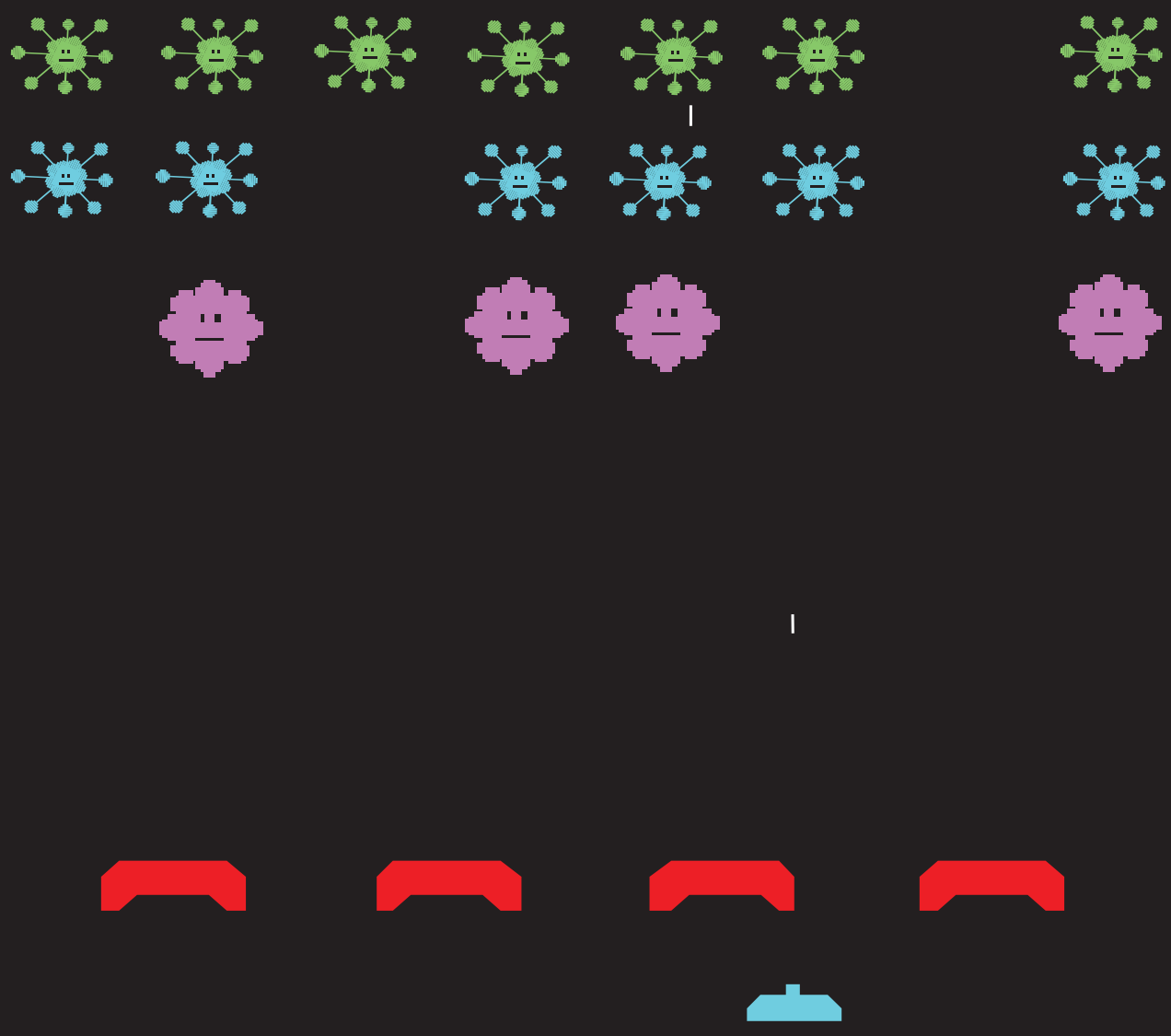
MilliporeSigma

Viral Invaders

Protection at the genetic level – a new line of defense against contamination in biopharmaceutical manufacturing



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INTELLIGENT VIRUS DEFENSE

Powered by MilliporeSigma's advanced gene-editing platform, **Centinel™ Intelligent Virus Defense** is a technology that provides **security and sustainability by offering reagents, cell-line engineering services and viral challenge assays to promote viral resistance and true progress**. We call it bioadvancement.

DEFEND TRUE BIOADVANCEMENT.

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The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

A Contamination-Free Future

Great progress has been made in the battle against adventitious viruses, but biopharmaceutical manufacturers must continually improve and add to their defenses.

Chinese hamster ovary (CHO) cells have been a staple of biopharmaceutical manufacturing since the 1980s. It's been three decades since scientists first started experimenting with CHO cell culture for protein production and, in that time, the industry has garnered a vast amount of knowledge and experience with CHO cells – but challenges remain. Working with living organisms is difficult; cells do not always behave as intended, nor as you would like them to – and, to make matters worse, adventitious viruses are a continual threat against the process.

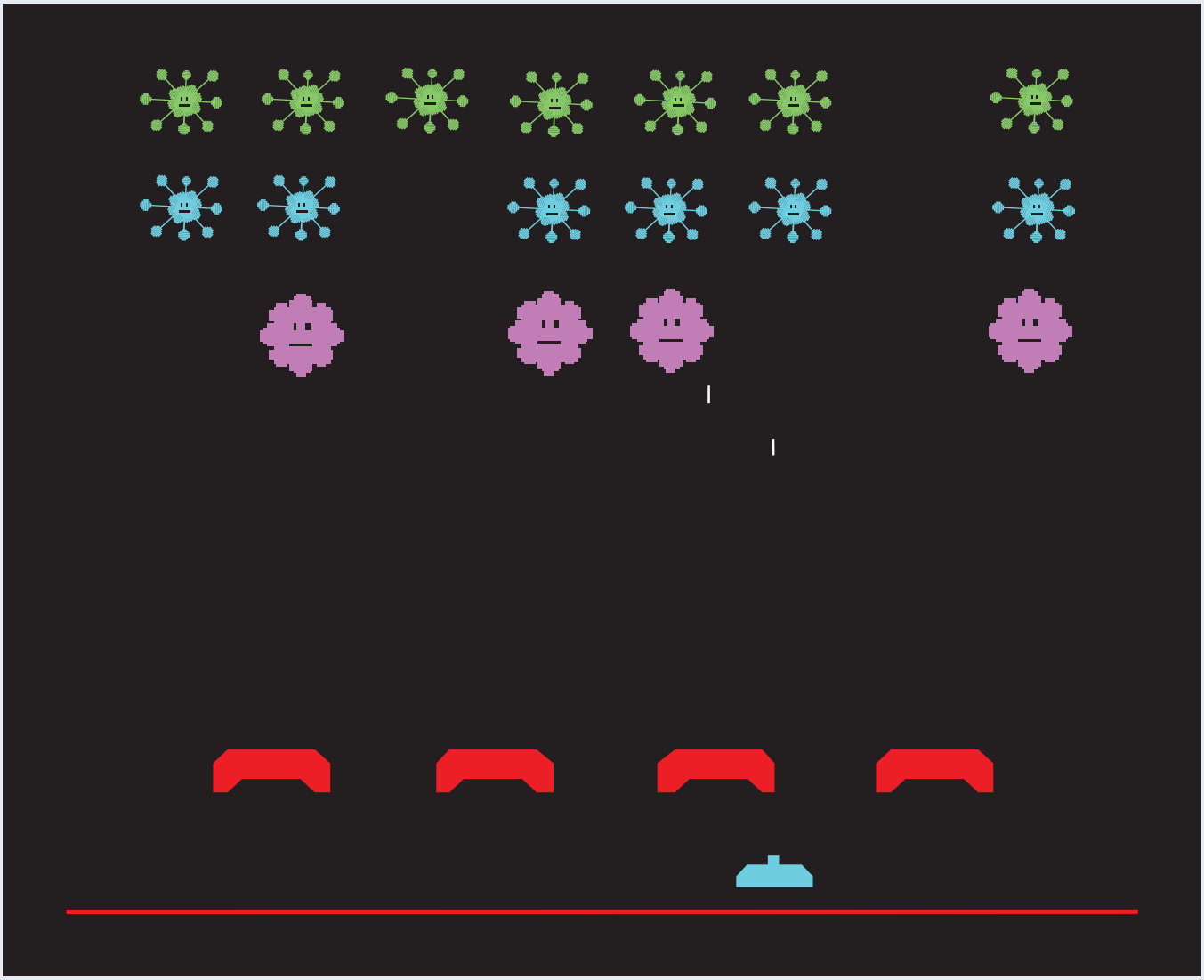
Though viral safety in biomanufacturing is better than ever before, the industry must not grow complacent. A contamination event can shut down a plant for weeks or months while the problem is resolved – generating huge costs and potentially even causing shortages of life-saving medications. Technologies in this area are constantly advancing to help reduce viral contamination, but no company is immune to the risks, as highlighted by high-profile contamination incidents at large, global companies. Millennia of evolution have made viruses the ultimate survival machines, and no single strategy can guarantee total elimination of viral risk.

