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Feature Article

DNA Vaccines Inch Toward Human Use

West Nile Virus Equine Product Gains Foothold for a Burgeoning Field

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VGXI is developing a more effective way to manufacture a high-concentration multicomponent DNA vaccine.

A DNA vaccine targeting West Nile virus in equines was launched in December by the Fort Dodge Animal Health division of [Wyeth](#). That vaccine was reportedly the first DNA vaccine for any species to be registered with a government regulatory body. It was also one of the many milestones and advancements in DNA vaccine development and manufacturing methods outlined by speakers at the recent International Society of DNA Vaccines conference in Las Vegas, organized by [BioConferences International](#).

Wyeth's development program proved that DNA vaccines can be as efficacious as traditional vaccines. "This was proof of concept for DNA vaccines as a class," explained Hsien-Jue Chu, DVM, Ph.D., executive vp, animal health research and development. This is the fourth vaccine for West Nile virus to reach the market since Wyeth's West Nile Innovator, launched in 2001, and two subsequent recombinant-based vaccines.

In the early part of this decade, experimental DNA vaccines seemed effective in rodents, where "it was easy to induce an immune response. It was difficult to make it work in large animals, however," Dr. Chu recounted. Success came when an immune-modulator was added to the plasmid DNA, which probably allowed the intramuscularly-injected DNA to target the cells more effectively. Now, the DNA-based West Nile vaccine confers at least one year immunity—the same duration of immunity that is demonstrated by traditional vaccines, he said.

The lessons learned from the West Nile DNA vaccine program are being applied to rabies and other vaccines, he said, and work is under way to see whether it is possible to lengthen the conferred immunity. Programs at other companies, Dr. Chu said, involve DNA vaccines to treat melanoma in dogs and to target a particular pathogen in fish that affects salmon farms.

Even before Wyeth launched its equine DNA vaccine targeting West Nile virus, companies were working to create the technologies needed to produce and scale-up aspects of DNA vaccine development.

Multicomponent Manufacturing

[VGXI](#) is developing a more effective way to manufacture a high concentration multicomponent DNA vaccine. The benefit, according to Robert J. Juba, senior director of manufacturing and technical services, is that combination DNA therapies elicit elevated immune responses to multiple viral components or disease indications.

VGXI's manufacturing approach is based upon fermentation methods that generate high plasmid yield, purity, and concentration. The company uses a 500 liter fermentation reactor that generates an average of 50 kilograms of paste and yields 50 grams of plasmid per cycle. A high-throughput recovery system relies upon continuous flow from lysis to capture, recovering 50 grams of plasmid in eight hours, Juba said.

The lysis step uses high-shear, low residence cell lysis in combination with the VGXI Airmix® column to

neutralize and separate the solution. "It's very gentle," Juba insisted. Multistage in-line filtration separates genomic DNA and cell debris for high plasmid purity. "No hold times are required," he stressed.

The company uses ion exchange and hydrophobic interaction membrane chromatography for plasmid enrichment and to remove undesired plasmid forms and residual endotoxin. Most importantly, it maximizes supercoiled forms of the plasmid and minimizes plasmid shearing. Plasmid concentration is achieved by recirculating the solution, after buffer exchange, through tangential flow filtration membranes, and sterile-filtered to achieve concentrations of up to 15 mg per mL.

This manufacturing process has been used successfully for a smallpox vaccine that contained eight different plasmid components that was developed by VGXI. The company is also developing multi-component DNA vaccines using this technology for HPV and influenza.

Directed Molecular Evolution

In an effort to increase the quantities of useful, functionally diverse molecules, researchers embraced rational design philosophies, building upon established knowledge. [Maxygen](#) has taken what Robert Whalen, Ph.D., director of infectious disease, calls "the diametrically opposite approach" and adopted the concept of directed molecular evolution, based upon genes' natural diversity creation using a method derived from nature's process of gene recombination.

"Evolution has been very successful, and typically produces improvements in organisms," Dr. Whalen noted. The "directed" part of the procedure prevents the process from becoming a vast scavenger hunt.

Once a product opportunity has been identified, Maxygen finds related genes and places them in a test tube where the genes are fragmented and those fragments are further pulled apart. Those fragments recombine, when the tube cools, at homologous sites. The fragments are then extended, and the genes are recombined to create a library of full-length genes. Those genes are expressed as proteins, resulting in one thousand to one million variants, which are then screened, based on scientists' understanding of what the ultimate product will require, according to Dr. Whalen.

The recombination process doesn't cause dramatic changes, he elaborates, and results in a high proportion of functional, useful, genes. "We've had some successes," Dr. Whalen said, outlining Maxygen's preclinical work developing vaccines for HIV, hepatitis B, and influenza, as well as one vaccine that induces antibody reactions against all four types of Dengue fever.

Purification

[BIA Separations](#) has developed a process for purifying supercoiled plasmid DNA at milligram and larger scales. The process is based on its high productivity CIM HiP2 Plasmid Process Pack™. "The process contains a selective precipitation and two chromatographic steps on monolith columns for anion exchange and hydrophobic interaction," according to Matjaz Peterka, Ph.D., manager, molecular biology laboratory.

"In our process, all the chromatographic steps are done on CIM monoliths," Dr. Peterka said. That takes advantage of the monolith's flow—independent dynamic binding capacity and separation, high flow rates, flow independent resolution, and low pressure drop, he explained. The process successfully separates supercoiled plasmid DNA from structurally related impurities, like RNA, host chromosomal DNA, and lipopolysaccharides.

"As a direct consequence of high capacity, buffer consumption is low. Because there is almost no limitation on flow rate, purifications can be done in a short time." These factors result in a more than 50% lower production price per milligram of plasmid DNA.

