmaceutical, seriously compromising low-cost goals. If sufficiently dehydrated, and stored sufficiently dry, it should be possible to avoid the need for any preservatives. However, the standard food and “non-solid dosage form” pharmaceutical preservatives (those used for vaginal and dermatological topical gels), could be used if needed. These include sorbic acid, methyl and propyl paraben, benzoic acid, and EDTA. Alone, or in combination, these are very likely to be successful in maintaining a low bioburden during shelf life. Straightforward and inexpensive testing is done with USP microbial limits testing as part of the release testing and stability testing. In contrast, for sterility testing, even a single colony is “failure.” And, not infrequently, failed sterility tests are due to laboratory contamination rather than product contamination, causing needless expense, delay, and rejected lots. Sterility is a very expensive and unnecessary standard for an oral product.

### *Manufacture*

There are several important manufacturing issues with respect to plant-derived vaccines. These include:

- Definition of detailed production protocols
- Specification of facilities and equipment
- Detailed calculation of the costs of production

#### **Definition of detailed production protocols**

Commercial production of oral vaccines in transgenic plants will require uniform and stable products from otherwise perishable tissues. It is anticipated that variability of expression among plants or even in tissues of a single plant will always be a factor. The direct use of freshly harvested produce—such as fruit, tubers, roots, foliage or any other plant tissue—is limited due to the relatively short shelf life of these biological materials. It will be necessary, therefore, to focus efforts on finding inexpensive sample stabilization technology, primarily by drying plant materials to yield batch quantities of temperature stable material that can be stored, shipped, and administered at ambient temperatures. A lead strategy would be to

utilize one or more food processing technologies to reduce freshly harvested, antigen-containing plant tissues to a stable formulation, whereby the protein of interest is encapsulated within the preserved plant cell in a dehydrated state. The resulting material would address issues of homogeneity, stability, antigen concentration, and the addition of adjuvants in a final formulated product.

The utility of plant-derived vaccines will depend on the ability to provide a consistent dose. Appropriate clinical data to support the minimal and maximal doses to be applied must be determined. Batch manufacturing and quality assurance can only be validated if the vaccine material provides consistent antigen concentration within an acceptable range. One advantage of applying food processing techniques is the concentration of protein achieved. Material mass can be reduced by as much as 94% (11% of the initial weight for potato, 6% for tomato fruit). Additionally, the physical qualities of this powdered material are convenient to allow formulation for oral intake by palatable methods, such as in gelatin capsules or reconstituted into liquid. Several processed formulations expressing model antigens of interest have been tested for ambient stability of the desired protein at regular periods up to 12 months.

Ongoing studies utilizing food-processing techniques lead to the conclusion that standardized and ambient-stable material can be derived from any perishable plant source. The processing stages provide convenient opportunities to add excipients, such as adjuvants, buffers, antioxidants, or other protein stabilizers, to easily create a final formulation as desired for administration or storage. Mixing batches of dry material could produce multivalent or multicomponent vaccines.

Moisture content may be important for both bioburden and antigenicity, and therefore it may be necessary to employ a system that will preserve a low-water activity state. For developing country applications, stability in high moisture (not just high temperature) environments will often be necessary. Heat will likely be better tolerated by a dry product. Low product moisture achieved at manufacture can be maintained even in high humidity environments by packaging in foil-laminate pouches. These can be formed at low cost with the “horizontal-flow-wrapper” machines use to wrap granola bars, Pop Tarts, and candy

bars. Multiple doses could be packaged in one pouch, then used within some shorter period of time (weeks to months) after opening the pouch. Laminates that include a true foil (rather than metalized plastic laminates) will be needed. Simple gelatin capsules inside

plastic bottles or blister packs may be insufficient, since over time moisture will pass through those partial barriers and increase the water content of the vaccine. Additional techniques (e.g., co-packaging with a desiccant) can be employed if needed.



**Figure 1.**

Freshly harvested plant leaves were freeze-dried to remove about 90% of the fresh weight, ground into a powder and packed into gelatin capsules. One standard size #24 capsule—the largest that can be comfortably swallowed—can hold about 0.8 grams dry material. Ongoing plant expression studies indicate that it is possible to achieve a target oral antigen level of 1-2 mg in one capsule by optimizing leaf gene expression.