One of the major threats for manufacturers using CHO cells is minute virus of mice (MVM) contamination, which can be shed anywhere mice are found, including warehouses, factories, farms and shipping containers. Because it is so small, MVM removal is difficult and just one virus particle per liter can infect a whole bioreactor – costing millions of dollars. The contamination often goes undetected until a lack of productivity in CHO cell cultures is noticed in the bioreactor. Regulators now expect manufacturers to test every bulk harvest for MVM, and there is a firm expectation that no detectable virus will be found.

It is crucial to keep building viral defenses, and developing new ways to detect, destroy and evade MVM. To that end, the Medicine Maker is delighted to co-present this compendium on viral risk mitigation with MilliporeSigma and allow them to introduce Centinel™, a means of generating MVM-resistant CHO cells. Centinel was developed using MilliporeSigma's zinc finger nuclease gene-editing technology, and represents another layer of defense for manufacturers. In the following pages, experts in virology and gene editing discuss how the problem of contamination has changed over the years, describe how gene-editing technology was used to engineer a safer form of CHO cell, and consider the future potential of productivity-enhancing alterations.

Stephanie Sutton
Editor of the Medicine Maker

Stephanie Sutton



A New Line of Defense

Viral contamination events are rare in biopharmaceutical manufacturing, thanks to a variety of risk mitigation strategies. But when prevention fails, the costs are staggering. Now, there's a new way to guard cells against a key viral threat.

Companies are understandably reluctant to discuss contamination events. "There are probably at least twice as many events happening than are reported publically," says Martin Wisher, Global Head of Regulatory Affairs at BioReliance. Wisher is an expert in viral risk mitigation, with over 30 years in biopharmaceutical manufacturing safety and quality control. A 15-year analysis of in vitro viral screening assays in unprocessed bulk drug substance from BioReliance showed that just 0.04 percent of tests were positive for a viral contaminant.

"These are still rare events. And some companies make the mistake of thinking it won't happen to them. But the chances of a contamination event are certainly much higher than an earthquake or fire, which all companies are insured against," says Wisher.

Contamination costs

Published viral contamination events at Genzyme, Amgen, Genentech and others have drawn attention to the high costs

of contamination (1–3). "Genentech staff have estimated the cost of their minute virus of mice (MVM) contamination event at millions of dollars," says Wisher, while Genzyme's well-publicized challenges with Vesivirus 2117 have racked up estimated costs as high as $1 billion (4), and caused some patients to go without treatment for months.

"There should be no detectable virus in your production process. There is no leeway as far as virus safety is concerned."

The FDA issued a consent decree at Genzyme's manufacturing facility in Massachusetts soon after the event in 2010, which has only recently been lifted. Regulators take a dim view of any contamination, says Wisher, "The expectation for manufacturers is that there should be no detectable virus in your production process. There is no leeway as far as virus safety is concerned."

The costs increase the longer the contamination goes unnoticed, ranging from lost raw materials to cleaning and additional regulatory audits. If the product manages to reach patients, you can add the cost of product recalls, lost market share and brand damage to the list.

Most companies are well aware of all the risks, and there has been a strong drive to improve processes to detect, remove and inactivate viruses. Sophisticated viral detection and elimination measures have proved very effective in preventing any danger to patients. Indeed, says Wisher, "The processes we have in place for biotechnology recombinant products have an extremely good safety margin built into them."

But risks remain, so many companies have also joined the MIT Consortium on Adventitious Agent Contamination in Biomanufacturing, which offers confidential collection and analysis of industry data to increase understanding of adventitious organisms – and disseminate "lessons learned".

Reducing the risks

What are the main ways that companies can cut the risk of a catastrophic contamination? Safety starts with the raw materials used in manufacturing. Animal products, such as bovine serum used in media, are a major risk factor, but are increasingly being phased out by biopharmaceutical makers in favor of animal-origin free or chemically defined media. However, "animal-product free" does not mean "risk free". Raw materials are not produced in sterile conditions – plant products, for example, come from farms where they are exposed to rodents and other animals. High-risk raw materials, such as serum, can be screened to ensure they contain no viral hitchhikers and gamma irradiated to inactivate any particles present. Before being used in bioreactors, media can be UV sterilized, heat-treated or undergo nanofiltration to inactivate or remove adventitious agents. During cell culture, testing can identify a viral infection before it contaminates the downstream process, while purification processes typically include viral inactivation or removal steps plus viral clearance testing, to ensure that the final product is safe for patients.

Several advances have been made in recent years, both in testing and viral clearance strategies, and as the technology

Regulatory expectations

Regulators require that an overall safety margin (for example, less than one virus particle per million doses) must be used to demonstrate the virus safety of the manufacturing process. Drug manufacturers are required to qualify the virus "load" in the process.

For biotech products derived from Chinese hamster ovary (CHO) and murine NS0 cells, this typically translates to around 12–18 $\log_{10}$ clearance for endogenous retroviruses and around 6 $\log_{10}$ removal for other adventitious viruses.

US FDA asks manufacturers of biotech products that use murine cell lines to demonstrate clearance capability of their manufacturing processes with one relevant retrovirus (murine retrovirus) before starting Phase I studies.

