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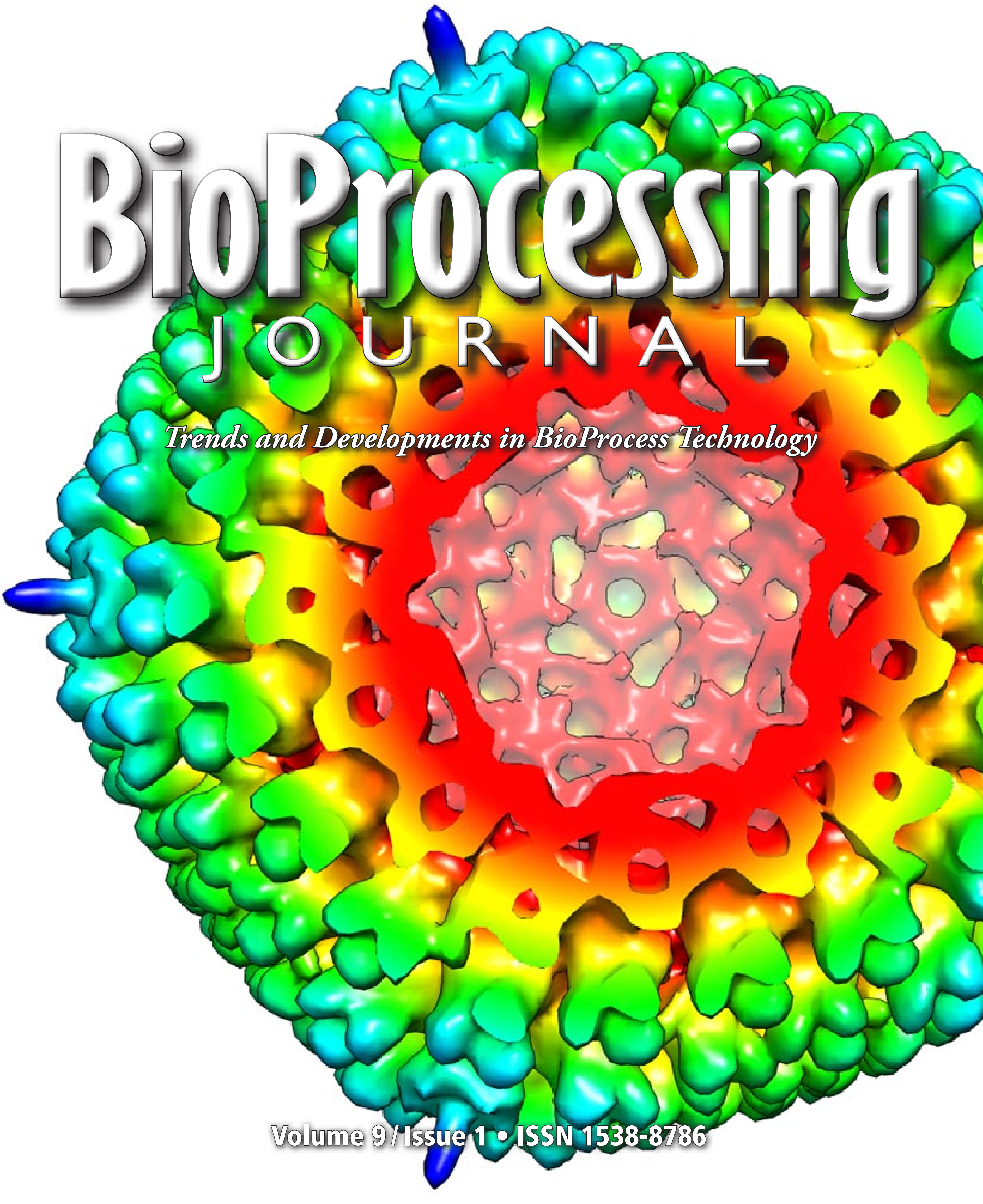

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Improving Process Economics When Developing a Vaccine Portfolio

By Philip BALL and David SIMPSON

Introduction

Over the last few years, the challenges of vaccine development have created perhaps an unprecedented level of scrutiny, not just within the biotech industry, but also in the consciousness of the general public. This was certainly the case during the recent H1N1 influenza outbreak. The demand to know when a vaccine would be available, and if producers could meet the global demands consistently made front page news.

The challenge of rapid and scalable manufacture is of course nothing new in biopharmaceutical development. In many respects, monoclonal antibodies are leading the way as the industry moves toward the required level of industrialization. At the heart of this is the use of generic platforms to develop and manufacture discrete antibody products, therefore accelerating development, mitigating risks, and providing incremental returns on capital investment. These attractive benefits have certainly contributed to the current situation of an industry increasingly dominated by this particular product class.

By comparison, there has been traditionally less synergy between vaccine products, placing considerable logistical, technical, and capital demands on a company wishing to develop a portfolio of diverse vaccines products. This article will examine this disparity further but also explain how modern approaches are resulting in improved process economics when establishing and maintaining a diverse vaccine portfolio. The resulting consequences not only challenge us to rethink how vaccine companies should best operate but also provide opportunities to better meet the ever-increasing global demand for this important product class.

The Benefits of Rapid Development – A Risk-Based Approach

One of the most important considerations when developing a novel vaccine is of course time involved in meeting market forces and keeping up with global supply demand. But fast development is also beneficial commercially as time is inherently linked to the value (perceived or otherwise) of the product under development.

