GENOMIC CHARACTERIZATION OF HIV-1 PROVIRAL QUASISPECIES OF PATIENTS UNDERGOING FIRST-LINE THERAPY BY NEXT-GENERATION SEQUENCING

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INTRODUCION

Increased access to highly active antiretroviral therapy (HAART) by individuals infected with the human immunodeficiency virus (HIV) has become a reality worldwide. In the face of the 90-90-90 challenge proposed by UNAIDS, rates of diagnosis, treatment and therapeutic success are aimed at 90%. In this context, several countries, such as Brazil, established free access to antiretroviral therapy (ART) to all HIV+ individuals, currently covering more than half of the infected individuals (Figure 1).

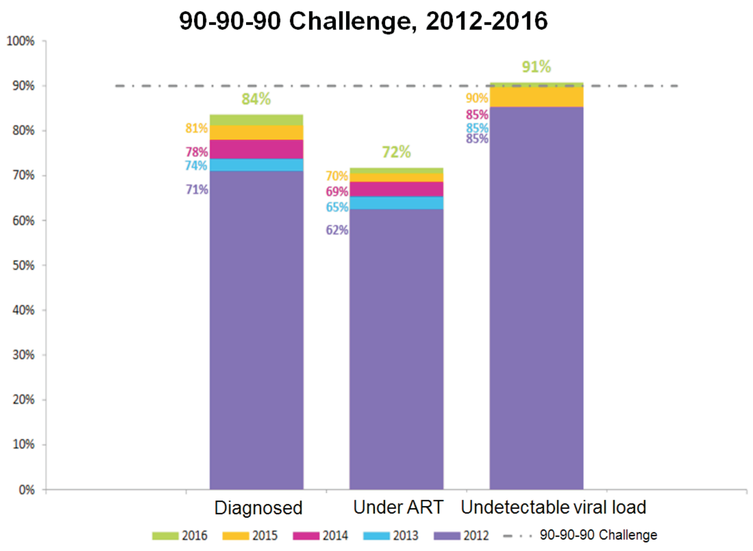

Figure 1. Brazilian progress of 90-90-90 challenge. Cascade of continuous care from 2012 to 2016. Source: MS/SVS/Departamento de DST, Aids e Hepatites Virais/Sistema de Controle de Exames Laboratoriais (Siscel) e Sistema de Controle Logístico de Medicamentos (Siclom) (MINISTÉRIO DA SAÚDE, 2017)

HIV-1 has a remarkable genetic variability resulted from several mutational events, the lack of a proofreading function by reverse transcriptase enzyme and high rate of replication. In such context of continuous intrahost variability, several highly related viral variants, called quasispecies, are generated. The HIV quasispecies may influence viral persistence and pathogenicity, representing a challenge for treatment. However, the clinical relevance of minority variants and its implication on the evolution to therapeutic failure are still uncertain.

OBJECTIVES

Thus, in this study we determined the archived proviral sequences, the viral subtype and the ARV-resistance mutations of a Brazilian cohort of HIV+ patients with undetectable viral load submitted to HAART as first-line treatment option through next-generation sequencing (NGS) to obtain HIV near full-lenght proviral genome (NFLG).

METHODOLOGY

This study included 32 patients from Hospital de Ipanema, RJ and 12 from HUCFF-UFRJ, RJ..

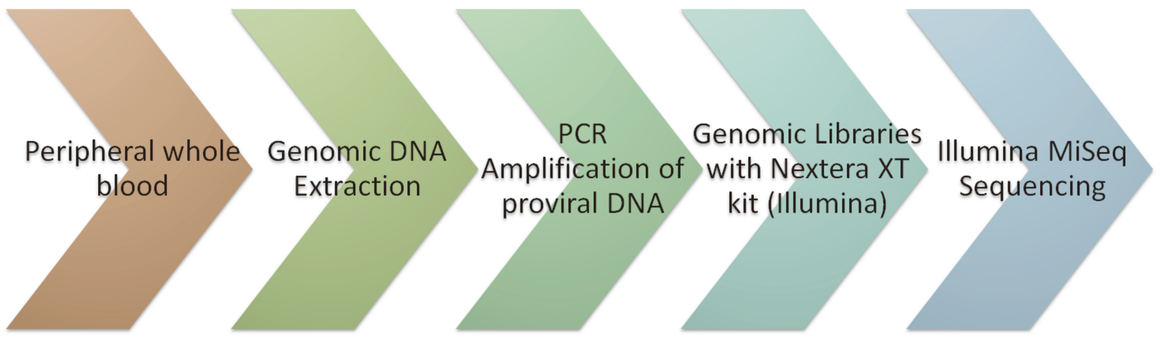

Figure 2. Representation of the methodology to be developed in the study.

The analyses were carried out in Geneious, where the reads were used in assemblies with reference. From the consensus sequence extracted of each sample, it was possible to define the infecting HIV subtype through phylogenetic analysis. Viral tropism was determined using the Geno2Pheno algorithm. The presence/frequency of variants resistant to antiretrovirals was determined based on the IAS consensus and the Stanford database.

RESULTS

In total, 32 samples were successfully sequenced (11 NFLG consensus sequences were obtained). Phylogenetic analyses indicated the predominance of subtype B (81%, 26/32) and viruses with CCR5 tropism (84%, 25/32). Of these, 23 patients had at least one mutation able to confer resistance to ARVs.

Table 1. Clinical and epidemiological characteristics of HIV + patients included in the study.