“We also developed a new HIC monolith called CIM C4 HLD—high ligand density—to separate plasmid DNA isoforms and genomic DNA,” Dr. Peterka said. “Because the monoliths’ structures are the same across all sizes—1, 8, 80, 800, and 8,000 mL columns—scale-up is easy.” And, they are shipped ready to use. Column packing and handling is unnecessary, he added.

Scaled-Up PCR

[Vandalia Research](#) has developed a PCR system that scales DNA sequence production from the bench to commercialization. Triathlon™, a continuous, large-scale PCR system, is the heart of this system. As Derek Gregg, vp of business development, explained, “DNA vaccines have a lot of potential advantages, especially in manufacturing, but they are mainly based on plasmid DNA, which is propagated in bacteria. Therefore, removing the bacteria remnants is important.” And, he added, “Often, only one-third of the plasmid is used because much of the sequence is required for replication in bacteria and isn’t used by the vaccine.”

Using PCR to amplify the DNA, however, could eliminate many of the challenges associated with plasmid purification. As yet, PCR hasn’t become popular for this use because of the many heating and cooling steps, the large quantities of plasmids needed, and the time required for processing.

Triathlon resolves those issues through a continuous process that currently produces about 50 mg of DNA per day. The PCR reagents are transported through tubing that wraps around a cylinder, which rapidly heats and cools the solution, saving 40 to 90 seconds per cycle. The flow is continuous, eliminating the need to pool products from plates. The process results in reduced labor, decreased turnaround time, and reduced risk of contamination. The Triathlon is scalable, and the next-generation machine is expected to be able to produce up to 1 g or more of DNA per machine, per day, Gregg noted.

PCR also produces linear DNA, so researchers can work with only the fragments of interest. “Some studies have shown that it’s easier to get smaller DNA fragments into the cell, which allows researchers to get better expression,” Gregg said. “The resulting vaccine also may be more transient,” he continued, allowing the DNA fragment to do its job and leave.

Speed is a major benefit of this approach to plasmid production. Scaling up for manufacturing can be accomplished in a few days, versus the weeks or months needed for fermentation processes, Gregg said. “Therefore,” he pointed out, “this method could be particularly valuable in a pandemic. Vandalia offers large-scale PCR as a service.”

Vectors

[VIRxSYS](#) is working to boost DNA delivery efficiency using lentiviral vectors, which, using its system, can deliver therapeutic payloads into human cells with more than 90% efficiency, Franck Lemiale, Ph.D., director of vaccines and immunology, said. Other vectors used for gene therapy range from 20 to 50% efficiency, he noted.

According to Dr. Lemiale, VIRxSYS is seeing good results using the HIV virus as a vector. Obviously, “the vector is non-pathogenic.”

One of the benefits of this approach is that “people have immunity against adenoviruses, but not against HIV,” explained Gary McGarrity, Ph.D., executive vp of research and clinical affairs. Consequently, an HIV vector can deliver its payload without the immune response that thwarts adenovirus and other vectors.

Payloads have been delivered successfully to several targeted human cell types. Results so far have shown highly efficient gene transfer and genetic stability of the payload in dividing and nondividing cells. And,

from a production standpoint, the HIV viral vector can be manufactured readily.

One trial involving VRX496 as an AIDS treatment is in Phase II trials now. It uses antisense as a payload in a similar lentiviral backbone. Patient safety monitoring to date shows no safety concerns, Dr. McGarrity said. VRX496 inhibited HIV replication by more than 99% in laboratory studies, and data from clinical trials is encouraging. Early preclinical studies for an HIV vaccine have been completed, and Dr. Lemiale says VIRxSYS expects to enter clinical trials for that vaccine by 2010. Results from nonhuman primate studies are showing that the approach provides immunity against HIV. He added that the question remains as to whether the degree of immunity is sufficient to provide protection.

Regulatory Considerations



BIA Separations' CIM HiP2 Plasmid Process Pack contains a selective precipitation and two chromatographic steps on monolith columns for anion exchange and hydrophobic interaction.

Those results in vaccine development clearly transfer to human development, as do the lessons learned from presenting other new therapeutics to regulatory agencies. "FDA is eager to bring new approaches to unmet medical needs; approaches that are safe and effective," noted E.J. Brandreth, vp of quality and regulatory affairs at [Althea Technologies](#). The issue, he said, is that, for new approaches, "The rules aren't yet written."

Knowledge regarding impurity profiles, safety, and other parameters must be based upon data from related products and good scientific judgment, and augmented over time with data from long-term studies and analyses. The company that introduces the therapy or diagnostic sets the bar.

"FDA is extremely easy to work with in situations like this," Brandreth said. "The systems have come a long way." This often occurs under FDA's Orphan Drug program and under a rolling biologic license application, in which clinical data is separated from manufacturing data, letting companies provide partial submissions as the data becomes available. That approach could speed the process by six months, he said.

New vaccines are benefiting from manufacturers' decisions not to use mammalian cell lines for production. That approach eliminates the risk of adventitious viral contamination, and thus makes production faster and easier than the traditional processes that use animal-derived components.

In terms of DNA vaccines, Brandreth suggested that the differences between linear and super-coiled fragments need to be understood as they relate to the particular vaccine. If the different forms affect potency, "setting up meaningful specifications regarding the percent of supercoiled DNA that is needed is important," he said.

The bottom line, Brandreth insists, is to break down each step and understand its clinical and manufacturing ramifications during process characterization and validation studies. Be aware that changes made in Phase III "can't simply meet the specifications from earlier clinical previous processes. We'll be expected to meet tighter specifications. Therefore, avoid changes in Phase III. Also," Brandreth said, "it's extremely beneficial to manufacture the Phase III material in the actual facility to be used for the commercial launch. Plan ahead."

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