ID Week Oral Presentation #869

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
  - Takeda
  - Biogen (board)
  - Regenxbio
  - Ring Therapeutics (board)
  - Canine Rabies Treatment Initiative (board)

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

PAMP, pathogen-associated molecular patterns; dsRNA, double-stranded RNA

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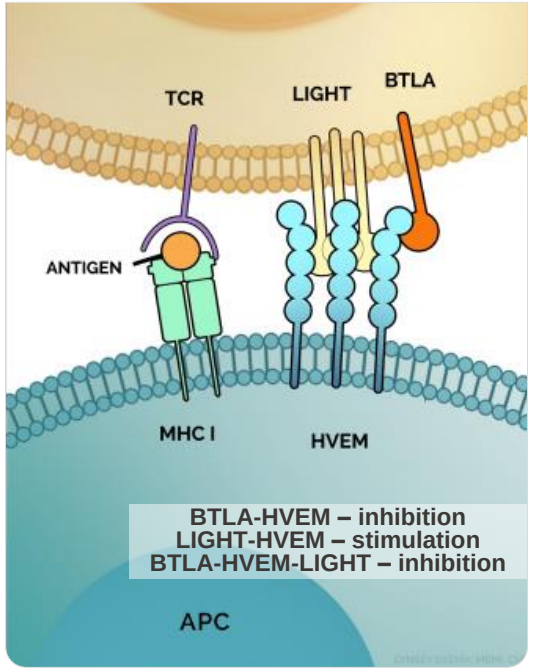
Herpes Simplex Virus Glycoprotein D

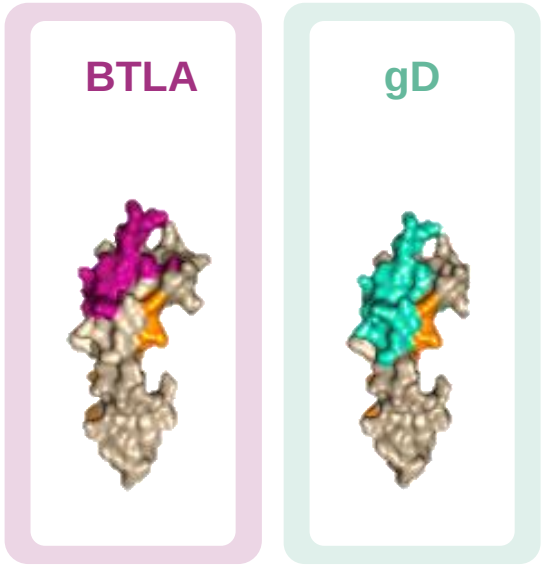
The Genetically Encoded Checkpoint Modifier Adjuvant[1,2]

HVEM Complex in Regulating T cell Activation

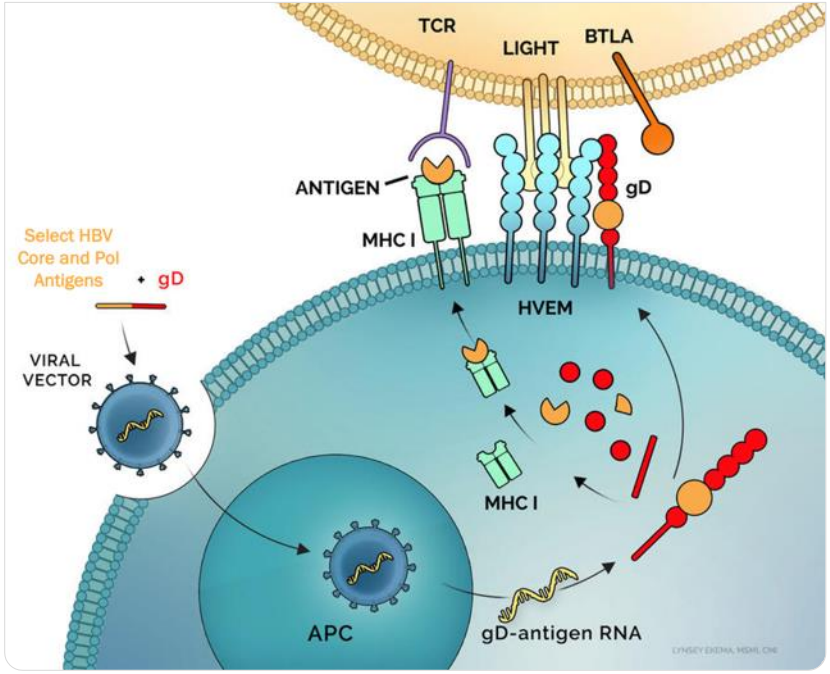


gD & BTLA Share HVEM Binding Site

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

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, 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.

