Pat Steele DVM
Matt McGee DVM
Paul Fox DVM
Sarah Dunleavy DVM

LifeVax™
An Autologous Canine Cancer Vaccine

OVERVIEW

The canine dendritic cell vaccine is an autologous living vaccine that utilizes a dog's own blood cells and tumor antigens. When a dog is diagnosed with a cancerous neoplasia, the tumor will be surgically removed and is processed on site. Once the tumor sample is received, it will be processed into a lysate through a series of proprietary manufacturing steps. The tumor lysate is then stored frozen at -80ºC. The dog will be allowed to recover from surgery and, in the interim, may be administered standard of care chemotherapy and radiation treatment by the referring veterinarian as appropriate if directed.

After a recovery period of two weeks to two months (depending on the prescribed chemotherapy/radiation treatment regimen if applicable), approximately 50ml of peripheral blood will be collected in heparinized tubes by veni-puncture from the dog. A red top tube for serum (to be a component of cryopreservation media) will also be collected along with the 50mL peripheral blood unit. The blood sample will be processed on site to isolate peripheral blood mononuclear cells (PBMC). The PBMC will be placed into tissue culture with selected canine cytokines to induce the differentiation of monocytes into dendritic cells (DC). After one week, the DC will be harvested from the cultures and mixed with thawed tumor lysate for one additional day to load the cells for antigen presentation. The tumor antigen-loaded DC will be cryopreserved in aliquots appropriate for injection.

The frozen vials of antigen-loaded DC will be available to the referring veterinary clinic where the veterinarian will inject the cells intra-dermally into the patient. Approximately one-half million thawed cells will be administered per treatment and at least 3 treatments will be given every two weeks (initial injections). If sufficient vaccine is available, further booster injections will be administered at intervals to be determined. Once injected, the antigen-loaded DCs are expected to migrate to local lymph nodes and activate a variety of tumor-specific T cells, including cytotoxic T cells. These cytotoxic T cells will destroy the residual tumor masses that typically kill the patient.

INTRODUCTION TO DENDRITIC CELL BASED IMMUNOTHERAPY FOR CANCER

Immunotherapy for cancer utilizes the power of the immune system to eliminate existing cancer cells and prevent the outgrowth of new cancer cells. The immunotherapy concept has been tested in many clinical trials and has demonstrated its potential for altering the disease course, extending measurable progression of disease, and increasing survival of cancer patients.

One avenue for activating the immune system is through ex vivo preparation of the cells that are responsible for initiation of immune responses, the Dendritic Cells (DC). Dendritic Cells can be readily manufactured from circulating precursor cells, known as monocytes, for the generation of a patient-specific cellular product. To initiate immune responses against the cancer cells, the DC are loaded with cancer cell antigens prior to injection of the live cell product back into the patient.

LifeVax™, consists of autologous DC loaded with cancer cell antigens derived from the surgically resected autologous tumor. A single manufacturing batch yields sufficient product for several injection doses, which are kept cryopreserved in small single dose aliquots.

THE IMMUNE SYSTEM

The immune system comprises both initiation and effector mechanisms, which have the power to deal effectively with foreign substances such as infectious organisms and cancer cells. The innate immune system, which is largely non-specific, comprises various cell types such as macrophages, neutrophils and DC as well as humoral components in the form of circulating antibodies with broad specificity. The innate immune system can clear low level or localized infections, and works in concert with the adaptive immune system to eliminate spreading infections and even cancerous tissues. This adaptive system is capable of mounting a powerful, targeted and highly specific response, and is comprised of cellular and humoral components.

Dendritic Cells are also included in the adaptive immune system as they underlie the induction of specific immune responses. Other components include T cells and B cells, as well as specific antibodies and factors of the complement system. Immunotherapy aims at activating the adaptive immune system to attack and destroy cancer cells.