European regulatory agencies require manufacturing processes to be evaluated to clear non-enveloped parvoviruses in addition to retroviruses.

Before marketing authorization, manufacturers must assess clearance of multiple model and relevant viruses in their manufacturing processes.

Read more at Merck Millipore's online Virus Safety Learning Center (http://bit.ly/2arwcLM)

Virus	*Cell*	*Year*	*Company*	*Reported by*
EHDV	CHO	1988	Bioferon GmbH	Bioferon GmbH
MVM	CHO	1993	Genentech	Genentech
MVM	CHO	1994	Genentech	Genentech
Reovirus	Homo 1 Kidney	1999	Abbott Labs	FDA
Reovirus	CHO	Not Disclosed	Not Disclosed	BioReliance
Cache Valley	CHO	1999	Amgen/CMO	Amgen
Cache Valley	CHO	2000	Not Disclosed	BioReliance
Vesivirus 2117	CHO	2003	Boehringer-Ingelheim	Boehringer-Ingelheim
Cache Valley	CHO	2003	Not Disclosed	BioReliance
Cache Valley	CHO	2004	Not Disclosed	BioReliance
Hu Adenovirus	HEK 293	Not Disclosed	Eli Lilly	Eli Lilly
MVM	CHO	2006	Amgen	Amgen
Vesivirus 2117	CHO	2008	Genzyme, Belgium	Genzyme
Vesivirus 2117	CHO	2008	Genzyme, USA	Genzyme
Vesivirus 2117	CHO	2009	Genzyme, USA	Genzyme
MVM	CHO	2009	Merrimack	Merrimack
PCV-1	Vero	2010	GlaxoSmithKline	GlaxoSmithKline
PCV-1/PCV-2	Vero	2010	Merck	Merck

Table 1. Reported major viral contamination events in biopharmaceutical manufacturing.

evolves, regulatory expectations tighten.

Physically filtering out the virus using nanofilters is one of the most effective techniques, and modern nanofiltration devices, like MilliporeSigma's Viresolve®, can capture even the smallest viruses. High-temperature, short-time (HTST) techniques and UVC irradiation are increasingly deployed for inactivation.

In terms of detection, Wisher points out, "The problem with media contamination is it's always at low level, so you don't notice it until the virus has a chance to infect a cell and multiply." The first sign of a problem may be a drop in viable cell count or a decrease in production. Moreover, the old maxim applies to traditional testing – you only find what you are looking for, which makes new or unknown viral contaminants a bigger threat. Massively parallel sequencing techniques allow detection of even unknown viruses at very low levels, so that more specific PCR or in vitro assays can be developed. These next-generation sequencing approaches have already led to the identification of several novel viruses infecting mammalian cell culture. Using such techniques, BioReliance discovered a new parvovirus in bovine serum (BAAV-2) that is able to infect human and animal cells (5). It can establish "silent" infections, so any cells that have been exposed to bovine serum in the past need to be screened.

Small but deadly

One virus has emerged as a particular threat for biomanufacturers using

Chinese hamster ovary (CHO) cells: MVM – a small, non-enveloped parvovirus that can contaminate a whole range of raw materials via the urine and feces of infected mice. Anywhere mice are found (warehouses, factories, farms and shipping containers), MVM can be shed, which means the virus can find its way into even animal-origin free raw materials or media.

"MVM most likely comes from non-animal components of media, such as glucose or amino acids. Media manufacturers may have stringent GMP, good manufacturing practices, high quality standards and good rodent control in their facilities, but typically they buy the raw materials for their media from the food industry. It's not easy for manufacturers to keep their facilities completely rodent free and, in fact, there's no requirement for that," explains Wisher.

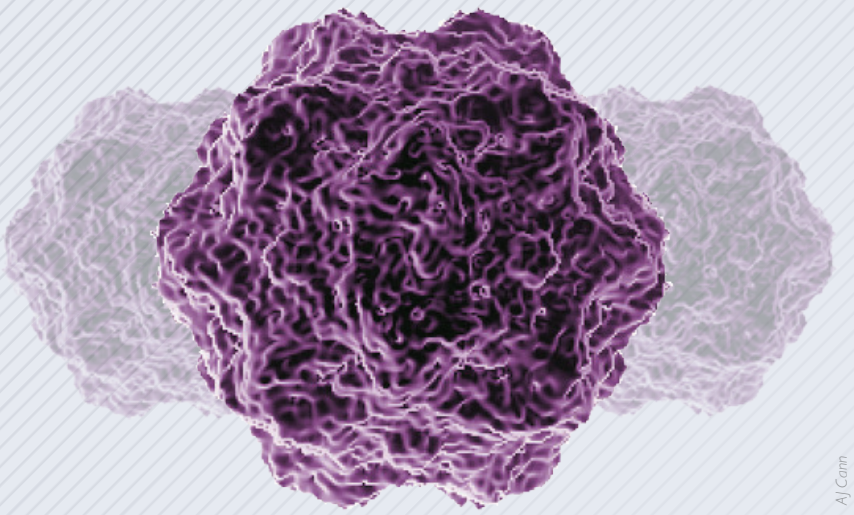
The small size of MVM makes it hard to remove by filtration, and it is extremely tough and virulent. MVM is more resistant to heat and irradiation treatments than most other viruses, and if even just a few viral particles make it through to the cell culture stage, it can spell disaster – just one viral particle per liter is enough to generate a contamination event. MVM specifically targets rapidly dividing cells, and can quickly take down a bioreactor full of CHO cells, as shown by a number of major contamination events (see Table 1).

"Having already dealt with more than one case of MVM contamination, regulators now expect manufacturers to test every bulk harvest for MVM," says Wisher. As the industry increasingly moves to chemically-defined/animal-origin free media, MVM is likely to be one of few remaining viral threats.

And now for something completely different...

Centinel™ technology enables the generation of a MVM-resistant CHO cell line. It applies MilliporeSigma's

FDA's MOST WANTED



AJ Cann

Full name: Minute Virus of Mice (MVM)

Alias: Mouse Minute Virus (MMV)

Genus: Protoparvovirus

Family: Parvoviradae

First Isolated: 1966

Description: A small (<20 nm) non-enveloped virus with a 5 kb linear, ssDNA genome.

Natural history: Hosts are wild and laboratory mice, but hamsters and rats can be infected. It requires mitosis to replicate so targets rapidly dividing cells in areas like the gut. Infections in adult mice are asymptomatic, but in pregnant females can cause miscarriage or stillbirth of pups.

Wanted for: Infecting CHO cell cultures for production of recombinant proteins. Damage amounting to millions of dollars in lost product and cleanup operations. Just one virus particle/liter can infect a whole bioreactor.

Centinel in brief

- No detectable MVM replication after viral challenge
- No drop in productivity or quality
- Suitable for production of asialylated recombinant proteins
- Centinel technology can be applied to multiple cell types and gene targets

gene editing technology, zinc finger nucleases (ZFNs), to generate CHO cell lines that are resistant to infection by MVM. The result of a collaboration between BioReliance's viral safety experts and MilliporeSigma's cell-line gene editing team, Centinel represents an entirely new way to prevent viral contamination. Cell lines can be engineered using ZFN technology to suppress expression of sialic acid, a key component for MVM cell binding and entry. With no sialylated glycoproteins or glycolipids on the cell surface, MVM has no means of entering the cell, and so cannot replicate.

