Intrahepatic CD8+ T cells correlate with significant declines in HBV viral load and S antigen following a single vaccination with VRON-0200 in an AAV mouse model

M HASANPOURGHADI,[1] A LUBER,[2] S CURRIE,[2] X ZHOU,[1] and HCJ ERTL[1]

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

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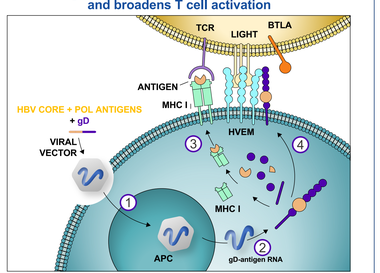
INTRODUCTION

- Despite HBV preventative vaccines, chronic HBV infection remains a high unmet need:
  - Most of the ~296M people with chronic HBV go undiagnosed and untreated[1]
  - 1 in 4 people with chronic HBV will die prematurely from liver cirrhosis, HCC, or liver failure[2]
  - Antivirals rarely achieve functional cure and require lifelong therapy[3,4]
- Chronic HBV infection is characterized by highly impaired and exhausted CD8+ T cells
  - Current investigational treatments target viral and antigen suppression or immune modulation in the hope of restoring the same immune response that failed previously
  - Restoring the same failed CD8+ T cell response is limited by:
    - Irreversible epigenetic changes and terminal exhaustion of CD8+ T cells, which can blunt antiviral, small molecule, and checkpoint inhibitor interventions
    - Vaccines aimed at:
      - stimulating memory CD8+ T cells are unable to expand those that are already exhausted
      - naïve HBV-specific CD8+ T cells have a limited number of available naïve cells, since most people with a prolonged infection have already had these cells activated
- AdC6-gDHBV2 (VRON-0200) is a therapeutic vaccine for HBV functional cure that contains a genetically encoded checkpoint modifier, glycoprotein D (gD),[5–8] designed to enhance, broaden, and prolong CD8+ T cell responses to HBV core and polymerase
  - gD lowers the activation threshold by inhibiting binding of the B and T lymphocyte attenuator (BTLA) to the herpes virus entry mediator (HVEM) on APCs (**Fig 1**)
    - This results in T cell activation to sub-dominant epitopes[9]
      - These T cells are generally not stimulated by an infection,[10,11] are less susceptible to suppression or exhaustion, and thereby remain functional and work for longer periods of time[12]
- We hypothesized that by eliminating HBV-infected hepatocytes, S antigen declines would occur without directly targeting S by vaccine-induced T cells

AIM

To evaluate the immunogenicity and correlations between intrahepatic CD8+ T cell responses and HBV viral load and S antigen declines in blood in an AAV mouse model following a single i.m. injection of AdC6-gDHBV2

Figure 1. Early checkpoint modifier – gD mechanism of action[5–8]



APC, antigen-presenting cell; BTLA, B and T lymphocyte attenuator; CD, cluster of differentiation; gD, glycoprotein D; HBV, hepatitis B virus; HVEM, herpes virus entry mediator; i.m., 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.

METHODS: IMMUNOGENICITY

- A chimpanzee adenovirus vector of serotype 6 (AdC6) was used to express sequences of:
  - Polymerase and core within gD (AdC6-gDHBV2)
  - Polymerase and core without gD (AdC6-HBV2)
  - Controls – no insert, HIV gag (without gD), or unvaccinated animals
- C57Bl/6 mice (groups of 5–10 animals) received a single i.m. dose of 1 x 10^{10} vp of AdC6-gDHBV2, Ad6C-HBV2, or a control treatment
- The frequencies of insert-specific CD8+ T cells were determined in blood and spleen by intracellular cytokine staining (ICS) for IFNγ 4 and 8 weeks after injection, respectively
- 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 IFNγ) 8 weeks after treatment
- Immunogenicity was tested in the presence and absence of an AAV8-1.3HBV challenge (described below)
- In the AAV8-1.3HBV challenge studies:
  - T cells from livers and spleens were tested at Week 12 post-vaccination
  - Frequencies and phenotype of CD8+ T cells to one immunodominant epitope within the N-terminus of Pol were tested for by staining with an MHC I tetramer and antibodies to various markers

