# EVIDENCE OF DISTINCT PHENOTYPES OF HCV SUBTYUPES 1A AND 1B CONFERRING RESISTANCE TO DIRECT-ANTIVIRAL AGENTS IN ISOLATES FROM BRAZILIAN THERAPY-NAIVE PATIENTS

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## Introduction

Natural dominant resistance mutations against direct antiviral agents (DAAs) for hepatitis C virus (HCV) may represent an important factor that limits the effectiveness of therapy. Analysis of viral sequences from different geographical regions may show significant differences in the frequencies of resistance mutations to DAAs.

## Objective

The aim of this study was to investigate non-structural genes mutations associated with resistance to DAAs in viral isolates circulating in Brazil

## Methods

A total of 390 sequences from therapy-naive Brazilian patients chronically infected with HCV genotype 1 were studied. Viral RNA was extracted, and the region encompassing the non-structural genes of NS3/4A, NS4B, NS5A and NS5B were RT-PCR amplified and sequenced.

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References:

[1] McPhee F, Hernandez D, Yu F, Ueland J, Monikowski A, Carifa A, et al. Resistance analysis of hepatitis C virus genotype 1 prior treatment null responders receiving daclatasvir and asunaprevir. **Hepatology** 2013; 58(3):902-11.

[2] Walker J, Crosby R, Wang A et al. Preclinical characterization of GSK2336805, a novel inhibitor of hepatitis C virus replication that selects for resistance in NS5A. Antimicrob Agents Chemother 2014; 58: 38–47.

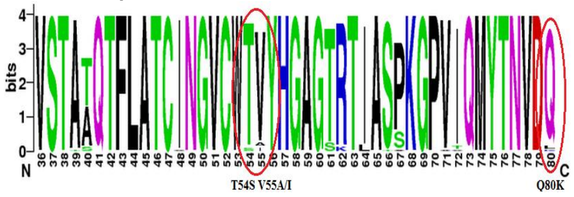
[3] Zeuzem S, Buggisch P, Agarwal K, Marcellin P, Sereni D, Klinker H, et al. The protease inhibitor, GS-9256, and non-nucleoside polymerase inhibitor tegobuvir alone, with ribavirin, or pegylated interferon plus ribavirin in hepatitis C. **Hepatology** 2012; 55(3):749-58.

[4] Tong X, Le Pogam S, Li L, Haines K, Piso K, Baronas V, et al. In vivo emergence of a novel mutant L159F/L320F in the NS5B polymerase confers low-level resistance to the HCV polymerase inhibitors mericitabine and sofosbuvir. **J Infect Dis** 2014; 209(5):668-75.

## Results

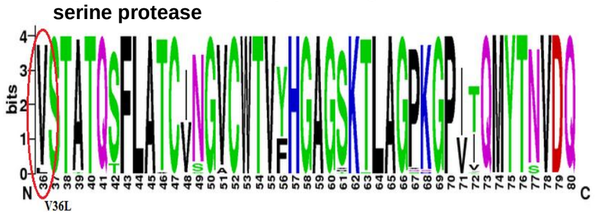
### NS3/4A protease

**Fig 1-a: Amino acid sequence alignment of the HCV-1a serine protease**




In HCV-1a, a T54S variation was found in two sequences (4,16%); at position 55, the variant V55I was found in 4,16% and other 4,16% present the V55A variantion. At position 80, the mutation Q80K which confers resistance to simeprevir was found in only 2,08% of HCV-1a Brazilian sequences.

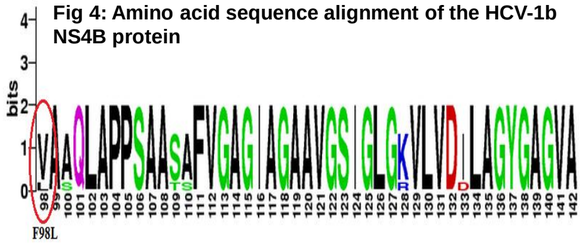
**Fig 1-b: Amino acid sequence alignment of the HCV-1b serine protease**




In HCV-1b, the V36L variation was found in three isolates (5,6%)

### NS4B protein

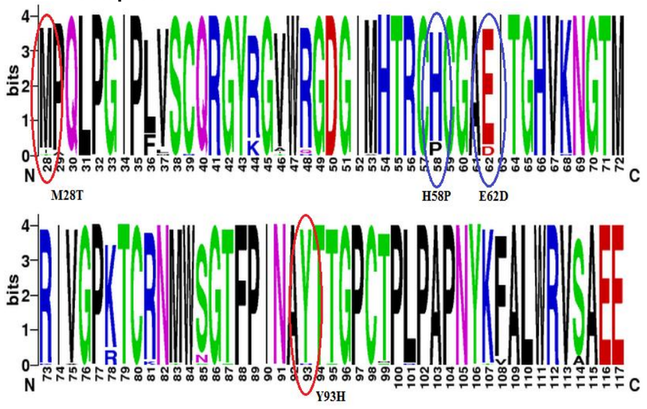
**Fig 4: Amino acid sequence alignment of the HCV-1b NS4B protein**




Only one HCV-1b isolate presented in the dominant viral population the F98L variant which confers resistance to PTC725, AP80978 and sylibin.

