Review

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The use of *Drosophila* S2 cells in R&D and bioprocessing

Drosophila Schneider 2 (S2) cells have been available for approximately 40 years. Their use has intensified over the past 15 years: resolution of the whole *Drosophila melanogaster* genome and the amenability of S2 cells for siRNA-based studies are some of the reasons for their growing use. This review covers recent publications on use of S2 cells for research and manufacturing and points to some possible future developments in their use in the vaccine field. Relatively few groups have systematically developed the system to enable expression of challenging proteins. They demonstrated that these cells can constitute a robust, efficient protein expression system, with specific advantages such as homogeneous glycosylation profile; reproducibility between production runs; options for cultivation modes, including perfusion; and no cell lysis, leading to relatively low levels of contaminating host cell proteins. The platform has shown to be particularly well adapted for the production of challenging viral, malaria and immunotherapy antigens.

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Recently, Moraes *et al.* have reviewed the factors that impact on growth and protein expression using Scneider 2 (S2) cells, comparing them to other expression systems [1]. The focus of this review is on the application of S2 cells in the production of vaccine products in the clinic. A brief mention of use of these cells in early R&D is made, as this use will potentially enable improvements in S2 cell-based manufacturing as well as further applications of the system in the future.

The *Drosophila* S2 cell expression system was developed in the early 1970s by Schneider [2] and has been used in research for almost 40 years. The applications of the system have mostly been in fundamental research, however a renewed interest can be deduced from the increased number of scientific references that refer to the system. A search in the PubMed database for *Drosophila* S2 cells, clearly illustrates this intensification of use: from 1970 to 2000, there are 148 publications referring to the system, while from 2001 to 2012, 884 hits were found. In the last 2 years, 183 publications refer to use of *Drosophila* S2 cells. The availability of the whole genome sequence of *D. melanogaster* and the suitability of the S2 system for modern techniques in molecular and cell biology has sustained this interest.

Use of insect cell lines for recombinant protein production

Several examples in the scientific literature can be found where insect expression systems have been engineered into high yielding heterologous protein producers, typically for the production of protein-based veterinary biopharmaceuticals, such as the Porcilis® pesti (Intervet [NJ, USA], 2000) and Bayovac CSF E2 (Bayer [Munich, Germany], 2001) vaccines for pigs, and Vibragen (Virbac [Cedex, France], 2001) for the treatment of parvovirus infections. The production of commercial biopharmaceuticals for humans, however, is relatively new. Insect cells are particularly good at expressing complex proteins and they provide a eukaryotic environment

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Key Term

Perfusion: In perfusion mode, a specialized continuous bioreactor setup, media components and metabolites are continuously exchanged, allowing product recovery throughout the culture period. The cells are retained and the constant flow of fresh nutrients and removal of waste products assist in the formation of high cell densities, leading to higher productivities compared with a traditional continuous bioreactor. As such, a steady state can be reached and may be sustained for several months. The continuous harvest features shorter product residence time compared with fed-batch, thus making degradation less likely.

conducive to the post-translational modifications required for biological activity. Expression systems based on insect cells have a number of significant advantages over other methods of recombinant protein production, including lack of adventitious agents that could infect and replicate in mammalian cells or humans. However, the lower regulatory track record compared with other expression systems and the short history of commercial use may be the reason for the slower uptake of insect cell expression systems.

Insect cell expression can be divided into two different expression modes:

» Lytic expression, for example, Baculovirus expression vector system (BEVS). Insect cell lines used with BEVS are derived from insects including the fall army worm (*Spodoptera frugiperda*; Sf9 and Sf21 – a clonal derivative of Sf9) and the cabbage looper (*Trichioplusia ni*; Hi-5) and derivatives thereof, such as Protein Sciences' (CT, USA) expresSF+ cell line (Sf9) and GSK's Hi-5 cell line (Hi-5 Rixx4446). The advantages of BEVS are that Baculoviruses are harmless to humans and allow relatively quick access to biologically active proteins. However, there are also a number of disadvantages to using BEVS and lytic expression: the protein may be proteolyzed (due to its lytic nature); baculovirus infection is an extra step in the process; heterogeneous glycosylation patterns; difficulties in reproducibility and consistency between production runs; and production in perfusion mode is not possible. The application of BEVS to pharmaceutical production/development has recently been reviewed [3].

» Stable insect cell lines and nonlytic expression relies on a different mechanism for expression. In this mode of expression, an expression vector is stably integrated into the chromosome of the insect cell and the recombinant protein is expressed without subsequent cell lysis (comparable with traditional protein expression in mammalian cells). Cell lines used with this expression system include Sf9, Sf21, Hi-5 and *D. melanogaster* cell lines such as the S2. Stable S2 insect cell systems have not been widely explored in the biopharmaceutical industry, most likely due to the previous intellectual property position held by GSK, even though they have several advantages over BEVS. Lytic expression, for example, BEVS, where cell lysis occurs after infection yielding a recombinant Baculovirus comprising/containing the gene of interest, or stable expression, where insect cells are transfected with a (shuttle?) vector containing an expression cassette, and later screened for high yielding polyclonal or monoclonal cell lines. The disadvantage compared with BEVS is that establishment of stable cell lines is needed unless transient transfection is pursued.

» S2 cells

S2 cells are relatively small (8–10 µm) in diameter and very robust. The cells grow readily in serum-free medium without adaptation and form either loose monolayers or grow in suspension as single cells in static environments. The believed macrophage origin of the S2 cell line could explain the preference for the cells to grow in suspension. Cell cultures of S2 generally reach cell densities up to 70 million cells/ml; orders of magnitude greater when compared with other insect and mammalian cell lines in batch processes.

Applications of S2 cells in fundamental R&D

» S2 cells are particularly well suited for RNAi studies

Since its discovery in the 1990s [4], the study and applications of RNAi in fundamental research have provided important insights into fundamental processes relating to regulation of gene expression and the function of specific genes. RNAi, in combination with high-throughput screening technologies and advanced analysis technologies, such as imaging and expression profiling, enables the study of the effects of knocking down specific genes. These discoveries are of great value both for fundamental understanding of pathways involved in protein expression and secretion, as well as future efforts to engineer S2 cells for high level protein expression. Several groups have established S2-based platforms for RNAi studies, [5–7], and there have been significant advances to reduce the number of false negatives [8]. In 2008, Cherry reviewed the use of S2 cells and RNAi in the study of host–pathogen interactions [9]. Effects of specific gene knockdowns on the viral replication, intracellular bacterial survival and growth, as well as recognition, phagocytosis and uptake by S2 cells are discussed. In a further advance, Haley *et al.* demonstrated the application of miRNA silencing in S2 cells using high-throughput compatible expression vectors [10]. This advance should facilitate novel strategies for manipulation of gene expression levels in S2 cells.

