Mode of Action Studies on HBV RNA Destabilizer AB-452

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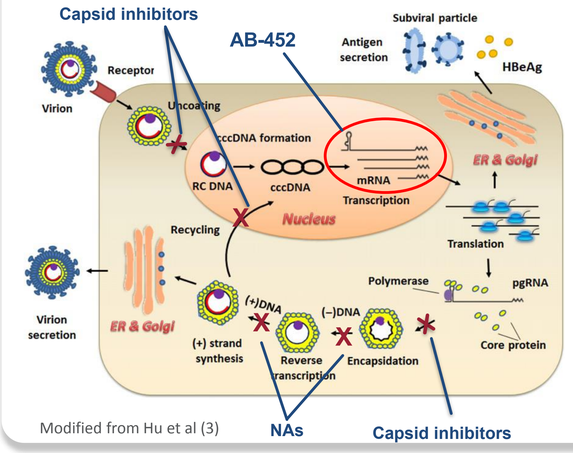
HBV INTERNATIONAL MEETING

Arbutus BIOPHARMA *Curing Chronic Hepatitis B*

BACKGROUND

The available treatments for chronic hepatitis B infections include nucleoside/tide analogs (NAs) and pegylated interferon-alpha (PegIFN-alpha). The inability of these approved treatments to achieve a functional cure for HBV infection warrants the discovery of antivirals with novel mechanisms. We believe that the combination of novel agents with different mechanisms of action will result in increased HBV cure rates with reduced duration of therapy. AB-452 is a HBV RNA destabilizer that promotes vRNA degradation and thus affects production of HBV proteins, encapsidation of pgRNA, viral DNA replication and virion release. It also shows additive to synergistic effects in inhibiting HBV DNA replication when combined with other anti-HBV agents *in vitro* and *in vivo.* AB-452 is specific to HBV as evidenced by its inactivity against a panel of RNA and DNA viruses. AB-452 significantly inhibits both HBV replication and antigenemia in an immunocompetent AAV based mouse HBV model (1-2).



Modified from Hu et al (3)

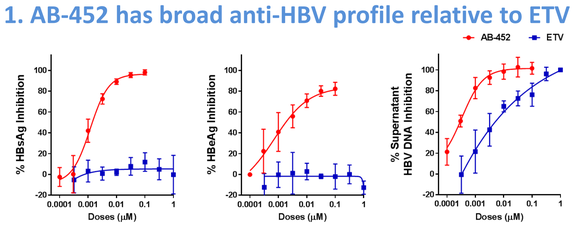
OBJECTIVES

To demonstrate AB-452:

i) antiviral activities *in vitro*,
ii) mechanism in the promotion of HBV RNA degradation,
iii) antiviral selectivity and
iv) complementary mechanism with capsid inhibitors.

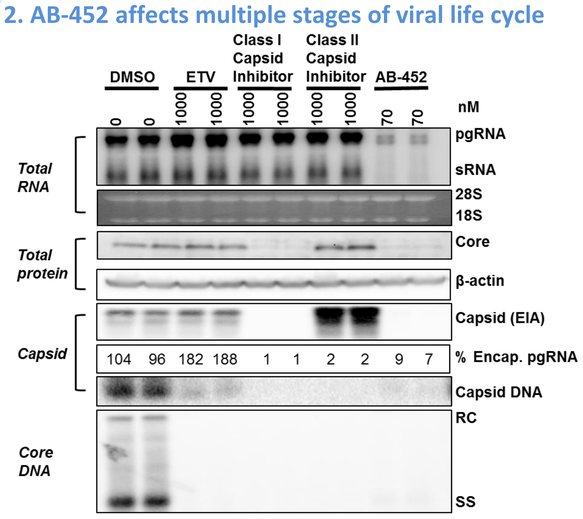
RESULTS

1. AB-452 has broad anti-HBV profile relative to ETV



In HepG2.2.15 cells, AB-452 inhibits HBsAg, HBeAg and HBV DNA production with EC_{50} values ranging from 0.3 to 2.9 nM

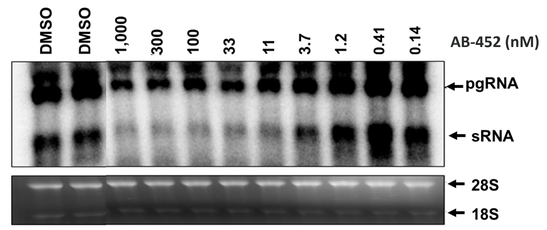
2. AB-452 affects multiple stages of viral life cycle



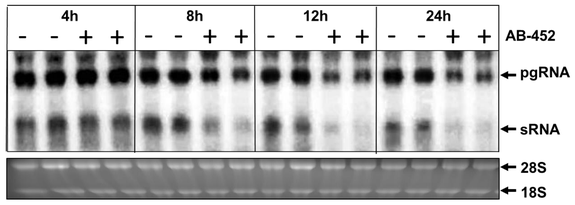
Distinct from NA and Capsid inhibitors, AB-452 affects production of HBV proteins, encapsidation of pgRNA and viral DNA replication

