Checkpoint Modification of Early T cell Activation Provides Enhanced Anti-Tumor Activity and Survival Benefits in a Fast-Growing Preclinical Tumor Challenge Model

Mohammadi M[1], Zhou X[1], Xiang Z[1], Giles-Davis W[1], Luber A[2], Currie S[2], Ertl HCJ[1]

[1]The Wistar Institute, Philadelphia, PA, USA; [2]Virion Therapeutics, LLC, Philadelphia, PA, USA

Abstract #454

SITC 38th Annual Meeting;
San Diego, CA, USA;
November 1–5, 2023

BACKGROUND

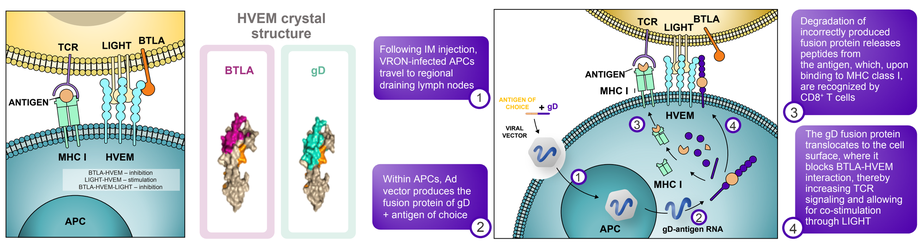
CHECKPOINT INHIBITORS

- Checkpoint inhibition by mAbs against PD-1 or 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 act earlier by enhancing 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

GLYCOPROTEIN D (gD) – A NOVEL EARLY CHECKPOINT MODIFIER (CPM)

- **Multifunctional:** Herpes simplex virus type 1 gD, when genetically expressed as a fusion protein with tumor antigens, serves as a checkpoint modifier of the BTLA-HVEM pathway, which acts **early** during T cell activation (**Figure: Early CPM – gD Mechanism of Action)**
  - T cell responses driven by an antigen in the presence of gD are more **potent** and **durable**, and are **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]

Early CPM – gD Mechanism of Action[2,3,5,6]



APC, antigen-presenting cell; BTLA, B- and T-lymphocyte attenuator; CD, cluster of differentiation; gD, glycoprotein D; HVEM, herpes virus entry mediator; IM, intramuscular; 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; VRON, Virion-specific immuno-oncology therapy.

PURPOSE

- To report the immunogenicity and efficacy of a gD-CPM immunotherapy in a highly malignant, fast-growing preclinical tumor model 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

VECTOR CONSTRUCTS

- An **HPV-16 *E7652* gene construct** was generated containing immunogenic fragments of E7, E6, E5, and E2 derived mainly from HPV-16
  - The *E7652* gene was fused into gD and then inserted into an E1-deleted, partial E3-deleted AdC6 vector (AdC6-gDE7652)
- **Control vectors** included:
  - *E7652* gene construct **without gD (AdC6-E7652)**
  - Challenge models were controlled by **AdC6 vectors expressing HIV gag fused within gD** (**AdC6-gDgag**) or with **multi-epitope melanoma antigens fused within gD** (**AdC6-gDMelapoly**)

IMMUNOLOGIC ASSESSMENTS

Immunogenicity evaluations

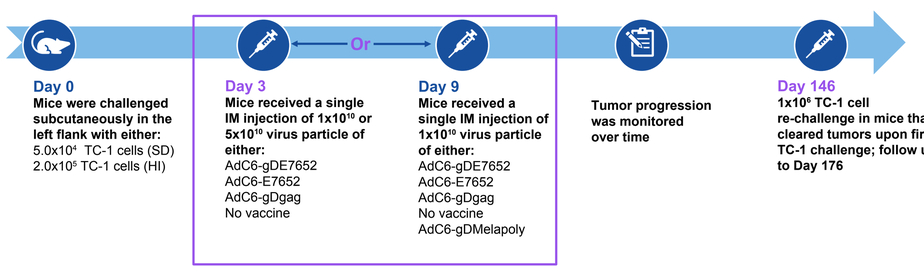
- C57Bl/6 mice (n=5 per group) were evaluated
- Mice received a single IM injection of $5x10^{10}$ virus particles comprising:
  - AdC6-gDE7652
  - AdC6-E7652; or
  - No vector (control; naïve)
- Frequencies of insert-specific CD8+ T cells were determined by ICS for IFNγ 2 weeks after injection

