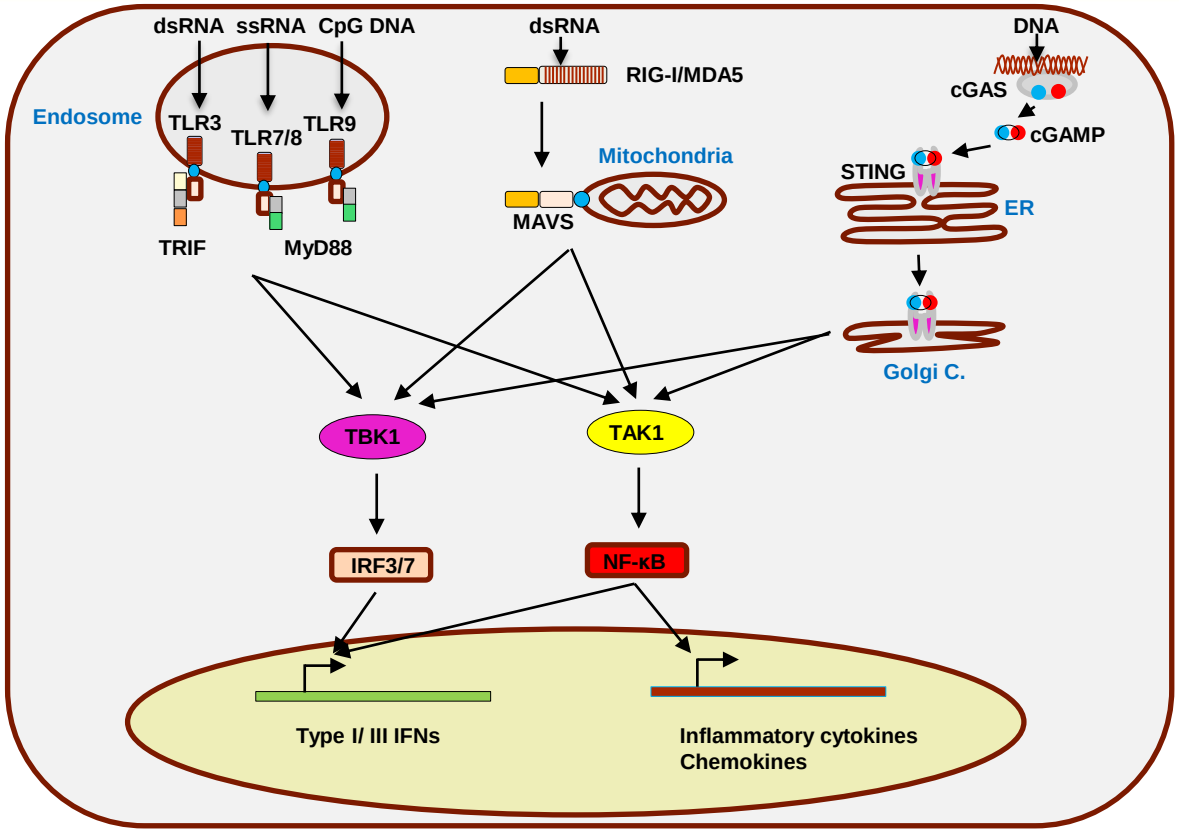
# HBV-TAG

## 2021 CONFERENCE

# Innate Immunity: TLR, RLR and STING

Ju-Tao Guo, M.D.
Baruch S. Blumberg Institute
Doylestown, Pennsylvania

# Pattern recognition receptors (PRRs)-mediated innate immune responses play important roles in viral infections



# Multiple PRRs are expressed in human liver cells

| PRRs | Hepatocytes | KC | LSEC | HSC | LMNC |
|---|---|---|---|---|---|
| TLR3 | + | - | + | - | + |
| TLR7 | - | - | - | - | - |
| TLR8 | - | - | + | + | + |
| TLR9 | - | - | + | - | - |
| RIG-I | + | + | + | + | + |
| MDA5 | + | + | + | - | + |
| cGAS | -/+ | + | - | - | - |
| STING | -/+ | - | - | + | + |

**KC**, Kupfer cells; **LSEC**, liver sinusoidal endothelial cell; **HSC**, Hepatic stellate cells; **LMNC**, total nonparenchymal liver mononuclear cells.

