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TITLE: A Novel HBV Drug for Effective Cure of Chronic Hepatitis B

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CONTRACTING ORGANIZATION: HBVtech, Frederick, MD

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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14. ABSTRACT

Hepatitis B and evaluation of curative hepatitis B therapies are listed in FY20 PRMRP Topic Areas and the Areas of Encouragement, respectively. This proposal aims to test a new HBV cure therapy and is encouraged by FY20 PRMRP.

Current HBV drugs can inhibit HBV replication, but rarely cure chronic hepatitis B (CHB). The reason for this failure is the persistence of HBV cccDNA (covalently closed circular DNA) in infected hepatocytes. A global panel of 35 HBV experts publishes a special paper titled "A Research Agenda for Curing Chronic Hepatitis B Virus Infection", in which they state that" the surest way to cure HBV is to eliminate or permanently silence its cccDNA". This recommended cure strategy clearly assumes no cccDNA clearance with current HBV treatment.

However, both nucleot/side analogues (NA) and interferons (IFN) treatments can reduce intrahepatic cccDNA level by 1 to 2.94 log. For instance, average cccDNA copies were reduced from 1.05 copies per cell at treatment initiation to one copy per 250 cells (0.004 copies per cell) after 48-week of IFN + adefovir therapy, implying >99% of infected cells clear HBV cccDNA.

15. SUBJECT TERMS

None listed.

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TABLE OF CONTENTS

1. Introduction

Hepatitis B and evaluation of curative hepatitis B therapies are listed in FY20 PRMRP Topic Areas and the Areas of Encouragement, respectively. This project aimed to determine if blocking *de novo* infection with a sustained high level of anti-HBs antibody after a liver is fully infected, can lead to HBV cure in HBV infected uPA/SCID mice.

2. Key Words

Chronic hepatitis B therapy, HBV Cure, HBV de novo infection, cccDNA replenishment.

3. Accomplishments

- **I. What were the major goals of the project?**

This project only contains a single specific aim, which is to determine if a combination of entecavir (one of FDA approved anti-HBV drugs) with anti-HBs antibody expressed by AAV-anti-HBs vectors can lead to HBV functional cure in HBV infected uPA/SCID mice, including:

1. Conduct an animal experiment
2. Collect and analyze blood and liver samples

- **What was accomplished under these goals in the 1st year (5/1/2021 to 4/30/2022)**

1. **Completed the major activities and objectives**:

1). Manufactured two AAV vectors: one expresses anti-HBs antibody (Product ID: HBVZ40); and the other expresses malaria antibody as treatment control (Product ID: 1882.2). The amount of each manufactured vector after purification and concentration reached >1E13 genomic copies and the expressed antibody level was >1μg/ml in the transduced 293 cells.

2). Gained the approvals of IACUC and ACURO.

Thus, milestone 1 was achieved.

3). Purchased 24 uPA/SCID mice

4). Conducted and completed the animal experiment

5). Started analyzing blood samples

6). The request for one year extension was approved.

Thus, the major experiment required for reaching milestone 2 was completed and the remaining tasks to complete analyses of blood and liver samples, will be done in the 2nd year (5/1/2022 to 4/30/2023).

2. **Preliminary Results as of May 31, 2022**

A. <u>The Anti-HBs antibody levels expressed by AAV-anti-HBs vector were comparable to the levels expressed by control AAV vector that expressed malaria antibody</u>.

The antibody levels expressed by control AAV vector that expressed malaria antibody ranged around 20-100μg/ml (Blue in figure 1) and comparable levels of antibody levels were expressed by AAV-anti-HBs vectors (Red in figure 1). The results support that the AAV-anti-HBs vectors can express sustained antibody after a single intramuscular injection of 1E11 genomic copies.

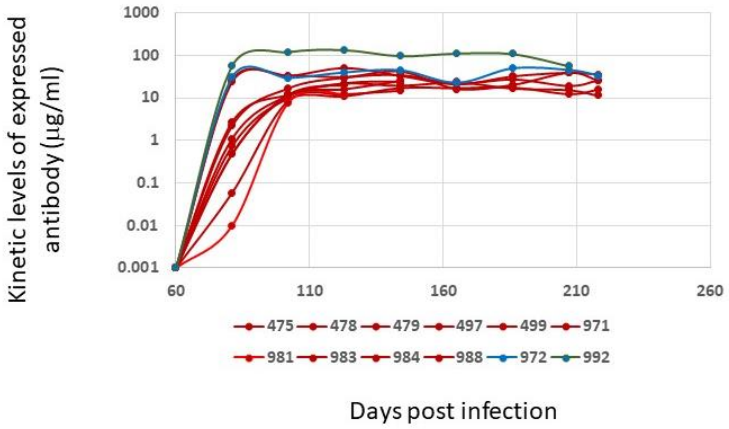

Figure 1. Kinetic levels of antibodies expressed by AAV-malaria antibody vector (blue) and AAV-anti-HBs vectors (red).

