Added value of Ultra Deep Sequencing in patients with HIV-1 Transmitted Drug Resistance mutations in the Reverse Transcriptase

P-127

N Chueca[1], M Alvarez[1], V Guillot[1], J López-Bueno[1], C Pou[2], Salanueva I[3], R Boulmé[4], J Hernández-Quero [1], M Alvarez-Tejado[3], R Paredes[2], D Gonzalez [5], F García [1].

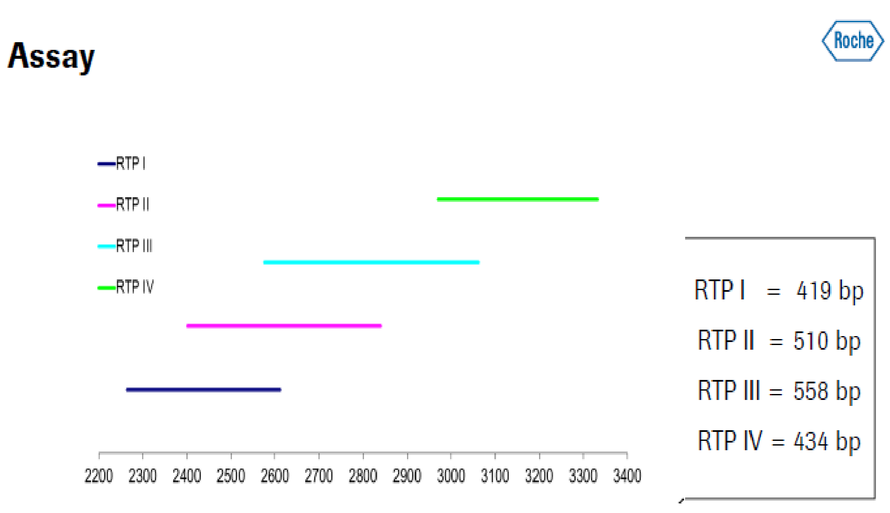
[1] Hospital Universitario San Cecilio, Granada, Spain; [2] IrsiCaixa AIDS Research Institute, Badalona, Spain; [3] Roche Diagnostics, Barcelona, Spain; [4] Advanced Biological Laboratories SA, Luxembourg, Luxembourg; [5] Advanced Biological Laboratories TE Spain SL, Barcelona, Spain

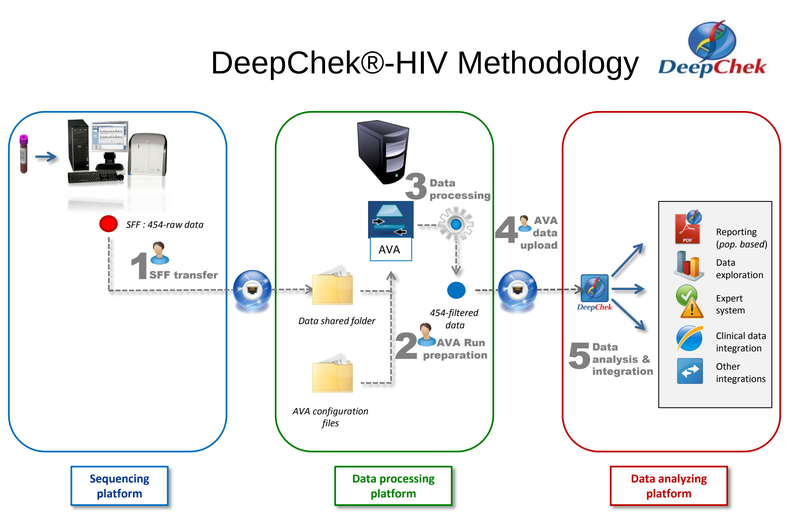
Aim

In this report we have investigated if additional mutations can be detected as viral minor mutants using the GS Junior System (Roche) in a background of Sanger detected HIV-1 transmitted drug resistance mutations (TDR) and how they can impact resistance interpretation.