RESULTS

3. AB-452 reduces viral RNAs dose dependently

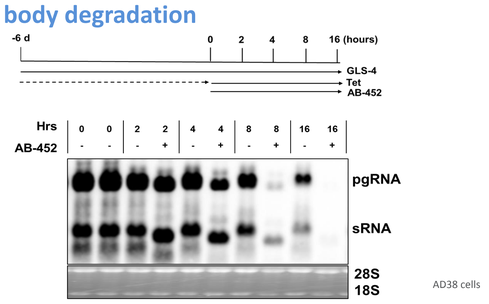


4. HBV RNA degradation by AB-452 observed at 8 hours and beyond

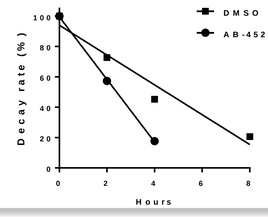


HepG2.2.15 cells were treated with 70nM AB-452

5. AB-452 treatment leads to RNA shortening and RNA body degradation

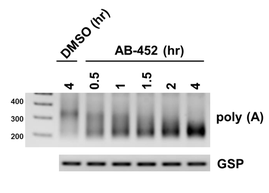


RNA shortening is observed at 2hrs and beyond, RNA body degradation becomes more apparent at 8hrs and beyond

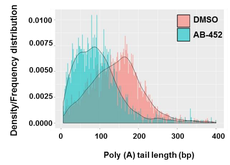


pgRNA $T_{1/2}$ is reduced from 4.5hr to 2.4hr (via qRT-PCR)

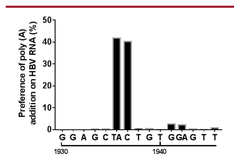
6. AB-452 shortens the poly (A) tail of HBV RNA prior to the RNA body degradation



HBV RNA shortening can be detected at 0.5hr under AB-452 treatment by RT-PCR



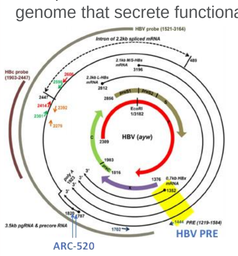
HBV RNA poly(A) tail is ~100 bases shorter after AB-452 treatment at 4hrs



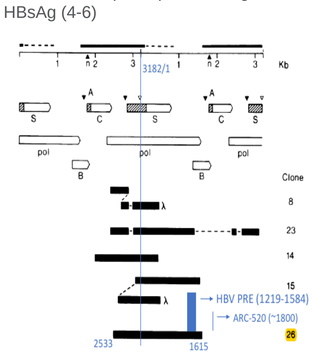
More than 80% of HBV RNA ends at nucleotides 1935-1937 (TA/C)

7. AB-452 inhibits HBV HBsAg derived from integrated HBV DNA

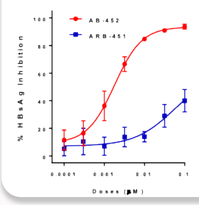
HBV patient-derived Alexander cells with multiple copies of integrated HBV genome that secrete functional HBsAg (4-6)



Modified from Yang et al, 2014 (7) and Wooddell et al, 2017 (8)

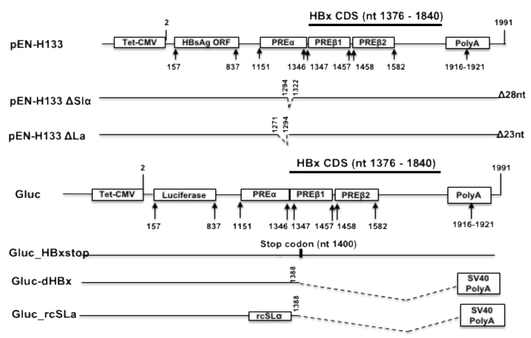


Only DNA from clone 26 generates immunoreactive HBsAg



AB-452 inhibits HBsAg with EC_{50} 1.6 ± 0.4 nM

8. Stem loop alpha (SLα) of HBV post-transcriptional element (PRE) is required for AB-452 activity, but HBV protein is not required for AB-452 activity



AB-452 (EC_{50}, nM)			ARB-451* (EC_{50}, nM)		
H133	ΔLa	ΔSlα	H133	ΔLa	ΔSlα
2.5 ± 0.61	4.3 ± 3.5	>100	>100	>100	>100

AB-452 activity requires SLα in HBV PRE

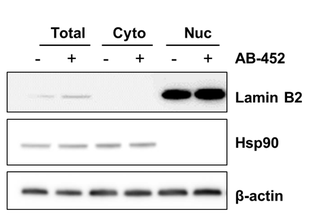
AB-452 (EC_{50}, nM)				ARB-451* (EC_{50}, nM)			
Gluc	HBxstop	ΔHBx	rcSLa	Gluc	HBxstop	ΔHBx	rcSLa
10 ± 0.6	6.2 ± 2	4.2 ± 0.8	>100	>100	>100	>100	>100

HBV viral protein is not required for AB-452 activity. A luciferase construct has been developed to evaluate RNA destabilizers. AB-452 remains inactive when HBV PRE SLα sequence in inverted.

