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Compensatory mutations in HCV NS5A/B coevolve in patients failing NS3 inhibitors

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Background

The monocistronic nature of HCV genome may imply a coevolution of different genes during protease-inhibitors (PI) failure, allowing the development of mutations outside NS3, potentially affecting future treatment strategies.

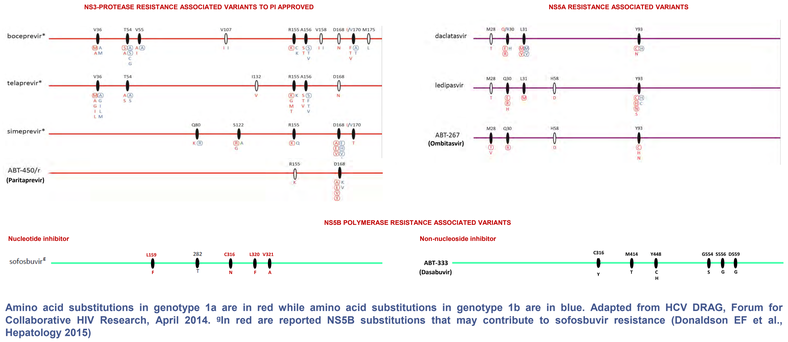
Aim of the study

The aim of this study was to analyze the simultaneous evolution of NS3, NS5A and NS5B in 20 PI-failing patients, from baseline to failure.

Methods

- Twenty patients (15=GT-1a; 5=GT-1b) experiencing virological-failure with peg-IFN/ribavirin+boceprevir (N=5), +telaprevir (N=13) or +simeprevir (n=2) were analyzed.
- NS3-protease, and full-length NS5A and NS5B sequences were obtained in all patients at baseline and at PI-failure, by population sequencing.
- Phylogenetic analysis was performed to evaluate concordance of subtype assignment among NS3, NS5A and NS5B.
- Presence of NS3-protease, NS5A and NS5B resistance associated variants (RAVs) was analyzed in all three genes at baseline and at PI-failure.

MAIN RESISTANCE MUTATIONS ASSOCIATED WITH DIRECT ANTIVIRAL AGENTS CURRENTLY APPROVED



Amino acid substitutions in genotype 1a are in red while amino acid substitutions in genotype 1b are in blue. Adapted from HCV DRAG, Forum for Collaborative HIV Research, April 2014. §In red are reported NS5B substitutions that may contribute to sofosbuvir resistance (Donaldson EF et al., Hepatology 2015)

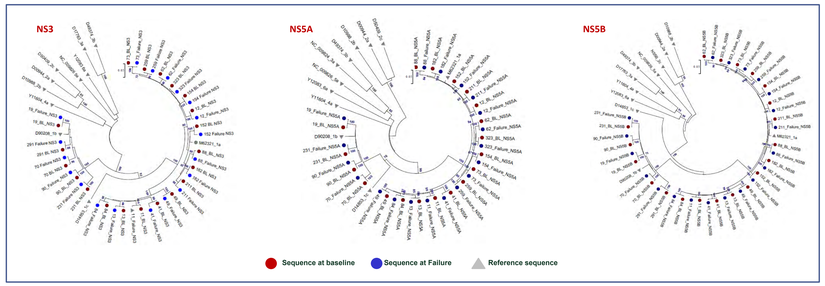
Results

BASELINE CLINICAL CHARACTERISTICS OF THE 20 PI-FAILING PATIENTS INCLUDED IN THE ANALYSIS

	HCV-1a	HCV-1b	Overall
Patients, N	15	5	20
Males, N(%)	12 (80.0)	3 (60.0)	15 (75.0)
Age (years), Median (IQR)	47 (41-53)	42 (42-44)	44(42-53)
Patients with available IL-28B genotype, N(%)			
CT	9 (60.0)	1 (20.0)	10 (50.0)
TT	1 (6.7)	2 (40.0)	3 (15.0)
Unknown	5 (33.3)	2 (40.0)	7 (35.0)
Cirrhotic patients, N(%)	7 (46.7)	2 (40.0)	9 (45.0)
Virological outcome to previous SOC, N(%)			
Non-responder	3 (20.0)	1 (20.0)	4 (20.0)
Null-responder	9 (60.0)	1 (20.0)	10 (50.0)
Partial-responder	3 (20.0)	0 (0.0)	3 (15.0)
Breakthrough	0 (0.0)	1 (20.0)	1 (5.0)
Relapse	0 (0.0)	2 (40.0)	2 (10.0)
Baseline HCV-RNA (log IU/ml), Median (IQR)	6.1 (5.9-6.7)	5.6 (5.4-6.1)	6.1 (5.8-6.6)
Baseline ALT, Median (IQR)	76 (65-110)	76 (66-101)	77 (65-106)
PI-regimen			
Telaprevir	10 (66.7)	3 (60.0)	13 (65.0)
Boceprevir	3 (20.0)	2 (40.0)	5 (25.0)
Simeprevir	2 (13.3)	0 (0.0)	2 (10.0)

