



Figure 1: 1802 caricature of Edward Jenner vaccinating patients who feared it would make them sprout cow-like appendages

The Cow-Pock — or — the Wonderful Effects of the New Inoculation! — etc. the Publication of S. A. & Co. Society

Vaccine Evolution

Phil Ball and Maria Lusk at Eden Biodesign discuss the latest technologies and regulatory initiatives that aim to enhance vaccine development

Humans have utilised vaccines in rudimentary forms for centuries. Smallpox was the first disease against which people tried to protect themselves through intentional exposure to infected material (variolation), in a practice that originated in China or India over a thousand years ago. There was relatively limited advancement until the late 1700s, when the English physician Edward Jenner inoculated a young boy with cowpox to prevent him from catching smallpox. Since then, vaccine development has progressed to the extent that, in 2006, the vaccination rates against six major diseases (diphtheria, measles, pertussis, polio, tetanus and tuberculosis) had reached 80 per cent (1).

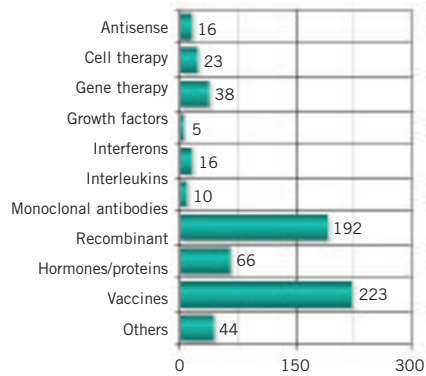
GROWTH DRIVERS IN VACCINE DEVELOPMENT

In both the public eye and around the board tables of large pharmaceutical companies, vaccines are undergoing something of a renaissance. Vaccines demonstrated significant market growth in 2007, outperforming most other sectors of the industry, predominantly due to the success of Merck’s Gardasil and continued strong performance of Prevnar from Wyeth. This growth and a market of approximately \$16.3 billion in 2007 (with projections of a \$100 billion market by 2024 based on a similar compound annual growth rate), have certainly attracted the attention of companies not traditionally associated with vaccines (2). The vaccine industry is at present an oligopoly, dominated by major

players: Merck, Sanofi-Aventis/Pasteur, GSK, Novartis and Wyeth (Pfizer). However, the high performing potential of vaccines now represents an attractive prospect for other large pharma companies with concerns over diminishing returns through their traditional pipelines.

In addition to corporate organisations, the cost-effectiveness of vaccination rather than treatment is widely recognised by international health authorities. Further sources of funding from philanthropic organisations have boosted the development and availability of paediatric vaccines in developing countries. Likewise, there continue to be high levels of biodefence funding for vaccine research and development. In the 2009 US fiscal year, there was a budget of \$8.01 billion for civilian biodefence programmes, bringing the total funding from 2001 to 2009 to \$49.66 billion (3).

Figure 2: Biotechnology medicines in development by product category



Each of these factors represents a driver for growth in vaccine development, which perhaps explains why there are more vaccine products (prophylactic and therapeutic) under development than any other class of biological medicine (4). This growth will only be sustained through innovation and strategic development, and although the biopharmaceutical industry has come a long way since the earliest days of vaccine manufacturing, the challenges remain fairly constant: what is the most reliable and cost effective manufacturing method? How does one ensure that the starting materials and final product are both

consistently safe and of high quality? Who can manufacture the amount of vaccine needed for clinical trials and, eventually, commercial production? This article will highlight some technological innovations and regulatory perspectives that may potentially facilitate expedited and streamlined development within this traditionally conservative industry sector.

VALUE-ADDING ENABLING TECHNOLOGIES DURING VACCINE DEVELOPMENT

Expression Technologies

As for all biopharmaceuticals, there are key value-adding stages during the development of a vaccine. However, as vaccines are probably the most disparate of all biological medicines, both in terms of biochemical composition and the methods of production, there are currently only a limited number of platform manufacturing methods applicable to distinct vaccines. Likewise, there is no single expression system suitable for numerous vaccine candidates, as they range from relatively simple polypeptides to entire cells.

The criteria for choosing an expression system are the same as for any other biopharmaceutical – it is essential that the system produces a product of consistent safety and quality. It is also necessary to ensure that the yield of the production method is fully optimised, as cost-of-goods for vaccines are critically important, particularly for those medicines intended for supply to developing nations.

