Substantial presence of natural NS3, NS5A and NS5B HCV-resistance in real practice, and their impact on direct-acting antiviral treatment in genotypes 1-4

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Introduction

- High SVR rates (>90%) are achieved by different approved IFN-free DAA combination treatment regimens (SMV/SOF, DCV/SOF, LDV/SOF, ASV/DCV, PTVr/OBV±DSV with and without RBV)*
- Natural resistance associated substitutions (RASs) decrease SVR rates between ≈1-50% dependent on the DAA regimen and other factors like HCV genotype/-subtype, previous treatment experience and/or presence of cirrhosis.
- The prevalence of baseline RASs among HCV patients is not well defined, especially among certain sub-populations.
  - Non-1 HCV infected patients
  - Patients with previous IFN experience
  - Patients with cirrhosis
- We still have no clear results on the impact of specific RASs on drug-efficacy, especially in NS5A and in real-life settings

*ASV, asunaprevir; DAA, direct acting antiviral; DCV, daclatasvir; DSV, dasabuvir; IFN, interferon; LDV, ledipasvir; OBV, ombitasvir; PTVr, paritaprevir/ritonavir; RBV, ribavirin; SMV, simprevir; SOF, sofosbuvir.

AIMS

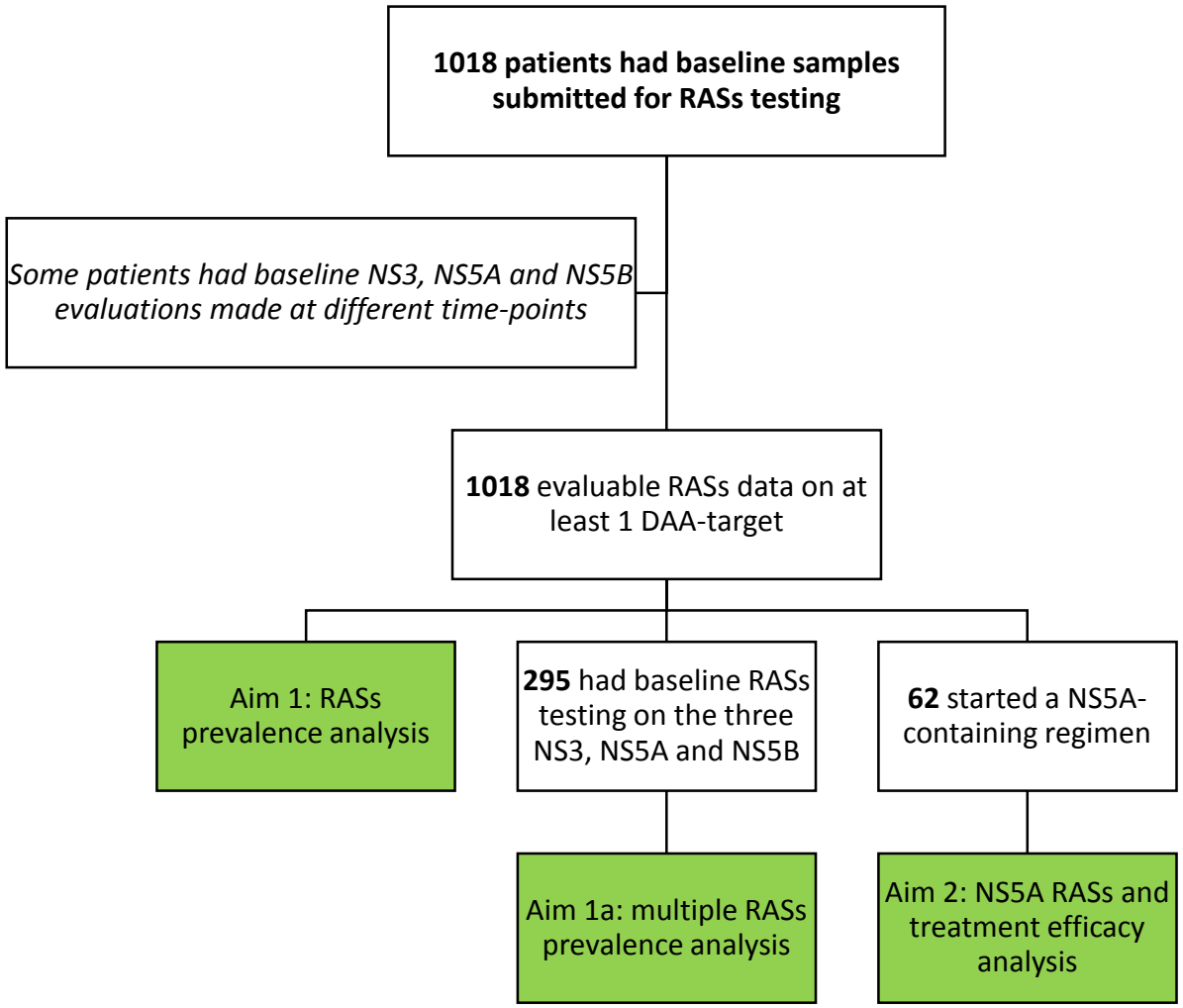
1. To analyze the frequency of natural NS3, NS5A and NS5B RASs in a large real-life Italian multicenter database, within the 4 main HCV GTs.
   a. Prevalence of patients with RASs on all 3 drug targets.

2. To investigate the impact of natural NS5A RASs on NS5A-inhibitors efficacy.

Methods

- Multi-center, observational study of patients undergoing HCV treatment in routine clinical practice.
- All patients were DAA-naïve for the target analyzed (NS3, NS5A, NS5B).
- HCV resistance testing was performed on baseline samples using an home made sequencing assay based on Sanger sequencing.
- RASs were classified according to fold-change reduction in efficacy of different drugs in different HCV-genotypes:
  - Low-level resistance: fold-change 2-100
  - Intermediate-level resistance: fold-change 100-1000
  - High-level resistance: fold-change >1000

Patients


1018 patients had baseline samples submitted for RASs testing
Some patients had baseline NS3, NS5A and NS5B evaluations made at different time-points
1018 evaluable RASs data on at least 1 DAA-target
Aim 1: RASs prevalence analysis
295 had baseline RASs testing on the three NS3, NS5A and NS5B
62 started a NS5A-containing regimen
Aim 1a: multiple RASs prevalence analysis
Aim 2: NS5A RASs and treatment efficacy analysis

