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Review

The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors

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The baculovirus expression vector system (BEVS) platform has become an established manufacturing platform for the production of viral vaccines and gene therapy vectors. Nine BEVS-derived products have been approved – four for human use (Cervarix®, Provenge®, Glybera® and Flublok®) and five for veterinary use (Porcilis® Pesti, BAYOVAC CSF E2®, Circumvent® PCV, Ingelvac CircoFLEX® and Porcilis® PCV). The BEVS platform offers many advantages, including manufacturing speed, flexible product design, inherent safety and scalability. This combination of features and product approvals has previously attracted interest from academic researchers, and more recently from industry leaders, to utilize BEVS to develop next generation vaccines, vectors for gene therapy, and other biopharmaceutical complex proteins. In this review, we explore the BEVS platform, detailing how it works, platform features and limitations and important considerations for manufacturing and regulatory approval. To underscore the growth in opportunities for BEVS-derived products, we discuss the latest product developments in the gene therapy and influenza vaccine fields that follow in the wake of the recent product approvals of Glybera® and Flublok®, respectively. We anticipate that the utility of the platform will expand even further as new BEVS-derived products attain licensure. Finally, we touch on some of the areas where new BEVS-derived products are likely to emerge.

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1 Introduction

The baculovirus expression vector system (BEVS) is far from new. For thirty years researchers have been using this platform to express recombinant proteins, and thousands of proteins have been successfully expressed and purified. However, for much of this time, BEVS was relegated to the ranks of research tool. What we have seen in the last decade is the elevation of BEVS from research tool to an established manufacturing platform for production of novel biologic products.

Ten years ago there were only two commercial products manufactured using the BEVS manufacturing platform and both of these were veterinary vaccines to prevent classical swine fever in pigs. Since then, seven new products have been licensed, four of which are for humans, including vaccines and therapeutics, and many more products are in development (Table 1) [1, 2]. We have passed a tipping point where BEVS-derived products are becoming mainstream, and the BEVS platform is being actively utilized by major players in the biotechnology industry to develop new products.

Although the BEVS platform has distinct features that make it an attractive platform for the production of many biologics, it is not ideally suited for all products.

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Abbreviations: **AcMNPV**, *Autographa californica* multiple-capsid nuclear polyhedrosis virus; **BEVS**, baculovirus expression vector system; **GOI**, gene of interest; **HA**, hemagglutinin; **M1**, matrix 1; **NA**, neuraminidase; **rAAV**, recombinant adeno-associated virus; **VLP**, virus-like particle; **WVB**, working virus bank

Table 1. BEVS-derived products licensed for commercial use

Product/Indication	Manufacturer	Product Type
Veterinary Vaccines (pigs)		
Classical swine fever		
– Porcilis® Pesti	MSD Animal Health	subunit
– BAYOVAC CSF E2®/Advasure[a)]	Bayer AG/Pfizer Animal Health	subunit
Porcine circovirus type 2		
– Circumvent® PCV	Merck Animal Health	VLP
– Ingelvac CircoFLEX®	Boehringer Ingelheim Vetmedica	VLP
– Porcilis® PCV	MSD Animal Health	VLP
Human Vaccines		
Human papillomavirus		
– Cervarix®	GlaxoSmithKline	VLP
Influenza		
– Flublok®	Protein Sciences Corporation	subunit
Human Therapeutics		
Prostate cancer		
– Provenge®	Dendreon	immunotherapy
Lipoprotein lipase deficiency		
– Glybera®	uniQure	rAAV-based gene therapy

a) Discontinued

Factors such as protein complexity, post-translational modification, scale and cost must be considered collectively when selecting a manufacturing platform; these have been extensively reviewed elsewhere [2–4]. In this review, we explore the BEVS platform by looking at how the system works and the advantages and limitations of the platform from a manufacturing and regulatory perspective. The opportunities to develop new products using BEVS are abundant, and we review the latest developments in the gene therapy and influenza vaccine fields as examples. Finally, we consider some newer areas where BEVS-derived products show promise for the future.

2 The BEVS platform: How it works from cloning to protein production

The BEVS platform has been previously described [1, 2, 5–8]. The platform takes advantage of baculoviruses' natural propensity to infect insect cells. In nature, there are more than 500 different types of baculoviruses, all of which have a host range restricted to invertebrates [9]. In the laboratory and for manufacturing purposes, the most commonly used baculovirus is *Autographa californica* multiple-capsid nuclear polyhedrosis virus (AcMNPV), a virus with a double-stranded DNA genome of approximately 134 kb [10]. The large size of its genome gives the baculovirus ample capacity to accommodate large amounts of foreign DNA, including multiple genes, an advantage over other expression vectors such as vaccinia and adenovirus [2].

To begin the BEVS process, a recombinant baculovirus is constructed comprising the desired gene(s) of interest (GOI) (Fig. 1). First, the GOI is cloned into a transfer plasmid, typically behind the strong polyhedrin or p10 promoter that can drive protein expression to high levels in insect cells [11, 12]; notably, these promoters are not very active in *E. coli* and, therefore, can be stable expression cassettes. The GOI is flanked by AcMNPV DNA, e.g. the polyhedrin promoter on one side and a portion of the essential gene ORF1629 on the other. Insect cells are then co-transfected with a mixture of the transfer plasmid and parental AcMNPV DNA that has been linearized such that the parental polyhedrin gene and portion of ORF1629 are missing, rendering it non-infectious [13]. The plasmid and parental DNA undergo homologous recombination to generate de novo recombinant baculoviruses. These baculoviruses are plated and individual plaques purified to isolate a single, pure plaque of recombinant baculovirus. This plaque is subsequently passaged through multiple rounds of insect cell infection to generate a high-titer stock and establish a working virus bank (WVB) that can be utilized for protein production.