The adoption of novel expression systems has to be carefully considered. Although there are benefits in applying innovation in order to shorten development times and be the first to market, the vaccine industry is traditionally quite conservative. This is because the medicines are typically administered to healthy patients and, as a result, there is a strong emphasis on product safety. In addition, the complex nature of many vaccines minimises the available options for risk-reduction through extensive product characterisation.

As vaccines are typically administered in smaller doses, market supply may consist of grams rather than kilograms of material per annum, diminishing the net requirement for large-scale production. The drive for large-scale production involving high-titre expression systems has, therefore, been less for vaccine manufacture than for other biological molecules, such as monoclonal antibodies. With all of these considerations, there has been reluctance in the vaccine industry to adopt novel expression systems, but rather a preference for working with well-established systems of a known and accepted safety profile.



Table 1: Drivers to move from eggs to cell culture

Eggs	Cell culture
Keeping chicken flocks segregated and disease free is challenging.	Cell culture and subsequent product purification represent a rapid means of production that can be validated for process and product consistency.
The logistics of egg procurement may be a limiting factor, as shipment and delivery of the eggs must be controlled. Timelines must be carefully monitored to ensure availability allows enough time to produce vaccine and hit the delivery dates to the clinics for trial commencement.	A precedent of performing cell culture processes at hundreds or thousands of litres scale means there is the potential to provide all of the required product in only a few runs.
If there is a flu pandemic caused by a strain of virus that was not anticipated, it will be difficult to utilise the slow (approximately six months) egg production process to manufacture enough vaccine in time to protect the general population.	Several biopharmaceutical companies already have in-house cell culture capability, and for those who do not, there are contract manufacturing organisations (CMOs) who can provide everything from process development services and cGMP manufacturing capacity to stability testing and support for regulatory filing.

There are examples of economic and regulatory influences, however, that are encouraging the adoption of new expression technologies for vaccine development. A high profile example is the move from eggs to cell culture in the production of the influenza vaccine (see Table 1). There is likely to be further adoption of new expression systems, especially those with associated benefits for reducing overall development times.

There are very few expression systems that are specifically targeted towards vaccines, although there are examples that are particularly applicable to this market sector. These include the PER.C6® cell line, and the associated AdVac/Virosome technology, available from Crucell/DSM, and the avian-derived cell lines from Vivalis (EBx®) and ProBioGen. Another technology with potential benefits is the Pfenex Expression Technology™ from Dow, which has been applied to generate high levels of vaccine antigens. Insect cells also represent an alternative system for vaccine production, with examples including production of antigen for the Provenge™ cellular vaccine (Dendreon) and Ceravix™ (GSK). Protein Sciences Corp has developed a patented baculovirus protein expression system (BEVS), for production of proteins and vaccines in variant Sf9 cells.

Process Development and Advances in Vaccine Production

As described, vaccines are complex and diverse biomolecules ranging from recombinant subunit antigens to live organisms. Correspondingly, therefore, a range of production technologies are required to manufacture sufficient quantities of these products, including the use of eggs, cell factories, roller bottles,

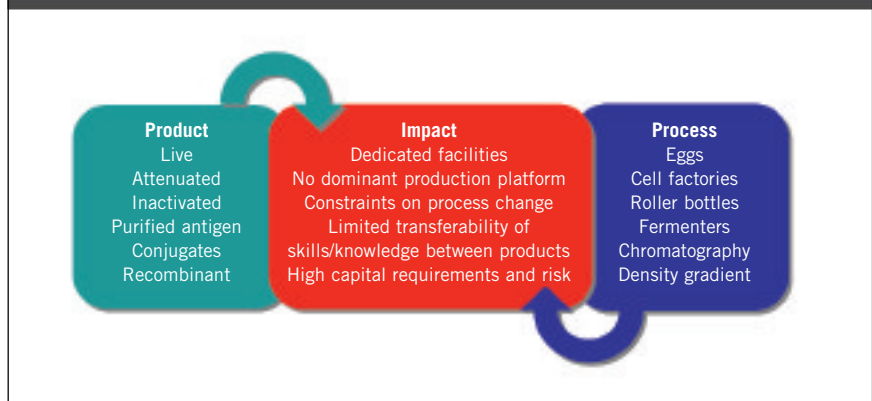
shake/tissue culture flasks and bioreactors. This wide range of production methods can be problematic, as each one requires specific capital expenditure, specialised development approaches and operator expertise. As a result, there is a growing trend to reduce the number of production platforms by implementing manufacture in suspension culture bioreactors wherever possible. For vaccines manufactured in mammalian cell culture, it is necessary to establish well-characterised expression permissive cell lines that have been adapted to grow in suspension culture, and (where appropriate) in a serum-free media. An example of this is the emergence of suspension HEK293 cultures for the production of adenovirus viral vectors, where the development of chemically defined and serum-free formulations has been influential in increasing titres and generating fully scalable processes (5). Similarly, microbial systems are being optimised for the manufacture of vaccines, especially those in which co-expression is beneficial to provide multi-valency.

