Characterization of Cavrotolimod, a TLR9 Agonist for the Treatment of Chronic Hepatitis B

HJ. Ramos [1], J. Deval [1], JC. Stanton[1], N. Shulman [1], and H. Javanbakht [1]
1. Bluejay Therapeutics, San Mateo, CA, United States.

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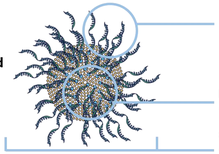
Background

- Restoring HBV-specific immune responses is central to achieving a cure for chronic hepatitis B.
- Cavrotolimod (CAVRO) is a novel spherical nucleic acid (SNA) Toll-Like Receptor 9 (TLR9) agonist oligonucleotide designed to activate innate and adaptive immunity.
- CAVRO features a unique design aimed at enhancing antiviral immune responses
  - (1) TLR9 targeting for selective activation of plasmacytoid dendritic cells (pDC) and B-cells
  - (2) SNA formulated delivery of a TLR9 agonist to increase endosomal uptake and improve potency compared to other TLR agonists.
- CAVRO has been evaluated in a first-in-human Phase 1 study in healthy subjects (Daniel et al., 2022), demonstrating safety and tolerability across various doses.



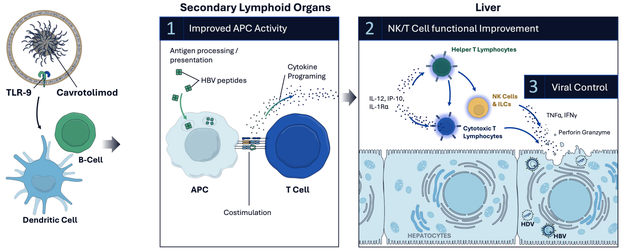
TLR9 agonist oligonucleotides – potent immunomodulators
- Cytokines/chemokine signaling (IL-12, IP-10)
- T cell activation
- NK cell activation

Benign lipid nanoparticle
- Scaffold for SNA structure

Oligonucleotides + nanoparticle = SNA
- Increased cellular and endosomal uptake

→ Endosomes: Locations of TLR9 target

Cavrotolimod Mode of Action



CAVRO signals through TLR9 on plasmacytoid dendritic cells (pDCs) and B-lymphocytes, potentially driving distinct innate and adaptive immune responses to support an HBV functional cure: **1** – direct signaling enhances antigen-presenting cell (APC) activity, including (**A**) antigen processing and presentation, (**B**) co-stimulatory receptor induction, and (**C**) type-1 cytokine programming; **2** – Indirect activation of NK and antigen-specific T-cell subsets leads to improve functional characteristics, including the induction of cytotoxic pathways (perforin, granzyme, TNFα, IFNγ). **3** – Facilitation of viral control.

Objective

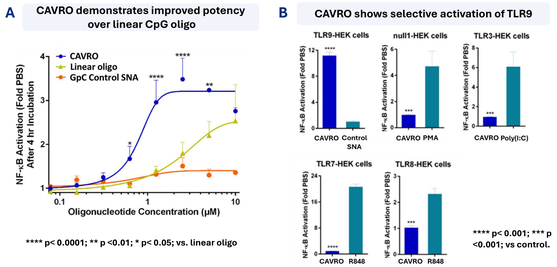
- To assess pharmacodynamic activity in both *in vitro* and *in vivo* assays and to evaluate the potential for therapeutic intervention in CHB-infected patients.

Methods

- *In vitro* studies were conducted to demonstrate the potency and selectivity of CAVRO.
- Human PBMCs from healthy or HBV+ donors were obtained and treated with CAVRO or control oligos to assess activity and cytokine response.
- Pharmacodynamic (PD) effects of subcutaneously administered CAVRO in cynomolgus monkeys(non-human primate, NHP) were assessed across a range of doses.