B. <u>HBV infection levels suppressed by anti-HBs antibody monotherapy</u>

Three HBV infected uPA/SCID mice were treated with mouse anti-HBs antibody alone that was administrated through intraperitoneal injection at a dose of 250μg/mouse started at day 57 post inoculation (pi) triweekly for 8 consecutive times. Viremic levels in all 3 mice were suppressed by up to 100-fold with anti-HBs antibody treatment compared to untreated mice (Red vs blue in figure 2). The results support the concept proposed by the PI that HBV infection level is maintained by new rounds of infection, as blocking new rounds of infection with anti-HBs antibody alone lowered HBV infection level by up to 100-fold.

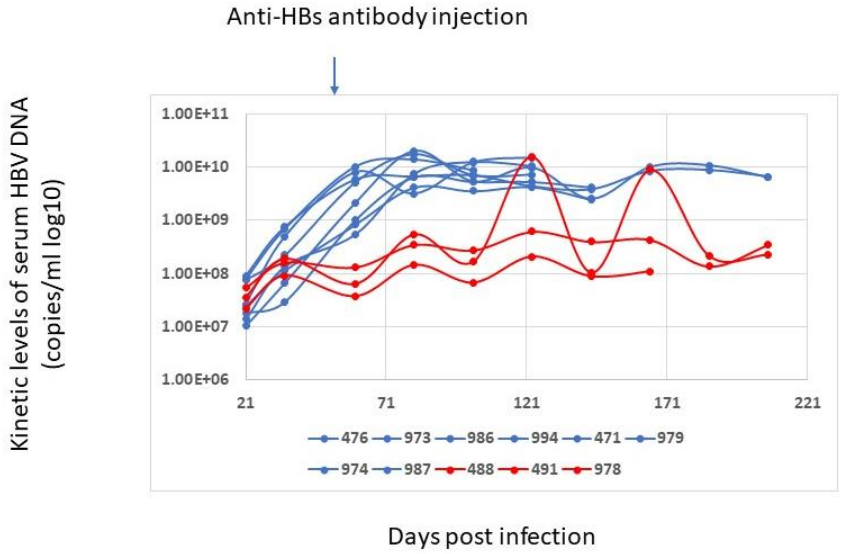

Figure 2. Anti-HBs antibody monotherapy suppressed HBV viremia level by up to 100-fold (red) compared to untreated mice.

C. <u>Serum HBV DNA levels were potently inhibited by entecavir monotherapy or in combination of AAV-anti-HBs vectors</u>.

HBV infected uPA/SCID mice were treated at week 6 pi either with entecavir alone or in combo with AAV-anti-HBs vectors. Entecavir was administrated through intraperitoneal injection of 0.3mg/kg, three times a week for 16 consecutive weeks. AAV-anti-HBs vector was given through a single intramuscular injection of 1E11 genomic copies at week 6 pi. Three of the 4 mice in the combo group died shortly after starting the treatment and 4 of 6 mice in the monotherapy group also died before termination. Nevertheless, HBV DNA level was inhibited by 3-4 logs in both treated groups (red and orange in figure 3) during the ETV treatment duration. As expected, HBV DNA level was rebounded after ETV withdrawal in ETV monotherapy group. Unfortunately, all 4 mice in the combo group died prior to the ETV discontinuation. We are unable to know the differences in HBV DNA levels after day 144 between two groups.

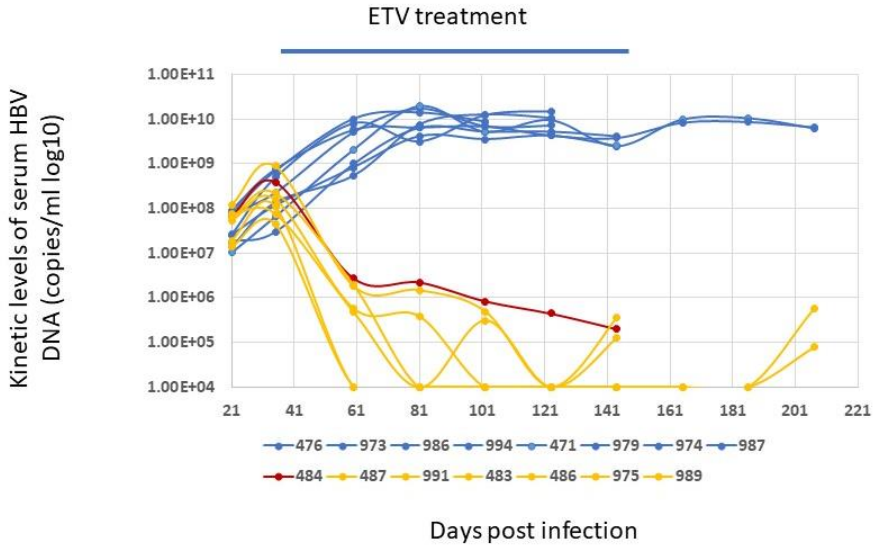

Figure 3. HBV DNA level was inhibited by 3-4 logs during up to 16 weeks of entecavir treatment. **Blue**: untreated. **Orange**: ETV monotherapy and HBV DNA rebounded after ETV withdrawal. **Red**: ETV combination with AAV-anti-HBs vector. The lower limit of detection: 10,000 copies/ml.

