Abstract #1303

Immunogenicity and Efficacy of an HBV Vaccine with an Intrinsic Checkpoint Inhibitor

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Introduction

- CD8⁺ T cell responses are necessary to clear a chronic hepatitis B virus (HBV) infection; their effectiveness is limited by HBV's ability to evade immunosurveillance and by T cell exhaustion.
- Therapeutic interventions to restore immune functions have shown little clinical benefit and the usefulness of anti-PD-1/L1 antibodies is limited by frequent serious adverse events.
- HSV glycoprotein D (gD) is an immune checkpoint inhibitor that when expressed as a fusion protein with target antigens:
  - Blocks BTLA, an inhibitory signal of early CD8⁺ T cell activation resulting in the production of highly potent and durable antigen-specific CD8⁺ T cell responses.
  - Broadens T cell responses to sub-dominant epitopes [1], which are more resistant to immune exhaustion.
  - Minimizes risk for immune-related "off target" side effects by its delivery by replication-defective viral vectors that encodes gD in its target cells.
- Here we describe the immunogenicity and efficacy in an AAV8-HBV mouse model of a novel pan-genotypic therapeutic HBV T cell vaccine that couples gD with key HBV core and polymerase (Pol) antigens.

Methods

Vaccine Constructs

- Consensus amino acid sequences from core and Pol of HBV genotypes A, B, C & D were selected from 8,629 publicly available viral genomes [2] and optimized using HLA prediction algorithms.
  - Regions of HBV core and Pol associated with prevention of viral escape and/or hepatic flares upon antiviral discontinuation were prioritized for inclusion [3-7].
- Core and two regions of Pol (N-terminus (PolN) and C-terminus (PolC)) were cloned, genetically fused into gD and inserted into either AdC6 or AdC7, two serologically distinct chimpanzee adenoviral vectors (e.g. AdC6 vector plus gD plus PolN, "AdC6-gDPolN").
- Humans have limited pre-existing neutralizing antibodies to AdC6 and AdC7 vectors.
- The two vectors are not cross-neutralized by antibodies, thereby allowing for their use in effective prime and boost regimens.

Immunogenicity

- C57Bl/6 mice (n=5 per group) were injected with various doses of AdC6 and AdC7 vectors expressing HBV core, PolN or PolC fused into gD. Two months after the first injection, AdC6 vector-immunized mice were boosted with AdC7 vectors containing the same insert.
- The frequencies of insert-specific CD8⁺ T cells were determined by intracellular cytokine staining (ICS) for IFN-γ at various time points after injection.
- Frequencies and phenotype of CD8⁺ T cells to one immunodominant epitope within PolN were tested for by staining with an MHC I tetramer.
- The breadth and specificity of CD8⁺ T cell responses to individual peptides within a target sequence was performed via epitope mapping of splenocytes (CD8⁺ T cells tested by ICS for IFN-γ).

Efficacy

❖ **AAV8-1.3HBV Vector Studies**

- C57Bl/6 mice (n=8) were challenged intravenously via their tail vein with 1x10^10 viral genomes (vg) of AAV8-1.3HBV and 4 weeks later received a single IM injection of 5x10^9 viral particles (vp) of AdC6-gDPolN.
- HBV DNA viral titers were evaluated by qPCR; pre- and post-vaccination changes from baseline (log_{10} copies/mL) are reported.

❖ **Impact of chronic HBV virus exposure on CD8⁺ T cell antigen recognition over time**

- The effect of AAV8-1.3HBV on vaccine-induced hepatic CD8⁺ T cells was assessed.
- The epitope profile in splenocytes of naïve mice immunized with a single IM injection of $5x10^9$ vp of AdC6-gDPolN was determined 4 weeks after vaccination.
- Mice challenged with 1x10^10 and 1.5x10^11 vg of AAV8-1.3HBV and subsequently vaccinated with 5x10^9 vp of AdC6-gDPolN 4 weeks later had CD8⁺ T cell epitope profiles in splenocytes performed 10 weeks after vaccination (14 weeks after AAV injection).
- Epitope profiles between AAV-naïve and AAV-treated vaccinated animals were compared.
- PolN-specific CD8⁺ T cells from liver were analyzed for differentiation markers.

