Characterization of BJT-778, an anti-HBsAg neutralizing monoclonal antibody for treatment of hepatitis B virus and hepatitis D virus infections

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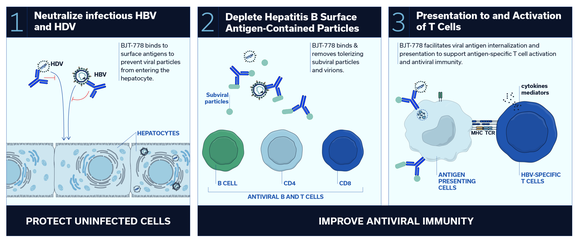
Background

- An estimated 300 million people worldwide are infected with chronic hepatitis B (CHB).
- Approximately 12 million of these individuals are also chronically coinfected with hepatitis D (CHD).
- BJT-778 is a high-potency, fully human immunoglobulin G1 (IgG1) monoclonal antibody (mAb), targeting the hepatitis B surface antigen (HBsAg).
- Neutralizes hepatitis B and D virions and depletes HBsAg-containing subviral particles, which may help reconstitute antiviral immunity and contribute to a functional cure for CHB.
- Features a fully functional IgG Fc domain that facilitates Fc-gamma mediated antigen uptake and antigen-specific T cell activation (**poster SAT-536**).
- BJT-778 is currently being evaluated in healthy volunteers and subjects with CHB and CHD (**posters WED-374/LBP-001**).

BJT - 778 has Multiple Modes of Action



Objective

To evaluate the specificity and *in vitro* an *in vivo* characteristics of BJT-778 in pre-clinical assays and models of HBV and HDV infection.

Methods

- The binding affinity of BJT-778 to HBsAg from various serotypes, genotypes, and clinical mutants was evaluated using surface plasmon resonance (SPR) and ELISA assays.
- Ability of BJT-778 to neutralize HBV and HDV infection was determined in an *in vitro* primary human hepatocyte model.
- The *in vivo* efficacy of BJT-778 was also investigated in a humanized Fah/Rag2/IL-2rγ triple knockout (FRG) mouse model of chronic HBV infection.

Results

Table 1: Affinity Profile of BJT-778 to HBsAg

	HBsAg: Serotype ad Mean±SD			HBsAg: Serotype ay Mean±SD		
	k_a (1/Ms)	k_d (1/s)	K_d (nM)	k_a (1/Ms)	k_d (1/s)	K_d (nM)
BJT-778	1.59E+6 ±3.54E+4	3.54E-4 ±1.34E-5	0.22 ±0.003	9.90E+5 ±7.78E+3	3.85E-4 ±5.66E-6	0.39 ±0.009

HBsAg=hepatitis B surface antigen; Ms-molar seconds; SD=standard deviation.

BJT-778 binds with high affinity to the HBsAg protein of serotypes ad and ay. The kinetic rate and binding affinity of BJT 778 were assessed using surface plasmon resonance using purified HBsAg from HBV ad and ay serotypes. Both affinity (Kd) and kinetic rate of binding (Ka) were assessed. BJT-778 binds with pM affinity across both serotypes of purified HBsAg proteins tested in these experiments. The binding affinities (Kd) of BJT 778 are 0.22 nM and 0.39 nM for HBsAg serotypes ad and ay, respectively.

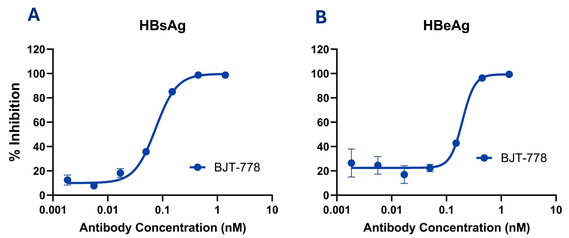
Table 2: BJT-778 Binds to HBsAg from Major HBV Genotypes

Antibody	IC_{50} (nM) Mean±SD			
	Genotype A	Genotype B	Genotype C	Genotype D
BJT-778	0.07±0.08	0.02±0.003	0.02±0.003	0.07±0.05
LJP-537	>67	>67	>67	>67

HBsAg=hepatitis B surface antigen; IC50=half maximal inhibitory concentration; SD=standard deviation.

BJT-778 binds with high affinity to the HBsAg protein of the major HBV genotypes. BJT-778 or a negative control antibody, recognizing human cytomegalovirus, LJP-537 were evaluated for binding to representative HBsAg isolates from the 4 major genotypes of HBV (A, B, C, and D), via enzyme-linked immunosorbent assay (ELISA). BJT-778 bound to all genotypes with half maximal inhibitory concentration (IC_{50}) values ranging from 0.02 to 0.07 nM. In contrast, a negative control antibody targeting cytomegalovirus (LJP 537) did not bind to any HBsAg proteins in this assay at the highest concentration tested.