There has also been significant investment in the provision of disposable bioreactor

systems, which have potential applications in vaccine development and manufacture (6). Disposable systems have particular benefits in multi-product environments, as there is a diminished requirement for cleaning validation activities, and they have the potential to be rapidly deployed in emergency situations. Although the use of disposable systems (stirred tank or rocking platform) is gaining momentum for vaccines manufactured in mammalian cell culture, further development is necessary to provide the mass transfer required for microbial cultures.

Purification of vaccines, particularly those comprising whole cells or complex macromolecules, has traditionally been relatively inefficient and technically challenging. For example, the production of viruses and virus-like particles (VLPs) has commonly involved a primary purification step by density-gradient centrifugation, a labour-intensive process that is not readily scalable. An alternative strategy is to apply column chromatography, which, until recently, had been confined mainly for use as a polishing step during the production of

Figure 3: Impact of product and process complexity on vaccine development



viruses and VLPs. The binding capacity of conventional porous chromatography resins for large molecules such as vaccines during primary capture is relatively low because of steric restriction to the active binding sites. This capacity can be increased when using membranes or monolithic columns, as these have a wider pore or channel diameter, and thus a more accessible active chemistry than the porous beads within packed beds (7). Membranes and monoliths can also be operated at high flow rates, resulting in smaller columns and shorter cycle times. These combined benefits are making chromatographic purification of certain vaccines an increasingly viable option.

Characterisation and Formulation

For many complex vaccines, the true proof of principle can only be gained by clinical testing, and during early development the product features that influence efficacy may be poorly defined. It is important, however, to elucidate the physicochemical features of the vaccine as far as practicable. As vaccines are traditionally quite complex, detailed characterisation has necessitated the development of novel analytical methods such as those based on spectroscopy and mass spectrometry (8). This in turn has provided opportunities to design and evaluate vaccine manufacturing processes with greater consistency.

Designing formulations that enhance stability on storage and maintain or optimise the subsequent immune response is of critical importance during vaccine development. Vaccines, perhaps more than any other class of biopharmaceutical, require storage across a wide range of temperatures, especially when administered in developing nations or in military environments. Likewise, the complexity of vaccines often causes them to be relatively unstable by comparison with other pharmaceutical products that have a low turnover and frequently require a long shelf life.

A more established way of achieving stability is through lyophilisation. If stability cannot be achieved in liquid formulation, removal of the solvent via freeze- or spray-drying represents another manner in which product degradation can be minimised. For this reason, many vaccines are lyophilised prior to storage

and reconstituted in water for injection (WFI) immediately prior to administration. The delivery of vaccines can be further improved by utilising technologies that involve controlled product release from microspheres. Spray-drying technology is particularly suited to vaccines, as alum is preserved during spray-drying, and the resulting powder can be combined stoichiometrically during the preparation of multi-valent vaccines.

Many vaccines are formulated with adjuvants, which help modulate and stimulate the immune response (9). These compounds bind to the vaccine and aid retention at the site of injection or delivery to the lymph nodes. As a result, the release of the antigens to the surrounding tissues is slowed and a stronger immune response is achieved than would be generated by the vaccine alone. The predominant adjuvants are 'alum'-based, containing a mixture of aluminum salts. However, there are novel adjuvants under investigation that can provide benefits over alum, such as the technology developed by Antigenics: QS-21, an adjuvant derived from tree bark (10). The uptake of new adjuvants may be restricted, as they themselves will require testing and regulatory approval, similar to the vaccine itself.

REGULATORY INITIATIVES TO STREAMLINE VACCINE DEVELOPMENT

A predominant focus of regulatory agencies such as the FDA is to ensure the safety of the recipients, as vaccines are typically administered to healthy patients. For this reason, as discussed, the vaccine development industry has always been considered relatively conservative and risk-averse.

However, recent initiatives designed to streamline the development and approval of all biopharmaceuticals are having an impact on vaccine development. These, in combination with initiatives specifically aimed at addressing situations of pandemic or biodefense, have provided a regulatory environment in which there is now increased scope to expedite vaccine development.