Extensive testing by BioReliance, both with laboratory strains and with viruses isolated from biopharma producers, show that the cells are highly resistant to MVM, with no adverse effects on protein production. It's impossible to completely remove the risk of viral contamination, but using cells that are resistant to one of the worst threats should give even the most comprehensive viral safety program an added boost. "Centinel ramps up resistance to the most common type of viral infection we've seen to date; it's a very positive development that will certainly reduce viral contamination risk," says Wisher.

Centinel technology is an extension of MilliporeSigma's CHOZN® platform, which uses cells that are modified by ZFNs to remove endogenous glutamine synthetase expression, allowing more stringent, higher performing clone selection. Wisher believes gene editing of CHO cells could lead to better manufacturing all round, "I think this is all part of developing a much more sophisticated production base. The decision to use CHO cells for biomanufacturing wasn't really a scientific one – it was just a cell line that was available and produced proteins at a high level. Now, we're beginning to rationalize use of that production system by engineering in viral safety and high expression of the product."

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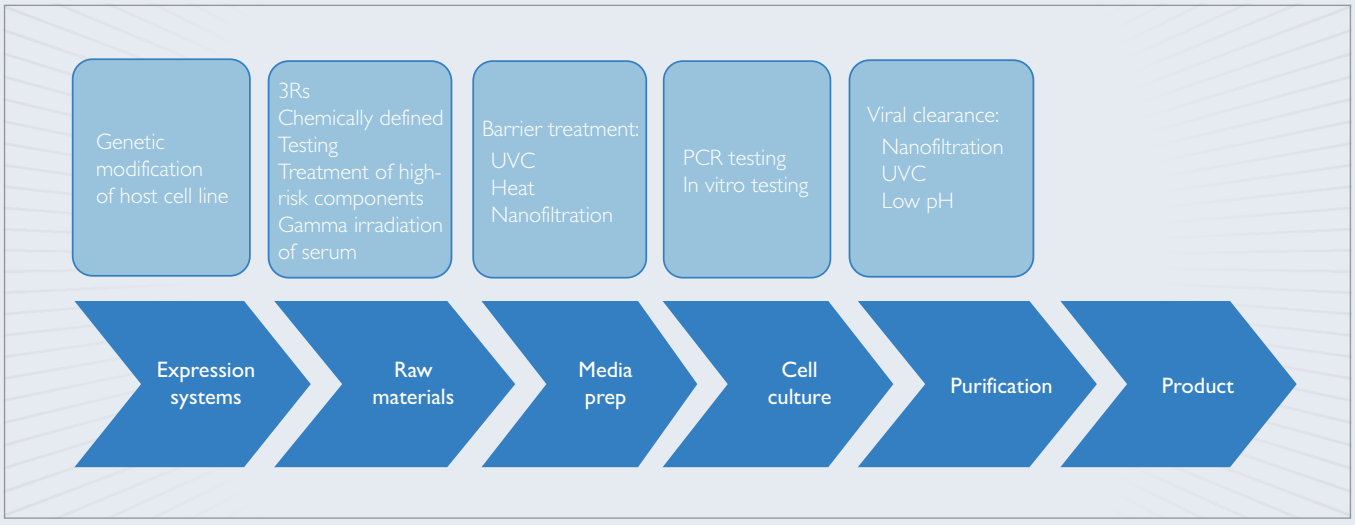

Figure 1. Viral safety during the biopharmaceutical manufacturing process.

CELL LINE CHARACTERIZATION

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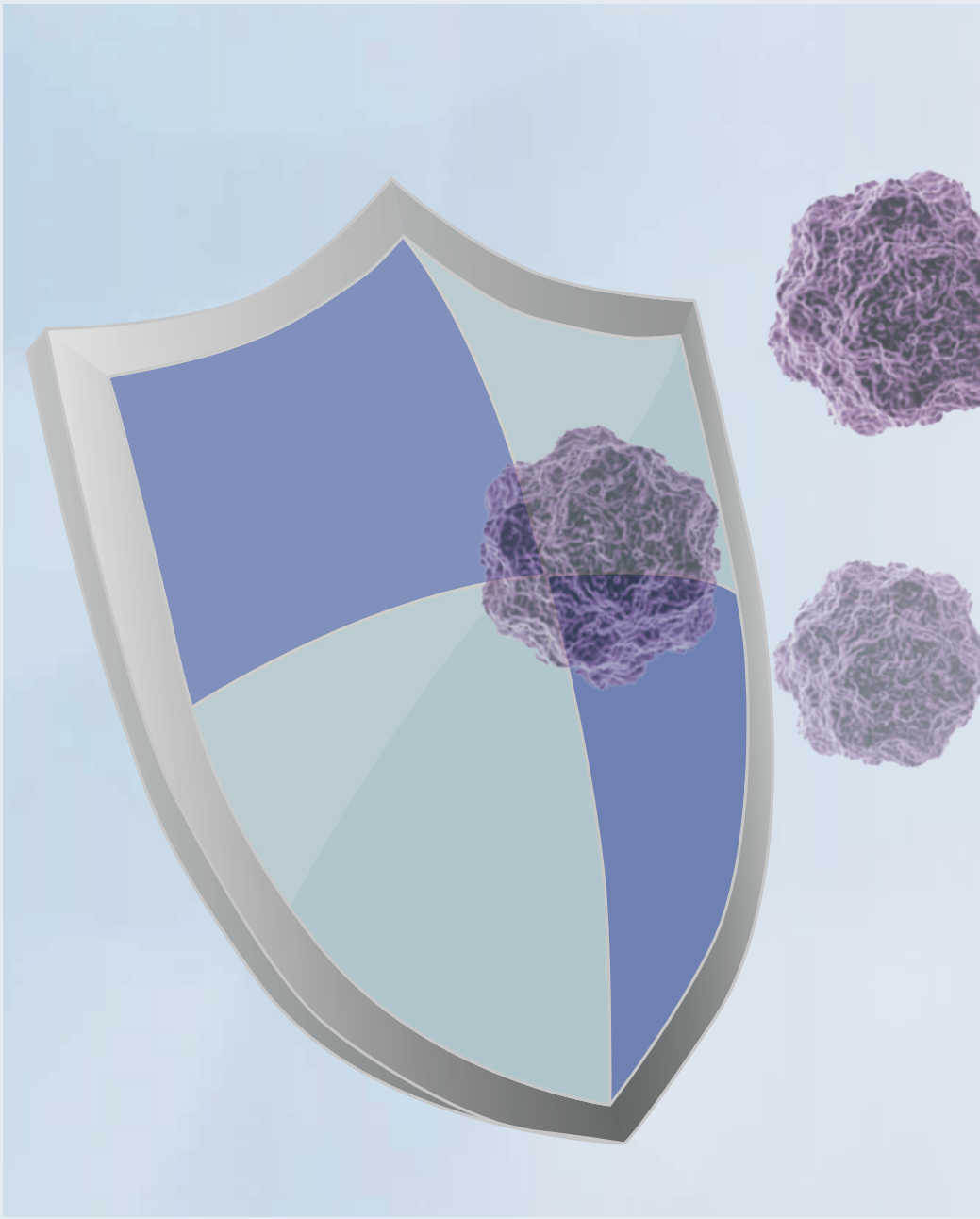
Lightening the (Viral) Load

Joaquina Mascarenhas, Trissa Borgschulte and Henry George tell the story behind Centinel™, the technology that enables the creation of a virus-resistant CHO cell line.



