Activation of STING Mediates Antiviral Effects in a Mouse Model of Chronic Hepatitis B

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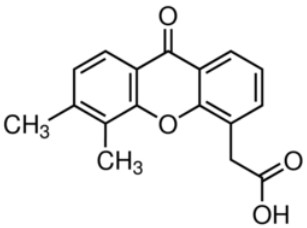
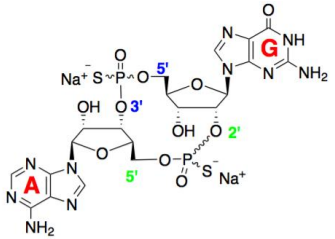
STING: Stimulator of Interferon Genes

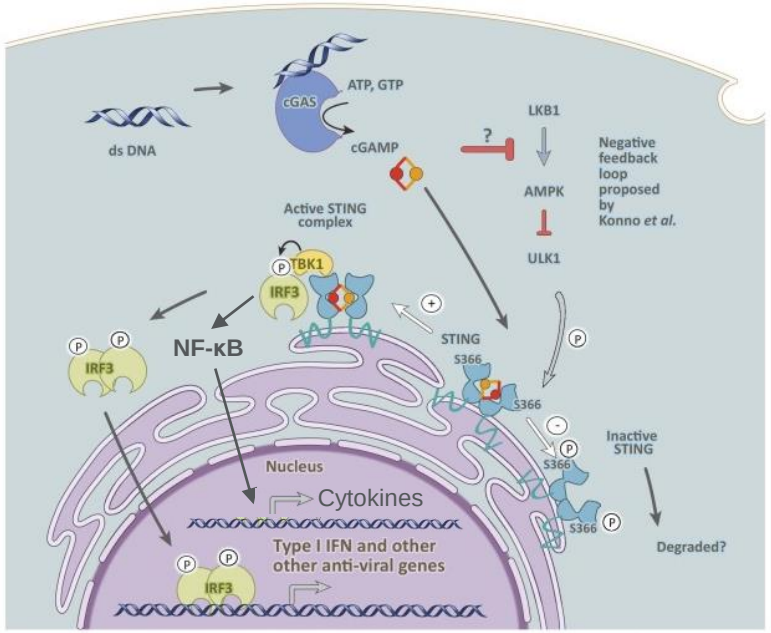
- **An innate immune adaptor regulating responses to viral dsDNA**
  - **Leads to Type I IFN and activation of NF-κB pathway**
- **Activated by CDNs and small molecules**
- **Role in viral infection and cancer**
  - **HCV NS4B[1,2], DENV NS2B-NS3 serine protease[3] and HBV Pol RT/RNase H domains[4] bind to/cleave STING and inhibit activation of IFN pathway**
  - **Therapeutic STING activation able to break tumor immune tolerance and promote tumor control[5] (intra-tumor admin)**



DMXAA
Small molecule



2'3' cGAM(PS)2
Cyclic dinucleotide (CDN)



Modified from Diner and Vance, Trends in Immunology, 2014

1: Nitta, Hepatol, 2013; 2: Ding, J Hepatol, 2013; 3: Aguirre, Plos Pathog, 2012;
4: Liu, JVI, 2015; 5: Corrales, Cell Reports, 2015