Transcription profiling of S2 cells has been used for study in a wide variety of fields [11–14]. For example, to study the mechanisms of insect resistance to the non-

steroidal ecdysteroid agonist methoxyfenozide, representing a group used as novel biorational insecticides in the control of insect pests [11]; or the effect of *Drosophila* juvenile hormone on S2 cells, which is involved in regulating larval development and adult reproductive processes in *D. melanogaster* flies [13]; as well as using S2 cells as a model to study human innate immunity [14].

Gene knockdown is a useful tool to understand biological and physiological processes. The availability of genome-wide RNAi tools in *Drosophila* S2, as well as genome-wide sequence profiling offers the opportunity to study the effect of knockdown of any gene on gene transcription [15,16]. Foglietti *et al.* demonstrated the biological effects on genome-wide gene transcription, cell growth and cell cycle, by knocking-down specific *Drosophila* histone deacetylases [16], while Ben *et al.* studied the effects of dSelK knockdown [15].

Combining transcriptomic, lipidomic and targeted functional genetic analyses, Castorena *et al.* [17] studied the role of glycerophospholipid metabolism in Flock House virus (FHV) RNA replication. It was found that FHV induces transcriptional upregulation of lipid metabolism and altered the phospholipid levels in S2 cells. Targeted knockdown of 32 genes were studied for the effect on FHV replication, and possible novel targets for drugs to inhibit positive-strand RNA viruses were found.

Microscopy can be used to gain deeper insight into S2 cells' physiology. A recent article showed how cell motility and growth could be measured on the single cell level [17], while super-resolution 3D microscopy on live whole S2 cells further advances the information that can be gained [18]. Great advantages have also been achieved through combining RNAi and microscopy [19–22]. Screening of RNAi against 156 proteins with endoplasmic reticulum retention or retrieval signals was performed, and the cells were scored for organization of the transitional endoplasmic reticulum-Golgi units using microscopy. The study identified novel proteins influencing the organization of the early secretory pathway [22]. A whole-genome RNAi screen using automated computational image analysis identified genes involved in S2 cell spreading on ConA-coated plates. This powerful technique identified unknown and important genes involved in cell spreading and the automated analysis methodology should be applicable to other studies [21].

» Further understanding of S2 fundamental biology

A recent technology applied to S2 cells, chromatin immunoprecipitation sequencing (CHiP-seq) (ChiP-sequencing), consists of CHiP along with massively parallel DNA sequencing, and is used as a tool for the study of transcription factors and chromatin-associated proteins and their influence on mechanisms relating to phenotype [23,24]. Progress has also been made in large-scale proteomic studies of S2 cells. A selection of studies looked at far-ranging applications, such as the phosphoproteome [25,26], mucin-type O302 glycosylation [27], shotgun proteomics of the cytosolic fraction [28], the active cysteine protease content in S2 cells [29], and proteomic analyses of released microvesicles [30]. Further advances in phosphoproteome analysis using high-field asymmetric waveform ion mobility spectrometry coupled with LC–MS have recently been reported [31].

» Impact of use of S2 cells for research protein production

S2 cells can transiently express proteins and generate sufficient yields for screening assays within 3–4 days. This allows for rapid screening and can accelerate selection of protein candidates. Once the candidates have been selected, generation of a host cell line(s) and later, the protein production, are both bottlenecks in the future development. Using S2 cell lines can, compared with Chinese hamster ovary (CHO)-based systems, significantly reduce the time from several months to a couple of weeks, by the use of stable polyclonal cell lines. This is possible due to one-step gene amplification that occurs during selection, yielding high-expressing cell lines without amplification, often required in CHO-based systems. Additionally, with S2 cells, the time-consuming clonal isolation can be eliminated from the discovery phase. This makes the S2 expression platform superior for protein evaluation and characterization in R&D, as different proteins and/or variants thereof can be evaluated in a short timeframe.

» S2 cells have enabled significant advances in vaccine development

Vaccine development is one of the areas where *Drosophila* S2 cells as an expression platform have demonstrated their strength (Table 1). Their use has enabled development of new vaccine candidates in areas of need, and the *Drosophila* S2 system delivers very well to the most innovative trends in vaccine development. The interest in S2 cells for vaccine development stems from the fact that the system responds to some of the current industry concerns, such as suitability of the standard chicken egg production platforms to serve current needs. Furthermore, it has shown to be a good fit as a manufacturing platform for leads emerging from rational vaccine design projects and offers the potential for controlling the costs of manufacturing through utilization of, for example, single-use technology. S2 cells

Key Terms

Virus-like particle: Resemble viruses, but are noninfectious since they do not contain any viral genetic material. Often used in studies to identify protein components required for viral assembly and are also a useful tool for the development of vaccines.

Subunit vaccine: Include only the antigens that best stimulate the immune system instead of the entire microbe. 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 one to 20 or more antigens, and are manufactured using recombinant DNA technology.

have, so far, delivered promising results in several vaccines projects.

Use of S2 cells for the development of recombinant vaccines & virus-like particles

Rational design of vaccines makes use of a set of interdisciplinary tools that drive design and manufacturing towards what are expected to be more efficient and safer vaccines. These new approaches rely on recombinant technologies to deliver the vaccine either as recombinant DNA, or the resulting recombinant protein, which may or may not assemble in a particle, including particles that mimic viruses (virus-like particles [VLPs]). New vaccines, such as Prevnar® (recombinant subunit; Wyeth, NJ, USA), Cervarix® (GlaxoSmithKline [GSK]; London, UK) and Gardasil® (Merck MSD; Hoddesdon, UK), both VLPs, have demonstrated the market potential of innovative approaches to vaccine design and technology.

The following sections describe recombinant subunit vaccine strategies that have been enabled through use of S2 cells.

Drosophila S2 cells have been used to express recombinant viral proteins from Dengue Virus (DENV), Japanese Encephalitis Virus (JEV) and West Nile Virus (WNV) in vaccine development projects. The DENV [32] and the WNV [33,201] vaccines have been tested in Phase I, which were considered successful. It is expected that each of these vaccines will continue further clinical studies in the coming year.