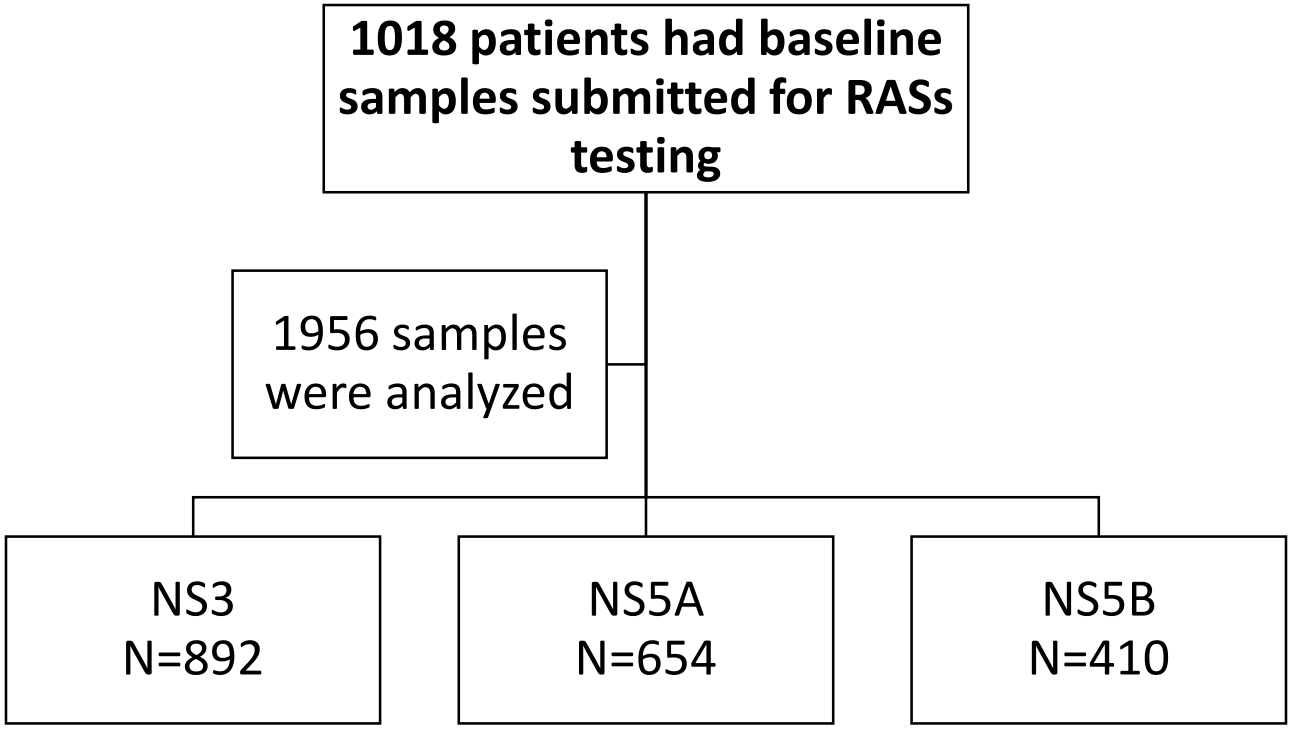
Demographics and Baseline Characteristics Analysis of DAA-Naïve Patients (N = 1018)

		HCV genotype and subtype						Overall
		1a	1b	2c	3a	4a	4d	
Patients included, N		**330**	**454**	**53**	**112**	**10**	**59**	**1018**
Males, N(%)		268 (81.2)	245 (54.0)	29 (54.7)	94 (83.9)	8 (80.0)	45 (76.3)	689 (67.7)
Age (years), Median (IQR)		51 (45-56)	64 (54-71)	68 (61-75)	53 (48-57)	52 (50-53)	53 (49-58)	55 (49-65)
BMI (Kg/m^2), Median (IQR)		25 (23-29)	26 (24-29)	23 (23-23)	24 (22-25)	27 (27-27)	23 (21-26)	25 (23-28)
IL-28B genotype[a]	CC	29 (23.6)	19 (11.0)	0 (0.0)	8 (50.0)	1 (33.3)	1 (7.1)	58 (17.5)
	CT	79 (64.2)	111 (64.2)	2 (100)	6 (37.5)	2 (66.7)	8 (57.1)	208 (62.8)
	TT	15 (12.2)	43 (24.9)	0 (0.0)	2 (12.5)	0 (0.0)	5 (35.7)	65 (19.6)
Cirrhosis, N(%)		158 (47.9)	270 (59.5)	30 (56.6)	66 (58.9)	3 (30.0)	42 (71.2)	569 (55.9)
Liver transplant, N(%)		13 (3.9)	27 (5.9)	0 (0.0)	13 (11.6)	1 (10.0)	3 (5.1)	57 (5.6)
HCC, N(%)		2 (0.6)	30 (6.6)	3 (5.7)	8 (7.1)	0 (0.0)	2 (3.4)	45 (4.4)
IFN naive, N(%)		104 (31.5)	122 (26.9)	27 (50.9)	46 (41.1)	2 (20.0)	15 (25.4)	316 (31.0)

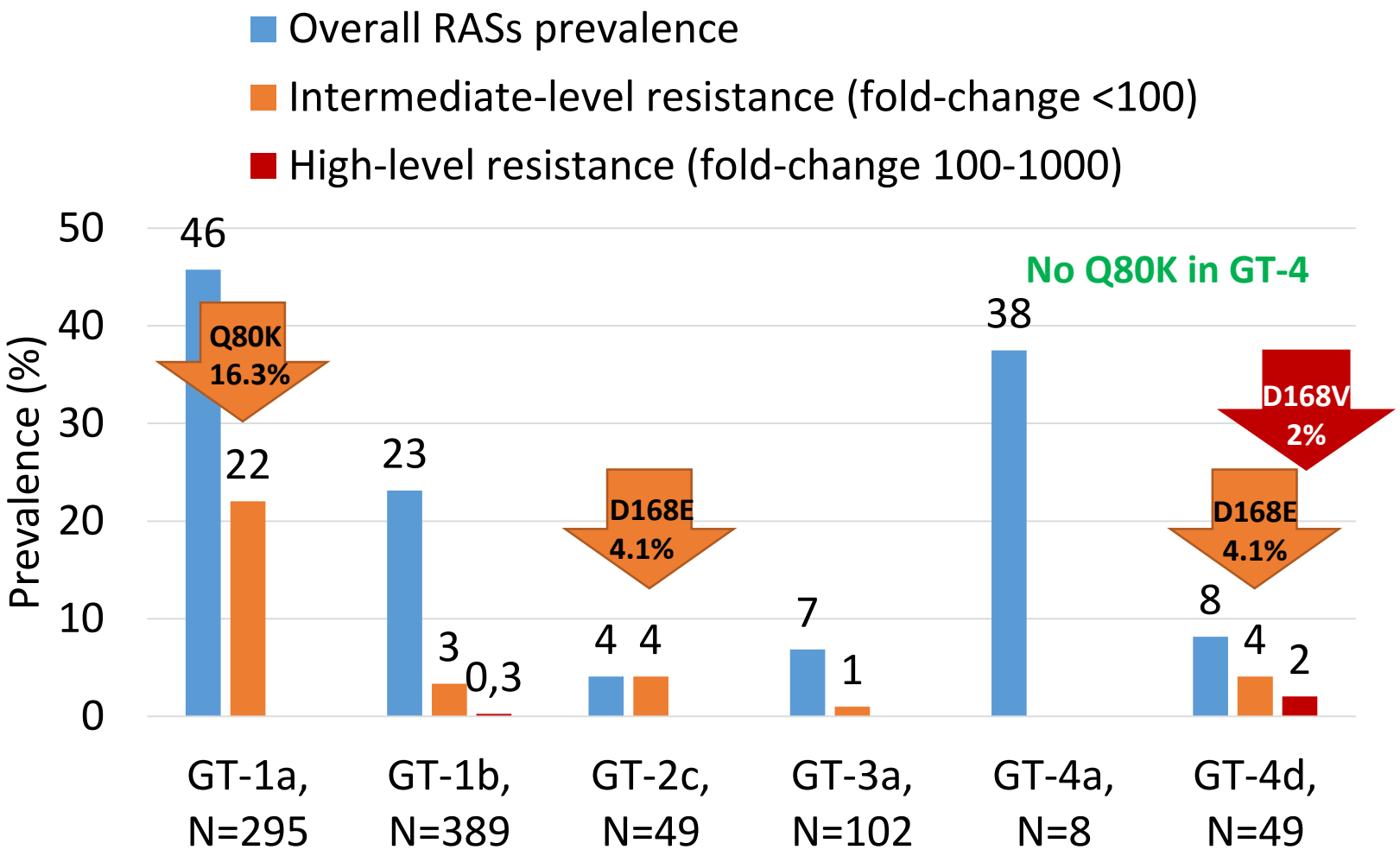
[a] Available for 123 GT-1a, 173 GT-1b, 2 GT-2c, 16 GT-3a, 3 GT-4a and 14 GT-4d patients.
IQR, interquartile range; BMI, body mass index; HCC, hepatocellular carcinoma; IFN, interferon

