Technology evaluation: DCVax, Northwest Biotherapeutics
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DCVax, a dendritic cell-based immunotherapy, is an active immunization platform being developed by Northwest Biotherapeutics for the potential treatment of multiple malignancies, including hormone-refractory metastatic prostate cancer, non-small-cell lung cancer, renal cancer, and glioblastoma multiforme. The DCVax platform is tailored to a specific cancer type with either purified tumor-specific antigen or tumor cell extracts derived from patients at the time of resection. Phase I clinical trials of DCVax-Prostate have been completed, and phase III clinical trials have recently been initiated. DCVax-Brain is currently undergoing phase II clinical trials, and DCVax-Lung recently received approval from the US FDA for phase I clinical trials.

Introduction
Dendritic cells (DCs) are potent professional antigen-presenting cells that can activate multiple effectors of both the adaptive and innate immune systems. Under normal conditions, in the absence of inflammatory signals, DCs normally reside in the body in an immature state, where they play an active role in attenuating immunity against self-antigens. Since malignancy is not associated with strong inflammatory signals, endogenous DCs do not readily mature during active disease. In fact, in some malignant states the number of circulating immature DCs is amplified, thus protecting the tumor from T-cells that could become activated due to overexpression of antigens to which the immune response is normally unaccustomed to seeing [459784]. Maturation or differentiation of DCs induced by various factors, such as cytokines or microbial products, leads to the accumulation of mature DCs that have lost the ability to negatively regulate the immune response. As a result, DCs can directly activate cytolytic T-cells (CTLs), CD4 helper T-cells, natural killer (NK) cells and NK-T cells. Methods have now been developed that allow for the ex vivo maturation of DCs that are capable of eliciting functional immune responses in vivo following infusion.

Northwest Biotherapeutics is harnessing the power of DCs to specifically treat a variety of malignancies, including prostate [447090], lung [441755], brain [449480] and renal carcinoma [374117]. Therapies are tailored to each individual cancer type by pulsing DCs with malignancy-specific antigens. DCVax-Prostate (phase III) and DCVax-Renal (preclinical) are produced using purified recombinant antigens, while DCVax-Lung (phase I) and DCVax-Brain (phase II) are produced with tumor cell peptide extracts derived from resected autologous tumor.

Synthesis and SAR
DCVax is constructed using each patient's autologous peripheral blood mononuclear cells (PBMCs) derived by leukapheresis. In prior reports and in current clinical trials, DCs are derived by maturation of immature peripheral blood DCs using granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-4 and bacillus Calmette-Guerin (BCG). BCG promotes maturation, can be used to monitor the development of an immune response, and provides additional helper activity during immunization [447090]. During maturation, DCs remain adherent to the culture flask and can be easily separated from non-adherent lymphocytes. In one report, the DC cultures obtained using this platform were typically 86% DC, 7% B-cells, 4% T-cells and 0.7% NK cells [450636]. Following maturation, DCs are tailored to immunize against specific malignancies as previously mentioned.

DCVax-Prostate, the most advanced of the DCVax family of therapies, is tailored for the treatment of prostate cancer with recombinant prostate-specific membrane antigen (PSMA). PSMA is a 730-amino-acid type 2 transmembrane glycoprotein expressed on prostate epithelium [459789]. Expression of PSMA is highly specific for prostate tissue and is only weakly expressed on other normal tissue, such as brain and small intestine [459789]. Expression is greatly upregulated on malignant epithelium and increases with disease progression and androgen independence [459789]. To prepare DCVax-Prostate, recombinant PSMA whole protein is pulse into the matured DCs prior to re-infusion into the patient. DCVax-Brain is produced from autologous DCs pulsed with peptide epitope acid-elicited from autologous glioblastoma multiforme tumor cells obtained at the time of tumor resection [444989]. DCVax-Lung is produced similarly using non-small lung tumor cells derived at the time of resection [444755].