Results

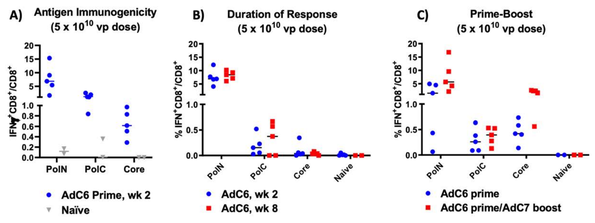
Immunogenicity

- Vaccination induces robust and sustained CD8⁺ T cell responses to PolN (median frequencies over all circulating CD8⁺ T cells: 6.0%) and lower responses to PolC and core (median frequencies: 1.0% & 0.4%, respectively; Fig 1A/B).
- Boosting at 8 weeks increases responses to all regions with significant changes being observed for core (p=0.007) (Fig 1C).
- Vaccination induces broad epitope recognition by CD8⁺ T cells that is further enhanced after boosting (27% to 34%; Fig 2).
- At week 12 following AdC6-gDPolN vaccination, AAV8-1.3HBV-infected vaccinated mice show a preferential increase in hepatic CD8⁺ infiltrates (Figs 3 and 4), a decreased presence of vaccine-induced HBV-specific CD8⁺ T cells (Fig 3) and reduced levels of T-bet (suggestive of loss of effector functions) (Fig 5).
  - However, no clear pattern of cellular markers suggestive of T cell differentiation to an exhaustion phenotype was observed between vaccinated AAV1.3HBV-infected and -uninfected mice (Fig 5).

Efficacy

- Following a single IM injection of the AdC6-gDPolN vector, AAV8-1.3HBV-infected mice had multi-log HBV DNA declines in serum that persist throughout the 8-week post vaccination period (Fig 6).
- Post vaccination, median declines in serum HBV DNA viral load levels at four and eight weeks were 0.86 and 2.69 log_{10} cps/mL, respectively (Fig 6A).
  - At week 8, all animals had a > 1 log_{10} cps/mL, 6/7 (86%) had > 2 log_{10} cps/mL, and 2/7 (29%) had > 3 log_{10} cps/mL declines from baseline (Fig 6B).

Figure 1 - Vaccine Induced HBV-Specific CD8⁺ T cells



Data derived in each Fig from separate experiments

Figure 2 - Breadth of CD8⁺ T cells (Splenocytes)

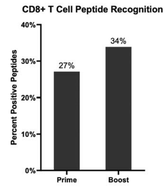
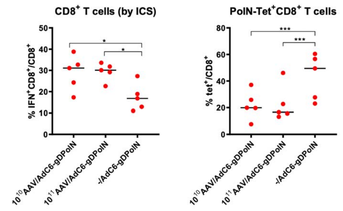
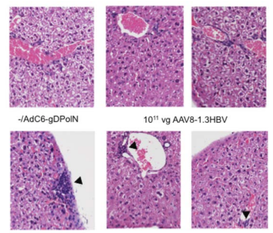


Figure 3 – Vaccine-Induced HBV-Specific CD8⁺ T cells in Liver



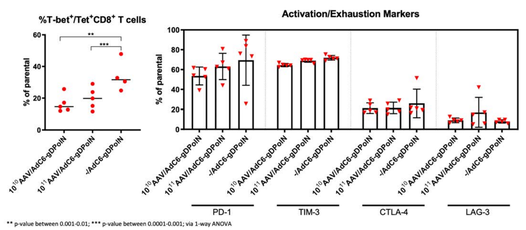
* p-value between 0.01-0.05; *** p-value between 0.0001-0.001; via 1-way ANOVA

Figure 4 - Vaccine-Induced HBV-Specific CD8⁺ T cells in LIver



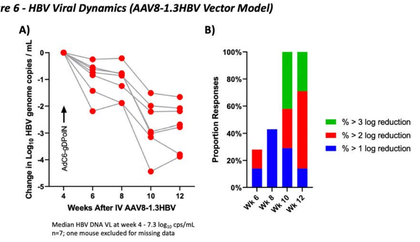
10^{11} vg AAV8-1.3HBV / AdC6-gDPolN
Hematoxylin & Eosin stain; 20x
Arrows show areas of lymphocytic infiltrates

Figure 5 – Vaccine-Induced Markers of CD8⁺ T cell Activation/Exhaustion in Liver



** p-value between 0.001-0.01; *** p-value between 0.0001-0.001; via 1-way ANOVA

Figure 6 - HBV Viral Dynamics (AAV8-1.3HBV Vector Model)