22/1018 (2.2%) with HBV co-infection
42/1018 (4.1%) with HIV co-infection

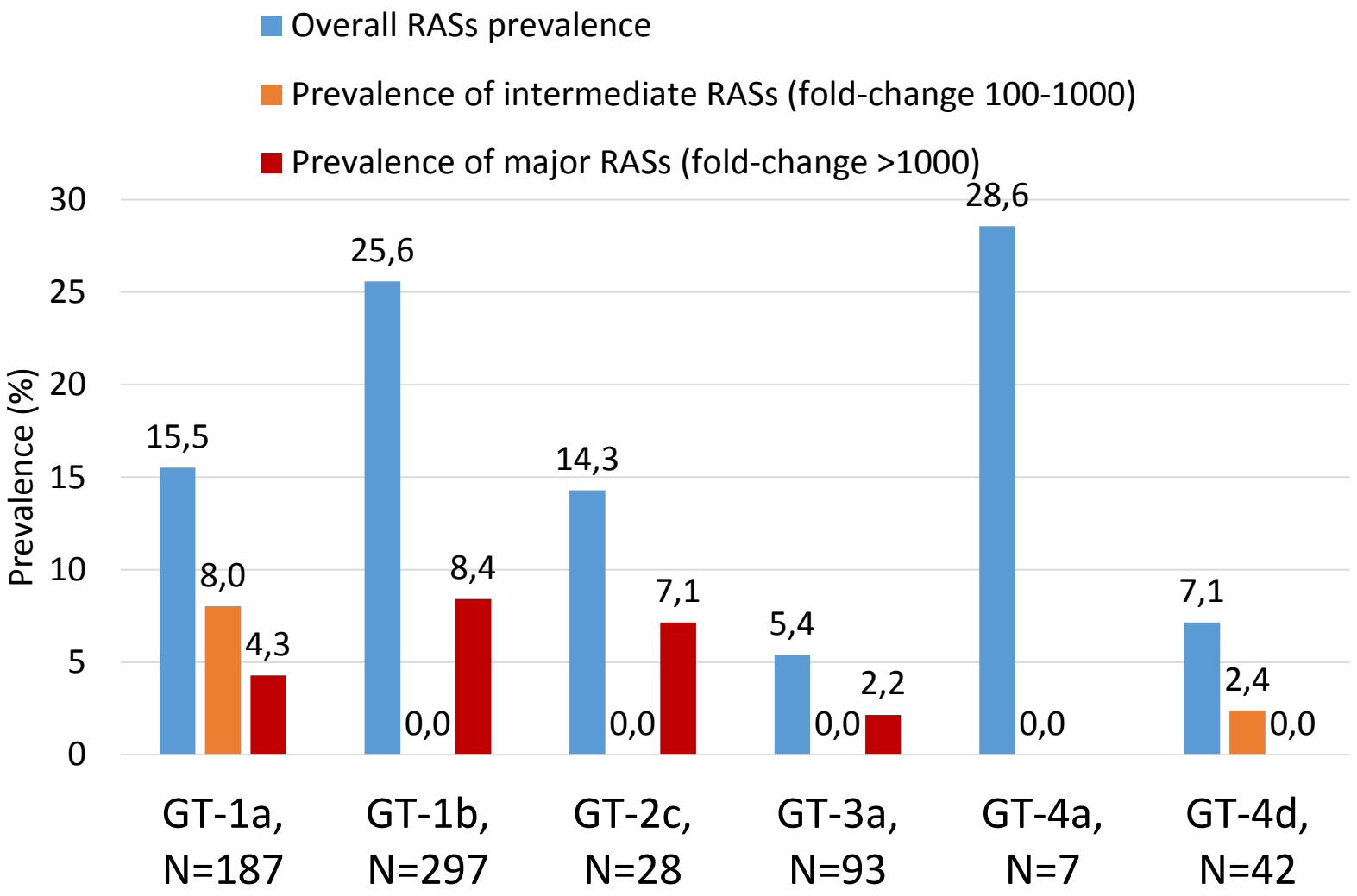
Aim 1: Prevalence of NS3, NS5A, and NS5B RASs



