Farshad Guirakhoo, PhD
fguirakhoo@geovax.com
678-384-7229

Development of MVA-VLP Vectors for Cancer Immunotherapy

Executive Summary

Cancer is the second most common cause of death in the US, exceeded only by heart disease. Its global burden is expected to rise to 22 million new cases by 2030.

Currently, there is only one FDA approved cancer vaccine, PROVENGE® (sipuleucel-T). PROVENGE® is a personalized therapy for prostate cancer patients which prolongs survival times by about 4 months. However, the field of immune-oncology has received new momentum with the discovery and initial launch of monoclonal antibodies (Mabs) called immune checkpoint inhibitors (ICIs). Tumors hijack the body's natural immune checkpoints by overexpressing immune checkpoint ligands (proteins that bind to and activate the inhibitory activity of immune checkpoints), as a mechanism of immune resistance, especially against T cells that are specific for tumor antigens and can kill cancer cells. ICIs block the interaction of Immune checkpoints with their ligands on tumor cells, allowing poorly functional T cells to resume proliferation, cytokine production and killing of tumor cells.

The first known immune checkpoints were Cytotoxic T-Lymphocyte-Associated antigen 4 (CTLA4), programmed cell death protein 1 (PD1) and the PD1 ligand (PDL1). The approval of the first anti CTLA4 Mab, YERVOY® (ipilimumab), in 2011 for treatment of melanoma, was followed by anti PD1 Mabs, OPDIVO® (nivolumab) and KEYTRUDA® (pembrolizumab), in 2014 for melanoma and then in 2015 for non-small cell lung cancer (NSCLC). ICIs are currently being combined with many types of oncology products such as chemotherapies, small molecules, therapeutic vaccines, cell based therapies, or even with other ICIs to show improvements in safety and efficacy over monotherapies.

Unlike conventional therapies (e.g. radiation, chemotherapy, antibody, etc.), cancer vaccines have the potential to induce responses that not only result in the control and even clearance of tumors but also establish immunological memory that can suppress and prevent tumor recurrence. Convenience, safety, and low toxicity of cancer vaccines make them invaluable tools to be included in future immunotherapy approaches for treating tumors. Currently, there are only a few vectored cancer vaccines being tested in combination with ICIs, all of which are in early clinical stages.

GeoVax has established a collaboration with Dr. Olivera Finn, a leading expert in cancer immunotherapy at the University of Pittsburgh. She was the first to show that many tumors express an abnormal form of cell surface-associated Mucin 1 (MUC1) protein that could be recognized by the immune system as foreign. Given this, we are using our Modified Vaccinia Ankara virus-Virus Like Particle (MVA-VLP) vaccine platform to deliver abnormal forms of MUC1 (e.g. hypo-glycosylated forms found in tumors) with the goal of raising protective anti-tumor antibodies and T cell responses in cancer patients.

We are also collaborating with ViaMune, Inc. who has developed a fully synthetic MUC1 vaccine candidate (MTI). The collaboration will assess each companies' vaccine platform, separately, and in com-

bination, with the goal of developing a tumor MUC1 vaccine that can produce a broad spectrum of anti-tumor antibody and T cell responses. The resulting MUC1 vaccine will be combined with CPIs as a novel vaccination strategy for cancer patients with advanced MUC1+ tumors.

In the last 2 decades, MUC1 has been used as a tumor associated antigen (TAA) in more than 2000 patients in clinical trials. Despite the fact that most patients were in late stages of cancers, some encouraging results were observed, but only in less immunosuppressed patients [1]. The mechanisms of immunosuppression in these trials involved the presence of ICIs, low frequencies of tumor-specific cytotoxic T cells and lack of T helper responses leading to impaired antibody responses (IgM, but not IgG).

GeoVax's approach to cancer immunotherapy will utilize a combination approach, for which each component has already shown some promising results in preclinical models. Selected vaccine regimen will be used to elicit antibody and T cell responses to MUC1 in the patients' own bodies. Prior to vaccination, patients will undergo their Standard of Care (SOC) treatments, such as chemotherapy or radiation (Fig. 1). ICIs will be used to activate suppressed T cells and enable the patients' immune system to respond to VLP-delivered MUC1 antigens, with the goal of causing tumor regression.

