In Vivo Study of a LNP siRNA Investigational Agent Applied Sequentially with Immunomodulatory Treatments for Chronic Hepatitis B Infection

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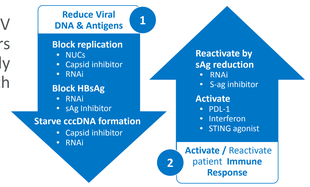
Arbutus BIOPHARMA *Curing Chronic Hepatitis B*

OBJECTIVES

- Here we examine, in a mouse tolerance model of chronic HBV (CHB) infection, drug combination strategies utilizing a HBV antigen-reducing LNP siRNA agent with the goal of sustained off-treatment viral control
- Reducing HBV proteins, particularly surface antigen (HBsAg), may be required to abrogate viral suppression of immune function as a prerequisite to reinvigoration of a host defense
- Markers of humoral and cell-mediated immunity were examined in this study for possible correlation with off-treatment viral control

THERAPEUTIC APPROACH

Developing a HBV cure for chronic HBV infection must address multiple factors involved in viral persistence and likely will require combination of drugs with complementary modes of action.

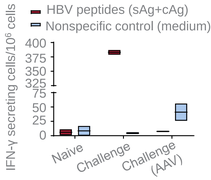


1. ARB-1467 and ARB-1740 are lipid nanoparticle (LNP)-delivered siRNA agents clinically validated for HBsAg reduction in both eAg-Pos and eAg-Neg CHB patients[1]
2. In this *in vivo* study we combined antigen-reducing LNP siRNA with two immune-boosting treatments: checkpoint blockade and vaccination

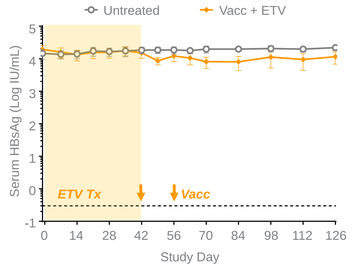
MOUSE MODEL OF CHRONIC HBV

- Immunocompetent C57BL/6 mice produce HBV from their livers via a 1.2× overlength genotype D sequence on an adenovirus associated vector (AAV)[2]
- Stable long term HBV biomarker levels similar to CHB patients *(table below)*
- Exhibits HBV immune tolerance characteristics *(bottom left of this panel)*

Model	Subpopulation	Serum HBsAg (Log_{10} IU/mL)	Serum HBV DNA (Log_{10} Copies/mL)
AAV-Mouse	eAg-Pos	3.8 (2.2-4.3)	7.3 (4.6-8.2)
CHB Patient	eAg-Pos	4.0 (1.8-5.0)[3]; 4.4 (±0.6)[4]	9.1 (±0.9)[4]



AAV mice have tolerance to HBV. Liver T/NK cell responses to HBV antigens are muted in AAV-HBV1.2 treated mice. Fluorospot IFN-γ assessment after challenge by hydrodynamic injection of HBV expression plasmid pHBV1.3. Data are mean ± range of technical duplicates from pooled liver lymphocytes (n=4 mice/group).



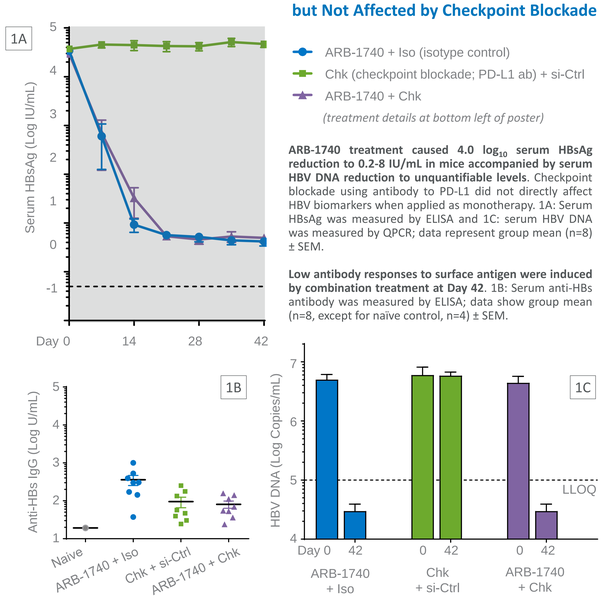
AAV-mouse treatment using vaccine + ETV does not lead to off-treatment viral control, similar to outcomes in chronically infected CHB patients. Serum HBsAg measured by ELISA; group mean (n=4) ± SEM.