The seeds of our project to create a virus-resistant Chinese hamster ovary (CHO) cell line were sown when a proposal was sent from David Onions (then Chief Scientific Officer at BioReliance) to Kevin Kayser at MilliporeSigma in 2013. BioReliance testing had revealed that minute virus of mice (MVM) contamination events were still occurring in biopharmaceutical manufacturing using CHO cells, even when animal-origin free processes were in place. Sigma-Aldrich had acquired BioReliance the year before, and David spotted an opportunity to use our zinc finger nuclease (ZFN) gene editing and cell engineering technology to prevent MVM from taking hold. He reasoned that if we could eliminate the receptor that MVM uses to gain entry, the virus may be unable to infect the cell.

ZFN technology was the obvious choice of gene editing technique for us. ZFNs have a clear intellectual property position and the clinical precedent of ZFN engineered cells makes them attractive to biopharmaceutical manufacturers. ZFNs typically recognize a 36 base pair DNA sequence within the genome, which can lead to better identification of unique target sites and increased specificity compared with some other gene editing techniques.

The CHOZN® platform, a glutamine synthetase (GS) gene knockout expression platform that simplifies cell line generation, had proven popular, and we were keen to expand the program. Viral safety is an issue that our customers – and the industry as a whole – take very seriously, and several major biopharmaceutical companies have championed efforts to improve viral safety in the industry. Centinel technology has benefited greatly from a collaboration with the industry.

Targeting sialic acid

It is well documented in the literature that MVM gains entry into the cell line via a sialic acid receptor. Sialic acids represent

AJ Cann

more than 50 sugars that are widespread in mammalian tissues, often found at the end of glycan chains anchored to the cell surface. Several other pathogens are known to target cell-surface sialic acids to gain entry into the cell.

In a proof-of-concept study, based on previous literature reports, we first treated CHO cells with an enzyme that removes the sialic acid bound to the cell surface, then tried to infect them with MVM. The treated cells proved resistant to infection, which gave us confidence to focus our efforts on genetically modifying the machinery that generates cell surface sialic acid.

"We reasoned that if we could eliminate the receptor that MVM uses to gain entry, the virus may be unable to infect the cell."

We experimented with knocking out a variety of genes involved in synthesis of sialylated glycoproteins and glycolipids, before narrowing down our target: solute carrier family 35, member A1 (*Slc35A1*) – a transporter protein that is responsible for the transport of sialic acid into the Golgi apparatus, where it is added to glycoproteins. Knocking out the *Slc35A1* gene resulted in loss of the enzyme activity, eliminating sialic acid-linked glycostructures from the cell surface.

One might expect that disabling the cell's ability to produce sialylated molecules would have some phenotypic effects. After all, sialic acid is abundant on the surface of CHO cells and sialylated glycoproteins and other glycoconjugates are thought to play a role in cell recognition, cell–cell attachment and signaling. Indeed, we thought there would likely be some minor changes in cell clumping or stickiness. However, rather to our surprise, exhaustive testing revealed no detectable detrimental effects whatsoever on cell performance. When we introduced a recombinant protein into the *Slc35A1*-modified cell lines, we found the same levels of growth and productivity in *Slc35A1* knockout cells compared with unmodified cells. We also took a CHO clone already producing a monoclonal antibody and knocked out *Slc35A1*, and again found no significant difference in growth and productivity when compared with the original cell line.

Since sialylated cell surface glycoproteins and glycoconjugates are largely involved in various forms of cell–cell communication, we speculated that they are simply not needed in a clonal isolated, independent cell line. In biopharmaceutical manufacturing processes, CHO cells, which never encounter another cell type, must have little need for these communication channels.

Collaboration and validation

Success meant that we were forging an entirely new path. Validation of viral resistance was the next challenge. There is little academic research in this area and the biopharma industry rarely publishes details of contamination events. Here, our sister group at BioReliance, an industry leader in viral testing, was able to draw on experience gathered from many years of conducting viral detection and clearance assays for biopharmaceutical manufacturers. With their help, we came up with an industrially relevant protocol, covering a range of possible scenarios in real-life contamination events.

We confirmed MVM resistance with a qPCR assay, testing at 0 hours, 24 hours

Choosing CHO

How Chinese hamster ovary cells became a biopharma stalwart.

1919 Chinese hamsters, native to Northern China and Mongolia, are used by Chinese scientists to identify pneumococcal strains, sparking a nationwide breeding program.

1948 The first successful breeding colony outside China is established in the US. Their low chromosome number and rapid reproduction make them popular for cytogenetic and tissue culture studies.

1957 Theodore Puck of the University of Colorado is the first to isolate and culture CHO cells.

1967 The discovery of several mutant CHO cell lines paves the way for the use of CHO cells to produce selected proteins.

1980 Richard Axel and colleagues at Columbia University file patents for the use of DHFR-mutant CHO cells to express proteins – patents later worth millions of dollars. Biotech companies soon begin to experiment with CHO cell culture for recombinant protein manufacture.

1987 Genentech launch the first human drug produced in CHO cells – cardiovascular therapy Activase. To scale up production, Genentech had to develop a new way of culturing the cells – growing them in large fermenters rather than the roller bottles used previously.

1998 Enbrel and Herceptin hit the market, both made using CHO cells.

2000s A raft of new biologics produced in CHO cells are approved. Biopharma is booming, and new, high-yield CHO cell lines start to be developed.

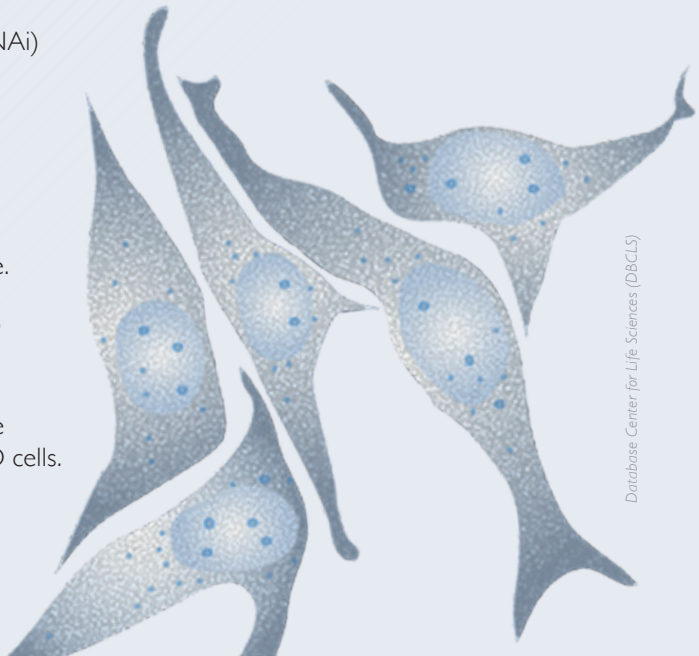
2009 Alnylam Pharmaceuticals apply RNA interference (RNAi) technology to silence select CHO cell genes, and enhance productivity.