1. Xiang ZQ, et al. ASCO-SITC Clinical Immuno-Oncology Symposium 2020, Abstract No. 71; 2. Stiles KM, et al. J Virol. 2010;84:11646–60.

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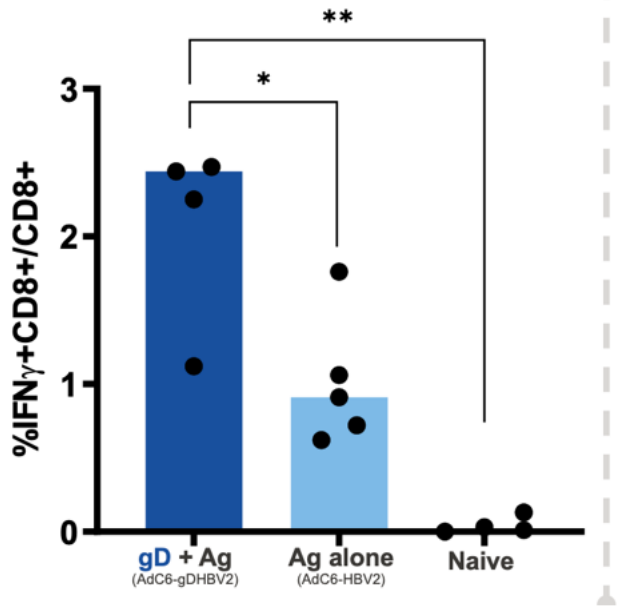
Methods: Basic Experimental Design

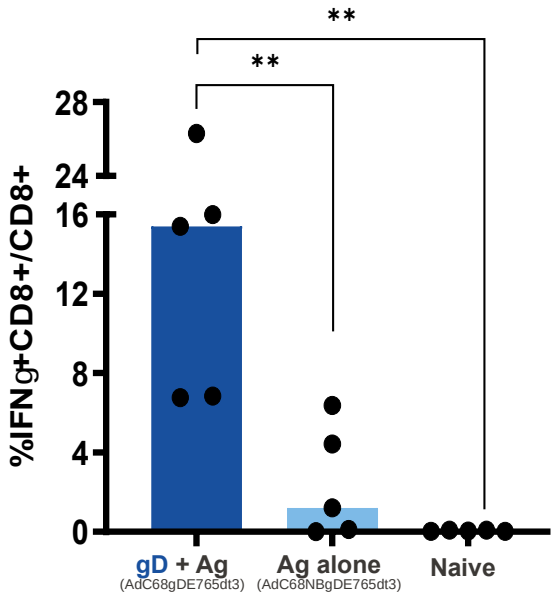
- **Step 1**
  - Clone antigen into the C-terminus of gD
- **Step 2**
  - Express the gD-antigen fusion protein by an adenovirus vector
- **Step 3**
  - Test the vector expressing the fusion protein compared with a vector expressing antigen only
    - In vitro QC (e.g., protein expression)
    - $CD8^+$ 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

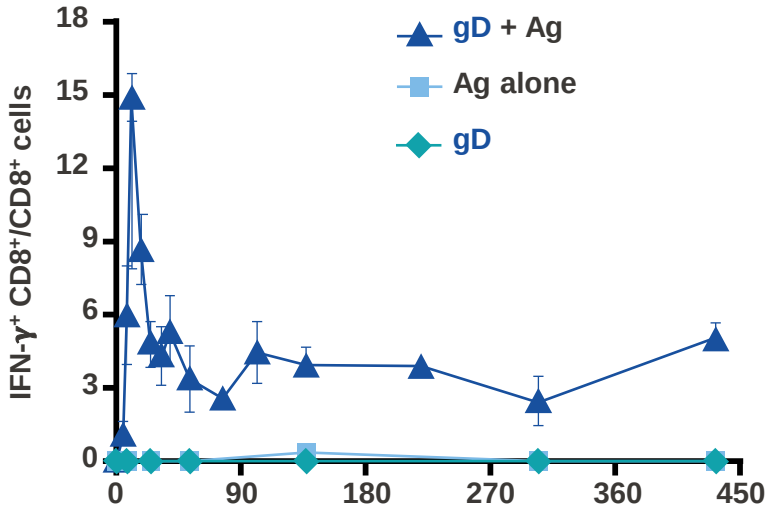
HBV





Response to HBV polymerase & core[1]