An example of a general programme is the FDA's 2004 Critical Path Initiative (11). The focus of this project is to work with

pharmaceutical companies, academic institutions and others to "stimulate and facilitate a national effort to modernize the scientific process through which a potential human drug, biological product or medical device is transformed from discovery or proof of concept to a medicinal product". Essentially, the initiative responded to the fact that, although the science behind drug discovery continues to advance, the number of innovative medical products submitted for approval has actually decreased (12). It is argued that this is because the current pathway for the development of biopharmaceuticals is becoming increasingly challenging, inefficient and costly.

The FDA stresses that further tools are required to demonstrate more quickly and cheaply the effectiveness and safety of new products, as a lack of these tools may be one reason that many products fail during clinical testing. Recognising their unusual position in witnessing the development challenges of many companies, the FDA is working on the initiative to identify the most pressing problems and those areas where there are the greatest opportunities for rapid improvement and public health benefits. This is referred to as the Critical Path Opportunity List (13). Within this initiative, there are goals directly applicable to vaccines, including:

- The development and use of better evaluation tools – the FDA is working with NIAID to develop rapid *in vitro* tests for adjuvant development that are based on relevant human cells, as many adjuvants harbour components that may produce negative side-effects when administered.
- Streamlining clinical trials – the FDA proposes using sponsor-supplied data to establish immunogenicity thresholds for vaccine approval, particularly if a large scale clinical trial is not possible. In addition, work is ongoing to develop a new vaccine efficacy trial design, to help alleviate the burden of a large-scale trial, especially for a new vaccine that is similar to a product that is already approved.
- Utilising bioinformatics – the FDA is working to move away from paper systems and use electronic systems, particularly for data mining. The



‘Sentinel System’ is a collaboration between the FDA and the industry to develop a tool to monitor the safety and effectiveness of several biologics, including vaccines (14). This system will utilise public and private databases.

- Development of products to address urgent public health needs – fast preparation of vaccines for diseases such as AIDS and pandemic influenza is of paramount importance.
- Modernising manufacturing – initiatives by both the FDA and the EMEA are focused on introducing quality by design (QbD) (15) into the manufacturing space, with the aim of providing regulatory flexibility around process analytical technology (PAT), formal experimental design and the design space of a process.
- Focusing on at-risk populations – the FDA is working to define ways to support at-risk populations, such as infants and children.

Quality by Design

The QbD framework is a risk-based, quality-enabling approach driven by a

thorough understanding of the product and the process. The rationale is that the product developer must understand the relationship between the critical process parameters (CPPs) and their influence on the product’s critical quality attributes (CQAs), and likewise the influence of these CQAs on product safety and efficacy. This understanding allows a multi-dimensional ‘design space’ to be defined, from within which the developer and the regulatory authorities have confidence that the process will consistently achieve the required quality level to meet the desired clinical performance. A benefit of the QbD approach is that quality is built into a process, not tested for at the end of manufacturing. In turn, this provides opportunities for regulatory flexibility, such as reduced regulatory oversight when changes to the process are involved.

The challenge for implementing QbD with biological products is the extensive process and product characterisation required to create the design space. For complex molecules such as vaccines, it is a

difficult to assign definitively the CQAs during early development, especially if the product is a novel biomolecule and hence there is no precedent allowing for comparison, or if the mode of action has not been fully elucidated. However, major vaccine companies such as Wyeth have been champions of QbD from the onset, as they recognise the potential benefits that it may provide.

In addition to more general initiatives, the FDA and other regulatory agencies have implemented formal mechanisms to expedite the approval of vaccines under emergency situations, be these for emerging diseases or for biodefence reasons. This regulatory approach is important because advances in genomics and vaccinology now allow the scientific community to provide potential vaccine candidates rapidly in response to a natural threat or human action. Such scientific advancements need to be complemented by a streamlined regulatory environment, and therefore ‘Fast Track’ designation and ‘Accelerated Approval’ pathways have been introduced (16).

Fast Track designation has been designed to facilitate the development and review of new biopharmaceuticals, and includes more frequent communication with the FDA who provide advice and case studies through their outreach programme. Also, a product may be granted 'priority review' status in certain circumstances.