In a recent preclinical study, Northwest Biotherapeutics reported that incorporation of IFNγ into their DC production platform improves maturation of Th1 helper cell-inducing capabilities [447093]. The addition of IFNγ during maturation specifically upregulates co-stimulatory and MHC molecules, and increases production of IL-12, a potent Th1-inducing cytokine. This is of importance, as Th1 cells are the critical T-
cell population for the development and maintenance of a
cell-mediated inflammatory response. Although this
technology may be employed in new clinical trials using
DCVax platforms, no plans have been announced and current
trials continue to use GM-CSF, IL-4 and BCG as maturation
factors. The synthesis of DCVax-Renal is still under
development, but press releases in 2000 indicated that the
renal cancer-specific antigen G250 had been selected for
further testing with the DCVax platform [374117].

Pharmacology
DCVax is delivered intradermally to patients. Dosing varies
depending on the platform. DCVax-Prostate is delivered as
two monthly injections, and DCVax-Brain is given in a series
of three bi-weekly injections. It is currently unclear what
percentage of the injected DCs migrate to the lymphoid tissue,
but studies in mice and primates suggest that only 0.2% can
reach a local lymph node. The remainder of the DCs usually
remain at the injection site. Longevity of DCs, once migrated
to the lymph node, is not well understood. Migration and
longevity are two notable areas of DC biology that are actively
being studied for improvement. DC-based immunization and
therapeutics will greatly benefit from continued improvements in this area.

Metabolism
No data are currently available.

Toxicity
Immune-based vaccines and therapeutics continue to
demonstrate a low-level of adverse events that are usually
only of grade 1 or 2. No serious adverse events have been
reported with the use of DCVax-Prostate [447090] and
DCVax-Brain [449480]. Side effects also appear to be minor
(see below), while long-term effects are not known.

Clinical Development
DCVax-Prostate
Phase I/II studies of the DCVax-Prostate have been
completed and recently reported at the 93rd Annual
Meeting of The American Association for Cancer Research
[447090]. The trial examined the safety, immunogenicity and
clinical outcome of four monthly intradermal injections at
one of three different doses: 5, 10 or 20 x 10^6 PMSA-loaded
autologous DCs. Patients with low volume (three bone
metastatic lesions) androgen-independent prostate cancer
with no other treatment options were eligible for study
[459787]. Other inclusion criteria included progressive
disease, a Zubrod or ECOG performance status of 0 to 1,
lymph node lesions of < 3 cm, and adequate hematological,
hepatic and renal function. Assisik and co-workers reported
that out of 30 patients that were screened for eligibility, 24
met the criteria and began therapy [447090]. All 24 of the
patients received at least one vaccination, and none of the
patients demonstrated adverse events. 80% Of patients
demonstrated either humoral or cellular immunity to PSMA
as a result of immunization. At the time of reporting, 19
patients in the above trial had reached 6 months of follow-
up. Of those 19 patients, four had no signs of disease
progression as measured by PSA levels and radiography.
Another six patients had stable disease as determined by
radiography, but showed increasing PSA levels. This
translates to a disease stabilization in 53% of the patients.

Data from the complete phase I trial of DCVax-Prostate are
currently not published in their entirety.

In another report of the same DCVax-Prostate phase I/II
clinical trial, Shankar and colleagues reported the
immunogenicity of DCVax-Prostate in 33 patients from the
two clinical sites [459792]. T-cell responses to PSMA
were examined using proliferation assays and antibody responses
with PSMA-specific ELISA. Of 33 patients examined, 74%
developed significant proliferative responses to PSMA with
a median stimulation index of 18.5 and a range of 2.1 to 136.
The stimulation index is defined as the ratio of the T-cell
growth in response to antigen and the basal growth rate.
52% Of patients also developed PSMA-specific antibodies
with a median peak antibody titer of 1:10,240 (range 1:160 to
1:2,400). Overall, 72% of patients developed immunological
responses, suggesting that the vaccine strategy was highly
effective at overcoming tolerance to PSMA.

