Novel Factories for Novel Vaccines

PEER REVIEWED

Summary

Vaccination is considered one of the most successful strategies for fighting infectious diseases, an area where there still remain significant unmet medical needs. In the past, it has been difficult for business to find opportunities in this area due to the long development times and to the risks involved in testing, for what could be relatively low returns, as many of the development opportunities were in low income countries. This makes it hard to build the right business proposition – right product price – that could bring a return on the high-risk investment. A relief in this status has been observed recently: the establishment of clear needs in terms of vaccines at global level has given the right degree of predictability to commercial entities. This predictability removes some of the risk. Better business models that can be applied to these indications, better understanding of the disease mechanisms, along with application of novel technologies have enabled new vaccine projects that address diseases with great need. Globalisation has also meant that diseases are not "local", and their ability to spread fast globally has been seen in recent years. The traditional methods for preparing vaccines are not suitable for the fast response which is needed to face global epidemics. This opens the opportunity to develop more efficient and better adapted platforms. Manufacturing platforms and technology have been aspects that have proven very relevant both in translating the science into a product, and in responding to the challenging need to control manufacturing costs. Faster, more flexible, less capital-intensive platforms are needed, that can fulfill the potential of rationally designed vaccines. The importance of understanding and including the right adjuvant has been increasingly recognised, triggering considerable research in this area. The focus of this short review will be on recent alternative manufacturing platforms for novel vaccine production. Examples from the development pipeline of vaccines to Dengue fever, West Nile and Japanese Encephalitis virus will be used to illustrate the issues.

Introduction

Although vaccination is demonstrably one of the most efficient ways of fighting infectious diseases, there are still significant neglected needs for a number of diseases, despite recent important advances. For example, following the introduction of pneumococcal conjugate vaccine, an estimated 211,000 serious pneumococcal infections and 13,000 deaths were prevented during 2000-2008. Routine rotavirus vaccination, implemented in 2006, now prevents an estimated 40,000-60,000 rotavirus hospitalisations each year[1]. Launched in 1974, the Expanded Programme on Immunization (EPI) was first designed to deliver vaccines against diphtheria, tetanus, pertussis, polio, measles and tuberculosis (BCG). Together, these vaccines prevent close to 2.5 million deaths every year[2]. Diseases most prevalent in poorer parts of the world have lagged behind as targets for vaccine development, certainly because they are challenging to tackle, but also for lack of the right business model that provides an acceptable level of risk for the investment commitments that are required.

On the other hand, recent years have provided interesting returns to vaccine manufacturers, with sales of vaccines reaching US$25.3 bn in 2010. This figure is predicted to increase at a compound annual rate of 9.3% during 2010–2015, to reach US$39.5 bn in 2015 as new product introductions continue and usage of current products expands further[3]. Promising new areas for development include RSV and Dengue, but also *S. aureus*, and other nosocomial infections, and immunotherapies for neurological and metabolic diseases, as well as cancer.

The changing environment, pressure from payers to cut costs, and the implications of certain decisions - for example, as already indicated by the insurance companies, the possible exclusion of nosocomial diseases from insurance repayment - have a real impact on business opportunities, and illustrate clearly how societal factors condition the opportunities beyond the science and investment.

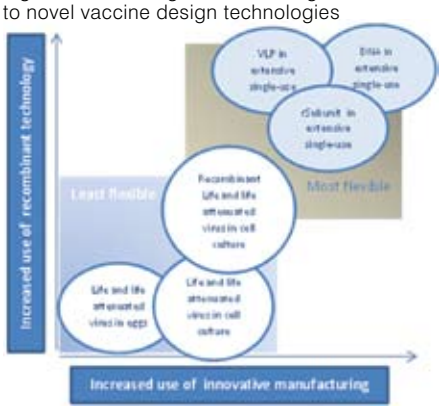
In order to represent a real business opportunity, the development of vaccines needs to have the right science, but politics and financing also need to come together. Politics have a role in the effectiveness of implementation and extent of vaccine campaigns, for example, in allowing access to vaccination centres and for follow-up.

Making a Vaccine

There are three main "science-based" components in a vaccine — antigens, adjuvants and delivery system/ administration schedule. Production platform and supply chain efficiency are two strategically enabling and differentiating factors.

