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Genotypic and phenotypic analysis of the NS5B polymerase region from viral isolates of HCV chronically infected patients treated with BI 207127 for 5 days' monotherapy

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ABSTRACT

Background: BI 207127, a specific and potent non-nucleoside inhibitor of the hepatitis C virus (HCV) RNA-dependent RNA polymerase *in vitro*, has been studied in patients with chronic genotype (GT) 1 HCV infection for 5 days as monotherapy. The antiviral activity of BI 207127 reached a maximal effect (median 3.8 $\log_{10}$ viral load [VL] decline) for the group receiving 800 mg every 8 hours (Q8H) with no viral breakthrough observed during treatment. NS5B genotypic and phenotypic characterization of viral isolates at baseline and after BI 207127 dosing has been performed.

Methods: HCV RNA was collected and extracted at baseline and at timepoints following the last dose from 60 patients treated with either placebo, 100, 200, 400, 800 or 1,200 mg BI 207127 Q8H. Population sequencing was performed on the NS5B region. Clonal sequencing permitted the quantification and identification of major and minor variants (lower limit of quantification: 5%) in the NS5B region. Chimeric HCV subgenomic replicons were constructed to determine BI 207127 EC_{50} values against patient-derived NS5B polymerase sequences.

Results: Phenotypic analysis of the NS5B segment derived from patient samples at baseline reveal finalized mean BI 207127 EC_{50} values[a] of 43±42 nM for GT-1a and 17±22 nM for GT-1b derived samples. Population sequence analysis of baseline and post-treatment samples revealed the selection of NS5B amino acid substitutions in the thumb-pocket 1 domain, consistent with *in vitro* resistance studies, in 6/25 BI 207127 treated GT-1b infected patients and in 0/21 BI 207127 treated GT-1a infected patients. Reduced phenotypic susceptibility to BI 207127 was associated with the emergence of variants encoding substitutions at position 495 (P495L/Q/S) of the NS5B region, which occurred in 5 patients. Clonal analysis confirmed the amino acid substitutions as the predominant variants in the patient samples. NS5B phenotyping of baseline and posttreatment samples revealed that the five viral isolates encoding P495 changes exhibited up to 400-fold decreased susceptibility to BI 207127.

Conclusion: BI 207127 resistant mutants that encode P495 substitutions were observed in only 5/46 (11%) patients who received 5-day monotherapy. These NS5B variants do not alter the sensitivity to interferon or an NS3 protease inhibitor and support the further investigation of BI 207127 in combination with PegIFN/RBV and/or other direct acting antivirals for the treatment of chronic HCV infection.

[a]Revised from submitted abstract with updated values

INTRODUCTION

- BI 207127 is an orally available, reversible, thumb-pocket 1 non-nucleoside inhibitor of the hepatitis C virus (HCV)-RNA dependent RNA polymerase
- BI 207127 exhibits potent and specific inhibition of the HCV-RNA-dependent RNA polymerase with cell-based HCV subgenomic replicon EC_{50} values of 23 and 11 nM for genotype (GT)-1a and GT-1b, respectively
- The first clinical trial of BI 207127 in HCV-infected patients studied antiviral activity in treatment-naïve (TN)-chronic, GT-1 HCV-infected patients treated with 100, 200, 400, 800 or 1,200 mg every 8 hours (Q8H) as monotherapy for 5 days.[1] In these studies:
  - a rapid and dose dependent viral load (VL) decline was observed with the first dose of BI 207127
  - antiviral activity against HCV GT-1 that correlated with BI 207127 plasma exposure, reaching a maximum effect at the 800 mg Q8H dose with a median VL reduction of 3.8 $\log_{10}$
  - no viral breakthrough was observed during treatment
  - after the treatment period, VL rebounded slowly during the 48-hour follow-up
- Investigational procedures for the genotyping and phenotyping of clinical isolates were developed in order to:
  - monitor for any pre-existing variants with reduced phenotypic sensitivity to BI 207127
  - monitor the emergence and persistence of viral resistance during the administration of HCV NS5B polymerase inhibitors and following drug administration

METHODS

Genotyping and phenotyping methods

- Samples for clinical virology analyses were collected at screening, pretreatment (baseline) and pre-specified patient visits that included follow-up for 10–14 days after the 5 day treatment period