ROLES OF DENDRITIC CELLS

Dendritic Cells reside in the skin and lining of mucosal tissues, and circulate in the blood and in the lymph. They ingest and destroy foreign substances such as bacteria and viruses, in response to what are sometimes described as 'danger signals'. The bacterial cell wall is interpreted by the DC as a danger signal. Other danger signals include: secretions by cells infected with viruses; specific factors associated with the malignant transformation of cells leading to tumor growth; and cytokines produced by macrophages in an inflammatory response. Thus, the DC continually guard against infection and cancer in a process called immune surveillance. In the absence of immune surveillance, the body would be much more susceptible to cancer, as has been demonstrated in experimental animal systems where key surveillance mechanisms are disabled.

Following ingestion and destruction of the foreign substance, the DC processes it into small fragments. Simultaneously, the DC undergo a maturation process which results in down-regulation of the antigen uptake and processing machinery. Also, the specific cell surface molecules and soluble cytokines essential for the initiation of an adaptive immune response are up-regulated. These processes take place while the DC migrate from, for example, the skin to the draining lymph nodes. In these nodes, DC interact with helper T cells (Th), the next component in the immune cascade.

The processed antigen components or peptides are displayed on the cell surface of the DC, where they are bound to components of the major histocompatibility complex (MHC). The MHC-peptide complexes are recognized by specific receptors on the surface of Th cells. This recognition, paired with the interaction of complementary pairs of co-stimulatory molecules on the surfaces of both cells, and the binding of the DC-secreted cytokines, leads to activation of the Th cells. Other immune cells, such as B cells and cytotoxic T cells (CTL), also interact with the DC, and are dependent on factors secreted by the Th cells for activation. The antigens originally ingested and processed by the DC provide the specificity of the ensuing immune response, as the activated Th and CTL, as well as the generated antibodies, are all specific for these tumor antigens.

EFFECTOR MECHANISMS OF THE ADAPTIVE IMMUNE SYSTEM

Activation of the adaptive immune system by DC leads to the generation of CTL and antibodies. CTL are particularly effective against virus-infected and cancer cells. Antibodies are effective against circulating invaders such as bacteria and viruses, as well as against virus-infected and cancer cells.

CTL recognize abnormal cells through their T cell receptor (TCR). The TCR recognizes such cells because they display the same peptide-MHC complexes as are displayed by the DC. This recognition results in the release of toxic substances by the CTL. The activity of these substances can be through direct lysis of the membrane of the target cells, or the activation of the death pathways that every cell is equipped with.

The antibodies produced by B cells recognize antigens in their free form; i.e., not in the form of a peptide-MHC complex, which is why they are effective in inducing clearance of circulating microorganisms. Binding of antibodies to microorganisms results in their uptake and destruction by macrophages and DC. Binding of antibodies to cells displaying the target antigens, such as cancer or virus-infected cells, results in activation of the complement system which leads to killing of the cell through lysis of the cell membrane.

Th cells also contain effector mechanisms, of which the secretion of interferons are the most well known. Interferons are small protein molecules produced by Th and other cells. These proteins both enhance and direct the immune response, and have direct, toxic effects on cancer cells and on viruses.

The most effective immune responses comprise all components of the immune system. The use of ex vivo generated DC is universally recognized as the best method to produce this type of immune response.

IMMUNE SUPPRESSION BY CANCER CELLS

Once established, cancer cells have evolved mechanisms to suppress the induction of immune responses and escape established responses. They produce substances that interfere with the normal functions of DC and down-regulate cell surface molecules such as those of the MHC complex. Further, they evade immune recognition by masking those antigens that make them recognizable as cancer cells. These mechanisms are particularly effective once a tumor has achieved a certain size, which is why immunotherapy is often preceded by or combined with more traditional forms of cancer therapy (e.g., chemotherapy or radiation) that reduce tumor burden.

The immune suppressive mechanisms of tumor cells are effective, but not absolute. Measures to overcome such suppression can be employed in the generation of products for cancer immunotherapy. Ex vivo manufacturing overcomes the suppressive effects on DC generation. Likewise, using the broad antigen repertoire present in whole tumor cell lysate, instead of a single antigen or epitope, minimizes the risk of immune escape through down-regulation of cancer antigen expression. Lastly, effective activation of Th cells leads to the production of interferons which, in turn, leads to up-regulation of MHC antigens on the tumor cells and better targeting by CTL.

