# Therapeutic Vaccination With HPV-16 Oncoproteins Fused Into a Checkpoint Modifier of Early T-cell Activation Protects Against HPV-associated Tumors in a Preclinical Model

Currie S[1], Zhou X[2], Xiang Z[2], Giles-Davis W[2], Luber A[1], Mohammadi M[2], Ertl HCJ[2]
1 Virion Therapeutics, LLC, Newark, DE, USA; 2 The Wistar Institute, Philadelphia, PA, USA

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## BACKGROUND

- Checkpoint inhibition by mAbs against PD-1 and its ligand, CTLA-4, and other immuno-inhibitors has revolutionized cancer treatment
- However, current checkpoint inhibitors target activated T cells that are differentiating towards exhaustion[1]
- Immunotherapies that can enhance CD8⁺ T-cell activation, have the potential to increase and broaden T-cell responses to various cancers[1–3]
- There remains a need to produce novel cancer treatments, alone or in combination, that:
  - Have better safety and tolerability
  - Provide more potent and prolonged T-cell responses
- **Multifunctional**: Herpes simplex virus type 1 gD, when genetically expressed as a fusion protein with tumor antigens, serves as a checkpoint inhibitor of the BTLA HVEM pathway, which acts **early** during T-cell activation (**Figure: Early Checkpoint Modifier – Glycoprotein D Mechanism of Action**)
  - The resultant antigen-driven responses are more **potent** and **durable**, and **broadened** to include CD8⁺ T cells to sub-dominant epitopes, which are more resistant to exhaustion
- **Safety profile**: Low risk for "off-target" adverse events; **gD is only expressed** locally at the injection site and in regional draining lymph nodes
- **Checkpoint inhibitor combination data**: Preclinical studies have shown improved efficacy using gD-based immunotherapies plus anti-PD-1 mAbs[4]

## PURPOSE

- To report the immunogenicity and efficacy of a gD-based immunotherapy in a highly malignant, fast-growing preclinical tumor model (e.g., HPV-16 TC1) using a chimpanzee adenoviral vector (AdC6) expressing a novel sequence derived from early proteins 2, 5, 6, and 7 of HPV-16 fused into gD (AdC6-gDE7652)

## METHODS

- An **HPV-16 *E7652* gene construct** was generated containing immunogenic fragments of E7, E6, E5, E2
  - The *E7652* gene was fused into gD and then inserted into an E1-deleted, partial E3-deleted AdC6 vector
  - Two separate constructs were evaluated using two different forms of gD: AdC6-gD$_{v1}$E7652 and AdC6-gD$_{v2}$E7652
- **Control vectors** included:
  - *E7652* gene construct **without gD (AdC6-E7652)**
  - Challenge models had **AdC6 vector expressing HIV gag fused within gD (AdC6-gD$_{v2}$gag)**

### IMMUNOGENICITY ASSESSMENTS

- C57Bl/6 and HLA-2 transgenic mice (n=5 per group) were evaluated
- Mice received a single IM injection of 5x10$^{10}$ virus particles comprising:
  - AdC6-gD$_{v1}$E7652
  - AdC6-gD$_{v2}$E7652
  - AdC6-E7652; or
  - No vector (control; naïve)
- Frequencies of insert-specific CD8⁺ T cells were determined by ICS for IFN-γ 2 weeks after injection
- Breadth and specificity of CD8⁺ T-cell responses to individual peptides within a target sequence were performed via epitope mapping of splenocytes (CD8⁺ T cells tested by ICS for IFN-γ) 5 weeks after treatment

### EFFICACY ASSESSMENTS

- **TC-1 cells:** A tumor cell line from primary lung epithelial cells of C57Bl/6 mice were immortalized by HPV-16 E6 and E7 and then transformed with an activated *ras* oncogene
- **Treatment model:** C57Bl/6 mice (n=10 per group)

**Day 0**
**Mice were challenged subcutaneously in the left flank with:**
5.0 x 10$^5$ TC-1 cells (SD)
2.5 x 10$^5$ TC-1 cells (HI)