METHODS: EFFICACY

AAV8-1.3HBV viral dynamics study

- C57Bl/6 mice (n=5–10) were challenged intravenously via their tail vein with 1 x 10^9 (low-dose) or 1 x 10^{10} (high-dose) viral genomes (vg) of AAV8-1.3HBV and 4 weeks later received a single i.m. injection of 1 x 10^{10} vp of AdC6-gDHBV2, AdC6-HBV2, or no vaccination
- HBV DNA viral load and HBsAg titers (high-dose AAV8-1.3HBV study only) were evaluated in sera at different time points out to 16 weeks post-vaccination by qPCR and ELISA, respectively; pre- and post-vaccination serum HBV DNA (copies/mL) and HBsAg levels (IU/mL) are reported

Correlations between intrahepatic lymphocytes and splenocytes and viral dynamics

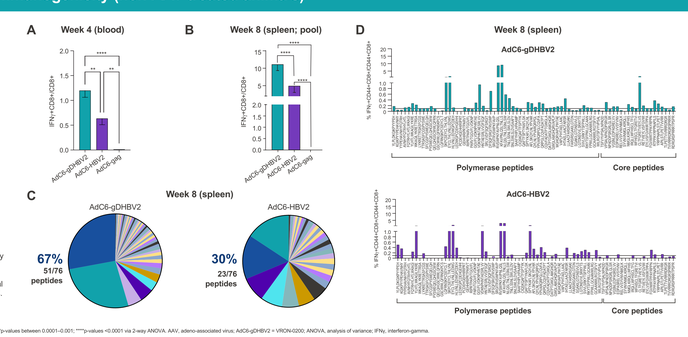
- The relationship between HBV DNA viral load and HBsAg titers in sera at Week 16 post vaccination and the frequencies of vaccine-induced IFNγ-producing CD8+ T cells in liver and spleens were assessed by Spearman's rank correlations in high-dose AAV8-1.3HBV-treated animals

RESULTS

IMMUNOGENICITY: NON-AAV-TREATED ANIMALS

- AdC6-gDHBV2-vaccinated animals had significantly higher frequencies of vaccine-induced IFNγ-producing CD8+ T cells in blood (**Fig 2A**) and were >2x as frequent, recognizing a higher number of epitopes in spleens (**Fig 2B/C**) than those observed in AdC6-HBV2-treated mice
  - Responses to some individual peptides were comparable between treatments, while others were only elicited by the AdC6-gDHBV2 vaccine (**Fig 2D**)
  - No T cell responses were observed in splenocytes of AdC6-HIV gag control-vaccinated animals (data not shown)

Figure 2. Immunogenicity (non-AAV treated animals)

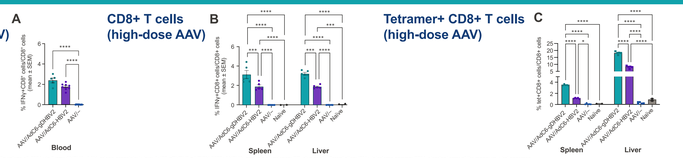


**p-values between 0.001–0.01; ***p-values between 0.0001–0.001; ****p-values <0.0001 via 2-way ANOVA. AAV, adeno-associated virus; AdC6-gDHBV2 = VRON-0200; ANOVA, analysis of variance; IFNγ, interferon-gamma.

IMMUNOGENICITY: AAV-TREATED ANIMALS

- Similar frequencies of vaccine-induced IFNγ-producing CD8+ T cells were observed in the blood of AdC6-gDHBV2- and AdC6-HBV2-vaccinated animals (low-dose AAV8-1.3HBV study) (**Fig 3A**)
- Significantly higher vaccine-induced IFNγ-producing CD8+ T cells were observed in the livers and spleens of AdC6-gDHBV2-vaccinated animals by both ICS and tetramer stain (**Fig 3B/C**) (high-dose AAV8-1.3HBV-treated groups)
- CD4+ T cell responses in vaccinated animals in both AAV8-1.3HBV and non-AAV8-1.3HBV studies were minimal or non-detectable in all treatment groups (data not shown)

Figure 3. Immunogenicity (AAV-treated animals)