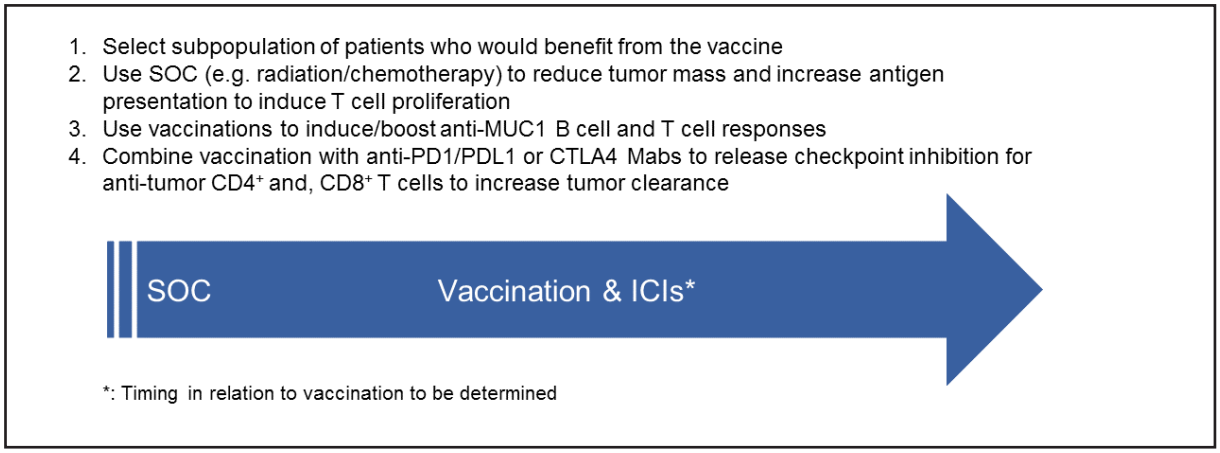

Fig 1. GeoVax Therapeutic Cancer Vaccine Strategy

We have already produced MVA-VLP-MUC1 vaccine candidate, demonstrated VLP production by electron microscopy using MUC1 immunogold staining and showed that the VLPs express hypo-glycosylated from of MUC1 in human 293T cell lines.

Proof of Concept is being demonstrated in preclinical, in collaboration with ViaMune and University of North Carolina, at Charlotte, using engineered murine human MUC1 models. It is anticipated that within 2 years we will be able to file an IND with the FDA and initiate a Phase 1 trial in a limited number of cancer patients.

Problem and GeoVax Solution

The cancer vaccine market represents a compounded annual growth rate of 20% from 1.7 billion in 2010 to 5 billion in 2015; and, is expected to reach $7.1 billion by 2018.

The therapeutics portion represents the most rapidly expanding area, growing from $48 million in 2010 to more than $4.8 billion by the end of 2018, according to GlobalData Healthcare. Currently, there is only one FDA approved cancer vaccine, PROVENGE®. PROVENGE® is an autologous cellular

immunotherapy indicated for the treatment of castration-resistant prostate cancer. Recently cancer immunotherapies have received a boost with the approval of ICIs such as YERVOY® (an anti CTLA4 Mab) as well as OPDIVO® and KEYTRUDA® (anti PD1 Mabs) originally for melanoma and more recently for NSCLC. Both the cancer vaccine PROVENGE® and ICIs prolong patients' survival time by only 4-6 months and are expensive (>$100,000 per patient per year), limiting their broad applications. Moreover, PROVENGE® requires complex manufacturing involving multiple leukapheresis of blood, sending blood to manufacturing facilities, stimulation of antigen presenting cells in vitro, and infusion of stimulated cells back into the donor patient. ICIs also cause various adverse reactions at the same or higher rate than seen in chemotherapy patients [2].

Although other cancer vaccines have been tested in advanced clinical trials, none have had a significant impact on tumor regression or overall survival. TG4010 (MVA-MUC1+IL2) and Prostvac (vaccinia-PSA+TRICOM), both poxvirus based prostate cancer vaccines being tested in Phase 2/3 trials, may not be highly effective without the addition of ICIs. Numerous Phase 1 clinical trials are currently in progress, evaluating combinations of ICIs with other cancer drugs, chemotherapies, small molecules, and vaccines. Vaccine and ICI combinations constitute only 12% of such trials (Fig 2), and only a few utilize a viral vector or DNA vaccine approach (e.g. ADXS-PSA, ADXS-HPV based on Listeria, and poxvirus vectored vaccines such as MVA-p53 and ALVAC-NY-ESO) (Fig. 3) [3].

