RESISTANCE ANALYSIS IN HCV-3 INFECTED PATIENTS WITHIN THE ITALIAN NETWORK VIRONET-C

#00565

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on behalf of HCV Virology Italian Resistance Network (Vironet C)

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Background

- Hepatitis C virus (HCV) genotype (GT) 3 is one of the most common genotype and is estimated to account for 54.3 million (30.1%) cases globally (Messina JP et al. Hepatology 2015)
- Despite the high efficacy of new interferon and ribavirin (RBV) free direct-acting antiviral (DAAs) regimens, GT3 is still considered a "difficult to treat genotype" and even with new pan-genotypic combinations needs tailored treatment-protocols.

Aim of the study

This study aimed to investigate the presence and role of resistance-associated-substitutions (RASs) in HCV GT3.

Methods

- Within the Italian network VIRONET-C, 539 GT3 infected patients were analyzed (417 DAA-naïve and 135 DAA-failures, of whom, 13 at both baseline and DAA-failure)
- NS3-protease (aa 1–181), NS5A domain I (aa 1-213) and NS5B (aa 1-591) proteins were tested on available samples by population-sequencing, using in house protocols in 12 Italian laboratories.
- Phylogenetic analysis was performed to exclude contamination and confirm GT using Geno2Pheno reference strains (Kalaghatgi P 2016). RASs reported to confer >2-fold increase in EC50 were analyzed, following recent literature data (Sorbo et al. 2018.).
- Multivariable logistic regression analysis was performed to evaluate factors independently associated with treatment-response by adjusting for the following variables: cirrhosis, previous IFN-treatment, baseline HCV-RNA ≥800.000 IU/ml, at least one regimen-related NS5A-RAS, treatment duration, ribavirin use, patient age and gender.

Results

Baseline characteristics of 539 GT3 infected patients included in the analysis

Patients, N		539
Males, N (%)		428 (79.4)
Age, Median (IQR)		52 (46-56)
HCV genotype/subtype, N (%)	3a	530 (98.3)
	3b	2 (0.4)
	3g	1 (0.2)
	3h	6 (1.1)
HIV coinfection, N (%) [a]	HIV	72 (13.9)
Risck factor, N (%) [b]	Drug addiction	264 (91.4)
	Sexual	10 (3.5)
	Transfusion	9 (3.1)
	Parenteral	5 (1.7)
	Iatrogenic	1 (0.3)
Liver transplant, N (%) [c]		16 (4.1)
HCC, N (%) [d]		24 (4.9)
Cirrhosis, N (%) [e]		263 (49.3)
Stiffness at baseline (Kpa), Median (IQR) [f]		11.7 (7.1-20.9)
Fibrosis score, N (%)	F0-F1	34 (9.9)
	F2	21 (6.1)
	F3	49 (14.3)
	F4	238 (69.6)
Baseline HCV-RNA (logIU/ml), Median (IQR)		5.9 (5.3-6.4)
Baseline ALT (IU/ml), Median (IQR)		88 (60-147)
DAA naïve patients, N		417
DAA failing patients, N [h]		135

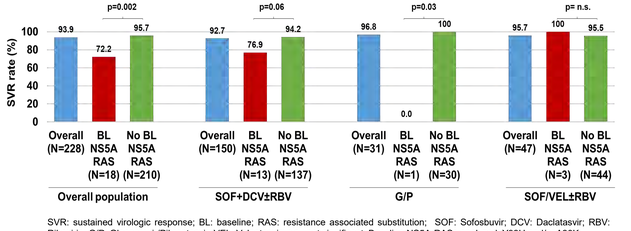
IQR, interquartile range; HCV, Hepatitis C Virus; HCC, Hepatocellular Carcinoma; IU, international units; ALT, alanine transaminase. [a]Data available for 516 patients; [b]Risk factor available for 289 patients; [c] Data available for 389 patients; [d]Data available for 485 patients; [e] Data available for 533; [f]Data, available for 347 patients; [g] Data available for 342 patients; [h] 13 had both baseline and failure sample;

In 39/539 (7.2%) patients previously classified with an indeterminate or a non GT3 genotype, HCV-sequencing identified a GT3 infection