Table 1. Examples of products produced in insect cells or in clinical development.

Company/manufacturer	Product	Stage of clinical development
Baculovirus-based products on the market		
GlaxoSmithKline (London, UK)	HPV vaccine – Cervarix®	Market
Dendreon (WA, USA)	Prostate cancer – Provenge®	Market
Protein Sciences Corp. (CT, USA)	Influenza Vac. – Flublok®	Market
Drosophila *Schneider 2 products in clinical development*		
Pharmexa (Horsholm, Denmark)	HER-2+ breast cancer vaccine	Phase II
TxCell (Valbonne, France)	Crohn's disease	Phase II
Merck Inc. (MSD)	Tetravalent Dengue fever vaccine	Phase I
Hawaii Biotech (HI, USA)	West Nile virus vaccine	Phase I
Pharmexa	Bone metastatic cancer vaccine	Late PC
Copenhagen University (Copenhagen, Denmark)	Placental malaria vaccine	Late PC

» Production of a DENV antigen in S2 cells

DENV causes 100 million infections per year. There is no specific therapy for DENV, and no vaccine has been licensed yet, although a number of candidates are in development, including a Phase III candidate (for a recent review of the pipeline of DENV vaccines, see [34]). There are four viral serotypes, and the vaccines in development must address them all, in order to effectively confer immunity. One of the most advanced candidates, in Phase I, consists of a recombinant subunit approach where the envelope (E) subunits of all four DENV serotypes were expressed in the *Drosophila* S2 expression system [35–37]. The subunits were expressed at levels up to 10–50 mg/l and all had native-like conformation. The authors refer to the fact that previously expressed recombinant flavavirus products, using mammalian or the BEVS, yielded low expression levels and/or improper confirmation [38]. Another viral protein, the NS1 protein, was also expressed in the *Drosophila* S2 system, and tested in conjunction with the 80E subunits [38,39]. This vaccine program is currently part of the Merck Inc. pipeline.

» Expression of JEV proteins in S2 cells

JEV is the most common agent of viral encephalitis causing an estimated 50,000 cases annually, of which 15,000 result in death. Half of the survivors suffer from severe sequelae. A vaccine containing inactivated JEV grown in Vero cells has recently been licensed (IXIARO, Intercell). Two of the candidates in the pipeline include antigens produced in S2 cells. It is known that the envelope glycoprotein E is the main trigger of host immune response. Infected cells replicate and release the virus, and also viral sub particles, containing two viral proteins: membrane (M) and protein E, but no nucleic acid or capsid protein. These two proteins have been expressed in mammalian cell lines and in the BEVS system [40]. Zhang and co-workers have used *Drosophila* S2 cells to express both E and M proteins and have suggested that the relative advantages of the *Drosophila* S2 systems over the other two, for production of this type of vaccine, are the potential to be a cheaper and less hands-on platform [40]. The team describes the production of sub-viral JEV particles in S2 cells transfected with the two proteins (E and M). The authors confirmed that the ability of the *Drosophila* S2-produced sub-viral particles to induce in-mouse

specific antibodies, could be a possible path for developing a vaccine to protect against JEV.

The NS1 protein is a nonstructural virus protein with roles in virus cycle and pathogenesis with a high degree of sequence homology across viral strains. This protein was produced in the supernatant of S2 cells [41]. These authors observed one form of approximately 300 kDa JEV NS1 purified from the culture supernatant by size exclusion column, corresponding to the hexameric form of full-length NS1. The protein produced in monoclonal S2 cells gave a yield of 2–5mg/l of >90% pure protein. Antibodies specific for the pure recombinant protein were raised in mice. According to the authors, these studies will contribute to better characterizing NS1 protein produced in S2 cells and in understanding its role in neuropathogenesis. Based on animal data, it is believed that NS1 contributes to the induction of protective immunity, and is an important component of a potential vaccine [42].

» Use of S2 cells in a WNV vaccine program

Another member of the Flavavirus, WNV is transmitted by mosquitos to birds. Humans and horses are considered dead-end hosts for the virus. The disease was not considered a problem for humans until a first fatal case of encephalitis was described in 1994. Since this time, and in particular after the introduction of the virus in New York in 1999, the disease has been considered a major human and veterinary health problem. There are vaccines available for veterinary use (in horses) but none for humans. Several vaccines are in development, including a recombinant subunit option, where the protein subunits are produced in a *Drosophila* S2 system [42,43]. Truncated forms of the E protein and the N protein were produced with yields of approximately 10–25 mg/l. Both proteins were shown to be in the right conformation. The recombinant truncated E protein mixed with an adjuvant was used to immunize monkeys, and shown to be 100% efficacious against WNV [43]. This protein is part of Hawaii Biotech Inc. (HI, USA) development pipeline.

» Use of S2 cells to develop a placental malaria vaccine

A particular form of malaria affecting pregnant woman – placental malaria – causes the death of 200,000 infants and 10,000 pregnant women each year. Placental malaria has no effective cure and has been associated with a significant decrease in infant birth weight, especially in primigravidea. Infants born to mothers with placental malaria may be at an increased risk of anemia, malaria and mortality during their first year of life. Women with malaria infection of the placenta also have a higher risk of passing HIV infection to their newborns. An effective vaccine would have many benefits in communities affected by malaria. Several malaria vaccines are in development, all targeting severe malaria in children, and none of these are likely to be effective against placental malaria because of the molecular mechanism that is unique to placental malaria. VAR2CSA was identified by the team at the Center for Medical Parasitology at Copenhagen University (Copenhagen, Denmark), and is the main antigen candidate in the current efforts to develop a pregnancy-associated malaria vaccine [44–46]. The stable insect cell lines and nonlytic expression has the advantages already discussed, such as reproducibility of production runs and more options for cultivation, such as perfusion. It is expected that these advantages will lead to reduced cost. The robustness of S2 cells and processes facilitates transfer to current good manufacturing practice facilities for manufacturing and decreases process transfer time, while increasing the likelihood of success. CHO and other insect cells are less robust and more sensitive to process changes than S2 cells. In this project, the ability of a proprietary S2 platform (ExpreS^2 by ExpreS^{2}ion Biotechnologies) to be used in selecting the appropriate antigen variant, and an assessment on how it performs on large scale so as to produce the needed malaria antigen within the cost constraints needed was successfully tested [47]. It was demonstrated to be an appropriate platform to screen more than 30 variants. The protein was produced in the right conformation, and at yields compatible with the development of a large-scale process [47,48].