If we consider "value" in these terms, ultimately it relates to costs (development or subsequent manufacturing/marketing) and future income (total market/share of that market). However, each year this cash flow is discounted back to its present value according to a pre-determined interest rate. The sum (or net) of these present values is a common measure by which to evaluate the viability of an investment and is inherently linked to time (Figure 1.)

Let us consider two products with the same total development costs, but one in which the development costs are accrued over a shorter period of time. This product will have a greater

$$PV = \frac{C_t}{(1+i)^t} \qquad NPV = \sum_{t=0}^{n} \frac{C_t}{(1+i)^t}$$

PV = Present value	*i = Interest rate*
C_t = Cash flow (revenue – costs)	*t = Time (years)*
	n = Total duration

FIGURE 1.

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net present value (NPV) and will break even sooner (Figure 2). Also, as costs can be capitalised over time, it makes financial sense to reduce the time over which the costs are incurred. However, we must also be pragmatic and understand that the majority of novel biopharmaceuticals in pre-clinical development will not proceed to the later stages of clinical development, never mind approval and commercial launch (Figure 3).[1, 2] There are many reasons for this, including commercial viability, but in many cases it is simply due to biology manifesting in unwanted safety or efficacy profiles.

Biopharmaceutical development is therefore an inherently risky business and ultimately it will not be solely boiled down to process development strategy determining if a product proceeds. For this reason, there is often a temptation to move quickly through early development to the point of first-in-man studies. This is especially true for vaccines where there are no animal models as the only real proof of concept will come during clinical trials. At this point, you will know if there is a viable product or not and therefore if further investment in manufacturing and testing is required.

It is generally possible to move though early phases of clinical development without expending too much time on process development. This is because the amount of product required during Phase I and Phase II studies is often quite low. However, there is usually a considerable increase in material requirements as you move into later phase clinical development, particularly for vaccines where large efficacy studies are often required. For example, Prevnar, Rotarix, and Rotateq all required significantly more patients in their pivotal Phase II trials. Even though vaccines tend to be relatively low dosage, as the product moves into later phases of development, the existing manufacturing process will be challenged. In some cases it may simply be impossible to scale-up to meet with demands. The consequence is that the process will likely require further refining in order to capture the scalable elements required. What is the net outcome? Firstly, in the event that the production cell line is changed, this will have clear implications on the purification strategy. Furthermore, a process change may manifest in a different impurity profile requiring further analytical development and possible modification to the entire testing regime.

Regulatory guidelines are quite clear in stating that any significant process redevelopment will likely necessitate a comparability exercise.[3] This will involve physicochemical and biological characterisation at a minimum and a clinical bridging study may also be required (Figure 4).

All of this is not only costly but time consuming, resulting in lost years of potential revenue/market share. But for a novel vaccine, there is

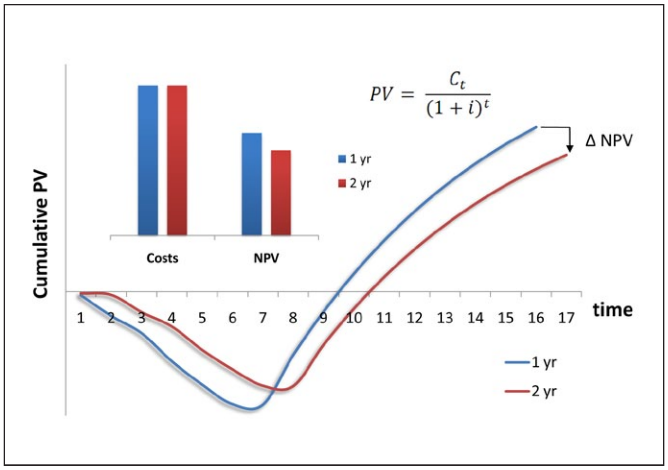
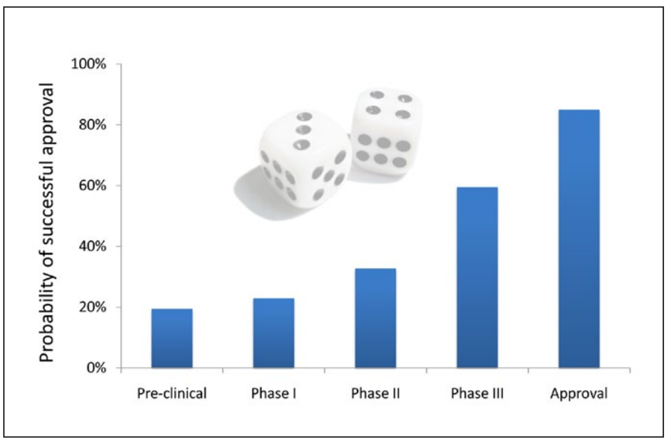


FIGURE 2.



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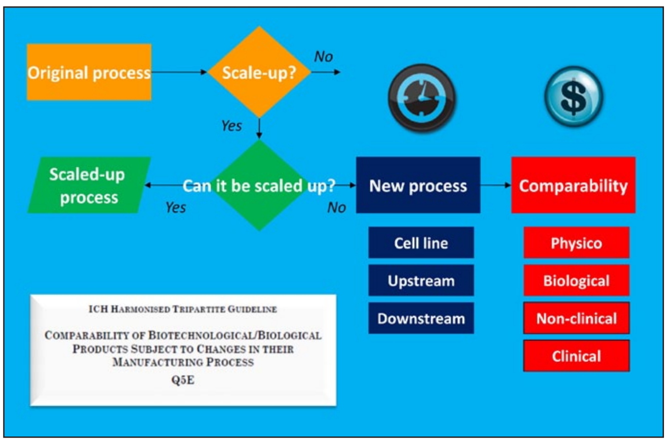


FIGURE 4.

a risk of not being the first to market which ultimately further reduces the NPV (Figure 5).