For manufacturing purposes, it is important that WVBs be stable and retain integrity as virus passages are scaled up. Laboratory kits such as Bac-to-Bac® (Life Technologies) that employ bacmid technology have been developed that allow researchers to quickly and easily construct recombinant baculoviruses in *E. coli* rather than

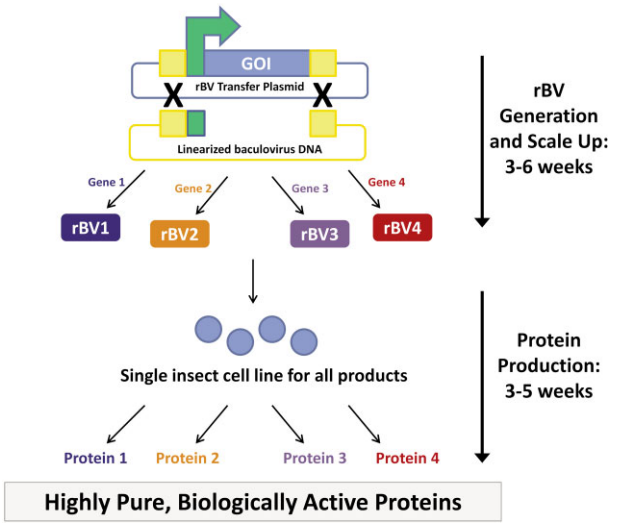

Figure 1. The BEVS platform. The BEVS platform is an efficient process for producing a wide variety of proteins in a streamlined manner. A gene of interest (GOI) is cloned into a transfer plasmid behind a strong promoter (green arrow) and surrounded by DNA homologous to the parent baculovirus (yellow and green boxes). A library of recombinant baculoviruses (rBV) can be made using standard cloning techniques and varying the GOI. Construction of an rBV takes eight days. To generate protein, the appropriate rBV is scaled up (taking on average two to five weeks) and used to infect insect cells, which programs the cells to generate large quantities of recombinant protein that can subsequently be purified to high levels using standard techniques. A single insect cell line can be used to produce all proteins. Protein production averages three to five weeks and yields highly pure, biologically active products.

by homologous recombination in insect cells. These tools are useful for small-scale research purposes; however, the recombinant gene cassette in bacmid-derived baculoviruses is inherently unstable and may easily be lost during virus passaging [14]. Long-term stability of the baculovirus is an important consideration if large-scale protein production is envisioned.

Once a high-titer WVB has been established it is used to infect insect cells and stimulate protein production. Cells are seeded in culture flasks (for small-scale production) or bioreactors (for large-scale production) and the WVB added to infect the insect cells when they are in their logarithmic growth phase. The baculoviruses reprogram the cellular machinery to produce the recombinant protein(s). Following protein expression (typically 48–96 hours post-infection), the cells and/or supernatant are harvested, depending on whether the product is intracellular or secreted, respectively, and the proteins are purified according to standard techniques such as ultracentrifugation or column chromatography.

Many different insect cell lines have been used for BEVS but the most common are derived from ovarian cells of the Fall Army Worm, *Spodoptera frugiperda* (e.g., Sf-21, Sf-9 and *expres*SF+® [Protein Sciences Corporation]), and the Cabbage Looper, *Trichoplusia ni* (High Five™ Cells, Life Technologies) [5, 15, 16]. High Five Cells are used to manufacture the licensed human papillomavirus vaccine, Cervarix® (GlaxoSmithKline), and Sf-21 cells are used to produce the antigen used in the prostate cancer immunotherapy, Provenge® (Dendreon). *expres*SF+ cells are used to manufacture three licensed products: Flublok® influenza vaccine (Protein Sciences Corporation), Glybera® gene therapy for the treatment of familial lipoprotein lipase deficiency (uniQure), and Ingelvac CircoFLEX® veterinary vaccine to protect against porcine circovirus type 2 (Boehringer Ingelheim Vetmedica). Large scale manufacturing and commercial production require specific cell line characteristics such as scalability, high yields, the ability to grow in low-cost, serum-free media, and qualification to meet regulatory agency (e.g. FDA, EMA, etc.) requirements for purity and safety.

3 BEVS platform features: Advantages and considerations

Characteristics of the BEVS platform are summarized in Fig. 2. These features as well as other important considerations about the technology are discussed below. The choice of an expression system for manufacturing is a complex decision that must balance many facets, including attainment of specific product features, the demands of the therapeutic indication, and the needs of the manufacturer. Comparisons of the BEVS platform to other expression systems such as bacteria, yeast, mammalian cells and plants have been made and are useful to consider when selecting a platform [2–4].