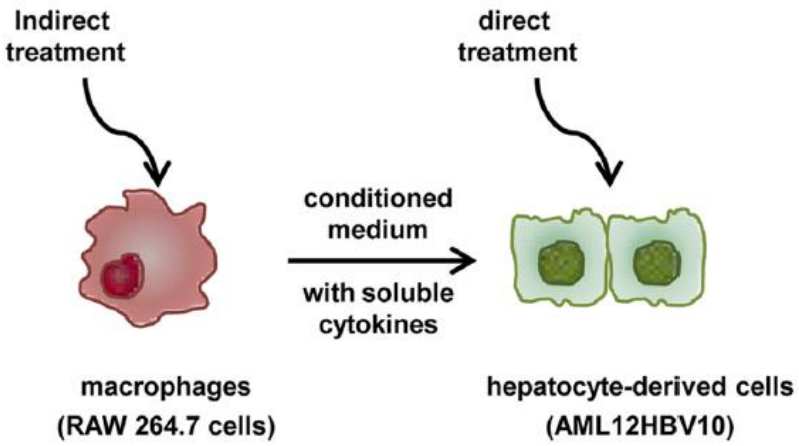
Faure-Dupuy et al., J Innate Immunity, 2018, DOI: 10.1159/000489966

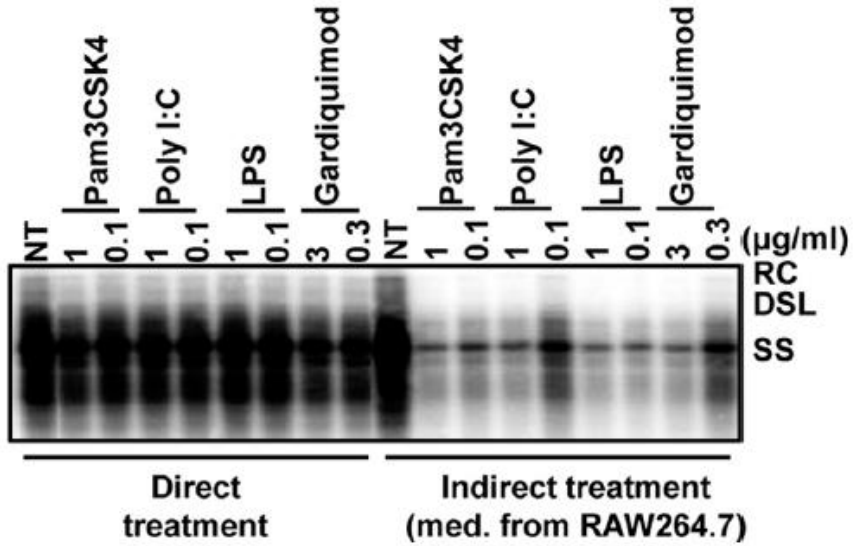
# Therapeutic activation of PRRs in hepatocytes potently suppresses HBV replication via multiple mechanisms

- **While it remains controversial on whether HBV infection activates pattern recognition receptors in hepatocytes, ectopic activation of RIG-I/MDA5 as well as STING potently inhibits HBV replication.**
  - Guo, H., et al. **Activation of pattern recognition receptor-mediated innate immunity inhibits the replication of hepatitis B virus in human hepatocyte-derived cells**. Journal of Virology. 2009, 83(2):847-58.
  - Guo, F., et al. **Activation of Stimulator of Interferon Genes in Hepatocytes Suppresses the Replication of Hepatitis B Virus.** Antimicrob Agents Chemother. 2017. 22;61(10):e00771-17.

- **Treatment of hepatocytes with RIG-I agonists inhibited HBV cccDNA synthesis and induced the decay of established cccDNA.**
  - Lee S., et al. **Suppression of hepatitis B virus through therapeutic activation of RIG-I and IRF-3 signaling in hepatocytes**. iScience. 2021. 24(1):101969.