ID Patient	Risk factor	Previous genotype assignment	Genotype determined by sequencing and phylogenetic analysis	Reason for sequencing
834		Indeterminate	3a	Genotype assignment
926		Indeterminate	3a	Genotype assignment
1693		Indeterminate	3a	Genotype assignment
2160	Drug addiction	Indeterminate	3a	Genotype assignment
2436	Trasfusion	Indeterminate	3h	Failure: DCV+SOF
2583	Drug addiction	Indeterminate	3a	Genotype assignment
2751	Drug addiction	Indeterminate	3a	Genotype assignment
2826	Drug addiction	Indeterminate	3a	Genotype assignment
3072		Indeterminate	3a	Genotype assignment
3324		Indeterminate	3h	Genotype assignment
2353		1	3a	Failure: 3D
828	Drug addiction	1a	3a	BL resistance test
1497		1a	3a	Failure: 3D+RBV
1534	Drug addiction	1a	3a	
1784	Drug addiction	1a	3a	
2150	Drug addiction	1a	3a	Failure: 3D+RBV
3924	Sexual	1a	3a	Failure: 3D+RBV
889	Drug addiction	1b	3a	
1424		1b	3a	Failure: 3D+RBV
2068		1b	3a	Failure: 3D
2140		1b	3a	Failure: 3D+RBV
2860		1b	3a	Failure: LDV/SOF+RBV
2986		1b	3a	
3246		1b	3a	Failure: DCV+SOF
3729		1b	3a	Failure: LDV/SOF+RBV
3771		1b	3a	Failure: 3D
3772		1b	3a	Failure: 3D
3956		1b	3a	Failure: 3D
3067		2	3a	
1043	Drug addiction	2a	3a	
3179		2c	3a	
8884		4	3a	Failure: SIM+SOF
3728		1a/1b-3h	3h	Failure: 3D
875	Drug addiction	1b/3	3a	
3770		1b/5a	3h	Failure: 3D
2624		2/1b	3a	Failure: 3D
3249		3/6	3a	
3255		3/6	3a	
3446		3/6	3a	

228 GT3a DAA-naïve patients had an available treatment-outcome and baseline NS5A RASs testing. Of these, 93.9% (214/228) achieved a SVR

Virological outcome according to baseline presence of NS5A-RASs in GT3a infected patients treated with recommended DAA-regimens



SVR: sustained virologic response; BL: baseline; RAS: resistance associated substitution; SOF: Sofosbuvir; DCV: Daclatasvir; RBV: Ribavirin; G/P: Glecaprevir/Pibrentasvir; VEL: Velpatasvir; n.s.: not significant. Baseline NS5A-RASs analyzed: Y93H and/or A30K.

ID Patient	Cirrhosis	OLT	BL HCV RNA LogIU/ml	NS5A Baseline RASs	Treatment	Treatment duration (weeks)	Outcome	RASs at failure
2294	No	No	5.3	Y93H	DCV+SOF	12	SVR	-
1858	Yes	No	6.6	A30K	DCV+SOF	24	Relapse	A30K
1905	Yes	No	6.0	Y93H	DCV+SOF	24	Relapse	Y93H
931	Yes	No	6.3	Y93H	DCV+SOF+RBV	24	SVR	-
1506	Yes	No	6.6	A30K	DCV+SOF+RBV	24	SVR	-
1790	Yes	No	4.7	A30K	DCV+SOF+RBV	24	SVR	-
2362	Yes	No	6.7	A62L+Y93H	DCV+SOF+RBV	24	SVR	-
2485	No	Yes	6.8	Y93H	DCV+SOF+RBV	24	Relapse	Y93H
3774	Yes	No	4.7	Y93H	DCV+SOF+RBV	24	Relapse	Y93H
331	No	No	3.1	A30K	SOF/VEL	12	SVR	-
332	Yes	No	5.8	Y93H	SOF/VEL	12	SVR	-
336	No	No	5.3	Y93H	SOF/VEL	12	SVR	-
3552	No	No	6.6	A30K	G/P	16	Breakthrough	A30K+Y93H

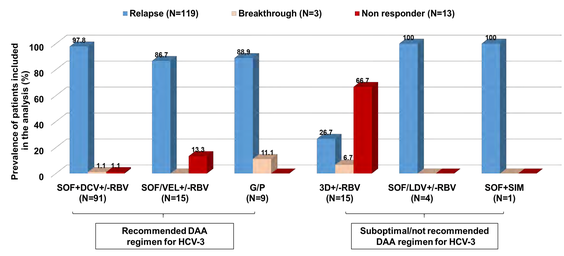
OLT: Orthotopic Liver Transplantation; BL: Baseline; IU: International Unit; RAS: resistance associated substitutions; DCV: Daclatasvir; SOF: Sofosbuvir; RBV: Ribavirin; SVR: Sustained Virologic Response; G/P: Glecaprevi/Pibrentasvir; SOF/VEL: Sofosbuvir/Velpatasvir.

Baseline characteristics of 228 GT3 infected patients with an available treatment-outcome and baseline NS5A RAS testing

Baseline characteristics	SVR (N=214)	Failure (N=14)	p value+
GT3a, N (%)	214 (100)	14 (100)	-
Male, N (%)	158 (73.8)	12 (85.7)	0.74
<65 years, N (%)	207 (96.7)	13 (92.9)	0.40
HIV coinfection, N (%)	44 (20.6)	0 (0.0)	0.08
Cirrhosis, N (%)	99 (46.3)	9 (64.3)	0.27
HCC, N (%)	11 (5.7)	1 (7.1)	0.58
Liver transplant, N (%), N (%)	4 (3.1)	1 (10.0)	0.32
HCV-RNA ≥ 800.000 IU/ml*, N (%)	98 (46.4)	9 (64.3)	0.27
Previous IFN experience[g], N (%)	129 (63.5)	6 (46.2)	0.26
BL NS5A-RASs, N (%)	14 (6.5)	4 (28.6)	**0.01**
BL NS5A-RASs+HCV RNA≥800,000 IU/ml, N(%)	6 (2.8)	3 (21.4)	**0.01**
BL NS5A-RASs+Cirrhosis, N(%)	9 (4.2)	3 (21.4)	**0.03**
≥ 2 risk factor for failure, N(%)	88 (41.1)	10 (71.4)	**0.05**