So in short, there is a risk-benefit balance during early phase development. Considering on one hand the inherent risk of failure in the clinics, it does not make sense to over-develop a process, especially when the manufacturing requirements are likely to be quite low for early clinical trials. On the other hand, proceeding with a non-scalable process may introduce otherwise avoidable delays and negatively impact the product's value.

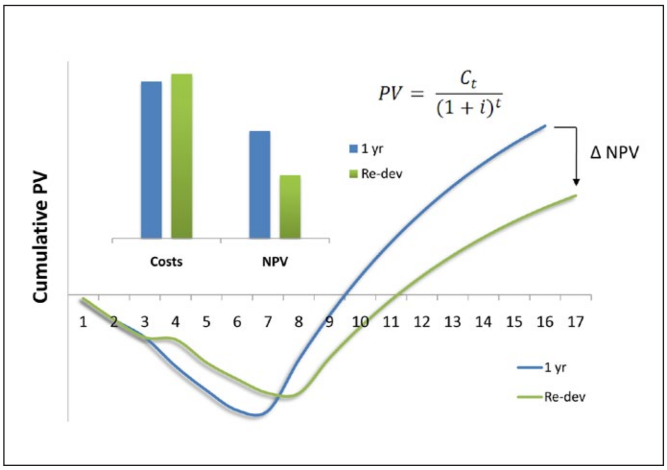
However, in reality there are strategies that can be implemented from day one in a development programme that will increase the value of the product by reducing the risk requirement for wholesale redevelopment, minimise regulatory concerns, and improve cost of goods without adding significant time to the development plan.

Scalable Solutions for Vaccine Manufacturing

Before discussing scalable processes, it is important to consider what we mean by "scalable." To change the scale of something means to make it bigger or smaller by keeping the proportions the same. An example of this would be the proportional expansion of a bioreactor from 10 to 100 L or increasing a column diameter from 10 to 30 cm. Another common (but less accurate) method of process scale-up is to increase the number of units of the same size. For example: more eggs, more tissue culture flasks, more bioreactors, more membranes, etc.

It is generally regarded that when a unit operation is examined in isolation, a greater economy of scale is achieved by increasing unit size rather than unit numbers. This is because when the number of units is increased, the labour and capital costs also increase in a linear fashion. However, by comparison, the capital costs are not proportional to the increase in scale when larger units are used (Figure 6).[4, 5] For this reason, scale-up typically involves increasingly larger bioreactor/column/membrane sizes as the product progresses to commercial stage. Large-scale bioreactors in excess of 10,000 L have been widely accepted in the monoclonal antibody industry, driven by the fact that there are multiple products with an annual demand in excess of 100 kg per year (Figure 7).[6]

One of the reasons why we have seen such growth in scale is because there is a predominant cell used in large-scale manufacture (CHO) which has been adapted to grow in suspension culture at high densities in large-scale reactors. However, one of the challenges when developing scalable manufacturing processes for vaccines is that they represent a complex and diverse set of products, all with particular manufacturing requirements and therefore, potential issues of scalability. As compared to monoclonal antibodies,



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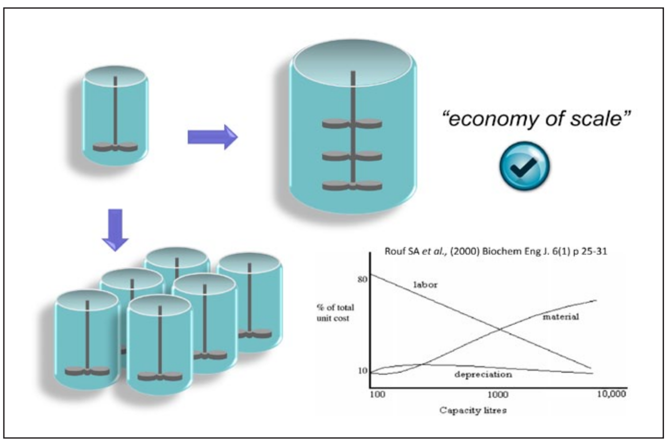

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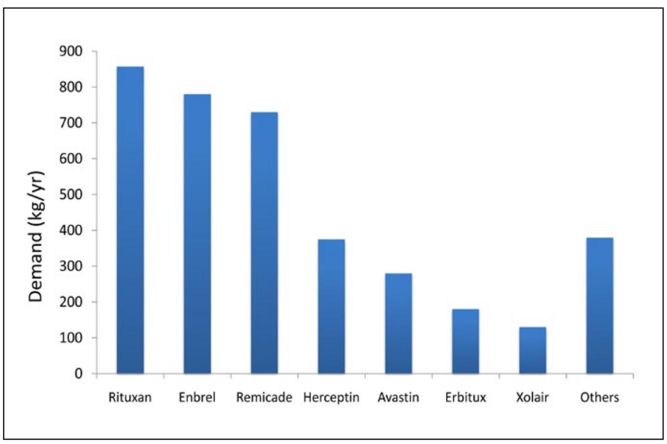