It would be useful to compare three means of subunit vaccine production. First, root culture utilizes fermentation technology that is entirely contained during all aspects of production. Studies could provide definitive data on the cost of goods from this approach. Second, one could utilize a non-food crop (tobacco) for oral delivery. This may require a partial purification step to remove toxic alkaloids prior to product formulation. Third, it would be useful to evaluate the use of tomato fruit, since this crop is routinely produced in greenhouse containment systems around the world, and physical isolation (plus potential genetic isolation through male sterility systems) can be used to ensure crop isolation from food production systems.

#### **Facility specifications and detailed calculation of the costs of production**

An important way to justify additional investment in plant-derived vaccines would be to establish an economic advantage to their production when compared with other methods such as fermentation. Work to-date seems to support the following assertions:

- Production of protein subunit antigens in plants is highly efficient. For example, with the current state of the technology, enough HBsAg antigen to

vaccinate all of the world's babies each year could be grown on roughly 200 acres of land; all of the HBV vaccine required annually for China could be produced on a 40-acre plot. Significant further advances in the efficiency of protein accumulation are being obtained.

- Plant-produced vaccine production costs would be lower because they do not require capital-intensive pharmaceutical manufacturing facilities and associated high staff expenses. For example, enough antigen for one dose of HBV vaccine can be produced in unprocessed plant material at an estimated cost of about \$0.005. *This is the US production cost; expenditure would be reduced in developing countries.* [Final product cost will be higher; it is necessary to determine final costs to assess with greater precision end-product savings over traditional vaccine production.]
- Development can proceed immediately in developing countries. One reason new vaccines are not introduced promptly into developing countries is because of the high initial dose cost. The initial price of a new vaccine is set to allow the manufacturer to recoup the high cost of R&D, expense of the production facility, market development and other associated costs. Developed country

producers can afford to offer prices at “marginal cost of production” for developing countries only after a large profitable market is established from which prior investment can be recouped. With plant-derived vaccines the need to recoup capital investments could be much lower than with conventionally produced vaccines, therefore it may take considerably less time before the vaccine can be sold at a price close to the marginal cost of production.

It would be very valuable to evaluate the cost of producing plant-derived vaccines. These studies could include the following variables:

- Plant in which the vaccine is produced
- Scale of production
- Country of production, particularly developed vs. developing countries
- Whether the resulting product is taken orally or injected
- The method of final product preparation
- Effect of different kinds and levels of regulatory requirements

A major study of these issues is underway and the results will be available in early 2006.

### *Management of IP<sup>1</sup>*

As with most biotechnology products, the intellectual property (IP) situation in plant-derived vaccines and pharmaceuticals is complex. Managing IP and tangible property rights presents added challenges—and expense. The main IP aspects that need authoritative management are:

- Protecting new IP generated during the development process, including out-licensing
- Obtaining freedom-to-operate (FTO) by the time products are ready to be commercialized
- Ensuring availability of products to the poor for humanitarian purposes

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<sup>1</sup> This section is in addition to a confidential supplement which, understandably, will be shared on a need-to-know basis only. That document contains a brief review of specific proprietary elements related to plant-derived vaccines (tangible and intangible property), and a more detailed analysis of the Hepatitis B virus vaccine (IP related to the HBV vaccine, plant transformation and antigen production in plants, broad PMP patents, and bioprocess facility aspects). The document concludes with a forward looking section on the management of new IP as well as IP cost implications.

The task at hand is complex, not least because plant-derived vaccines build on several distinct areas of innovation, including:

- Protein engineering and specific antigens (including immunogenicity, specific genes encoding antigenic proteins, etc.) Many patents in this area are the same as those applicable to vaccine production through conventional means.
- Antigen production and accumulation in plants (including expression of foreign genes, optimization of genes, etc.) The technologies associated specifically with the expression of antigenic determinants in plants are the subject of several issued patents.
- Genetic transformation of plants (including transformation protocols and equipment, marker technologies, etc.). Basic plant transformation technologies have been under development for over 20 years. The procedures commonly in use today are covered by a range of issued and pending patents. Virtually all of the groups that have been involved in plant-derived vaccine activities have utilized the *Agrobacterium*-mediated approach to plant transformation. Also included here are promoters, terminators, enhancers, and vectors for use in plant transformation, as well as molecular biology “tool kit” items that mediate the delivery and expression of genes in plant cells, selectable marker systems that allow for the identification of plant cells that have successfully taken up the DNA comprising the gene expression systems, and the “optimization” of genes to ensure that plants recognize and express them.
- Related technologies (such as adjuvants, product formulation, immunomodulatory technologies, etc.).
- Bioprocess engineering for extraction and processing.