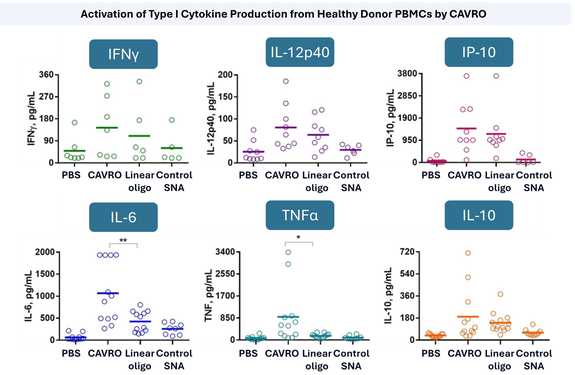
Results

Figure 1: CAVRO stimulates a potent and selective activation of TLR9



Cavrotolimod activates NF- κB in a dose-dependent and TLR-9 specific manner. The ability of CAVRO to activate TLR9 specific signaling was evaluated in HEK-293-blue reporter cells overexpressing TLR9 or off-target TLRs, TLR3, TLR7, and TLR8. (**A**) HEK-293 TLR9 cells were incubated with 0.1 – 10μM CAVRO, linear oligo, or Control GpC SNA, and NF- κB signaling was assessed after 4 hours in a reporter assay. CAVRO elicited a response at EC_{50} ~ 3-fold greater than linear oligo. (**B**) The specificity of TLR9 activation was further evaluated by assessing activation of cell lines expressing TLR3, TLR7, TLR8, or TLR9 with (5μM) CAVRO or positive TLR-ligand controls, Poly(1:C) (TLR3); R848 (TLR7/8) after 24 hrs. of stimulation. Shown is the fold change vs PBS. CAVRO elicited potent NF- κB induction on TLR9 HEK cells but not TLR7, 8, 3, or null control cell lines.

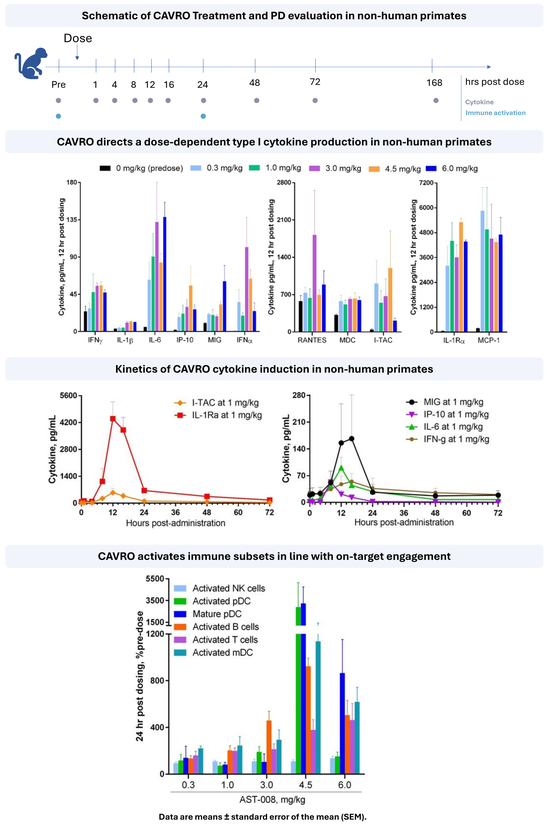
Figure 2: CAVRO Elicits A Th1/Tc1 Cytokine Profile from Human PBMCs



* p< 0.05; ** p <0.01; Cavro vs Linear oligo control.