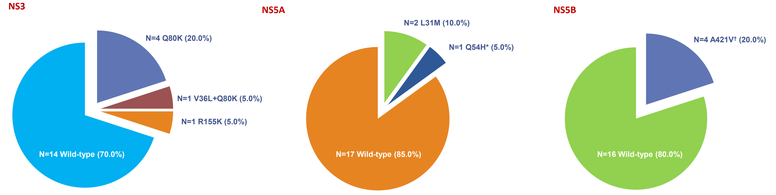
IQR, interquartile range; SOC, standard of care; IU, international units; PI, Protease Inhibitor

PHYLOGENETIC ANALYSIS OF NS5A AND NS5B ALWAYS CONFIRMED SUBTYPING AS DEFINED BY NS3-PROTEASE, IN LINE WITH THE MONOCISTRONIC CHARACTERISTICS OF HCV-GENOME



The first number represents the patient's identification, BL and failure represent the time-point of the sample, NS3 or NS5A or NS5B the gene. Reference sequences are reported with the relative GenBank accession number. The scale bar indicates 5% nucleotide sequences divergence. The reliability of the branching patterns was evaluated by bootstrapping (1.000 replicates). Only bootstrap values >70 are reported. Genetic distances were calculated using the Kimura two-parameter model of MEGA v5.1.

AT BASELINE, NATURAL RESISTANCE VARIANTS WERE FOUND IN NS3, NS5A AND NS5B, RESPECTIVELY



NS3-Protease Inhibitor	Subtype	Fold change for each NS3 RAV		
		V36L	Q80K	R155K
Telaprevir*	1a	2.2	4.6	5.9
	1b	2	0.9	7.4
Boceprevir*	1a	2.9	0.6	6.3
	1b	n.a.	n.a.	4
Simeprevir*	1a	n.a.	9.3	88
	1b	n.a.	7.7	30
ABT-450* (Paritaprevir)	1a	n.a.	n.a.	43
	1b	n.a.	n.a.	n.a.

*Kieffer TR et al., 2014; n.a., not available

NS5A Inhibitor	Subtype	Fold change for each NS5A RAV	
		L31M	Q54H
Daclatasvir*	1a	341	n.a.
	1b	3	1
Ledipasvir†	1a	>100	n.a.
	1b	2.5-100	n.a.
ABT-267* (Ombitasvir)	1a	2	n.a.
	1b	0.9	n.a.

‡Kohler J et al.,2014; †Gao M et al., 2013; *Krishnan P et al., 2015; n.a., not available

NS5B Inhibitor	Subtype	Fold change for NNI-1 RAV
		A421V
Deleobuvir*	1a	5.8
	1b	n.a.
BMS-791325†	1a	2
	1b	n.a.

*Larrey et al., 2013; †Pelosi LA et al., 2012;n.a., not available

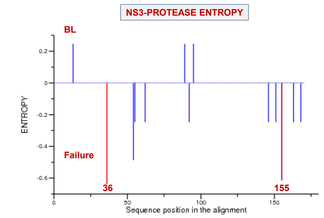
Baseline $_{NS3}$RAVs were detected in 6/20 patients, all infected with HCV-1a (1=V36L+Q80K, 4=Q80K, 1=R155K). In addition, 3/20 patients presented $_{NS5A}$RAVs (1 HCV-1a and 1 HCV-1b=L31M, 1 HCV-1b=Q54H) and 4/20 $_{NS5B}$RAVs to Non-Nucleosidic Inhibitors (NNI; 3 HCV-1a=A421V, 1 HCV-1b=A421V).
Only one HCV-1a infected patient showed natural resistance simultaneously in NS3 (Q80K) and NS5A (L31M).

AT FAILURE, ALL 15 HCV-1a AND 4/5 HCV-1b PI-FAILING PATIENTS PRESENTED MAJOR NS3 RESISTANCE VARIANTS

ID	Genotype	Previous response	PI	Outcome	NS3 Mutations at baseline	NS3 Mutations at failure	NS5A Mutations*	NS5B Mutations*
11	1a	Partial-responder	TVR	Partial-responder	-	R155K/R T54T/S	-	A421V
12	1a	Partial-responder	TVR	Breakthrough	-	R155K/R	-	-
13	1a	Null-responder	TVR	Partial-responder	-	V36V/M R155K	-	-
41	1a	Non-responder	TVR	Breakthrough	-	R155K	-	-
49	1a	Null-responder	TVR	Breakthrough	-	V36V/M R155R/K	-	-
62	1a	Null-responder	BOC	Partial-responder	Q80K	Q80K V36V/M R155K/T	L31M	-
73	1a	Null-responder	TVR	Partial-responder	V36L, Q80K	Q80K V36L R155M	-	-
84	1a	Null-responder	TVR	Breakthrough	-	V36M R155K	-	A421V
88	1a	Null-responder	TVR	Partial-responder	-	V36V/M R155K	-	-
152	1a	Null-responder	SMV	Null-responder	R155K	V36M R155K	-	-
154	1a	Null-responder	SMV	Relapse	Q80K	Q80K V36M R155K	-	-
182	1a	Null-responder	BOC	Partial-responder	-	V36M R155K	-	A421V
211	1a	Non-responder	TVR	Partial-responder	-	R155K	-	-
259	1a	Partial-responder	TVR	Partial-responder	Q80K	Q80K V55A R155T	-	-
323	1a	Non-responder	BOC	Partial-responder	Q80K	Q80K R155K/R T54A/T	-	-
19	1b	Relapse	BOC	Relapse	-	T54T/A V170V/A	-	A421V
70	1b	Null-responder	TVR	Breakthrough	-	none	-	-
90	1b	Relapse	TVR	Partial-responder	-	A156T/A F43C T54T/S	L31M	-
231	1b	Breakthrough	BOC	Partial-responder	-	T54S A156A/T V170A/V	-	-
291	1b	Non-responder	TVR	Partial-responder	-	A156T	Q54H	-