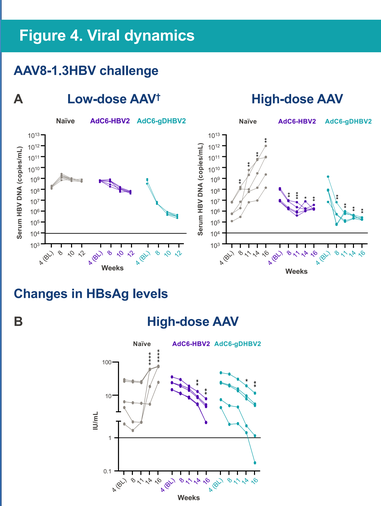


***p-values between 0.0001–0.001; ****p-values <0.0001 via 2-way ANOVA. AAV, adeno-associated virus; AdC6-gDHBV2 = VRON-0200; ANOVA, analysis of variance; CD, cluster of differentiation; IFNγ, interferon gamma; SEM, standard error of the mean; vg, viral genomes.

ANTIVIRAL ACTIVITY

- Following a single i.m. injection:
  - *AdC6-gDHBV2:* HBV viral load declines of >3 log10 copies/mL were observed at Week 8/12 post-vaccination in both the low- (n=10) and high-dose (n=5) AAV8-1.3HBV mice upon vaccination (**Fig 4A**)
  - *AdC6-HBV2 and unvaccinated (control) mice:* In contrast, these mice experienced <1.5 log10 copies/mL, and no change or increase in viral loads, respectively (**Fig 4A**)
- Levels of HBsAg showed:
  - Increases in unvaccinated (control) mice
  - Significant decreases by Week 12 after vaccination in both the AdC6-gDHBV2- and AdC6-HBV2-treated groups (**Fig 4B**)
- HBsAg antibodies at Week 16:
  - Detected in all (100%) of AdC6-gDHBV2-vaccinated mice (n=5), albeit at low levels
  - In contrast, only 1 of 5 (20%) of AdC6-HBV2-vaccinated animals had a detectable level (data not shown)

Figure 4. Viral dynamics



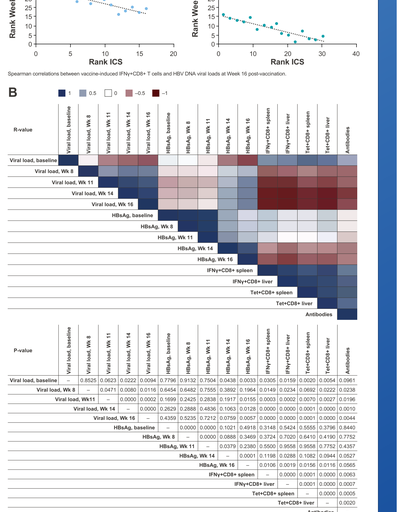
**p-values between 0.001–0.01; ***p-values between 0.0001–0.001; ****p-values <0.0001 via 2-way ANOVA.
†Presented in part: EASL 2021: Abstract OS-2478.

AAV, adeno-associated virus; AdC6-gDHBV2 = VRON-0200; ANOVA, analysis of variance; BL, baseline; HBsAg, hepatitis B surface antigen; vg, viral genomes.

CORRELATIONS BETWEEN INTRAHEPATIC AND SPLENIC LYMPHOCYTES AND VIRAL DYNAMICS

- At Week 16, the frequencies of vaccine-induced HBV-specific CD8+ T cells in spleens or livers strongly inversely correlated with HBV DNA viral load (**Fig 5A**)
  - Other correlations are shown in **Fig 5B**
  - HBsAg levels inversely correlated with CD8+ T cell frequencies in both the liver and spleen and directly correlated with HBV DNA copies/mL in the blood

Figure 5. Correlations between Intrahepatic lymphocytes and splenocytes and viral dynamics



Spearman correlations between parameters via heatmap (top) and p-values for respective relationships (bottom).
AAV, adeno-associated virus; CD, cluster of differentiation; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ICS, intracellular cytokine staining; IFNγ, interferon gamma; tet, tetramer; vg, viral genomes; Wk, week.