T cell functions and exhaustion marker evaluations

- T cells were tested by ICS, which involved peptides representing the vaccine antigen in the presence of a secretion inhibitor, followed by surface staining for T cell markers and intracellular staining for cytokines and lytic factors. Cells were then analyzed by flow cytometry
- Using ICS, T cells were analyzed from spleens or tumors of mice with growing tumors. In addition, T cells responding to an immunodominant E7 epitope were tested for differentiation and exhaustion markers (PD-1, TIM3, LAG3, CTLA-4)

EFFICACY ASSESSMENTS

- **TC-1 cells**: A tumor cell line was established from the primary lung epithelial cells of C57Bl/6 mice upon transduction with HPV-16 E6 and E7 and the v-Ha-*ras* oncogene
- **Treatment model**: C57Bl/6 mice (n=5–10 per group)

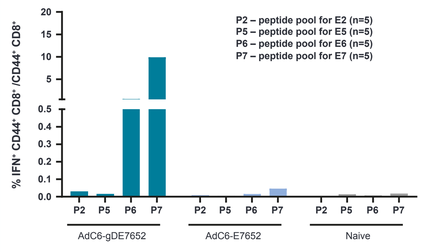


AdC6, chimpanzee adenoviral vector; gD, glycoprotein D; HI, high-dose challenge; IM, intramuscular; SD, standard-dose challenge.

RESULTS

gD-CPM (AdC6-gDE7652) Significantly Enhanced CD8+ T cell Responses to E7 by 10-fold in C57Bl/6 Mice

Response to HPV Insert (C57Bl/6 mice): CD8+ T Cells 14 Days After Treatment[7]

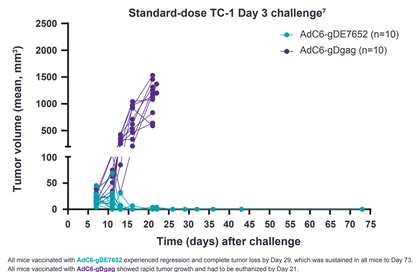


AdC6, chimpanzee adenoviral vector; CD, cluster of differentiation; CPM, checkpoint modifier; gD, glycoprotein D; HPV, human papillomavirus; IFN, interferon.

gD-CPM (AdC6-gDE7652) Resulted in Tumor Regression and Sustained Complete Tumor Loss

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

Standard-dose TC-1 Day 3 challenge[7]



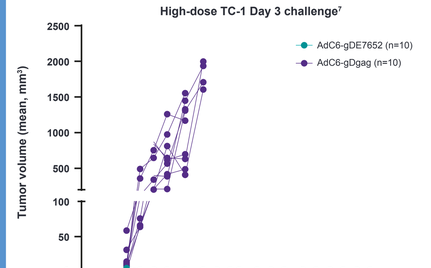
All mice vaccinated with AdC6-gDE7652 experienced regression and complete tumor loss by Day 29, which was sustained in all mice to Day 73.
All mice vaccinated with AdC6-gDgag showed rapid tumor growth and had to be euthanized by Day 21.

AdC6, chimpanzee adenoviral vector; CPM, checkpoint modifier; gD, glycoprotein D; IM, intramuscular.

gD-CPM (AdC6-gDE7652) Needed for Tumor Regression and Sustained Tumor Loss

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

High-dose TC-1 Day 3 challenge[7]

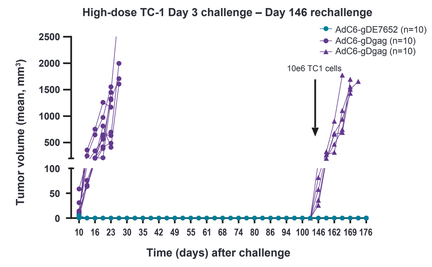


All mice vaccinated with AdC6-gDE7652 experienced regression and complete tumor loss by Day 13, which was sustained in 10 of 10 (100%) mice through Day 67.
All mice vaccinated with AdC6-gDgag showed rapid tumor growth and had to be euthanized by Day 27.

gD-CPM (AdC6-gDE7652) Shows Complete Protection Against High-Dose Tumor Re-Challenge

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

High-dose TC-1 Day 3 challenge – Day 146 rechallenge

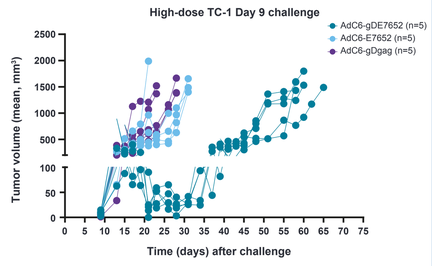


AdC6, chimpanzee adenoviral vector; CPM, checkpoint modifier; gD, glycoprotein D; IM, intramuscular.