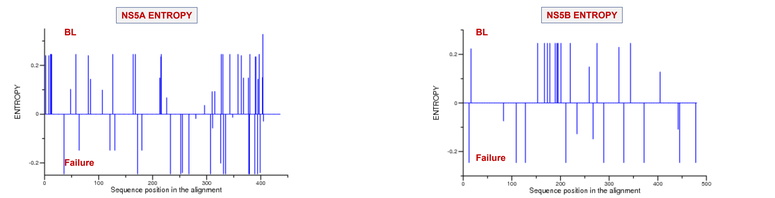
PI, Protease inhibitor (Telaprevir , TVR; Boceprevir , BOC; Simeprevir SMV); *NS5A and NS5B mutations at baseline and at failure.

STATISTICALLY SIGNIFICANT DIFFERENCES IN ENTROPY WERE OBSERVED IN NS3 RAV POSITIONS, COMPARING BASELINE AND FAILURE SEQUENCES



Shannon entropy differences between sequences of NS3 at baseline and failure. Sites displaying a statistically significant difference in entropy between baseline and failure are indicated in red. The analysis was performed starting from sequences of 15 HCV-1a infected patients.

DIFFERENTLY, NO STATISTICALLY SIGNIFICANT DIFFERENCES IN ENTROPY WERE OBSERVED IN BOTH NS5A AND NS5B, COMPARING BASELINE AND FAILURE SEQUENCES

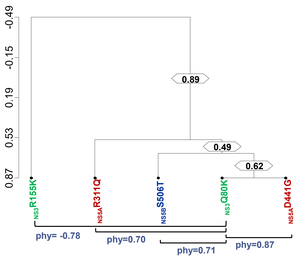


Shannon entropy differences between sequences of NS5A or NS5B at baseline and failure. No sites displaying a statistically significant difference in entropy were observed in the two groups of baseline and failure sequences, in both NS5A and NS5B. The analysis was performed starting from sequences of 15 HCV-1a infected patients.

Some new NS5A and NS5B variants were observed at failure: 13/20 patients developed de novo NS5A mutations (median[IQR]number=2[1-3]), even if putative IFN-resistance associated NS5A-regions remained unaffected, and 15/20 patients developed also de novo NS5B mutations (median[IQR]number=1[1-4]). Overall, none of these new mutations outside NS3 was found in more than one patient.

DIFFERENT PATTERNS OF MUTATIONS WERE IDENTIFIED BY COVARIATION ANALYSIS ACROSS ALL 3 GENES AMONG 15 HCV-1a INFECTED PATIENTS

Cluster of NS3, NS5A and NS5B mutations



The dendogram reports the hierarchical clusters analysis performed including NS3, NS5A and NS5B mutations. Bootstrap values are reported into boxes.

Significantly correlated pairs mutations

Mutation	Prevalence[a], no. (%)	Correlated mutation	Prevalence[a], no. (%)	Covariation fequency[b] (%)	p-value[c]	Phy[d]
Q80K (NS3)	5 (33.3)	D441G (NS5A)	6 (40.0)	5 (100)	0.001	0.87
		R311Q (NS5A)	5 (33.3)	4 (80.0)	0.016	0.70
		S506T (NS5B)	3 (20.0)	3 (60.0)	0.022	0.71
R155K (NS3)	13 (86.7)	S506T (NS5B)	3 (20.0)	1 (7.7)	0.028	-0.78

[a] The prevalence was determined in 15 HCV-1a failing patients at failure
[b] Percentages were calculated in patients containing each specific classical mutation
[c] P values were determined by Fisher exact test.
[d] Phi is the binomial correlation coefficient that indicates the strength of a correlation between the variables. Phi for positive correlations ranges from 0 to 1. The highest the phi is and the strongest the correlation is.

Conclusions

The present study shows that mutations in NS5A and NS5B may coevolve with NS3 RAVs during PI-treatment, potentially acting as compensatory mutations for viral fitness.

The clinical relevance of such mutations may deserve future attention, particularly in the scenario of future treatment-strategies.