2012 Sigma-Aldrich (now MilliporeSigma) introduce the CHOZN® platform.

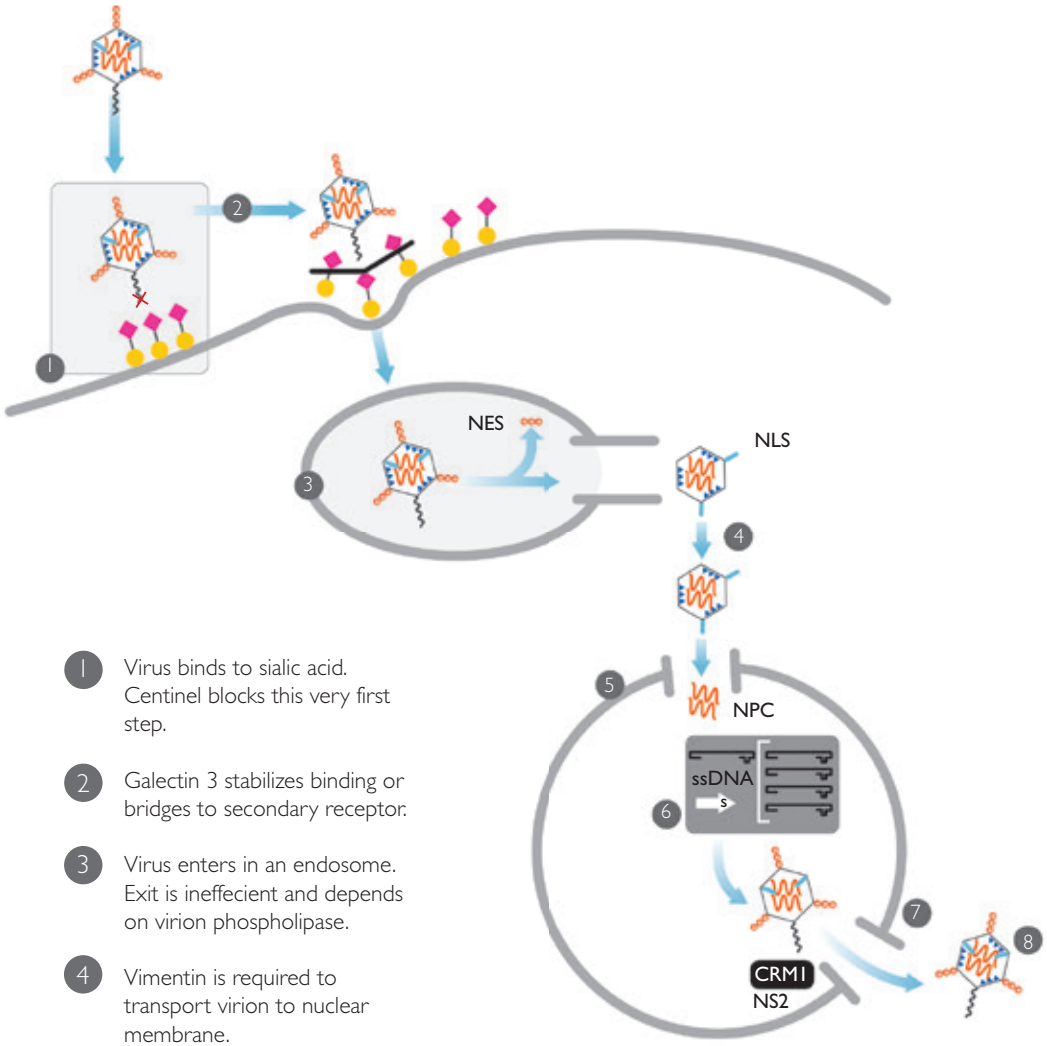
2013 The Chinese hamster genome is published in Nature.

2014 Researchers in Denmark demonstrate CRISPR/Cas9 editing of CHO cell genome.

2016 Launch of Centinel™, the first commercially available technology for engineering viral resistance into CHO cells.



Database Center for Life Sciences (DBCLS)



1. Virus binds to sialic acid. Centinel blocks this very first step.
2. Galectin 3 stabilizes binding or bridges to secondary receptor.
3. Virus enters in an endosome. Exit is ineffecient and depends on virion phospholipase.
4. Vimentin is required to transport virion to nuclear membrane.
5. Entry into nucleus requires breakdown of lamin, which is facilitated by Caspase 3.
6. Replication in nucleus of mitotically active cells.
7. Exit of viruses is an active process and requires breakdown of actin filaments.
8. Final processing of progeny virions in Golgi involves phosphorylation of the capsid.

NLS = Nuclear localization sequence
NPC - Nuclear pore complex
NES = Nuclear export signal
NS2 = Non-structural protein 2
CRM1 = Chromosome region maintenance 1

Figure 1. MVM entry into CHO cells.

and 5 days after introduction of MVM into a growing CHO cell culture. As expected, wild-type CHO cells were very susceptible to infection by MVM and almost immediately produced high levels of virus and suffered a loss of viability. The *Slc35A1* knockout cell line, however, continued to grow and propagate, with no detectable viral production via this sensitive assay. A very high level of resistance is crucial for biopharmaceutical production, where any detectable virus means the loss of the whole batch.

As well as a standard laboratory strain of MVM (i.e., MVMp), we were fortunate to have the opportunity to test Centinel's defenses against a real-world wild-type strain. We enlisted the help of a collaborator, who gave us access to a strain they had isolated from a previous contamination event. Centinel performed equally well against this wild-type strain, which gave us added confidence that knockout of *Slc35A1* activity offers protection against MVM infection.

One concern that we had at the beginning of the project was whether the virus might change or mutate in some way – finding a way around our engineered defenses. But with no detectable sialic acid on the cell surface, we believe such change is highly unlikely; after all, with no means to enter the cell, the virus cannot replicate, so there is no evolutionary pressure to drive MVM genetic modifications.

The next step

All glycoproteins produced by *Slc35A1* knockout cells are asialylated, including any recombinant glycoprotein product that may be produced from such a cell line. Around 80 percent of the current biopharmaceutical market consists of monoclonal antibodies of the IgG type, most of which – with only two N-glycosylation sites per fully assembled dimer – have a low sialic acid content (1–2 percent), and due to this low content are most likely to be unaffected by asialylation. For these products, Centinel is ideal.

In more complex therapeutic glycoproteins, elimination of sialic acid could have an impact on bioactivity and/or circulating half-life, so these products will need a different approach. With that in mind, we're already working on a next-generation Centinel cell line, which will maintain viral resistance while enabling sialylation of its protein product.

As well as more sophisticated options for viral resistance, we're working on other modifications that add value to the biopharmaceutical industry; for example, by increasing productivity or reducing the immunogenicity of recombinant proteins being produced in such cell lines – we sometimes joke that our mission is to create a "Super CHO" cell line.