DCVax-Brain
DCVax-Brain is Northwest Biotherapeutics' second most
advanced product using the DCVax platform. Interim results
of a phase I clinical trial being conducted at the UCLA School
of Medicine have been reported [449480]. Eligibility criteria
included newly diagnosed grade IV or low-grade recurrent
glioblastoma multiforme, which had not been treated
previously with either chemotherapy or radiation. In
addition, for the purposes of antigen extraction, the tumors
had to be surgically resectable. The results for nine patients
have been reported. Patients were treated with three bi-
weekly intradermal injections of autologous DCs pulsed with
peptide epitopes eluted from the tumors. The objectives of the
trial were to demonstrate safety and immunogenicity. No
serious adverse events were observed. Furthermore,
neuropsychological testing and magnetic resonance imaging
indicated that the therapy does not detectable harm to brain or
other components of the central nervous system. In this
interim analysis, immunological responses to DCVax-Brain
were not published. The progression rate was 30% for those
receiving DCVax-Brain. Patients seen at UCLA not enrolled in
the study had a progression rate of 85%. Eight out of nine
patients were still alive at the time of the report, with a
survival range of 7.9 to 25.1 months (median 15 months).

DCVax-Lung
DCVax-Lung is currently undergoing phase I trials [441755],
[459794], although no data from these trials have been
reported. The clinical trial will enroll a total of 12 patients
with stage III non-small-cell lung cancer, and will evaluate
different doses of DCs pulsed with resected brain
tumor antigen. The trial is designed to evaluate toxicity and
establish the maximum tolerated dose. Clinical responses
will also be examined at days 30, 60, 90 and 120.

Side Effects and Contraindications
Side effects for DCVax-Brain have been reported and
include lymphadenopathy, nausea/vomiting, headache,
fatigue and low-grade fever [449480]. Contraindications for
DCVax-Prostate are inadequate hematological, hepatic or
renal function [459787]. Contraindications for DCVax-Brain
and DCVax-Lung are prior history of malignancy (except for
adequately treated basal or squamous cell skin cancer, in situ
cervical cancer or other cancer in which the subject has been
disease free for at least five years) and inadequate
hematological, hepatic or renal function [459794], [459816].
Current Opinion

The generation of an immune response against tumor is, in essence, an attempt to generate a tissue-specific (eg, tumor) autoimmune response that is sufficient in magnitude to completely destroy the target tissue, but is limited enough to prevent direct damage to non-malignant normal tissues. One major obstacle to overcome when applying immunological approaches to treat and prevent cancer is tolerance. We now know that tolerance to specific self-antigens can be overcome with the use of strong adjuvants that promote the generation of a broad immune response that includes the activation of multiple effectors of both the innate and adaptive immune responses. DCs are the body's natural adjuvant that can break tolerance to self-antigens in animal models [438488], [438489], and even result in eradication of existing malignancy [357832].

The development of methodologies for the ex vivo expansion of human DCs is now enabling the clinical testing of DCs as natural adjuvants to induce tumor-specific immune responses that can effectively and specifically destroy malignant cells. DCVax represents a pioneering effort to commercialize a DC-based platform that has potential as a strategy for both treating existing malignancy and preventing relapse. The successes observed so far in trials of DCVax-Prostate and DCVax-Brain are encouraging, and the approach can expect to benefit from a better understanding of many areas of DC biology that are being actively explored, such as antigen loading, DC maturation, routes of injection and in vivo turning.

Antigen-loading represents an important control point that could potentially impact the breadth of the immune response elicited. The antigenic milieu should be sufficient to generate both a CD8 as well as a CD4 T-cell response. CD4 T-cells are central to both the adaptive and innate immune response. CD8 T-cell immunity cannot be sustained without additional help, which is illustrated in recently reported adoptive T-cell therapy trials. For example, in a recent study, Dudley and colleagues assessed the safety, feasibility and clinical response of adoptive T-cell therapy of melanoma using ex vivo expanded CTL clones specific for an HLA-A2-binding peptide derived from the melanoma antigen gp-100 [459800]. 12 Patients were treated with multiple infusions of gp-100-specific T-cell clones, with an average of 1 x 10^9 cells per infusion. The T-cell clones were selected based on their apparent avidity for peptide antigen as assessed by the magnitude of antigen-specific cytokine release in vitro. Although the clones secreted large amounts of IFNγ and recognized HLA-A2+ melanoma cell lines, only two patients had minimal partial responses. An important finding from this study was that the T-cells disappeared rapidly and were undetectable at 2 weeks even though the patients received concomitant intravenous IL-2. Persistence of CD8 T-cells is largely dependent on the concomitant generation of CD4 T-cell immunity, which creates an environment appropriate for the generation of immunological memory [459803], [459805].