HPV



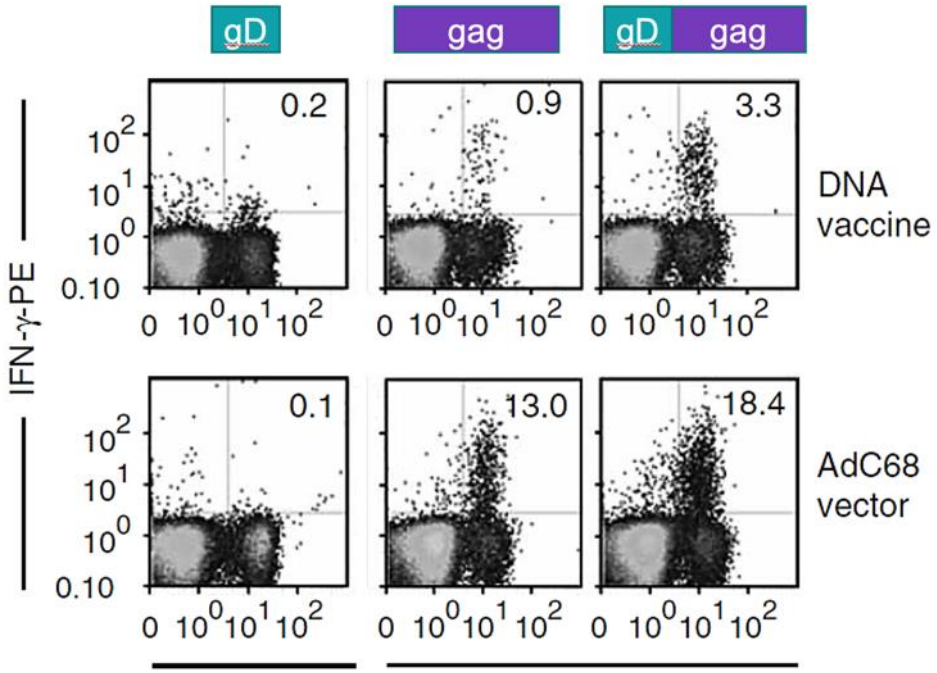
Response to immunodominant epitope within E7[2,3]

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.

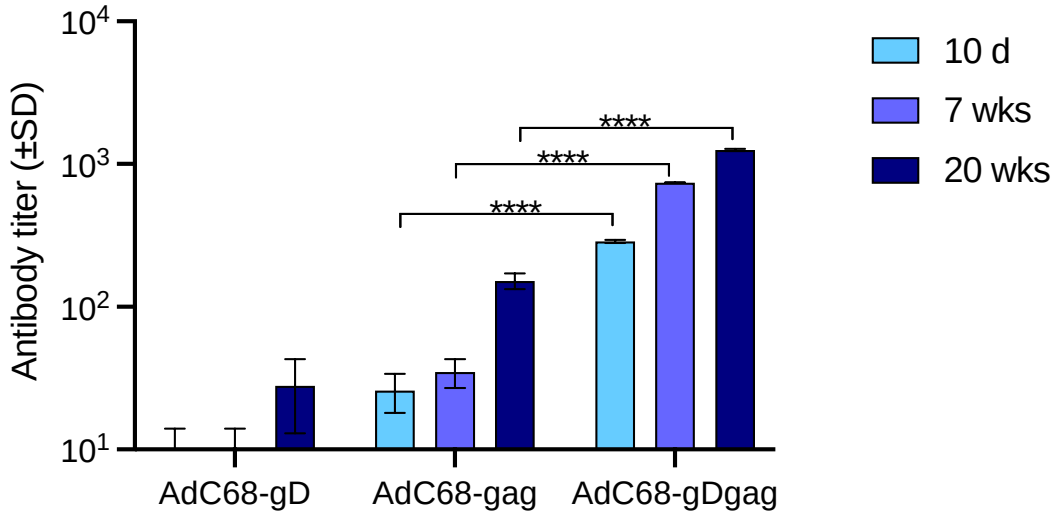
1.Hasanpourghadi M, et al. EASL 2021: Abstract OS-2478; 2. Zhang Y, et al. J Immunol 2014;193:1836–46; 3. Xiang Z, et al. ASCO-SITC Clinical Immuno-Oncology Symposium 2020: Abstract #71;
4. Hasanpourghadi M, et al. Curr Trends Microbiol 2021; 15:1-28.
HBV, hepatitis B virus (gDHBV2 – gD with N- and C-terminus 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[1]



**** p<0.0001

*Mice were immunized by i.m. either with 100 µg DNA or 1x10[10] virus particles of AdC68.

