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# You Can Teach an Old Dog New Tricks: VERO Cells for Virus-Based Cancer Vaccine Therapies

## The Old Dog: Vero Cells

Vero cells, a continuous monkey kidney cell line, have proven to be a relative constant in the vaccine industry for over 3 decades. These cells are an established cell line with a long history of safe vaccine production for a multitude of human vaccines such as those for polio, rotavirus, and smallpox. Growing these cells is relatively easy in that they have the capability to grow very efficiently in flasks, cell factories, and microcarriers in bio-reactors. Vero cells are a favored cell factory for the production of herpes, measles, and vaccinia-based viral vectors. This, along with the dependability of Vero cells, has made them a desirable cell line for use in new virus-based cancer vaccine therapies.

## The New Trick: Cancer Therapy

Virus-based cancer therapies are emerging as a potent treatment of cancer, with numerous therapies currently in clinical evaluation. The approach is straightforward. Using high doses of genetically-modified virus as a delivery vehicle, patients can be immunized against tumor antigens associated with a particular tumor type or other markers of malignant cellular transformation. Alternatively, a patient's immune system can be activated via expression of immunostimulatory proteins that are localized via the direct injection of virus into the tumor mass. These types of virus-based approaches have demonstrated impressive responses in several different tumor types including brain, prostate, lung, colon, and other cancers.

The viral vector is responsible for delivering the vaccine, and is therefore a critical component in a functional cancer vaccine. Each viral vector has unique characteristics that can be beneficial for the delivery of transgene(s). For example, pox viruses, vaccinia, modified vaccinia Ankara (MVA), and fowlpox are large viruses that can encode multiple transgenes for their expression inside target cancer cells. Using this multi-gene delivery strategy, the immunostimulatory proteins B7-1, ICAM-1, and LFA-3 generate the poxvirus-delivered TRIad of COstimulatory Molecules (TRICOM). When expressed by the infected cell, these proteins greatly enhance the immune response to a target antigen. The ProstVac (Bavarian Nordic) vaccine, which utilizes the TRICOM-based immune stimulation strategy, also expresses carcinoembryonic antigen (CEA). Other pox virus approaches by Bavarian Nordic include vectors that express TRICOM in combination with CEA and MUC-1. Transgene's TG4010 targets the MUC-1 antigen and expresses the T cell-stimulatory cytokine IL-2.

Herpes based vectors (Herpes simplex, cytomegalovirus) are large and can also package/carry multiple proteins for delivery. Similar to pox vectors, these viruses are oncolytic after several days of infection which allow more time for recognition of immunostimulatory factors or cancer antigens prior to cellular lysis. Among the leading herpes based vaccines is Amgen's T-Vec/ OncoVex HSV/ talimogene laherparepvec. This GM-CSF-containing virus is the first vaccine to demonstrate therapeutic benefit against melanoma in a 400 patient phase III trial. The overall response rate was 26% among patients in the T-VEC group compared to 6% among patients in the GM-CSF treatment group. Phase II studies showed that a regimen of 4 doses of 10e6, 10e8, 10e8, and 10e8 PFU (Plaque Forming infection Units) resulted in a 76.5% relapse-free rate during the follow up period. Other herpes based vaccines in development include vaccines produced by Tomegavax, Virttu Biologics, and others.

Measles virus vaccines also show promise as an effective approach for cancer vaccine therapy. MV-NIS vaccine (Mayo Foundation) is directly oncolytic and is engineered to express the human sodium iodide symporter (NIS). The expression of NIS allows for the noninvasive monitoring of viral infection and spread and potentially enhances anti-tumor therapy by the selective uptake of radiolabeled iodine. Local administration of MV-NIS into MPNST-derived tumors resulted in significant regression of tumor and improved survival in an animal-xenograph model.