# Activation of PRRs in liver non-parenchyma cells/or immune cells induces antiviral cytokine responses




Guo, F., et al. Antimicrob Agents Chemother. 2015 Feb;59(2):1273-81. doi: 10.1128/AAC.04321-14

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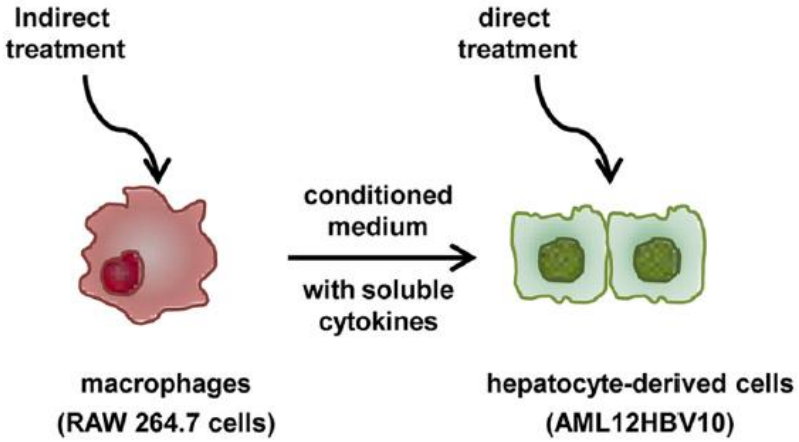
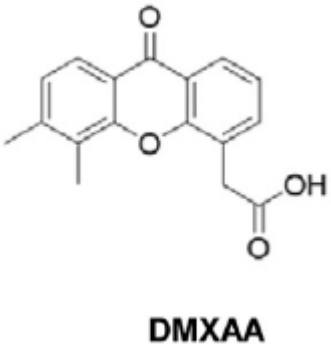
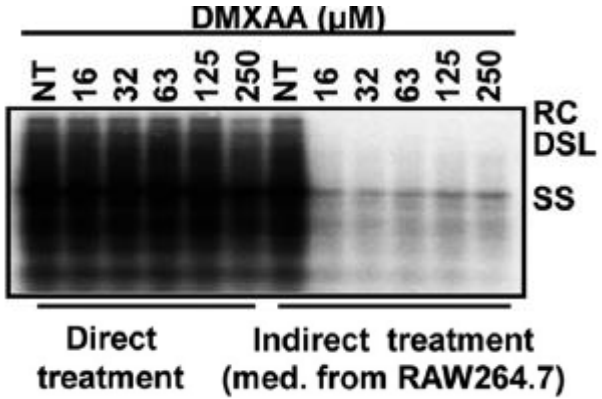