An additional complication is that most plant-derived vaccine projects build on the collaboration of a range of research institutions, including private companies and academic centers. Materials will have changed hands during the development, possibly with material transfer agreements that contain restrictions. Collaborative research agreements will need to be developed for each collaboration that deal with new inventions. These must specifically address what will happen if such inventions are joint ones. Further, nasal administration may require access to a number of patents that may be difficult to obtain.

Despite the complexity, the task is doable. First, corporations always manage their IP strategically; obtaining FTO is an integral component of any product development plan. Public institutions are generally less experienced in focusing on FTO and a shift in mindset will be inevitable. The work should take advantage of existing flexibilities, such as the freedom in the United States to undertake research without a license on patented technologies when the goal is to generate data for FDA regulatory requirements.

Second, the goal of facilitating the availability of products for humanitarian uses is gradually becoming an integral part of the policies and strategies of many public institutions. As this mindset gains hold, it will become easier to manage large projects and align the policies of different collaborators, including private entities.

Third, while there is a thicket of patents for plant-derived vaccines in industrialized countries, very few of these patents have been filed in developing countries. This will simplify matters somewhat where humanitarian use is concerned. It also applies to commercial applications in developing countries but does not reduce the overall need for IP management and strategies to obtain FTO.

Finally, there are several models of humanitarian use licensing where patent rights were effectively pooled. An example would be the biotech rice containing pro-vitamin A, “Golden Rice.” Many of the FTO issues that will be faced for plant-derived vaccines arose in the development of Golden Rice. An FTO revealed that Golden Rice included over 70 issued patents and patent applications owned by over a dozen institutions[25]. Yet thanks to the publicity surrounding Golden Rice and the importance of vitamin A deficiency in developing countries, it took only a few months to resolve the constraints. Public and private organizations that hold relevant patents have made them available at no cost through a standard agreement to the inventor who, in turn, can grant one single license for all necessary IP to developing country institutions. Golden Rice thus serves as a useful model of how to approach the community of owners or assignees of relevant proprietary technologies for royalty-free access for humanitarian purposes.

One important difference between the nutritionally-enhanced rice and plant-derived vaccines is that vectors and gene expression components used to produce Golden Rice were assembled without advance consideration of IP and FTO. Preliminary analysis and continued review and update of the IP landscape are essential elements of the development of plant-derived vaccines. Whereas it is relatively easy to put the different pieces into place, the challenge is to manage the process parallel to the advance of the science and the development of the product.

In sum, and based on a preliminary review of a specific plant-derived vaccine, we find that a) the IP issues are fairly clear although additional FTO analysis will be required to address specific cases, b) the issues can be addressed with straightforward IP management approaches, and c) the impact on cost of finished vaccine is expected to be minimal. If a great deal of the work is conducted in developing countries, the IP management issues will be significantly simplified because a number of the patents for which licenses may be required have not been filed in developing countries.

Plant-derived vaccines should be developed along with IP management strategies so that their availability by the poor in developing countries can be better ensured. The negotiating strategy and contract terms are illustrated in Table 2, which is taken from the MIHR Handbook[26]. The table also compares the public sector approach with “traditional” ways of handling these issues.

### *Domestic and International Markets*

The introduction and use of plant-derived vaccines in individual countries must be planned carefully. The issues will be fundamentally different between developed and developing countries. Especially important will be policy research that assesses the views and policies of all key participants in the establishment and sustained use of vaccines in public and private systems [27]. In developing countries, these include ministry of health personnel, the national regulatory authorities, the ministry of finance (responsible for allocating funds for purchase), members of national medical societies and government advisory committees, staff of public and private sector vaccine distributors, and others.

