

Resistance Profile Of ABT-333 And Relationship To Viral Load Decrease In Patients Treated In Combination With Peg-interferon And Ribavirin For 28 Days.

Tim Middleton, Yupeng He, Jill Beyer, Rajeev Menon, Cheri E. Klein, Daniel Cohen and Christine Collins

Abbott Laboratories, Abbott Park, IL, USA

Background

Introduction

ABT-333 is a non-nucleoside HCV NS5B polymerase inhibitor with potent in vitro activity against genotype 1a and 1b NS5B replicons. Amino acid variants conferring resistance to ABT-333 in cell culture have been identified at positions 316, 414, 448, 556 and 559. The resistance profile generated in subjects from a 28 day, phase Ib clinical study is presented here.

Study Objectives

Assess safety, tolerability, pharmacokinetics, and antiviral activity of ABT-333. Assess emergence of resistant virus.

Methods

- Dose-ranging study in HCV genotype 1-infected patients.
- Two days of monotherapy followed by 26 days of combination with pegIFN α -2a and ribavirin.
- Doses of ABT-333 used were 300 mg BID, 600 mg BID and 1200 mg QD.
- Samples taken before the first dose, and 5, 10, 17, 24 and 28 days after initial dosing.
- Susceptibility to ABT-333 determined in vitro by transferring isolated NS5B genes to a subgenomic replicon, followed determination of EC_{50} in a transient transfection assay.
- For selected samples, variants conferring resistance were detected by nucleotide sequencing of mixed species and of molecular clones derived from the mixed species.

Study Design

Arm (n)	Group 1: ABT333 300 mg BID (n=8)	Group 2: Placebo (n=2)
Arm 1 (n=10)	ABT-333 300 mg BID + pegIFN-2a + RBV for 26 days	Placebo + pegIFN-2a + RBV for 26 days
Arm 2 (n=10)	Group 3: ABT333 600 mg BID (n=8)	Group 4: Placebo (n=2)
Arm 3 (n=10)	ABT-333 600 mg BID + pegIFN-2a + RBV for 26 days	Placebo + pegIFN-2a + RBV for 26 days
Arm 4 (n=10)	Group 5: ABT333 1200 mg QD (n=8)	Group 6: Placebo (n=2)
Arm 5 (n=10)	ABT-333 1200 mg QD + pegIFN-2a + RBV for 26 days	Placebo + pegIFN-2a + RBV for 26 days

All subjects were eligible to receive pegIFN α -2a + RBV therapy for up to 48 weeks

Baseline Characteristics

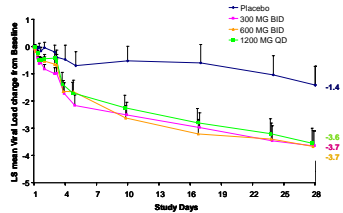
Characteristic	Placebo (No=1)	ABT-333 (No=24)
Age, mean (SD)	48.2 (7.31)	48.0 (10.42)
Gender, % male	83.3%	88.7%
Race, %		
White	83.3%	91.7%
Black	16.7%	8.3%
Ethnicity, % Latino/Hispanic	0%	41.7%
BMI, mean (SD) kg/m ²	26.9 (4.81)	26.4 (3.51)
Baseline HCV RNA, mean (SD) log ₁₀ IU/mL	6.47 (0.27)	6.29 (0.48)
Genotype, N (n/N)	4/2	21/3

References

Rodriguez-Torres, M. et al., *Hepatology*, DEC 2009, vol. 50, no. 6, p. 5A
Koev, G. et al., *Journal of Hepatology*, 2009, vol. 50, no. Suppl. 1, p. S346-S347

In Vivo Response To Treatment

Mean HCV RNA Change from Baseline M10-380



- Average viral load decrease of 3.7 log₁₀ across ABT-333 dose groups
- 2.3 log₁₀ greater decrease in presence of ABT-333 than for pegIFN/RBV alone
- No significant difference between groups dosed with ABT-333

In Vitro Resistance to ABT-333

Susceptibility to ABT-333 of Replicons Carrying Resistant Amino Acid Variants

- Resistance selection studies in vitro identified variants at five amino acid positions that accounted for nearly all of the resistance to ABT-333. The variants are listed in the table, along with the fold loss of susceptibility incurred in replicon transient transfection assays.
- The appearance and phenotype of variants at these positions was monitored in the clinical samples.