P-value	Viral load, baseline	Viral load, Wk 8	Viral load, Wk 11	Viral load, Wk 14	Viral load, Wk 16	HBsAg, baseline	HBsAg, Wk 8	HBsAg, Wk 11	HBsAg, Wk 14	HBsAg, Wk 16	IFNγ+CD8+ spleen	IFNγ+CD8+ liver	Tet+CD8+ spleen	Tet+CD8+ liver	Antibodies
Viral load, baseline	–	0.8525	0.0623	0.0222	0.0094	0.7796	0.9132	0.7504	0.0438	0.0033	0.0305	0.0159	0.0020	0.0054	0.0961
Viral load, Wk 8	–	–	0.0471	0.0090	0.0116	0.6454	0.6482	0.7555	0.3892	0.1964	0.0149	0.0234	0.0692	0.0222	0.0238
Viral load, Wk 11	–	–	–	0.0000	0.0002	0.1699	0.2425	0.2838	0.1917	0.0155	0.0003	0.0002	0.0070	0.0027	0.0196
Viral load, Wk 14	–	–	–	–	0.0000	0.2629	0.2888	0.4836	0.1063	0.0128	0.0000	0.0000	0.0001	0.0000	0.0010
Viral load, Wk 16	–	–	–	–	–	0.4359	0.5235	0.7212	0.0759	0.0057	0.0000	0.0000	0.0001	0.0000	0.0044
HBsAg, baseline	–	–	–	–	–	–	0.0000	0.0000	0.1021	0.4918	0.3148	0.5424	0.5555	0.3796	0.8440
HBsAg, Wk 8	–	–	–	–	–	–	–	0.0000	0.0888	0.3469	0.3724	0.7020	0.6410	0.4190	0.7752
HBsAg, Wk 11	–	–	–	–	–	–	–	–	0.0379	0.2080	0.5500	0.9658	0.9658	0.7742	0.4357
HBsAg, Wk 14	–	–	–	–	–	–	–	–	–	0.0001	0.1198	0.0288	0.1082	0.0944	0.0527
HBsAg, Wk 16	–	–	–	–	–	–	–	–	–	–	0.0106	0.0019	0.0156	0.0116	0.0565
IFNγ+CD8+ spleen	–	–	–	–	–	–	–	–	–	–	–	0.0000	0.0001	0.0000	0.0063
IFNγ+CD8+ liver	–	–	–	–	–	–	–	–	–	–	–	–	0.0001	0.0000	0.0007
Tet+CD8+ spleen	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0000	0.0005
Tet+CD8+ liver	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0020
Antibodies	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

CONCLUSIONS

- **These are the first preclinical HBV therapeutic vaccine data that show correlations between intrahepatic CD8+ T cells and HBV viral load declines in serum**
- **Clearance of infected hepatocytes with AdC6-gDHBV2 correlates with lower HBsAg levels**
- **The addition of gD, a novel checkpoint modifier of early T cell activation:**
  - Markedly enhanced the breadth of CD8+ T cell responses
  - Significantly improved T cell frequencies within the liver
- **A Phase 1b clinical trial of AdC6-gDHBV2 for HBV functional cure is scheduled to begin in Q3 2023**

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ABBREVIATIONS

AAV, adeno-associated virus; Ad, adenovirus; AdC6, chimpanzee adenoviral vector; ANOVA, analysis of variance; APC, antigen-presenting cell; BL, baseline; BTLA, B and T lymphocyte attenuator; CD, cluster of differentiation; ELISA, enzyme-linked immunosorbent assay; gD, glycoprotein D; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV gag, human immunodeficiency virus group specific antigen; HVEM, herpes virus entry mediator; ICS, intracellular cytokine staining; IFNγ, interferon-gamma; i.m., 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; qPCR, quantitative polymerase chain reaction; SEM, standard error of the mean; TCR, T cell receptor; tet, tetramer; vg, viral genomes; vp, viral particles; VRON-0200 is AdC6-gDHBV2.

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DISCLOSURES

Dr A Luber is CEO, Virion Therapeutics, LLC and owns shares in the company. He has no other conflicts to report.

FOR MORE INFORMATION

Contact Dr A Luber at: aluber@virionx.com & www.VirionTx.com for permission to reprint and/or distribute.