DIFFERENT APPROACHES

The optimal way to arrive at the most effective immune response is through the control of the DC: if you control the DC, you control the immune system. Most traditional immunization approaches, including traditional virus, specific antigen or peptide vaccines, as well as some that are used for immunotherapy of cancer, try to control the already existing DC in the body, or modulate only one arm of the immune system. The immunogen - the vaccine component mainly responsible for provoking the immune system - is usually injected into the body in a formulation that aims at targeting and activating local DC. In these instances, it is left to chance as to whether the immunogen arrives at the DC, whether the DC are properly activated and whether they effectively migrate to the lymph nodes to produce an effective immune response. We believe that the failure of several recent human clinical trials is the result of the inability of these other approaches to effectively mount a natural and clinically meaningful immune response.

Treatments that use only a single division of the immune system may employ only large amounts of T cells, or a single (monoclonal) antibody. While monoclonal antibody treatments can be effective, their success is often of limited duration and restricted to patients that do not become resistant to the treatment. LifeVax™ has an advantage in that it activates all aspects of the immune response, both cellular and antibody, thus potentially providing a broader and longer-lasting immune and clinical response.

The LifeVax™ product consists of pure, activated DC, which are loaded with the immunogen as would naturally occur and that are capable of migrating to lymph nodes. The result is a robust and full immune response consisting of both a specific cellular T cell response and a specific antibody response against the cancer-associated antigens as demonstrated in human Phase I and Phase II clinical trials, and that is translated into a clinical benefit.

THE HUMAN STORY

Northwest Biotherapeutics, Inc. is a biotechnology company focused on the clinical development of autologous immunotherapy products for cancer and offers a range of treatments analogous to the LifeVax™ treatment in humans. Their technology platform, DCVax®, similarly uses a patient's own dendritic cells—the starter engine of the immune system—which are loaded with tumor proteins or antigens. Injection of these cells back into the patient initiates a potent immune response against cancer cells, resulting in delayed time to progression and prolonged survival. Their two lead product candidates, DCVax®-Prostate and DCVax®-Brain, are entering FDA-allowed late-stage clinical trials from which licensure is possible. They also have additional biological products in their pipeline that are also FDA-allowed and ready to enter Phase I clinical trials.

The human product DCVax®-Brain, using the same Dendritic Cell technology platform, has shown amazing efficacy data in its clinical trials with no toxic effects. In the Phase I and Phase I/II clinical trials for patients with GBM (Glioblastoma Multiforme) with the human product DCVax®-Brain, and follow-up long-term data, 84% of patients who received DCVax®-Brain in these trials have so far lived longer than the median survival of 14.6 months under standard of care, 68% have so far lived more than 2 years, 47% have so far lived more than 3 years, and 31% have so far lived more than 4 years, with patients surviving as long as 8 years to date. The median survival in the patients from these trials is now 36.4 months, under a standard Kaplan Meier analysis.

DCVax®-Brain Two Phase I Trials
Newly Diagnosed Glioblastoma Multiforme

	Standard Treatment	NWBT Treatment
Time to Progression	8.1 Months	18.1 Months
Median Survival	17 Months	36.4 Months

DCVax®-Brain Two Phase I Trials
DCVax-Brain doubles 2- and 3-year survival rates

	% Alive at		
Patient Population	1 Year	2 Years	3 Years
DCVax-Brain Treated	**95**	**68**	**47**
Institutional Controls	69	34	21

DCVax®-Brain Two Phase I Trials
DCVax-Brain significantly decreases rate of progression

	% Not Progressed at		
Patient Population	6 Months	1 Year	2 Years
DCVax-Brain Treated	**90**	**73**	**49**
Institutional Controls	64	26	4

Equal if not more significant, the long term data from the above clinical trials shows that more than 80% of the patients who received DCVax®-Brain showed a clinical response. In contrast, the typical response rates for cancer drugs are in the range of 20 to 25% of patients, and have been as low as 13% with some approved cancer drugs.