1. Lasaro M, et al. Nat Med 2008;14:205–12.

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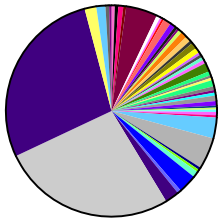
Checkpoint Modifier HSV gD Broadens CD8+ T Cell Responses

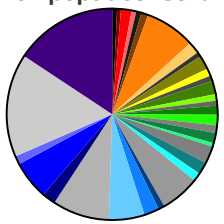
HBV

CD8+ T cell responses after priming, Week 8

Proportion of responses to Individual peptides

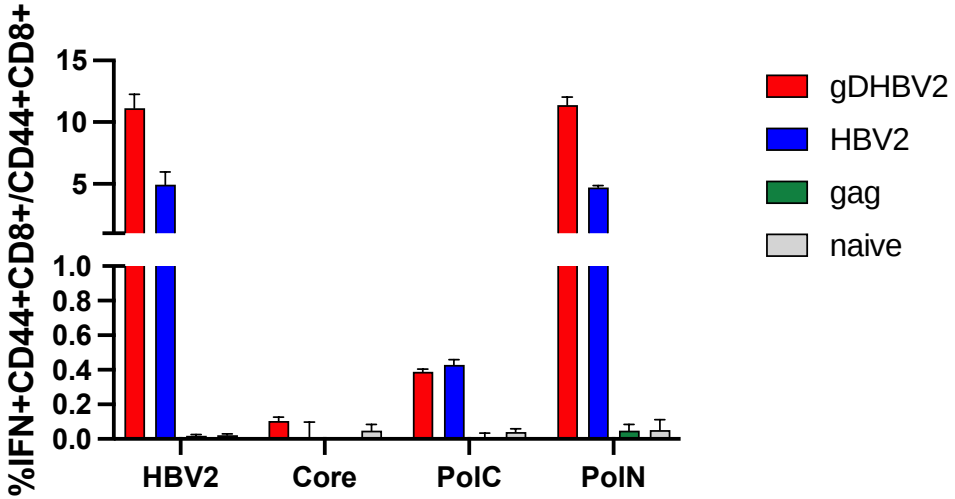


Total response to 51 peptides: 33%



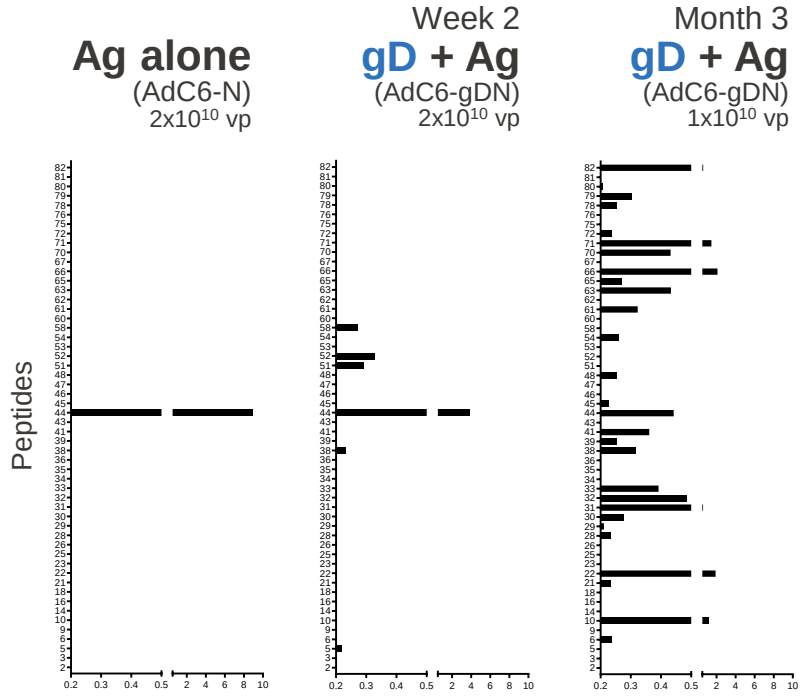
Total response to 33 peptides: 19%

Response to pools



COVID-19 (Nucleoprotein)

CD8+ T cell responses after priming



All data from splenocytes

Hasanpourghadi M, et al. Virol J 2021;18:242–56; Novikov M et al. bioRxiv 2022; doi.org 10.1101.