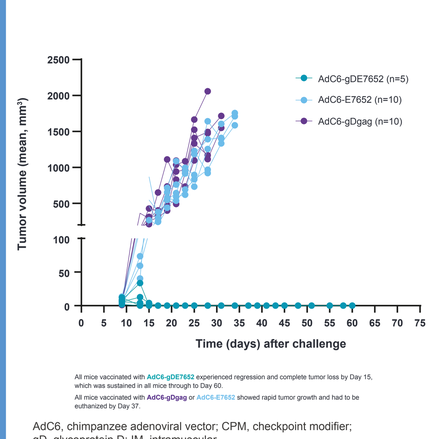
gD-CPM (AdC6-gDE7652) Delays Tumor Progression and Doubles Survival Time

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

High-dose TC-1 Day 9 challenge



AdC6, chimpanzee adenoviral vector; CPM, checkpoint modifier; gD, glycoprotein D; IM, intramuscular.



All mice vaccinated with AdC6-gDE7652 experienced regression and complete tumor loss by Day 15, which was sustained in all mice through to Day 60.
All mice vaccinated with AdC6-gDgag or AdC6-E7652 showed rapid tumor growth and had to be euthanized by Day 37.

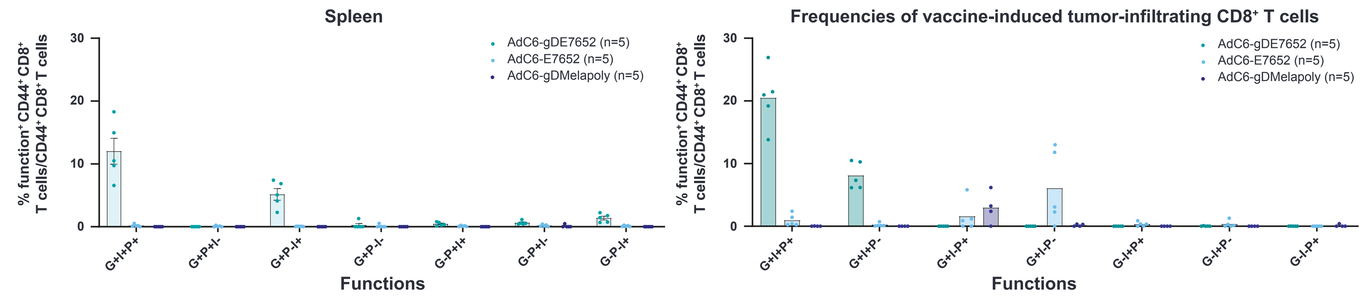
AdC6, chimpanzee adenoviral vector; CPM, checkpoint modifier; gD, glycoprotein D; IM, intramuscular.

TC-1 CHALLENGE MODEL

In mice vaccinated with gD-CPM (AdC6-gDE7652):

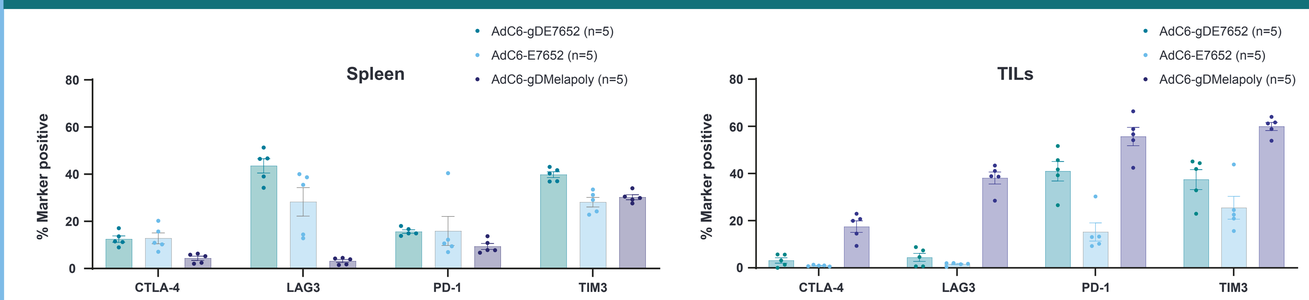
- **Three days post TC-1 transplantation:**
  - 25/25 (100%) mice cleared their tumors, remained tumor free; all were alive at study end vs;
  - 25/25 (100%) of control-vaccinated mice showed rapid tumor progression; none were alive at study end
  - AdC6-gDE7652-vaccinated mice that cleared their tumors (n=10) remained tumor free upon a high-dose TC-1 rechallenge
- **Nine days after TC-1 challenge:**
  - Tumor growth was delayed, and survival times doubled vs non-gD AdC6-E7652-vaccinated animals (~30 vs ~60 days)