Another human product DCVax®-Prostate, also developed by Northwest Biotherapeutics, combines the company's DCVax platform with the cancer-associated antigen PSMA to target hormone independent (i.e. late stage) prostate cancer. A Phase I/II clinical trial in 33 patients with non-metastatic and metastatic hormone independent prostate cancer indicates that: Among the 11 non-metastatic hormone independent prostate cancer patients, there has been an increase in survival from 36 months for the natural course of the disease to >54 months for DCVax®-Prostate treated patients. There also has been a delay in time to metastases from 28 to 36 weeks, for the natural course of the disease, to 59 weeks with DCVax®-Prostate treated patients. In the above 11 patients, none had progressed at 28 weeks and only five had progressed at 59 weeks.

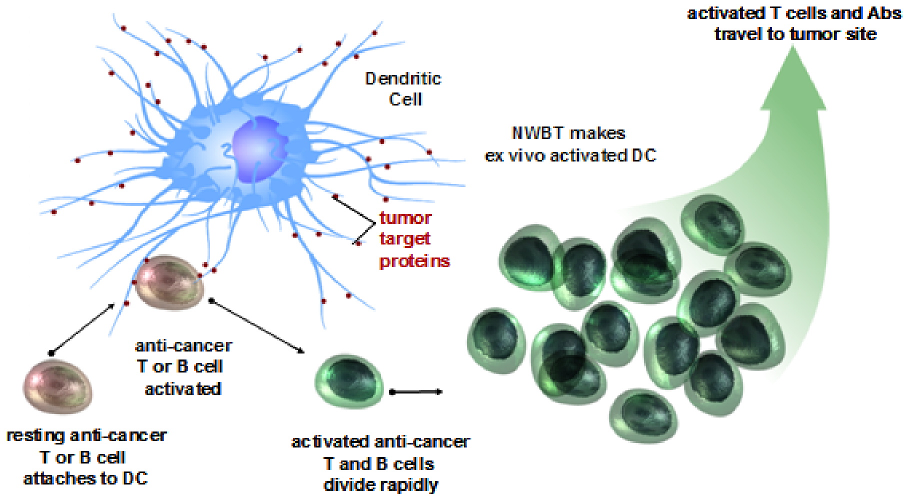
Among the 22 hormone independent prostate cancer patients with metastases, there was an increase in median overall survival from 18.9 months for standard of care to 38.7 months for DCVax®-Prostate treated patients. Patients in this study had a six-fold greater chance of being alive at 36 months compared to patients treated with the standard of care.

With similar products showing convincing safety data and extraordinary efficacy data in the above clinical and nonclinical studies, in various types of cancer and in multiple species, we are convinced that LifeVax™ is a potentially ground breaking therapeutic vaccine for various canine solid malignant tumors. LifeVax™ offers dog owners the potential of significantly extending their dogs' lives cancer free.

DENDRITIC CELL-BASED IMMUNOTHERAPY PLATFORM: LifeVax™

LifeVax™ is a novel platform technology applicable to unmet medical needs for multiple cancers. It combines a patient's own dendritic cells (DC) with cancer-related proteins or antigens to induce immune responses against a patient's cancer cells. Early stage clinical trial data from human clinical trials clearly demonstrate that Dendritic Cell vaccine technology has the ability to significantly delay disease progression, as well as prolong patient survival, while maintaining an excellent quality of life. We believe that LifeVax™ has the following characteristics, that in total make the LifeVax™ product an attractive therapeutic addition to current treatments.

One Activated Dendritic Cell
Activates Hundreds of Anti-Cancer Cells



EFFECTIVELY ACTIVATES THE NATURAL IMMUNE SYSTEM

The LifeVax™ platform is designed to naturally elicit a powerful, prolonged immune response against a patient's cancer. This response is effectively accomplished through the injection in the skin of DC that are educated to teach the immune system to recognize and kill cancer cells. Human clinical trials have shown that the DC vaccine technology platform activates a natural immune response consisting of specific and high titer antibody and T cell responses.