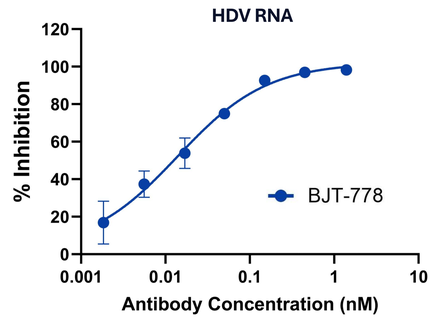
Figure 1: Neutralizing Potency of BJT-778 Against HBV



Inhibitor	Mean EC_{50} (nM)±SD	
	HBsAg	HBeAg
BJT-778	0.09±0.02	0.11±0.07
Bulevirtide	1.09±0.58	1.45±0.6

BJT-778 neutralizes HBV in a primary human hepatocyte infection assay. The ability of BJT-778 to neutralize HBV infection was assessed using *de novo* infection of primary human hepatocytes (PHH). PHH were treated with a mixture of BJT-778 and HBV inoculum and co-incubated for 8 days. At day 8, HBsAg and HBeAg were measured by ELISA. Representative HBsAg (**A**) and HBeAg (**B**) inhibition by BJT-778 from 1 of 3 experiments are shown in the figure. Average half maximal effective concentration (EC_{50}) data from n=3 experiments is shown in the table; EC_{50} values from Bulevirtide, an entry inhibitor[3] run in the assay are shown for comparison. BJT-778 showed potent neutralization of infectious HBV, with EC_{50} values of 0.09 and 0.11 nM for HBsAg and HBeAg, respectively. In contrast, bulevirtide,, prevented HBV infection with EC_{50} values ranging from 1.09 to 1.45 nM

Figure 2: Neutralizing Potency of BJT-778 Against HDV



Inhibitor	Mean EC_{50} (nM)±SD
BJT-778	0.01±0.003
Bulevirtide	0.77±0.39

EC50=half-maximal effective concentration; HDV=hepatitis D virus; RNA=ribonucleic acid; SD=standard deviation

BJT-778 neutralizes HDV in a primary human hepatocyte infection assay. The ability of BJT-778 to neutralize HDV infection was assessed using *de novo* infection HBV/HDV co-infection of primary human hepatocytes (PHH). PHH were treated with a mixture of BJT-778 and HBV and HDV inoculum, and co-incubated for 8 days. Representative HDV RNA inhibition by BJT-778 from 1 of 3 experiments is shown in the figure. Average half maximal effective concentration (EC_{50}) data from n=3 experiments is shown in the table; EC_{50} values from Bulevirtide, an entry inhibitor[3] run in the assay are shown for comparison. BJT 778 showed potent neutralization of infectious HDV, with an EC_{50} value of 0.01 nM. In contrast, Bulevirtide, prevented HDV infection, with EC_{50} values of 0.77 nM.

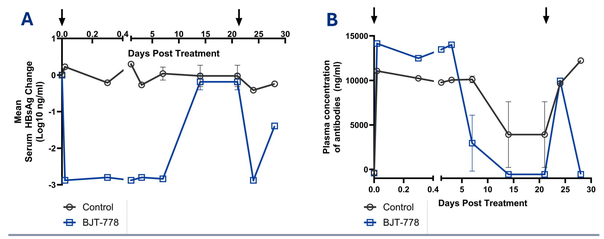
Table 3: Binding profile of BJT-778 against HBsAg with clinical mutations

HBsAg Protein	BJT-778 IC_{50} (nM) Mean±SD	LJP-537 IC_{50} (nM) Mean±SD
WT (Ayw)	0.14±0.11	>67
G145R	>67	>67
D144A	0.99±0.08	>67
T126S	0.03±0.01	>67
M133L	0.03±0.04	>67

HBsAg=hepatitis B surface antigen; IC50=half maximal inhibitory concentration; SD=standard deviation; WT=wild type

BJT-778 binding profile to HBsAg across a range of variants with clinically relevant mutations. The ability of BJT-778 to bind to HBsAg proteins with a single mutation that represents each of the 4 previously described clinical mutations observed in HBV was assessed by ELISA. BJT-778 binding to HBsAg harboring single mutations D144A, T126S, and M133L, with IC_{50} values ranging from 0.03 to 0.99 nM, was observed. However, BJT-778 did not bind to HBsAg protein harboring a G145R mutation at the highest concentration tested, 67 nM. LJP 537, a negative control, did not bind to any HBsAg mutants.

Figure 3: BJT-778 depletes HBsAg in a humanized mouse model of chronic HBV infection



BJT-778 effectively depletes HBsAg in a humanized mouse model of chronic HBV infection. The efficacy and pharmacokinetics (PK) of BJT-778 were investigated in a humanized FRG mouse model of HBV chronic infection. HBV-infected humanized Fah/Rag2/IL-2rγ triple knockout mice (FRG) were dosed at day 0 or day 21 (arrows) with 20 mg/kg, BJT-778, or an irrelevant negative control antibody. Animals were monitored for control of infection by measuring HBsAg in serum (**A**) or antibody exposure by detection of total human IgG1(**B**). BJT-778 demonstrated rapid depletion of HBsAg from serum and maintained depletion of HBsAg for up to 14 days. The IgG control, the negative control antibody targeting BK virus, had minimal activity against HBsAg in this model. Depletion of HBsAg by BJT-778 was tightly correlated serum concentration (PK) of the antibody.

Conclusions

- BJT-778 binds to and neutralizes HBsAg with broad coverage across major genotypes.
- It demonstrates picomolar (pM) potency in controlling HBV and HDV infection *in vitro* and rapidly depletes HBsAg in a humanized CHB murine model.
- The unique design, including retained functional IgG Fc activity, enhances BJT-778's ability to serve as a cornerstone in Bluejay's HBV functional cure and HDV therapeutic strategies.
- BJT-778 is currently being evaluated in subjects with CHB and CHD.

References

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