A Fully Integrated and Simplified HIV Clinical Genotyping Solution Using 454 Ultra-Deep-Sequencing and the DeepChek™-HIV system

D Gonzalez[1], M Noguera[2], C Rodríguez[2], M Fernández Martínez[3], M Álvarez Tejado[3], C Pou[2], D Dalmau[4], R Delgado[5], F Garcia[6], R Boulmé[1], C Sayada[1], B Clotet[2], and R Paredes[2]

1 Advanced Biological Laboratories SA, Luxembourg; 2 IrsiCaixa, AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; 3 Roche Diagnostics, Sant Cugat del Vallès, Spain; 4 Hospital Universitari MutuaTerrassa, Terrassa, Spain; 5 Hospital 12 de Octubre, Madrid, Spain; 6 Hospital San Cecilio, Granada, Spain

Background

Ultra Deep Sequencing (Roche® 454 Life Sciences) (UDS-454®) detects low-level drug resistant HIV variants, which is not possible with commercially available sequencing assays. An integrated genotyping solution incorporating UDS-454® and a powerful software system to process large sequencing information is warranted to generate clinically meaningful genotyping reports (*Figure 1*).

Method

One hundred thirty treatment-experienced HIV positive failing patients (viral load >1,000 copies/mL) were administered rescue treatment (PRIUS Study). TruGene® and UDS-454® sequencing were performed prior to initiation of treatment. OpenGene® and Amplicon Variant Analyzer (Roche® UDS) sequencing information was analyzed in DeepChek™ software (part of TherapyEdge™, a global HIV database system, *see Figure 2*) to confirm its usefulness. The prevalence of low abundance drug resistant variants was compared using UDS-454® and DeepChek™ versus TruGene® at several thresholds (20, 15, 10, 5 and 1%) along with its impact on drug susceptibility using the Stanford HIVdb interpretation algorithm.

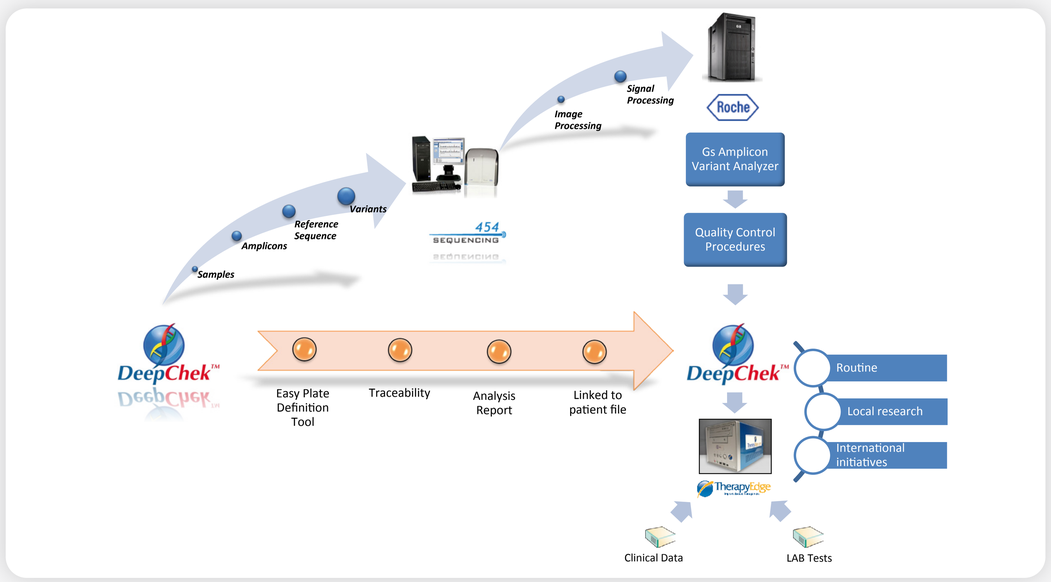

Figure 1 : Overview of the integrated genotyping solution incorporating UDS-454® and DeepChek™-HIV.