FIGURE 7.

there is not a single pre-dominant manufacturing platform for vaccines (Figure 8). For marketed vaccines, manufacturing technology is broadly split into those derived from viruses or those comprised of or expressed in microbial systems.[7]

Vaccines derived from microbial systems can either be whole cell products or filtrates in which the microbe is inactivated or attenuated. A significant number are derived from either polysaccharides (conjugated or otherwise) or toxoids from microbial culture. Hepatitis B vaccines are also virus-like particles (VLPs) expressed in yeast. Those derived from viruses can be live, live-attenuated, or inactivated. As influenza vaccines are so predominant, the major manufacturing platform is embryonated chicken eggs where the virus is inactivated post-production. However, there are a number of products such as those for measles, mumps, rabies, and yellow fever that are produced in chicken egg cell culture systems, whilst other viral based vaccines can also be propagated in cell lines (typically WI-38, MDCK, Vero, or MRC-5). These different propagation systems are also associated with various production methods, each with their own readiness to scale-up (Figure 9).

Outside of biocontainment issues, scale increases are generally more readily achievable for those products consisting of or derived from microbial culture. Although not the focus of this article, by following classical bioprocessing paradigms with regard to dissolved oxygen, impeller speeds, etc., it is possible to scale-up fermentation in a linear fashion. For example, pertussis has been manufactured at the 15,000 L scale by sanofi, and VLPs expressed in *S. cerevisiae* have also been manufactured at large scale.

Scaling-up production of viral vaccines has been more problematic. For example, increasing the capacity of influenza production in eggs is the most extreme example of where scale-up is achieved by increasing the number of units rather than the size of the unit. It is remarkable that the global capacity to produce flu in eggs is so high (approximately 250 million doses for seasonal flu) – and this has been achieved, at least in part, by automation. However, it is generally recognised that production in eggs is far from ideal, and there have been numerous calls for a change to comparative cell culture systems. Of course, this is not just because of scale and capacity issues, but also because of supply chain issues involving the sourcing and handling of vast quantities of eggs.

The scale-up of cell culture is in itself challenging as the predominant cells lines (Vero, MDCK, MRC-5, and WI-38) used to propagate viral growth of approved products are all anchorage-dependent cell lines. Anchorage-dependent cell line scale-up is more difficult than those adapted to grow in suspension culture. As such, a variety of methods focus mainly on increasing unit operations or surface areas including tissue culture flasks, roller bottles, cell factories, and cell cubes (Figure 10). Scale-up for commercial production using these methods is achieved by increasing the number of units rather than volume. It becomes problematic to significantly increase scale in this way because of product handling. And unless expensive automation is installed, there

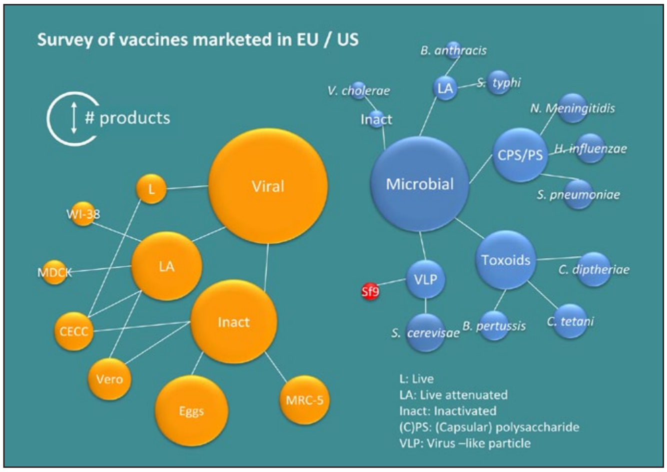


FIGURE 8.

FIGURE 9.

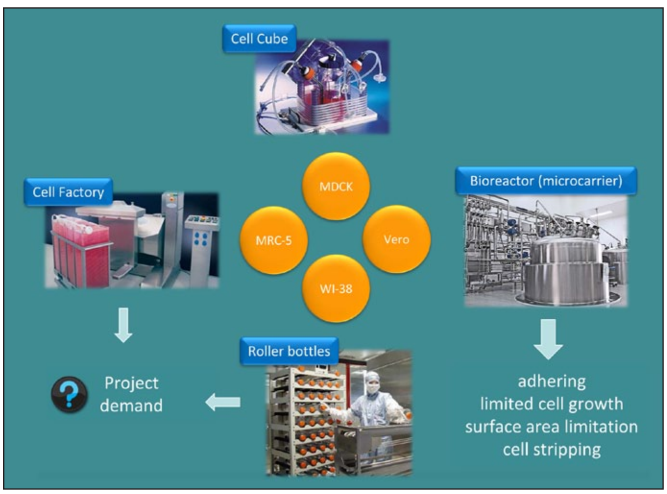


FIGURE 10.

is a maximum scale at which these multi-unit systems can be effectively operated. If total annual product requirement projections exceed what can be achieved using roller bottles or cell stack/cubes, it is recommended that a process be developed based upon scalable cell culture systems in stirred tanks or rocking platforms at the earliest stage.

For anchorage-dependent cells, it is usually impossible to fully adapt the cell line to suspension culture. Therefore, further support for the cells is often required (*e.g.,* by using microcarriers).[8, 9] Batch culture using microcarriers provides many of the same benefits as classical adherent cell culture. Productivity is increased through volumetric increases in a nearly linear fashion as long as accepted bioprocessing methodologies are followed. Through development and optimisation, it is now possible to realise large-scale manufacture of both Vero[10-13] (6,000 L, Baxter) and MDCK[14-15] cells (2,500 L, MedImmune).