3.1 Recombinant technology offers speed, specificity and control

BEVS employs recombinant technology, giving researchers and product developers a level of control over the production process that is not possible with other techniques. A good example is found with vaccine manufacturing. Traditionally, vaccines are made by cultivating large volumes of the pathogen against which protection is desired to generate the "raw materials" required for the product. This can be accomplished by infecting substrates such as embryonated chicken eggs or mammalian cells, both of which are used to manufacture the majority of the vaccines recommended for routine immunization [1, 17]. The pathogen is either weakened and administered live as a live attenuated vaccine, or is killed or inactivated with reagents such as formalin or heat prior to being formulated into vaccine. These methods yield safe and effective vaccines; however, important shortcomings have been observed. The first concerns specificity. For example, traditional influenza vaccine production involves virus propagation in eggs. As influenza viruses are RNA viruses, they have a tendency to mutate to optimize their

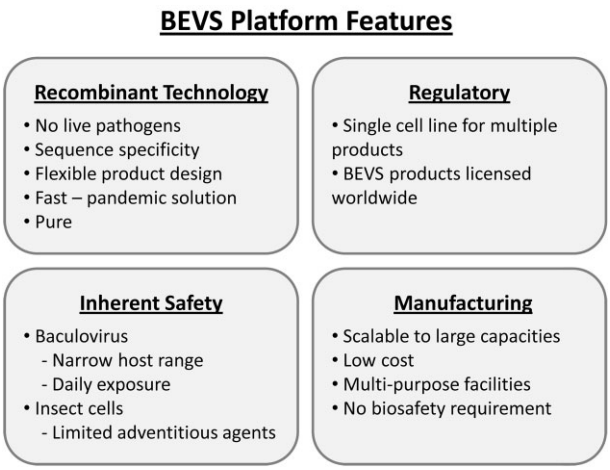

Figure 2. BEVS platform features. A summary of the features of BEVS technology as discussed in the text.

growth [18]. Generally, the changes introduced into the virus sequence have little impact on vaccine efficacy. However, since the virus receptors for birds and mammals differ, these changes can be meaningful, and it has been documented that in some cases the changes have rendered egg-based influenza vaccines ineffective [19]. The BEVS process does not involve pathogen growth and, therefore, avoids this complication. Rather than cultivate a pathogen to collect "raw materials", a recombinant baculovirus is constructed that codes for the antigen required for protection. Since accommodations for growth do not need to be made, the antigen can be an exact sequence match to the human pathogen. This has the potential to solve the ineffectiveness observed for some egg-adapted vaccines as was described by Skowronski et al. (2014) [19]. Moreover, specific point mutations can be introduced to enhance features such as stability. For example, it has recently been reported that purified hemagglutinin protein, the protective antigen in influenza vaccines, appears unstable in the SRID potency assay due to cross-linking of specific cysteine residues in the protein [20]. By mutating these cysteine residues to non-thiol residues, it was possible to prevent cross-linking and enhance stability [21].

A second shortcoming with traditional vaccine manufacturing is process length. Again, influenza serves as a good example. Influenza epidemics occur every year and annual influenza vaccination is recommended by the Centers for Disease Control and Prevention [22]. In addition, pandemic outbreaks occur occasionally, the most recent of which was the 2009 H1N1 swine flu (A/California/07/2009) [23, 24]. Timeliness of vaccine manufacture is critical to ensure that adequate vaccine supply is available. Egg-based influenza vaccine manufacturing takes on average about six months, as the process of creating a high-producing, egg-adapted seed virus is slow [17]. This means that manufacturing of seasonal influenza vaccines must begin the winter prior to an epidemic, before that season's strain prevalence is definitively known, to guarantee adequate supply, and this has resulted in seasons where the available influenza vaccines have not matched the circulating influenza strains [25, 26]. Moreover, the President's Council of Advisors on Science and Technology (PCAST) reported that pandemic influenza vaccine in 2009 was not readily available until after the pandemic peaked, a major concern for public health and safety (www.whitehouse.gov/sites/default/files/microsites/ostp/PCAST-Influenza-Vaccinology-Report.pdf). PCAST identified recombinant technology and freedom from virus growth and egg adaptation as the solution to this problem. BEVS-derived influenza vaccines do not require seed viruses and can be produced in as little as 45 days [5].

In addition to specificity and speed, recombinant technology offers advantages with respect to purity and product design. With respect to purity, recombinant products are free of pathogens, eggs and many chemicals (such as formalin and antibiotics) that can be undesirable or allergenic [27]. For product design, recombinant techniques make it possible to engineer proteins with desired features, such as fusion proteins that increase immunogenicity or include multiple antigens and truncated proteins with deleted domains to improve yields and ease purification [28–32]. One example of this application is the antigen used in Provenge immunotherapy. The antigen is a fusion glycoprotein consisting of prostatic acid phosphatase (PAP) linked to granulocyte-macrophage colony-stimulating factor (GM-CSF) and is used to stimulate autologous antigen presenting cells [33]. The GM-CSF activates the antigen presenting cells and enhances cell viability while the PAP serves as the target antigen. Another example is the Cervarix® vaccine manufactured by GlaxoSmithKline. The vaccine is a bivalent virus-like particle (VLP) comprised of the human papillomavirus L1 proteins (strains 16 and 18). By expressing C-terminally truncated L1 proteins, the manufacturers are able to prevent intracellular VLP self-assembly, which would complicate purification. Instead, the L1 subunit proteins are purified to a high degree and VLP assembly is achieved in vitro [28].

