ABSTRACT OS-0490 Oral Presentation

Novel Early Checkpoint Modifier Demonstrates Broadened and Enhanced CD8⁺ T Cell Responses Across Multiple Preclinical Studies

Luber A¹, Currie S¹, Ertl HCJ²
¹Virion Therapeutics, Newark, DE; ²The Wistar Institute, Philadelphia, PA

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Author Disclosures

• Co-founder of Virion Therapeutics

• Advisor roles with:
  • Freelance, Inc
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Background: Use of an Early Checkpoint Modifier as a Vaccine Adjuvant

• **Traditional vaccine adjuvants:**
  • **Purpose:** Enhance, prolong or broaden immune responses to an antigen, delivered by a vaccine
  • **Function:** Increase adaptive responses by activating the innate immune system resulting in inflammation-associated side effects
  • **Adjuvants in use:** Mineral salts (aluminum hydroxide), liquid particles (MF59), microparticles (polylactic acid), immune modulators (PAMPS, e.g., dsRNA)

• **Herpes simplex virus (HSV-1) glycoprotein D (gD) adjuvant:**
  • Checkpoint modifier of early CD8+ T cell activation
  • Lowers the activation threshold – producing potent, prolonged, broad and highly functional antigen-specific CD8+ T cell responses

PAMP, pathogen-associated molecular patterns.

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Herpes Simplex Virus Glycoprotein D
The Genetically Encoded Checkpoint Modifier Adjuvant

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.

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

Within APCs, Ad vector produces the fusion protein of gD + antigen of choice.

Following IM injection, VRON-infected APCs travel to regional draining lymph nodes.

APC, antigen presenting cell; BTLA, B- and T-lymphocyte attenuator; 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; pol, polymerase; TCR, T cell receptor; VRON, Virion specific I/O therapy.

Methods: Basic Experimental Design

• Step 1
  • Clone antigen into the C-terminus of gD

• Step 2
  • Express the gD-antigen fusion protein by an adenovirus vector

• Step 3
  • Test the vector expressing the fusion protein compared with a vector expressing antigen only
    • In vitro QC (e.g. protein expression)
    • CD8⁺ T cell responses
      • Magnitude
      • Breadth
      • Duration
    • B cell responses
    • Vaccine efficacy studies

• Antigens tested for immunogenicity
  • HPV-16 – E7
  • HBV – core & polymerase
  • Melanoma – multi-epitope vaccine (Melapoly)
  • SARS-CoV2 – nucleoprotein
  • HIV – gag

• Vaccine efficacy studies
  • HPV-16 E7 – transgenic mouse model
  • HBV – AAV8-1.3HBV
  • Melanoma – transplantable tumor model (B16.F10)
Checkpoint Modifier HSV gD Enhances CD8+ T Cell Responses

**HBV**
(sequences of N-terminus of polymerase)

**MELANOMA**
(epitopes from Trp1, Trp2, gp100 and Braf)

**HPV**
(sequences of early oncoproteins)

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Results reported as medians. HBV and HPV analysis via one-way ANOVA; Melanoma via two-ANOVA with Sidak correction. *p-value between 0.001–0.01; **p-value between 0.001–0.01; ***p-value >0.001. NBgD has a deletion to gD eliminating the herpes virus entry mediator binding site.

HBV, hepatitis B virus (gD-polN); HPV, human papillomavirus (gD-E7/6/5 detox); Melapoly: melanoma antigens (Trp-1, Trp-2, gp100, mutated BRAFV600E, antigen)

CD8, Cluster of Differentiation 8; gD, glycoprotein D; IFN, interferon; tet, tetramer.
**Checkpoint Modifier HSV gD Enhances Both B and T Cell Responses (HIV gag)**

CD8+ T cell responses to vectors expressing antigens fused to gD*

- gD
- gag
- gD gag

Gag-specific antibody response after immunization with AdC68 vectors expressing gD, Gag, or gD-Gag

* Mice were immunized i.m. either with 100 μg DNA or 1x10^10 virus particles of AdC68.
CD8^+ T Cell Responses

**MELANOMA**
- **Ag alone** (Melapoly)
- **gD + Ag** (Melapoly)

**HBV**
- **Single dose gD + Ag**
  (AdC6-gPolN)
- **Total IFN^+CD8^+**/CD8^+ = 4.4%
  # Peptides: 18

**COVID-19 (Nucleoprotein)**
- **CD8^+ T cell responses after priming**
  - **Ag alone** (AdC6-N)
    2x10^10 vp
  - **gD + Ag** (AdC6-gDN)
    2x10^10 vp
  - **gD + Ag** (AdC6-gDN)
    1x10^10 vp


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Checkpoint Modifier HSV gD Enhances Vaccine Efficacy

**Enhanced HBV virus decline in mice**

- **Naive**
  - HBV
- **VRON-0200**

Enhanced HBV virus decline in mice.

**Anti-tumor activity in mice**

- **Naive**
- **gD-E7**
- **gD-E7/Melapoly**

Anti-tumor activity in mice.

**Improved survival in mice**

- **Naive**
- **gD + Ag**
- **Ag alone**

Improved survival in mice.

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**References**


**Abbreviations**

- HBV, hepatitis B virus; HBV2, HBV core & pol; VRON-0200, gD fused to HBV core & pol; HPV, human papillomavirus; Ag, antigen; gD, glycoprotein D; Melapoly, melanoma antigens (Trp-1, Trp-2, gp100, mutated BRAFv600E); E7, HPV E7 oncoprotein.

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**Figure Legends**

- Serum HBV DNA (copies/mL) over time for naive, HBV2, and VRON-0200 groups.
- Anti-tumor activity with gD-E7 and gD-E7/Melapoly compared to naive.
- Improved survival with gD + Ag and Ag alone compared to naive.

**Statistical Significance**

- Melapoly versus naive: p=0.0001; gD-Melapoly versus naive: p=0.0001; Melapoly versus gD-Melapoly: p=0.0018.
Conclusions

These preclinical data, using various infectious disease and cancer antigens/animal models, demonstrate the benefits of using a genetically encoded checkpoint modifier as an adjuvant:

- **Multifunctional:** Most adjuvants only increase the magnitude of response; HSV gD does more
  - **Key addition:** Broadens CD8+ T cell responses to include sub-dominant epitope recognition
- **Safety profile:** Low risk for “off target” adverse events
  - **gD adjuvant:** Only expressed locally at the site of injection, and in draining lymph nodes
- **Inexpensive:** No additional costs over that of the adenovirus vector alone
- **Scalability:** Millions of SARS-CoV-2 adenoviral vaccines produced

Initial gD-containing vaccine against chronic HBV infection to enter the clinic at end of 2022
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**FOR MORE INFORMATION**: Contact H. Ertl at: ertl@wistar.org