However, when compared to batch suspension culture, there are further limitations with microcarrier use. For example, it can be difficult to adhere cells to the microcarriers, cell growth can be limited, and collision of solid microcarriers can lead to lowered cell growth, stacking, and subsequent stripping of the cells. As such, there has always been a limitation to the maximum volume that can be achieved. For this reason, interest has increased in pre-adapted suspension cultures such as ProBioGen's AGE1 avian cell lines[16] or Crucell's PerC6®.[17] Likewise, interesting recent developments in the generation of suspension-adapted Vero[18] and MDCK[19] cells could provide further opportunities for scale-up.

But a key question is: How far do we need to go with scale-up? Probably the biggest demand in routine viral manufacture is for influenza. By current estimates, between 100 and 200 kL of cell culture could satisfy the typical demand for seasonal flu and this would be spread out between various manufacturers. For other vaccines, the demand is far less so it is unlikely that multiple > 10,000 L bioreactors will be required for MAb production (Figure 11). Also, we should learn from the industrialised MAb industry where there is a trend toward utilizing smaller tanks and disposable technologies that are increasing productivity. Finally, an integrated approach should be taken when evaluating scale-up options. Fermentation or cell culture should not be the only considerations as purification can account for 80% of production costs. Obviously, increasing the culture volume is going to place strain on the downstream purification stages, especially if it requires the harvest and pooling of multiple discreet units from microcarriers at large-scale.

Vaccine Purification

The purification of vaccine products often has a set of challenges unique to complex macromolecules such as VLPs or viruses. The classical approach when designing a purification process is to exploit the greatest difference in the properties of the product and impurities. Therefore, the larger size of viruses and VLPs have been targeted during the development of filtration and size-exclusion chromatography approaches (Figure 12).

Filtration is critical for purification of all viruses, from harvesting and clarification to

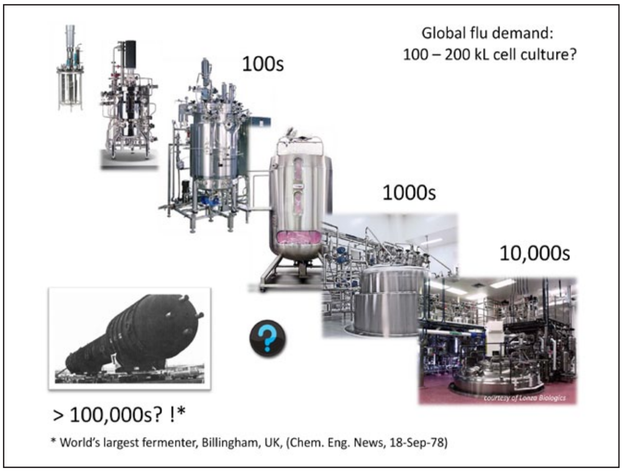


FIGURE 11.

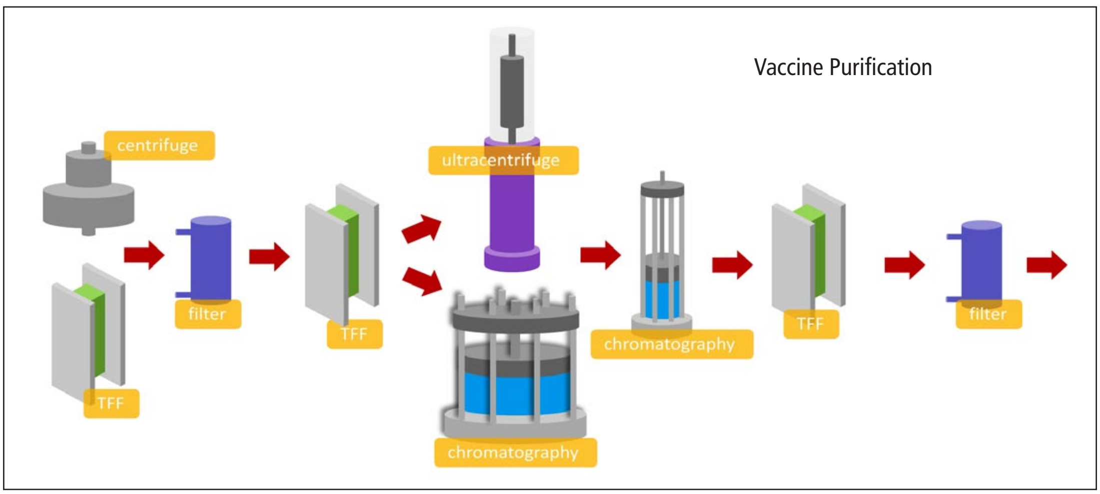


FIGURE 12.

removal of contaminants. Ultrafiltration/diafiltration (UF/DF) is commonly used as a concentration step where high molecular weight cut-off membranes can be used to significantly reduce the impurities whilst retaining the larger vaccine product. Hollow fibre technology is particularly appropriate in vaccine purification as the relatively open flow path limits shear damage.