Natural NS3 RASs by HCV genotype



Natural NS5A RASs by HCV genotype



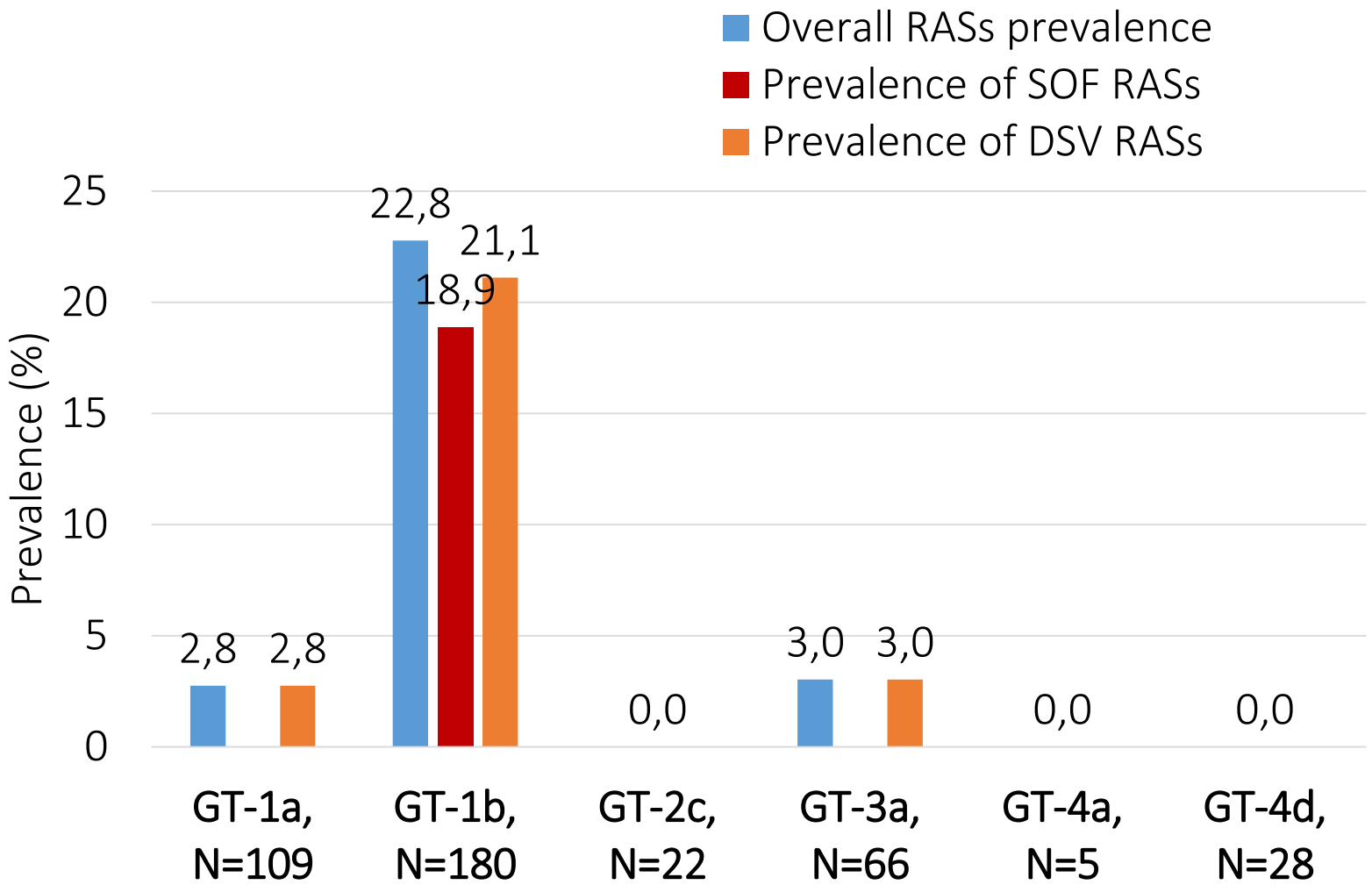
Natural NS5A RASs by AA position

	Natural NS5A RAS prevalence, N (%)						
	GT-1a, N=187	GT-1b, N=297	GT-2c, N=28	GT-3a, N=93	GT-4a, N=7	GT-4d, N=42	Overall, N=654
High-level resistance (fold-change >1000)							
Q30H	1 (0.5)	-	-	-	-	-	1 (0.2)
Q30R	4 (2.1)		-	-	-	-	4 (0.6)
L31M	8 (4.3)		1 (3.4)	-	-	-	9 (1.4)
Y93C	1 (0.5)		-	-	-	-	1 (0.2)
Y93H	1 (0.5)	25 (8.4)	1 (3.4)	2 (2.2)	-	-	29 (4.4)
Y93N	1 (0.5)	-	-	-	-	-	1 (0.2)
Intermediate-level resistance (fold-change 100-1000)							
M28V	7 (3.7)	-	-	-	-	-	7 (1.1)
R30S	-	-	-	-	-	1 (2.4)	1 (0.2)
Low-level resistance (fold-change <100)							
K24R	1 (0.5)	-	-	-	-	2 (4.8)	3 (0.5)
L28M	-	8 (2.7)	-	-	-	-	8 (1.2)
A30K	-	-	-	2 (2.2)	-	-	2 (0.3)
L30R	-	-	-	-	2 (28.6)	-	2 (0.3)
R30H	-	1 (0.3)	-	-	-	-	1 (0.2)
R30Q	-	17 (5.7)	-	-	-	-	17 (2.6)
L31F	-	-	1 (3.4)	-	-	-	1 (0.2)
L31M	-	10 (3.4)	-	-	-	-	10 (1.5)
L31P	-	-	-	1 (1.1)	-	-	1 (0.2)
P58L	-	2 (0.7)	-	-	-	-	2 (0.3)
P58S	-	11 (3.7)	1 (3.4)	-	-	-	12 (1.8)
E62D	7 (3.7)	-	-	-	-	-	7 (1.1)
A92T	-	13 (4.4)	-	-	-	-	13 (2)