A recent analysis by Frost & Sullivan

Figure 1: Matching manufacturing innovation to novel vaccine design technologies



on the vaccine market[4] highlights that the most significant technical challenges in the field of vaccine development at present are: selection of appropriate expression systems for recombinant or subunit vaccines, cold chain storage requirements, and production challenges with egg-based vaccines. This latter is triggering interest in developing new types of platforms, for example, the use of plants to produce virus-like particles (VLPs).

Choice of manufacturing platform is strategically important in vaccine design, and will condition cost and ability to respond to need. Figure 1 depicts the interconnection between modernisation in both technology for antigen development and manufacturing units of operation. At one end are the traditional life attenuated vaccines, produced in the most traditional platforms, such as chickens' eggs; at the other end of the spectrum are innovations in facility design and in unit operations that have enabled and are adapted to innovative recombinant technology platforms. Traditional steel-based technology represents high investment and low flexibility, whilst the more modern platforms, based on recombinant technologies and facilities that include single-use technologies, allow for greater manufacturing flexibility. Development of new antigens has also benefited from increased platform versatility, which have made possible rational vaccine design.

An examination of the current vaccine R&D portfolio activity reveals that it is still highest in antigenic component development followed by delivery systems. Adjuvant research is highly fragmented, but is now being recognised by vaccine developers as essential for making vaccines with high efficacy.

Adjuvants are now considered an area of opportunity for development, in particular for the newer indications in non-infectious contexts such as cancer, neurology and metabolic diseases, because of their interaction with the immune response. Adjuvant development is challenging, not only because the exact mechanisms by which stimulation of the immune system occurs, and the exact type of response sought, are still poorly understood, but also (and consequently) because the path to approval is long and challenging. Thus, often the better known adjuvants continue to be used, although some of the newer technologies such as use of oligonucleotides are finding good grounds to advance. The potential of adjuvants in permitting dose reduction or overcoming non-response clearly marks the importance of continuing to understand the role of these important vaccine components. Table 1 summarises the current adjuvants in use and in development[5]. Amongst the most recent advances, two are highlighted for their originality: use of Lactococcus "shells", or BLPs (bacterium-like proteins) being developed by the Dutch company Mucosis and the very early stage and intriguing use of the protozoan *Tetrahymena thermophila* and its ability to produce naturally occurring protein nanoparticles by Tetragenetics which the company markets as their G-SOME™ vaccine technology[6].

Table 1: Adjuvants in use and in development

Adjuvant	Major immunostimulating component(s)
In the market	
Alum	Aluminium salts
MF59 (Novartis) and AS03 (GSK)	Squalene-in-water emulsions
AS04 (GSK)	MPL plus alum
Widespread experimental use or late-stage vaccines	
Poly-IC (also Poly-ICLC)	Synthetic derivatives of dsRNA
MPL and formulations (AS01, AS02)	MPL and QS-21
Flagelin, flagelin-Ag fusion proteins	Flagelin from *S. typhimurium*
Imiquimods	Imidazoquinoline derivatives
CpG oligonucleotides and formulations (IC31, CB10)	Synthetic P-thioate-linked DNA Oligonucleotides with optimised CpG motifs
CAF01	Trelose dimycolate (cord factor)
ISCOMS and ISCOMATRIX	Saponins
IFA (and Montanide formulations)	Mineral or paraffin oil & surfactant
CFA	IFA plus peptidoglycan, trehalose dimycolate
MUCOSIS	BLPs, mucosal immunisation
TETRAGENETICS	G-SOME™ platform, naturally made nanoparticles

Modified from Coffman, Sher, Seder, Immunity 2010

The Mucosis BLPs, derived from food-grade bacteria, are adapted to mucosal delivery of vaccines. According to Mucosis, there are two modes of application of the BLPs. The first is an add & mix approach in which the immune-stimulating properties of the BLPs are used to improve existing vaccines and to enable a mucosal route of administration. The second is a bound approach in which the BLPs not only act as an immunostimulant but also as a carrier for the subunit antigen(s). The binding of the antigens is achieved by a linker domain called Protan that binds to the surface of the BLPs. Mucosis is developing its adjuvant technology in a vaccine pipeline that includes FluGEM, an influenza vaccine using the BLP technology, which is in Phase I clinical trials[7].