» S2 cells for production of rabies virus glycoproteins

Rabies is a viral disease that causes acute encephalitis in mammals. Recently, recombinant viral proteins have been tested as potential vaccine candidates for rabies. The major surface protein of rabies virus is the glycoprotein, responsible for eliciting the production of neutralizing antibodies. Extensive studies were performed in producing the rabies virus glycoprotein (RVGP) in *Drosophila* S2 cells [49–51]. RVGP synthesis was optimized by the expression/selection vector's design, cell subpopulations selection, chromatin exposure and culture medium employment. The recombinant viral protein was used to immunize mice, and has shown protection against rabies virus in experimental challenge studies with rabies virus [49].

» Production of VLPs in S2 cells

VLPs resemble the shape of a virus, but are noninfectious because they do not contain genetic material (DNA or RNA). The objective is that the immune system responds to a VLP vaccine in a similar man-

Key Term

Glycoengineering: Method to engineer or change the glycosylation profile of a cell.

ner to the live virus, because the VLP presents viral antigens in a structure that imitates it. The advantage of this strategy is that immune response occurs without the risk associated with virus replication, hopefully making the technology an efficient and safer advance for vaccines.

HIV-1 VLP production in S2 cells

Recently, Yang *et al.* published their success in making recombinant HIV-1 VLPs [52]. The rationale for using VLPs was that an ideal vaccine may require a structural component that initiates a broader antibody response, and thereby binds the envelope spikes of the particle. Such a response could aid pools of memory T cells recognizing multiple epitopes on the virion. There have been HIV-1 VLPs produced in a variety of expression systems, including mammalian and yeast systems. However, the low yields obtained in these systems make them unsuitable for larger animal studies, and consequently, for clinical development. Expression of the HIV-1 proteins in BEVS yielded higher amounts of VLP. However, there are major drawbacks with these expression systems: it is not possible to separate the HIV-1 VLPs from recombinant baculovirus; the gp160 is not properly cleaved, and its effect on a vaccine is not known; and finally, the lytic nature of the BEVS system necessitates that a new lot of cells producing VLPs is created with potential variations from lot-to-lot, which makes production more complex and expensive. Thus, the S2 system was tried as an alternative that would not have the limitations above. In these cells, the gp160 envelope precursor was cleaved properly [52]. To test this hypothesis, Yang and collaborators co-transfected S2 cells with plasmids encoding an envelope glycoprotein (consensus B or consensus C), a Rev-independent Gag (Pr55) protein and a Rev protein, along with a selection marker. After the stably transfected S2 clones were established, the production and characteristics of the HIV-1 envelope proteins produced were evaluated. After demonstrating by western blot that the expected proteins were produced, with the correct size, a larger scale production using WAVE (GE) bioreactors was performed. In fed-batch fermentations, after 11 days of fermentation the authors found that Gag55 was detected in the supernatants at a concentration of 9.5 mg/l and the gp120 was found in a concentration of 7.5 mg/l. According to the authors, these yields are comparable to those obtained when producing VLPs with the BEVS system, and much higher than the yields obtained with mammalian cell systems. These authors believe this was possible because S2 cells can be grown to a much higher density than mammalian cells. The authors comment that the S2 system therefore has a yield advantage in relation to the mammalian systems tested, and advantages in relation to both BEVS because the HIV-1 VLPs produced in the stable S2 system are not contaminated with recombinant baculovirus.

The morphology of both the VLPs formed and of their spikes was examined by cryo-electron microscopy and electron tomography, and shown to be round and with diameters ranging from 95 to 185 nm. This compares well with the average diameter of SIV and HIV-1 virions, which average 109 and 110 nm, respectively. Also, similarly to what is observed in the virions, the recombinant VLPs had spikes,ranging from 13 to 20 spikes per VLP and they were periodically spaced on the surface of the VLP. The immunogenicity of the VLPs was assessed using a panel of antibodies and a variety of tests (enzyme-linked immunosorbent assay and western blot). The results showed that all but one of the immunogenic epitopes was preserved on the spikes of the HIV-1-VLPs produced in S2 cells. The authors further demonstrated immunogenicity of the S2-produced HIV-1-VLPs in mice, using a prime-boost approach with heterologous DNA [52]. The S2-produced recombinant VLPs elicited both antibody response and T-cell response in immunized mice. The authors used a combination of constitutive and inducible promoters to express the proteins for VLP formation, and observed that their strategy led to the formation of two populations of VLPs, which was undesirable, and led them to comment that further refinement of the expression strategy would be needed. Another aspect highlighted by these authors was the impact of insect glycosylation on the ability of a broadly neutralizing antibody for HIV-1 to recognize glycosylated epitopes. In this study, the authors found that one of the antibodies used could not recognize its epitope, most likely due to different glycosylation. Glycosylation engineering has been done in expression organisms, namely *Saccharomyces cerevisiae* and Sf9 cells (Mimic™, see 'Glycoengineering of S2 cells' section) [53].

Use of S2 cells in immunotherapy projects

An early publication describes the use of S2 cells in immunotherapy [54]. The authors have transfected S2 cells as primer in a Phase I trial of immunotherapy against melanoma. The purpose was to immunize cytolytic T lymphocytes (CTLs) against a single melanoma epitope. *Drosophila* cells transduced with HLA-A2.1, CD80 and CD54 (intracellular adhesion molecule-1) were used for priming patients that further received injections of the immunized CTLs. The treatment was non-toxic, but the CTLs were not specifically localized

to the tumors. To the authors' knowledge, the work was not continued. In 2003, Pharmexa A/S (Horsholm, Denmark), had two HER-2 positive breast cancer immunotherapy programs, AutoVac™, in development: a DNA- and a protein-based vaccine program. HER-2 is present in many cancer forms, including breast, ovary, uterus, stomach, bladder, prostate, colon and lung cancers. The protein vaccine aimed to establish a high level of antibodies.