Small but mighty

Over the past three years, those of us working on Centinel have come to appreciate the remarkable properties of MVM. Large, framed images of MVM particles adorn our office walls – its structure is beautiful in its simplicity (at least to a biologist). However, we are acutely aware of the havoc it can wreak. It's no accident that our viral lab is located half a mile from our other facilities. Just a few particles of this tiny virus can take down a full bioreactor, costing a biopharmaceutical company millions of dollars and delaying production of life-saving medicines. We may have a grudging respect for its tenacity, but if our work helps eliminate MVM from biopharmaceutical manufacturing, we will consider it a job well done.

We see Centinel as another layer of protection in a company's overall viral mitigation program. After all, why take a risk that you don't have to?

Joaquina Mascarenhas, Senior Research Scientist/Team Lead, MilliporeSigma

"I have been with the company for about seven years, working primarily on CHO host cell line engineering for developing next generation expression systems for the manufacture of recombinant therapeutic proteins and vaccines. I started on this project three years ago, when Kevin Kayser and David Onions came up with an idea to create a virus-resistant cell line."

Trissa Borgschulte, Head of Cell Line Development and Engineering, MilliporeSigma

"I have been with the company for 11 years, working exclusively in R&D, focused on CHO cell line biomarker discovery, cell line engineering and recombinant cell line development processes. I lead our cell line development and engineering teams, including the group developing Centinel."

Henry George, Director of R&D Operations – Cell Sciences and Development, MilliporeSigma

"I have spent most of my 35-year career to date in the biopharmaceutical sector, and have been involved with Centinel from its inception. My focus is on cell-line engineering and gene editing – but I know enough virology to be dangerous!"

What is ZFN Technology?

Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations.

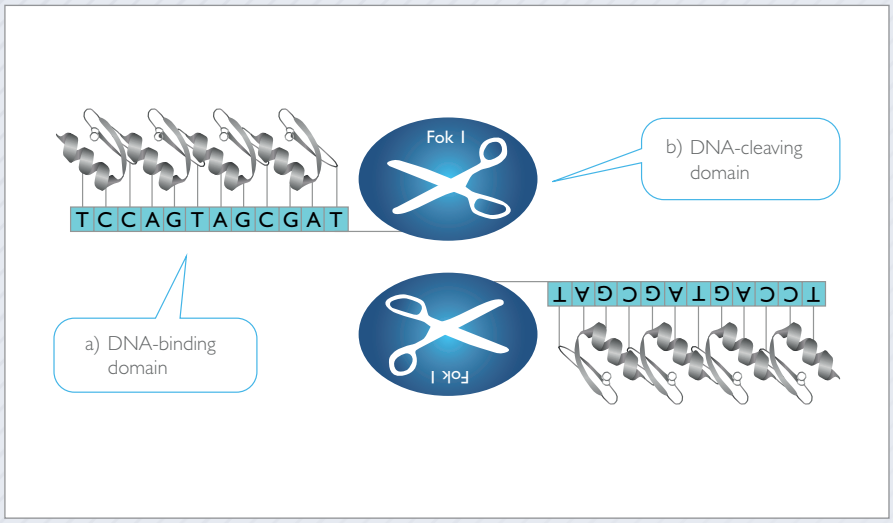


Figure 2: Each zinc finger nuclease (ZFN) consists of two functional domains: a.) A DNA-binding domain comprised of a chain of two-finger modules, each recognizing a unique hexamer (6 bp) sequence of DNA. Two-finger modules are stitched together to form a zinc finger protein, each with specificity of ≥ 24 bp. b.) A DNA-cleaving domain comprised of the nuclease domain of Fok I. When the DNA-binding and DNA-cleaving domains are fused together, a highly-specific pair of "genomic scissors" are created. Double-strand breaks are important for site-specific mutagenesis in that they stimulate the cell's natural DNA-repair processes, namely homologous recombination and non-homologous end joining (NHEJ). By implementing established, field-proven methods, these processes are harnessed to generate precisely targeted genomic edits, resulting in cell lines with targeted gene deletions, integrations, or modifications.

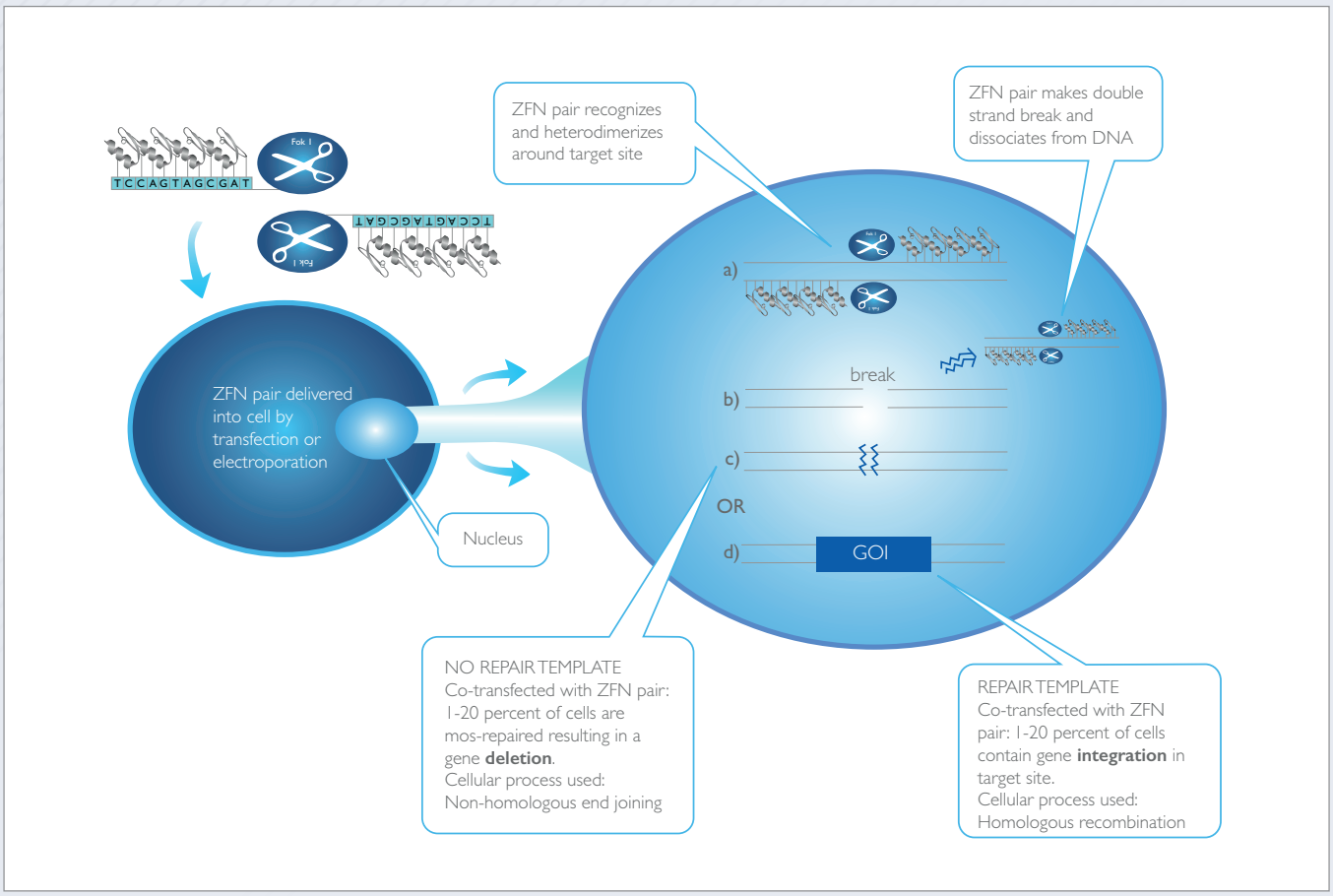


Figure 3: ZFN-mediated genome editing takes place in the nucleus when a ZFN pair targeting the user's gene of interest is delivered into a parental cell line, either by transfection, electroporation or viral delivery.