A mouse STING agonist



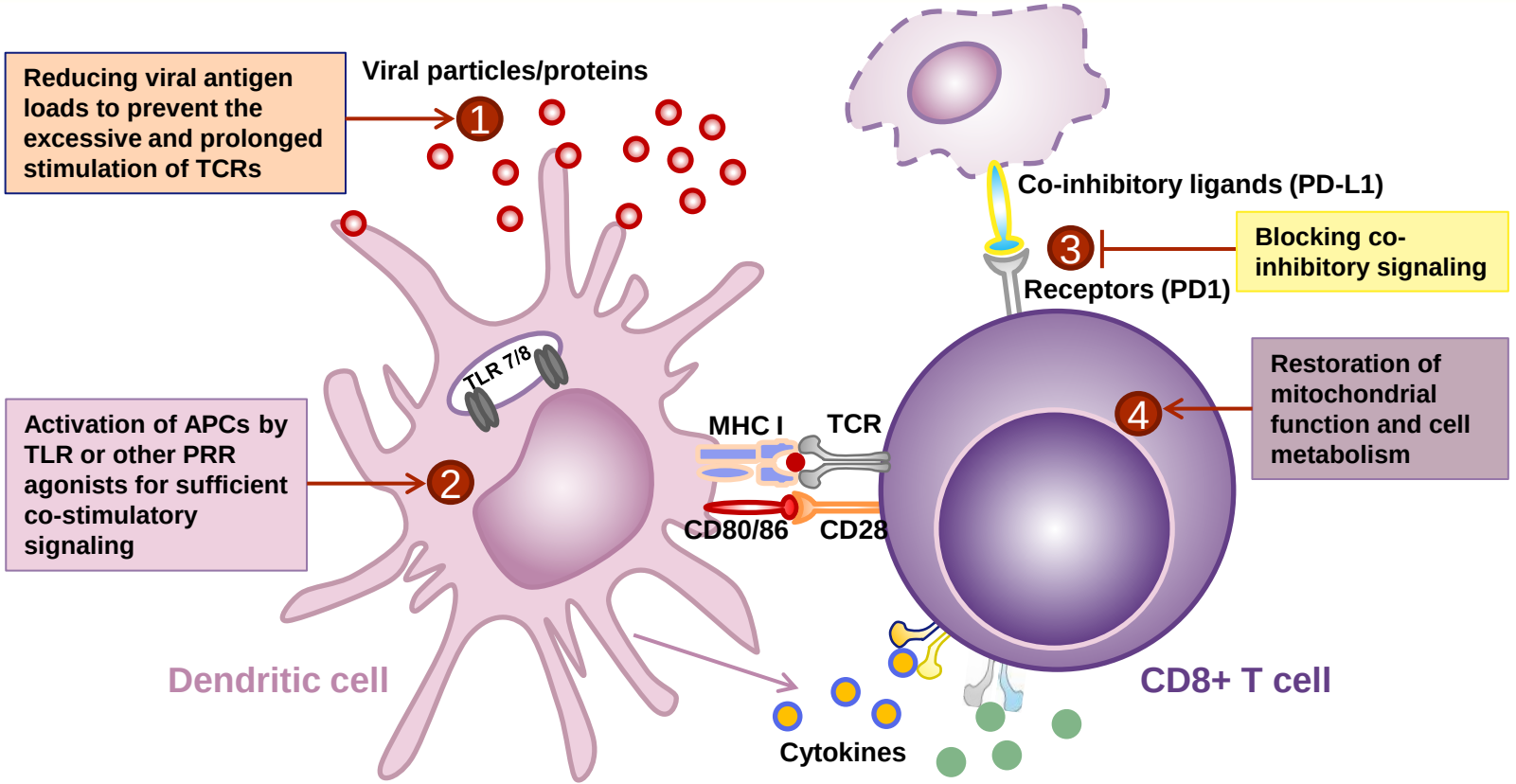

Guo, F., et al. Antimicrob Agents Chemother. 2015 Feb;59(2):1273-81. doi: 10.1128/AAC.04321-14

# GS-9620 activates an cytokine response in PBMCs that induces prolonged suppression of HBV replication in hepatocytes

- GS-9620 had no antiviral activity in HBV-infected PHHs.
- Conditioned media from human peripheral blood mononuclear cells (PBMCs) treated with GS-9620 (GS-9620-CM) induced prolonged *(35 days after cessation of treatment)* reduction of HBV DNA, RNA, and antigen levels in PHHs.
- GS-9620-CM inhibited HBV replication in PHHs *via* type I IFN-dependent mechanism.
- Type I IFN induced by GS-9620 durably suppressed HBV in human hepatocytes without reducing cccDNA levels. In contrast to IFN-α, TNF-α did not induce prolonged suppression of viral RNA in HBV-infected PHH once treatment was stopped.
- GS-9620-CM also induced expression of immunoproteasome subunits and enhanced presentation of an immunodominant viral peptide in HBV-infected PHHs.

Niu, C., et al. J Hepatol. 2018 May;68(5):922-931. doi:10.1016/j.jhep.2017.12.007.

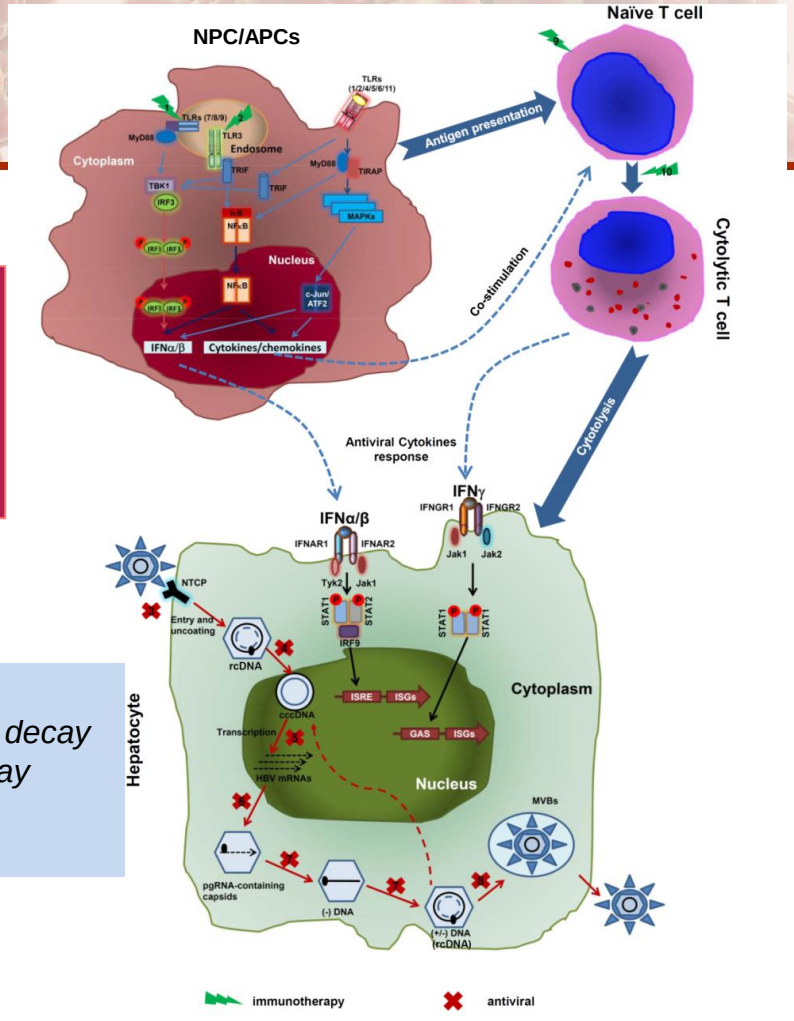
# Activation of PRRs in antigen presentation cells promotes the activation of adaptive immune response against HBV