D. <u>HBsAg was lost in the combo therapy while HBsAg remained positive in the monotherapy group</u>
As expected, serum HBsAg level kept rising and stayed at steady levels in entecavir monotherapy group (Orange in figure 4). But serum HBsAg level was not increased in animal 484 with combo therapy and became undetectable from day 81 to day 144 (the last timepoint, red in figure 4). The results suggest that the combination of entecavir with AAV-anti-HBs vectors helped effectively achieve HBsAg loss, which is the most difficult part of HBV functional cure and the current HBV drugs can't achieve HBsAg loss despite years of treatment. We only had one animal in the combo group because of animal losses. However, the identical results were generated in another project in which a complete serum HBsAg loss was achieved in 8 of 8 mice treated with high level of anti-HBs antibody and HBV functional cure was established in 5 of the 8 mice with a serum HBsAg loss (data not shown). Taken together, our results provide the preliminary proof that this combinational therapy is much more effective in achieving HBsAg loss and HBV functional cure compared to entecavir monotherapy.

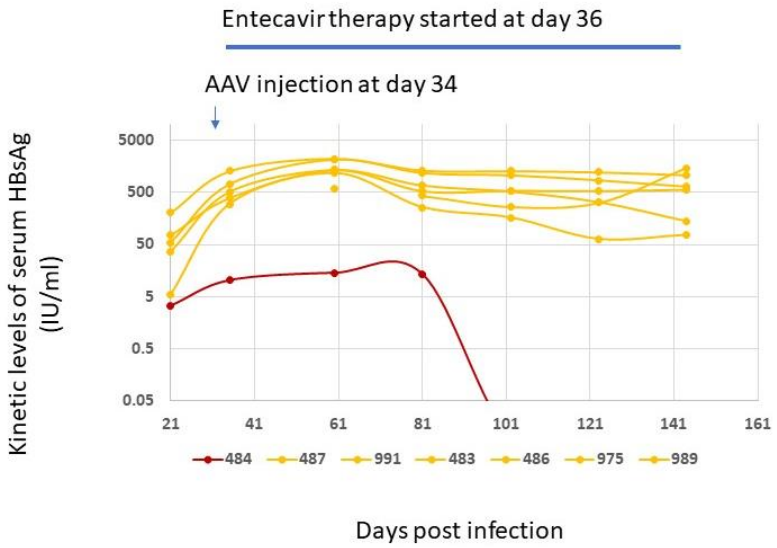

Figure 4. A serum HBsAg loss was established in animal 484 with combo therapy (Red) while serum HBsAg level was not significantly affected in entecavir monotherapy (Orange).

- **What opportunities for training and professional development has the project provided?**

Nothing to report.

- **How were the results disseminated to communities of interest?**

Nothing to report at this time.

- **What do you plan to do during the next reporting period to accomplish the goals?**

1. Complete analyses of all serologic markers in all blood samples
2. Complete analyses of intrahepatic HBV DNA including cccDNA in all liver samples
3. Submit an abstract to scientific conference

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

The results from this project together with another project as briefly discussed above are expected to establish proof of concept that a combination of one of the currently approved HBV drugs with this AAV-anti-HBs vector delivers efficient serum HBsAg loss and a complete anti-HBs seroconversion, leading to a higher rate of HBV functional cure (5 of 8 mice) in a period of months, compared to the rarity of the current therapies mediated HBV cure that usually take years.

Our results are expected to prompt HBV field to evaluate this combo therapy in human trials. Once confirmed in clinical trials, a more effective HBV functional cure will be a reality under this combo therapy.

- **What was the impact on other disciplines?**

The concept of new rounds of infection covers all infection including HIV, TB, and malaria. Thus, this new treatment strategy and this new single injection-based gene therapy are expected to impact HIV, TB, and malaria curative treatment.

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

5. CHANGES/PROBLEMS

No change was made in performing this project including animal use.

The main problem is a high mortality rate of uPA/SCID chimeric mice. This animal model expresses high level of uPA gene product to destroy mouse liver, which is required for growing the transplanted human liver cells. However, the elevated uPA product digests off one of clotting factors, causing internal bleeding that is the main death cause. Currently, there is no effective solution to this problem.

6. PRODUCTS

1. Publications, conference papers, and presentations

Nothing to report at this time.

2. Inventions, patent applications, and/or licenses

Nothing to report at this time.

3. Other Products

Nothing to report at this time.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Yong-Yuan Zhang
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0001-9337-1553
Nearest person month worked:	4 months
Contribution to Project:	Performing this project

Name:	Bai-Hua Zhang
Project Role:	Scientist and lab manager
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9 months
Contribution to Project:	Analyzing samples and logistics support

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report.

- **What other organizations were involved as partners?**

Nothing to report.

- **Organization Name:**

HBVtech, LLC.

- **Location of Organization:** *(if foreign location list country)*

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- **Partner's contribution to the projec**t *(identify one or more)*

Nothing to report.