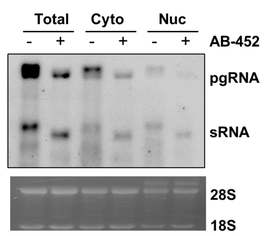
The selectivity and the specificity are contributed by the HBV PRE sequence and AB-452

*ARB-451 is the enantiomer of AB-452 (>1,000 fold less active)

9. AB-452 appears to reduce HBV RNA in the cytosol and nucleus simultaneously

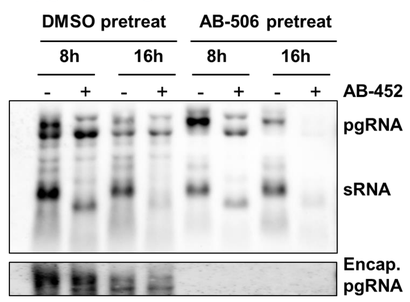


Nuclear and cytoplasmic fractions are well separated



pgRNA and sRNA are degraded in both cytoplasm and nucleus (70 nM AB-452)

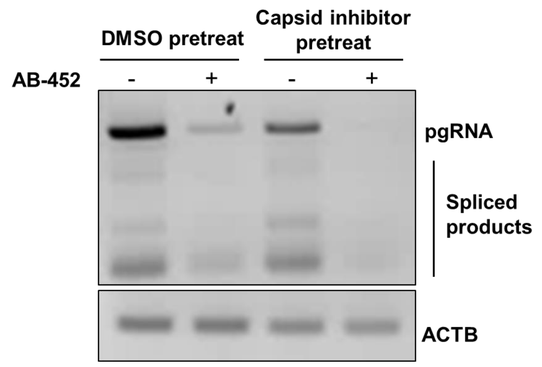
10. AB-452 in combination with capsid inhibitor enhances pgRNA degradation



Similar results for AB-452 (70nM) + Class I capsid inhibitor (1uM), data not shown

RESULTS

11. AB-452 in combination with capsid inhibitors enhances degradation of HBV spliced RNAs



Spliced HBV transcripts from pgRNA and preS2/S mRNA have been identified *in vitro,* in animal models and in chronically infected patients. Spliced HBV transcripts are implicated in the HBV life cycle and HBV infection associated pathogenesis (12-13)

CONCLUSIONS

- **AB-452 represents a novel class of antivirals with broad, potent anti-HBV effects.**
- **AB-452 is a HBV RNA destabilizer, promoting vRNA degradation and thus affecting production of HBV proteins, encapsidation of pgRNA, viral DNA replication and virion release.**
- **AB-452 can inhibit HBsAg derived from both integrated HBV DNA and cccDNA.**
- **AB-452 induces HBV RNA shortening and RNA body degradation.**
- **HBV PRE, but not HBV protein, is essential to AB-452 activity.**
- **AB-452 mediated HBV RNA degradation occurs in both the nucleus and cytosol.**
- **AB-452 treatment combined with capsid inhibitor(s) promotes the degradation of HBV pgRNA and splicing RNAs.**
- **Combining AB-452 with other anti-HBV agents, especially capsid inhibitors and NAs, may further inhibit HBV DNA replication and viral antigen production to achieve a higher cure rate with a defined and shortened treatment duration.**

REFERENCES

1. Gotchev D, et al. 2017, AASLD Poster.
2. Rijnbrand R, et al. 2018, EASL Oral presentation.
3. Hu YM, et al. 2013, Vol. 48: 265-281.
4. Ziemer M, et al. J Virol. 1985, Vol. 53: 885–892.
5. Freitas N, et al. J Virol. 2014, Vol. 88:5742-54.
6. Yang CC, et al. PLoS One. 2014, 9(10):e106683.
7. Wooddell C, et al. Sci Transl Med. 2017, 9(409).
8. Zhou T, et al. Antiviral Res. 2018, Vol. 149:191-201.
9. Huang J, et al. Mol Cell Biol. 1993, Vol. 13:7476-86.
10. Schwalbe M, et al. 2008, Vol. 36:1681-9.
11. Heise T, et al. Nucleic Acids Res. 2006, Vol. 34:353-63.
12. Lee GH, et al. Virus Res. 2008, Vol. 136:1-7.
13. Duriez M. J Hepatol. 2017, Vol. 67:687-699.