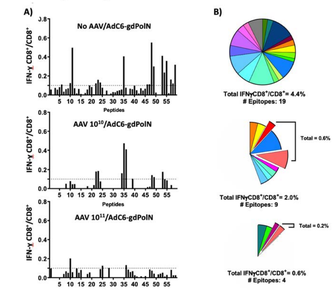


Median HBV DNA VL at week 4 - 7.3 log_{10} cps/mL
n=7; one mouse excluded for missing data

CD8⁺ T cell Recognition Patterns

- Following a single AdC6-gDPolN vector injection, distinct CD8⁺ T cell recognition patterns to PolN peptides in splenocytes are observed when AAV-HBV-infected and naïve mice are compared:
  - The percentage of functional HBV-specific CD8⁺ T cell responses are highest in naïve mice (4.4%, Fig 7B) but decrease in the presence of low and high dose AAV8-1.3HBV (2.0% & 0.6%; respectively, Fig 7B).
  - AAV8-1.3HBV-uninfected animals show strong CD8⁺ T cell responses to a number of epitopes, which are decreased and shifted in AAV-HBV-infected animals to include T cell recognition of new epitopes - these T cells represent roughly a third of the detectable CD8⁺ T cell responses (Fig 7A/B).

Figure 7 - Impact of AAV-Induced HBV on CD8⁺ T cell Responses



Each slice/color represents an individual epitope with size showing proportion of total, only responses >0.1% were included. Pullouts – epitopes only recognized in AAV8-1.3HBV infected mice.

Discussion

Here we describe an HBV therapeutic vaccine that targets early CD8⁺ T cell activation using gD as a genetically encoded checkpoint inhibitor. In the current studies, vaccine constructs containing gD:

- Induce potent and durable CD8⁺ T cell responses to key HBV antigens (Fig 1),
- Stimulate very broad CD8⁺ T cell responses (Fig 2) that include sub-dominant epitope recognition (Figs 7 A/B),
- Achieve sustained multi-log HBV DNA viral load reductions in an AAV mouse model (Fig 6) with preferential trafficking of functional CD8⁺ T cells to the liver (Figs 3 & 4).

The optimal antigens for HBV immune-based treatments and the impact of immune exhaustion on T cell antigen recognition over time is unknown - both factors could impact the design and efficacy of T cell-based treatments. In our AAV studies, AAV-induced HBV infection causes loss of CD8⁺ T cell recognition to the dominant epitopes of PolN following vaccination with AdC6-gDPolN (Fig 7 A/B). We theorize that it is the breadth of the CD8⁺ T cells induced by gD and their ability to recognize subdominant epitopes that leads to a sustained immune response and multi-log suppression of HBV. If this finding translates to human chronic HBV disease it may be important to target sub-dominant HBV epitopes as opposed to primary/dominant epitopes for optimal clinical benefit.

Vaccine constructs containing all three regions (core, PolC, PolN) have been created:

- One series is based upon sub-dominant epitope recognition patterns from the AAV studies,
- One series is created using the overall immunogenicity in the absence of AAV-induced infection,
- Both constructs are fused into gD and inserted into AdC6 and AdC7 chimpanzee adenoviral vectors, which allows testing of prime and boost regimens,
- Each vaccine construct is currently undergoing preclinical testing.

Conclusions

Vaccination using optimized HBV core and Pol antigens fused into the novel checkpoint inhibitor gD:

- Induces potent, broad and highly durable CD8⁺ T cell responses
- Produces sustained multi-log HBV DNA viral load declines in an AAV8-1.3HBV vector mouse model

A Phase 1b trial is in planning

[1] Zhang, Y, et al. J. Immunol. 2014; 193: 1836-46.; [2] Khakpoor, A, et al. J. Virol. 2019; 93(4): e1057-18.; [3] Cheng Y, et al. Sci Immunol. 2019; 4: 1-16.; [4] Schuch A, et al. Gut. 2019; 0: 1-11.; [5] Hoogeveen R, et al. Gut. 2019; 68: 893-904.; [6] Rivino L, et al. J Clin Invest. 2018; 128(2): 668-681.; [7] Boni C, et al. Gastoenterology. 2012; 143(4): 963-993.; [8] Hayer J, et al. Nucelic Acid Res. 2013; 41: D566-571.