*FC DCV =24-145
*FC LDV > 1000
*OMB = 77

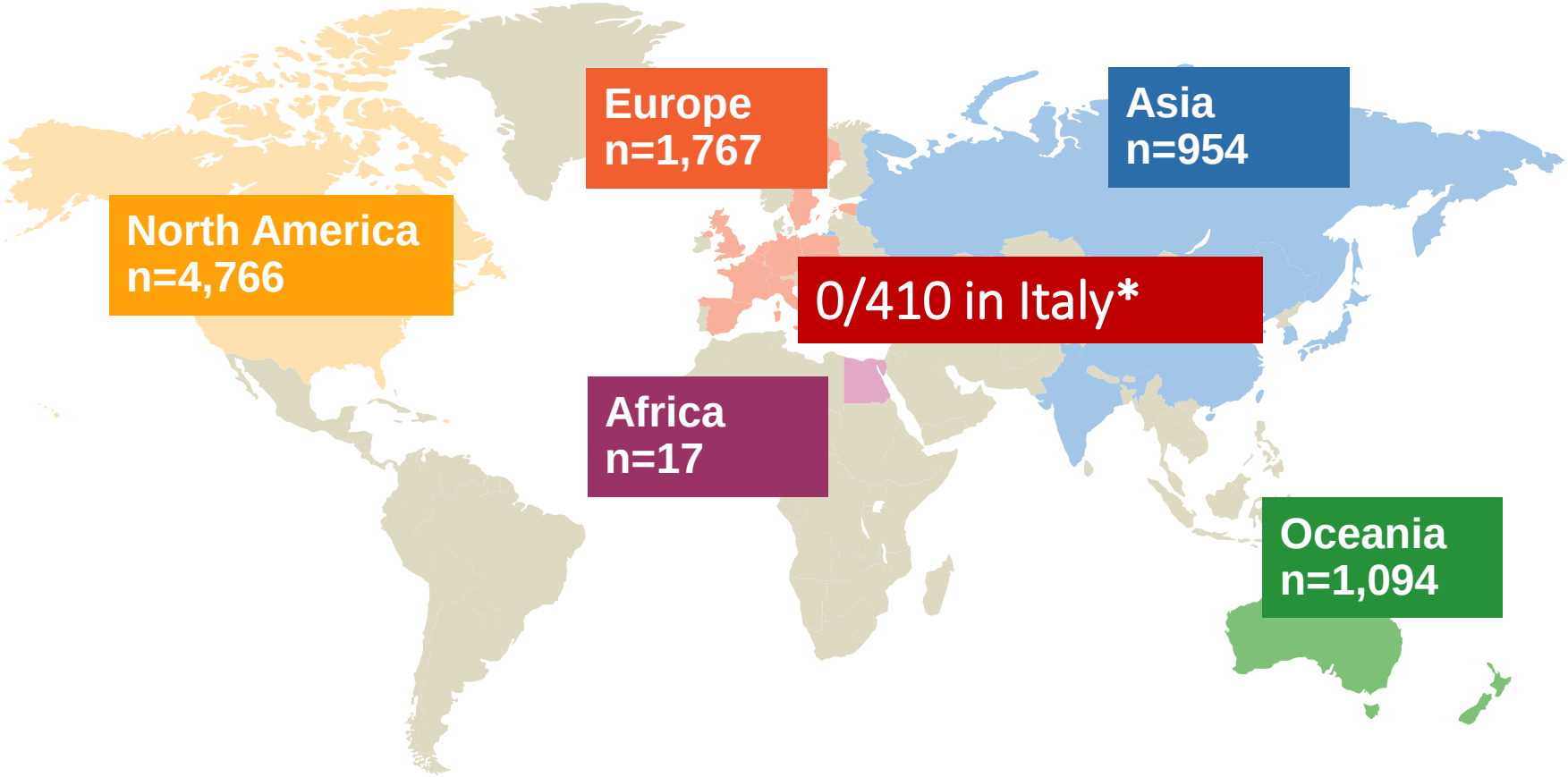
NS5A RASs are reported according to genotype-specific wild-type amino acid.

Natural NS5B RASs by HCV genotype



Baseline Prevalence of S282T Sofosbuvir RAS

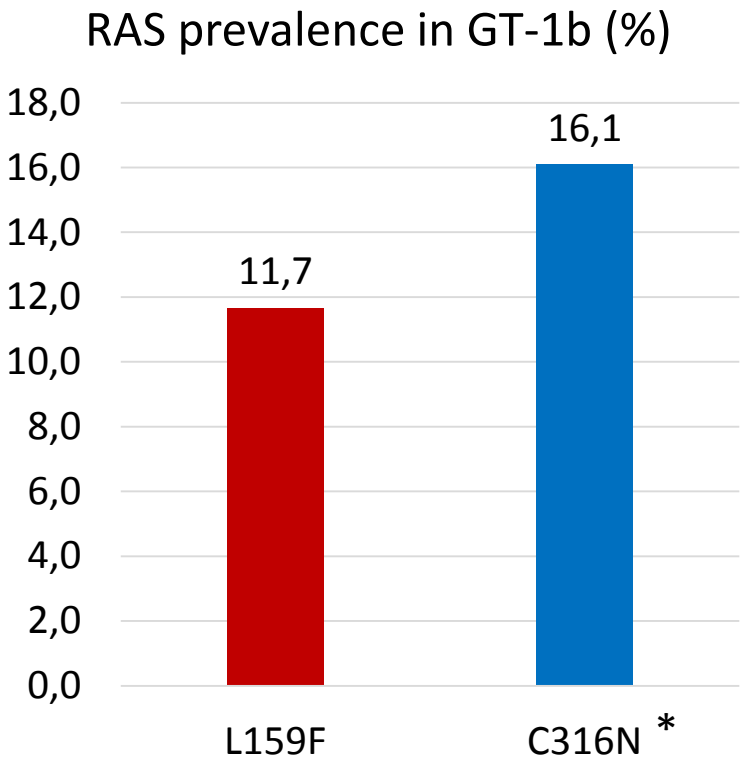
- No S282T (0/8598) was detected in any pretreatment patient samples