gD-CPM (AdC6-gDE7652) Produced a Higher Percentage of Polyfunctional T Cells Within Spleens and TILs



AdC6, chimpanzee adenoviral vector; CD, cluster of deafferentation; CPM, checkpoint modifier; I, IFNγ; G, granzyme B; gD, glycoprotein D; P, perforin; TILs, tumor-infiltrating lymphocytes.

gD-CPM (AdC6-gDE7652) Produced a Lower Percentage of Exhaustion Markers on T Cells Within TILs



AdC6, chimpanzee adenoviral vector; CPM, checkpoint modifier; CTLA-4, cytotoxic T lymphocyte antigen-4; gD, glycoprotein D; LAG3, lymphocyte-activation gene 3; PD-1, programmed death 1; TILs, tumor-infiltrating lymphocytes; TIM3, T cell immunoglobulin and mucin domain-containing protein 3.

CONCLUSIONS

- In a fast-growing tumor cell challenge model, the addition of the CPM of early T cell activation, gD, when fused to HPV-16 proteins 2, 5, 6, and 7, demonstrated:
  - Markedly improved immunogenicity (~10-fold)
  - Enhanced tumor clearance, with improved progression-free survival, upon TC-1 rechallenge
  - Higher percentages of tumor antigen-specific T cells within spleens and tumors with polyfunctional activity and lower levels of exhaustion marker expression
- A clinical trial evaluating a gD-based IM vaccine for advanced solid tumors is in development
  - A Phase 1B study in infectious disease using gD is currently enrolling (NCT06070051)

REFERENCES

1. Lee J, et al. *For Immunopathol Dis Therap* 2015;6:7–17.
2. Xiang ZQ, et al. ASCO-SITC Clinical Immuno-Oncology Symposium 2020; Abstract #71.
3. Virion Therapeutics, September 2021. Data on file.
4. Zhang Y, et al. *Cancer Cell* 2017;32:377–91.
5. Stiles KM, et al. *J Virol* 2010;84:11646–60.
6. Luber A, et al. ESMO TAT 2021; Abstract 143.
7. Currie S, et al. ESMO TAT 2023; Poster #36P.

ABBREVIATIONS

Ad, adenoviral; AdC6, chimpanzee adenoviral vector; APC, antigen-presenting cell; BTLA, B- and T-lymphocyte attenuator; CD, cluster of differentiation; CPM, checkpoint modifier; CTLA-4, cytotoxic T lymphocyte antigen-4; G, granzyme B; gD, glycoprotein D; HI, high-dose challenge; HIV gag, human immunodeficiency virus group-specific antigen; HPV, human papillomavirus; HVEM, herpes virus entry mediator; ICS, intracellular cytokine staining; IFNγ, interferon-gamma; IM, intramuscular; LAG3, lymphocyte-activation gene 3; 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; P, perforin; PD-1, programmed death 1; *ras*, rat sarcoma; SD, standard-dose challenge; TIM3, T cell immunoglobulin and mucin domain-containing protein 3; TILs, tumor-infiltrating lymphocytes; TCR, T cell receptor; VRON, Virion-specific immuno-oncology therapy.

ACKNOWLEDGMENTS

We would like to thank the Ertl laboratory at The Wistar Institute, and, specifically, the following:

- Mohsen Mohammadi
- Amara Saha
- Xiang Zhou

Medical writing assistance was provided by Cynthia Umukoro, Ph.D., and Alison Lovibond, Ph.D., of BOLDSCIENCE Inc., and was funded by Virion Therapeutics.

Funding was provided by Virion Therapeutics, LLC for this study.

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@viriontx.com; and www.VirionTx.com for permission to reprint and/or distribute.