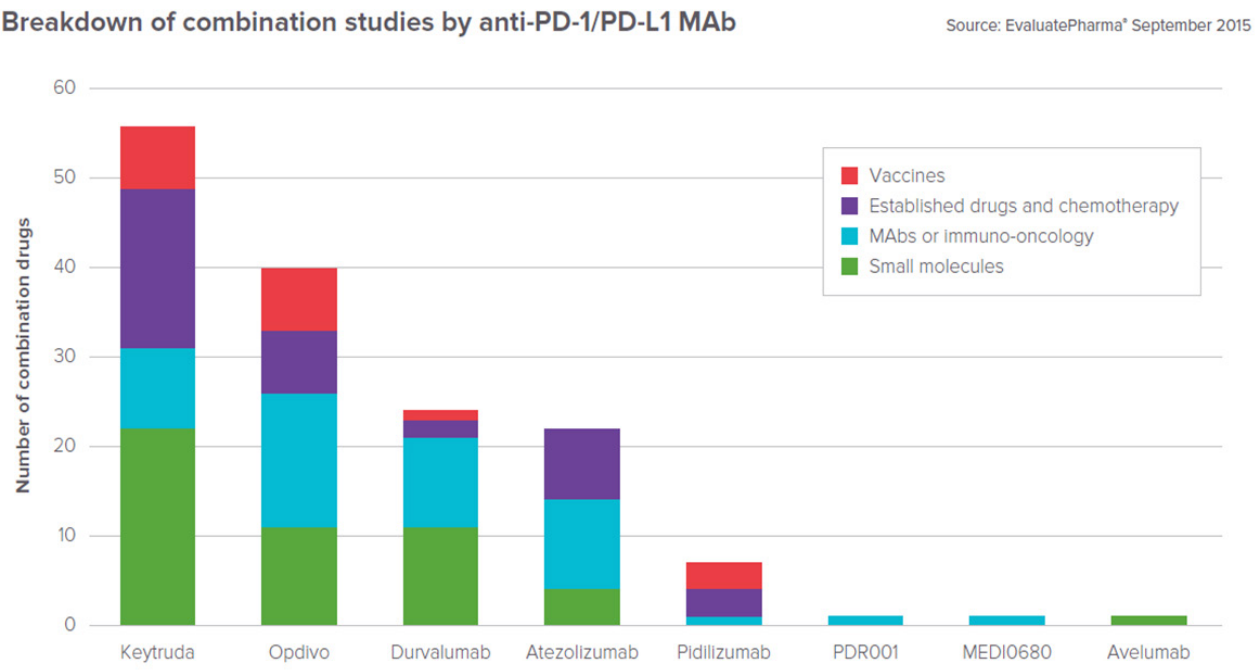

Fig 2. Combination of ICIs with other cancer therapy approaches

Anti PD-1/PD-L1 Mab combinations with vaccines

Source: EvaluatePharma® September 2015

	Combination vaccine	Pharmacology class	Indication	Sponsor
Keytruda – Merck & Co (PD-1 MAb)				
	ADXS-PSA	Anti-PSA vaccine	Metastatic, castrate-resistant prostate cancer	Advaxis
	G100	Vaccine	Non-Hodgkin's lymphoma	Immune Design
	LV305	NY-ESO-1 cancer vaccine	Melanoma	Immune Design
	OncoTICE/Tice BCG	BCG vaccine	Bladder cancer	Merck
	MVI-816	pTVG-HP plasmid DNA vaccine	Metastatic, castrate-resistant prostate cancer	NCI (NIH)
	MVA vaccine expressing p53	Modified vaccinia virus	Solid tumours	NCI (NIH)
	6MHP	6 melanoma helper peptide vaccine	Melanoma	University of Virginia
Opdivio – Bristol-Myers Squibb (PD-1 MAb)				
	ALVAC(2)-NY-ESO-1 (M)	Cancer vaccine	Melanoma	Bristol-Myers Squibb
	DC Vaccine	Dendritic cell vaccine	Glioma	Bristol-Myers Squibb
	Viagenpumatucel-L	Cancer vaccine	NSCLC	Heat Biologics
	ISA101	HPV vaccine	Solid tumours	ISA Pharmaceuticals
	GM.CD40L	Cancer vaccine	Lung cancer	Lee Moffitt Cancer Center
	GVAX Pancreas and CRS-207	Mesothelin cancer vaccines	Pancreatic cancer	Sidney Kimmel Cancer Center
Pidilizumab – Medivation (PD-1 MAb)				
	Dendritic Cell/Myeloma Vaccines	Vaccine	Multiple myeloma	Beth Israel Deaconess Medical Center
	Provenge	T-cell vaccine	Prostate cancer	Georgia Regents University
	DC/RCC fusion vaccine	DC/RCC fusion vaccine	Renal cell carcinoma	NCI (NIH)
Durvalumab – AstraZeneca (PD-L 1 MAb)				
	ADXS-HPV	HPV vaccine	HPV-associated cervical and head & neck cancers	Advaxis