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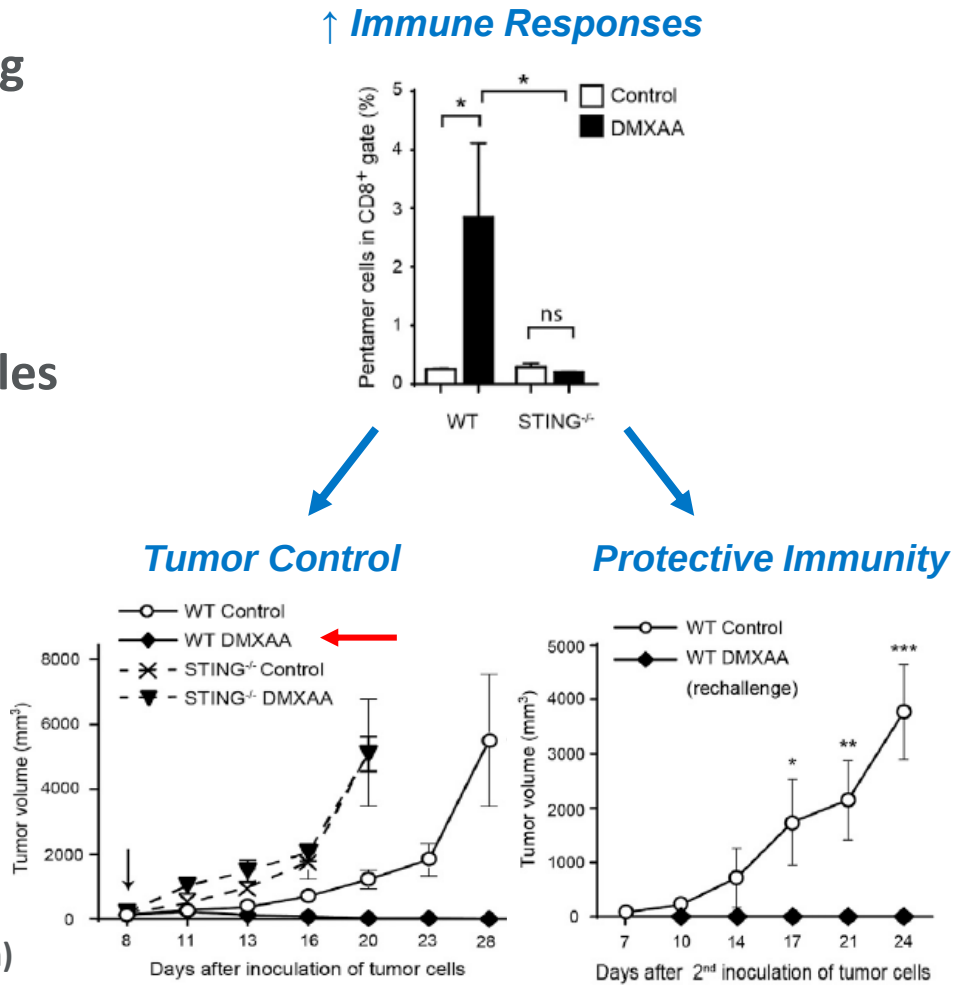

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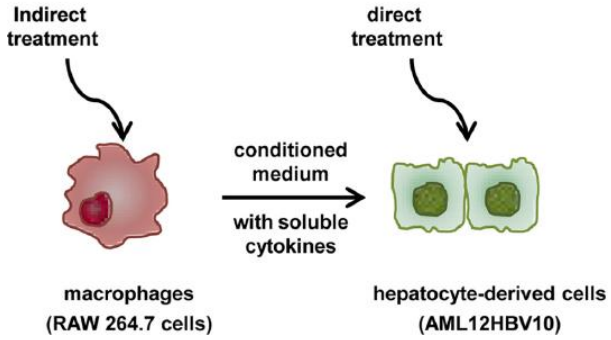
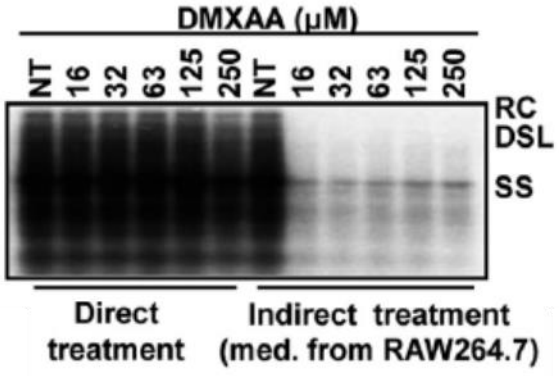
STING and HBV Antiviral Mechanism

- Chronic HBV infection is mediated by viral immune tolerance
- **STING activation may help break HBV immune tolerance and induce antiviral responses**
  - **Direct activation** of infected hepatocytes
  - **Indirect effects** through activation of antigen presenting cells, T cells and production of antiviral cytokines that affect HBV in hepatocytes & trigger T cell priming
- STING expressed in hepatocytes (low level), antigen presenting cells and T cells



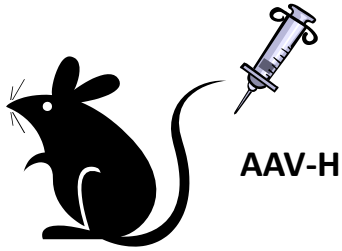
Hepatocyte-derived AML12HBV10 cells



Guo, Antimicrob Agents Chemother, 2015

What is the effect of STING activation in an immune tolerance mouse model of HBV?