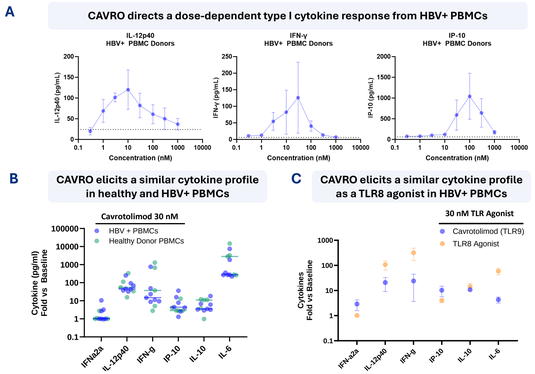
Cavrotolimod elicits a Th1/Tc1 type cytokine profile in human PBMCs from healthy donors. The cytokine profile produced by peripheral blood mononuclear cells (PBMCs) after incubation with CAVRO or control nucleic acids was assessed. Primary human PBMCs from healthy donors were treated with 2.5 μM CAVRO, linear oligonucleotide, or a control SNA at concentrations. The cytokine concentrations in the cell culture supernatant were measured using human multiplex cytokine arrays at 24 hours post stimulation. Sixteen cytokines were assessed (GM-CSF, IFNα, IFNγ, IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-17, IP-10, RANTES, and TNFα). CAVRO elicited a Th1/Tc1 cytokine profile with general higher responses than linear oligo and control SNA indicating the potential for responsiveness in patients.

Figure 3: CAVRO elicits an on-target PD profile in Non-human Primates



Cavrotolimod Induces dose-dependent cytokine induction in non-human primates (NHP). The in-life kinetics and PD response of CAVRO was evaluated after administration of a single dose to cynomolgus macaques (NHP). (**A**) NHP male (n=2) and female (n=2) were administered CAVRO at 0.3, 1, 3, 4.5 or 6 mg/kg and cytokine (**B**,**C**) and cell activation (**D**) was determined at the timepoints shown in panel A. (**B**) Cytokine induction at 12 hr. post dosing in NHP demonstrates a dose-dependent induction of type I cytokines including IFNγ, IFNα, IP-10, IL-6, and IL-1Ra. (**C**) Kinetics of cytokine response from 1 – 7 2 hrs. post dosing with 1 mg/kg CAVRO. Cytokine response generally peaked between 12 and 24 hrs. post treatment. (**D**) Immune activation at 24 hrs. post dosing of 4.5 mg/kg in NHP. Cavro elicits an on-target dose – dependent expansion of DC and B – cell subsets in NHP.

Figure 4: CAVRO elicits a cytokine profile that supports the potential for safe and efficacious response in HBV$^+$ patients



Cavrotolimod elicits a cytokine profile in PBMCs from HBV+ donors comparable to healthy PBMCs and with similar profile to TLR8 agonism. Primary human PBMCs from HBV+ donors or healthy donors were treated with CAVRO or a TLR8 agonist at doses expected to cover the range of exposure expected in patients (0.3 – 100 nM). (**A**) CAVRO elicits a dose-dependent type I cytokine response in HBV+ donors, including IL-12p40, IFN-γ, and IP-10. (**B**) CAVRO elicits a similar magnitude and cytokine profile in HBV+ PBMCs compared to healthy donor PBMCs. (**C**) CAVRO induces a cytokine profile comparable to TLR8 agonism in HBV+ donors and suggests the potential for beneficial immune activation in HBV+ patients.

Conclusions

- CAVRO exhibits a selective and potent TLR9 activation profile *in vitro*, effectively inducing a type I cytokine response in human PBMCs.
- The immune activation and cytokine profile in PBMCs from HBV+ donors closely resembles that of TLR8 agonism.
- In non-human primates, CAVRO administration demonstrated target engagement and triggered pharmacodynamic responses linked to type I cytokine production and immune cell activation.
- Taken together, these data support the development of Cavro as part of a potential functional cure regimen for chronic hepatitis B.

References

1. **Daniel W et al.** Pathogenesis of hepatitis B virus infection. A first-in-human phase 1 study of cavrotolimod, a TLR9 agonist spherical nucleic acid, in healthy participants: Evidence of immune activation *Frontiers in Immunology. 2022;13: 107377*

Acknowledgements and Disclosures

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