Longitudinal analysis on the evolution and genetic diversity of HIV-1 proviral quasispecies of patients undergoing HAART

BRUNNA M. ALVES[1], ORNELLA BOTELHO[1], ISABEL M. PRELLWITZ[1],HECTOR N. SEUÁNEZ[2], MARIANNE M. GARRIDO[3], SAYONARA ROCHA[3], MARCELO A. SOARES[1], ESMERALDA A. SOARES[1]

[1]Programa de Oncovirologia, Instituto Nacional de Câncer,Rio de Janeiro, Brazil;

[2]Programa de Genética, Instituto Nacional de Câncer, Rio de Janeiro, Brazil;

[3] Hospital Federal de Ipanema, Rio de Janeiro, Brazil

Introduction

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).

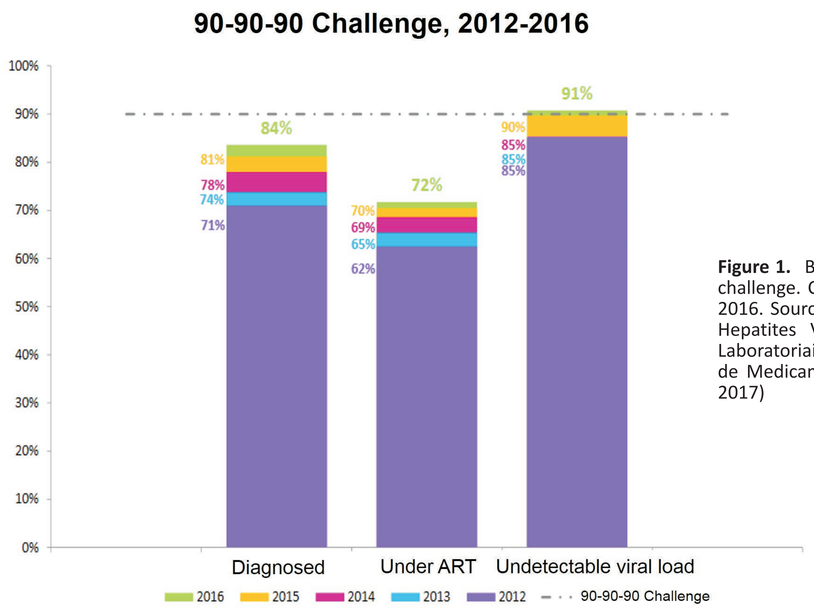

Figure 1. Brazilian progress of the 90-90-90 UNAIDS challenge. Cascade of continuous care from 2012 to 2016. Source: MS/SVS/Departamento de DST, Aids e Hepatites Virais/Sistema de Controle de Exames Laboratoriais (Siscel) e Sistema de Controle Logístico de Medicamentos (Siclom) (MINISTÉRIO DA SAÚDE,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

HIV-1 has a remarkable genetic variability resulted from mutational events, the lack of a proofreading function by the viral reverse transcriptase enzyme and a 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.

In this context, the determination of the proviral sequences from a well-studied Brazilian cohort of patients with chronic HIV will strongly support the understanding of the association between minority resistance variants and the occurrence of therapeutic failure, besides allowing longitudinal analysis of viral diversity. Our first study was able to evidence a high prevalence of drug resistance mutations in this cohort, despite the therapeutic success achieved by its carriers (ALVES et al., 2017).

Objectives

In this study we aim to determine the near full-length proviral genomes (NFLG) of the archived proviral sequences from a well characterized chronic HIV+ Brazilian cohort and to compare them with HIV data obtained from the same patients collected two years ago.

Methodology

This study intends to include new samples of the 32 patients attending the Hospital de Ipanema, in Rio de Janeiro, which were previously included and analyzed by our group.

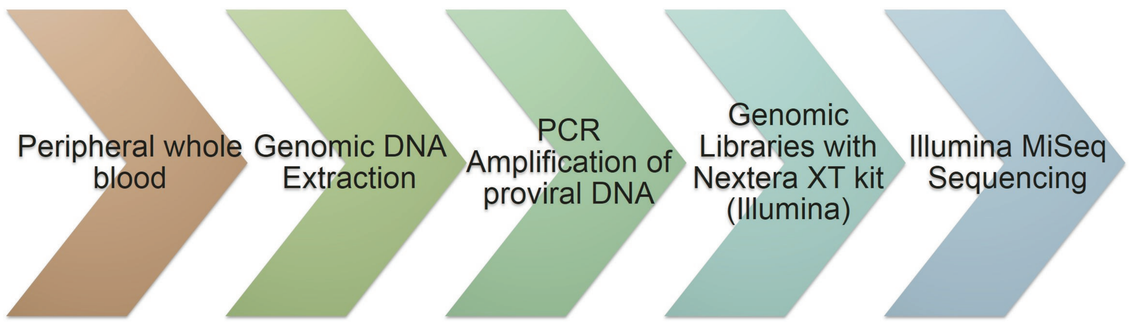

Figure 2. Representation of the methodology to be developed in the study.

