ABT-493, a Potent HCV NS3/4A Protease Inhibitor With Broad Genotype Coverage

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BACKGROUND

- HCV NS3/4A protease inhibitors are proven therapeutic agents used in treating chronic HCV infection
- First generation protease inhibitors (boceprevir and telaprevir)
  - Need to be combined with pegIFN/RBV
  - Demonstrate reduced potency against some HCV genotypes
  - Are associated with adverse event profiles that add significantly to that of pegIFN/RBV
  - Show drug interactions and require restrictions of common concomitant medications
- ABT-450
  - Is a second generation HCV NS3/4A protease inhibitor identified in a collaboration between AbbVie and Enanta
  - Has been studied extensively in phase III clinical trials in IFN-free regimens containing ABT-267 (NS5A inhibitor), ABT-333 (NS5B non-nucleoside inhibitor) with or without RBV
  - Is more potent than first generation inhibitors against genotype 1, but has reduced potency against genotypes 2 and 3
  - IFN-free regimens containing ABT-450/ritonavir demonstrate much improved tolerability and adverse event profile compared to pegIFN/RBV regimens containing first generation protease inhibitors
- ABT-493
  - Is a next generation HCV NS3/4A protease inhibitor identified in a collaboration between AbbVie and Enanta
  - Demonstrates higher potency and broader genotype coverage in *vitro* than earlier generation protease inhibitors
  - Is active *in vitro* against commonly observed variants that confer resistance to earlier generation protease inhibitors
  - Has entered phase II clinical development and will be studied as part of an IFN-free regimen for the treatment of patients chronically infected with HCV of different genotypes

OBJECTIVES

- To characterize the *in vitro* properties of ABT-493 in HCV replicon cells, including antiviral activity, genotypic coverage, and cytotoxicity
- To determine the *in vitro* resistance and cross-resistance profile as well as the genetic barrier to resistance of ABT-493

DISCLOSURES

Teresa Ng, Thomas Reisch, Tim Middleton, Keith McDaniel, Dale Kempf, Liangjun Lu, and Tami Pilot-Matias are employees of AbbVie. Guoqiang Wang, Lijuan Jiang, and Yat Sun Or are employees of Enanta. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie and Enanta participated in the interpretation of data, review, and approval of the publication.

METHODS

- The antiviral activity, cytotoxicity, and resistance profile of ABT-493 were evaluated using subgenomic HCV replicons expressing NS3 or NS3/4A from different HCV genotypes in transient transfection assays or in stable cell lines
- The genetic barrier to resistance in HCV genotype 1 and 3a replicons was measured in resistant colony selection assays using concentrations at various multiples over the EC_{50} value of ABT-493 in the respective replicon
- Variants that emerged during selection were identified by population sequencing
- Phenotypic analyses of the variants were performed by introducing the variants into their corresponding wild-type replicons and testing them in transient replicon assays

RESULTS

Table 1. ABT-493 is a Potent HCV Inhibitor Exhibiting Broad Genotype Coverage *in Vitro*

HCV Replicon	Mean EC_{90}, nM
GT 1a	0.85 ± 0.15
GT 1b	0.94 ± 0.35
GT 2a[a]	2.7 ± 1.1
GT 3a	1.6 ± 0.49
GT 4a	2.8 ± 0.41
GT 6a	0.86 ± 0.11

[a]Study conducted at Southern Research Institute.
GT: Genotype.

- ABT-493 demonstrates comparable activity against HCV replicons containing NS3 genes from genotype 1a, 1b, 2a, 3a, 4a, and 6a
- Of note, ABT-493 is potent (EC_{50} = 1.6 nM) against genotype 3a; many other NS3/4A protease inhibitors have significantly lower potency against this genotype
- Cytotoxicity (TD_{50}): 72,000 nM in Huh-7 cells; therapeutic index: 85 000-fold (relative to GT 1a EC_{50})

Table 2. Activity of ABT-493 Against Human Proteases in Biochemical Assays

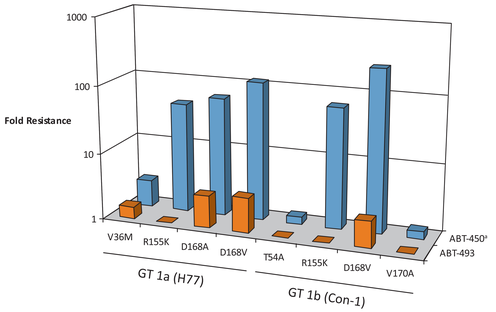
Human Protease	IC_{50}, nM	Selectivity, -Fold[a]
Chymase	>200 000	>320 000
Elastase	>200 000	>320 000
Cathepsin B	>200 000	>320 000
Chymotrypsin Type II	>200 000	>320 000
Chymotrypsin Type VII	>200 000	>320 000
Kallikrein	>200 000	>320 000
Urokinase	>200 000	>320 000

[a]Calculated from the ratio of the IC_{50} value for the human proteases relative to the HCV GT 1a protease IC_{50} = 0.63 nM.

- No inhibition of human proteases at the highest concentrations tested

RESULTS (CONTINUED)

Figure 1. ABT-493 has Potent Activity Against Common HCV Genotype 1 NS3 Resistance-associated Variants *in Vitro*



[a]ABT-450: AbbVie's earlier generation protease inhibitor.
[b]Pilot-Matias et al., 2011.