Historically, chromatography has been used primarily for polishing in either size exclusion or ion exchange flow-through mode. It has been less commonly used as a primary capture stage because conventional resins are suboptimal for the purification of complex molecules such as vaccines and are subject to capacity limitations due to steric hindrance. As such, the main purification step in many products has been ultracentrifugation (using a CsCl or sucrose cushion) which remains the process of choice for many products, including flu. However, although developments have taken place to provide continuous ultracentrifugation, there is a limit to the volume that can be processed at any one time, and large-scale production may require installation of multiple units.

Centrifugation will likely continue to be utilized as a purification tool in vaccine manufacture. However, vaccine companies are starting to investigate emerging technologies. These include wide pore media and membrane adsorbers or monoliths,[20] all of which have wider diameter pores/channels and therefore a greater surface area of accessible active chemistry. The potential benefit of these technologies is that in theory, they are scalable either by increasing the number of membrane units or by using larger columns.

For those technologies that utilise convective mass transfer rather than diffusion, there is also the added benefit that high flow rates can be applied, leading to reduced processing time. This is particularly important if the product is unstable during processing. Likewise, there are increasing opportunities to reduce the number of unit operations, as in some cases there is the potential to combine clarification and capture in one step.

Finally, many of the emerging technologies are available as single-use products in a pre-validated, pre-sterilised form. Disposable technologies with sterile connections are also directly applicable for processes in which sterile filtration is not possible and aseptic processing is required.

So in summary, the scalability of the entire process must be considered from the earliest stage of development. In general, the best economies of scale are achieved from stirred tank reactors, especially in suspension culture. However, it is important to take an integrated systems approach and not just focus on upstream processes. Finally, novel technologies are emerging to further enable scalable and efficient purification.

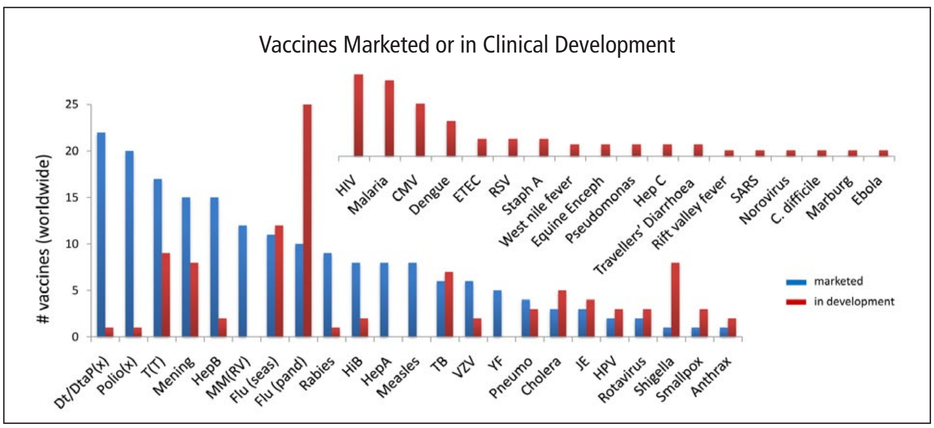
Developing a Vaccine Portfolio

As described, vaccines are an extremely disparate sector of biopharmaceuticals. A survey of the vaccines marketed for use in the US and Europe indicates there are over 20 single indications for which there are more than 50 vaccines. Additionally, there are over 20 marketed combination vaccines including variants of hepatitis A, hepatitis B, DTaP, and MMR.

As you would expect, the most prominent class is the influenza vaccine. Some are for pre-pandemic influenza, approved via fast-track regulatory pathways. Also, well represented are variants of the combination vaccines based around DTaP, hepatitis A, hepatitis B, and MMR. The remaining 50% of marketed products, however, represent vaccines for almost 20 indications (Figure 13).

This is likely to be further amplified as many vaccines enter development, either as improvements to existing vaccines or for diseases where there are unmet needs.[21, 22] Innovative technologies are being further developed to realise novel or better vaccines which are reflected in the manufacturing platforms that are required. These include DNA vaccines, peptide mixtures, novel VLPs, gene therapy vectors, and rationally designed recombinant proteins.

With this complexity in mind, a company wishing to develop a portfolio of vaccine candidates must invest in a diverse set of technologies and manufacturing platforms. This, along with the traditional view that vaccines are low value/low margin products, may help explain why the vaccine industry has been consolidated in its present situation where the majority of vaccines are manufactured by an oligopoly of major players (Figure 14). There are, in short, a multitude of unique development, manufacturing, and logistics requirements which in many cases are not



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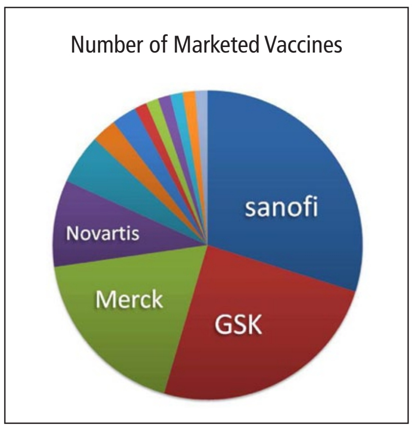

FIGURE 14.

transferable between different vaccines. Let us compare this to monoclonal antibodies, for example, that also have a wide array of current and potential medical applications but are almost generic in their method of manufacture and analysis. From a portfolio developer's standpoint, this provides opportunities for incremental return on investment (ROI) as the processes developed are generally transferable between products. Multiple products can be developed without the necessity for further significant capital reinvestment. Tried and tested scalable platform processes and analytical methods provide potentially shortened development times with fewer risks.

