19-21 GIUGNO ROMA
UNIVERSITA' CATTOLICA DEL SACRO CUORE CENTRO CONGRESSI EUROPA
RESEARCH and CARE: FROM BENCH, TO BEDSIDE, TO COMMUNITY
Promosso da SIMIT
16° CONGRESSO NAZIONALE ICAR Italian Conference on AIDS and Antiviral Research
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POSTER 4 YOU ICAR 2024

Comparison of Sanger sequencing and amplicon-based NGS approaches for the detection of HIV-1 drug resistance mutations

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Introduction

- Huge progresses have been made in the field of antiretroviral therapies for HIV-1, but the development of drug resistance continue to be a major problem for a subset of infected individuals.
- The standard method to test HIV-1 drug resistance is Sanger sequencing, but the availability of commercial next-generation sequencing (NGS) kits will probably lead to its replacement to test HIV-1 drug resistance.

Study Design

- We compared the ability of detecting drug resistance mutations among three different sequencing methods on a panel of 28 clinical isolates.
- The 28 plasma samples had at least one resistance mutation on PCR amplicons including PR, RT (aminoacids 1-400) and IN coding regions.
- We performed Sanger sequencing and two different PCR amplicon-based NGS approaches: one using home-made amplicons and one based on the commercial CE IVD "HIV-1 solution v2" kit by Arrow Diagnostics.

Methods

- In the homemade NGS approach, the amplicons used for Sanger sequencing were also used for tagmentation, indexing and library preparation (Illumina DNA Prep kit). To perform NGS according to the "HIV-1 solution v2" kit, viral RNA of each sample was amplified based on the manufacturer's instructions. Both NGS libraries were loaded on Nano 2x250 bp v2 flowcells and run on a MiSeq platform by Illumina.
- FASTA files from Sanger sequencing were analyzed through the HIVdb Program v9.6 (HIVdb Stanford), while FASTQ files from NGS were analyzed through the HIVdb-NGS (beta) program (HIVdb Stanford) using ≥100 as minimum read depth and 5% as mutation detection threshold. Viral subtype on consensus sequences for each sample was determined through the COMET HIV-1 tool.

Table 1. Comparison of additional mutations identified among the three sequencing methods with their relative frequency

Sample	Sanger	NGS: homemade NGS	NGS: HIV-1 solution v2
155826		**PR:** I50IV (29%) **NRTI:** V75VI (6.5%)	**PR:** I50IV (24%)
155974		**NRTI:** K219KQ (7.1%) **NNRTI:** V106VI (10%)	-
155979	**NRTI:** M41L	**PR:** I54IT (10%) **NRTI:** M41L **IN:** A128AT (6.2%)	-
156436		**NNRTI:** K238KT (7.8%) - N348NI (6.8%)	-
156471	**NRTI:** D67N, K219KE		**NRTI:** E44EA (15%) - D67DN (19%)
156493		**NNRTI:** V179VID (I 85%, D 7%) - Y181YC (34%)	**NNRTI:** Y181YC (25%)
156570		**PR:** K20KT (14%)	**PR:** K20KT (9.7%)
156592		**NNRTI:** K101KE (40%)	**NNRTI:** K101KE (14%)
156669		**IN:** E157EQ (7.1%)	**NNRTI:** Y181YC (5,8%) **IN:** E157EQ (11%)
156813		**PR:** G73GS (7.3%)	**NNRTI:** K103KN (7%)
156817		**NNRTI:** V106VI (5.4%)	-
156835		**NRTI:** M41ML (30%)	**PR:** M46MI (5.2%) NRTI: M41ML (19%)
156880			**NNRTI:** V179VIT (I: 12%; T: 5.6%)
157347		**NNRTI:** V106VI (16%) **IN:** T97TA (6.2%)	**NNRTI:** V106VI (15%)
157312		**NRTI:** S68SG (52%) - T69Δ **NNRTI:** Y181YC (55%)	**NRTI:** S68SG (7.8%) **NNRTI:** Y181YC (68%)

PI: Protease Inhibitors; NRTI: Nucleosidic Reverse Transcriptase Inhibitors; NNRTI: Non Nucleosidic Reverse Transcriptase Inhibitors; IN: Integrase Inhibitors

Table 2. Cases with different prediction of drug susceptibility among the three sequencing approaches

Sample	Antiretroviral	Sanger	NGS: homemade NGS	NGS: HIV-1 solution v2
155974	DOR - RPV - ETR	S	PLLR	S
155974	ABC - TAF	I	I	LLR
	DOR	LLR	LLR	PLLR
156471	TAF	R	I	R
156493	DOR	PLLR	I	I
	RPV - ETR	S	I	I
156592	DOR	S	LLR	I
	RPV	I	R	R
	ETR - EFV	PLLR	LLR	I
	NVP	PLLR	I	I
156669	RPV - ETR	S	S	I
156813	ATV	S	PLLR	S
156817	DOR - RPV - ETR	S	PLLR	S
156835	ATV - LPV	S	S	PLLR
157347	DOR	LLR	I	I
	ETR	I	I	R
157312	3TC/FTC	LLR	I	LLR
	DOR	LLR	I	I
	RPV	LLR	R	R
	ETR - EFV	LLR	I	I
	NVP	I	R	R

ATV: atazanavir; LPV: lopinavir; DRV: darunavir; ABC: abacavir; TAF: tenofovir; DOR: doravirine; RPV: rilpivirine; ETR: etravirine; EFV: efavirenz; NVP: nevirapine; 3TC/FTC: lamuvidine/emitricitabine S: susceptible; PLLR: "potential low-level resistance; LLR: "Low-level resistance; I: intermediate resistance; R: resistance

Results

- The 28 plasma samples had a median viral load of 4.8 [IQR 4.4-5.3] $\log_{10}$ HIV-1 RNA copies/mL. Subtype B was identified in 17 (61%) cases, CRF02_AG in 4 (14%) and F1 in 3 (11%).
- Comparable median read depth was obtained through the homemade NGS and the "HIV-1 Solution v2" kit (2189 [IQR 1842-7809] reads vs. 4634 [3056-6472] reads, p=0.143).
- Regions with coverage depth <100 reads were detected in 2/28 and 17/28 cases with homemade NGS and the HIV-1 Solution v2 kit, respectively. This occurred in 9 B and 8 non-B subtypes, mainly affecting codons 14-49 and 260-319 of RT, codons 1-75 and 201-288 of IN.
- NGS based methods globally identified additional mutations with respect to Sanger in 15/28 (54%) cases, with agreement between methods in 7 cases (**Table 1**). Resistance mutations identified only by NGS had a frequency <20% in all but 7 cases for the homemade NGS and in all but 3 cases for the "HIV-1 solution v2" kit (**Table 1**).
- NGS also showed a different prediction of drug susceptibility in 11/28 (39%) with respect to Sanger. This prediction was in agreement among the two NGS methods in 18/28 (64%) samples (**Table 2**).

Conclusion

NGS systems showed fair agreement to detect additional resistance mutations that were not identified through Sanger sequencing. However, the sensitivity of detection of minority mutations may be affected by low coverage issues that are frequently observed with the "HIV-1 Solution v2" kit.