VAST MAJORITY OF DETECTED NS5A RESISTANT VARIANTS ARE NOT AMPLIFIED IN HCV PATIENTS DURING 3-DAY MONOTHERAPY WITH THE OPTIMIZED NS5A INHIBITOR PPI-668

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48th Annual Mtg of the European Association for the Study of the Liver
Amsterdam, The Netherlands - Apr 2013

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Abstract

BACKGROUND: HCV NS5A inhibitor PPI-668 was assessed in a 3-day Phase 1b, placebo controlled, monotherapy trial in HCV gt-1 patients using QD oral doses of 40, 80, 160 and 240 mg, with 8/10 patients receiving PPI-668 per cohort. Mean maximal viral load reductions of 3.54-3.75 $\log_{10}$ IU/mL were observed with doses ≥80 mg QD. **METHODS:** Comprehensive monitoring of HCV resistant variants was performed during dosing and post-dosing periods. HCV RNA was extracted from patient serum samples, RT-PCR amplified and the resulting DNA subjected to population sequencing. Clonal sequencing was performed to determine if detected substitutions were genetically linked. PPI-668 susceptibility was assessed in transient transfection assays using HCV replicons encoding specific substitutions or population NS5A gene inserts from clinical samples. **RESULTS:** Known NS5A resistance substitutions at residues 28, 30, 31, 58 and 93 were detected early during PPI-668 monotherapy. Among the 40 enrolled gt-1 patients, eight had detectable NS5A resistance substitutions at baseline. One patient (gt-1b 240 mg) was a non-responder exhibiting high level resistance at baseline, with 100% of his circulating virus encoding genetically linked R30Q+L31I+Y93H substitutions. The other seven PPI-668 treated patients with baseline resistance substitutions responded well (RNA reductions of 2.23 to 3.95 $\log_{10}$ IU/mL). Resistance substitutions became detectable in all but one PPI-668 treated patient within 24-48 hr, as WT virus was rapidly eliminated. Importantly, these observed substitutions were not further amplified with continued monotherapy, suggesting that PPI-668 concentrations were sufficient to suppress these single-substitution variants. Susceptibility (EC_{90}) of replicons encoding resistance substitutions detected in PPI-668 treated patient samples were generally at or below C_{min} levels, confirming the advantageous PK profile of PPI-668 and its ability to cover single-substitution resistant variants. No significant differences were observed in the overall resistance patterns across the four PPI-668 treated gt-1 cohorts. **CONCLUSIONS:** NS5A resistance variants frequently pre-exist among HCV patients, emphasizing the need for combination therapy and use of optimized NS5A inhibitors, such as PPI-668, that achieve plasma/liver levels high enough to suppress single substitution HCV variants. Further studies of PPI-668 in combination with other DAAs are warranted.

Introduction

- PPI-668 is a potent and selective HCV NS5A inhibitor, with pan-genotypic activity in replicon assays and an optimized PK profile
- Phase 1b study was conducted in treatment-naïve HCV-g1 patients
  - Three-day treatment period with 40, 80, 160 or 240 mg of PPI-668 QD
  - Well tolerated at all dose levels, no AEs attributed to PPI-668
  - Predictable and consistent PK profile in humans, with steady-state reached after a single dose
  - Rapid, marked HCV RNA responses seen in 31/32 HCV gt-1 patients
  - Nearly equivalent activity observed in both HCV gt-1a and HCV gt-1b patients

