Abstract

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 60 mg paired with 30 mg and 31 mg for others. Mean resistant virus load reductions of 3.1-4.7 log10 IU/mL were observed with doses 80-160 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 amplicons of the resulting RNA subjected to population sequencing. Genomic sequencing was performed to determine if detected substitutions were genetically linked. PPI-668 resistome was obtained in in vitro resistance assays using HCV replicons encoding specific substitutions or population HCV genome inserts from clinical samples. RESULTS: Known NS5A resistance substitutions at positions 28, 30, 31, 58 and 93 were detected during PPI-668 monotherapy. Among the 40 mg cohort, eight had detectable NS5A resistance substitutions at baseline. One patient (gt-1b) had a 240 mg dose was a non-responder exhibiting high level resistance at baseline, with 100% of its circulating virus encoding genetically linked R580K/H58S substitutions. The other four PPI-668 treated patients with baseline resistance substitutions responded well (RNA reductions of 2.2 to 3.5 log10, 0.7-3.0 log10). Resistance substitutions became detectable at all four PPI-668 treated patients within 24-48 h of RT-PCR was rapidly amplified. Importantly, these observed substitutions were not further amplified with continued therapy, suggesting that PPI-668 monotherapy is capable of blocking emergence of resistance substitutions. Susceptibility (S) or exclusion of resistance substitutions detected in PPI-668 treated patients were generally at or below Cmin levels, confirming optimized NS5A inhibitors, such as PPI-668, that achieve plasma/liver levels high enough to inhibit NS5A substitutions. CONCLUSIONS: NS5A resistance variants frequently pre-exist among HCV patients, emphasizing the need for combination therapy and optimized NS5A inhibitors, such as PPI-668, that achieve plasma/liver levels high enough to suppress single substitution NS5A variants. Further studies of NS5A in combination with other DAAIs are warranted.

Introduction

PPI-668 is a patient and selection HCV NS5A inhibitor, with pan-genotypic activity in replicon assays and an optimized PK profile.

Phase Ia study was conducted in treatment-naive HCV gt-1 patients - Three treatment periods with 0, 40, 80, 160 mg of PPI-668 QD - Well tolerated at all dose levels, no ADs attributed to PPI-668 - Predictable and consistent PK is in a linear, washout-related profile - Rapid, early HCV RNA responses seen in 11/19 (60%) gt-1 patients - Markedly fewer substitutions observed in PPI-668-treated vs. HCt controls.

Resistance Monitoring

• Comprehensive analysis performed on serum samples from all patients
• Multiple resistance tests were performed on at least one baseline sample and at least several subsequent treatment time points
• HCV NS5A substitutions were recorded in genotypic (patient) and phenotypic (STL) assays, with the latter 18 substitutions from multiple samples were present to determine if they were genetically linked
• Phenotype (S) determined for individual and linked substitutions emerging on therapy.

Emerging Substitutions

• Resistance substitutions were present at Baseline in 8/17 PPI-668 treated patients and became detectable within 24-48 h in all patients except patient 2-26 (gt-1b 240 mg), who had no evidence of resistant substitutions
• With the exception of patient 2-10 (320 mg, 100%), of its Baseline circulating virus encoding genetically linked L31M/H58D substitutions, all patients exhibited 2.2 to 4.0 log10 IU/mL drops in their HCV RNA levels
• Despite the rapid emergence of resistance substitutions, patients with baseline substitutions of L31M/H58D, L31I/H58D, Q54D/N, L31M/H58N substitutions were monitored up to 72 h in therapy

Emerging Substitutions

Substitution Frequencies During Dosing

• Resistance substitutions observed at a high frequency in HCV gt-1 patients include D28E, D28N, D28A, L31I, L31M, V30A, V30L, V30I
• High frequency of resistance substitutions L31M and H58D observed in all 17 patients
• HCV genotype 1a Q54D/N substitutions were observed in 5/17 (30%) patients
• Number expected was 1-2 substitutions for HCV gt-1b (n=3)

Conclusions

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

Percent of Linked Substitutions

• Coronal analysis (n=100) of 72 patient samples was performed to determine the frequency of genetically linked substitutions among every resistant substitution 28, 30, 31, 58 and 93
• Overall, percentage of linked substitutions was low at 72 h if substitutions were not pre-existing baseline
• No obvious trend observed over this relatively short dosing period 24-48 h in patients without baseline substitutions
• Resistant variants encoding linked substitutions likely needed to escape baseline PPI-668 concentration X and X expressed 160 and 240 mg dosing levels

Percent of Linked Substitutions

Plasma Cmin

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Percent of Linked Substitutions

Plasma Cmin

Activity in Patients with Baseline Resistance

• PPI-668 was well tolerated and exhibited strong antiviral activity in a Phase 1b clinical trial in gt-1 patients receiving 3-day QD monotherapy with 40 mg, 80 mg, 160 mg or 240 mg (AASLD 2013)
• The optimal PK properties of PPI-668 enabled all patients with single gt-1a baseline resistance substitutions to required well to treatment
• One gt-1b patient (240 mg), harboring a homogenous population of virus containing 23 linked NS5A resistance substitutions, was highly resistant to PPI-668 and was the only non-responder (0.13 log10, IU/mL).
• With H77 virus rapidly eliminated, low level pre-existing resistant variants at Baseline became detectable in 32/32 patients, but did not show a significant increase on treatment, indicative of inhibition during the treatment period
• Despite the appearance and increase of resistance substitutions at 48 hr, a subsequent decline of 0.4-1.0 log10, IU/mL in 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 various PPI-668 treatment cohorts evaluated
• Like other classes of DAAIs, combination therapy will be required to suppress the emergence of resistance

Resistant Phenotypes

• Percent (S) of substitutions selected on therapy were at (gt-1a) and below (gt-1b) 24 hr
• Single substitutions Q80K and Y93H in gt-1a and Y93H in gt-1b under the highest levels
• Ability to correlate replication of single substitution variants during monotherapy appears to be the dominant factor in the degree of resistance.