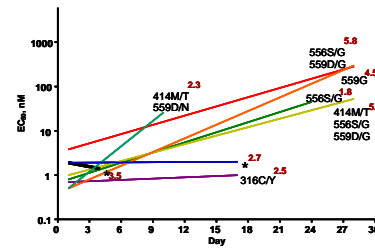
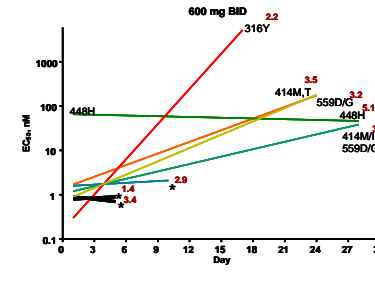
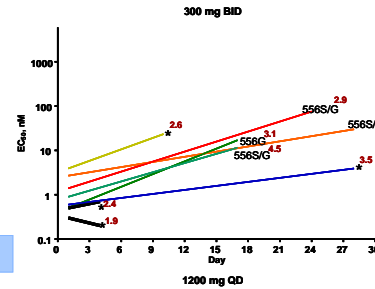
	Fold loss relative to wild type	
	GT 1a	GT 1b
C316Y	1600	1400
M414T	36	26
Y448H	1000	37
S559G	15	8
D559D	150	100
Wild type EC_{50}	7.7 nM	1.8 nM

Resistance Development In Vivo

Phenotypic and Genotypic Summary of Mixed Populations

Samples taken before dosing, or at days 4, 10, 17, 24 or 28 days after the initial dose were analyzed for viral titer, ABT-333 EC_{50} against NS5B isolates cloned into replicons, and for the presence of variants previously demonstrated to confer resistance to ABT-333.

- A, B, C: plots by dose group, showing:
 - Baseline and maximum EC_{50} observed during the treatment period (beginning and end of lines)
 - Known resistance variants observed at time of maximum EC_{50} (* = no variant noted)
- *Viral load at time of maximum EC_{50} (log₁₀ IU/mL in red)
- Subjects with short time courses plotted (black lines) had viral titers that were too low to permit amplification of the NS5B gene at later time points



Summary

- 28 of 30 baseline samples had EC_{50} between 0.3 – 3.9 nM. One baseline EC_{50} = 10 nM, with no known resistance variants detected. The remaining baseline isolate EC_{50} = 60 nM; this isolate encoded a histidine at amino acid 448, a known resistance variant.
- Variants noted at later time points at amino acids 414, 448, 556 and 559 were associated with EC_{50} values from 10 – 400 nM.
- An isolate with complete conversion from Cys to Tyr at amino acid 316 increased EC_{50} to >3000 nM.
- Known resistance variants were associated with an increase over time in EC_{50} .
- Resistance variants were distributed throughout the range of viral load decreases observed, indicating that the presence of resistance variants did not impact the response to therapy.

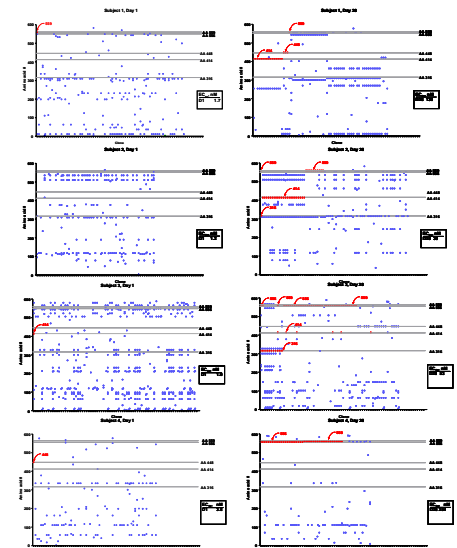
Clonal Sequence Analysis

Clonal isolates of the polymerase gene were sequenced to:

- Assess the extent of evolution of quasispecies with ABT-333 treatment.
- Determine whether signature resistance variants identified by population sequencing were linked in clones.
- Establish the predictability of in vitro resistance selection for in vivo studies with ABT-333

Summary

- An average of 72 clones were sequenced per sample tested. The plots show examples from four subjects, with a comparison of each clone to the consensus sequence for all of the clones from that isolate for days 1 and 28.
- Variants were relatively uniformly distributed between the five amino acid positions.
- Resistance variants accumulated with time of treatment. Six percent of clones from baseline isolates contained resistance variants, with 19% of clones at day 10 and 36% at days 24 or 28 containing resistance variants.
- The average number of variants associated with a sample also increased over time of treatment (1.6, 25 and 39% at days 1, 10 and 24/28). There was a general increase in EC_{50} as the proportion of resistance variants increased, though number or position of variant did not predict potency loss. Note, for instance that subject 3 on day 28 contained resistance variants in 46 of 66 clones, including 11 clones encoding C316Y, yet EC_{50} only increased from 1 nM at baseline to 53 nM at day 28.
- Clonal sequencing showed a shift in the quasispecies found in the population on treatment with ABT-333 for most of the subjects.
- Linkage of resistance variants occurred in a few clones, but was relatively rare. In most instances where multiple resistance variants were noted in an isolate by mixed population sequencing, clonal sequencing indicated that the variants were found on different clones.



Summary and Conclusions

- ABT-333 in combination with pegIFN/RBV resulted in a 2.3 log₁₀ greater decrease in viral titer than pegIFN/RBV alone.
- Resistant variants emerged on treatment with ABT-333, but the emergence of resistant variants did not appear to impact continued response to therapy, suggesting that resistant variants are still susceptible to the combination of ABT-333 + pegIFN/RBV.
- In vitro resistance selection implicated variants at amino acids 316, 414, 448, 556 and 559. Variants at these positions were found in almost all of the isolates where phenotypic resistance was observed, though this analysis does not preclude the existence of other variants that may have contributed to the loss of susceptibility to ABT-333.