The preliminary data from a Phase I trial with the HER-2 protein AutoVac vaccine in ten breast cancer patients in the USA indicated that the vaccine was safe and induced antibodies in patients [202]. The company discontinued the clinical trials after completing a Phase II clinical trial due to corporate changes (the company was combined with Affitech AS [Oslo, Norway] in 2009) and pipeline rationalization. *Drosophila* S2 cells were used in yet another immunotherapy in clinical development: the technology being developed by the company TxCells (Valbonne, France), who are using *Drosophila* feeder cells to improve the stimulation and growth of Trl cell clones (Trl cells being TxCells proprietary technology platform). S2 cells were transfected with a transmembrane form of a murine antihuman CD3 antibody, with human CD80, human CD58, human IL-2 and human IL-4 [101]. A clinical Phase I study in an immunotherapy for Crohn's disease (CD) using the Trl cell clones grown by TxCell was performed. The authors of the study concluded that administration of antigen-specific Tregs to patients with refractory CD was well tolerated and had dose-related efficacy; and are planning further clinical studies with this therapeutic platform in refractory CD [55].

Expression of antibodies in *Drosophila* S2 cells

The first instance of monoclonal antibody production and characterization in S2 cells was in 1995 and made by researchers at SmithKline Beecham Pharmaceuticals (now GSK). The target of the monoclonal antibody was the antigen of respiratory syncytial virus [56]. Significantly, the cells were found to efficiently secrete monoclonal antibodies in transient transfections at levels similar to CHO and rat myeloma cells, and with substrate-binding activity identical to that produced in vertebrate cells lines. Research performed at the Karolinska Institute (Stockholm, Sweden) supports these findings, as the binding characteristics for IgG1 of the same clones expressed in S2 cells or mammalian cells were indistinguishable [57]. An *in vitro* infectivity study using HIV as a model similarly showed equivalent neutralization of the virus [57]. The effects of S2 paucimannose (±3-fucose) glycosylation on effector functions and half-life have, however, not been studied in detail. Monoclonal antibodies targeting other diseases/targets have also been produced in S2 cells, namely: African cassava mosaic virus [58], human polymeric immunoglobulin receptor [59], E-selectin [60] and melanoma [61].

Furthermore, S2 cells have been used to express other examples of fully functional monoclonal antibody IgG [57] and membrane-bound IgD [62], antibodies with modified binding [63], single-chain variable fragments [59–61], Fc chimeras [64] and Fab fragments [63]. Uniquely, both heavy chain dimers and light chains were secreted independently when expressed without the corresponding light or heavy chains in S2 cells [56,57]. Recently, an S2 perfusion culture was used to produce 1 g/l of monoclonal antibody in a WAVE bioreactor [65].

A perceived obstacle to commercial monoclonal antibody production using the S2 system is the matter of nonhuman glycosylation. This could theoretically lead to faster clearance of proteins produced in S2 cells through binding of terminal mannose to mannose receptors. The proportion of 3-fucose is also much lower in insect cells than for CHO-produced proteins, which would likely lead to improved antibody-dependent cell-mediated cytotoxicity activities of monoclonal antibodies. Strikingly, it has been shown that antibodies produced in insect cells (*Spodoptera* Sf21) have the same level of ADCC as a glycoengineered GnTIII-overexpressing HEK293T cell line, which reduces the percentage of 3-fucose present on glycans. Both the insect-produced material and the glycol-engineered HEK293 strains had 50–100-fold enhanced ADCC levels compared with CHO-produced material [66].

S2 cell glycosylation

Currently used production hosts, such as CHO and HEK93 cells, can result in nonterminally sialylated glycans. These glycans often have truncated and hybrid forms ending in GlcNac, Gal or both [67]. CHO cells can also add nonhuman *N*-glycolylneuraminic acid instead of sialic acid. S2 cells have been shown to produce paucimannose (MAN-3) *N*-glycosylation **(Figure 1)** [68]. This was confirmed in studies focused on determining the glycosylation pattern of S2-produced hEPO and human transferrin proteins [69,70], which showed predominantly paucimannose glycosylation with and without non-immunogenic 3-fucose. This is in agreement with results by Gårdsvoll [71] and findings in the authors' laboratory [Dyring C, Adriaan de Jongh W, Salgueiro S, Unpublished Data].

» Advances in glycoengineering of S2 cells

Immune responses to different immunogenic glycoforms have been extensively reported for enzyme re-

placement therapies and antibody treatments [72,73]. The aim of glycoengineering would therefore be to alter the native insect glycosylation machinery to allow for the production of 'mammalianized' patterns. This would enable the use of insect cells to broaden from current vaccine applications to the production of human therapeutics. Extensive glycoengineering of Sf9 cells have been ongoing for more than 15 years [74–76], and humanized glycosylation has now been achieved for Sf9 cells [77]. One of these cell lines is commercially available under the brand name Mimic™.

Similar approaches have been applied to S2 cells to engineer novel glyco-modified S2 insect cell lines. Initial work showed enhanced activity of recombinant beta-secretase and recombinant cyclooxygenase 1 from *Drosophila* melanogaster S2 cells transformed with cDNAs encoding human β1,4-galactosyltransferase and Galbeta1,4-GlcNAc α2,6-sialyltransferase [78,79]. Unfortunately, detailed analysis of the glycoforms was not conducted. A significant finding in the field was the discovery of the 'fused lobes' gene as a main stumbling block to extended bi-antennary glycoforms through enzymatic *N*-acetylglucosaminidase activity [80]. This knowledge was exploited to show that downregulation through dsRNA or chemical suppression of beta-*N*-acetylglucosaminidase (GalT) led to the extension of the paucimannose glycan to bi-antennary terminal GlcNac. The terminal GlcNac glycans constituted up to 11% of the total glycoforms detected [81]. Concomitant expression of β-1,4-galactosyltransferase and suppression of β-N364 acetylglucosaminidase led to 22% of the glycans being in the form of a single-antennae extended to terminal Gal, with the other antennae not extended and ending in annose [82]. However, the main glycoform remained as paucimannose, while bi-antennary terminal Gal glycans were also detected in very low amounts. The authors suggest that more efficient knockdown or deletion of the fused lobes gene could lead to more complete glycoforms.