**Table 2. Illustrations of best practices for licensing to meet public sector goals**

Topic	Basic concept	Public sector consideration
<b>Field of use</b>	This clause specifies the limitations on the application of the patent in developing products. The simplest approach is to grant the licensee an exclusive right to all possible applications of the patent, including not only those specified in the patent but others that may emerge as further research and development proceeds.	The clause could grant an exclusive license only for those products that the licensor actually wishes to pursue. Also, it could grant an exclusive license only for those products that were unlikely to have a significant market among the poor in developing countries.
<b>Territory</b>	This clause specifies the geographic areas in which the licensee has the right to exercise the patent. The simplest approach is to grant the licensee an exclusive right to all possible territories. Usually a license is valid only in the countries where a patent has been filed, but the license can give the licensee the right, at the licensee’s expense, to file for patent protection in additional countries.	The clause could grant an exclusive right to a major portion of developed countries (e.g., North America). The licensor could grant another exclusive limited license to Europe. Finally, the licensor could grant non-exclusive licenses to both licensees for an agreed list of developing countries. Then the two primary licensees would have to compete for sales to developing countries.
<b>Price</b>	In most licensing agreements, there will be no conditions with respect to price. The licensor assumes the licensee will determine the best price to ensure the greatest return on investment.	<p>The licensor can consider several options of setting the price to the public sector in developing countries.</p> <ul style="list-style-type: none"> <li>▪ The price could be specified, e.g., \$0.30 per dose. This is feasible only when the licensor has detailed technical knowledge of the production, marketing and distribution costs.</li> <li>▪ The price could be set at cost of production plus a reasonable mark-up (e.g., 15% of cost of production). This is feasible when the licensor has a reasonable expectation of being able to monitor the cost of production.</li> <li>▪ The price could be set at “no higher than the lowest price offered to any private sector buyer.” This may be preferred in cases where it is expected there will be large bulk purchases by private sector buyers who are good at negotiating the very best price.</li> </ul> <p>Price is probably the most difficult area for a licensor. Some believe that licensors should avoid this issue because of its complexity.</p>

*continued...*

Topic	Basic concept	Public sector consideration
<b>Labeling</b>	In most licensing agreements, there will be no conditions about labeling. The licensor assumes the licensee will prepare labeling in conformity with national drug regulatory agency requirements.	The licensor can help ensure that the product is licensed properly, especially in developing countries where national regulatory agency requirements for labeling may not be rigorous or enforced. For example, if some of the research that led to the patent was supported by WHO, the license can specify that the name of WHO cannot be used without prior written approval of WHO. Additionally, the license could state that any claims for the use, safety, and effectiveness of the product should receive prior written approval.
<b>White Knight condition</b>	This concept has been developed by the U.S. NIH. It calls for the licensee to undertake some specific actions that will benefit the public sector.	The licensor can ask for a number of actions, such as the donation of product for clinical evaluation in public sector research programs, joint efforts to develop markets in developing countries, free supply under specified condition to developing countries, etc.
<b>Royalties</b>	Usually a licensee will negotiate the largest royalty in order to maximize revenue from the license.	The licensor can specify that royalties apply to sales only in developed countries. Sales to the public sector in developing countries would be free of royalties. The impact for the licensor would normally be minimal. For example, 5% of a sale in a developed country of a million doses of vaccine at \$10 per dose would be \$500,000. Five percent of the same quantity at \$0.10 per dose for a developing country sale would be \$5000.

Source: Mahoney (2003).

Mahoney and Maynard constructed a strategic approach to the introduction of new vaccines in developing countries[28]. The approach lays out the following five essential overlapping and complementary elements for success. Each element concerns one or more aspect(s) of policy analysis to support the formulation and implementation of effective policy.

1. Measurement of disease burden and computation of vaccination cost-effectiveness
2. Conduct of large scale vaccine-introduction trials (model programs)
3. Establishment of international and national consensus on the need for the vaccine and recommended use practices
4. Assurance of adequate and competitive supply
5. Creation and sustenance of funding mechanisms to procure the vaccine

Measuring disease burden and computing cost-effectiveness are essential for national and international policy-makers to be able to determine the pri-

ority to be accorded to various vaccines. In the context of scarce resources, they must decide the relative priority of various vaccines, such as hepatitis B, *Haemophilus influenzae* type b, Japanese encephalitis, and others. Large-scale vaccine-introduction trials are essential to determine appropriate policies for the delivery of vaccine at the community, provincial, and national levels [29]. The establishment of international consensus and recommended practices is needed to ensure that critical agencies, such as WHO, UNICEF, the World Bank, and others, can help developing countries introduce vaccines consistently and effectively. Ensuring adequate and competitive supply depends on establishing effective policies of cooperation between users and producers.

