Abstract #1303

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

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Introduction

- Immune control of Hepatitis B virus (HBV) is necessary for viral clearance and limited by low antigen immunogenicity and CD8⁺ T cell exhaustion.
- Investigational HBV therapies designed to restore immune functions have shown little clinical benefit and anti-PD1/PDL-1 monoclonal antibody checkpoint inhibitor therapies are limited by their known risk for serious adverse events.
- Glycoprotein D (gD) is a novel, first-in-class genetically encoded checkpoint inhibitor that when expressed as a fusion protein with target antigens:
  - Blocks 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, which are more resistant to immune exhaustion.
  - Through administration via replication-defective viral vectors, gD is only expressed at the injection site and regional draining lymph nodes thereby minimizing the risk for serious "off target" side effects.
- Here we describe the immunogenicity and efficacy in an AAV-HBV mouse model of a novel pan genotypic HBV T cell vaccine that couples gD with key HBV core and polymerase antigens.

Methods

Vaccine constructs

- Key immunogenic regions of HBV core and polymerase were identified and areas associated with prevention of viral escape and/or hepatic flares upon antiviral discontinuation were prioritized for inclusion [1-6].
- Consensus amino acid sequences from core and two regions of polymerase (N-terminus (PolN) and C-terminus (PolC)) of Clades A through D were selected from 8,629 publicly available HBV viral genomes [7] and enhanced using HLA prediction algorithms.
- Each region (core, PolC and PolN) was separately fused with gD and inserted into two different serologically distinct chimpanzee adenoviral vectors (AdC6 and AdC7) for evaluation (e.g. AdC6 vector plus gD plus PolN - AdC6-gDPolN).
- In humans, AdC6 and AdC7 vectors have limited pre-existing neutralizing antibodies that could blunt their immunogenicity and have no cross-immunity, thereby allowing for prime and boost evaluations.

Immunogenicity

- C57Bl/6, BALB/c, and HLA-A2 transgenic mice (n=5 each) were injected with various concentrations of AdC6 and AdC7 containing core, PolN or PolC inserts genetically fused into gD. Two months after the first injection, AdC6 mice were boosted with AdC7 containing the same insert.
- The frequencies of insert-specific CD8⁺ T cells was determined by intracellular cytokine staining (ICS) for IFN-γ at various time points after injection.
- The breadth and specificity of CD8⁺ T cell responses to individual peptides and peptide regions 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 in absolute HBV genome copies/mL over time and $\log_{10}$ copies/mL from baseline are reported.

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

- 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 as described above.
- 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.

Results

Immunogenicity

- Vaccination induces robust and sustained CD8⁺ T cell responses to PolN and lower responses to PolC and core (Fig 1A). (Results for C57Bl/6 mice using AdC6, similar results observed with BALB/c and HLA-A2 transgenic mice and AdC7; data not shown).
- Boosting at 8 weeks with AdC7 vector containing the same antigenic inserts increases responses to core but had minimal impact on PolN and PolC (Fig 1B) (C57Bl/6 mice data shown). Increases in responses following boost were mainly seen at vector doses that upon priming induce suboptimal responses (e.g. 1x10^9 vp).
- Vaccination induces very broad T cell responses; 18%, 30%, and 15% of all of the peptides delineated from the vaccine inserts were recognized by CD8⁺ T cells of C57Bl/6, HLA-A2 transgenic, and BALB/c mice, respectively (Fig 2). Boosting with the AdC7 vector increases the breadth of responses to 31% in C57Bl/6 mice and 22% in BALB/c mice, but does not improve responses in HLA-A2 transgenic mice.

Efficacy

- Following a single IM injection of AdC6-gDPolN, AAV8-1.3HBV infected mice (n=8) had rapid and durable multi-log HBV DNA declines in serum that persist throughout the 16-week study period (Fig 3A).
- At weeks 10 and 16, mice had median declines in HBV DNA viral load serum levels of -1.98 and -1.88 $\log_{10}$ cps/mL, respectively (Fig 3B) - 8/8 (100%), 3/8 (38%) and 1/8 (12%) animals had > 1 $\log_{10}$ cps/mL, > 2 $\log_{10}$ cps/mL and > 3 $\log_{10}$ cps/mL declines at week 16, respectively (Fig 3C).
- Evaluation of livers of AAV-HBV-infected vaccinated mice show preferential trafficking of functional CD8⁺ T cells to liver; in contrast, AAV-HBV-uninfected unvaccinated mice show no hepatic infiltrates or functional CD8⁺ T cells (Fig 4A/B).

CD8⁺ T cell Epitope Recognition Patterns

- Following vaccination with AdC6-gDPolN, distinct CD8⁺ T cell recognition patterns to PolN peptides are observed when AAV-HBV-infected and AAV-HBV-uninfected mice are compared:
  - Overall, CD8⁺ T cell responses are blunted in AAV-HBV-infected vs AAV-HBV-uninfected animals (Fig 5A/B).
  - AAV-HBV-uninfected animals show strong responses to dominant peptide pools E, F and O (Fig 5A), whereas these responses substantially decrease and disappear in low and high-dose AAV-HBV-infected animals, respectively (Fig 5B).
  - The breadth of CD8⁺ T cells to sub-dominant epitopes is increased in AAV-HBV-infected animals and shifts with higher AAV-HBV-exposures (e.g. peptide pools J through M; Fig 5B).
  - Individual peptides are listed in the heat map; loss of dominant epitopes and shifting of responses in untreated vs low- and high-HBV-AAV infected animals are shown.