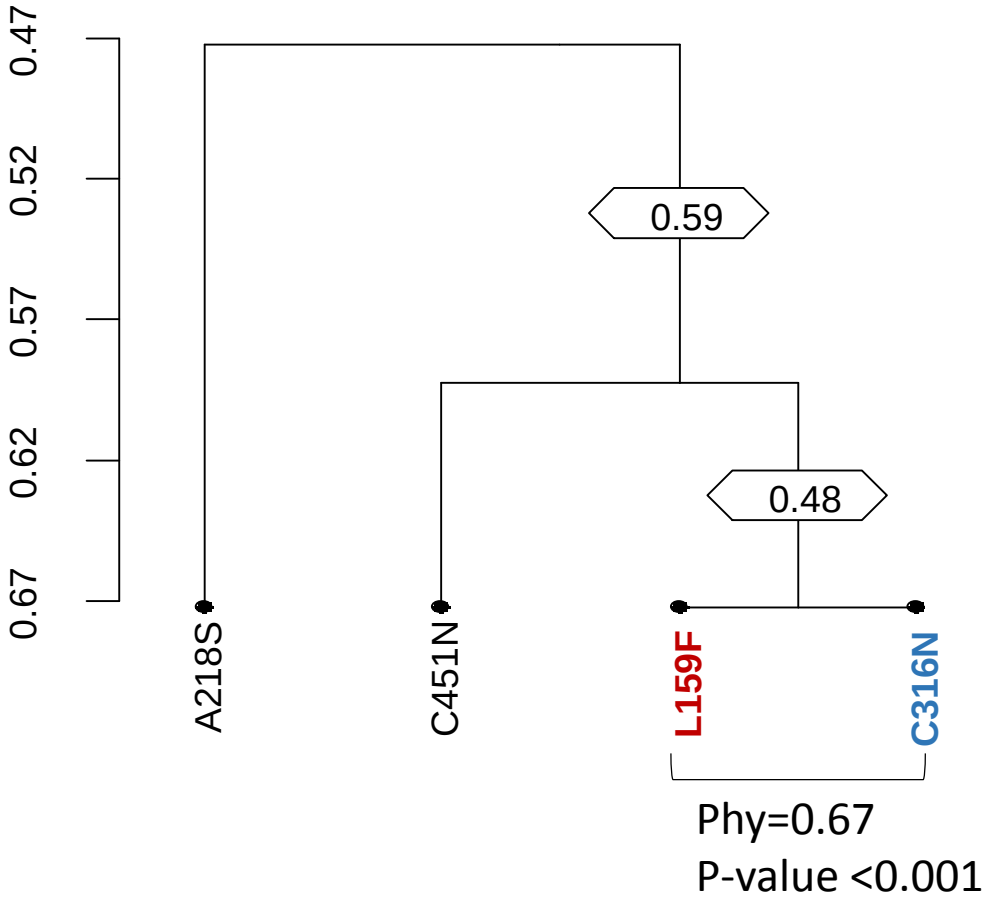
Gane EJ et al., AASLD 2015

* Our data

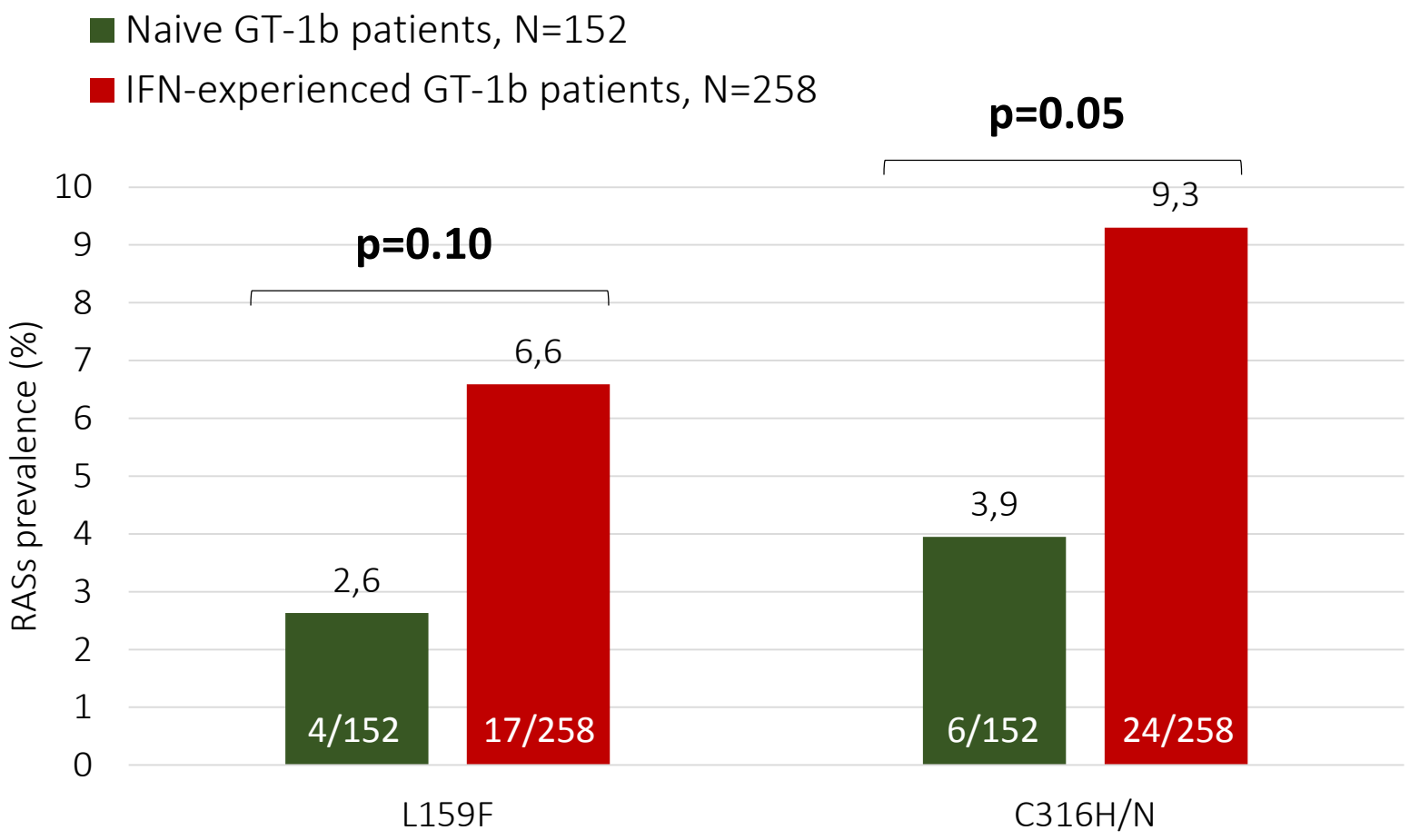
L159F and C316N SOF-RASs have high prevalence and are ofter associated in GT-1b NS5B-naive patients



* C316N showed a fold-change of 5 for dasabuvir, and it was found in dasabuvir-failing patients (Kati W. Et al., AAC, 2015; Zeuzem NEJM, 2014; Sarrazzin 2015 HCV J of Hepat).

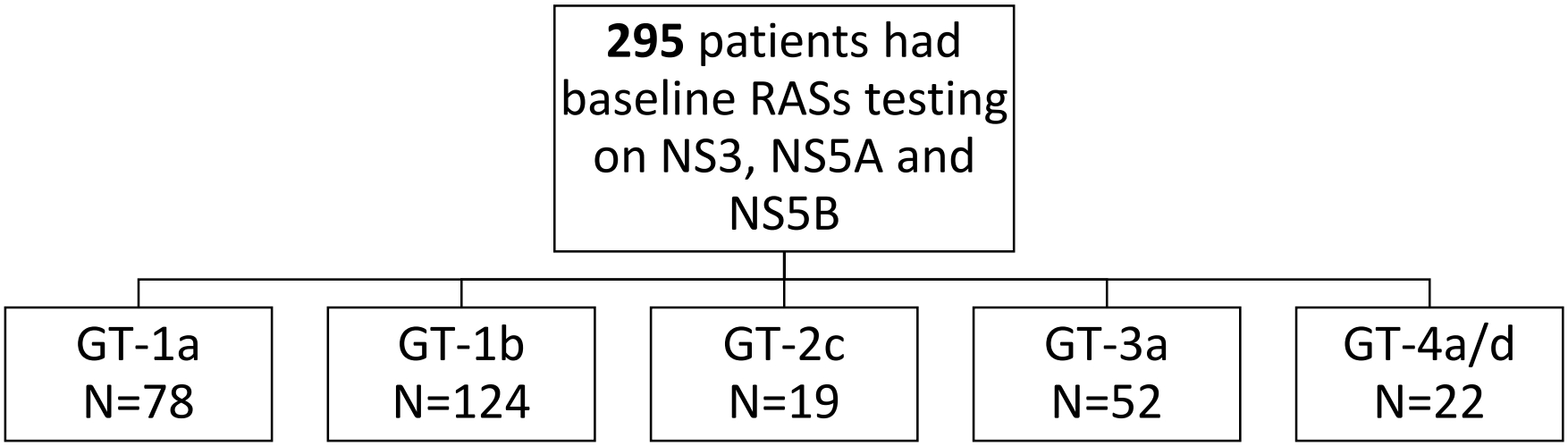


Natural NS5B RASs L159F and C316N were more frequently detected in IFN-experienced vs. naive GT-1b patients