# PRR agonists usually target many distinct cell types

1. *Induction of antiviral cytokines, particularly type I/III IFNs*
2. *Promotion of DC maturation and antigen presentation*
3. *Modulation of T and B cell activation/differentiation*
4. TLR-7/8 is expressed by B lymphocytes and its activation results in polyclonal expansion and differentiation towards immunoglobulin-producing plasma cells

1. *Inhibition of cccDNA synthesis/promotion of cccDNA decay.*
2. *Suppression of cccDNA transcription and promotion of viral RNA decay*
3. *Inhibition of pgRNA packaging and acceleration of nucleocapsid ay*
4. *Inhibition of virion secretion*
5. *Increasing hepatocyte presentation of viral antigens*



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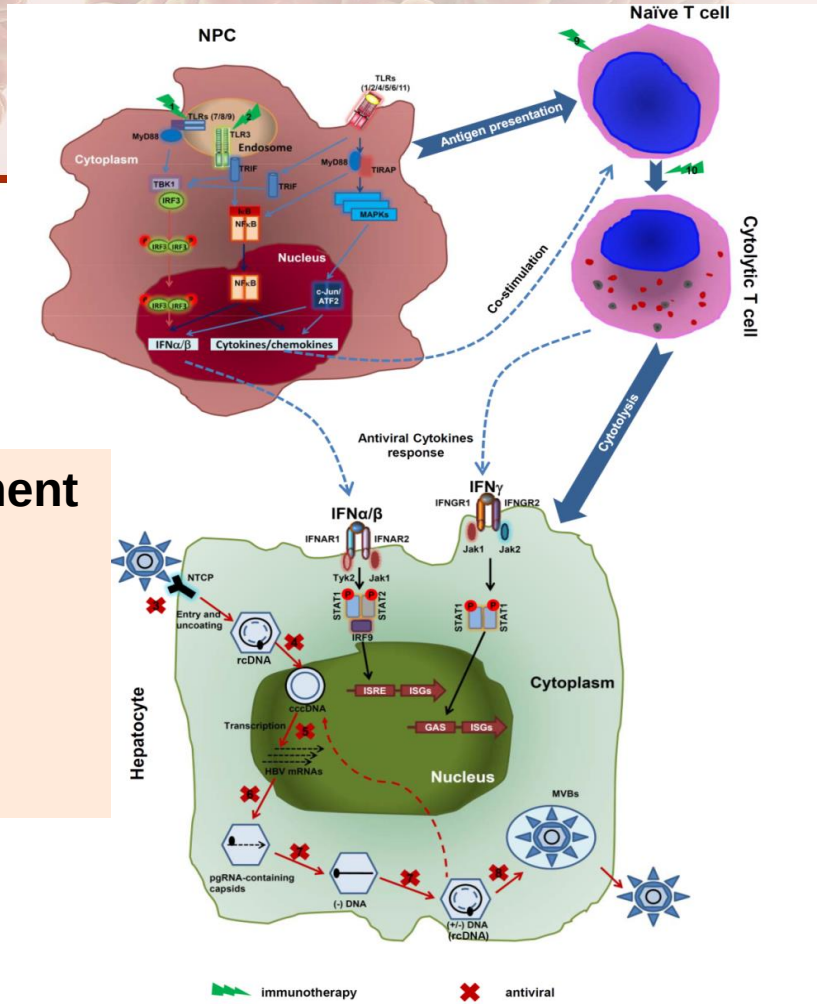