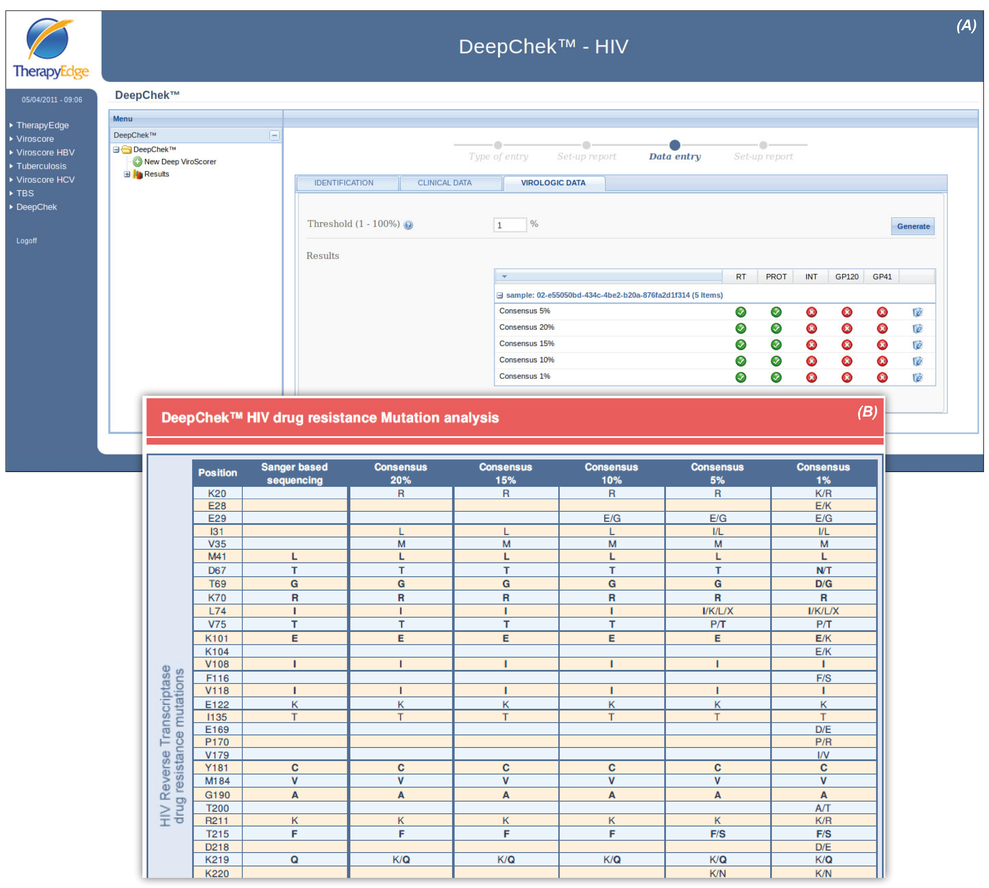

Figure 2 : Overview of the DeepChek™-HIV general interface (A) and report (B).