Under the Accelerated Approval pathway, the vaccine approval is based upon a decision that the effect of the vaccine on a surrogate endpoint, such as the production of protective antibodies, is likely to predict its clinical benefit before true efficacy is demonstrated. Although it undoubtedly expedites the process, the approach of administering a vaccine to humans without complete confirmation of efficacy is far from traditional. For it to be successful, there is considerable emphasis on post-approval surveillance studies to demonstrate that the vaccine indeed prevents the targeted disease. This pathway was implemented during the approval of Fluvarix in 2005.

A formalised 'fast-track' mechanism is less evolved in Europe, although the EMEA has introduced the concept of submitting dossiers for 'mock-up' influenza vaccines, in which the process of manufacture is approved but the mock-up strain is substituted following an officially declared pandemic situation (17). In theory, this could be expanded to include other vaccines where an expedited approval mechanism is required.

CONCLUSION

While demand and funding for vaccines is currently high, the only way this industry can continue to grow is through innovation – not only in the development of enabling technologies, but also in the approach taken by regulatory authorities.

Although they are often relatively complex, scientific advancements in many cases now allow developers to design, manufacture and characterise vaccines to no less degree than they would for products such as monoclonal antibodies. The outcome of this is increased levels of both expectation and flexibility from the regulatory authorities.

In short, as the science of vaccinology evolves, so does the regulatory environment. It is therefore vitally important that vaccine developers, contract organisations, technology providers and regulatory agencies continue to work closely together to meet the growing demand for safe and effective vaccines.

References

1. WHO vaccine-preventable diseases: monitoring system, Global Summary, 2007
2. Kalorama Information: www.kaloramainformation.com
3. Franco C, Billions for Biodefense: Federal Agency Biodefense Funding, FY2008-FY2009, *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science* 6(2): p131, 2008
4. Report on biotechnology medicines in development, *PhRMA*, 2008
5. Durocher Y, Phama PL, St-Laurent G, Jacob D, Cass B, Chahal P, Laub CJ, Nalbantoglu J and Kamena A, Scalable serum-free production of recombinant adeno-associated virus type 2 by transfection of 293 suspension cells, *Journal of Virological Methods* 144: p32, 2007
6. Clarke HRG and Compton BJ, Comparing Mammalian Expression Systems The First Rate-Limiting Step in Making Products for Clinical

Testing, *BioProcess International* p24, November 2008

7. Gagnon P, Monoliths Emerge as Key Purification Methodology, *Genetic Engineering and Biotechnology News* 28(14), 1st Aug 2008
8. Arnaud CH, Analyzing Vaccines, *Chemical & Engineering News* 84(14): p52, 3rd April, 2006
9. Scott C, Formulation Development, Making the Medicine, *BioProcess International* 42, March 2006
10. Sjolander A, Cox JC and Barr IG, ISCOMs: an adjuvant with multiple functions, *Journal of Leukocyte Biology* 64: p713, 1998
11. www.fda.gov/oc/initiatives/criticalpath
12. Innovation/Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products, FDA, March 2004
13. <http://www.fda.gov/oc/initiatives/criticalpath/opportunities06.html>
14. <http://www.fda.gov/oc/initiatives/advance/sentinel/>
15. Somma R and Signore A, Embracing Quality by design, *Contract Pharma*, October 2008
16. Baylor NW, Regulatory Issues: Pandemic Preparedness Pacing Up, *Biopharm International*, August 2007
17. EMEA: Guidelines on Influenza Vaccine Prepared from Viruses with a Pandemic Potential

About the authors



Phil Ball is Technical Director, US, at Eden Biodesign, Inc in North Carolina. Phil is responsible for leading technical development and strategic business analysis in terms of proprietary technology. Previously, Phil was the Analytical and Purification Development Manager, responsible for managing the development of products spanning the full range of biopharmaceuticals. Prior to this he was a consultant, providing technical and regulatory evaluation of early to late-stage biotech products. Phil gained his PhD in protein biochemistry from the University of Liverpool.
Email: philip.ball@edenbiodesign.com



Maria Lusk is Director of Client Management at Eden Biodesign, Inc in North Carolina. Maria joined Eden Biodesign in January 2009 and is responsible for project management and client interface for North America and also supports the business team. Prior to joining Eden, Maria worked as a project manager for a pharmaceutical consulting firm in Cary, NC, and was also previously at Diosynth Biotechnology in Research Triangle Park. Maria's background is in project management, business development, quality assurance and quality control. Maria graduated with a BS in Life Sciences from North Carolina State University in Raleigh, NC, and is PMP certified.
Email: maria.lusk@edenbiodesign.com