In Vivo Experimental Approach



AAV-HBV

C57BL/6

Model	Sub-type	HBsAg (log IU/mL)	HBV DNA (log copies/mL)
AAV	HBeAg+	3.8 (2.2-4.3)	7.3 (4.6-8.2)
CHB Patient	HBeAg+	4.0 (1.8-5.0)[1]; 4.4 (±0.7)[2]	9.2 (±0.8) [2]

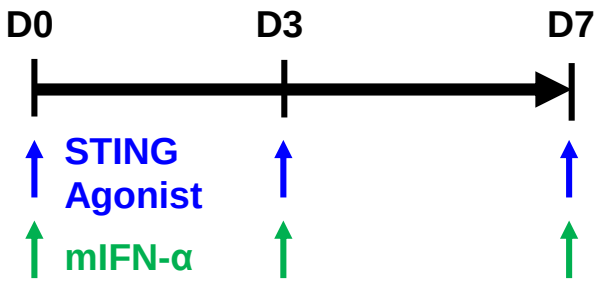
- **Immunocompetent model results in long term (> 200 day) stable marker expression**
- **Exhibits HBV immune tolerance similar to CHB patients** (Zhu 2016 J Immunol.)

Treatments:

Untreated
Vehicle
mIFN-α 1000 U/g Standard dose in literature
STING agonist 5 mg/kg DMXAA
STING agonist 12.5 mg/kg DMXAA/cGAM(PS)2
STING agonist 25 mg/kg DMXAA/cGAM(PS)2

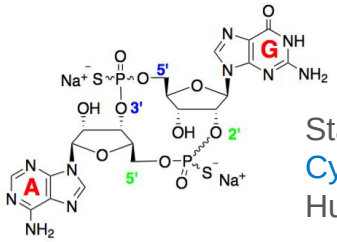
Assess HBV DNA, cytokines, Interferon stimulated genes (ISG) mRNA

Sacrifice & analyze 6 h after each dose (n=4)

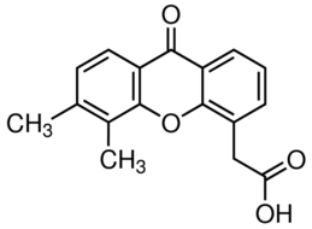


1: Seto et al. HEPATOLOGY 2013, 2: P. Arends et al. Journal of Viral Hepatitis 2014

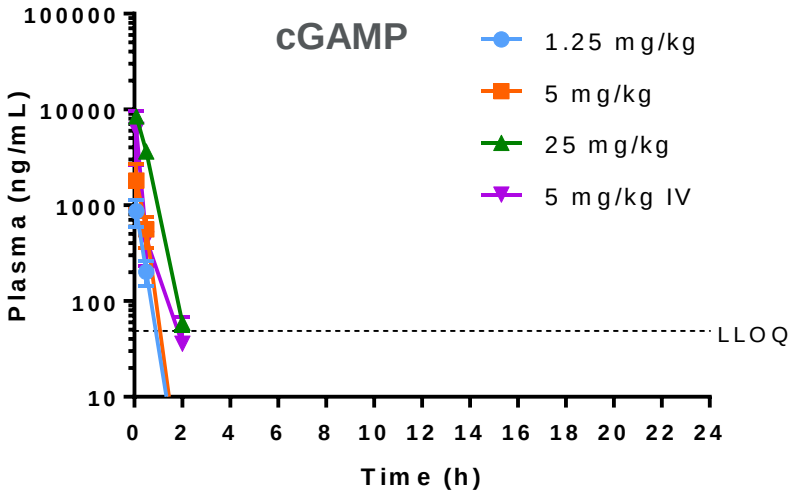
STING Agonist Classes Show Distinct Pharmacokinetic Profiles