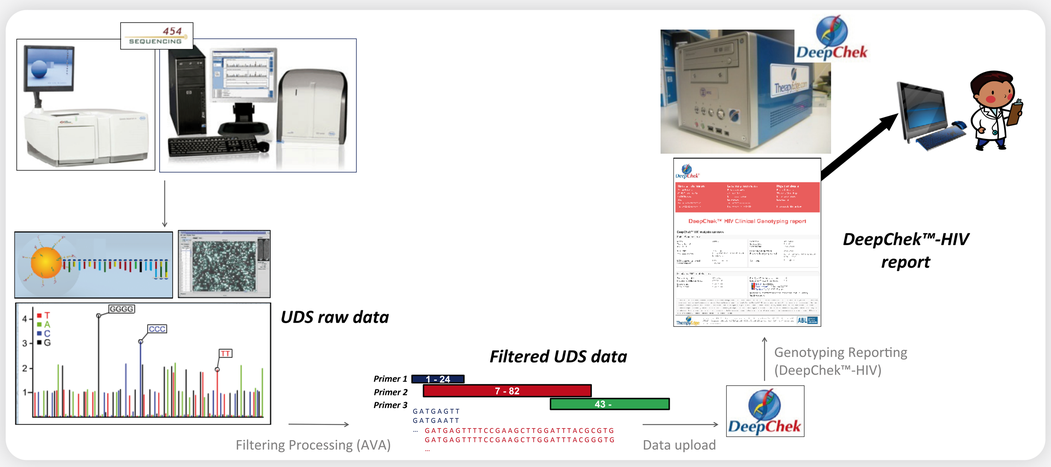

Figure 3 : General data workflow of the UDS-454® and DeepChek™-HIV drug-resistance genotyping system.

Results

On a subset of 47 samples corresponding to a cohort of treatment-experienced patients (median time on therapy of 9 years – Q1=4,25;Q3=12), harboring a median viral load of 43,100 copies/mL (Q1=9,988;Q3=130,000 respectively) and a median CD4 count of 140 cells/µL (Q1=38,5;Q3=298 respectively), the ratio of the median number of resistance mutations detected by UDS-454® compared with TruGene® at the different thresholds (20, 15, 10, 5 and 1%) were 1.11, 1.17, 1.27, 1.38 and 1.70, respectively (*Figure 4*). Higher ratios of decreased drug susceptibility (1.00, 1.18, 1.42, 1.84 and 2.62) were observed using UDS-454® with the three drug susceptibility comparisons performed [Susceptible (S) to Intermediate (I); I to Resistant (R); S to R] at each of the thresholds (20, 15, 10, 5 and 1%), respectively (*Figure 5*). The full workflow of database processing (*Figure 3*), analysis and reporting using DeepChek™ was 10 minutes/sample (around 50,000 sequences per sample and a DeepChek™-HIV analysis to be performed on 5 different thresholds as well as with the Sanger-based comparative option activated) comparable to the TruGene® system. Statistical testing and impact of baseline resistant variants on clinical and virological outcomes will be assessed following availability of complete dataset.

	TruGene	T20	T15	T10	T5	T1
Number of reports	45	45	45	45	45	45
Mean	1	1,28	1,39	1,5	1,72	2,03
Median	1	1,11	1,17	1,27	1,38	1,7
Ratio >4		0	0	0	2,22	6,67
Ratio : 3 - 4		2,22	2,22	6,67	6,67	6,67
Ratio : 2 - 3		6,67	11,11	8,89	17,78	24,44
Ratio 1.5 - 2		13,33	15,56	20	20	37,78
Ratio 1 - 1.5		64,44	66,67	66,67	55,56	22,22
Ratio 0.8 - 1		24,44	11,11	4,44	2,22	0
Ratio 0.6 - 0.8		2,22	0	0	2,22	0
Ratio 0.4 - 0.6		2,22	2,22	2,22	0	0
Ratio 0.2 -0.4		0	0	0	0	0
Ratio <0.2		0	0	0	0	6,67