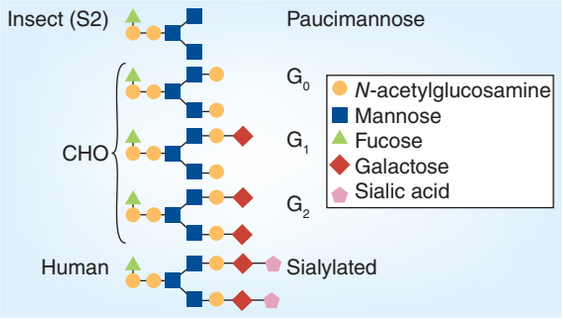

Figure 1. Glycosylation profiles of proteins produced by various expression systems. Glycosylation in Schneider 2 cells differs from that of mammalian cells in that it is shorter and ends on mannose (paucimannose structure) while that of Chinese hamster ovary cells is G0, G1 or G2, depending on the number of terminal galactoses.
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S2 cells in bioproduction

Drosophila S2 cells are extremely well suited for bioproduction. The system allows stable, nonlytic, high-level expression of a wide range of recombinant proteins. The system is particularly well suited to secreted protein production and yields of more than 500 mg/l have been achieved for complex proteins, for example, rHA from influenza in batch culture [ExpreS²ion Biotechnologies, Unpublished Data] and 1 g/l of monoclonal antibody in perfusion culture [65]. The nonlytic nature of the cells allows batch, fed-batch and perfusion technology to be applied. These high yields combined with the cost advantages of perfusion makes the system particularly attractive in cost-sensitive vaccines for neglected diseases. Several recent publications have focused on understanding the basic principles influencing S2-based protein production processes. Specifically, there has been significant focus on transmembrane RVGP-producing S2 cell lines [83] and processes to improve RVGP production.

Detailed studies of growth and metabolism of a RVGP-producing S2 cell line [84], as well as respiratory activity [85] during production, has been performed with implications for increased protein production, process control and scale-up. Specifically, the abundance of amino acids glutamine, serine and cysteine was shown to be important to avoid protein degradation, while proline, glutamine and glucose addition could increase final cell densities when using SF900II medium [84]. It should be stressed that alternative media can be expected to have different limiting nutrients. The importance and influence of different culture media and media components [86,87] and the effects of serum-containing or serum-free media [86,88,89] on wild-type and/or RVGP-producing S2 strains have also been explored. Insights gained through detailed studies of S2 processes have led to a 9.3-fold increase in RVGP yield by lowering the production temperature to 22°C compared with the initial process conducted at 28°C [90]. Following this study, a detailed analysis of the effect of culture temperature on RVGP production between 16 and 28°C demonstrated that the best production was attained at 20°C while feeding limiting amino acids, as suggested by the study of Swiech *et al.* [91]. An interesting 60% increase in RVGP production was seen for the addition of *Lonomia oblique* hemolymph [92]; it was shown that the positive effect was mainly early in the culture and was related to an increased maximum specific productivity of 60 ng/cell/day ver-

sus 24 ng/cell/day for the control culture [93]. Further factors investigated included inducible versus stable expression [51], cell line reselection [94] and the addition of sodium butyrate [95] on RVGP productions. Significant work has also been performed on the expression of secreted truncated DENV envelope proteins (Den80-E) [96] as well as the E and NS1 from WNV [42]. Both these viral vaccine projects have progressed to successful Phase I clinical trials.

As mentioned in the introduction, a thorough review of S2-based bioprocess development has recently been published, where important factors relating to recombinant protein production in bioreactors were discussed. Specifically, the effects of culture temperature, dissolved oxygen, medium, culture additives, amino acid supplementation, carbohydrate and amino acid metabolism, lactate and ammonium accumulation, inoculum concentration, pH and hydrodynamic forces are detailed [1]. In the following sections we will therefore focus on new bioreactor systems and cultivation modes.

Key advantages of S2 versus other expression systems

The key advantages of the S2 expression system are listed below:

- Fast to robust protein production process;
- No scalability or good manufacturing practice issues reported or experienced by the authors;
- Suitable for constitutive and inducible expression;
- Very high cell densities without aggregation or toxic metabolite issues;
- Highly suitable for perfusion, since the cells grow to very high cell densities without aggregation;
- Grows readily in standard equipment and a variety of capacities;
- Cells and processes are robust;
- The risk of human viral contaminants is reduced due to the insect origin;
- Culture conditions are flexible (batch, fed-batch and perfusion);
- Regulatory acceptance.

» Fast to robust protein production process

The rate-limiting step is usually generation of host cell line(s) and protein production for downstream development and animal experiments once a desired protein has been identified. By using the S2 expression system, medium- to high-level transient expression can be obtained in 3–4 days, which allows fast screening and preselection of protein candidates for further development. Time from DNA to protein can be reduced from months to weeks by using stable polyclonal cell pools. The one-step gene amplification that occurs during selection generates high-expressing cell lines without amplification, which is often needed in CHO-based systems, and is a hallmark of the S2 system. These polyclonal pools are usually stable for months – another advantage of the S2 system, and in the discovery phase the cumbersome and time-consuming step of clonal isolation to produce recombinant proteins can be completely eliminated. This unique feature of the S2 protein expression system is particularly useful to express many different proteins or protein variants in a short timeframe for evaluation and characterization. By using stable pools of transfected S2 cells for protein production, upstream and downstream process development can be significantly faster and potentially established once the monoclonal cell line has been ascertained. This approach shortens the timelines, saves money, and exploits the company's human and financial resources in the best possible way.

» No scalability or good manufacturing practice issues

Upstream and downstream processes using S2 cells have been established and transferred to contract organizations for good manufacturing practice manufacturing for clinical Phase I and II trials. The processes are regulatory-friendly and scalable and no process-related issues were reported (or experienced by the authors). The processes developed at Pharmexa using S2 cells were robust and reproducible, which is crucial for transferring to current good manufacturing practice manufacturing. The upstream processes can also be successfully downscaled to 500 ml fermentations to facilitate optimization and minimize development costs. Furthermore, downscaled process protocols can be used in laboratory-scale process tests to support process validation for clinical Phase III trials.

» High cell densities without aggregation or toxic metabolite issues

S2 cell cultures reach very high cell densities – in batch mode typically more than tenfold higher than cell densities obtained from other insect or mammalian cell lines in batch processes (up to 85 million in shake flasks). High volumetric productivity can be obtained in batch modes facilitating ease of produc-

Table 2. Expression platforms used for recombinant dengue protein expression.