Additional viral vectors include adenovirus and orthomyxovirus. Adenovirus is typically a quick, directly lytic virus. Aduro's GVax and GC0070 shows selectivity for replication only in dividing cells. Other adenovirus-based therapies also show selectivity for tumor cells. Shanghai Sunway Biotech's ONCORINE is engineered to selectively replicate in p53 deficient tumor cells. Other selective adenovirus vector approaches include pSIOxus's EnAd. Other viral vector approaches in development include orthomyxovirus (Wellstat Biologics) and parvovirus (Oryx). Adenovirus is typically produced in HEK-293, PERC.6, or other cell lines.

## Keeping the Dog Happy: Vero-based Cell Culture Media

The approaches used to elicit a potentially curative immune response against tumor-specific antigens vary greatly. As expected, so do the methods used to produce these novel cancer vaccines. The majority of these vaccines are produced in mammalian cell systems, but the cell lines can vary from Vero cells to HEK-293 cells and beyond. Some viruses, such as MVA and fowlpox vectors, are replication defective in mammalian cells. While this feature provides a margin of safety in the prevention of unwanted viral spread, these vectors must be produced using non-mammalian cells such as primary avian cells or established avian cell lines.

Not surprisingly, the old dog Vero has found itself producing many of these life changing therapies given its dependability, safety profile, and wide acceptance in industry. This makes the medium used to propagate these cells critically important. Traditionally, these cells have been expanded in serum-containing medium. Even with current advancements in serum-free media formulations, some vaccines are still produced in the presence of fetal bovine serum because of this legacy. Modern medium formulations avoid serum and animal components due to the increased risk of animal-origin pathogen contamination of the final product. Further, due to the fluctuating serum price and supply, obtaining a reliable source of reasonably-priced serum can be difficult, or impossible. And, serum varies from lot-to-lot which leads to variable manufacturing outcomes.

For these reasons, the vaccine industry is moving toward defined animal-free media (excludes components from either animal-origin or human-origin) to propagate Vero cells and produce vaccines. Several animal-free Vero media are currently marketed, including Life Technology's VP-SFM. Unfortunately, most of these media are outdated in that they are designed for flask-based production and do not contain the depth of nutrients needed for high density bioreactor-based viral production methods.

InVitria has a portfolio of key components that are critical in producing an animal component-free Vero medium that is microcarrier capable. These are used by vaccine developers who formulate their own cell culture medium. One component, Cellastim™, is a lipid-rich recombinant human serum albumin used to enhance the production of numerous viruses via increased virus stabilization and enhanced Vero cell growth and production. Optiferrin is another key component. It is an animal-free recombinant transferrin used in many cell therapies and vaccine production mediums to provide iron to cells via the transferrin receptor. It is also used in combination with recombinant insulin found in the Animal-Free ITSE supplement. These reagents can be combined to produce an animal free protein supplement that can be added to a basal medium such as DMEM/F12 and animal-free cytokines to efficiently propagate Vero cells for virus production.

Of course, many researchers will continue to utilize serum in the experimentation process. The inclusion of a product like Zap-SR (Serum-Reducer), reduces the amount of serum needed, while maintaining high performance in Vero cell culture. This product is cost-effective and relieves pressures on serum supply.

## Concluding Remarks

The favorable characteristics of the old reliable Vero platform have been utilized in the generation of novel vaccines that can elicit impressive and sustainable anti-tumor responses in patients. Given the clinical successes observed thus far, it will be critical to update cell culture processes and techniques of the Vero platform in order to meet demand for these novel virus vectors and at the same time enhance patient safety. Therefore, current serum-based Vero cell culture processes should be improved to high cell density animal-free microcarrier-capable media. Cellastim™ and Optiferrin are well suited to this task. In summary, virus-based cancer vaccines have tremendous promise as an emerging life-saving therapy for countless cancer patients, and Vero cells could prove to be the platform of choice.

# Product Ordering Information

InVitria Cellastim Recombinant Human Serum Albumin - 10G (10847-718), 100G (10847-716), 1KG (10847-792)

InVitria ITSE Animal-Free - 10ML (10847-714), 100ML (10847-794), 10x10ML (75784-766)

InVitria Optiferrin Recombinant Human Transferrin - 1G (10847-770), 10G (10847-778)

InVitria Zap-SR Serum Reducer - 50ML (10847-772), 5x50ML (75784-776)



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