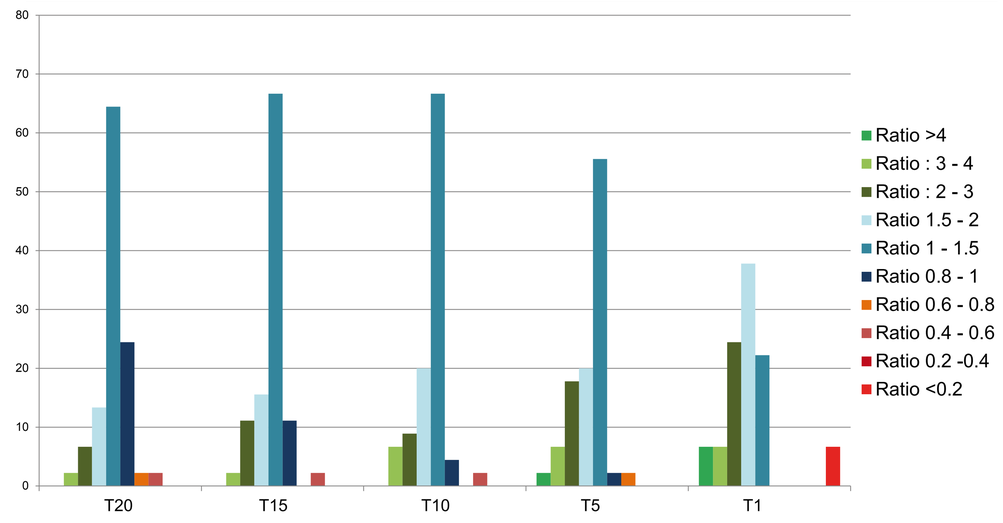

Figure 4 : Ratio of mutations of interest detected by UDS-454® compared to TruGene® at different thresholds (T20, T15, T10, T5 and T1).

	T20		T15		T10		T5		T1	
	Inc. res.	Dec. res.	Inc. res.	Dec. res	Inc. res.	Dec. res	Inc. res.	Dec. res	Inc. res.	Dec. res
Number of reports	45	45	45	45	45	45	45	45	45	44
TOTAL	45	45	53	38	64	34	83	31	118	24
Mean	1	-1	1,18	-0,84	1,42	-0,76	1,84	-0,69	2,62	-0,55
Median	0	0	0	0	0	0	0	0	1	0

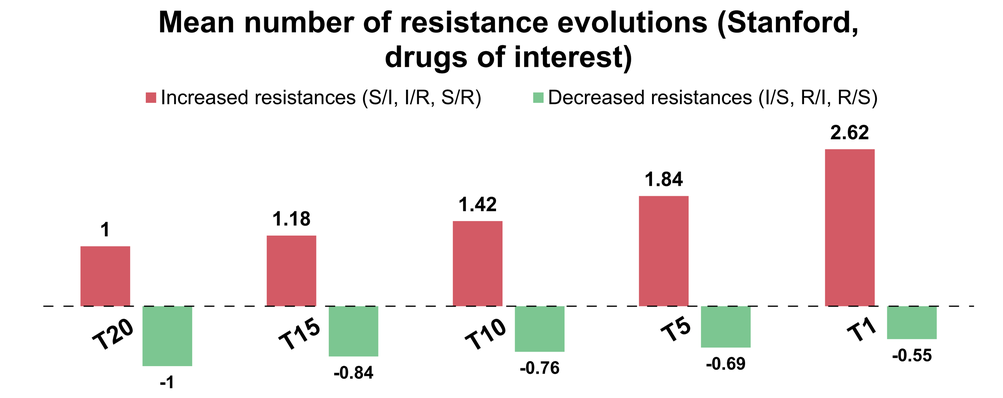

Figure 5 : Number of drug resistance evolutions (increased resistance "Inc. Res." or decreased resistance "Dec. res") given by UDS-454® compared to TruGene® for all the drugs defined in Stanford guidelines (v6.0.8) except ddI, ddC, dlv, nfv, idv and apv at different thresholds (T20, T15, T10, T5 and T1).

Conclusion

UDS-454® identified the same drug resistant mutations as standard sequencing at the prevalence rate of 20%, but had consistently greater sensitivity in identifying resistant variants at <20%. DeepChek™ offered a simplified global database system wherein UDS-454® data were fully integrated to analyze its impact on clinical and biological outcomes with statistical input. This next generation and fully integrated drug resistance genotyping system can easily be used with routine and research applications.

To contact us : **http://www.therapyedge.com - deepchek@therapyedge.com**

This study was supported by the CDTI (Centro para el Desarrollo Tecnológico Industrial), Spanish Ministry of Science and Innovation (IDI-20080843) FMC was supported by the Marie Curie European Reintegration Grant number 238885, 'HIV Coevolution', funded by the European Commission - Framework 7 Program.