Fig 3. Combination of ICIs with cancer vaccines in early clinical trials

In the last 15 years, GeoVax has developed an MVA-based VLP platform and successfully tested it in preclinical models for HIV and Ebola vaccines as well as in Phase 1 and Phase 2 clinical trials for an HIV vaccine. Competitive advantages of MVA-VLP vs. other poxviruses for delivery of MUC1 include; VLP formation, expression of hypo-glycosylated forms of MUC1 in vaccinated individuals, immunogenicity, safety and transgene stability (MUC1 gene is inserted between MVA essential genes so that empty vectors cannot outcompete the vaccine vectors during manufacturing) (Table 1).

Table 1. Competitive advantage of MVA-VLP vs. other poxviruses for delivery of MUC1

Vactor	VLP Formation	Priming capacity	Transgene Stability	Hypogly-cosylated MUC1	Immuno-genicity	Non-replicating	No Pre-existing Immunity	Thermal Stability	Self Adjuvanted
MVA-VLP	++	++	++	++	++	++	++	++	++
MVA	-	-	?	?	+	++	++	++	++
Vaccinia	-	++	-	?	++	-	+	++	++

++ High ++ Medium - Low

GeoVax's novel therapeutic cancer vaccine strategy is based on the MVA-VLP platform to deliver the TAA MUC1 in a highly immunogenic format (e.g. VLP), in combination with SOC and ICIs. The MVA-VLP-MUC1 recombinant virus was created to express (1) a sequence consisting of the MUC1 extracellular ectodomain with multiple variable number of tandem repeats (VNTRs), the transmembrane domain of a matrix protein, and the intracellular domain of MUC1. The ectodomain of MUC1 is thereby expressed in cells and on the surface of VLPs and serves as target antigen for the vaccine (Figure 4).

In healthy individuals, MUC1 transmembrane protein is heavily glycosylated, lines the epithelial surfaces, protects the body from pathogens, and is involved in cell signaling. Over-expression and hypo-glycosylation of MUC1 is associated with multiple myeloma as well as all human adenocarcinomas including breast, colon, ovarian, prostate, pancreatic, and lung. Because MUC1 is abnormally glycosylated in tumor cells, it is subject to immune surveillance resulting in spontaneous induction of anti-tumor antibodies and T cells. The presence of antibodies to altered MUC1 at diagnosis is associated with clinical benefits [4]. Since its discovery as a TAA, MUC1 has been used as a promising antigen for passive (e.g. antibody) and active immunizations (e.g. vaccines) in a number of clinical trials, with some success [1]. Success has been limited by the immunosuppressive microenvironment of advanced cancer that affects cytotoxic and helper T cell responses, upregulation of checkpoint inhibitors (e.g. high expression of PDL1 by tumors and PD1 on responding T cells) and consequently, production of low levels of anti-MUC1 IgG antibodies. Immunizations against MUC1 induces some CD8+ and CD4+ T cell responses in humans and causes tumor regression in preclinical models [5]. DNA vaccination with MUC1 has also shown efficacy in preclinical models [6-8]. Moreover, MVA delivered MUC1 +IL2 (TG4010) was tested in NSCLC, prostate, renal cell carcinoma, and lung cancer, which yielded the best results (6 months improvement vs. chemotherapy but only in Phase 2 studies) [9]. Currently, MUC1 antigen is being tested in > 60 trials. However, most of these are early phase trials, with only a few in Phase 2b and none in combination with a DNA vaccine, vectored VLP, or ICIs.