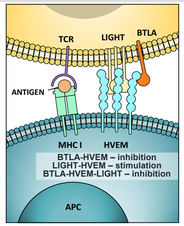
**Day 3**
**Mice received a single IM injection of 5x10$^{10}$ virus particle of either:**
AdC6-gD$_{v1}$E7652
AdC6-gD$_{v2}$E7652 (HI only)
AdC6-gD$_{v2}$gag

**Tumor progression was monitored over time up to:**
Day 73 (SD group)
Day 67 (HI group)

## RESULTS

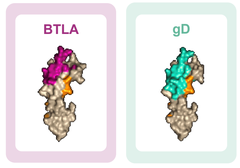
### Early Checkpoint Modifier – Glycoprotein D Mechanism of Action[2,3,5,6]

**HVEM Complex in Regulating T cell Activation**




**gD & BTLA Share HVEM Binding Site**

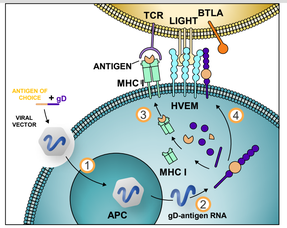
HVEM Crystal Structure




**gD BTLA-HVEM Blockade Enhances and Broadens T cell Activation**

Following IM injection, VRON-infected APCs travel to regional draining lymph nodes (1)

Within APCs, Ad vector produces the fusion protein of gD + antigen of choice (2)



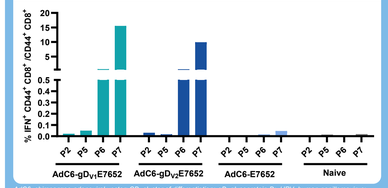

Degradation of incorrectly produced fusion protein releases peptides from the antigen, which, upon binding to MHC class I, are recognized by CD8⁺ T cells (3)

The gD fusion protein translocates to the cell surface, where it blocks BTLA-HVEM interaction, thereby increasing TCR signaling and allowing for co-stimulation through LIGHT (4)

*APC, antigen presenting cell; BTLA, B- and T-lymphocyte attenuator; CD, cluster of differentiation; gD, glycoprotein D; HVEM, herpes virus entry mediator; LIGHT, lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes; MHC, major histocompatibility complex; TCR, T cell receptor.*

### gD-based Immunotherapies Significantly Enhanced CD8⁺ T Cell Responses to E7 by 10- to 15-fold in C57Bl/6 Mice

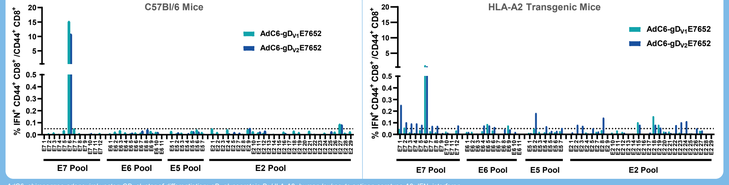
**Response to HPV Insert (C57Bl/6 mice): CD8⁺ T Cells 14 Days After Treatment**




*AdC6, chimpanzee adenoviral vector; CD, cluster of differentiation; gD, glycoprotein D; HPV, human papillomavirus; IFN, interferon.*

### gD-based Immunotherapies Induced T Cell Responses to All Regions of the Immunotherapy, Predominantly E7

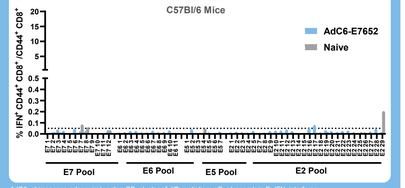
**Breadth of CD8⁺ T Cell Responses in Splenocytes of Mice That Received gD-based Immunotherapy**




*AdC6, chimpanzee adenoviral vector; CD, cluster of differentiation; gD, glycoprotein D; HLA-A2, human leukocyte antigen serotype A2; IFN, interferon.*

### Immunotherapies Without gD Showed Little to no Immunogenicity

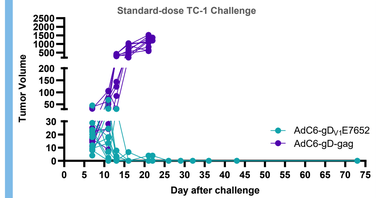
**CD8+ T cell Breadth of Response in Splenocytes of Mice Treated with Immunotherapy Without gD**




*AdC6, chimpanzee adenoviral vector; CD, cluster of differentiation; gD, glycoprotein D; IFN, interferon.*