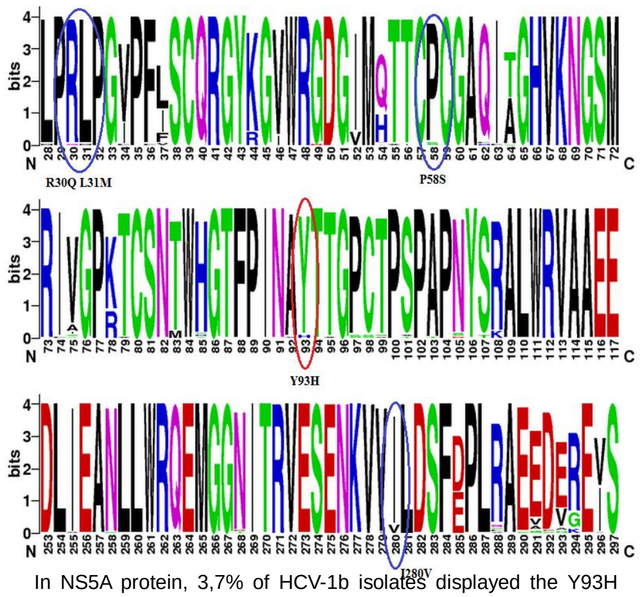
### NS5A protein

**Fig 2-a: Amino acid sequence alignment of the HCV-1a NS5a protein**




In NS5A protein, 3,85% of HCV-1a isolates present the M28T and Y93H mutations, which confers resistance to daclatasvir inhibitor and 13,6% present the secondary substitution H58P, E62D or E62D-H58P, which enhance primary resistances and could influence the emergence of resistant variants with possible consequences for clinical outcome [1].

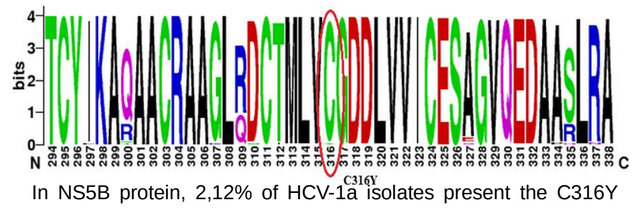
**Fig 2-b: Amino acid sequence alignment of the HCV-1b NS5a protein**




In NS5A protein, 3,7% of HCV-1b isolates displayed the Y93H and 14.8% exhibited the secondary mutations R30Q, L31M, P58S and I280V [2].

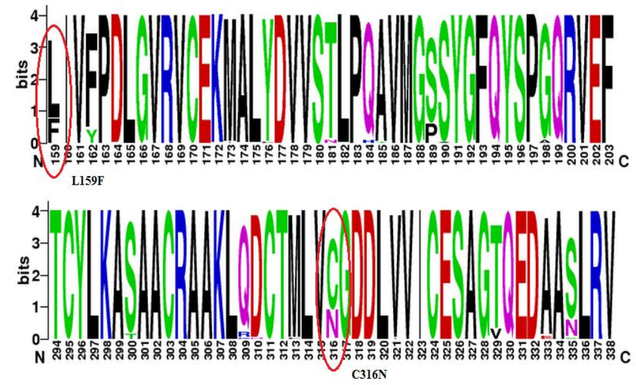
### NS5B protein

**Fig 3-a: Amino acid sequence alignment of the HCV-1a NS5B polymerase**




In NS5B protein, 2,12% of HCV-1a isolates present the C316Y variation, associated with resistance to ABT-033, ABT-072 and tegobuvir [3].

**Fig 3-b: Amino acid sequence alignment of the HCV-1b NS5B polymerase**




In NS5B protein, 29% presented the C316N variation (associated with resistance to ABT-033, ABT-072 and tegobuvir [3] and 25% of HCV-1b sequences present de L159F variation (associated with resistance to mericitabine and sofosbuvir [4]).

## Conclusion

The data on natural polymorphisms of the HCV non-structural regions obtained in the present study showed that mutations conferring resistance to DAAs are present in isolates from Brazilian therapy-naive patients with chronic HCV infection. Information regarding sequence variations may be important for choosing the best therapeutic approaches using the DAAs in different regions of the world.