## Applications of PRR agonists in CHB treatment

1. *Therapeutics to restore antiviral innate and adaptive antiviral immune responses*

2. *Adjuvants to therapeutic vaccinations*

# PRR agonists currently under development for treatment of chronic hepatitis B

| Drug | Target | Company | Development Stage |
|---|---|---|---|
| GS-9620 (**Vesatolimod**) | TLR7 | Gilead Sciences | Terminated |
| AL-034 | TLR7 | J&J/Alios | Phase 1 |
| RG-7854 | TLR7 | Roche | Phase 1 |
| RG7020531 | TLR7 | Roche | Phase 1 |
| GS-9688 **(Selgantolimod)** | TLR8 | Gilead Sciences | Phase 2 |
| Inarigivir | RIG-I (?) | Spring Bank | Terminated |
| Formula 7 (F7) | RIG-I/IRF3 | ?? | Preclinical |
| BNBC (B6) | STING | RimmSTING | Preclinical |

# TLR agonists induced antiviral immune responses and sustained suppression of viral replication in preclinical animal models

| Mice models | Woodchucks | Chimpanzees |
|---|---|---|
| Hydrodynamic (HD) mice model | WHV Chronically infected Woodchucks | HBV chronically infected chimpanzees |
| AAV-HBV mice | | |
| Transgenic mice | | |

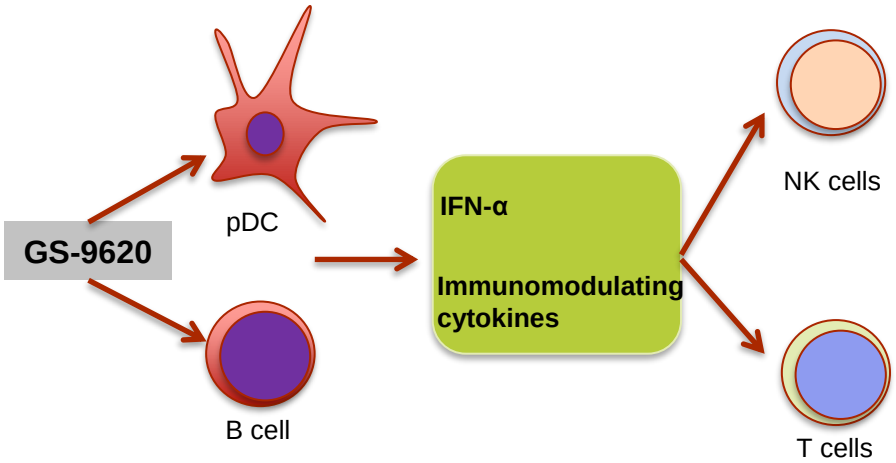
## Comments

| | | |
|---|---|---|
| Mouse STING agonist DMXAA had been demonstrated to induce ISGs in the liver and significantly reduced viral load in HD and HBV-AAV mice models (1). | • GS-9620 induced a sustained antiviral efficacy and seroconversion (2);<br>• Liver-targeted TLR-7 agonist (APR002) combined with Entecavir promotes a functional Cure (3);<br>• GS-9688 induces sustained antiviral efficacy (4) in WHV chronically infected woodchucks. | • GS-9620 Induces prolonged suppression of HBV in CHB chimpanzees (5).<br>• The antiviral response to GS-9620 in CHB chimpanzees was associated with an intrahepatic IFN response and formation of lymphoid aggregates in the liver (6). |


(1) Guo, F., et al. Antimicrob Agents Chemother. 2015 Feb;59(2):1273-81. doi: 10.1128/AAC.04321-14.
(2) Menne, S., et al. J Hepatol . 2015 Jun;62(6):1237-45. doi: 10.1016/j.jhep.2014.12.026.
(3) Korolowizc, K.E., et al. Hepatol Commun. 2019 Jul 8;3(10):1296-1310. doi: 10.1002/hep4.1397.
(4) Darffs, S., et al. Hepatology. 2021 Jan;73(1):53-67. doi: 10.1002/hep.31255.
(5) Lanford, R.E., et al., Gastroenterology. 2013 June ; 144(7): 1508–1517.e10. doi:10.1053/j.gastro.2013.02.003.
(6) Li, L., et al. J Hepatol. 2018 May;68(5):912-921. doi: 10.1016/j.jhep.2017.12.008.