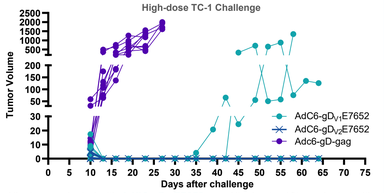
### gD-based Immunotherapies Resulted in Tumor Regression and Sustained Complete Tumor Loss

**Post-treatment Efficacy Following a Single IM Dose in TC-1 Challenge Models**




All mice (n=10) vaccinated with **AdC6-gD$_{v1}$E7652** experienced regression and complete tumor loss by Day 29, which was sustained in all mice to Day 73

All mice (n=10) vaccinated with **AdC6-gD$_{v2}$gag** showed rapid tumor growth and had to be euthanized by Day 21



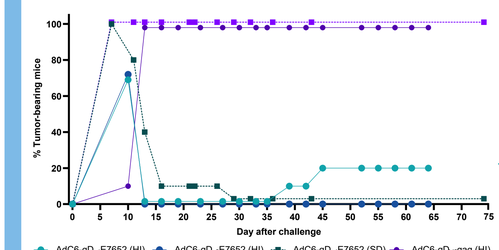

All mice vaccinated with **AdC6-gD$_{v1}$E7652** (n=10) or **AdC6-gD$_{v2}$E7652** (n=10) experienced regression and complete tumor loss by Day 13, which was sustained in 18 of 20 (90%) mice through to Day 67

All mice (n=10) vaccinated with **AdC6-gD$_{v2}$gag** showed rapid tumor growth and had to be euthanized by Day 27

*AdC6, chimpanzee adenoviral vector; gD, glycoprotein D; IM, intramuscular.*

### gD-based Immunotherapy Results in Improved Anti-tumor Activity and Survival

**Overall Post-treatment Efficacy Following a Single IM Dose in TC-1 Challenge Model**




**AdC6-gD$_{v2}$gag**
- 0/20 tumor-free (0%)
- 0/20 alive (0%)

All control mice (n=20) experienced rapid tumor growth and death

**gD-based immunotherapy**
- 28/30 tumor-free (93%)
- 30/30 alive (100%)

All mice (n=30) treated with a single gD-based immunotherapy were alive and 93% tumor-free at the end of study

*AdC6, chimpanzee adenoviral vector; gD, glycoprotein D; IM, intramuscular; HI, high-dose; SD, standard-dose.*

## CONCLUSIONS

- **These are the first preclinical data of a novel early checkpoint modifier construct, AdC6-gDE7652, which demonstrated:**
  - **The addition of the checkpoint modifier, gD, to an immunotherapy markedly improved immunogenicity**
  - **Treatment responses (tumor regression and sustained complete tumor loss) were durable and reproducible**
    - **These types of responses have not been previously observed with other checkpoint inhibitors or immunotherapies[7]**
- **These data suggest that a gD-based immunotherapy could have clinical applications for treating various cancers, and they support further exploration with different combinations of checkpoint inhibitors and other immunotherapies:**
  - **A clinical study evaluating a gD-based immunotherapy for cancer is in development**

## REFERENCES

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## ABBREVIATIONS

AdC6, chimpanzee adenoviral vector; APC, antigen presenting cell; BTLA, B- and T-lymphocyte attenuator; CD, cluster of differentiation; CTLA-4, cytotoxic T lymphocyte antigen-4; gD, glycoprotein D; HIV gag, human immunodeficiency virus group specific antigen; HLA-A2, human leukocyte antigen serotype A2; HPV, human papillomavirus; HVEM, herpes virus entry mediator; ICS, intracellular cytokine staining; IFNγ, interferon-gamma; IM, intramuscular; LIGHT, lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes; mAb, monoclonal antibody; MHC, major histocompatibility complex; PD-1, programmed death 1; pol, polymerase; *ras*, rat sarcoma; TCR, T cell receptor; VRON, virion specific I/O therapy.

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## DISCLOSURES

Dr. Currie is COO, Virion Therapeutics, LLC and owns shares in the company. She has no other conflicts to report.

## FOR MORE INFORMATION

Contact S. Currie, at: scurrie@virionbx.com; and www.VirionTx.com for permission to reprint and/or distribute.