IU, international unit; IFN, interferon; BL, baseline; RAS, resistance associated substitution. *Data available for N=211 SVR patients and all failure patients; [g]Data available for N=203 SVR patients and N=13 failure patients; +p value was calculated by fisher exact test; ≥ 2 risk factors for failure (cirrhosis, high viral load [≥800.000 IU/ml], previous IFN experience, or baseline NS5A-RAS). HCC, hepatocellular carcinoma (Data available for 193 SVR and 14 failure patients); Liver transplant information was available for 128 SVR and 10 failure patients.

Overall, 135 patients experienced a virologic failure to a DAA interferon-free regimen



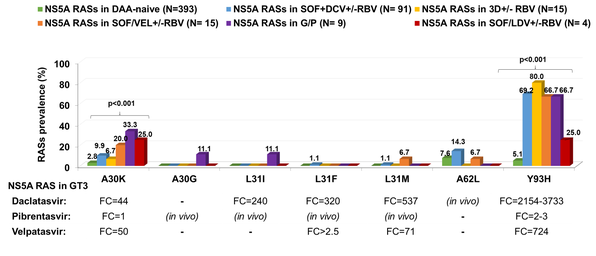
Multivariate logistic regression analysis identified baseline presence of NS5A-RAS as the only independent factor negatively associated with the achievement of SVR

Univariate and multivariate logistic regression performed to evaluate factors independently associated with treatment-response

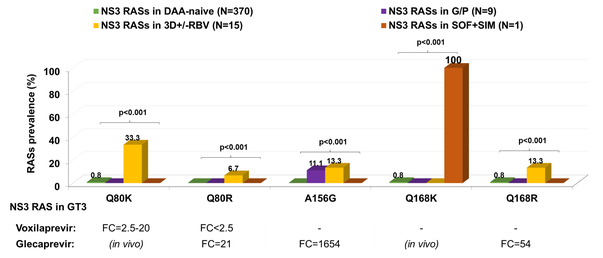
Variables	Univariate OR	Univariate 95% C.I. Lower	Univariate 95% C.I. Upper	Univariate P-value	Multivariate OR	Multivariate 95% C.I. Lower	Multivariate 95% C.I. Upper	Multivariate P-value
Baseline NS5A-RASs	**0.17**	**0.05**	**0.63**	**0.01**	**0.19**	**0.05**	**0.74**	**0.02**
Male	2.09	0.45	9.67	0.34	1.60	0.30	8.68	0.59
<65 years	0.40	0.05	3.58	0.41	0.18	0.02	1.94	0.16
HCV-RNA ≥800.000 IU/ml	0.48	0.16	1.48	0.20	0.48	0.15	1.59	0.23
Cirrhosis	0.47	0.15	1.45	0.19	0.76	0.21	2.80	0.68
Previous IFN-treatment	0.58	0.20	1.73	0.33	0.84	0.26	2.73	0.78
Ribavirin Use	0.39	0.13	1.18	0.10	0.52	0.14	1.89	0.32
Length of retreatment	0.35	0.11	1.16	0.09	0.63	0.14	2.85	0.55

C.I., confidence interval; OR, odds ratio; BL, baseline; RAS, resistance associated substitution; IU, international units; IFN, interferon.

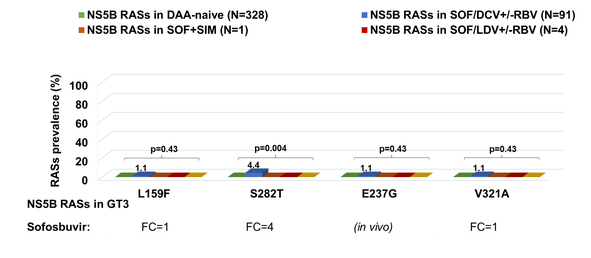
GT3 NS5A resistance analysis



GT3 NS3 resistance analysis



GT3 NS5B resistance analysis



Conclusions

- Overall, these results highlight how to perform HCV sequencing before first-line or second-line DAA regimens allows a personalization of DAA regimen by both definition of viral resistance profile, and assessment of "correct" HCV genotype, avoiding suboptimal and inappropriate treatments that may lead to multiple RASs development.
- In this large cohort of GT3 infected patients, the majority of failures harbored resistant HCV variants carrying one or two NS5A RASs, the most frequent being Y93H.
- The presence of natural NS5A RAS before treatment was associated with failure.
- Further analyses are needed to confirm this observation, particularly for the new current regimens.

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