3.2 BEVS safety

The BEVS platform has inherent safety measures built in that are attractive from a regulatory perspective. Baculoviruses have a narrow host range restricted to specific insects and are considered safe to use as biological pesticides with no negative impact on plants, mammals, birds, fish or non-target insects [9]. People are exposed to baculoviruses daily by consuming fresh vegetables. For instance, a serving of coleslaw may contain hundreds of millions of baculoviruses [34]. Baculovirus vectors have also been explored as gene therapy vectors. These studies have demonstrated that baculoviruses cannot repli-

cate in mammalian cells and cannot express a gene cassette unless it is driven by a mammalian promoter [9, 35–38].

A major consideration for regulatory agencies when evaluating a novel cell substrate is the potential to harbor adventitious agents that could threaten patient safety. There are very few adventitious agents that can replicate in both insect and mammalian cells. A notable exception is arboviruses that can be transmitted to humans via insect bites and can cause complications such as encephalitis and hemorrhagic fever [39]. To mitigate this risk, cells from non-biting insects such as the fall armyworm and cabbage looper have been used with BEVS as noted above. Nonetheless, adventitious agents have been detected in some insect cell lines. For instance, the *Trichoplusia ni* High Five cell line, BTI-TN-5B1-4, used to make Cervarix®, was found to be latently infected with an *Alphanodavirus* that was induced by recombinant baculovirus infection [40]. In addition, other insect cell lines, such as those generated from *Drosophila melanogaster*, have been shown to harbor innate retroelements derived from retroviruses that could potentially be infectious [41]. More recently, a possible insect-specific virus Sf-rhabdovirus was identified in *Spodoptera frugiperda* cells [42]. Although studies showed that this virus could not enter or replicate in human cell lines and, therefore, is unlikely to be a risk, novel cell lines will most likely need to be characterized and monitored for the presence of this virus as they are for nodaviruses, retroviruses and others. In general, because adventitious agents are a potential threat, cell substrates of all origins (including insect and others) must be thoroughly tested for the presence and infectivity of such agents before they are allowed by regulatory agencies for manufacturing use.

3.3 Regulatory features of BEVS

In addition to the safety considerations just discussed, there are two important regulatory features associated with the BEVS platform that should be considered. The first is that BEVS is a transient protein expression system; the recombinant baculoviruses used vary based on their foreign gene cassettes but a single cell line can be used for the expression of all proteins (Fig. 1). Qualifying a cell line is no small feat. It can take years of work to adequately ensure purity and safety in addition to high productivity. Stable cell lines have to be independently qualified each time their genetic composition changes [43]. In contrast, cell lines used with BEVS and other transient expression systems remain constant and, consequently, need to be qualified just once.

A second feature is the growing number of BEVS-derived products that have been approved by regulatory agencies worldwide (Table 1). As is the case for all technologies, prior regulatory approvals remove barriers for future product approvals. The technology becomes more mainstream and less novel with each approval, and the safety database of patients that are administered products without complication continues to grow. Nine BEVS-derived products have been licensed, providing regulators confidence with the platform.

3.4 BEVS manufacturing is scalable and cost efficient

The following characteristics make the BEVS platform appealing for commercial manufacturing: scalability, biosafety, flexibility and existing manufacturing capacity. Insect cells are grown in suspension, so if the cells and baculoviruses have been optimized for large scale and multiple passages, the culture size is only limited by the size of the bioreactor. For example, *expres*SF+ cells have been used to produce recombinant proteins at scales ranging from two to 21 000 L [44]. The cost of goods for BEVS production is largely dependent on capital costs and yields. As discussed by Cox (2012), vast global bioreactor capacity (~500 000 L) already exists and presents the opportunity to minimize the investment needed to establish BEVS manufacturing facilities [1]. Moreover, opportunities for yield improvements are abundant and include genetic and fermentation-based approaches; these are described elsewhere [1]. Unlike some other production facilities, BEVS facilities can be multi-purpose and used to produce a variety of BEVS-derived products, especially when disposable or single-use technology is employed [45]. This is because a single cell line can be used for production of different products. This feature is especially meaningful for regions of the world where limited manufacturing capacity exists. A single BEVS facility could, for example, be used to produce vaccines for diseases endemic to a region and quickly be converted to produce pandemic influenza vaccine in an emergency. Finally, because the BEVS platform does not require the handling of live, potentially dangerous pathogens, requirements for biocontainment that can be very costly are reduced.

3.5 Other considerations: Post-translational modifications and other expression systems

The BEVS platform is a versatile technology useful for the manufacture of many products; however, other platforms may be better suited for the production of certain proteins. For instance, small proteins that do not require post-translational modifications are best made in *E. coli* that can quickly generate high yields at low cost [4]. Yeasts such as *S. cerevisiae* can also produce high yields of protein at low cost and are capable of some post-translational modifications [4]. Insect cells are capable of many post-translational modifications but proteins that require complex post-translational modifications and folding may best be made in mammalian expression systems. For

example, the glycosylation patterns produced by insect and mammalian cells are related, and glycoproteins produced in insect cells are often correctly folded, biologically active and immunogenic [46]. However, insect cells generate less complex N-glycans than mammalian cells and this can negatively impact biological function [46, 47]. Some developments have been made to address this limitation, including engineering transgenic insect cell lines that stably express mammalian glycosylation enzymes or co-expressing such enzymes with the gene of interest in a single baculovirus [48–51]. Whether this is required must be assessed on a protein by protein basis.

