

Lentiviral Vectors: Not Just For Gene Therapy

Lentiviral vectors are well-known for their use in gene therapy – but they also have potential in a number of other applications, including the creation of virus-like particles, protein production and as a research tool.



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Dr Boro Dropulic founded Lentigen in December 2004, and serves as the company's Chief Executive Officer. He is one of the leading experts in the research and development of lentiviral vector technology. From 1998 through to 2004, he was Chief Scientific Officer of VIRxSYS Corporation, a company which he founded and at which he successfully led a multidisciplinary team to initiate and complete the first ever lentiviral vector Phase I clinical trial in humans. Dr Dropulic is a member of the Infectious Disease Committee of the American Society for Gene Therapy. Dr Dropulic obtained a PhD from the University of Western Australia (Perth, Australia) and an MBA from The Johns Hopkins University School of Business.

Lentiviral vectors (LVs) are viral-based gene delivery systems that can stably deliver genes or RNAi into primary cells or cell lines with up to 100% efficiency. LVs bind to target cells using an envelope protein which allows for release of the LV RNA containing the gene or gene silencing sequence into the cell. The LV's RNA is then converted into DNA using an enzyme called reverse transcriptase by a process called reverse transcription. The DNA pre-integration complex then enters the nucleus and integrates into the target cell's chromosomal DNA.

Lentiviral vectors are the only genetic vector system that affords both high and stable gene delivery. Other vectors, such as adeno-associated viral vectors can persist in non-dividing tissues, but are cured from cells once they divide. Lentiviral vectors integrate their payload sequence into the chromosome of transduced cells so that it is copied along with the chromosomal DNA when the cell divides. The properties of lentiviral vectors make them highly suitable for efficient and stable gene delivery into cell lines and primary cells. One of the discriminating features of LVs is their ability to integrate into non-dividing cells; this is in contrast to other vectors that either don't integrate

efficiently into chromosomal DNA (for example, non-viral, adenoviral and adenoviral-associated vectors), or can only integrate upon cell division (for example, conventional retroviral vectors). (The structure and mode of action of a lentiviral vector is shown in Figure 1.)

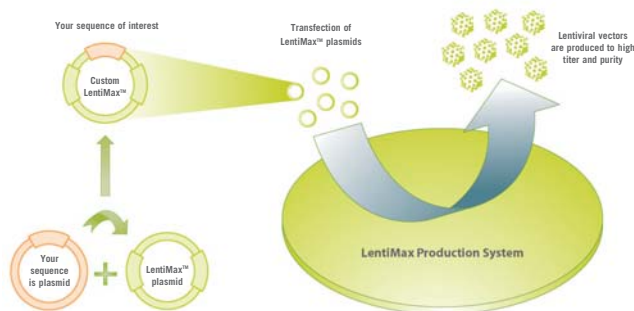
Most researchers still view lentiviral vectors as a gene therapy instrument – given their demonstrated safety and encouraging results in past and ongoing clinical trials – but in fact they have potential in many different applications. This article will provide additional detail on certain of these applications, including virus-like particle (VLP) creation, protein production and use as a research tool.

VIRUS-LIKE PARTICLES

Influenza viruses consist of influenza A, B and C, and Thogoto viruses; these are negative-strand RNA viruses from the family *Orthomyxoviridae*. The best studied and most well-defined are influenza A strains. The proteins of influenza A are encoded on eight RNA gene segments, and these viruses are widely distributed in nature. Influenza A virus subtypes are classified on the basis of the antigenicity of their surface glycoproteins – haemagglutinin (HA) and neuraminidase (NA); 16 HA and 9 NA subtypes are known to exist. All of them infect aquatic birds, but most infections are not associated with clinical disease. Only a few subtypes of influenza A virus have caused sustained outbreaks of disease in the human population. Currently, influenza epidemics are mostly caused by H3N2 and H1N1 influenza A and influenza B viruses.

Apart from seasonal influenza epidemics, influenza pandemics have been known to occur periodically. An influenza pandemic occurs when an influenza strain with a novel HA subtype (with or without a novel NA subtype) appears and spreads in the human population, which has little or no immunity to the novel HA. In this century, pandemics occurred in 1918, 1957 and 1968,

Figure 1: Lentiviral gene delivery





and were associated with extensive illness and death. The predictions are that, in the absence of effective interventions, in the US alone the next influenza pandemic could cause 89,000-207,000 excess deaths and 314,000-734,000 hospitalisations, as well as tens of millions of outpatient visits and additional illnesses.