The quality of the maturation state of the DC is also important, since it is becoming increasingly clear that not only are immature DCs unable to generate an antigen-specific immune response, but can actually diminish pre-existing immunity through several mechanisms, including the induction of regulatory T-cells to produce the immunosuppressive cytokine IL-10. Steinman and colleagues recently reported that immature DCs pulsed with influenza virus peptide can reduce peptide-specific CD8 T-cells for several weeks when infused into normal volunteers [459808]. In cancer patients, immature DC levels are pathologically increased, which could dampen any endogenous pre-existing immune response against the tumor [459784].

The route of administration in humans is also important, particularly if the desired outcome is the induction of a predominant Th1 cell-mediated immune response. Fong and colleagues recently reported results of a trial assessing the immunogenicity of DC immunization by three different routes: intradermal, intravenous and intralymphatic [459809]. In this study, prostate cancer patients administered with prostastic acid phosphatase-pulsed DCs by the intradermal or intralymphatic routes developed antigen-specific IFNγ responses, while those immunized by the intravenous route developed antibody responses but no IFNγ response. All patients, regardless of route, developed antigen-specific T-cell proliferative responses. These results suggest the route of administration can affect the Th1/Th2 balance of the resulting immune response.

In vivo migration of mature DCs to primary lymphoid tissue is required for activation of antigen-specific T-cell responses. Studies in mice have demonstrated that the magnitude of the immune response is directly dependent on the efficiency of migration, which is also thought to be one of the major rate-limiting steps for improving therapeutic efficacy. Studies in both mice and primates suggest that < 0.2% of injected DCs make the journey to the draining lymph node to activate T-cells [459810], [459811]. Efforts are under way in many laboratories to understand and improve in vivo DC migration.

The disease setting in which to apply the DC immune-based approaches is equally important in improving therapeutic efficacy. In general, it is agreed that a reduced tumor burden will greatly impact clinical efficacy of immune-based therapies. Established tumors are more likely able to evade immune effectors due to the clonal diversity of the tumor cells. For example, a tumor may contain clones that have reduced expression of antigen or effector recognition molecules such as MHC and CD1b [459812]. These clones would have a selective advantage during antigen-targeted immunotherapy. Clones that elaborate immunosuppressive cytokines, such as TGFβ or IL-10, may also be present [459814]. Thus, strategies such as DC vaccination, if used alone, may be most effective at preventing relapse in patients that have been rendered nearly disease free or who have minimal residual disease at most. In these settings, clonal diversity is minimized and the immunosuppressive tumor microenvironments are not well established. This prophylactic use of vaccine strategies is analogous to the use of viral or bacterial vaccines, which are used to prevent disease prior to, or shortly after exposure to the pathogenic agent. Although this is perhaps the preferred use, the initial application of novel strategies in the clinic is usually
confined to those patients who have limited life expectancies, and for whom treatment options are limited. It is impressive that DC-based strategies are safe and not associated with adverse events; however, a major challenge is to maintain this safety profile while improving clinical efficacy.

Based on the data available, the DCVax platform appears to have clinical potential, and as DC technology improves, the therapeutic efficacy of DCVax may also improve. Enhancements in DC-based therapeutics and vaccination in the clinical setting are dependent on a thorough understanding of the immune characteristics of the strategy, and efforts should be applied to rigorously identify the effector arms and magnitudes of the immune responses that are associated with clinical benefit. The identification of surrogates that can potentially predict clinical outcome will greatly streamline improvements in existing platforms and extend them to other disease settings, for which relapse-free and overall survival are of longer duration (eg, early stage disease).

