# DCVex™: A novel DC-targeted vector platform for cancer immunotherapy

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**Abstract #2817**

## Introduction

Dendritic cells (DCs) are essential for the initiation of T cell responses and are therefore an attractive target for cancer immunotherapy. The DC vaccine, Provenge®, as well as a number of on-going clinical trials, have validated this concept in principle. However, the currently pursued strategy of *ex vivo* immunization of DCs is time-consuming and costly. Here, we have developed a 3rd generation, integration-deficient lentivector platform, DCVex™, designed to deliver tumor antigen-encoding genes directly to DCs *in vivo* by targeting the DC-SIGN receptor (See Abstract #702).

Mice immunized with DCVex™ vectors developed strong, dose-dependent, multi-functional, and cytotoxic antigen-specific CD8 T cell responses, as assessed by intracellular cytokine staining and *in vitro* cytotoxic T lymphocyte assays. Repeated immunizations resulted in boosting of the T cell responses, indicating the absence of induction of inhibitory anti-vector immunity. Importantly, in stringent therapeutic tumor models (e.g., B16F10 footpad melanoma and CT26 lung metastasis models), immunization with tumor antigen-encoding DCVex™ vectors protected the majority of animals from death in a dose-dependent manner.

These findings demonstrate the potential of DCVex™ as a novel cancer vaccine platform suitable for *in vivo* DC immunization.

## Conclusions

A critical feature for an effective cancer immunotherapy is the induction of tumor-specific cytotoxic CD8 T cells. We show here that…

- ***DCVex™ targets dendritic cells***
  Targeted dendritic cells are transduced to present tumor antigen to CD8 T cells
- ***DCVex™ generates Ag-specific multi-functional CD8 T cell response and provides therapeutic anti-tumor protection in a dose-dependent manner***
  DCVex™ vectors efficiently induces the development of Ag-specific, multi-functional, cytotoxic CD8 T cells that mediate anti-tumor response
- ***DCVex™ induces limited persistence of antigen presentation***
  Among several safety measures, DCVex™ vectors are replication- and integration-deficient, designs to limit long-term residual vector activity

In sum, the DCVex™ platform combines the intrinsic preference of vector-delivered antigen for MHC-I presentation, the specialized ability of lentiviral vectors to carry significant genetic payloads and target non-dividing cells, and the immunological efficiency of DC-targeting to create a highly effective therapeutic vaccine platform.

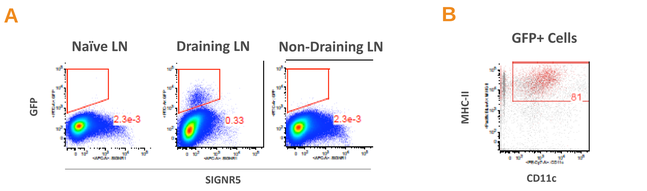
***Immune Design at a Glance***

- We are a clinical-stage company, headquartered in Seattle, WA, USA.
- Our Scientific Advisory Board Is comprised of leaders in immunology, vaccinology, and cancer immunotherapy, including members of the National Academy of Science and two Nobel Prize recipients in Medicine.

IMMUNE DESIGN

## Results

### DCVex™ targets dendritic cells and induces Ag-specific CTL activity

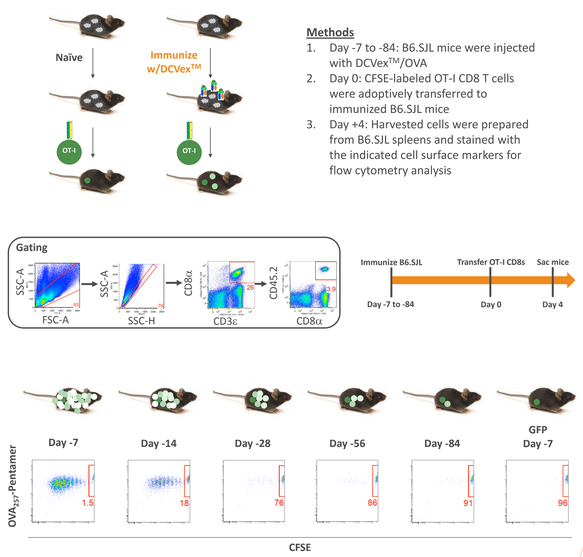



**Methods: A & B**
1. Day 0: B6 mice were injected with DCVex™/GFP
2. Day 4: Harvested cells were prepared from lymph nodes and stained with the indicated cell surface markers for flow cytometry analysis

**Methods: C**
1. Day 0: BALB/c mice were injected with DCVex™/NYESO1 or ctrl vector
2. Day 14: Harvested cells were prepared from spleens and cultured in the presence of NYESO1 peptide for a 5-day MLC to expand CTLs
3. Day 19: Cells post-MLC were incubated with PKH26-labeled target cells pulsed with NYESO1 peptide for 5 h per E:T ratio detailed in the figure; cells were then stained with a fixable live/dead dye and analyzed for cytotoxic killing by flow cytometry