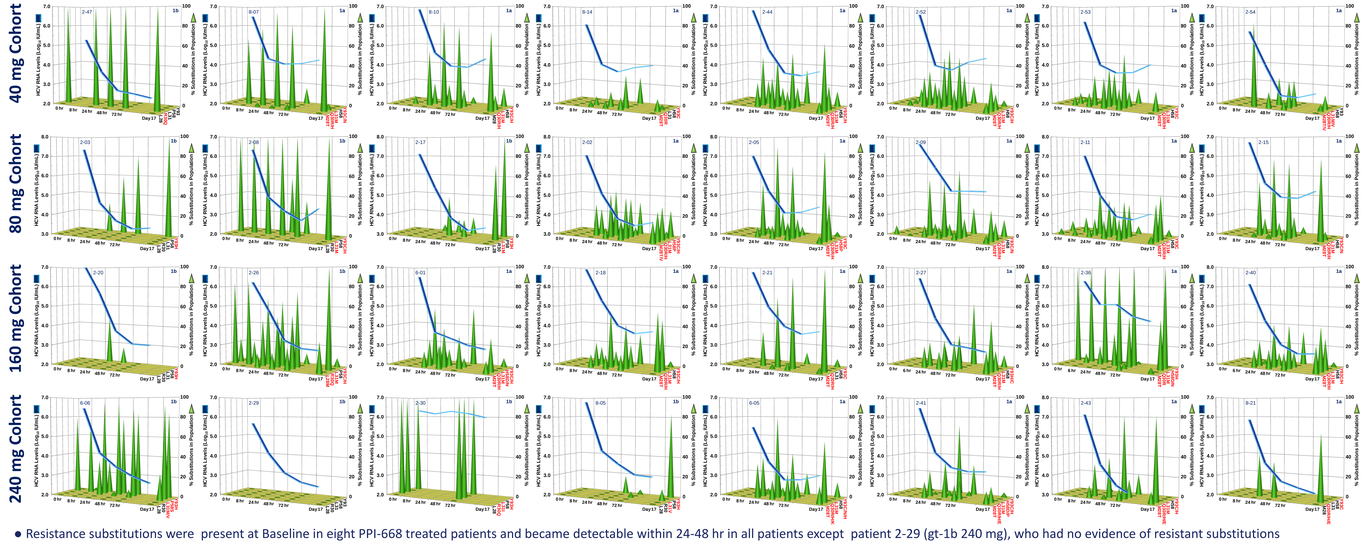
HCV Genotype	Dose (mg QD)	Maximum HCV RNA Reduction (Log10 IU/mL)	
		Mean	Range
gt-1a (n=7)	40	3.27	2.81 – 3.86
gt-1b (n=1)	40	2.89	Only 1 patient
gt-1a (n=5)	80	3.26	2.82 – 3.96
gt-1b (n=3)	80	4.00	3.95 – 4.12
gt-1a (n=6)	160	3.33	2.23 – 3.69
gt-1b (n=2)	160	3.93	3.47 – 4.39
gt-1a (n=4)	240	3.71	3.14 – 4.33
gt-1b (n=3)*	240	3.80	3.71 – 3.83

*Excludes a baseline resistant patient with 100% of his circulating virus encoding 3 genetically-linked substitutions (R30Q+L31I+Y93H)

Resistance Monitoring

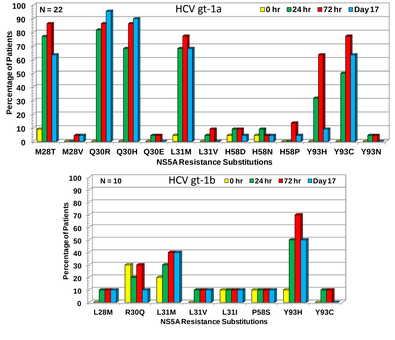
- Comprehensive analysis performed on serum samples from all patients
- HCV RNA was RT PCR-amplified from patients' samples and genotyped (population) at Baseline and at several subsequent treatment time points
- Clonal sequencing was performed on patient samples when multiple substitutions were present to determine if they were genetically linked
- Phenotypes (EC_{90}) determined for individual and linked substitutions emerging on therapy

Individual Patients - Antiviral Efficacy and Genotypic Monitoring for Resistance Substitutions



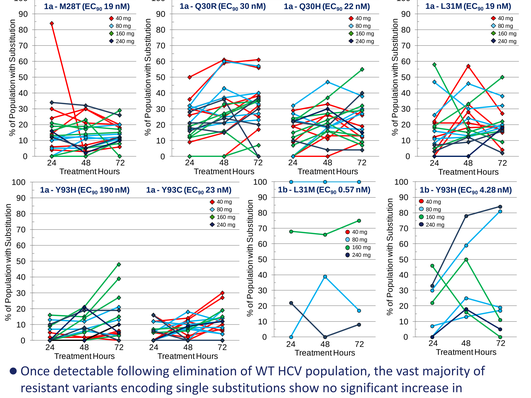
- Resistance substitutions were present at Baseline in eight PPI-668 treated patients and became detectable within 24-48 hr in all patients except patient 2-29 (gt-1b 240 mg), who had no evidence of resistant substitutions
- With the exception of patient 2-30 (gt-1b 240 mg, with 100% of his Baseline circulating virus encoding genetically linked R30Q-L31I-Y93H substitutions), all patients exhibited 2.2 to 4.4 $\log_{10}$ IU/mL drops in their HCV RNA levels
- Despite the rapid appearance of resistance substitutions, patients in the 160 mg and 240 mg cohorts experienced a further mean decline of 0.41 and 0.36 $\log_{10}$ IU/mL, respectively, in viral RNA levels between 24 and 72 hr on therapy