There are also opportunities to mitigate regulatory risk since agencies are familiar with reviewing data from MAbs and are clear on what they expect to see. Likewise, new initiatives like quality by design (QbD) are applicable to MAbs as many of the key product quality attributes (KPQAs) are known from prior development and clinical experience, and are readily characterisable in new antibodies.

There are also knock-on effects in terms of vendor activity and support services. Those supplying materials, technologies, and services recognise that it is a dynamic and growing market, and because of the generic nature of MAbs, they can be confident that the products, technologies, and services they develop will be broadly applicable to a wide range of clients. Therefore, we have a thriving and competitive industry built around MAbs. Ultimately this competition is to the benefit of the product developer as prices are reduced and differentiating technologies continuously emerge to increase productivity and decrease timelines.

There are many people, ranging from bench scientists to clinicians, who have experience in developing MAbs, which makes recruitment easier. It is also relatively simple to find high-quality partners with whom to outsource MAb development and production. This is the case throughout the value chain, from antibody design to large-scale routine production. In this case, increased capacity is also achievable via scaling out as well as scaling up.

All of this speaks to an economy of scope in which cost savings and competitive synergies are achieved by developing and manufacturing a range of products by a similar process. These are very attractive benefits and go a long way in explaining why more than 80% of the products in development are MAbs and more than 70% of the products in development are expressed in mammalian cell culture.[23] The barrier to entry has lowered and continues to decrease (Figure 15). By comparison, vaccines are manufactured by extremely diverse methods, limiting the options for incremental ROI because a new investment will be required for each new product. Traditionally, vaccines have not been as well characterised as recombinant products, and therefore the KPQAs are less well known. As such, vaccines have been primarily driven by the "process is the product" paradigm with emphasis on testing at the end of production and very little ongoing development of the manufacturing process once established.

It is a difficult industry for vendors and technology developers to penetrate due to the complexity of the products and the particularly challenging requirements in material handling. Finally, there are relatively few development and manufacturing outsource options (compared to the MAb sector). This is because very few contract companies have the manufacturing platforms required to support a diverse vaccine portfolio, and there are challenges to handling some of the products. As described, much of the expertise is locked within relatively few companies. So by contrast to MAbs, there are less economies of scope and the barrier to entry is higher.

The Way Forward – Platform Operations for Vaccines?

Which of the benefits enjoyed by the MAb industry are applicable to the vaccine industry? Undoubtedly, a major benefit is that different antibodies are made by an almost generic method of production. Of course, not all vaccines can be manufactured by the same method, but when developing a manufacturing process for a portfolio vaccine, you should rationalise wherever possible throughout the entire value

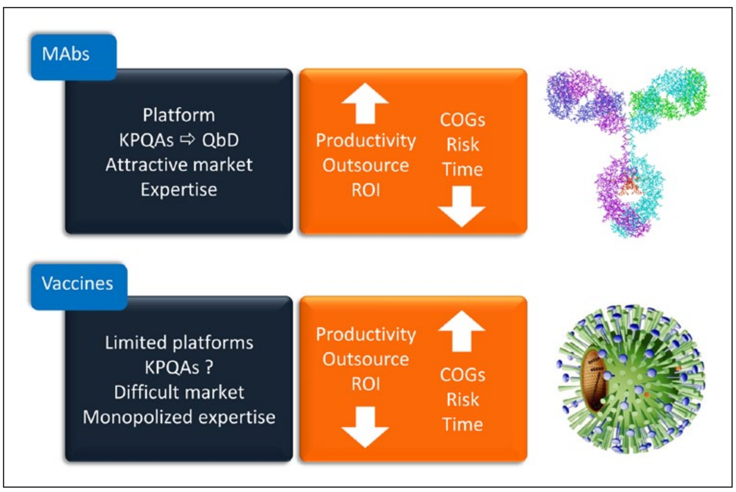

FIGURE 15.

chain. For example, with a novel virus vaccine this may mean evaluating a relatively small panel of cell banks that have already been adapted for growth in stirred tank culture, preferably in the absence of serum, and if at all possible, in suspension culture. From prior experience, the performance parameters of these cell lines will be known and will have the required documentation in place for regulatory compliance without any need for replication.

Of course, there are likely to be some cell lines that will not support viral propagation at all, and there also may be one in which the virus replicates at the highest titre. However, titre alone should not be the reason why a cell line is chosen. Other factors within an integrated development plan must be considered including whether or not existing portfolio products are produced in this cell line. This should be a weighted factor in the decision of which cell line to proceed with. Once multiple products are under development using a common cell line, platform processes and unit operations can then be applied. For example, at Eden we have developed platform processes for producing viral gene therapy vectors in HEK cells. We have adapted HEK from anchorage dependency to grow suspended in a stirred tank without serum. For a new project, we employ a rapid screening programme to establish the correct parameters such as multiplicity of infection (MOI), point of infection (POI), and point of harvest (POH) in shake flasks. We can then move seamlessly into stirred tanks since we already know that the cells are well suited to these conditions and we also have a baseline fermentation process. Finally, we apply these pre-optimised baseline conditions that require little or no modification to support the production of a high titre of viral particles (Figure 16).

We also adopt a platform approach to purification. Based upon membrane filtration and column chromatography, we have applied this platform approach to a range of viruses expressed in different cell lines (Figure 17). The benefit of this approach is that we can proceed through development quickly, and the risk is minimised as we have prior experience with the system and understand the critical parameters. We are also confident that this is a scalable process and is readily transferable to another party as long as they have access to a stirred tank reactor and a standard chromatography rig.