ANIMAL TREATMENTS

Abbreviation	Description	Treatment Regimen
ARB-1740	LNP-formulated triple siRNA agent targeting HBV **si-Ctrl** = negative control, targeting luciferase	1 mg/kg i.v. QW Days 0-35
Chk	Checkpoint inhibitor; αPD-L1 antibody clone 10F.9G2 **Iso** = isotype control, Rat IgG2b clone LTF-2	200 µg i.p. 2× weekly Days 0-41
ETV	Entecavir	1 µg/kg p.o. QD Day 0-56
Vacc	Engerix-B vaccine + ODN1826 TLR9 agonist	2 µg HBsAg + 50 µg ODN s.c. on Days 42 & 56

RESULTS

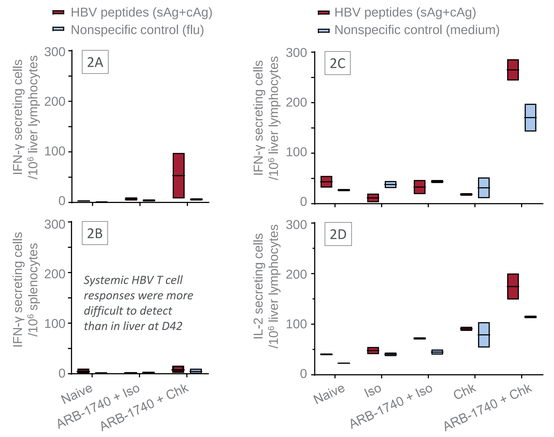
1. HBV DNA and Antigen Load Reduced by LNP siRNA but Not Affected by Checkpoint Blockade



ARB-1740 treatment caused 4.0 log_{10} serum HBsAg reduction to 0.2-8 IU/mL in mice accompanied by serum HBV DNA reduction to unquantifiable levels. Checkpoint blockade using antibody to PD-L1 did not directly affect HBV biomarkers when applied as monotherapy. 1A: Serum HBsAg was measured by ELISA and 1C: serum HBV DNA was measured by QPCR; data represent group mean (n=8) ± SEM.

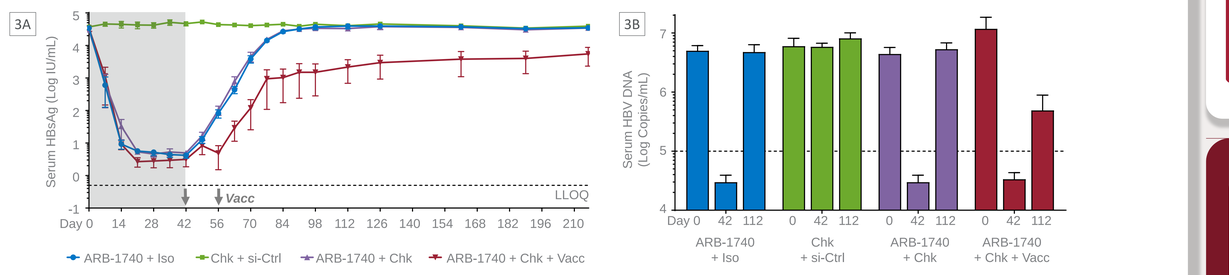
Low antibody responses to surface antigen were induced by combination treatment at Day 42. 1B: Serum anti-HBs antibody was measured by ELISA; data show group mean (n=8, except for naïve control, n=4) ± SEM.

2. Dual Combination of LNP siRNA + Checkpoint Blockade Resulted in T Cell Response Not Observed with Either Monotherapy



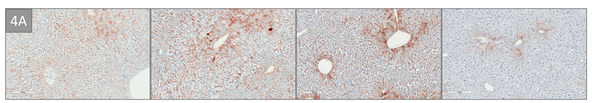
ARB-1740 plus antibody blockade against PD-L1 induced T/NK cell production of IFN-γ and IL-2 which are markers of antiviral function and proliferation, respectively. Fluorospot assays were conducted on Day 42 after 6 weeks of treatment. Data are means ± range of technical duplicates from pooled liver lymphocytes or splenocytes (n=4 mice/group). The left pair (2A, 2B) and the right pair (2C, 2D) of graphs represent two independent studies providing congruent results. HBV-specific T cell responses were stronger in liver than in spleen (2A vs 2B).

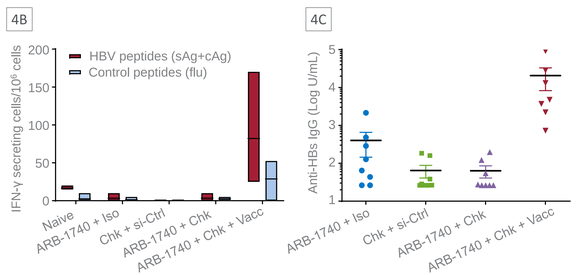
3. Despite T Cell Response, LNP siRNA + Checkpoint Blockade Dual Combination Was Insufficient for Off-treatment Viral Control → Addition of Vaccination (Sequential Triple Combination) Resulted in Sustained Off-treatment Suppression of HBV Antigen and DNA



Viral load was monitored in mice across a 6-week LNP siRNA treatment regimen followed by a 2-week prime-boost vaccination protocol and then a 23-week off-treatment observation phase. 3A: Serum HBsAg was measured by ELISA; data show group mean (n=8) ± SEM. 3B: Serum HBV DNA was measured by QPCR; data show group mean (n=8) ± SEM.