Tetragenetics recently announced preclinical study results showing that an investigational nanoparticle-based influenza vaccine using the G-SOME platform protects against a highly pathogenic H5N1 avian influenza strain. The study was conducted by scientists at the Institute for Antiviral Research of Utah State University in Logan, Utah and Tetragenetics, under a contract sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health.

The recombinant nanoparticle vaccine against a highly pathogenic avian H5N1 (A/Vietnam/1203/2004) strain at lethal doses led to 90% survival following immunisation and lethal challenge. The H5N1 vaccine candidate was comprised of the viral hemagglutinin linked to a scaffold protein from Tetrahymena thermophila that promotes assembly into remarkably homogenous nanoparticles in the 40-60nm size range (G-SOME™ particles[6]).

New Strategies for Antigen Design – Focus on Recombinant Subunits and VLPs

Rational design of vaccines has been one of the new areas of development that has given the vaccine field a new interest, with tools that allow the design and manufacturing of what is expected to be more efficient and safer vaccines. New vaccines such as Prevnar (recombinant subunits), Cervarix and Gardasil (VLP) have demonstrated the market potential of new technologies. New approaches that rely on recombinant technologies and that deliver the vaccine either in the shape of recombinant DNA, or the resulting recombinant protein, which in specific cases can assemble in a particle, include particles that mimic viruses: virus like particles, or VLPs. Amongst the new types of vaccines being developed, we will focus here on recombinant subunit vaccines and VLPs. In both cases, the products in development make use of industrial platforms for recombinant protein production.

The NIAID gives the following definition for recombinant subunit vaccines: *Instead of the entire microbe, subunit vaccines include*

only the antigens that best stimulate the immune system. In some cases, these vaccines use epitopes—the very specific parts of the antigen that antibodies or T cells recognize and bind to. Because subunit vaccines contain only the essential antigens and not all the other molecules that make up the microbe, the chances of adverse reactions to the vaccine are lower. Subunit vaccines can contain anywhere from 1 to 20 or more antigens, (..) [manufactured] using recombinant DNA technology.[8]

VLPs mimic the external protein structure of a virus without including the genetic material (DNA or RNA) necessary for viral replication. Without genetic material, VLP vaccines are incapable of causing infections themselves while at the same time presenting viral antigens in the most authentic configuration possible. The objective is that the immune system responds to a VLP vaccine as if to the live virus, without the risk associated with virus replication, hopefully making the technology an efficient and safer advance.

A variety of platforms have been used for production of VLPs, such as plants, mammalian and insect cell lines. In all cases, successful production of VLPs has been demonstrated, and its efficacy demonstrated through clinical trials. Recent advances using the VLP technology showed strong immune response for vaccines created from VLPs vs. traditional whole viron vaccines, as well as protein antigen-based vaccine. Further possibilities of VLPs were demonstrated by the recent RTS.S hybrid malaria-hepatitis B VLP, which showed 50% protection of children in Africa in Phase III clincal trials[1].

Developments in Manufacturing Process – Single-use Units of Operation

Manufacturing used to be – and still is to some extent – one of the most important entry barriers to the area of vaccines, with high initial capital investment required for building the production facilities. The introduction and development of single-use components in all unit operations for manufacturing, and the good match between these and the new platforms used in the production of the new vaccines, has reduced the size of this barrier. Widespread application of single-use technology is being welcomed by the industry and more widely adopted: in a recent industry survey[9], a majority of respondents indicated that biologics currently in the pipeline will likely be made in single-use bioreactors.

Flexibility in manufacturing is seen as one of the most important trends in innovation in this area, as illustrated by the recent award by the American Pharmaceutical Manufacturing Magazine to Sartorius Stedim Biotech (SSB) as a 2012 Innovations Honoree for its new FlexMoSys production platform design. The system combines SSB's broad, single-use and reusable product portfolio and G-Con's novel cleanroom pods, and thus offers a totally integrated production process platform. It enables GMP-compliant cleanroom space to be created that is faster, more flexible and significantly less expensive to set up and operate than a conventional, large purpose-built facility. Standard pods of the FlexMoSys cleanroom system can be conveniently tailored to a customer's specific needs, as the customer can choose from an entire suite of fully integrated bioprocessing equipment like SSB's FlexAct solutions. The FlexMoSys pods can be easily moved due to installed air bearings underneath each pod. This is important when modular unit operations must be placed in a different sequence or a pod has to be decontaminated[10].