Viral RNA extraction and PCR amplification

- Viral RNA was isolated from the plasma of HCV-infected patients at baseline, Day 6 and during follow-up
- Amplicons containing the NS5B region were generated by reverse transcription and PCR amplification
- Two PCR products containing the complete NS5B region or a 3' subfragment were generated in a second round of PCR
- The lower limit of detection of the RT-PCR amplification method restricted the analysis to patient samples with a VL ≥1,000 IU/mL

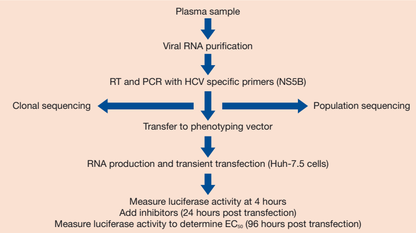
Sequence analysis

- The NS5B-containing DNA product was used for direct population-based sequencing of the NS5B region
- An NS5B subfragment (spanning codons 422–588) was used to generate the clonal sequences
- The resulting sequences were compared to reference sequences according to their respective subtypes; AF00906 served as the reference for subtype 1a and AJ238799 for subtype 1b

Drug sensitivity assays

- The first-round PCR products, from HCV-infected patient plasma samples, were used to amplify DNA fragments containing the NS5B region
- NS5B amplicons were ligated into HCV replicon shuttle vectors containing a luciferase reporter gene to generate NS5B chimeric replicons. The reconstituted plasmid DNA was used to generate HCV subgenomic replicon RNA transcripts
- *In vitro* transcribed RNA was electroporated into Huh-7.5 cells and luciferase activity was measured at 4 hours (signal used to evaluate transfection efficiency) and 96 hours post transfection, as a marker for HCV RNA replication. The replication capacity was calculated by quantifying the luciferase levels of the chimeric replicons and the wild-type (WT) (Con-1b) replicon control. All values were normalized for the RNA input (luciferase levels at 4 hours)
- Susceptibility to BI 207127 was revealed by quantification of luciferase activity across a range of inhibitor concentrations to determine the concentration giving 50% inhibition of HCV RNA replication (EC_{50})

FIGURE 1. Workflow for genotyping and phenotyping of viral HCV NS5B from plasma samples

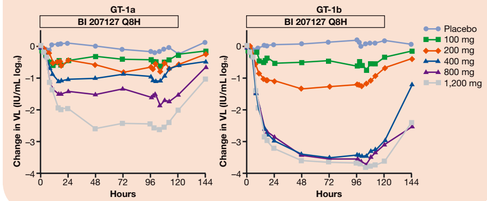


RESULTS

Antiviral activity

- Median VL reduction according to HCV subtype (change from baseline) for each dose group as a function of time is illustrated in **Figure 2**
- Significant antiviral potency in TN GT-1a and GT-1b HCV-infected patients
- Antiviral activity more pronounced in GT-1b than in GT-1a
- No VL breakthrough in any patient during BI 207127 5-day monotherapy

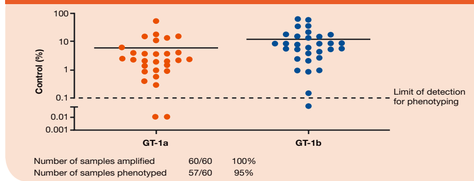
FIGURE 2. Median change in VL with each dose of BI 207127 for GT 1a and 1b HCV-infected patients



In vitro susceptibility of viral NS5B polymerase

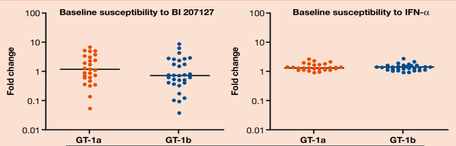
- Chimeric HCV subgenomic replicons constructed from baseline NS5B polymerase and used to evaluate drug susceptibility to BI 207127
- Replication capacity expressed as percentage control ranges from 0.05 to 52% and ≤0.01 to 61% with GT-1a and GT-1b chimeric replicons, respectively (**Figure 3**)