Characteristics	Hospital Federal de Ipanema (n=32)	HUCFF- UFRJ (n=12)
Men (%)	24 (75%)	8 (67%)
Age (anos) (Median ± SD)	40 ± 12.3	43.5 - 9.7
T-CD4 cell count median (cells/mm^3; IQR_{50})	712.5 (606.5-856)	1117[1]
T-CD8 cell count median (cells/mm^3; IQR_{50})	657.5 (529-1,047.25)	ND
Median time for HIV diagnosis (years; IQR_{50})	4.7 (3.9-6.5)	13.3 (7.7-14.8)
Median time from HIV diagnosis to the start of treatment (years; IQR_{50})	1.2 (0.6-2.8)[2]	1.7 (0.1-5.3)
Median time of ARV treatment (years; IQR_{50})	3.1 (2.4-3.9)	9.34 (4.6-11.4)

.[1] Information available for only two patients (1201 and 1033 cels / mm3),.[2] Data based on 31 patients; SD, standart deviation; IQR, interquartile range

Table 2. Drug resistance mutations (DRM) found at our cohort.

Patient	Drug resistance mutations (coverage, frequency, genomic region)	Patient	Drug resistance mutations (coverage, frequency, genomic region)
HI-1	M184V (11,979; 2.0%, RT), S147G (8,945; 1.1%, IN)	HI-19	A376S (6,663; 99.9%, RT)
HI-2	R263K (2,201; 6.2%, IN)	HI-21	L210W (12,583; 100.0%, RT)
HI-5	I47V (3,589; 10.3%, PR)	HI-22	A376S* (1,082; 94.9%, RT)
HI-6	A376S (8,471; 96.0%, RT)	HI-23	T97A (4,113; 2.1%, IN)
HI-8	E399D (2,701; 99.2%, RT)	HI-28	M41L (5,791; 99.5%, RT); D67N (6,465; 72.9%,RT); K70R (6,475; 77.2%, RT); T215Y (9,219; 38.7%, RT), E399D (2,051; 99.9%, RT)
HI-11	D30N (8,012; 2.8%, PR),M46I (8,639; 1.9% PR,), M41L (5,444; 99.8%, RT), T369V (9,726; 39.6%, RT), E399G (8,334; 4.9%, RT)	HI-29	E399D (5,209; 100.0%, RT), T66I (7,444; 31.0%, IN)
HI-13	E138K (4,909; 1.3%, RT), V38A (2,151; 3.0%, ENV)	HI-31	M184V (2,708; 1.3%, RT)
HI-14	R263K* (1,776; 11.0%, IN)	HI-32	V179D (7,146; 99.3%, RT); M184V (7,127; 8.0%, RT)
HI-15	E399G (14,872; 1.5%, RT)	HU-2	M41L (6,497; 99,1%, RT); M184I (10,197; 15,2%, RT); M230I (9,335; 16%, RT), E157Q (3029; 99,8%, IN)
HI-16	D30N (4,574; 49.3%, PR), M46I (4,378; 45.6%, PR)	HU-9	M46I (1,766; 38,6%, PR); I54T (1,708; 1,3%, PR), M184I (6,622; 20,1%, RT)
HI-17	M41L (3,070; 30,3%, RT)	HU-10	L74I (3,933; 100%, RT)
HI-18	A62V (4,116; 1.0%, RT)	-	-

PRO: protease; RT: reverse transcriptase; IN: integrase

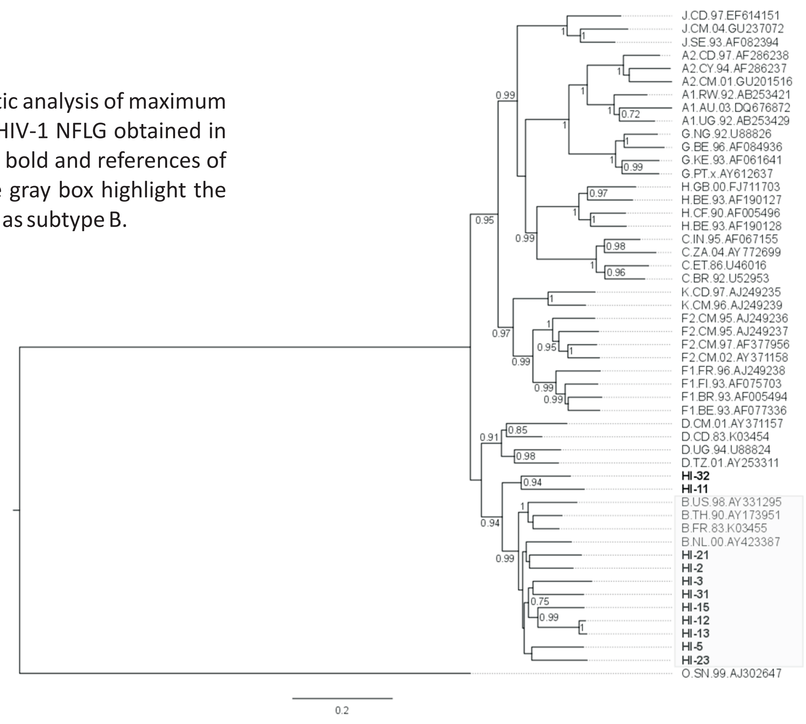

Figure 3. Phylogenetic analysis of maximum likelihood with the HIV-1 NFLG obtained in this study (shown in bold and references of HIV-1 subtypes. The gray box highlight the sequences classified as subtype B.

CONCLUSION

Together, these data highlight the importance to monitoring minority variants associated with antiretroviral resistance and its clinical impact, in order to assist future therapeutic switches, to complement the studies of proviral archived diversity and to contribute to the success of ART.

APOIO: Rede Hospitalar Federal no Rio de Janeiro; inct instituto nacional de ciência e tecnologia para controle do câncer

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

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