Current influenza virus vaccines consist of three components: an H1N1 (haemagglutinin [HA] subtype 1; neuraminidase [NA] subtype 1), H3N2 influenza A virus and an influenza B virus. Specifically, the 2005/06 vaccine formulation was made up of the A/New Caledonia/20/99 (H1N1), A/California/7/2004 (H3N2) and B/Shanghai/361/2002 viruses. Changes in the HA of circulating viruses (antigenic drift) require periodic replacement of the vaccine strains during inter-pandemic periods. The World Health Organization publishes semi-annual recommendations for the strains to be included for the northern and southern hemispheres. To allow sufficient time for manufacture, in the US the FDA must determine by the early spring which vaccine strains should be included in the following winter's vaccine.

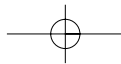
Most influenza virus vaccines used in the US and Europe consist of embryonated egg-grown and formaldehyde-inactivated preparations, which – after purification – are chemically disrupted with a non-ionic detergent. If 100 million doses of killed influenza virus vaccine are to be prepared, then the manufacturer has to procure 100 million embryonated eggs. Clearly, this manufacturing process is dependent on the timely availability of embryonated eggs and the vaccine seed strains to be used in a particular season. Unfortunately, only (high-yielding) influenza A viruses can be made in this way, and even with the A types, the 6:2 re-assortants- (HA and NA from recently circulating strains and the remaining six genes from A/PR/8/34 virus) are sometimes not easily obtained. This time-consuming process of re-assortment is then followed by repeated passage of the strain in embryonated eggs to allow for egg adaptation and growth enhancement. Although the manufacturing process is time-consuming, these killed influenza A and B virus vaccines are the workhorses for vaccination against influenza, and have been shown time and again to be highly effective.

The second major class of viral vaccines consists of live attenuated viruses; these cold-adapted influenza virus vaccines are easily administered by nasal spray. They induce local mucosal neutralising immunity and cell-mediated responses that may be longer-lasting and more cross-protective than those elicited by chemically inactivated (killed) vaccine preparations. Vaccine efficacy in vaccine-naïve children from six months to 18 years of age is high (range 73-96%). In children revaccinated for a second season, vaccine efficacy climbs to 82-100%.

In this case, lentiviral vectors can be used as a novel production system. After engineering the vectors to produce strain-specific genes, cell lines can be transduced to produce virus-like particles (VLPs) at high yields.

Advantages of using lentiviral technology for influenza vaccine production include the following:

- ◆ *Exact genetic match:* Issues with recombination and genetic drift of influenza virus strains means that the strain is constantly changing and one vaccine may not be protective for a second subtype strain. Multiple vaccines may then need to be produced. By cloning the influenza genes directly into the lentiviral vector, the exact strain of virus-like particle (VLP) vaccine can be made.
- ◆ *Maximal immunogenic potential:* Since the VLP is a particle, the influenza proteins are in the right 3D configuration, which maximizes their immunogenic potential.
- ◆ *Glycosylation:* Efficient mammalian cell production results in VLPs with proteins that are properly glycosylated, rather than being produced in non-mammalian cells which could result in glycosylation patterns that are not the most favourable for optimal immunogenicity.
- ◆ *No viral proteins:* Transduction with lentiviral vectors does not result in the expression of any lentiviral proteins as the vector is fully gutted. This is in contrast to other VLP technologies, such as the baculovirus vector system, where baculovirus proteins contaminate the VLP preparation.
- ◆ *Long harvest times:* Since transduction with 3-4 genes does not kill the cell (as is the case with live attenuated, live but later inactivated vaccines, or the baculovirus vector system), harvesting times are longer.
- ◆ *Lower cost:* Long harvest times allow for a cheaper vaccine since the same lot of cells can produce more VLPs than either live attenuated (LA vaccines kill the cells), or baculovirus VLPs (the transient nature of transduction makes for a limited harvest time).
- ◆ *Safety:* Lentiviral vectors are safer than live vaccines which are subsequently killed, since the VLP is genetically inactive. Mammalian cell culture allows for 'surge' capacity, which is not available with egg technology. Also, in the event of a flu pandemic, then the flock that produces the eggs are at risk.
- ◆ *Time factors:* Lentiviral technology allows for the generation of VLP-producing cell lines in a matter of weeks – not months as is the case with LA vaccines, since strains first need to be adapted to the culture system and a seed stock of virus



produced before it can efficiently infect the master cell bank to produce virus. For LentiVLP® technology, there is no adaptation time since the vector infects the producer cell very efficiently and expresses the influenza proteins with high efficiency, leading to excellent VLP yields.