FIGURE 3. Replication efficiency of NS5B chimeras



- Susceptibility was measured for those samples that displayed sufficient replication capacity in the phenotyping assay (**Figure 4**)
  - 57 NS5B were phenotyped at baseline (27 GT-1a and 30 GT-1b)
  - the average susceptibility of each baseline NS5B polymerase sample to BI 207127 is expressed as fold-shift relative to the WT control values of 23 and 11 nM for GT-1a and 1b non-chimeric GT-1a and GT-1b replicons. The values are distributed over a 2 $\log_{10}$ range with a mean of 43 nM for GT-1a and 17 nM for GT-1b. These values are within two-fold of the 23 and 11 nM EC_{50} obtained for BI 207127 in the GT-1a H77 and GT-1b Con-1 reference replicons, respectively
  - polymorphism at position 499 in 7 GT-1b patients (6 phenotyped) samples with 499A variant at baseline showed susceptibility to BI 207127 with EC_{50} ranging from 19–95 nM
- IFN-α was used as a control for the intrinsic variability of the assay. The mean EC_{50} value for IFN-α among GT-1a and GT-1b NS5B polymerase chimeras was 0.2 ± 0.06 and 0.2 ± 0.05 IU/mL, respectively

FIGURE 4. Susceptibility of GT-1a and GT-1b to BI 207127 and interferon alfa (IFN-α)



[a]Revised from submitted abstract with updated values

Genotyping

- NS5B was amplified at baseline, at Day 6 and during follow-up for all samples with VLs sufficient for PCR amplification (≥1,000 IU/mL HCV RNA). The sequences from all timepoints were compared to their respective baseline sequences. Amino acid changes known to confer resistance to this class of NS5B inhibitor[2] were identified by population sequencing
- Genotyping detected NS5B amino acid substitutions, relative to baseline, in the thumb-pocket 1 domain, consistent with *in vitro* resistance studies in only 6 out of 46 patients treated with BI 207127 (**Table 1**)

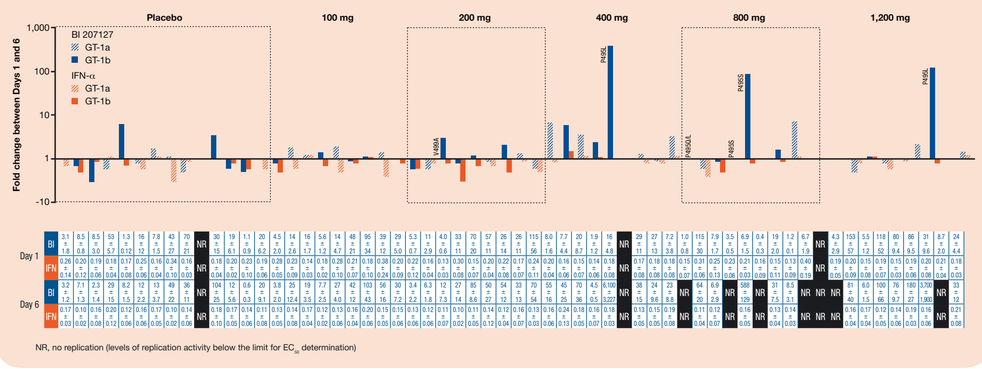
TABLE 1. Resistant mutants identified by population sequencing in HCV patients receiving BI 207127

Dose group	Frequency	Key NS5B change
Placebo	0/14	–
100 mg (n=9)	0/9	–
200 mg (n=9)	1/9	V499A
400 mg (n=9)	1/9	P495L
800 mg (n=9)	3/9	P495S, P495[Q/L]
1,200 mg (n=10)	1/10	P495L

The frequency is expressed as the number of patient samples with key amino acid changes relative to baseline sequence for the total number of samples succesfully PCR amplified in each dose group

Phenotyping of NS5B resistant variants

FIGURE 5. Susceptibility of NS5B chimeric replicons to BI 207127 and IFN-α, and fold change in EC_{50} between Day 1 and Day 6 among placebo controls and BI 207127-treated patients