An Ongoing Battle

Centinel™ is designed to form one part of a wider risk mitigation strategy. Here, we present further resources to reduce the risk from adventitious agents.

The Next Generation of Viral Safety Testing

Next Generation Sequencing (NGS, also referred to as "Massively Parallel Sequencing") can sequence all nucleic acids in a sample, setting it apart from standard testing (for example, by qPCR), which can only test for known adventitious agents. Incorporating NGS into a company's testing strategy can lend increased confidence to existing qPCR or in vitro tests, and speed up identification of the guilty pathogen in a contamination event. BioReliance has used NGS to discover several "silent" infections in bovine serum.

Ensuring the safety of vaccine cell substrates by massively parallel sequencing of the transcriptome – David Onions et al. http://bit.ly/2aazahS

Clearly Virus Free

Also known as a viral validation study (or evaluation), a viral clearance study tests the capacity of the manufacturing process to remove or inactivate viruses and, for some products, the prions causing transmissible spongiform encephalopathy (TSE). The effectiveness of individual steps in the manufacturing process are assessed separately, and combined to give a quantitative estimate of the overall level of virus clearance. Viral safety experts BioReliance have produced a handy guide to walk you through the process.

A guide to planning your Viral or TSE Clearance Study – BioReliance http://bit.ly/2a9AqT6

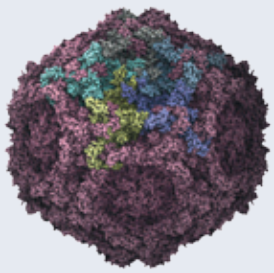
Joining Forces

Biopharmaceutical companies have generally shied away from publicizing their contamination events. This is understandable, but it means that potentially useful information on adventitious agents (and proven prevention methodology) is locked away in individual companies. To remedy this situation, MIT collaborates with 24 bio-manufacturers to maintain a central database for accumulated industry knowledge. Non-disclosure agreements create a safe space for companies to report data on contamination events – the resulting lessons learned are then disseminated, so that everyone can benefit.

Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) – MIT. http://bit.ly/2aHeQXm

Nanofilters for Nano-Viruses

It's not called "minute" for nothing – MVM is truly tiny at less than 20nm, and slips through many traditional filter membranes with ease. But improvements in nano-filtration mean that it's now getting harder and harder for MVM to evade capture. Viresolve(R) Pro Solution uses a patented dual-layer polyethersulfone (PES) membrane that offers a 4-plus log removal of parvoviruses, including MVM. Merck Millipore's Learning Center explains all you need to know to implement effective viral filtration.



Viresolve Pro Solution - MilliporeSigma http://bit.ly/2aawHDR
Virus Safety Learning Center – Merck Millipore http://bit.ly/2arwcLM

AJ Cann

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Scanning the Horizon

David Onions is an experienced virologist who helped kick-start the development of Centinel™. Here, he offers an overview of the viral contamination risk, talks about his involvement in the project and shares insight on what's next for the field.

How did you get involved in the biopharmaceutical industry?

I'm a virologist – and viral contamination is one of the major risk factors for product safety in the biotech industry. So when people first started using cells to make recombinant products, such as monoclonal antibodies, they would seek advice from my lab at Glasgow University on the viral risks associated with manufacturing biologics in living cells. As the demand for our services grew, we started a spin-out company: Q-One Biotech. It was fascinating to see the industry develop from the very beginning, and it meant that my colleagues and I had a hand in how the regulations were created and eventually introduced. I always knew that biotech was the future, but even I was surprised at just how quickly the industry grew.

How have the risks changed over time?

When I started out, viral contamination was a huge problem, but it has decreased for a number of reasons. The most important is elimination of serum and other animal reagents from manufacturing processes. Using massively parallel sequencing, we and others have shown that animal serum contains not only viruses that we commonly think of as potential contaminants, but many other really disturbing potential contaminants. So the move towards chemically defined media, led by vendors like MilliporeSigma, has been very important.

Nevertheless, contamination of fermenter systems still costs companies many thousands of dollars and, worse still, has the potential to interrupt the supply of medicines. BioReliance and other such companies test every batch of product, and the only contaminant that we see in Chinese hamster ovary (CHO) cell lines in animal origin-free systems is minute virus of mice (MVM).

How did the idea for an MVM-resistant CHO cell line come about?

The project was initiated following discussions I had with MilliporeSigma's Head of Upstream R&D – Kevin Kayser – at the European Society for Animal Cell Technology conference in Lille, France in 2013. It was a natural synergy; BioReliance brought an understanding of the problem from the virology point of view, while Kevin's group brought very sophisticated zinc finger nuclease (ZFN) cell engineering technology. When we put those two things together – chemistry and virology – we came up with a viable concept for Centinel.

Can we expect to see more genetic "upgrades" in future?

Centinel is just the beginning. The CHOZN® platform allows for "trait stacking" and there are several traits that could be open to engineering. The immediate next step is to humanize the glycosylation pattern and make the cell lines appropriate for highly sialylated proteins.

I think in the future there will be other changes that enhance both productivity and stability of these cell lines. For example, all mammalian cells contain genetic information to code for retroviruses, and the CHO line is no exception. The gamma retrovirus of Chinese hamsters is not a hazard but it is produced in large quantities and has to be removed during the purification process. Another application for ZFN engineering would be to remove those retroviral genes.

In general, you can strip down a lot of functions within cells without necessarily reducing their capacity to produce protein. By streamlining the cell's functions, we may be able to increase productivity.

How could Centinel change the industry?

I'll be bold and state that the introduction of Centinel is the most significant advance in viral safety since the removal of serum from manufacturing processes. I believe regulators will see this as an important addition to existing safety measures and be keen to see it adopted. I think we can expect to see momentum grow in the industry until it becomes an industry standard. In the long term, improvements in the safety of the cell lines will allow a change in the way downstream testing is done. I believe there will be a shift towards real-time testing on site, with simpler molecular-based assays. So it could really change the way the industry will function in 5–10 years' time.

"The introduction of Centinel is the most significant advance in viral safety since the removal of serum from manufacturing processes."

David Onions is an independent consultant to, and investor in, biotechnology & data analysis companies. Formerly, he was founder director of Q-One Biotech, and Chief Scientific Officer at BioReliance. Before joining the biotech industry he was Director of the Leukemia Research Fund's Virus Centre and Professor of Veterinary Virology at the University of Glasgow.

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