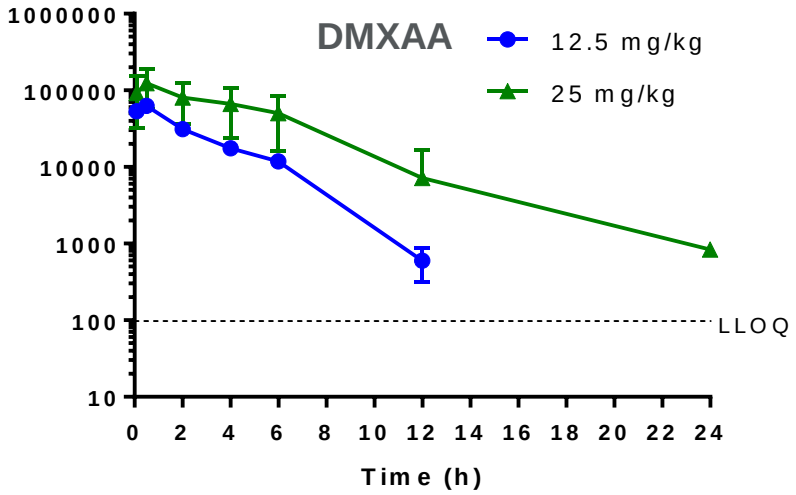


Stability-enhanced natural agonist
Cyclic dinucleotide
Human / multi-species active