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

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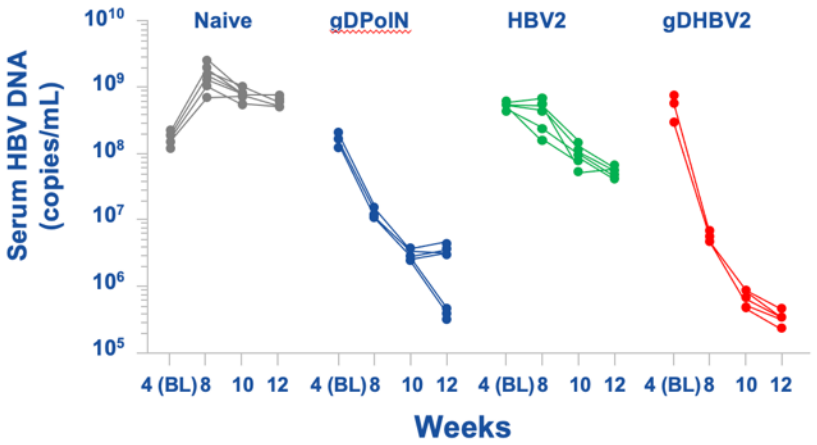
Checkpoint Modifier HSV gD Enhances Vaccine Efficacy

VRON-0200 HBV

Enhanced HBV virus decline in mice[1]



Correlations

r-values	CD8	CD4	vgc
CD8		-0.020	-0.491
CD4	-0.020		0.157
vgc	-0.491	0.157	

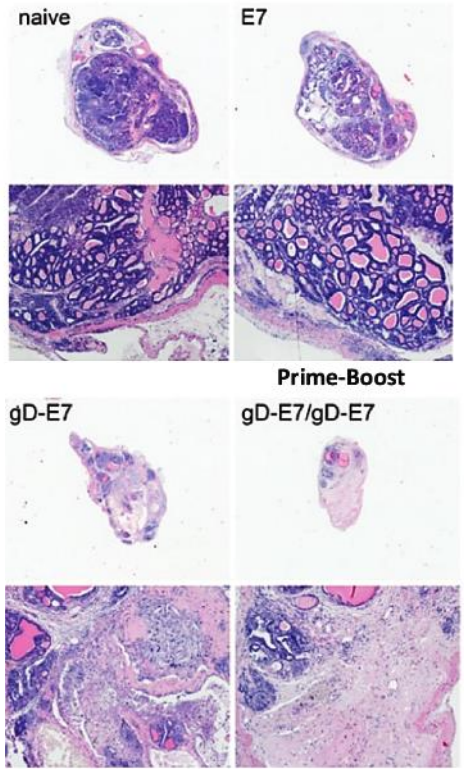
P-values	CD8	CD4	vgc
CD8		0.914	0.004
CD4	0.914		0.389
vgc	0.004	0.389	

Spearman Rank Correlation

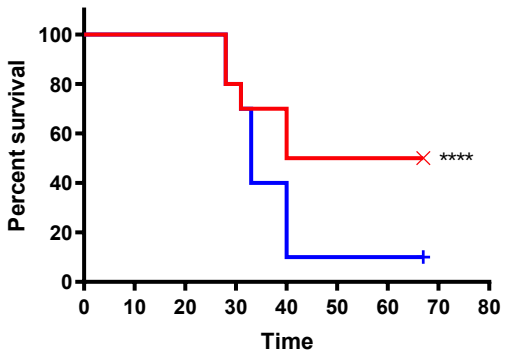
AAV8-1.3HBV – 1 x 10^9 vg IV at week 0 (n=10 per group)