Platform	Strengths	Weaknesses	Ref.
Escherichia coli	Easy to produce High level expression† of E domain III Low cost of goods	Lack of post-translational modifications – not able to support proper folding of E Low-level expression‡ of full-length or truncated E	[33–37]
Saccharomyces cerevisiae	Easy to produce High level expression of E domain III Low cost of goods	Hyperglycosylation Lack of native conformation for E → low immunogenicity Low-level expression of full-length or truncated E	[48]
Pichia pastoris	Easy to produce High level expression of E domain III Low cost of goods	Lack of native conformation for E → low immunogenicity Low-level expression of full-length or truncated E	[43,44] [Clements *et al.*, Unpublished Data]
CHO DHFR system	Capable of producing proteins with native conformation	Expression levels very low Moderate cost of goods	[Coller *et al.*, Unpublished Data]
Vaccinia expression in mammalian cells	Capable of producing proteins with native conformation Immunogenicity demonstrated in mice	Expression levels low Not a commercially viable production system (safety and expression)	[42]
Baculovirus in Sf9 or High Five cells	Capable of producing proteins with native conformation Immunogenicity demonstrated in mice	Expression levels moderate§ Moderate cost of goods	[30,39–43]
Stably transformed *Drosophila* S2 cells	Produces proteins with native conformation High expression levels for truncated E† Immunogenicity and efficacy in primates	Expression platform not yet registered for a commercial product Moderate cost of goods	[54,101]

†High expression level >10 mg/l.
‡Low expression level <1 mg/l.
§Moderate expression level >1 < 10 mg/l.
CHO: Chinese hamster ovary; S2: Schneider 2.
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tion and up-scaling in the discovery phase. In perfusion processes, cell densities above 350 million cells/ml have been obtained. S2 cells are exceptionally well suited for perfusion processes (see below) because the cells do not aggregate, even at very high cell densities. Cell aggregation may complicate process development as well as the manufacturing process due to, for example, lack of control with the culture because of differentiation in physical culture conditions, decreased cell viability and release of proteases and cell components from lyzed cells, which in turn makes the downstream processing difficult and may affect overall product recovery and homogeneity. Other cell lines, such as CHO, BHK and HEK cells usually form aggregates at high cell densities.

S2 cells are resistant to the toxic effects elicited by, for example, ammonia and lactate that are waste products in the fermentation. Ammonia is a catabolic by-product of glutamine in the medium, which is toxic to mammalian cells but does not usually accumulate in S2 cultures. However, the authors have shown that S2 cells tolerate very high concentrations of ammonia added to the culture medium. Lactate is not produced in cultures of S2 cells except under conditions of anoxic stress, as it is by CHO and other mammalian cells.

» Suitable for constitutive & inducible expression

S2 cells are amenable to constitutive as well as inducible expression. Inducible expression can be relevant for expression of toxic proteins. The *Drosophila* expression system from Invitrogen is based on the metallothionein (MT) promoter, which can be induced by Cu or Cd. In the past, the main vectors used for protein expression were the vectors from Invitrogen; the constitutive pAc vector based on the Actin5C promoter and the inducible pMT vector. The pAc vector is referred to in approximately 95% of the publications on expression in S2 cells. In the remaining publications, the pMT vector has been used. The pExpreS^2 vector series from ExpreS^{2}ion Biotechnologies has a hybrid promoter created from the Actin5C promoter and the hsp70 promoter. The vector series contain different sets of yield-enhancing elements as well as different antibiotic markers in order to provide flexibility, especially when expressing multiple proteins from the same cell.

» Highly suitable for perfusion

Perfusion mode is a cost-effective technology for protein production that provides high cell densities and allows for continuous harvest from the bioreactor. This is especially useful for proteins that are not stable in culture for longer periods of time. S2 cells do not form aggregates even at very high cell densities (>350 million cells/ml) and this is one of the reasons S2 cells are highly suitable for perfusion.

Perfusion is a production mode where culture medium is continuously exchanged and the supernatant containing the product is harvested throughout the production period. Continuous addition of fresh nutrients and removal of waste products provide the cells with the stable environment they require. Much higher cell densities can therefore be achieved, which leads to higher productivity. As such, a steady state can be reached and may be sustained for several months. The continuous harvest features shorter product residence time compared with fed-batch, thus degradation is less likely.

Fed-batch is a production mode where culture media and/or balanced nutrient solutions are added to the culture at fixed intervals or continuously. The product is only harvested once, namely at the end of the run. This is the preferred production mode for antibody production, since very high antibody levels can be achieved and the antibodies are usually stable in the culture. Because there is only one harvest, process characterization and downstream processing can be very efficient. Some of the potential disadvantages of fed-batch are:

- Accumulation of waste products (e.g., lactate and ammonia) as the culture degenerate during the course of the run, which may lead to formation of proteases and other degradation enzymes, which can act on the desired product;
- Exhaustion of key nutrients if these are not properly replaced;
- Degradation of the product prior to harvest since the conditions within the culture may be constantly changing, and cells go through several phases of growth, peaks and troughs of production.

Capital and start-up costs are lower for perfusion technologies than they are for fed-batch methods as smaller upstream and downstream capacity is needed. Also, the process uses fewer seed steps in the cultivations. In perfusion technologies, the cost of batch failure due to contamination is reduced. In case of a contamination, the product that was harvested prior to the contamination will not be affected and can still be used. Only a small volume of medium will have been used if the contamination occurs early in the run. If contamination arises later in the run, significant product will have been harvested. In both cases, the cost of failure is reduced compared with a contamination in a fed-batch culture where the whole run will generally be lost.

It is obvious that there are economic benefits to using perfusion, because these can last for months. Some of the benefits lie in the reduced labor requirements for bioreactor inoculation and turnaround, such as dismantling of the bioreactor, cleaning, re-assembly, autoclaving, sterile testing and so on. Pharmexa A/S has successfully produced vaccine proteins for human clinical Phase I and II trials using S2 cells and perfusion, and commercial production using perfusion includes ReFacto (Wyeth) and monoclonal antibodies, such as Remicade (Centocor [PA, USA]) and Simulect (Novartis [Basel, Schwitzerland).

» Grows readily in standard equipment & in a broad range of scales

S2 cells grow well in bioreactors used for mammalian cell lines and BEVS and, therefore, employing an S2-based production system does not require additional investments or changes to current equipment. In fact, employing perfusion can dramatically increase the capacity by minor investments in equipment and training of personnel. As mentioned elsewhere in this review, cultivation of S2 cells are compatible with a number of single-use bioreactors – both stirred tanks reactors – and cultivation bags.

» Cells & processes are robust

S2 cells are very easy to use due to the robustness of the cells, and are highly suitable for production processes, especially perfusion. It facilitates handling in that the cells grow readily and without adaptation in serum-free medium in suspension as single cells. This occurs not only in agitated cultures but also in static cultures as loose monolayers or as cells in suspension. Furthermore, S2 cells are more robust than other production cell lines (insect and mammalian) with respect to the following parameters: osmolality and shear; changes in pH, temperature and oxygen; and general handling. in addition, static cultures can be maintained in shake flasks for more than 1 week without maintenance, which reduces labor, compared with the usual 3–4 days interval for other cell lines.