4 Product opportunities using the BEVS platform

Licensure of the first BEVS-derived products has paved a regulatory pathway, reducing regulatory uncertainty. In this section we discuss opportunities in the areas of gene therapy and influenza vaccines spawned by the two most recent BEVS-derived product approvals, Glybera® (licensed by the EMA in 2012) and Flublok® (licensed by the FDA in 2013), respectively.

4.1 Gene therapy: BEVS-derived recombinant adeno-associated viruses (rAAVs)

The BEVS approach to gene therapy has largely involved the production of recombinant adeno-associated viruses (rAAVs) that house therapeutic DNA. The use of rAAVs as a gene delivery vector has gained popularity for several reasons, including long-term gene expression, lack of pathogenicity and ability to transduce a wide variety of cells, both dividing and non-dividing [52, 53]. Nine different rAAV serotypes (1–9) are most commonly used for rAAV-based gene therapy, each serotype with a different propensity for tissue-specific infection and infection kinetics [54].

Recombinant AAV-based gene therapies have been in development and shown promise for some time; however, a major limitation to their implementation had been the inability to scale up the manufacturing process to produce sufficient quantities of rAAVs. The original rAAV vectors were produced in mammalian tissue culture using adherent cells such as HEK293 cells, which required about 5000 175-cm^2 flasks to produce enough material for a large animal study or human clinical trial (~10^{15} rAAV particles) [55]. To overcome this limitation, scientists adopted and optimized the BEVS platform for production of large scale, high titer rAAVs [55–63]. By adjusting parameters such as multiplicity of infection, cell density and fermentation mode, rAAV yields on the order of 10^{14} vector genomes per liter have been reported [57].

The traditional BEVS production strategy for rAAVs requires the co-infection of insect cells with three different recombinant baculoviruses: Bac-Rep that expresses the major AAV replication enzymes (Rep78 and Rep52); Bac-Cap that expresses the AAV virion coat proteins; and Bac-GOI that expresses the gene of interest flanked by the AAV inverted terminal repeat elements that are required for the rescue, replication and packaging of the gene [55, 56]. No adenovirus helper is needed as is required for mammalian cell rAAV production [64, 65]. Due to some genetic instability of the Bac-Rep construct, a streamlined two-baculovirus system has further been developed where Rep and Cap proteins are expressed from a single baculovirus (Bac-RepCap) and Rep78 and Rep52 are transcribed from a single mRNA species that enhances stability [56]. Alternatively, genetic modifications have been made to the original Bac-Rep and Bac-Cap constructs to enhance stability and improve expression [58].

An important regulatory hurdle was overcome in 2012 when Glybera received marketing authorization in Europe, making it the first gene therapy product approved in the Western world and launching the BEVS platform into the spotlight as a preferred platform for rAAV manufacture. Glybera (alipogene tiparvovec) is comprised of the human gene LPL^{S447X} in a BEVS-derived rAAV serotype 1 vector and is used for the treatment of patients with lipoprotein lipase (LPL) deficiency [66]. Clinical studies have shown Glybera to be safe and effective [66, 67].

Glybera will likely be the beginning when it comes to rAAV-based gene therapy. The approach is relevant to the estimated thousands of monogenic diseases [68]. Treatments are actively being investigated in a diverse array of therapeutic areas and dozens of product candidates are in clinical development (summarized in Table 2). Hemophilia is one area where progress has been made (reviewed in [69]). There are four ongoing human clinical trials involving rAAV serotypes 8 or 2, all designed to express factor IX for the treatment of hemophilia B (Table 2). Factor VIII rAAV-based therapy is a target for the treatment of hemophilia A, the most common severe inherited bleeding disorder, and only a modest increase in plasma factor VIII levels is expected to be required to be clinically relevant [69]. rAAV-based treatments for retinal degeneration, including macular degeneration and Leber's congenital amaurosis type 2, are another area of intense investigation [70, 71]. Retinal treatments are ideal because of cell accessibility through intravitreal and subretinal injections and the ability to assess structure and function noninvasively. Serotype 2 is most commonly used for these therapies but types 2, 5, and 7–9 are all capable of infecting photoreceptors, the most prominent cell type for retinal degenerations [70]. Diseases of the central nervous system (CNS), such as Parkinson's and Alzheimer's diseases, are also actively being tested with rAAV-based therapies that are promising. AAVs exhibit a strong preference for neuronal transduction, making them a popular gene delivery vehicle for CNS therapies but vector improvements are still needed to optimize treatments (discussed in [72]). Final-

ly, Duchenne muscular dystrophy (DMD) is a therapeutic target where progress is being made. Although the single gene affected in DMD (dystrophin) has long been known, its large size has made gene therapy a challenge. Recent treatment approaches to overcome this include exon-skipping, trans-splicing and micro-and mini-dystrophin delivery strategies [53, 73].

