Novel checkpoint modifier lowers T cell activation threshold and enhances and broadens vaccine-induced responses to chronic viral infections

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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

- Therapeutic vaccines have historically produced inadequate immune responses for chronic infectious diseases

- **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 (e.g. PAMPS, 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
Herpes Simplex Virus Glycoprotein D
The Genetically Encoded Checkpoint Modifier Adjuvant1,2

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

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

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

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

APC, antigen presenting cell; BTLA, B-and T-lymphocyte attenuator; gD, glycoprotein D; HVEM, herpes virus entry mediator; IM, intramuscular; LIGHT, lymphotixin-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
  • SARS-CoV2 – nucleoprotein
  • HIV – gag

• **Vaccine efficacy studies**
  • HPV-16 E7 – transgenic mouse model
  • HBV – AAV8-1.3HBV
Checkpoint Modifier HSV gD Enhances CD8+ T Cell Responses

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

HBV, hepatitis B virus (gDHBV2 – gD with N- and C-terminus of polymerase and core antigens); HPV, human papillomavirus (gD-E7/6/5 detox); gD, glycoprotein D; IFN, interferon; NBgD, non-binding gD.
Checkpoint Modifier HSV gD Enhances Both B and T Cell Responses (HIV gag)

CD8+ T cell responses to vectors expressing antigens fused to gD*1

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

*<p><sup>*Mice were immunized by i.m. either with 100 µg DNA or 1x10<sup>15</sup> virus particles of AdC68. 1. Lasaro M, et al. Nat Med 2008;14:205–12.

**Ertl HCJ, et al. Oral presentation at ID Week 2022: Oral Presentation #869
**Checkpoint Modifier HSV gD Broadens CD8+ T Cell Responses**

**HBV**

**CD8+ T cell responses after priming, Week 8**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>%IFN+CD44+CD8+/CD44+CD8+</th>
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<tbody>
<tr>
<td>HBV2</td>
<td>10</td>
</tr>
<tr>
<td>Core</td>
<td>0.8</td>
</tr>
<tr>
<td>PolC</td>
<td>0.6</td>
</tr>
<tr>
<td>PolN</td>
<td>1.0</td>
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</tbody>
</table>

- Red: gDHBV2
- Blue: HBV2
- Green: gag
- Gray: naive

**All data from splenocytes**


HBV, hepatitis B virus (gDHBV2 – gD with N- and C-terminus of polymerase and core antigens)

**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

**Ertl HCJ, et al. Oral presentation at ID Week 2022: Oral Presentation #869**
Enhanced HBV virus decline in mice

Anti-tumor activity & survival in mice

AAV8-1.3HBV – 1 x 10^9 vg IV at week 0 (n=10 per group)


HBV, hepatitis B virus; HBV2, HBV core & pol; VRON-0200, gD fused to HBV core & pol; HPV, human papillomavirus; Ag, antigen; gD, glycoprotein D; E7, HPV E7 oncoprotein.; ***p<0.001

Ertl HCJ, et al. Oral presentation at ID Week 2022: Oral Presentation #869
These data demonstrate the benefits of using a genetically encoded checkpoint modifier as an adjuvant in various infectious disease antigens/animal models:

- **Multifunctionality**: Most adjuvants only increase the magnitude of responses; HSV gD does more
  - **Key addition**: Broadens CD8+ T cell responses to sub-dominant epitopes
- **Safety**: Low risk for “off target” adverse events
  - **gD adjuvant**: Only expressed locally at the site of injection, and in draining lymph nodes
- **Affordability**: No additional costs over that of the vaccine alone

**First-in-human gD-containing vaccine against chronic HBV infection to enter the clinic H1 2023**
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