Producers need to know projected levels of use at various price levels. By encouraging and fostering competition, public-sector policy-makers can help insure lower yet sustainable prices. Finally, global purchase systems, such as those of the Vaccine Fund

and the Global Fund to Fight AIDS, TB and Malaria (the Global Fund), are essential to meet the needs of poor countries. These face extraordinary challenges in mobilizing the required resources to purchase vaccines. The global funds operate using a complex set of policies that has already resolved many important issues, including such controversial matters as respect for intellectual property rights.

#### **Measurement of disease burden and computation of vaccination cost-effectiveness**

For plant-derived vaccines, it will be necessary to do cost-effectiveness studies to take into account the special characteristics of these vaccines. Of particular value will be to conduct analyses of the additional disease prevented in terms of both morbidity and mortality compared with traditional vaccines – cost per Disability Adjusted Life Years (DALYs) gained. For example, how many additional deaths could be prevented by increasing coverage thanks to the heat stability of plant-derived vaccines? Other analyses might look at the quantitative benefits of oral administration.

#### **Conduct of large scale vaccine-introduction trials (model programs)**

Model programs will be important for plant-derived vaccines to deal with professional and lay concerns about vaccines derived from genetically modified plants. Also, model programs will evaluate how the plant-derived vaccines with their different needs (e.g., no need for cold chain) fit with the other vaccines delivered in national immunization programs.

#### **Establishment of international and national consensus on the need for plant-derived vaccines along with recommended practices for use**

The development and introduction of plant-derived vaccines should be conducted with the close involvement of such key agencies as WHO, UNICEF, the governments of developing countries, producers, and others. Regular national and international meetings could help to air issues and generate consensus. Of particular importance will be interactions with

national vaccine regulatory agencies and with the relevant regulatory groups at WHO.

Plant-derived vaccines will face the concerns of individuals and organizations worried about genetically modified organisms. It will be necessary to ensure that clear, accurate, and complete information about the benefits and risks of plant-derived vaccines is made available. This work will have to be conducted in both developed and developing countries. One technical approach to this matter would be to focus on developing plant-derived vaccines in non-food or non-perennial crops. An additional tactic would be to accord priority to crops that can be grown in greenhouses with enough efficiency to preserve cost benefits. For all of these reasons, tobacco may be a good option.

Experience with microbicides indicates that it would be very valuable to prepare analyses that estimate the potential health impact of plant-derived vaccines. These studies could look at the potential savings to the immunization program (thereby freeing resources for other purposes), the increased coverage that could be obtained, and other benefits.

#### **Assurance of adequate and competitive supply**

As noted earlier, one of the potential comparative advantages of plant-derived vaccines could be the relative ease of establishing their production in developing countries. There should be a systematic program of assessing production opportunities in developing countries both through technology transfer and IP management. One useful strategy may be to form an independent public-private partnership in a developing country that will take on the detailed management of a consortium of developed and developing country public and private entities.

#### **Creation and sustenance of funding mechanisms for vaccine procurement**

These are long-term goals that will need to be addressed after plants have been demonstrated to be viable, attractive means to produce vaccines and after one or more vaccines are approaching the market.



## Conclusions

This Blueprint presents an overview of the issues facing the development of a new platform technology for the production of vaccines. This new platform promises to yield many benefits especially in facilitating access by the poor to existing and new vaccines. The establishment of plants as a method for producing large quantities of vaccines for oral administration can revolutionize the world of vaccination and help bring to the poor the promise of

vaccines for controlling infectious diseases. However, because of economic and market realities, the promise of plant-derived vaccines will be achieved only through the collaboration of the public and private sectors with significant investments by both parties. These investments however will yield rich dividends in the form of widely available, inexpensive, orally administered, heat stable vaccines.

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