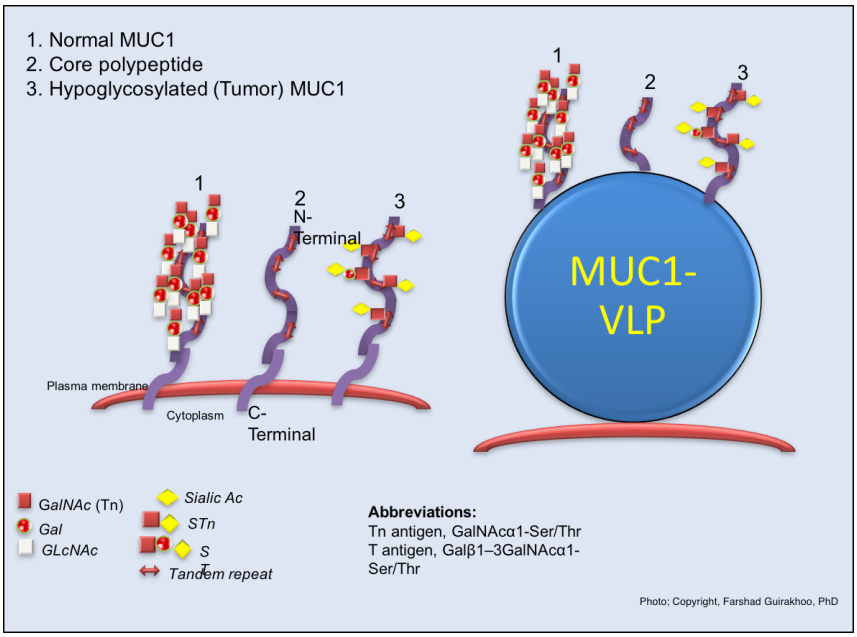

Fig 4. MVA-VLP-MUC1 vaccine

Because both MVA-MUC1 and newly approved ICIs have shown only modest efficacy as monotherapies for cancer treatment, GeoVax will combine its MUC1 vaccine with SOC and ICIs to maximize success (Fig 1). To our knowledge, there is currently no company in clinical testing utilizing a MVA-VLP vector approach in combination with SOC (e.g. Chemotherapy and radiation) and ICIs. This combination approach could enhance efficacy, potentially offering a cure for some indications such as prostate cancer, which currently kills one man every 18 minutes in the US.

Summary

GeoVax has used a novel approach to deliver "tumor like MUC1" antigens by over- expression of MUC1 on the surface of VLPs. Vaccine construct was made and evaluated *in vitro* for production of hypo-glycosylated forms of MUC1 by Western Blot and immunostaining using human 293T cells and antibodies recognizing tumor MUC1 proteins. VLP formation was demonstrated by immunogold electron microscopy. The vaccine candidates (MVA-VLP and MTI) are currently being evaluated for immunogenicity (induction of MUC1-specific IgG and T cell responses) and therapeutic efficacy (tumor model), separately, and in combination with ViaMune synthetic MUC1 vaccine candidate (MTI), using human MUC1.tg mice.

Upon completion of proof of concept studies in mice, GeoVax will explore the addition of other TAAs to its vaccine candidate to determine whether such antigens could further enhance efficacy for specific cancer indications. Filing an IND with the FDA and initiation of a Phase 1 trial is anticipated by 2019.

About GeoVax

GeoVax is a clinical-stage biotechnology company founded in 2001, located near Atlanta, Georgia. GeoVax' products are built on the MVA-VLP and DNA-VLP platforms and are currently being tested alone and in combination with recombinant protein vaccines. GeoVax has HIV vaccines in late clinical development, hemorrhagic fever vaccines in preclinical animal studies, and vaccines against Zika and other indications in early research studies. GeoVax currently has nine employees and expects to grow to a size of approximately 20 employees as its HIV vaccine enters a pivotal efficacy trial and new research programs progress into preclinical development.

About ViaMune

ViaMune, Inc., based in Athens, Georgia, is a pre-clinical stage company with a mission to enable immunotherapy for patients with advanced cancers. ViaMune offers a fully synthetic vaccine platform that generates robust immune responses to tumor-specific targets. ViaMune's patented technology provides a unique path to targeting structures found only on tumor cells. ViaMune is anchored by a leadership team with deep experience in oncology and pharmaceutical development.

Contact information:

Farshad Guirakhoo, PhD
Chief Scientific Officer
fguirakhoo@geovax.com
678-384-7229

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