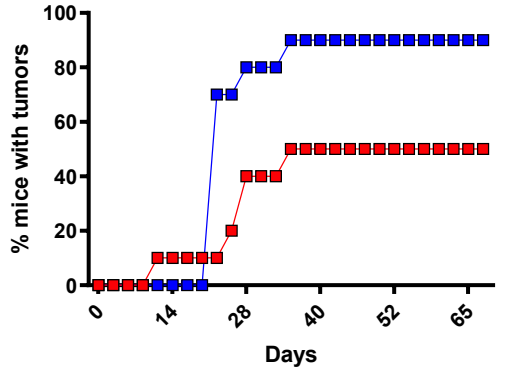
HPV

Anti-tumor activity & survival in mice[2,3]



HPV TC-1 Challenge Model





1. Hasanpourghadi M, et al. EASL 2021: Abstract OS-2478; 2. Lasaro M, et al. Mol Ther 2011;19:1727–36; 3. Xiang Z, et al. ASCO-SITC Clinical Immuno-Oncology Symposium 2020: Abstract #71
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

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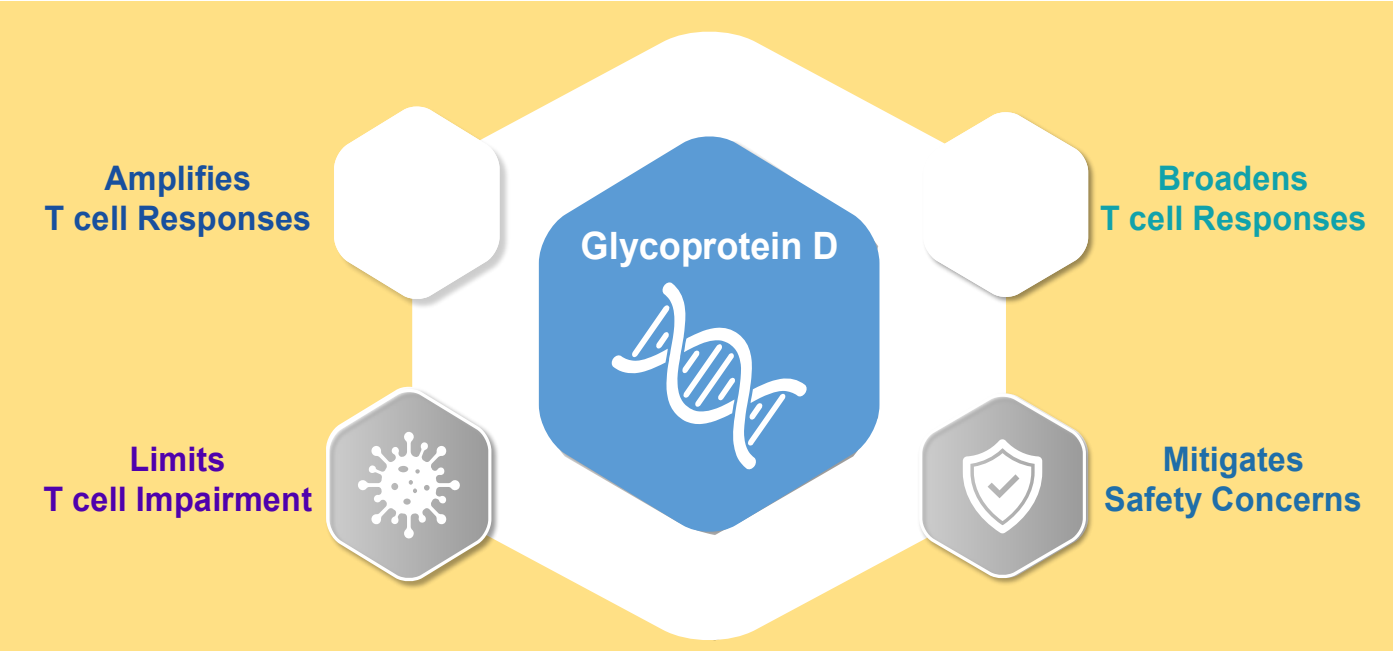
Take-away Points (Conclusion)

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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- **Melanoma model:** Ying Zhang
- **HBV model:** Mohadeseh Hasanpourghadi
- **COVID-19 model:** Mohadeseh Hasanpourghadi, Mikhail Novikov, Zhiquan Xiang
- **Vector production:** Xiang Zhou, Robert Ambrose, Dakota Newman

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