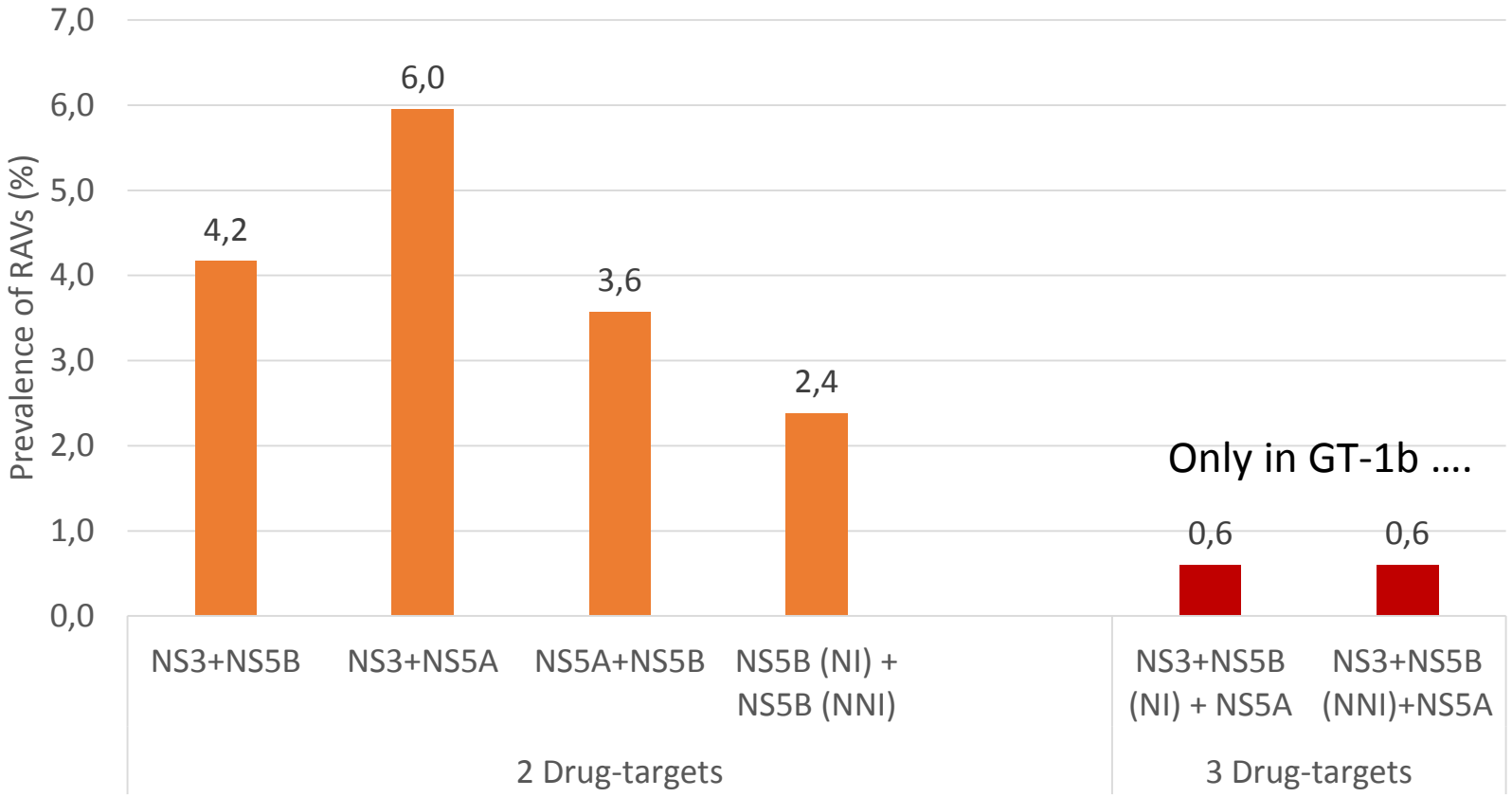


P-value was calculated by Fisher exact test

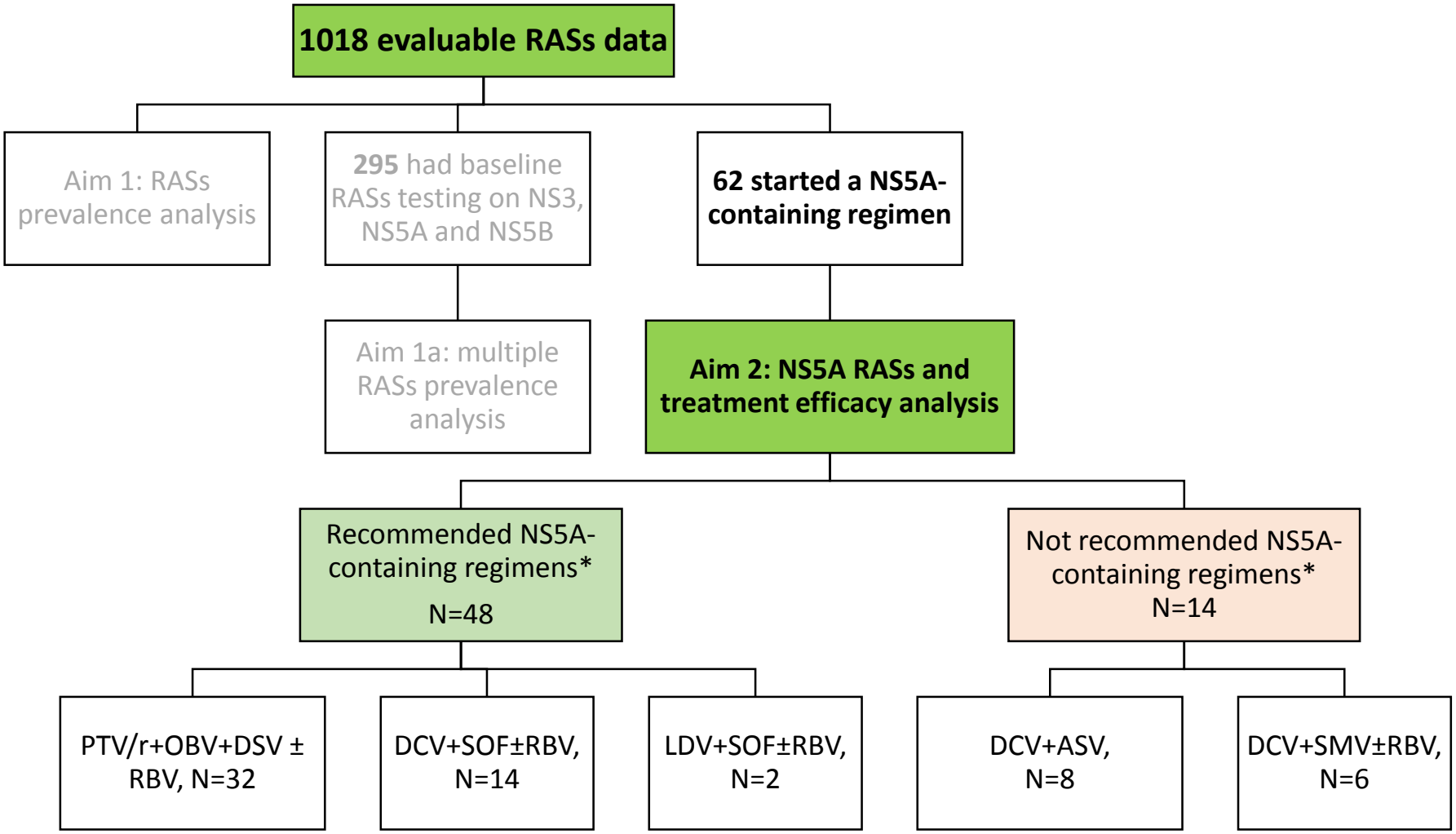
Aim 1a: Prevalence of NS3, NS5A, and NS5B RASs