Emerging Substitutions



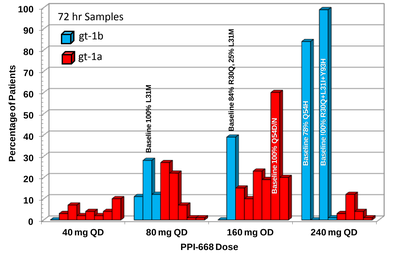
- Resistance substitutions observed at a high frequency in HCV gt-1a patients include M28T, Q30R, Q30H, L31M, Y93H and Y93C
- High frequency of resistance substitutions L31M and Y93H observed in HCV gt-1b patients
- Apart from the HCV gt-1a Y93H substitution, primary resistance substitutions tend to remain in circulation 2 wk post-treatment

Substitution Frequencies During Dosing



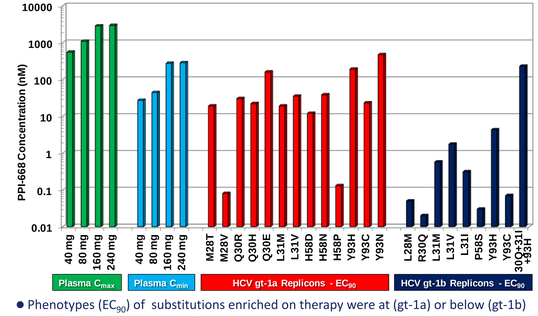
- Once detectable following elimination of WT HCV population, the vast majority of resistant variants encoding single substitutions show no significant increase in population percentage during continued treatment with PPI-668
- No obvious correlation with dose level, as all PPI-668 doses appear to provide exposure levels (peak and trough) needed to constrain expansion of single substitution variants

Percent of Linked Substitutions



- Clonal analysis (n=100) of 72 hr patient samples was performed to determine the frequency of genetically linked substitutions among amino acid residues 28, 30, 31, 58 and 93
- Overall, percentage of linked substitutions was low at 72 hr if substitutions were not pre-existing at Baseline
- No obvious trend observed over this relatively short dosing period (3 days) in patients without baseline substitutions
- Resistant variants encoding double/triple linked substitutions likely needed to escape formidable PPI-668 concentrations (C_{max} and C_{min}) established with 160 mg and 240 mg doses

C_{min} and Resistant Phenotypes



- Phenotypes (EC_{90}) of substitutions enriched on therapy were at (gt-1a) or below (gt-1b) PPI-668 plasma trough levels (blue bars)
- Single substitutions Q30E and Y93H/N in gt-1a and Y93H in gt-1b confer the highest levels of resistance in each genotype
- Ability to constrain replication of single substitution variants during monotherapy appears to be driven by the relatively high peak and trough levels achieved with all PPI-668 doses

Activity in Patients with Baseline Resistance

Genotype	Patient	Dose (mg)	Baseline Substitutions (%)	RNA Reduction ($\log_{10}$ IU/mL)
gt-1b	2-47	40	R30Q (100%)	2.89
gt-1b	2-08	80	L31M (100%)	3.95
gt-1a	2-09	80	M28T (9%)	2.82
gt-1a	2-11	80	M28T (6%), L31M (11%)	3.56
gt-1b	2-26	160	R30Q (84%), L31M (25%)	3.47
gt-1a	2-36	160	H58D (69%), H58N (31%)	2.23
gt-1b	6-06	240	P58S (79%)	3.82
gt-1b	2-30	240	Linked R30Q+L31I+Y93H (100%)	0.33

- PPI-668 was active in 7/8 gt-1 patients with pre-existing single NS5A substitutions at amino acid residues 28, 30, 31 and 58, with HCV RNA declines of 2.23-3.95 $\log_{10}$ IU/mL

Summary

- **PPI-668 was well tolerated and exhibited strong antiviral potency in a Phase 1b clinical trial in gt-1 patients involving 3-day QD monotherapy with 40 mg, 80 mg, 160 mg or 240 mg (AASLD 2012)**
- **The optimized PK properties of PPI-668 enabled all seven patients with single resistance substitutions at Baseline to respond well to treatment**
- **One gt-1b patient (240 mg), harboring a homogeneous population of virus containing 3 linked NS5A resistance substitutions, was highly resistant to PPI-668 and was the only non-responder (0.33 $\log_{10}$ IU/mL)**
- **With WT virus rapidly eliminated, low level pre-existing resistant variants at Baseline became detectable in 31/32 patients, but did not show a significant increase on treatment, indicative of inhibition during the treatment period**
- **Despite the appearance and enrichment of resistance substitutions by 24 -48 hr, a subsequent decline of ~0.4 $\log_{10}$ IU/mL in viral RNA levels was observed in patients treated with 160 mg and 240 mg PPI-668**
- **No significant differences observed in resistance frequency or degree across the PPI-668 treatment cohorts evaluated**
- **Like other classes of DAAs, combination therapy will be required to suppress the emergence of resistance**