- ABT-450, which has a resistance profile[b] that overlaps with that of many earlier generation HCV protease inhibitors, was used for comparison
- ABT-493 activity against common HCV genotype 1 NS3 resistance-associated variants is nearly equivalent to its activity against wild-type HCV replicon

Table 3. Selection of Resistant Colonies by ABT-493 in HCV Genotype 1 and Genotype 3 Replicons

		Selection Concentrations (Fold over EC_{90})		
		10X[a]	100X[b]	500X[b]
GT 1a	No. of Colonies Analyzed	28	17	1[c]
	NS3 Variants[d]	Q41R[e] (n = 13) I170V[e] (n = 10)	A156T (n = 8) A156V (n = 3) Q89R + A156T (n = 4)	NV (n = 1)
GT 1b	No. of Colonies Analyzed	25	25	24
	NS3 Variants[d]	A156T (n = 3) A156V (n = 7) P89L + A156V (n = 6)	A156V (n = 5) P89L + A156V (n = 5) A156V + D168V (n = 5)	A156V (n = 5) P89L + A156V (n = 6) A156V + D168V (n = 8)
GT 3a	No. of Colonies Analyzed	10	3[c]	ND
	NS3 Variants[d]	P89L (n = 2) P89S[e] (n = 2)	Y56H + Q168R (n = 2) A156G (n = 1)	ND

[a]1x 10^5 replicon cells for 10X selection in GT 1a or 1b; 1x 10^6 cells for 10X selection in GT 3a.
[b]1x 10^7 replicon cells for 100X or 500X selection in GT 1a, 1b, or 3a.
[c]Number of colonies that survived selection.
[d]Variants seen at NS3 positions previously reported in the literature to be associated with resistance to NS3/4A protease inhibitors.
[e]Variants with the designated mutation alone or with other mutation(s).
NV: Not viable (Colony formed but could not be expanded); ND: Not done.

- The most common variants selected by ABT-493 *in vitro* were at position A156

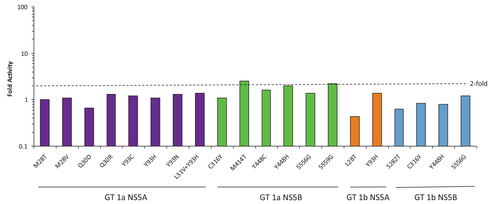
Table 4. Activity of ABT-493 Against HCV Replicons Containing NS3 Variants Identified by Resistant Colony Selection

GT	HCV Replicon	EC_{90}, nM	-Fold Resistance[a]	Replication Efficiency, %
1a	Wild-type	0.21	—	100
	A156T	290	1400	1.5
	A156V	NV	—	≤0.5
	Q89R + A156T	750	3700	0.7
1b	Wild-type	0.47	—	100
	A156T	300	630	40
	A156V	840	1800	9.2
	P89L + A156V	2100	4500	120
	A156V + D168V	1200	2500	16
3a	Wild-type	0.71	—	100
	A156G	1100	1500	45
	Y56H + Q168R	760	1100	43

[a]Calculated from the ratio of the EC_{90} values for variant/wild-type.
NV: Not viable (Replication efficiency ≤0.5%).

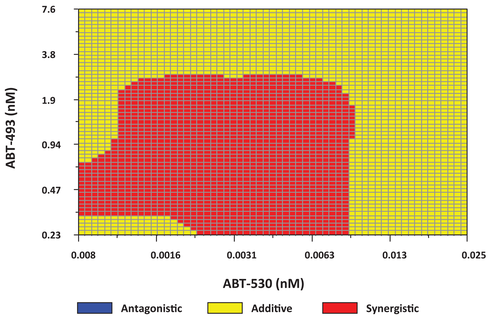
- Replication efficiency of all GT 1a variants selected by ABT-493 is low (≤2%)
- For GT 1b, most variants contain A156V **(Table 3)**. A156V by itself has low replication efficiency (9.2%), and tends to occur with an additional mutation (eg, P89L or D168V) to generate variants with higher resistance and replication efficiency

Figure 2. ABT-493 is Fully Active Against NS5A Inhibitor, NS5B NI, and NS5B NNI Resistance-associated Variants *in Vitro*



NI: Nucleoside/nucleotide inhibitor.
NNI: Non-nucleoside/nucleotide inhibitor.

Figure 3. Combination of ABT-493 With AbbVie's Next Generation NS5A Inhibitor ABT-530[a] *in Vitro*



[a]Please see ABT-530 poster presented at this conference.
[b]Prichard et al., 1990.

- ABT-493 demonstrates additive to synergistic activity in combination with ABT-530 in HCV GT 1b Con-1 replicon cells
- Results generated using Prichard and Shipman model[b]

CONCLUSIONS

- ABT-493 has a substantially improved *in vitro* virological profile compared to earlier generation HCV NS3/4A protease inhibitors
  - ABT-493 displays potent and broad genotype activity
  - Of note, ABT-493 is potent (EC_{50} = 1.6 nM) against genotype 3a; many other NS3/4A protease inhibitors have significantly lower potency against this genotype
  - ABT-493 retains potency against most of the clinically important resistance-associated variants (eg, R155 or D168 variants in genotype 1) selected by other NS3/4A protease inhibitors
- ABT-493 is fully active against variants resistant to NS5A or NS5B inhibitors *in vitro*
- ABT-493 demonstrates additive to synergistic antiviral activity *in vitro* when combined with NS5A inhibitor ABT-530
- ABT-493 is being developed for use in combination with other direct-acting antiviral agents in an IFN-free regimen for the treatment of patients chronically infected with HCV of different genotypes

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