Introduction

Vaccines are critical to the prevention of many infectious diseases, and they are being developed to prevent viral hepatitis. However, hepatitis B virus (HBV) continues to be a significant global health threat, with an estimated 240 million people chronically infected and 880,000 deaths annually. The current standard of care for chronic HBV infection is lifelong antiviral therapy, which is associated with significant side effects and costs. A vaccine capable of inducing long-lasting immunity to HBV could provide a safer and more cost-effective alternative. In this study, we investigated the immunogenicity and efficacy of a novel HBV vaccine containing an immune checkpoint inhibitor, Glycoprotein D (gD), and optimized HBV core and polymerase antigens in mice.

Methods

Vaccine constructs were designed in collaboration with Virion Therapeutics LLC. The vaccine constructs contained gD fused to optimized HBV core and polymerase antigens. The vaccines were administered intramuscularly as a prime and boost regimen, with the prime consisting of a single dose of the vaccine followed by a boost 4 weeks later. The immune response to the vaccine was evaluated by measuring the percentage of CD8+ T cells that recognize HBV antigens.

Results

The vaccine induced robust and sustained CD8+ T cell responses to HBV antigens, as measured by interferon-γ (IFN-γ) production in splenocytes from vaccinated mice. The vaccine also induced sustained multi-log HBV DNA viral load reductions in an AAV mouse model, with preferential trafficking of CD8+ T cells to the liver.

Conclusions

The vaccination using the novel checkpoint inhibitor gD resulted in optimized HBV core and polymerase antigens, with broad and highly durable CD8+ T cell responses. The vaccine produced sustained multi-log HBV viral load reductions in an AAV-1.3HBV mouse model, with preferential trafficking of CD8+ T cells to the liver. The optimal antigens for HBV immune-based treatments and the impact of immune exhaustion on T cell recognition over time are critical in determining the impact of T cell responses. In our AAV studies, AAV-induced HBV infection causes loss of CD8+ T cell recognition to the dominant epitopes of HBV. However, the breadth of CD8+ T cell responses is increased in AAV-infected animals and shifts with higher AAV-exposure. The current study provides evidence that the AdC6-gDPolN vaccine can induce robust and sustained CD8+ T cell responses to key HBV antigens, which may be beneficial in the treatment of chronic HBV infection.

Abstract #1303

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

Hasanpouroghdi, M1; Luber, A2; Magowan, C2; Zhou, X1; Ertl, HCJ1
1The Wistar Institute, Philadelphia, PA; 2Virion Therapeutics LLC, Newark, DE

Introduction

• Immunogen control of Hepatitis B virus (HBV) is necessary for viral clearance and limited by low immunogenicity and CD8+ T cell exhaustion.
• Investigational HBV therapies designed to restore immune functions have shown little clinical benefit and anti-PEG-PLD1 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.
  - Through administration via replicative-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 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 hepatitis 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 IL28A publicly available HBV viral genomes [7] and enhanced using HLA prediction algorithms.
- Each region (core, PolN and PolC) was separately fused with gD and inserted into different serologically distinct chimpanzee adenovectors (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-reactivity, thereby allowing for prime and boost evaluations.

Immunogenicity

- 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).
- Vaccination induces robust and sustained CD8+ T cell responses to WHV antigens and allow for prime and boost evaluations.
- A Phase 1b trial is in planning.

Efficacy

- Induces potent and durable CD8+ T cell responses to key HBV antigens (Fig 1).
- Achieves sustained multi-log HBV DNA viral load reductions in an AAV-1.3HBV mouse model (Fig 3A).
- Stimulates very broad CD8+ T cell responses (Fig 2) that include sub-dominant epitope recognition (Fig 5).
- 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.
- Overall, CD8+ T cell responses are blunted in AAV-HBV-infected as AAV-HBV-uninfected animals (Fig 5A/B).
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- The optimal antigens for HBV immune-based treatments and the impact of immune exhaustion on T cell recognition over time are critical in determining the impact of T cell responses. In our AAV studies, AAV-induced HBV infection causes loss of CD8+ T cell recognition to the dominant epitopes of HBV. However, the breadth of CD8+ T cell responses is increased in AAV-infected animals and shifts with higher AAV-exposure. The current study provides evidence that the AdC6-gDPolN vaccine can induce robust and sustained CD8+ T cell responses to key HBV antigens, which may be beneficial in the treatment of chronic HBV infection.

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=model (Fig 3A) 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 recognition over time are critical in determining the impact of T cell responses. In our AAV studies, AAV-induced HBV infection causes loss of CD8+ T cell recognition to the dominant epitopes of HBV. However, the breadth of CD8+ T cell responses is increased in AAV-infected animals and shifts with higher AAV-exposure. The current study provides evidence that the AdC6-gDPolN vaccine can induce robust and sustained CD8+ T cell responses to key HBV antigens, which may be beneficial in the treatment of chronic HBV infection.

Conclusions

• Vaccination using the novel checkpoint inhibitor gD fused to optimised HBV core and polymerase antigens:
  - Induces potent, broad and highly durable CD8+ T cell responses.
  - Produces sustained multi-log HBV DNA viral load reductions in an AAV-1.3HBV mouse model.

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 (AAV-1.3HBV Vector Model)

Figure 4A

Figure 4B

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

Figure 4A

Figure 4B

Figure 5

AAV-1.3HBV + HBeAg+ HBeAg- HBsAg+ HBsAg- Histology & Steatosis, HA-100x