4.2 BEVS-derived seasonal and pandemic influenza vaccines

For fifty years influenza vaccine manufacturing technology remained largely stagnant. That changed with the licensure of Flublok® in 2013. Flublok® is a trivalent BEVS-derived vaccine for seasonal influenza composed of 135 µg of recombinant hemagglutinin (HA) derived from the two A and one B influenza viruses selected for inclusion in the annual influenza vaccine by the World Health Organization; the vaccine is licensed by the FDA for adults 18 and older [74]. Influenza vaccines are standardized to contain a specific amount of HA, the major surface glycoprotein on the influenza virus [17]. BEVS-derived recombinant HA forms trimers that in turn oligomerize into immunogenic rosettes [75]. These proteins can be purified to high levels resulting in a vaccine that has been shown to be safe and effective in clinical studies [5, 76–79].

The advantages of recombinant BEVS vaccines for pandemic influenza are especially important. Vaccines for avian influenza viruses such as the H5, H7 and H2 subtypes are urgently needed because of these viruses' high pathogenicity and mortality rates in humans and the fact that H2 has previously demonstrated pandemic potential and human-to-human transmissibility [80, 81]. A monovalent variation of the Flublok® vaccine called Panblok® has been developed. In a Phase II study of H5 Panblok® (A/Indonesia/5/05), a two-dose schedule of vaccine at doses of 3.8–45 µg HA formulated with the adjuvant glucopyranosyl lipid A/stable emulsion (GLA/SE) had an acceptable safety and reactogenicity profile and elicited serologic responses meeting seroconversion criteria in adults 18–49 years old [82]. Moreover, an earlier study showed that people administered H5 Panblok® (A/Hong Kong/156/1997) in 1998 were primed for an enhanced immune response following administration of an antigenically variant vaccine strain in 2006 [83]. Liu et al. (2013) evaluated a different BEVS-derived H5 subunit vaccine candidate (A/goose/Guangdong/1/96) and showed that it protected against a lethal challenge in BALB/c mice and in specific pathogen-free and commercial chickens, suggesting it could be useful as both a human and animal vaccine [84].

Multi-component VLP vaccines for pandemic influenza are under development that are composed of recombinant HA, neuraminidase (NA) and matrix 1 (M1) proteins produced in Sf-9 cells [85–87]. Evaluation of an H5N1 (A/Indonesia/5/05) VLP vaccine in a Phase I/II study of adults 18–40 years old showed that two doses of unadjuvanted vaccine at 15, 45 or 90 µg HA/dose were generally well-tolerated and resulted in seroconversion [85]. Similarly, a Phase II study of an H1N1 (A/California/04/2009) VLP vaccine in adults 18–64 years old showed it was safe at doses of 5, 15 or 45 µg HA/dose and elicited high rates of seroprotection (82–92%) [86]. More recently, an H7N9 VLP vaccine was developed that was comprised of HA and NA proteins matched to A/Anhui/1/2013 (H7N9) and M1 protein matched to A/Indonesia/05/2005 (H5N1) [87]. This vaccine candidate was tested with or without saponin-based ISCOMATRIX adjuvant in BALB/c mice and was shown to protect against a lethal challenge. Antibodies against both HA and NA were elicited, with 3- to 4-fold higher responses in the ISCOMATRIX groups. Although the data are promising, a challenge for the development of multi-component VLP vaccines will be standardizing the vaccines for each of their components (e.g., quantity of HA vs. NA vs. M1).

An advantage of using recombinant technology for vaccine design is the opportunity to modularly add or subtract antigens to a formulation. Inclusion of recombinant NA in VLP vaccines has been shown to induce formation of anti-neuraminidase antibodies [87]. It has been noted that different vaccine compositions (e.g., VLP vs. subunit vs. whole virion) induce different immune profiles in BALB/c mice [88]. While the advantages of these various profiles are not yet clear, the flexibility of the BEVS platform enables catering towards different outputs. Besides inclusion in VLPs, recombinant NA can be individually produced via BEVS and may serve as a potentially efficacy-enhancing additive to influenza vaccines [89]. NA immunity is infection-permissive but can reduce infection severity and duration [90]. The potential benefits of including recombinant NA in influenza vaccines has recently been reviewed [90].

Other opportunities have emerged with BEVS as researchers begin pursuit of a so-called universal influenza vaccine. Licensed influenza vaccines offer limited cross protection to heterologous influenza viruses and, thus, there is the need for annual update of seasonal influenza vaccines and concern over pandemic preparedness. A successful universal influenza vaccine would offer long-lasting and broad protection against a range of different influenza virus strains. Approaches to universal influenza vaccine design include HA stalk-based constructs and chimeric HA-based vaccines that are composed of conserved stalk domains fused to "exotic" heads, usually of avian origin (these approaches are reviewed elsewhere [91]). The BEVS platform, being based on recombinant technology, offers the flexibility and genetic control required for the design and manufacture of these universal vaccine candidates.