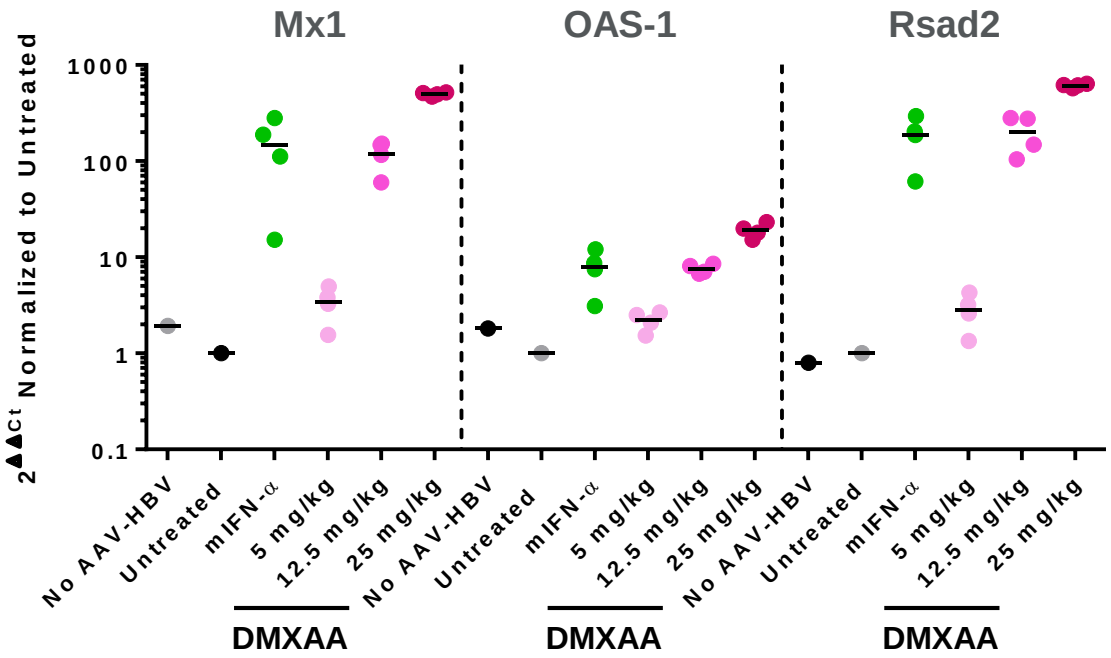
Mouse STING agonist
Small molecule

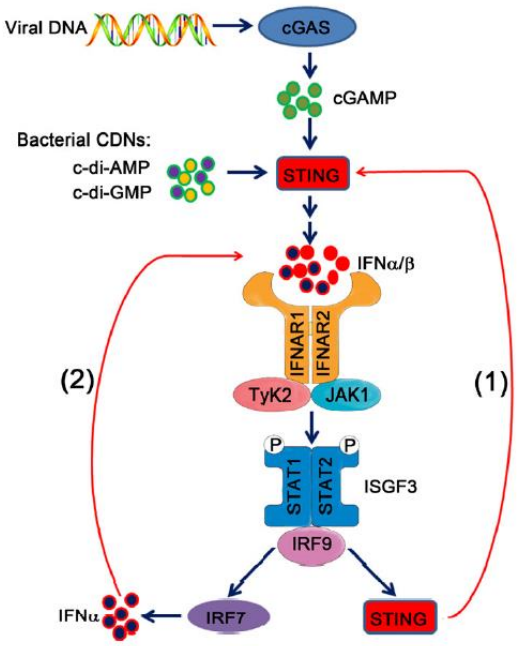




- For this mechanism of action (immune activation), achieving steady state levels of exposure may not be necessary

STING Activation Rapidly Induces Type I IFN Liver Response



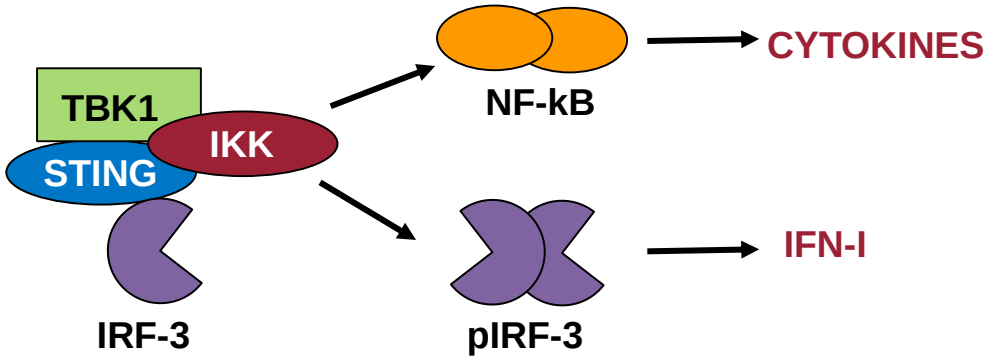


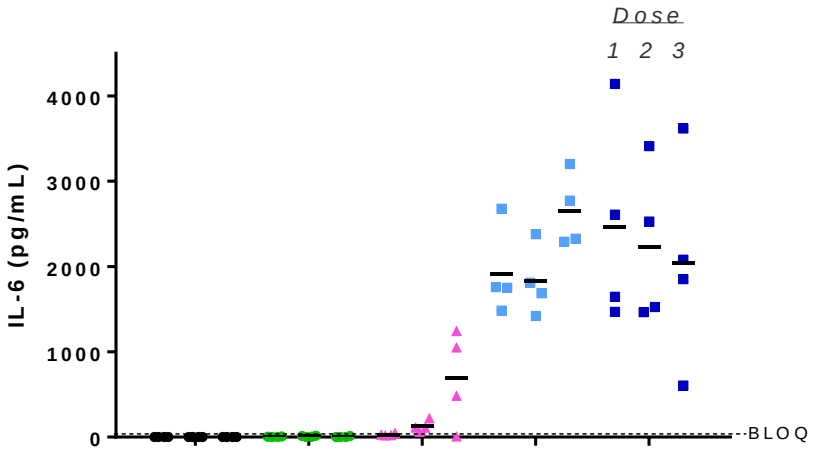
Ma, EMBO Rep, 2015

- **Strong ISG induction as soon as 6 h after administration**
  - **↑ by 1 to 3 orders of magnitude depending on the marker**
- **Magnitude of ISG induction from 1000 U/g mIFN-α as expected** (Harper 2013)
- **STING-triggered activation promotes positive feedback regulation of IFN responses**
- **Type I IFN responses can activate T cells and innate immune cells**