The robustness of the expression system leads to the processes developed using S2 cells also being robust and reproducible, which is crucial for transferring to current good manufacturing practice manufacturing. The upstream processes can also be successfully downscaled to, for example, 500 ml fermentations to facilitate optimization and minimize development costs. Furthermore, downscaled process protocols can be used in

laboratory-scale process tests to support process validation for clinical Phase III studies.

» The risk of human viral contaminants is reduced due to the insect origin

The relatively short track record of products produced in insect cells and the potential harmful properties of virus and cell contaminants in human use provide one of the potential risks of using insect cell production systems. However, electron microscopy, as well as *in vivo* and *in vitro* assays proven useful in ensuring safety of other cell lines used in production of biologics, can and do provide the same assurance of safety with insect cells. It is also worth noting that many potential serious human pathogens are unable to grow and replicate in insect cells. The risk that a virus can replicate in both human and S2 cells is remote and insect cells can therefore provide a distinct benefit with respect to these safety issues, unlike human or mammalian cell lines.

Pharmexa A/S commissioned a literature review and risk assessment of insect viruses in *Drosophila* S2 cells in preparation for the clinical development of the authors' HER-2 breast cancer vaccine. The Director of Technical Services and Regulatory Affairs, Q-One Biotech, carried out this assessment in January 2003. The report describes the known families and genera of viruses capable of infecting invertebrates. This is a relatively small number when compared with the diversity of virus families capable of infecting mammals, including humans. Each of these virus families and the likelihood of such a virus replicating in S2 cells and man is discussed, as well as techniques to appropriately test for, and eliminate, these viruses in cell culture.

In the light of the risk assessment and the proposed testing of the Pharmexa S2 cell bank and production bulk harvests, coupled with the downstream process design, the US FDA concluded that there was negligible risk from the use of the proposed S2 insect cells for the production of clinical material.

» Culture conditions are flexible (batch, fed-batch & perfusion)

When cultivating S2 cells in incubators, no buffer system is needed, such as the bicarbonate/CO_2 buffer system often used with mammalian cells, because S2 cells are insensitive to the pH changes that occur in the medium during growth. This eliminates the need for expensive humidified CO_2 incubators required for mammalian cells, and the cells can be cultivated in regular, cheaper and less maintenance-requiring incubators. Since no buffer system is required, S2 cells can be cultivated in tissue culture flasks with closed, unvented caps, which reduces the risk of contamination. Because of the low evaporation at the temperature where S2 cells are normally kept (23–28°C) and the use of unvented caps, S2 cells can be grown in cabinets without increased humidity of the air (usually 95% for mammalian cell cultures). Since S2 cells will grow at temperatures ranging from 16–30°C and even recover after excursions outside this range (e.g., up to 37°C or more), S2 cells can, in principle, be cultivated on the laboratory bench.

» Regulatory acceptance

Acceptance of the S2 expression system by regulatory authorities is crucial for uptake and use for commercial production of pharmaceutical products. The arrival of more and more insect-based products on the market will create a favorable environment for S2 commercialization. The presence of residual host cell proteins (HCPs) is one of the key issues with recombinant biopharmaceuticals. These are proteins that are produced during cell culture by the host cell. HCPs can cause an immune response in patients even when present at low levels. The level of such contaminating HCPs in the product must therefore be measured quantitatively according to the guidelines provided by the International Conference on Harmonisation and FDA, and assays for detection and characterization of HCP are important to establish. Accepted levels of HCP can be obtained through purification methods. The processes used with S2 cells are regulatory-friendly and scalable, and fully synthetic media are commercially available. Downstream processes, as well as methods for analysis and characterization, including assays for detection of host cell proteins, were successfully transferred.

Conclusion & future perspective

Drosophila S2 cells are a very useful tool in R&D, easily adapted to novel techniques and provide a useful model for a variety of studies. This cell system has been used in a number of vaccine projects. It has been shown to be well suited to production of insect-borne virus proteins such as flavavirus, and a variety of other proteins. The S2 cell system is therefore useful both in R&D and production. *Drosophila* S2 systems are flexible in production of biotherapeutics, crucially complementing existing standard protein production platforms such as CHO or BEVS, filling in existing gaps in bioproduction needs (Table 2). Advances in understanding glycosylation and impact of culture conditions on production are contributing to make this platform more useful – and used – for therapeutic recombinant protein production. The demonstrated high adaptability of the *Drosophila* S2 cells system to novel vaccine strategies and to novel production strategies, including very good results with single-use bioreactors, make it a platform worth keeping an eye on for the future.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

The authors are employed at ExpreS^{2}ion Biotechnologies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

Executive summary

Use of Drosophila *Schneider 2 in fundamental R&D*

- Developed in the 1970s, *Drosophila* Schneider 2 (S2) cells have, in the past decade, seen an increase in interest for use as tools to both understand fundamental biological processes, and to produce recombinant proteins.
- The resolution of the entire *Drosophila* genome and the flexibility of use of S2 cells have contributed to the increased use of S2 cell-based platforms in for example, understanding fundamental biology processes and as models to study human innate immunity.
- The interest at R&D level in S2 as a platform for recombinant protein expression stems from the relative ease of use of the cells in R&D laboratories (no need for specific equipment, robustness of the cells), its good complementation of other existing protein expression platforms, namely for production of secreted proteins, and the good quality of the proteins produced, which makes them well suited for crystallography studies.

Use of Drosophila *S2 cell in clinical development*

- The S2 cell system has been successfully scaled up. The robustness and ease of scalability of the system has been explored to study and develop a range of protein production processes, notably in a variety of single-use systems.
- Novel vaccine strategies that rely on viral or parasite protein antigen production, or in virus-like particles have been brought forward thanks to the ability of the S2 cell system to produce complex and challenging proteins. Vaccines for malaria, rabies, dengue fever and other hemorrhagic fever types, for which development has been a real challenge, are now being developed in S2 cell culture.
- S2 cells also demonstrated their role in other types of clinically relevant programs, such as cancer immunotherapies and autoimmune disease, as well as potential interest in antibody and antibody fragment production.
- Ongoing work on glycosylation modulation and on control and improvement of production processes will likely drive increased use of this system for production of novel vaccines and biotherapeutics.

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