HIV DRUG RESISTANCE ANALYSIS BY NEXT GENERATION SEQUENCING AMONG HIV INFECTED PREGNANT WOMEN FROM BUENOS AIRES, ARGENTINA

J.A. Sfalcin , A.G. Gomez , D. Cecchini , I. Zapiola , S. Fernandez Giuliano , L. Mammana , A. Seravalle , F.F. Fay , M.B. Bouzas

Centro de Diagnostico Medico de Alta Complejidad (CIBIC), Rosario, Argentina, Hospital Cosme Argerich, Buenos Aires, Argentina, Hospital de Infecciosas F. J. Muñiz, Buenos Aires, Argentina

Background

HIV-1 genotypic resistance testing is recommended in pregnant women to identify mutations associated with drug resistance, improving the selection of antiretroviral therapy (ART). Next Generation Sequencing (NGS) genotyping is able to detect minority variants (MVs) which cannot be detected by standard sequencing techniques as TRUGENE (Siemens). MVs have been shown to increase the risk of virological failure but its clinical use is still controversial. We aimed to evaluate the performance of NGS in a retrospective study from a cohort of HIV-infected pregnant women.

Methods and Material

Baseline plasma samples collected during 2008-2014 corresponding to 40 naive patients from a cohort of HIV-infected pregnant women were included in the present study. The median viral load was 4.2 log copies/mL [2.78, 5.38] and the median CD4 cell count was 390 cells/mm3 [30, 982]. All samples previously genotyped by TRUGENE HIV-1 Genotyping Kit were sequenced using a Public Health Agency of Canada (PHAC) protocol on a Miseq sequencer (Illumina). Bioinformatics analysis was performed using the HyDRA software for different thresholds of sensibility (NGS 1% and NGS 20%). Resistance mutations were identified according to WHO guidelines (WHO-DRMs) and Stanford University major mutation lists (SU-DRMs).

Results

100% of sensitivity and specificity was observed between TRUGENE and NGS at 20% threshold. In addition, at NGS 1% MVs were detected. All of VMs were found at a low frequency (<5%) within the viral population.SU-TDRMs and WHO-TDRMs are shown in table 1. MVs increased the number of patients from 8 (20%) to 14 (35%) for WHO-TDRMs and from 10 (25%) to 17 (42.5%) for SU-TDRMs.The highest number of MVs was observed in the protease gene (table 2). MVs in the protease gene increased the number of patient from 1 (2.5%) to 6 (15%), increasing protease gene mutation frequency 500% (Table 2). Thirty one patients received ART; of them 96.7% received boosted protease inhibitor (PI)-based therapy, with an adequate virological response in 86.6% of them.

Table 1: Samples with drug resistance mutations according to Stanford University major mutations list (SU-TDMRs) and WHO guidelines mutation list (WHO-TDRMs).