Patients and Methods

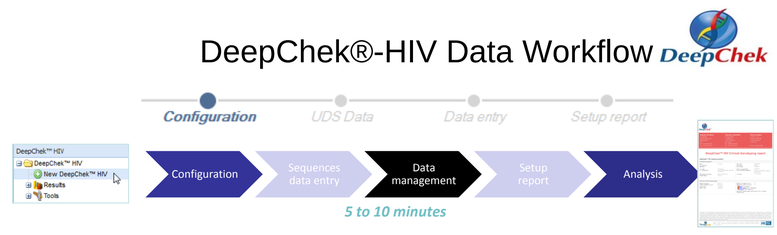
11 naïve patients with documented TDR by Sanger-Sequencing (Trugene, Siemens NAD) were retested using the 454 HIV Collaboration Initiative Primer Plates and GS Junior System from Roche, enabling ultra-deep sequencing (UDS) of HIV-1 reverse transcriptase and protease. The UDS 454 information were analyzed in the dedicated UDS DeepChek®-HIV (v1.1, CE-IVD marked, ABL-TherapyEdge SA) diagnostic software application, allowing analyzing in routine the frequency of detection and the mutational load for each minor variant. For interpretation of the resistance mutations, the Stanford algorithm was used. Only mutations detected over a 1% threshold were considered for resistance interpretation.





- Extraction: 1ml plasma with High Pure Viral Nucleic Acid Large Volume Kit-Roche.
- Library Quantification: QuantiFluor SINGLE TUBE

Baseline characteristics	
Viral load (median; IQR)	87900 [22283-1240000]
CD4 (median, IQR)	214 [42-610]
Males (%)	81.8
Age (median, IQR)	35 [32-43]
Country of Origin (Spain,%)	90.9
Subtype	9 B; 1 A1; 1 CRF07_BC



Results

The table shows, per each sample, the viral load in copies/mL; a list of mutations detected by Sanger and 454-DeepCheck with their corresponding frequencies and mutational load in copies/mL; a list of mutations detected only by 454-DeepCheck with their corresponding frequencies and mutational load in copies/mL, and the additional reductions in susceptibility predicted from the genotypic information provided only by 454-DeepCheck. Additional mutations detected by 454-DeepCheck were found at a median [IQR] frequency of 2.33% [1.01-4.94] and median mutational load of 1222 c/ml [348-24107].

Viral Load	Sanger (%, Mut Load)	Added 454-DeepChek (%, Mut Load)	Added Resistance*
87900	103S (99,43; 87399) 179I (58,96; 51826)	69N (1,39;1222) 103R (2,77;2435) 103N (2,42;2127)	D4T, DDI (PLL)
15957	103N (90,91; 14500)	230L (3,29; 525)	ETV, RPV (IR)
495000	138A (92.02; 455499) 179D (85,28; 422136)	65R (1,33; 6584) 103N (4,87; 24107) 115F (3,11; 15395)	D4T (LLR);3TC, FTC, DDI (IR); TDF, ABC (HLR); EFV, NVP (HLR)
22283	103N (34,98; 7795)	190E (1.56; 348)	ETV, RPV (PLL)
1240000	K103N (95,24;1180976) P225H (98.83; 1225492) A98S (99,28;1231072)	no	-
16600	E138A (37,63;6247)	M41L (4,94; 820) F77L (2.33; 387)	ABC, AZT, D4T, DDI, TDF (LLR)
27300	108I (97.77; 26691) 215L (97,02; 26486)	215R (1,75; 478)	-
752000	103N (97,03; 729666)	NO	-
500000	A98S (98,09/490450), K101Q (96.38/4819000), E138K (99,32/496600)	NO	-
341000	V90I (54,40/185538)	M41L (1,01/3444)	ABC, DDI, TDF (PLR); AZT, D4T (LLR)
32994	D67N (99,02/32671) T69N (99,24/32743) K219Q (91,36/30143)	225H (1,85/610)	EFV (IR), NVP (LLR)

*PLL, Potential low level resistance; LLR, Low Level Resistance; IR, Intermediate Resistance; HLR, High Level Resistance

Conclusion

Patients carrying TDR mutations detected by Sanger sequencing frequently carry also additional minor viral mutant populations above a 1% threshold, which can also be detected using UDS methods. In our study, most of the patients with TDR to NRTIs and or NNRTIs had resistance to additional drugs when UDS mutations were used for resistance analysis. These findings may have important implications for first and subsequent line therapy designs and decisions.