Figure 1 - HBV Core and Pol Specific CD8⁺ T cells (C57Bl/6 mice shown)

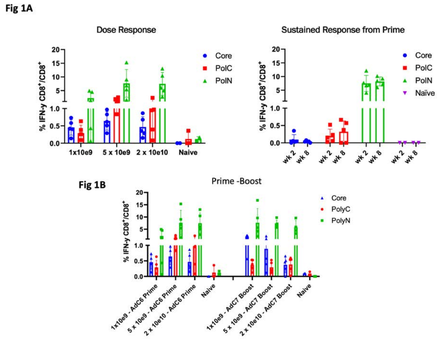


Figure 2 - Breadth of CD8⁺ T cell Responses (Splenocytes)

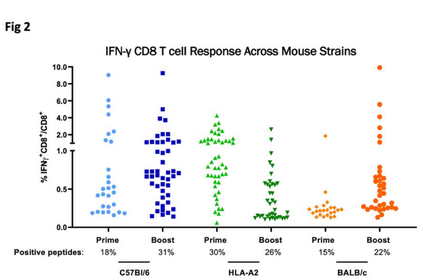
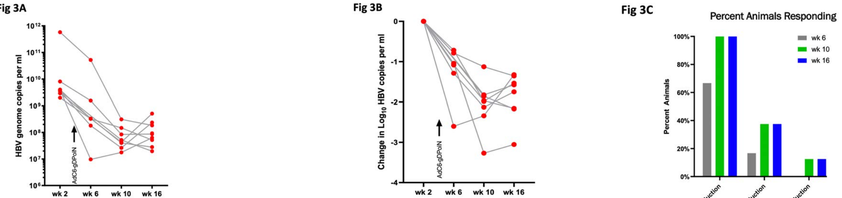


Figure 3 - Impact on HBV DNA Viral Dynamics Following a Single IM Vaccination of AdC6-gDPolN (AAV8-1.3HBV Vector Model)



	Week 2	Week 6	Week 10	Week 16
Median	3.71×10^9	3.27×10^8	4.75×10^7	8.72×10^7
Range (min, max)	1.98×10^9, 5.80×10^{11}	9.70×10^6, 5.24×10^{10}	1.77×10^7, 3.12×10^8	1.96×10^7, 5.12×10^8

HBV genome cps/mL

	Week 6	Week 10	Week 16
Median	-1.06	-1.98	-1.88
Range (min, max)	-0.72, -2.60	-1.13, 3.27	-1.32, -3.05

HBV DNA $\log_{10}$ cps/mL

Figure 4 - HBV CD8⁺ T cells in Hepatocytes Following Vaccination

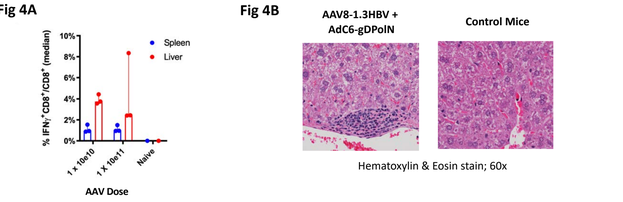
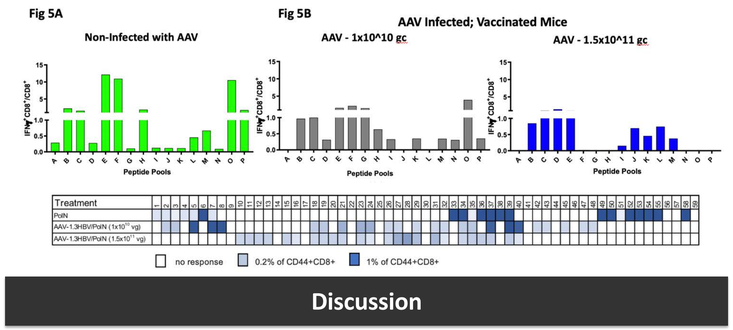


Figure 5 - Impact of AAV-Induced HBV on CD8⁺ T cell Responses



Discussion

Here we describe an HBV therapeutic vaccine that targets early CD8⁺ T cell activation using a genetically encoded checkpoint inhibitor, gD. 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 (Fig 5),
- Achieve sustained multi-log HBV DNA viral load reductions in an AAV mouse model (Fig 3A-C) with preferential trafficking of CD8⁺ T cells to the liver (Fig 4A/B).

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 5B). We theorize the reason the AdC6-gDPolN vaccine produced sustained multi-log viral load reductions is that despite this loss, the breadth of CD8⁺ T cells induced by gD to include recognition of sub-dominant epitopes allows for a sustained immune response. The clinical implication of these findings could be highly significant if this translates to human disease – e.g. targeting primary/dominant epitopes in the presence of chronic HBV infection could be of limited clinical benefit.

We are currently optimizing final vaccine constructs that will contain all three regions (core, PolC, PolN):

- One series will be based upon sub-dominant epitope recognition patterns from the AAV studies,
- One series will be determined by overall immunogenicity in the absence of AAV-induced infection,
- Both constructs will be fused into gD and inserted into AdC6 and AdC7 chimpanzee adenoviral vectors, which will allow for Prime and Boost evaluations.

Conclusions

- Vaccination using the novel checkpoint inhibitor gD fused to optimized HBV core and polymerase antigens:
  - 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] Khakpoor, A, et al. J. Virol. 2019; 93(4): e1057-18.; [2] Cheng Y, et al. Sci Immunol. 2019; 4: 1-16.; [3] Schuch A, et al. Gut. 2019; 0: 1-11.; [4] Hoogeveen R, et al. Gut. 2019; 68: 893-904.; [5] Rivino L, et al. J Clin Invest. 2018; 128(2): 668-681.; [6] Boni C, et al. Gastoenterology. 2012; 143(4): 963-993.; [7] Hayer J, et al. Nucelic Acid Res. 2013; 41: D566-571.

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