The trend also lowers considerably another traditional barrier holding off competition: the complexity of the supply chain. More flexible manufacturing, engendering lower capital investment for building factories, lowers the barriers for expanding manufacturing to areas in the world where it is difficult to make a case for capital-intensive investments in inflexible factories, such as the more traditional vaccine manufacturing facilities would require.

The advantages of single-use are being well documented, with thorough modeling indicating significant economic advantages, including the need for reduced capital investment, with improved returns, increased speed and flexibility due to reduced facility complexity, smaller footprint and portability, and improved process control. Single-use allows for greater flexibility in matching bioreactor size to actual need, since there is no need to decide and fix on a steel tank volume.

Extensive application of single-use is particularly well adapted to multiproduct facilities, since there is significant reduction in validation protocols, reduced downtime, faster turnaround, and significant reduction in cleaning and sterilisation between products/strains. Through elimination of need for cleaning in place and steaming in place, it was estimated that up to 50% of the energy costs and 8-20 times less water are needed when single-use is widespread[11]. Comparability of cell growth and productivity for a variety of platforms has also been demonstrated.

Bavarian Nordic uses a proprietary modified Vaccinia Ankara virus as the active ingredient of its products. For their smallpox vaccine, IMVAMUNE, the company collaborated with the US NIH for the development of a safe MVA-based smallpox vaccine. The success in developing this vaccine triggered an increased demand, and the company decided to invest in its own manufacturing facility, to complement its existing agreements in other manufacturing sites. The challenge was to build a facility capable of supplying 40 million doses annually in a relatively short period of time, at the same time as process industrialisation and optimisation. Single-use technology has been widely used during process development, and a decision was taken to continue to use it. This allowed building activities to be decoupled from process industrialisation, one of the requirements in the challenging timeline. The disposable work involved collaboration between the Bavarian Nordic staff, Sartoriu and Stedim (now Sartorius-Stedim), Wave Biotech, certain media suppliers, and the engineering company NNE. MVA-virus are too big to be filtered, so the whole process need to be aseptic from start to vial. Flexibility in the facility was also a requirement, and there was a decision to minimise

open handling. The implementation of single-use ensured minimised risk of cross-contamination and maximum flexibility. Regulatory facilities and auditors were consulted regarding the design of the facility, and they expressed interest in such extensive use of single-use technologies. Whenever a single-use solution was available, it was incorporated in the design. Bavarian Nordic used suppliers' validation packages and evaluated and selected single-use products based on a risk analysis approach that takes into account several factors, including how critical the process step is. The experience of Bavarian Nordic can be useful for other companies wanting to build new facilities that extensively integrate single-use. For this project, single-use allowed Bavarian Nordic to reach its objectives: short project timeline, highly flexible facility, adaptable to future manufacturing needs, and minimised contamination risk[12].

With the continuing success of single-use systems, there has been some consolidation in the industry, with large corporations acquiring smaller, innovative ones, where new single-use technology is developed. A relatively wide choice of single-use technologies and bioreactors is available and is being developed. Companies, such as Cellution Biotech[13], are developing bioreactor alternatives that are well adapted to enable flexibility in scaling-up and compatibility with cGMP.