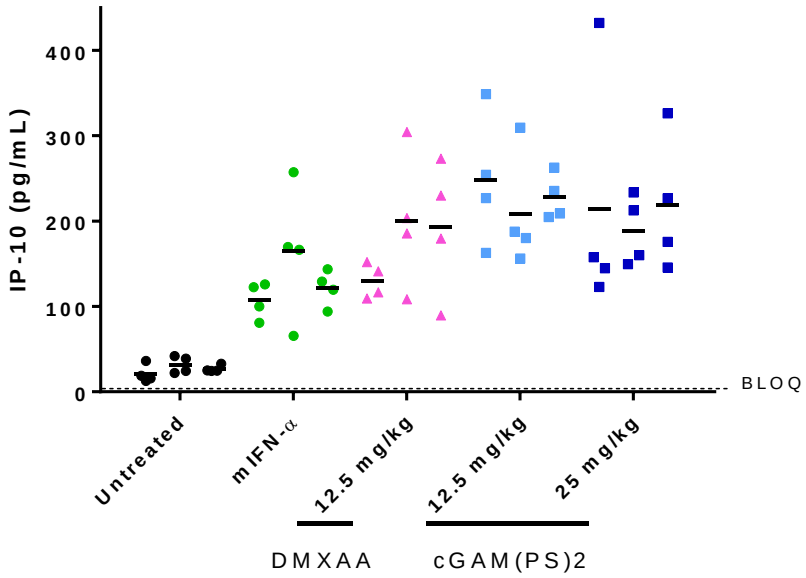
STING Activation Induces Cytokine Production

May result in both direct antiviral effects and T cell priming





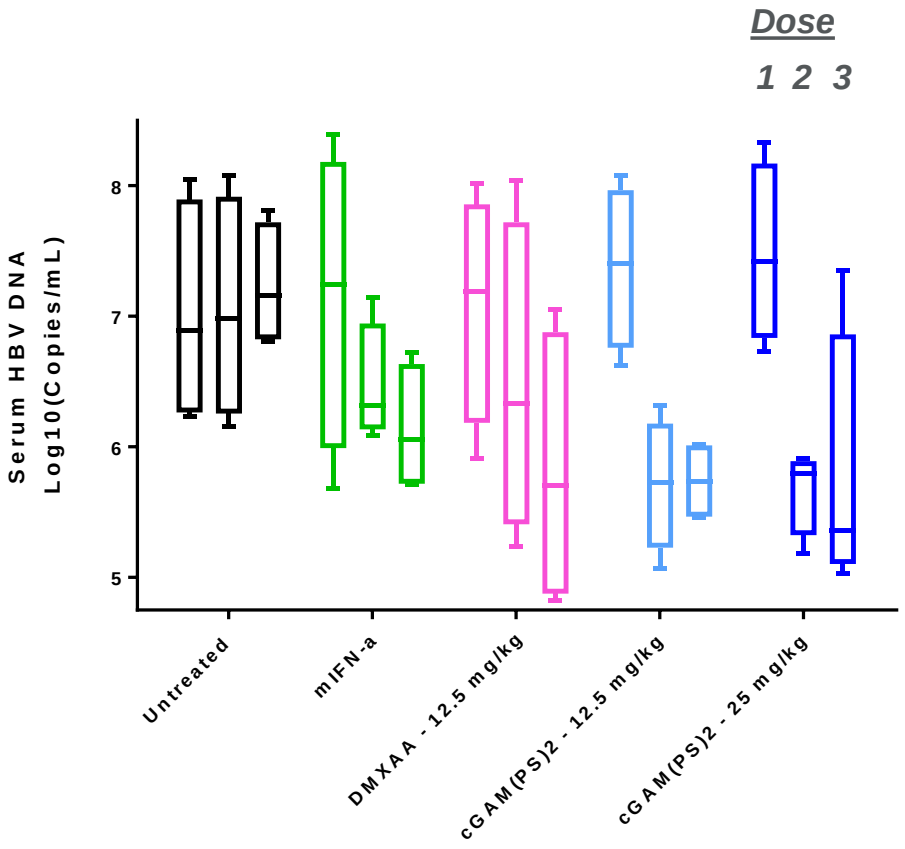
- Cytokines expected given bifurcated nature of STING signaling pathway
  - More potent stimulator of antiviral cytokine production than IFN-α
- Cytokines induced by STING may prime T cells, trigger maturation and migration (ex. IP-10)
- Activation of HBV-specific T cells can contribute to breaking HBV immune tolerance