Table 2. AAV-based gene therapy product candidates in clinical development

Indication	Product Description	Development Stage	ClinicalTrial.gov Identifier
Acute Intermittent Porphyria	Express PBGD		
	– rAAV2/5-PBGD	Phase 1	NCT02082860
Alpha 1-Antitrypsin Deficiency	Express alpha 1-antitrypsin (AAT)		
	– rAAV1-CB-hAAT	Phase 2	NCT01054339
	– rAAV2-CB-hAAT	Phase 1	NCT00377416
	– AAVrh.10halpha1AT	Phase 1	NCT02168686
Alzheimer's Disease	Express Beta-Nerve Growth Factor (NGF)		
	– AAV-NGF	Phase 1	NCT00087789
Aromatic Amino Acid Decarboxylase Deficiency	Express aromatic L-amino acid decarboxylase		
	– AAV2-hAADC	Phase 1/2	NCT01395641
Becker Muscular Dystrophy	Express follistatin		
	– rAAV1.CMV.huFollistatin344	Phase 1	NCT01519349
Choroideremia	Express gene encoding Rab-escort Protein 1 (REP1)		
	– rAAV2.REP1	Phase 1	NCT01461213, NCT02077361
Chronic Heart Failure	Express the sarcoplasmic reticulum calcium ATPase (SERCA2a)		
	– AAV1-CMV-SERCA2a	Phase 2	NCT00534703, NCT01966887, NCT01643330
Duchenne Muscular Dystrophy	Express mini-dystrophin		
	– rAAV2.5-CMV-minidystrophin	Phase 1	NCT00428935
Gastric Cancer	Express CEA		
	– AAV-DC-CTL	Phase 1	NCT01637805
Hemophilia B	Express factor IX		
	– AAV8-hFIX19	Phase 1	NCT0120801
	– AskBio009 (AAV8)	Phase 1/2	NCT01687608
	– scAAV 2/8-LP1-hFIXco	Phase 1	NCT00979238
	– AAV2-hFIX16	Phase 1	NCT00515710
HIV	Express gag, protease and part of the reverse transcriptase		
	– AAV-2 HIV vaccine (tgAAC09)	Phase 1	NCT00482027
	Express PG9 antibody		
	– rAAV1-PG9DP	Phase 1	NCT01937455
Inflammatory Arthritis	Express the TNFR:Fc Fusion Gene		
	– tgAAC94	Phase 1/2	NCT00126724
Late Infantile Neuronal Ceroid Lipofuscinosis	Express human CLN2		
	– AAVrh.10CUCLN2	Phase 1/2	NCT01414985
	– AAV2CUhCLN2	Phase 1	NCT00151216
Leber Congenital Amaurosis	Express RPE65		
	– AAV2-hRPE65v2	Phase 3	NCT00999609
	– rAAV2-CB-hRPE65	Phase 1/2	NCT00749957
	– tgAAG76 (rAAV 2/2.hRPE65p.hRPE65)	Phase 1/2	NCT00643747
	– rAAV2/4.hRPE65	Phase 1/2	NCT01496040
	– rAAV2-CBSB-hRPE65	Phase 1	NCT00481546
	– rAAV2-hRPE65	Phase 1	NCT00821340
Leber's Hereditary Optic Neuropathy	Express ND4		
	– scAAV2-P1ND4v2	Phase 1	NCT02161380
Limb Girdle Muscular Dystrophy	Express gamma-sarcoglycan		
	– AAV1-gamma-sarcoglycan vector injection	Phase 1	NCT01344798, NCT00494195

Table 2. AAV-based gene therapy product candidates in clinical development (continued)

Indication	Product Description	Development Stage	ClinicalTrial.gov Identifier
Macular Degeneration	Express soluble Flt1		
	– AAV2-sFLT01	Phase 1	NCT01024998
Parkinson's Disease	Express GAD		
	– AAV2-GAD	Phase 2	NCT00643890
	Express gene encoding NTN (CERE-120)		
	– AAV2-NTN	Phase 2	NCT00400634
	Express Human Aromatic L-Amino Acid Decarboxylase		
	– AAV-hAADC-2	Phase 1	NCT00229736
	– AAV2-hAADC	Phase 1	NCT01973543
	Express Glial cell line-derived neurotrophic factor (GDNF)		
	– AAV2-GDNF	Phase 1	NCT01621581
Pompe Disease	Express normal GAA		
	– rAAV1-CMV-GAA	Phase 1/2	NCT00976352
Spinal Muscular Atrophy	Express SMN		
	– scAAV9.CB.SMN	Phase 1	NCT02122952

5 Conclusion and future opportunities

In this review we have taken a close look at the BEVS platform, describing how the platform works, outlining the features and limitations of the technology and highlighting the growth opportunities that emerged from the two most recent BEVS-derived product approvals. We expect this growth to continue and expand as future BEVS-derived products attain regulatory approval.

The approvals of BEVS-derived Cervarix® and Flublok® vaccines have broadened the acceptance of the platform beyond its initial veterinary borders to use in healthy adolescents and adults. Human therapeutics is another area of use and gene therapy in particular is a growing area of interest; the approval of Glybera drew major attention to BEVS-derived rAAVs. Baculoviruses themselves can also be used as gene delivery vectors, and other recombinant protein complexes produced using BEVS are being explored for the delivery of various peptides and antigens, such as in the form of the newly characterized vault particles [92].