### Development history

<table>
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<tr>
<th>Developer</th>
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### Literature classifications

Key references relating to the technology are classified according to a set of standard headings to provide a quick guide to the bibliography. These are as follows:

**Chemistry**: References which discuss synthesis and structure-activity relationships.

**Clinical**: Reports of clinical phase studies in volunteers providing, where available, data on the following: whether the experiment is placebo-controlled or double- or single-blind; number of patients; dosage.

### Chemisty

<table>
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<th>Study type</th>
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<td>SAH</td>
<td>DC efficacy improved with addition of IFNγ during maturation.</td>
<td>447093</td>
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### Clinical

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<td>Toxicity, safety, immunogenicity and clinical response.</td>
<td>Phase I open-label study of nine newly diagnosed stage IV or low-grade recurrent glioblastoma multiformes.</td>
<td>Interim report demonstrated no serious adverse events, and minor side effects reported. Progression rate at one year was 20%.</td>
<td>449480</td>
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<tr>
<td>Toxicity, safety, immunogenicity and clinical response.</td>
<td>Phase III open-label dose-escalation study in 60 advanced-stage prostate cancer patients with low-volume androgen-independent disease.</td>
<td>No adverse events reported. &gt; 75% of patients developed immunity to target antigen and &gt; 53% had detectable disease stabilization.</td>
<td>447090</td>
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### Associated patent

**Title**: Method to increase class I presentation of exogenous antigens by human dendritic cells.

**Assignee**: Northwest Biotherapeutics Inc

**Publication**: US00187325 22-NOV-01

**Priority**: US00203758 12-MAY-00

**Inventor**: Seligman ML, Boyelon AL.

**438488** Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. Mayordomo JM, Zorina T, Storkus WJ, Zilvogel L, Celluzzi C, Falo LD, Melief CJ, Idstad ST, Kast WM, Delio AB et al. NAT MED 1995 1 1 1297-1302

- Seminar study demonstrating that peptide-pulsed DC could elicit a tumor-specific response.

**438489** Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. Celluzzi CM, Mayordomo JM, Storkus WJ, Lotze MT, Falo LD J EXP MED 1996 183 1 283-287

- Seminar paper describing that peptide-pulsed DC could generate an immune response capable of recognizing peptide-displaying tumor.

**441755** Northwest Biotherapeutics Inc to initiate phase I lung cancer trial. Northwest Biotherapeutics Inc PRESS RELEASE 2002 February 28

**444402** Northwest Biotherapeutics Inc announces fourth-quarter and full-year financial results. Northwest Biotherapeutics Inc PRESS RELEASE 2002 March 21

### Associated references

- **357832** Regression of tumors in mice vaccinated with professional antigen-presenting cells pulsed with tumor extracts. Nair SK, Snyder D, Rousse BT, Gilboa E INT J CANCER 1997 70 6 706-715
- Paper demonstrating that therapeutic immunity could be elicited to poorly immunogenic tumors in a mouse model.

- **374117** NWBio options kidney cancer technology. Northwest Biotherapeutics Inc PRESS RELEASE 2000 July 11

**Discussion**: Discusses biology of PSMA antigen used in DCVax-Prostate platform.


**Discussion**: Study documenting the fraction of injected DCs that reach local lymph nodes in mice.


**Discussion**: Study documenting the fraction of injected DCs that reach local lymph nodes in a primate model.

Role of dendritic cells in the induction and maintenance of autoimmune diseases. Ludwig B, Odermatt B, Ochsenbein AF, Zinkernagel RM, Hengartner H. *J IMMUNOL REV* 1999 169 45-54

**Discussion**: Loss of immune system recognition molecules is a mechanism by which tumors evade the immune response.


**Discussion**: Review of the literature describing how the tumor microenvironment suppresses the endogenous immune response against tumor.