There is also the option to use platform unit operations between entirely different products and cell lines. For example, at Eden we have used individual platform unit operations for a virus expressed in cell culture and a VLP expressed in *E. coli*.[24] Again, this process step was well-defined, and through a strong vendor relationship with exemplification through multiple product classes we were confident of the performance and scalability of this method.

Global Supply and Business Models

One final point to consider is how a platform approach can help alleviate global vaccine supply issues. As described, many of the methods used to manufacture existing vaccines

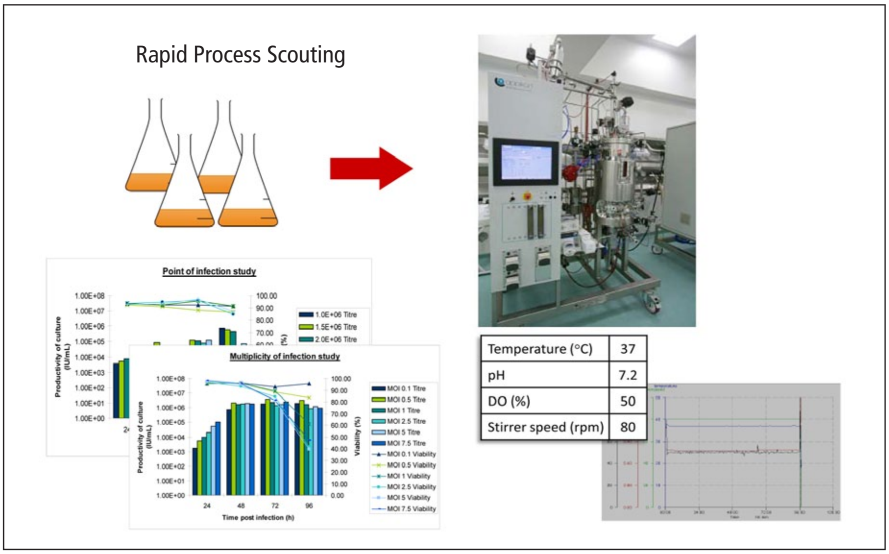

FIGURE 16.

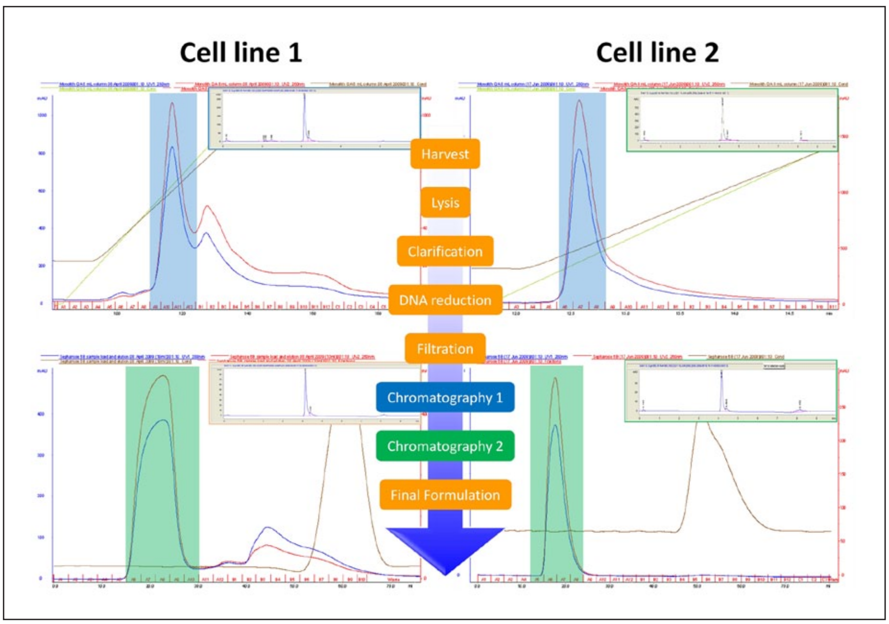

FIGURE 17.

are not readily transferrable to other manufacturing facilities. As such, during pandemic or emergency situations, the global community is reliant on existing manufacturers to scale-up their existing capacity, and as we know, this is not easy for some of the existing processes.

The general consensus is that there is available capacity in biomanufacturing.[25] In addition, some analysts think that this may be increasingly the case as productivities rise and there is less requirement for such large volume tanks. However, it does not appear to be readily available for vaccine production and therefore processes should be developed that are easier to transfer.

This does not just provide surge capacity, but also changes the way in which vaccine development companies can mature. In the past, the process was generally regarded as the product. As such, vaccine developers tended to become a fully integrated biopharmaceutical company (FIBCO) by investing in full-scale manufacturing facilities before moving into pivotal clinical trials.[6] Of course this required significant funding to not only build but also maintain these expensive facilities.

However, we now have better characterised processes, products, and platform approaches that allow developers to leverage the skills and knowledge of contract manufacturing organizations (CMOs). This in turn reduces the requirement for significant capital expenditure to build plants and may allow companies to go deeper into development without the need to be acquired or form strategic alliances. In summary, well-characterised vaccines that are manufactured increasingly by platform approaches may not only serve to address global supply chain issues but also change the way that we approach vaccine development.

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