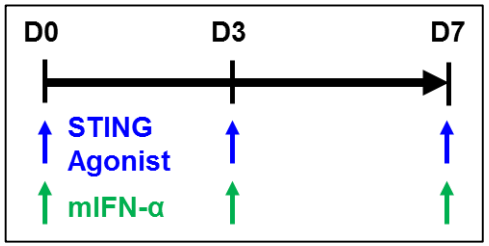


STING Activation Controls HBV Replication

Validated with 2 different classes of molecules

- First demonstration of cumulative reductions with repeat dosing
- Up to 2 log ↓ after 3 doses
- Better level of viral control than standard of care IFN-α
  (analogous to approved pegylated interferon drugs, in mouse)



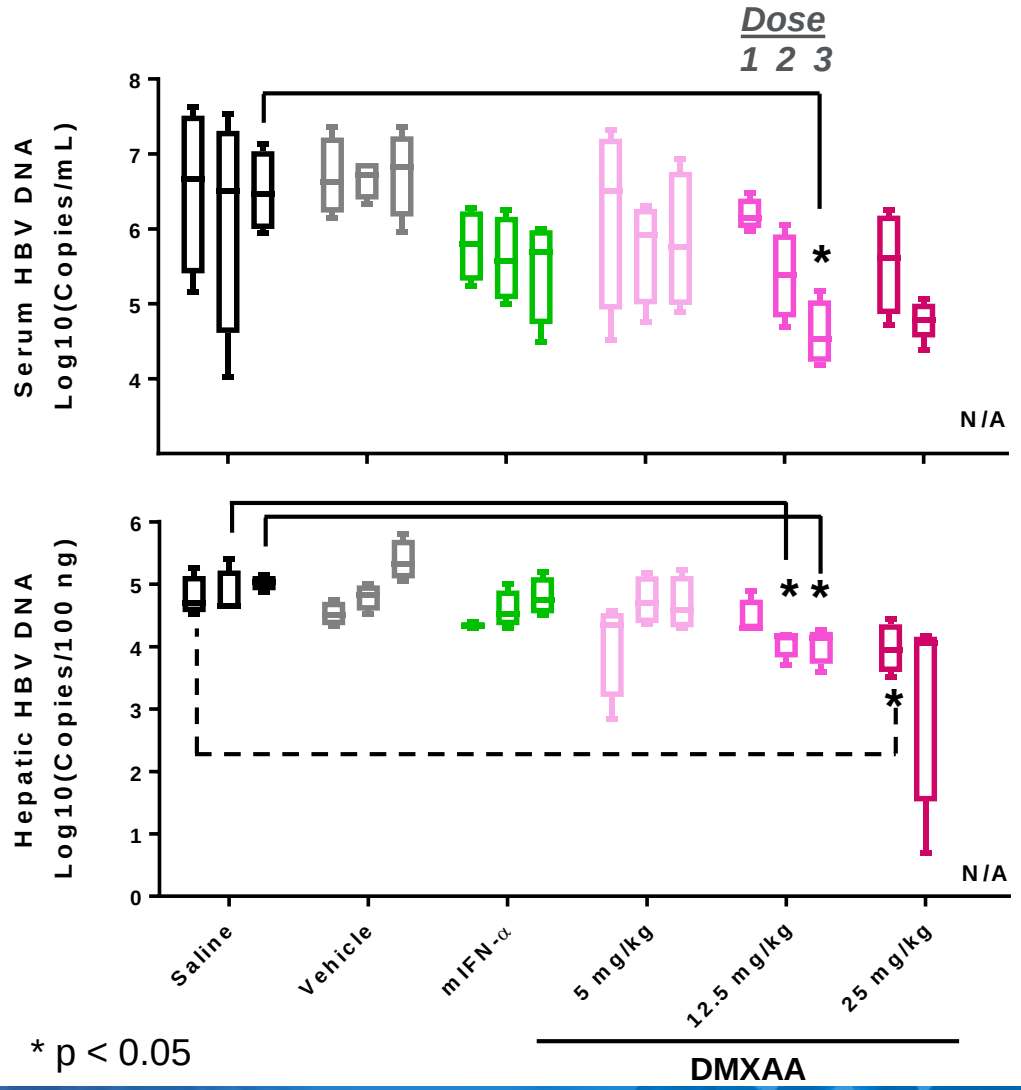


STING Activation Controls HBV Replication

Causes reductions in both liver and blood compartments

- 1 log ↓ serum HBV DNA and
- 1 log ↓ liver HBV DNA
- within 7 days of treatment

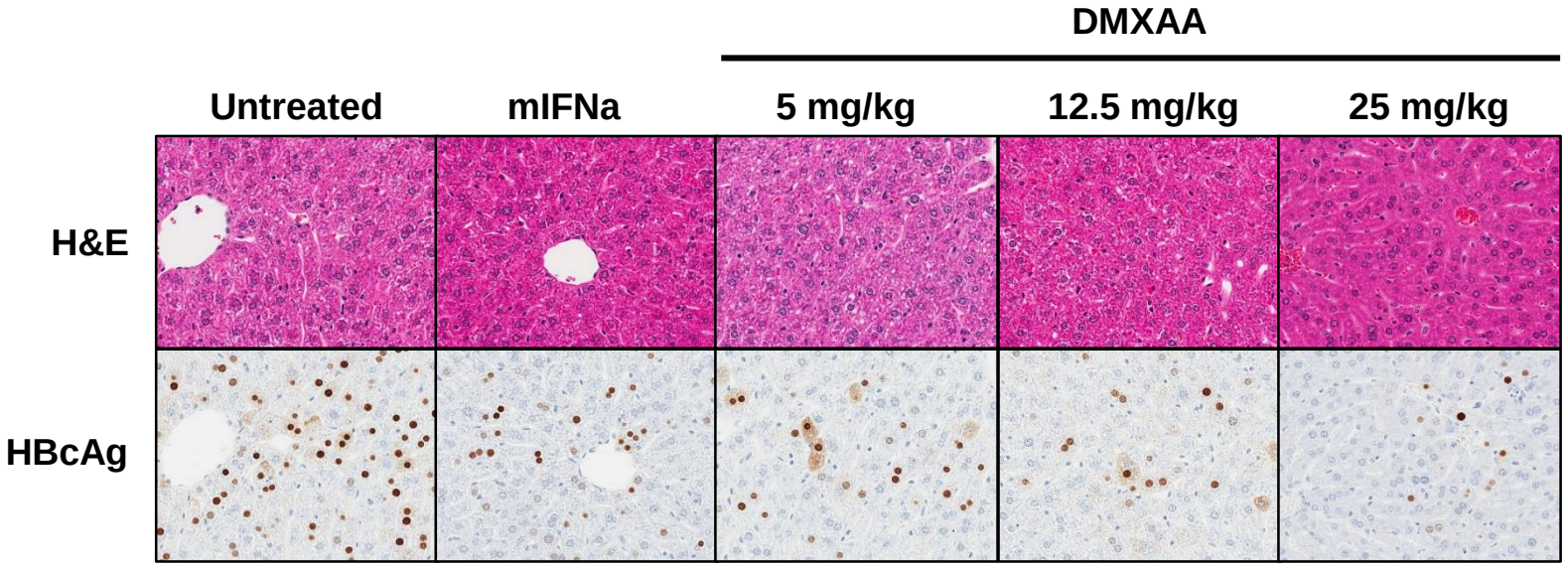
- Single dose consistent with published data[1] in hydrodynamic HBV mouse



* $p < 0.05$

1: Guo et al., Antimicro Agents Chemotherapy, 2015

Repeat STING Activation Reduces HBV Antigen in Liver



- Correlates with observed inhibition of viral replication
- Antigen control may potentiate HBV immune responses

Summary & Conclusions

- STING activation results in control of HBV replication
  - Demonstrated with two distinct chemical modalities (small molecule, CDN)
- Repeated STING activation yields cumulative reductions in HBV DNA, HBcAg across a 7-day course of study in an immune-tolerant animal model of HBV
- Type I IFN and cytokine induction may activate innate immune cells and T cell priming to potentially break HBV immune tolerance

Targeting STING in chronic HBV infection may benefit viral control and boost immunity

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