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Analysis of long-term persistence of resistance mutations within the hepatitis C virus NS3 protease after treatment with telaprevir or boceprevir

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ABSTRACT

Background: Telaprevir and boceprevir are highly selective hepatitis C virus (HCV) NS3/4A proteaseinhibitors in phase 3 development. Viral breakthrough during mono- and triple-therapies with PEG-interferon and ribavirin and relapse is associated with resistance.
Objectives: Potential persistence of resistance mutations during long-term follow-up should be analyzed.
Study design: Clonal sequence analysis of the NS3-protease gene was performed at long-term follow-up in HCV genotyp-1 infected patients who received telaprevir or boceprevir within phase-1b studies for comparison with resistant variants present directly after the end-of-treatment.
Results: After a median follow-up of 4.2 years in 28 of 82 patients HCV-RNA was still detectable. Resistance variants were detected in two of 14 telaprevir- and in four of 14 boceprevir-treated patients. For telaprevir patients two low-level (V36M, V36A) and one high-level (A156T) mutation associated with resistance were detected at low frequencies (4–9% of the clones). In five boceprevir-treated patients four low level mutations (V36A, T54A/S, V55A) were observed at low frequencies (1–10%) while in one patient additionally a combined variant (T54S + R155K) was detected at 94%. Presence of resistant variants at long-term follow-up was not predictable by variants detected at the end-of-treatment. In one patient a V55A variant which was dominant already at baseline was still detectable at long-term follow-up.
Conclusions: In the majority of patients after short-term treatment with telaprevir or boceprevir wild-type NS3-protease isolates are detectable by clonal sequencing at long-term follow-up. Detectable resistance mutations in single patients are not predictable by initial frequencies of variants.

1. Background

Chronic hepatitis C virus (HCV) infection is a serious public health problem affecting an estimated 130 million people worldwide.[1] The current standard-of-care, pegylated interferon-alpha (PEG-IFN) plus ribavirin, is of limited efficacy with eradication of the virus in approx. 50% of patients.[2] A large number of directly acting antiviral agents (DAA) targeting the nonstructural-(NS)3-protease, the NS5A-protein, the RNA-dependent RNA-polymerase NS5B, as well as host cell proteins are currently in phase-1 to -3 clinical trials.[3,4] Telaprevir and boceprevir, the most advanced agents, have completed phase 3 studies. Results of clinical studies have shown a significant improvement of sustained virologic response (SVR) rates with triple therapies with telaprevir or boceprevir in combination with PEG-IFN and ribavirin in treatment-naïve and -experienced HCV genotype-1 infected patients.[5–8]

For monotherapy with NS3-protease, NS5A-protein and non-nucleoside polymerase inhibitors rapid selection of resistant variants has been observed,[9,10] which represents one of the major problems of DAAs. Combination therapy with PEG-IFN and ribavirin or with different DAAs has been shown to reduce the likelihood of virologic break-through due to resistance developing.[7–14] However, even using triple therapy with pegylated interferon, ribavirin and NS3 proteaseinhibitors, still a significant number of patients experience viral breakthrough in association with selection of drug-resistant variants.[7,8,11,12,14] Furthermore, variants associated with resistance have been detected in patients with relapse after the end-of-treatment.[11,12,14] Finally, viral variants known to confer resistance to NS3-protease and NS5B non-nucleoside inhibitors have been detected in 0.2–2.8% of patients as the pre-existing dominant strain[15–18] and preexisting R155K variants seem

Abbreviations: HCV, hepatitis C virus; NS, non-structural protein; RNA, ribonucleic acid; WHO, World Health Organization; DAA, directly acting antiviral agent; TID, every 8 h; BID, every 12 h; wk, week; QID, every 6 h; PEG-IFNα, pegylated interferon-alpha; PCR, polymerase chain reaction; GT, genotype; bp, base pairs; SOC, standard-of-care; IU, international units; HIV, human immunodeficiency virus; HBV, hepatitis B virus; SVR, sustained virologic response.

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Table 1
Results of clonal resistance analysis and characteristics of patients who received TVR therapy.