Table 3: Summary of status for DenV, JEV and WNV (13, 14, 15, 16, 17, 18, 19)

Target Virus	Disease Background and Status
Dengue Fever Virus	There are an estimated 100 million cases of Dengue fever infection annually, caused by the Dengue fever virus DenV, of which there are 4 known serotypes. Infection by one serotype creates life-long immunity against that serotype, but there is a strong association between a second infection with a different serotype and more severe forms of the disease. There is still a need to fully understand the mechanisms underlying the disease, but from what is already known, there are implications for vaccine design, namely that any vaccine will need to immunize simultaneously against all four serotypes. To date, efforts to develop an effective vaccine capable of eliciting a balanced tetravalent immunity while maintain an acceptable safety profile, and with a convenient immunization schedule have failed. However, recent development of a tetravalent vaccine made using a recombinant YF that expresses Dengue antigens from SP and is in Phase III have renewed the hopes in the field. Other strategies such as the recombinant subunit vaccine developed initially by Hawaii Biotech and now owned by Merck, and a DNA vaccine being developed by the NAID/CVR also yielded promising results.
Japanese Encephalitis Virus	Japanese Encephalitis is characterized by high mortality (15%) and high frequency of neurological sequelae (WHO report 2006). The annual number of cases estimated at 50000 is likely an underestimation. Three billion people live in countries where JE is endemic REF United Nations The United Nations urbanization prospects: the 2005 revision. POP/DB/WUP/Rev.2005/1/F1. New York: United Nations; 2005. Approved vaccines are based on attenuated virus grown in Vero cells. Recently approved vaccines (IXIARO™, 2009, IMOJEV®,2010) are based on recombinant technology and has so far made sales in the order of 9 m€ ww in 2011. Estimate peak sales for this vaccine, in the order of 330 m€ (15).
West Nile Virus	WNV became endemic within 10 years of its introduction in North America. So far, in the United States between 1999 and 2010, 30 662 cases were confirmed and associated with 1163 deaths. The virus uses birds as replicating host, and opportunistically, infects animals and humans. Currently, there is no vaccine approved for use against WNV infection in humans, but there are vaccines for veterinary use. The frequency and severity of clinical illness increases with age. In about 20-30% of cases, the disease remains asymptomatic, but there is sudden onset of symptoms. Long term neurologic sequelae develop in about 50% of the cases. Disease manifestation is explained by neuronal damage in several regions of the brain. Patients with compromised immune systems, advanced age and underlying conditions such as diabetes, are at increased risk of death from the disease.

Table 2 summarises the current manufacturing landscape in terms of manufacturing platform and inclusion of single-use technology, using as examples VLP or recombinant subunit vaccine production, both strategies being amongst the most recent - and considered the most promising - technologies for vaccines. Both VLPs and subunit vaccines in development have been produced in a broad range of platforms, and all of the manufacturing platforms are well adapted to single-use. This example shows the good fit between the new manufacturing technologies and novel vaccine development. The following cases will show how the choice of the appropriate manufacturing platform enables the development of vaccines against a set of infectious agents, for which there is a strong unmet need, against Dengue Virus (DVE, Japanese Encephalitis Virus and West Nile Virus (WNV).

Table 2: Platforms for VLP and recombinant vaccine production

Both VLPs and Recombinant Subunits are successfully made using innovative platform and production technologies		
Producing platform	**Steel**	**Single use**
CHO	+	+
Other mamm	+	+
BEVS	+	+
S2	+	+
Plant cells	n.a.	+ [1]
Tetraynema	-	-
E. coli & other procaryots	+	+
Yeast	+	+

[1].VLPs were produced in whole plants. Where plant cells are used, there are reports of use of single-use reactors.

Case Analysis: Three Examples of Developments in Areas of Unmet Need

This section will use three viral infectious diseases as examples of how the new technologies are enabling strategies that make use of the most modern technologies to respond to current serious unmet medical needs. The three viruses belong to the same family and they are all insect-borne. Table 3 summarises the main disease characteristics and the specific challenges in developing a vaccine for these indications.

Of the three viral infections discussed, the only one for which a human vaccine is available is Japanese Encephalitis Virus. The vaccines marketed are all life-attenuated viruses. There are no marketed vaccines for humans

Table 4: Examples of production platforms for experimental vaccines for DenV, JEV, WNV