The speed at which recombinant proteins can be produced using BEVS makes it a particularly attractive platform to design safe and effective vaccines to be available to timely combat new infectious pathogens as they arise. Success with this approach has been demonstrated for influenza and can be applied to broader areas. For example, coronaviruses have been plaguing both people and animals especially in the last decade with lethal outbreaks of severe acute respiratory syndrome (SARS) in 2003, Middle East respiratory syndrome (MERS) in late 2012, and porcine epidemic diarrhea virus (PEDV) in 2013 [93–95]. All coronaviruses share a similar structure that includes the presence of the spike glycoprotein on the viral envelope that is the dominant immunogen [96–98]. Multiple coronavirus BEVS-derived vaccine candidates have been developed that include recombinant spike protein either alone as a subunit vaccine or together with recombinant envelope and membrane proteins as VLPs and have demonstrated efficacy in animal models [29, 99–107]. The spike protein has been shown to be immunogenic both as full length protein and as a truncated protein containing only the extracellular domain [29, 100], and multiple routes of vaccine administration have been examined [104].

Two recently emerged threats are chikungunya virus and Ebola virus, both of which can be addressed with BEVS-derived vaccines. Chikungunya virus causes a serious disease that involves severe joint pain and can be fatal; a recent outbreak appeared in Saint Martin in December 2013 and has since made its way to more than 20 countries or jurisdictions in the Americas, including the continental United States [108]. Chikungunya is an arbovirus, for which there are many opportunities to develop BEVS-derived vaccines (reviewed in [39]). Both subunit and VLP vaccine candidates expressing the chikungunya viral envelope glycoproteins have been developed using BEVS, with VLPs demonstrating higher immunogenicity in mice [109–111]. Ebola virus is a filovirus that causes lethal hemorrhagic fever in humans and is devastating Africa in an ongoing outbreak [112]. The Ebola virus glycoprotein has been shown to be the protective antigen and could be produced similar to a chikungunya virus vaccine [113].

In addition to glycoprotein-based vaccines, the BEVS platform has shown early promise for the production of

toxin-based vaccines. For example, the C-terminal heavy chain domain of clostridial botulinum neurotoxin, a highly toxic protein that causes botulism, has been produced with BEVS and has demonstrated immunogenicity and challenge protection in mice [114, 115]. Recombinant toxin vaccines for other diseases such as *Clostridium difficile* could also be possible [116, 117].

In conclusion, BEVS is a versatile platform whose potential is just beginning to be realized. The technology offers speed, flexibility, specificity and safety, and the use of a single cell line to manufacture multiple products makes BEVS an attractive platform to adopt. BEVS can be used to develop a wide variety of products and is especially well suited for combating rapidly emerging and dangerous pathogens. With new threats continually on the rise, tools such as BEVS offer an important defense.

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6 References

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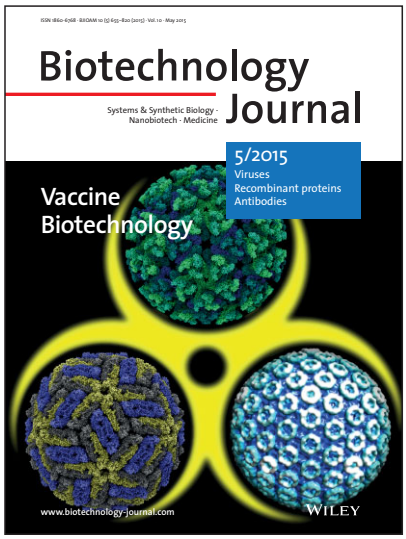

Cover illustration

Special issue: Vaccine Biotechnology. This Special issue edited by Reingard Grabherr and Udo Reichl includes articles on the design of cell lines for viral vaccine production, downstream processing of virus-like particles and plant-based production of vaccines. The cover shows particles of highly pathogenic viruses transmitted by mosquitoes: Chikungunya, dengue and Rift Valley fever virus. Image by Gorben Pijlman.

Biotechnology Journal – list of articles published in the May 2015 issue.

Research Article

Adjuvant poly(*N*-isopropylacrylamide) generates more efficient monoclonal antibodies against truncated recombinant histidine-rich protein2 of *Plasmodium falciparum* for malaria diagnosis

Reena Verma, Ramakrishnan Ravichandran, Naatamai S. Jayaprakash, Ashok Kumar, Mookambeswaran A.Vijayalakshmi, and Krishnan Venkataraman

http://dx.doi.org/10.1002/biot.201400386

Biotech Method

Bacterial cytoplasmic display platform Retained Display (ReD) identifies stable human germline antibody frameworks

Matthew D Beasley, Keith P Niven, Wendy R Winnall and Ben R Kiefel

http://dx.doi.org/10.1002/biot.201400560

Research Article

Heat shock protein 27 overexpression in CHO cells modulates apoptosis pathways and delays activation of caspases to improve recombinant monoclonal antibody titre in fed-batch bioreactors

Janice G.L. Tan, Yih Yean Lee, Tianhua Wang, Miranda G. S. Yap, Tin Wee Tan and Say Kong Ng

http://dx.doi.org/10.1002/biot.201400764

Research Article

Wheat enolase demonstrates potential as a non-toxic cryopreservation agent for liver and pancreatic cells

Mélanie Grondin, Mélanie Chow-Shi-Yée, François Ouellet and Diana A. Averill-Bates

http://dx.doi.org/10.1002/biot.201400562

Biotech Method

Purification and simultaneous immobilization of *Arabidopsis thaliana* hydroxynitrile lyase using a family 2 carbohydrate-binding module

Benita Kopka, Martin Diener, Astrid Wirtz, Martina Pohl, Karl-Erich Jaeger and Ulrich Krauss

http://dx.doi.org/10.1002/biot.201400786