DESCRIPTION OF LIFEVAX™

LifeVax™ consists of autologous, monocyte-derived DC loaded with tumor cell lysate antigen.

Monocyte-Derived DC

Monocytes are DC precursors that circulate in the blood. Large numbers of monocytes can be obtained through standard blood collection, from which specific cellular components are isolated from the other blood components. Purification of the monocytes is a stepwise process which includes a density gradient centrifugation to isolate mononuclear white cells, followed by a plastic adherence step after which the non-adherent cells can be removed by washing. The remaining cells, i.e., the monocytes, are then converted to DC through a 7 day incubation process with two small soluble proteins or cytokines: granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin – 4 (IL-4).

Tumor Lysate Antigen

The source of the tumor lysate antigen used in the manufacture of LifeVax™ is the tissue that is resected during the surgery which is part of the standard therapeutic practice for certain presenting tumors. Subsequently, the tumor tissue is ground to a fine suspension in an enzyme solution. The enzymes gradually break down the tumor tissue, resulting in a suspension of tumor cells which are then purified by centrifugation. The resulting cell pellet is then subjected to multiple freeze-thaw cycles, during which the forming ice crystals disrupt the membrane resulting in tumor cell lysis. Removal of all particulate material, through filtration, results in a clear suspension of tumor proteins which is then loaded onto the DC during the final manufacturing step.

Administration and Mechanism of Action

The loaded DC are cryopreserved in single dose vials in a small volume. LifeVax™ is administered through intradermal injection of the vial contents, with no additional manipulation required. After injection into the skin, the antigen-loaded DC migrate to the draining lymph nodes where they interact with the other cellular components of the adaptive immune system. This interaction results in an immune response directed against the tumor cells. The effectors of the ensuing immune response, including the CTL, antibodies and Th cells, migrate to the tumor site. They seek out and destroy remaining cancer cells, and use immune surveillance to guard against tumor re-growth.

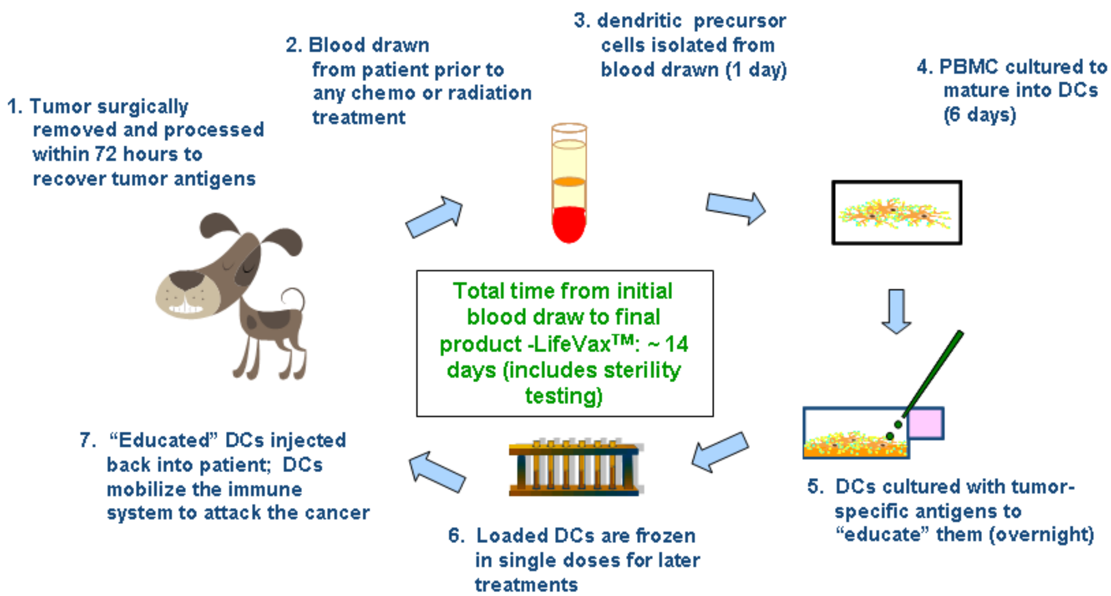
Typically, LifeVax™ is administered following surgery and either independently or in conjunction with other adjuvant therapies including radiation or chemotherapy. Such a regimen achieves the low tumor burden state that minimizes the immune suppressive effects of tumor cells. The first series of three immunizations are given at 2-week intervals (Weeks 0, 2 and 4), with the intent to rapidly initiate a broad immune response. This initiation phase may be followed by booster injections (if enough vaccine is available) aimed at further expanding and strengthening the immune response. This regimen is thought to provide the strongest and longest lasting protection from cancer relapse.