	Virus in Cell culture	VLP	Subunit	DNA	Viral vectors	Adjuvant examples
DV	In development	293 Yeast BEVS	S2 – Clinical Ph1 E. coli Yeast CHO	In development	MVA	Sanofi pasteur, Rec.virus Phase III, no adjuvant Gsk/WRAIR, rec Virus, AS03, Alum, AS01 NIAID/CVR/Vical, DNA vaccine is formulated in Vaxfectine®, a novel proprietary cationinc-lipid-based formulation Hawaii/Merck, subunit tetravalent, Iscomatrix® adjuvant
JEV	Marketed and in development	BEVS yeast	CHO S2	In development	-	Intercell -IXIARO® attenuated Virus, Alum Sanofi-pasteur Imojev®
WNV	Marketed vet and In development	yeast	COS S2 – Clinical Phase 1 BEVS	vet	VSV	Fort Dodge, DNA in MetaStim™, a proprietary lipid/surfactant adjuvant Hawaii, S2-rec. subunit in GPI-0100, a quillaja saponin semi.synthetic analog

against Dengue fever or West Nile Virus. A vaccine for veterinary use to protect against WNV is approved.

There has been a long history of failed attempts to develop a vaccine for Dengue Virus, and the current trials with tetravalent vaccines are drawing much excitement.

The chronology of vaccine strategies used for JEV illustrates well the impact of new technologies in allowing development of safer and potentially more efficacious vaccines: from an early approval of a vaccine extracted from animal tissue, to use of recombinant technology and safer manufacturing platforms, such as cultured cells, in the most recently approved vaccines, IXIARO™ and IMOJEV™.

Production Platforms

In view of the large unmet medical need, there has been a continued effort to find an appropriate vaccine strategy, and we can find in the pipeline for these three indications all of the most recent approaches to rational vaccine design, including DNA-based, recombinant virus, recombinant subunit and VLP vaccines, to cite some.

Amongst the platforms for recombinant protein production used in the development pipeline for these vaccines, we find that insect-based platforms feature in all three cases. Experimental subunit vaccines against JEV produced with the Balulouvirus Expression Vector system BEVS are in research stage, and recombinant subunit vaccines against DV and WNV produced in S2 cells have been tested in Phase I.

Over the past 10 years, insect-based platforms (BEVS and S2 cells) have moved from the research labs to larger-scale production. There are now insect-cell-made vaccine components in the market and in various stages of clinical development in a variety of indications. For a recent review of the use of BEVS, see[18], and for use of S2 cells, see for example[19]. The strengths of the S2 cell-based expression platforms can be summarised as: speed of production, allowing fast access to the required proteins, excellent expression capability, scalability, applicability to a variety of process choices, and regulatory friendliness. S2 cells have been used for expression and analysis of a broad range of protein classes. As the system is based on generation of stable production cell lines, it allows for a broader variety of upstream process options, compared to the obligatory batch process of the BEVS. Proteins have been produced in fed-batch and in perfusion using ExpreS^2, the platform developed by ExpreS^{2}ion Biotechnologies. Both methods led to high production yields, with perfusion allowing for smaller footprint installations, a factor that may allow for more flexibility in early stages of production[19].

In looking for more flexible, low capital cost investment alternatives to manufacturing, S2 cells again are an interesting alternative: the ExpreS^2 platform has been tested in Cellution's single-use bioreactors with success in a malaria vaccine in pre-clinical development[19]. S2 cells have also been tested for production of monoclonal antibodies in WAVE systems. The results showed that production in the order of 1g/L could be obtained and that the antibodies were functional[20].

The variety of manufacturing platforms being used in these three indications illustrates well the importance of developing a broad range of protein expression platforms to increase the potential for matching the needs for each protein.

In conclusion: rational design of vaccines with improved efficacy and safety profiles makes extensive use of recombinant technology. Amongst the new types of vaccines are recombinant subunit vaccines and VLPs. These technologies have opened new possibilities for developing vaccines for diseases where there are no

current products licensed, such as Dengue and Malaria. The availability of a broad range of manufacturing platforms has also contributed to the advancement of the field, allowing vaccine developers more options to express challenging proteins. It is very important that the production platform method has good industrialisation potential and compatibility with cGMP. Stable insect cell systems, such as S2-based, are well adapted to these needs.

The recognition of the important role adjuvants play has contributed to increased research in this area, but both the science and the regulatory path are current challenges to development. There are nevertheless several experimental vaccines in development making use of new adjuvant platforms. Matching the flexibility of the manufacturing technology with flexibility in the actual factory has been a recent development, with the broader introduction of single-use systems.

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