ID	Therapy	Resistance mutations at end of TVR treatment	SOC after TVR study	GT	Long-term follow-up				
					Years	VL [IU/ml]	Clones sequenced	Sensitivity of seq. analysis	Detected resistance mutations
1	3× 750 mg TVR	V36A/M 3% R155K/T 37% A156 6% V36A/M + R155K/T 51% V36A/M + A156T 4%	Yes (outcome: BT)	1a	3.5	570.000	21	4.8%	None
2	3× 450 mg TVR	Sequence data not available[a]	No	1b	4	12.000	20	5%	None
3	3× 450 mg TVR	V36A 62% T54A 28% V36A + T54A 3% V36A + R155L 1%	Yes (outcome: BT)	1b	4	1.200.000	20	5%	None
4	3× 450 mg TVR	Sequence data not available[a]	No	1b	4	3.700.000	27	3.7%	None
5	3× 750 mg TVR	Sequence data not available[a]	No	1b	4	1.400.000	27	3.7%	None
6	2× 1250 mg TVR	R155M/T/K/G 78% V36A/M + R155K/T 20% T54S + R155T 1%	No	1a	4	913.156	29	3.4%	None
7	3× 750 mg TVR	A156T/V 98% R155T/K + A156T 2%	No	1a	4	1.100.000	22	4.5%	None
8	3× 750 mg TVR	A156T/V/I 99% R155Q + A156T 1%	No	1b	4	2.200.000	21	4.8%	None
9	2× 1250 mg TVR	V36A/M 38% T54A 5% R155K 13% V36M + T54A 1% V36A/M + R155G/K/T 24% T54A + R155K 1%	No	1a	4	1.400.000	27	3.7%	V36M 4%
10	3× 750 mg TVR	Sequence data not available[a]	Yes (outcome: NR)	1b	4	380.000	25	4%	None
11	3× 750 mg TVR	Sequence data not available[a]	No	1a	5	19.126	23	4.3%	None
12	2× 1250 mg TVR	A156T/V 100%	No	1b	5	292.507	31	3.2%	None
13	3× 750 mg TVR	Sequence data not available[a]	No	1b	5	7.012.950	29	3.4%	None
14	2× 1250 mg TVR	V36M 5% T54S 1% R155M/T/K/G/S 64% V36M + T54S 2% V36M + R155T/K/G 9% T54S + R155T 1%	Unknown	1a	5	482.693	22	4.5%	V36A 9% A156T 5%

Patients were treated with telaprevir alone (450 mg TID, 750 mg TID, or 1250 mg BID).
[a] HCV RNA negative at end of treatment; TVR, telaprevir; SOC, standard-of-care treatment; GT, genotype; VL, viral load; BT, breakthrough during SOC; NR, non-response to SOC.

to be associated with a reduced response to boceprevir and telaprevir.[19,20]

2. Objectives

Preexisting or selected variants may affect virologic response to DAA. Here, we present long-term follow-up data on patients who were enrolled in phase-1 studies with telaprevir or boceprevir. After a median follow-up of 4.23 years clonal sequence analysis was performed in 28 patients with detectable HCV-RNA for analysis of potential persistence of viral variants (at amino acid (aa) positions 36, 54, 55, 155, 156, and 170) previously described to confer resistance to boceprevir or telaprevir.[21–27]

3. Study design

3.1. Patient population

Altogether 82 patients with chronic HCV genotyp-1 infection were enrolled in phase-1 clinical trials with telaprevir and boceprevir at Saarland University Hospital. Up to 5.5 years after termination of study treatment 42/82 patients could be contacted for a long-term follow-up visit. HCV-RNA was still detectable in 34/42 patients ($n = 6$ placebo; $n = 14$ telaprevir; $n = 14$ boceprevir).

3.2. Telaprevir

Thirty-three HCV genotyp-1 infected patients were enrolled into two randomized, double-blind, placebo-controlled phase-1b trials, which were described recently.[28,29] The patients were treated with telaprevir alone (450 mg TID, 750 mg TID, or 1250 mg BID) or in combination with PEG-IFN-alpha-2a (180 μg/wk) for 2 weeks (750 mg TID). Clonal sequence analysis of the NS3-protease gene could be performed in 14 available patients with ongoing HCV-infection during long-term follow-up. Clonal resistance analysis at end-of-treatment was obtained from a previous study.[21]

3.3. Boceprevir

Forty-nine HCV genotyp-1 infected patients were enrolled into randomized, double-blind, placebo-controlled phase-1b trials.[30,31] The patients were treated with boceprevir alone (200 mg BID, 400 mg BID, or 400 mg TID) or in combination with PEG-IFN-alpha-2b (1.5 μg/kg body weight/wk) for 2 weeks (400 mg