SUMMARY OF MANUFACTURING PROCESS

Preparation of LifeVax™ - Overview

LifeVax™ is a cellular immunotherapy based on the isolation, ex vivo manipulation, and re-injection of a patient's own tumor lysate antigen-loaded DC. LifeVax™ is prepared by ex vivo processing. The processing involves a blood collection from the patient, isolation of peripheral blood mononuclear cells (PBMC), purification of monocytes, generation of DC, and loading with tumor lysate antigen. Tumor lysate antigen is prepared from tumor material removed at surgery, including a freeze-thawing process, clarification through centrifugation, and sterile filtration. After processing, the tumor lysate antigen-loaded DC are cryopreserved and stored until administration.

The LifeVax™ product undergoes product sterility testing including Mycoplasma (prokaryotic microorganisms resistant to many antibiotics and when grown in close association with cells can alter cell characteristics), Endotoxin (found in the outer membrane of various Gram-negative bacteria and an important factor in their ability to cause disease) and Bioburden (The number of microorganisms with which an object is contaminated. This unit is measured in CFU (colony forming units) per gram of product). Vials of LifeVax™ are also inspected visually for particulate matter or discoloration prior to administration. If product sterility testing fails or abnormal particles or discoloration are observed, the product is not used. All LifeVax™ doses must be given intra-dermally.



LifeVax™ - USDA REGULATORY STATUS

LifeVax™ is an experimental treatment and **HAS NOT** been granted a Product License from the USDA.

The Veterinary Biologics Program of the USDA's Animal and Plant Health Inspection Service (APHIS) oversees the veterinary biologics industry in the United States. This Program consists of the Center for Veterinary Biologics (CVB) and allied services. The CVB requires that domestic manufacturers of veterinary biologics, for domestic use or for export, are required to possess a valid U.S. Veterinary Biologics Establishment License and an individual U.S. Veterinary Biologics Product License for each product produced for sale. In order to receive a US Veterinary Biologics Product License, rigorous clinical trials have to be carried out to test the safety and efficacy in a laboratory setting.

Meanwhile, under USDA exemption of the Code of Federal Regulations - Title 9: Animals and Animal Products 9 CFR 107.1, a veterinary biologic may be exempt from Federal regulation if the product was manufactured by veterinarians AND intended solely for use with their clients' animals under a veterinarian-client-patient (VCP) relationship. Such a relationship is considered to exist when: (1) the veterinarian has assumed the responsibility for making medical judgments regarding the health of the animal(s) and the need for medical treatment, and the client (owner or other caretaker) has agreed to follow the instructions of the veterinarian; and when (2) there is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s), and/or by medically appropriate and timely visits to the premises where the animal(s) are kept; and when (3) the practicing veterinarian is readily available for follow-up in case of adverse reactions or failure of the regimen.

The USDA, under 9 CFR 107.1(a)(2), would consider properly documented entries in the patient's medical records reflecting the Veterinarian-Client-Patient relationship, the need for the biologic and that the biologic was prepared on the veterinarian's premises at the direction of the veterinarian as sufficient to meet the regulation's record keeping requirements.

The LifeVax™ vaccine is being administered by veterinarians under the Title 9: Animals and Animal Products 9 CFR 107.1 exemption.