- All resistant mutants were identified in GT-1b infected patients
- The predominant mutants on treatment follow-up encoded P495L or S
  - P495L confers decrease in sensitivity of 120 and 380-fold, whereas the chimera with P495S is 85-fold less sensitive to BI 207127
- V499A (prevalent natural variant) was selected in only 1 patient following BI 207127 treatment with a sub-optimal (200 mg) dose. This variant only shifted the BI 207127 EC_{50} by three-fold
  - A499 is the consensus amino acid at this position in GT-1a patients
  - five GT-1b BI 207127-treated patients had pre-existing A499 at baseline and their initial VL decline was similar to GT-1a patients with A499
- The NS5B resistant variants remained sensitive to IFN-α
- No loss in sensitivity to the NS3 protease inhibitor BI 201335 was conferred by GT-1b replicons containing these mutations in NS5B (**Table 2**)

TABLE 2. In vitro GT-1b NS5B P495 replicon variants susceptibility to NS3 protease inhibitor BI 201335

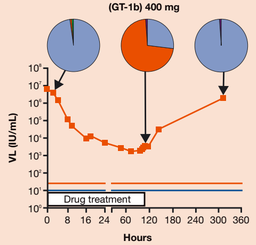
NS5B replicon	BI 201335 EC_{50} (nM)
Con-1 WT (1b)	5.0 ± 1.0
P495L	9.1 ± 2.2
P495S	6.7 ± 1.3
P495Q	3.6 ± 1.3

The average EC_{50} values were obtained from ≥6 values from at least two experiments

Longitudinal clonal analysis

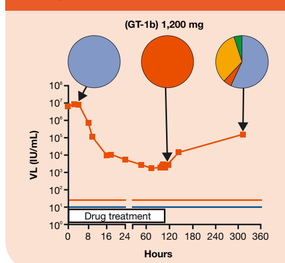
- Resistant mutants were monitored by clonal sequence analysis at the indicated timepoints at baseline and in follow-up to BI 207127 treatment. **Figures 6** and **7** depict two examples highlighting the prevalence of changes at amino acid 495
- There is rapid re-emergence of WT sequences from Day 6 to 14 for both patients with P495L variant suggesting that this variant may be less fit in the absence of drug pressure

FIGURE 6. Clonal sequence analysis of sample 400-5 at baseline, Day 6 and Day 14 following BI 207127 treatment



	Clones (%)		
	Baseline	Day 6	Day 14
WT	98	27	99
P495L	1	72	0
P495T	0	1	1
V499A	1	0	0

FIGURE 7. Clonal sequence analysis of sample 1,200-8 at baseline, Day 6 and Day 14 following BI 207127 treatment



	Clones (%)		
	Baseline	Day 6	Day 14
WT	100	0	57
P495L	0	100	5
P495S	0	0	33
V499A	0	0	5

SUMMARY AND CONCLUSIONS

- In this study with 5 days monotherapy, slight differences in mean BI 207127 GT-1a and GT-1b susceptibility were observed among diverse GT-1a and GT-1b NS5B from the isolates in this study, consistent with the preclinical profile of BI 207127 and with the observed clinical response
- HCV NS5B resistant variants that confer resistance to BI 207127 were selected in 6/46 patients treated with BI 207127 monotherapy. The predominant mutation in five of these GT-1b viruses encoded changes at P495
- The lack of persistence of P495 variants and rapid outgrowth of WT P495 during the follow-up suggests the fitness of this variant in the absence of drug pressure is substantially lower than that of WT – potential for a higher barrier to resistance
- The NS5B variants do not alter the sensitivity to IFN-α or a NS3 protease inhibitor and support the further investigation of BI 207127 in combination with peginterferon-α and ribavirin and/or other direct antivirals for the treatment of HCV infection

REFERENCES

1. Larrey DG, et al. BI 207127 is a potent HCV RNA polymerase inhibitor during 5 days monotherapy in patients with chronic hepatitis C. The 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, USA; 2009. Abstract 1599.
2. Marquis M, et al. Genotypic and phenotypic analysis of Hepatitis C Virus NS5B polymerase variants to BILB 1941 inhibition. The 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, USA; 2008. Abstract 1897.
3. Kukolj G, et al. Binding site characterization and resistance to a class of non-nucleoside inhibitors of the hepatitis C virus NS5B polymerase. J Biol Chem 2005;280:39260–39267.

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