The analyses of the files obtained on the Illumina MiSeq platform will be carried out with Geneious, where the reads will be used in the assemblies with reference. We will define the infecting HIV subtype through phylogenetic analyses. The presence/frequency of variants resistant to antiretroviral drugs will be determined based on the International Aintiviral Society - USA consensus and on the Stanford HIV drug resistance database.

Table 1. Clinical and epidemiological characteristics of HIV$^+$ patients to be included in the study. Data from the first collection point, approximately two years ago.

Characteristics	Hospital Federal de Ipanema (n=32)
Men (%)	24 (75%)
Age (years) (Median ± SD)	40 ± 12.3
Median T-CD4 cell count (cells/mm^3; IQR_{50})	712.5 (606.5-856)
Median T-CD8 cell count (cells/mm^3; IQR_{50})	657.5 (529-1,047.25)
Median time since HIV diagnosis (years; IQR_{50})	4.7 (3.9-6.5)
Median time from HIV diagnosis to start of treatment (years; IQR_{50})	1.2 (0.6-2.8)[1]
Median time of ARV treatment (years; IQR_{50})	3.1 (2.4-3.9)

[1]Data based on 31 patients; SD, standart deviation; IQR, interquartile range

Results

Currently, 11 of the 32 patients were already recruited during their regular clinical follow-up visits and had a blood sample collected. All patients maintained their therapeutic regimens and undetectable viral load. Of these, four had at least two fragments of HIV genome successfully amplified by PCR in duplicate. These samples were sequenced in a MiSeq platform.

Table 2. Comparison between drug resistance mutations (DRM) found at the first collection point, approximately two years ago, and at the second collection.

Patient	Drug resistance mutations at the 1st time point (coverage, frequency, genomic region)	Drug resistance mutations at the 2nd time point (coverage, frequency, genomic region)
6*	A376S (8,471, 96%, RT)	E138A (12,112, 31.8%, RT); A376S (14,406, 65.2%, RT)
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)	**M184V** (14,171, 1.8%, RT)
19*	A376S (6,663; 99.9%, RT)	**M230I** (9,287, 1.1%, RT); A376S (18,795, 99.7%, RT);
20	-[1]	-[2]

*NFLG obtained; PR: protease; RT: reverse transcriptase; bold represent DRMs associated with the ARTs in use;[1] except the env region; [2] except the protease region;

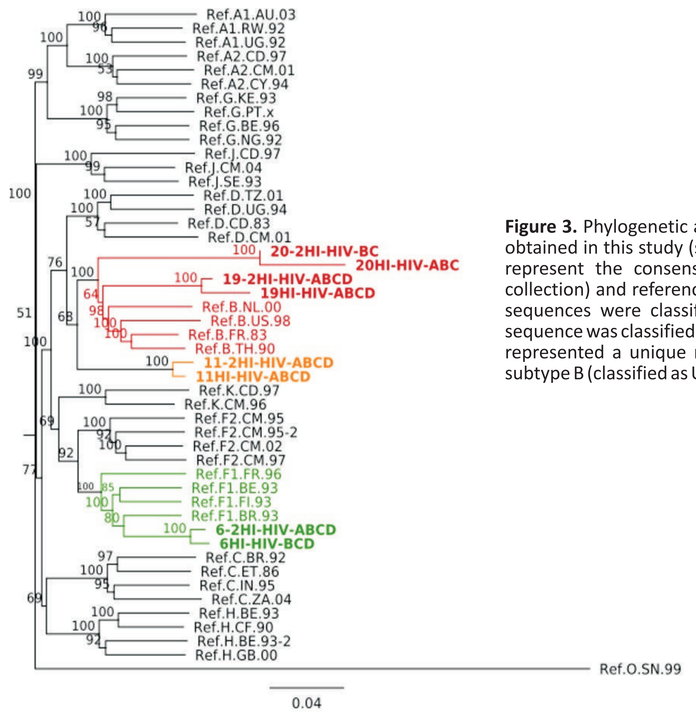

Figure 3. Phylogenetic analysis of the HIV-1 genomes obtained in this study (shown in bold, where the "-2" represent the consensus sequence of the second collection) and references of HIV-1 subtypes. The red sequences were classified as subtype B, the green sequence was classified as F1 and the orange sequence represented a unique recombinant form comprising subtype B (classified as URF_BF1 by simplot analysis).

Conclusions & Perspectives

- With the increased access to ARV therapy and the high rate of therapeutic success, it is critical to analyze the presence of ARV-resistant minority subpopulations. This study will enable a better understanding of the association between minority resistance variants and therapeutic failure.
- In addition, it will allow the longitudinal analysis of viral diversity, since in our first study we evidenced a high prevalence of drug resistance mutations in this cohort, despite the therapeutic success achieved by their carriers.
- Thus, it is expected that the study of HIV-1 variability of these patients may complement studies of viral diversity and therapeutic response, and may contribute to future therapeutic exchanges and maintenance of the success of HAART.

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

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

INCA SUS MINISTÉRIO DA SAÚDE