**A. DCVex™ targeted cells in draining but not non-draining lymph nodes**
**B. DCVex™ targeted dendritic cells *in vivo***
**C. Mice immunized with DCVex™ developed Ag-specific CTLs**

### DCVex™ promotes limited persistence of antigen presentation

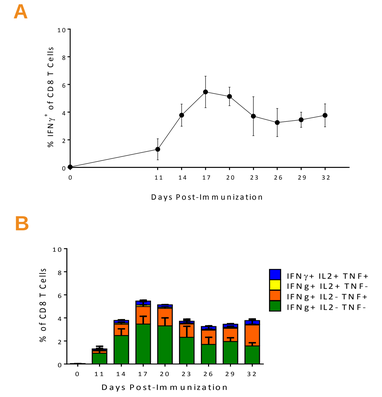



**Methods**
1. Day -7 to -84: B6.SJL mice were injected with DCVex™/OVA
2. Day 0: CFSE-labeled OT-I CD8 T cells were adoptively transferred to immunized B6.SJL mice
3. Day +4: Harvested cells were prepared from B6.SJL spleens and stained with the indicated cell surface markers for flow cytometry analysis

**DCVex™-induced Ag presentation was largely cleared by Day 28 post-immunization**

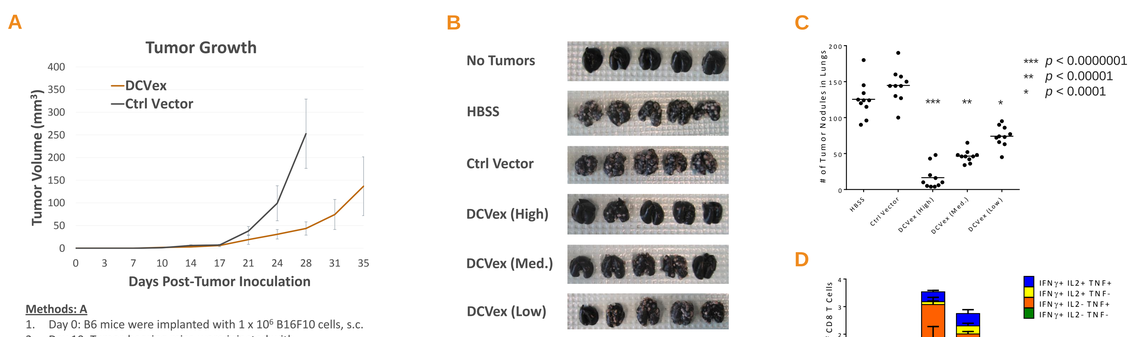
### DCVex™ induces multi-functional CD8 T cell responses

**Methods**
1. B6 mice were injected with DCVex™/mTRP1-A463M and sacrificed at the indicated days post-immunization
2. Harvested cells were prepared from spleens, stimulated with TRP1 peptide for 5 h, and stained for ICS and flow cytometry analysis; error bars represent SEM




**A. Mice immunized with DCVex™ developed Ag-specific CD8 T cells, which peaked at Day 17 post-immunization**
**B. DCVex™-induced Ag-specific CD8 T cells exhibited a multi-functional phenotype**

### DCVex™ generates Ag-specific CD8 T cell response and provides therapeutic anti-tumor protection in a dose-dependent manner

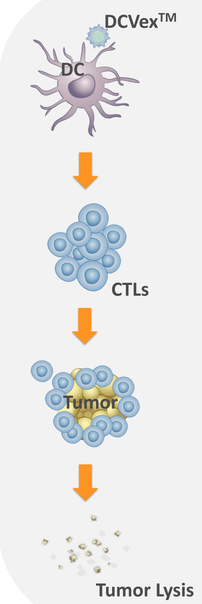



**Methods: A**
1. Day 0: B6 mice were implanted with 1 x $10^6$ B16F10 cells, s.c.
2. Day 10: Tumor-bearing mice were injected with DCVex™/mTRP1-A463M
3. Tumor growth was measured 2-3 times per week; tumor volume was calculated based on a modified ellipsoid formula: length x width$^2$ x π/6; error bars represent SEM

**Methods: B - D**
1. Day 0: BALB/c mice were implanted with 1.5 x $10^5$ CT26 cells expressing NYESO1, i.v.
2. Day 3: Tumor-bearing mice were injected with DCVex™/NYESO1
3. Day 18: Mice were sacrificed; lungs were isolated, stained with India ink, and fixed to enumerate tumor nodules; harvested cells were prepared from spleens, stimulated with NYESO1 peptide for 5 h, and stained for ICS and flow cytometry analysis; error bars represent SEM

**A. DCVex™ significantly delayed tumor growth**
**B-C. DCVex™ significantly decreased the development of metastatic lung nodules in a dose-dependent manner**
**D. Mice immunized with DCVex™ developed Ag-specific CD8 T cells in a dose dependent manner**

## Proposed Mechanism of Action




**DCVex™ transduces dendritic cells (DCs)**

**Transduced DCs present tumor Ag to CD8 T cells, inducing cytotoxic T lymphocytes (CTLs)**

**Activated CTLs recognize tumor cells…**

**… and target tumor cells for death**