SU-TDRMs							
SAMPLE ID	NGS 1% (FREQUENCY, %)			TRUGENE			ART
	PI	NRTI	NNRTI	PI	NRTI	NNRTI	
214463_S3	-	T215I(99,74)	-	-	T215I		3TC-AZT-SQV/R
219508_S4	-	-	G190A(99,78)	-	-	G190A	3TC-AZT-LPV/R
225212_S7	-	**D67G(1,28)**	-	-	-	-	3TC-AZT-SQV/R
225213_S8	**I85V(1,29), M46I(1,54)**	-	-	-	-	-	3TC-AZT-NVP
267312_S22	**L90M(2,27)**	-	**G190E(1,05)**, K103N(98,81)	-	-	K103N	3TC-AZT-LPV/R
268348_S23	**I50V(1,01)**	-	-	-	-	-	None
269554_S24	**I85V(1,0), M46I(1,3)**	-	-	-	-	-	3TC-AZT-LPV/R
271767_S25	-	M41L(79,8)	**G190S(1,25)**	-	M41L	-	3TC-AZT-LPV/R
287456_S35	-	-	K103N(98,57)	-	-	K103N	3TC-AZT-LPV/R
296464_S34	-	-	K103N(98,44)	-	-	K103N	NO RECIBIO
318024_S38	**I50V(1,03)**	-	-	-	-	-	3TC-AZT-LPV/R
322132_S39	V82A(99,71), L90M(99,45)	-	Y181I(99,59)	V82A, L90M	-	Y181I	ND
353362_S44	-	**M184I(1,02)**	-	-	-	-	3TC-AZT-LPV/R-RAL
357365_S45	-	-	K103N(97,78), P225H(93,83)	-	-	K103N, P225H	None
WHO-TDRMs							
SAMPLE ID	NGS 1% (FREQUENCY, %)			TRUGENE			ART
	PI	NRTI	NNRTI	PI	NRTI	NNRTI	
207260_S1	-	-	E138G(5,26)	-	-	-	3TC-AZT-SQV/R
210228_S2	-	-	E138A(99,04)	-	-	E138A	3TC-AZT-LPV/R
214463_S3	-	T215I(99,74)	-	-	T215I	-	3TC-AZT-SQV/R
219508_S4	-	-	G190A(99,78)	-	-	G190A	3TC-AZT-SQV/R
225212_S7	-	**D67G(1,28)**	-	-	-	-	3TC-AZT-SQV/R
225213_S8	**I85V(1,29), M46I(1,54)**	-	-	-	-	-	3TC-AZT-NVP
267312_S22	**L90M(2,27)**	-	G190E(1,05), K103N(98,81)	-	-	K103N	3TC-AZT-LPV/R
268348_S23	**I50V(1,01)**	-	-	-	-	-	None
269554_S24	**I85V(1,0), M46I(1,3)**	-	-	-	-	-	3TC-AZT-LPV/R
271767_S25	-	-	**G190S(1,25)**	-	M41L	-	3TC-AZT-LPV/R
282146_S30	-	-	E138A(75,08)	-	-	E138A	S/D
287456_S35	-	-	K103N(98,57), E138G(99,68), K238T (99,51)	-	-	K103N, E138G, K238T	3TC-AZT-LPV/R
296464_S34	-	-	K103N(98,44), E138K(51,97)	-	-	K103N, E138K	None
318024_S38	**I50V(1,03)**	-	-	-	-	-	3TC-AZT-LPV/R
322132_S39	V82A(99,71), L90M(99,45)	-	Y181I(99,59)	V82A, L90M	-	Y181I	S/D
353362_S44	-	**M184I(1,02)**	-	-	-	-	3TC-AZT-LPV/R-RAL
357365_S45	-	-	K103N(97,78)	-	-	K103N	None

Highlight cells are samples with MVs. Detected. All of the MVs were found at low frequency (<5%) within the viral population. ND: No data.

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Table 2: Number of patients with TDRMs detected by Trugene and NGS (1%).

DRUG AND MUTATION TYPE		TRUGENE (N, %)	NGS 1% (N, %)	INCREASE MUTATION FREQUENCY BY NGS (1%)
All	WHO-TDRM	8 (20)	14 (35)	+75%
All	SU-TDRM	10 (25)	17 (42,5)	+70%
IP	WHO-TDRM	1 (2,5)	6 (15)	+500%
IP	SU-TDRM	1 (2,5)	6 (15)	+500%
NRTI	WHO-TDRM	2 (5)	4 (10)	+100%
NRTI	SU-TDRM	2 (5)	3 (10)	+50%
NNRTI	WHO-TDRM	6 (15)	7 (17,5)	+17%
NNRTI	SU-TDRM	8 (20)	11 (27,5)	+37,5%

Highlight cells indicates the increased frequency mutation in the protease gene due to MVs detected.

Conclusions

- NGS increased the detection of resistance mutations, mostly in the protease gene.

- The mutations were present at a low frequency (< 5%) within the viral population and were not associated with virologic failure.

- Considering IP's high genetic barrier, detection of additional mutations by NGS may not predict an impact in the efficacy of boosted PIs-based ART on this population.

- Despite these considerations, NGS technology may improve drug resistance surveillance.

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