8.5% of patients tested on all 3 genes showed multiple RASs on multiple drug-targets, particularly in GT-1 and 4



AIM 2: Patients



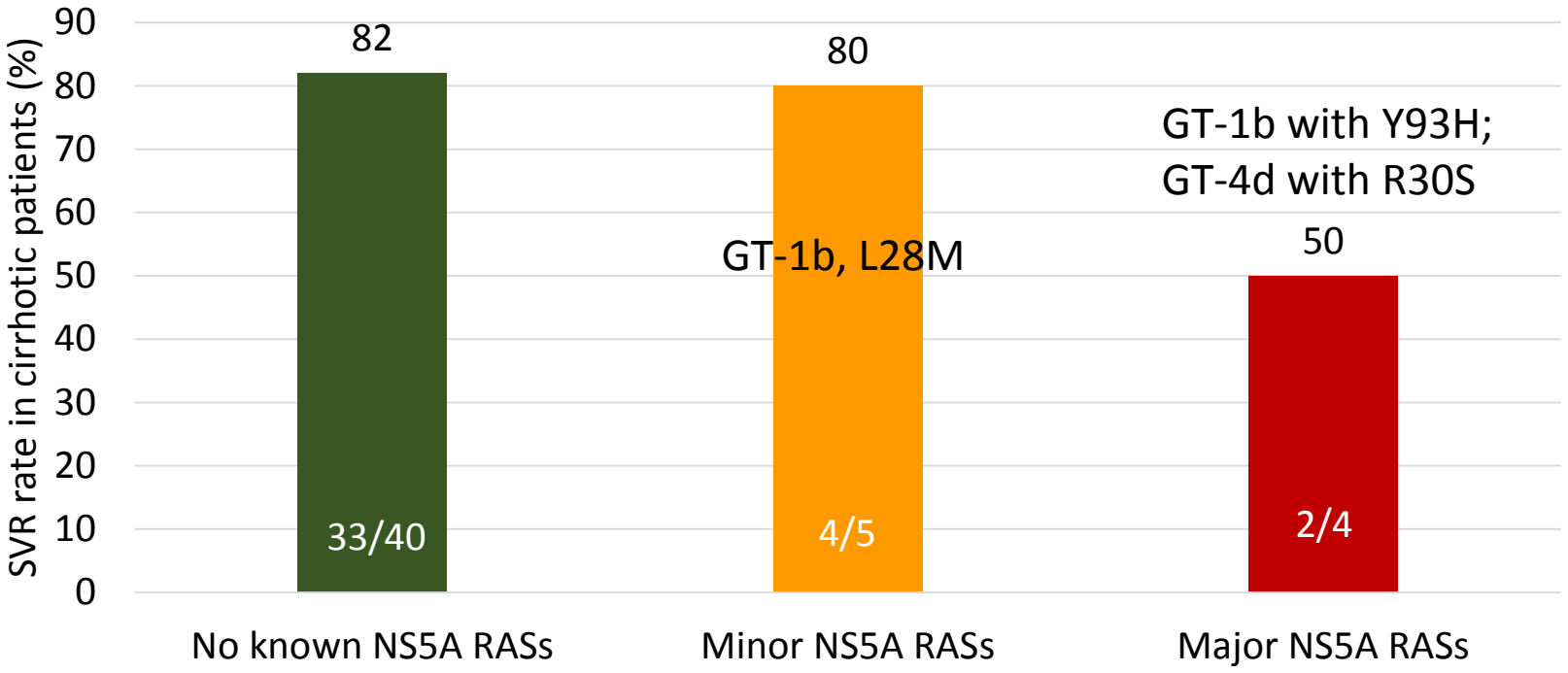
* Recommended regimens according to EASL and AASLD guidelines 2015.

62 patients tested for natural NS5A RASs were treated with an NS5A-inhibitor

No Cirrhosis	Recommended	Not recommended
	SVR (n=10)	SVR (n=3)
No natural RASs	7	2
Natural RASs	3	1

All 13 **non-cirrhotic** patients reached SVR, regardless minor RASs presence (R30Q, L31M, P58S, A92T).

In cirrhotic patients, SVR rates vary according to RASs presence …



Cirrhotic patients with major NS5A RASs experienced virological failure when treated with suboptimal regimens

Both failing patients with major NS5A RASs were treated with suboptimal regimens (DCV+ASU and DCV+SIM, **without ribavirin**). On the contrary, 2 patients with major NS5A RASs at baseline (GT-1b with Y93H and GT-1a with Q30R) reached SVR after 24 weeks of treatment with DCV+SOF**+RBV** or 3D**+RBV**, respectively.

Cirrhosis	**Recommended (n=38)**		**Not recommended (n=11)**	
	SVR (n=34)	**Failure (n=4)**	**SVR (n=5)**	**Failure (n=6)**
No natural RASs	29 (85.3)	3 (75.0)	4 (80.0)	4 (66.7)
Natural RASs	5 (14.7)	1 (27.0)	1 (20.0)	2 (33.3)
Minor/potential RASs	*3*	*1#*	*1*	
*Major RASs**	*2*			*2*

#GT-1b pt, previous failure to PI triple therapy, had L28M at baseline, and failed with L28M+Y93H.
*Y93H (N=2), R30S, Q30R

Conclusions

- Natural RASs are common across all HCV-genotypes in Italy, and 8.5% of patients presented double-class RASs.
- The putative NS5B SOF-RAS L159F reach 12% prevalence in GT-1b patients.
- NS5A RASs associated with substantial reduction of NS5A inhibitors activity are frequently detected.
- Major natural NS5A-RASs may affect treatment outcome in cirrhotic patients, when treated with suboptimal regimens (short duration and/or without ribavirin).

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