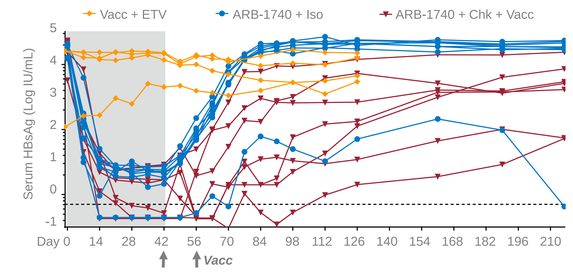
At end of study on Day 216, 23 weeks after the last treatment (vaccination), mice were examined for evidence of sustained off-treatment viral control, including reduction of viral load in liver as well as upregulation of T/NK cell and humoral responses. 4A: Immunohistochemistry was conducted on mouse livers to visualize HBsAg (brown) and counterstained with hematoxylin to visualize nuclei (blue). Images representative of each treatment group were selected (scale marker = 200 µm). 4B: T cell responses were measured using fluorospot IFN-γ assay; data are means ± range of technical triplicates (2 pools of 4 mice/group except for naïve which was 1 pool of 4 mice) from splenocytes; liver lymphocyte data were similar. 4C Serum anti-HBs antibody was measured by ELISA; data show group mean (n=8, except for naïve control, n=4) ± SEM.





4. Sustained Control of HBV in Liver and Blood Correlated with Increased T Cell and Humoral Responses at 23 Weeks Off-treatment

5. Wide Range of Individual Off-Treatment Responses to Sequential Triple Combo



Variation of individual animal responses *(graphed above, tabulated below)* **detected in this mouse tolerance model of chronic HBV infection**

- Antibody levels alone did not directly correlate with off-treatment control
- Achievement of low (HBsAg) antigen levels appeared to be a prerequisite but not a guarantee for sustained off-treatment viral control
- Further exploration of immune cell populations warranted

Group	Baseline		On-Treatment Response			Off-Treatment 8 Weeks (D112)		
	HBsAg	Anti-HBs Ab	Minimum HBsAg During Day 0-42		Anti-HBs Ab at D42	HBsAg		Anti-HBs Ab
	IU/mL	U/mL	IU/mL	Max Log Change	U/mL	IU/mL	Log Change	U/mL
Vacc + ETV	25,738	< 30	12,482	-0.3	42	15,934	-0.2	1,773
	25,434		9,458	-0.4	43	2,961	-0.9	23,081
	25,778		10,354	-0.4	43	22,656	-0.1	75
ARB-1740 + Iso	122		122	0.0	42	4,528	1.6	1,239
	41,637		3.3	-4.1	306	57,235	0.1	141
	44,298		4.5	-4.0	391	54,173	0.1	91
	40,424		7.1	-3.8	526	38,892	0.0	518
	16,199		0.9	-4.2	37	44,592	0.4	19
	40,692		4.4	-4.0	143	44,356	0.0	225
	35,958		0.2	-5.3	299	51	-2.9	441
	15,202		5.7	-3.4	171	45,071	0.5	90
	37,993		4.9	-3.9	999	29,966	-0.1	3,352
ARB-1740 + Chk + Vacc	54,671		0.2	-5.5	65	798	-1.8	1,042
	45,869		3.8	-4.1	606	5,324	-0.9	7,759
	50,330		5.9	-3.9	147	692	-1.9	37,260
	3,251		0.2	-4.3	59	178	-1.3	4,838
	29,403		7.5	-3.6	*No data; dropped out D21 (suspected mis-dose)*			
	23,420		0.2	-5.1	44	127	-2.3	16,928
	37,053		0.2	-5.2	50	12	-3.5	27,605
	23,813		0.2	-5.1	27	2	-4.1	99,989

Lowest — Highest

CONCLUSIONS

In an AAV mouse model of chronic HBV infection, sequential triple combination of LNP siRNA agent ARB-1740, checkpoint blockade and vaccination resulted in:

- ≥ 4 log_{10} serum HBsAg reduction to 0.2-8 IU/mL
- Induction of liver and systemic IFN-γ HBV T cell and NK cell responses
- Induction of anti-HBs antibody responses
- Sustained control of HBV in blood and liver for 23 weeks off-treatment

These data are consistent with the hypothesis that management of HBV surface antigen is a critical element in the development of curative therapy and that combination with agents boosting immune reactivation may be beneficial.

REFERENCES

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