Protein Production

Biopharmaceuticals have transformed the pharmaceutical industry – today, biotechnology drugs account for 10-15% of the pharmaceutical market, and it is estimated that 50% of new approved pharmaceutical products will be derived from biotechnological methods. Lentiviral vectors may prove useful in the development of new biological products, as well as the production of generic biologics.

Lentiviral vectors can create custom-engineered cell lines, capable of producing high yields of selected proteins. Master cell lines are simply mixed with the lentiviral vector, which are then transduced at high efficiency (approaching 100%). The transduction procedure can be adjusted to achieve multiple copies of the desired gene in the cells. The advantage of lentiviral transduction over other forms of cell-line generation is that the lentiviral vector integrates into sites of active chromatin, allowing for a high level of active transcription of the desired gene product. Lentiviral vectors are the only vector class capable of high and stable gene delivery – so the desired protein can be harvested from the cell line immediately and for long periods of time. The desired proteins are then isolated and purified by standard methods of protein purification.

One of the significant advantages of using a lentiviral system is that protein production can be undertaken in mammalian cell lines, particularly human cell lines. This is important for the overwhelming number of proteins that are glycosylated, since producing such proteins from non-human or non-mammalian cells will result in glycosylation patterns that are very different to those found in humans, and abnormal glycosylation will result in proteins that may not function correctly or be antigenic when injected in humans. Proteins that are antigenic will have a very short half-life in the body; they will not be able to be re-administered and therefore have low utility in biomedicine. In contrast, using lentiviral vectors is most efficient when delivering genes into human cell lines, and therefore proteins will be produced that have human glycosylation patterns resulting high functionality, low or no antigenicity and, therefore, high utility in biomedicine.

Research Applications

Genomics has generated remarkable opportunities for the pharmaceutical, biotechnology and health services industries. The study of functional genomics facilitates

the identification of genes within the genome along with the proteins they express – thereby helping to identify their various functions. However, translating the study of proteins into optimised drug targets poses substantial challenges. Hundreds of thousands of potential new protein targets have been identified, but the resources to validate them effectively are lacking.

A system to efficiently deliver these genes, or inhibitors of the expression of these genes, is needed to validate their function. Presently, there is no robust and reliable commercially available product to obtain stable long-term over-expression or 'knock-down' of genes without a labour-intensive process. Researchers have several options – but all of them suffer from serious limitations; as an example, plasmids have low delivery efficiency which is not suitable for sensitive cell analysis.

In this context, LVs offer significant benefits since they are capable of highly efficient and stable long-term gene expression in cells. In the drug discovery and validation sector of the market, the type of cells used for the screening of drug libraries has become critical for the identification of new lead candidate compounds and early determination of their potential toxicity before they are administered to humans. Lentiviral vector technology rapidly and efficiently generates cell-lines of any desired phenotype, including 'knocking out' specific gene function. These cells can then be used in drug discovery for the identification of new compounds that will have higher potency and lower toxicities than existing lead compounds. Also, lentiviral vectors can genetically modify cells directly from the body (primary cells), providing significant additional value since the drug screen is performed on cells that would be encountered by the drug in the body. Furthermore, the same cell lines can be used in target validation by creating cells that do not express the target gene of the lead drug compound; this is done to ensure that the lead compound does in fact specifically target the intended gene, and does not have any deleterious side effects. Therefore, LV technology can be used not only to accelerate drug discovery, but also to ensure that lead compounds are tested early for any signs of toxicity before significant resources are allocated to take the compound into clinical trials.

The power of lentiviral vector technology lies not only in its efficiency for stable gene delivery, but in its flexibility to deliver genes into a wide variety of systems. For example, once toxicity validation has been performed in cell lines, the same vector can be used to create transgenic animals for toxicity validation at the animal level. Already, HIV-based lentiviral vectors have been found to generate transgenic animals with efficiencies that greatly exceed other methods.

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