# GS-9620 increases responses of HBV-specific T cell and NK cells in CHB patients treated with NUCs

**Patients**: 28 HBeAg negative, HBV DNA negative, genotype D infected patients with CHB treated with NUC for at least 3 years. NUC-treatment naïve patients as well as subjects who resolved acute HBV infection served as controls.
**Treatment Schedule**: weekly dosing for 12 weeks and follow-up for 12 weeks.




- GS-9620 treatment significantly enhanced the frequency of total and CD56 bright NK cells as well as of NKT cells.
- TRAIL, HLA-DR, Ki67 and CD38 expression on CD56 bright and dim NK cells was progressively increased upon GS-9620 treatment.
- GS-9620 treatment increased expression of the activating NKp46, NKG2D and NKp30 and reduced inhibitory NKG2A receptors,

- Increased expansion, IL12/TNF-a/IFN-r production in response HBV peptide stimulation
- HBV-specific CD8 responses were improved more efficiently than CD4 responses

**Although twelve weeks administration of GS-9620 increased T-cell and NK cell responses and reduce the ability of NK to suppress T cells, the treatment had no significant effect on serum HBsAg levels.**

Boni, C., et al. Gastroenterology. 2018 May;154(6):1764-1777.e7.doi: 10.1053/j.gastro.2018.01.030.

# GS-9688 modulates immune cell phenotypes and functions

- GS-9688 activated dendritic cells and mononuclear phagocytes to produce IL-12 and other immunomodulatory mediators.
- GS-9688 increased the frequency of activated NK cells, CD4+ $T_{FH}$ and IFN-ɣ+ HBV-specific CD8+ T cells.
- GS-9688 induced NK cell expression of IFNɣ and TNFα and promoted hepatocyte lysis.
- GS-9688 reduced the frequency of CD4+ regulatory T-cells and monocytic myeloid-derived suppressor cells (MDSC).

Amin, O.E., et al. Hepatology. 2020 Dec 25. doi: 10.1002/hep.31695.

# Safety and pharmacodynamics of the oral TLR8 agonist selgantolimod in chronic hepatitis B *(Phase 1b study)*

**Treatment schedule:** **28** virally suppressed patients received once weekly 1.5 mg for two weeks or 3 mg for two or four weeks).
**10** viremic patients received once weekly 3 mg for two weeks

- 20/38 patients (53%) experienced an AE (mild or moderate in severity).
- 32/38 patients (84%) had laboratory abnormalities.
- The most common AEs were headache (32%), nausea (24%) and dizziness (13%).
- Selgantolimod induced transient dose-dependent increases in serum cytokines, including IL-12p40 and IL-1RA.

Gane, E.J., et al. Hepatology. 2021 Mar 11.doi: 10.1002/hep.31795

# Efficacy of 24 weeks treatment with oral selgantolimod: A phase 2 clinical trial

**Patients and Treatment schedule:** 48 patients (24 HBeAg-positive and 24 -negative) were randomized to to SLGN 3 mg, 1.5 mg, and PBO (2:2:1) once a week for 24 weeks while maintaining OAV.

## Virological response at week 48 (at the end of 24 week follow-up)

| HBV markers | SLGN 3mg | SLGN 1.5 mg |
|---|---|---|
| HBsAg loss | 1/19 (5%) | 1/20 (5%) |
| HBeAg loss | 2/9 (22%) | 1/10 (10%) |
| HBsAg decline > 0.1 $\log_{10}$ | 4/19 (21%) | 6/20 (30%) |

Gane, E.J., et al. Hepatology. 2020, 73:s52

# Summary

- Unlike DAAs, PRR agonists target multiple cell types to induce antiviral cytokines and modulate the function of immune cells to indirectly control viral replication in hepatocytes.
- TLR7 and TLR8 agonists can induce sustained control of viral infection or functional cure of chronic WHV infection in subsets of animals.
- TLR7 agonists elicited cytokine responses and improvement of HBV-specific T cell functions consistent with target engagement under tolerable doses in CHB patients. However, the activated immune responses failed to significantly reduce HBsAg antigenemia in the treated subjects.
- A modest antiviral efficacy in a subset of treated patients was demonstrated for TLR8 agonist (GS-9688) in a phase 2 clinical trail.