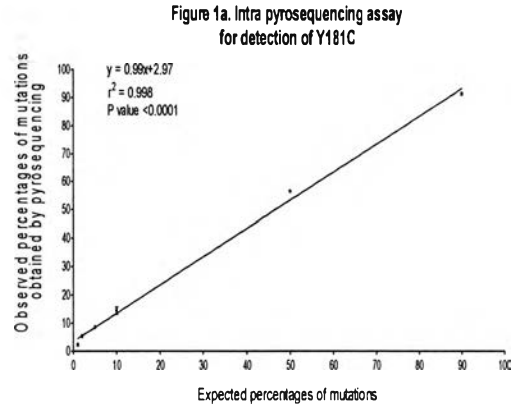
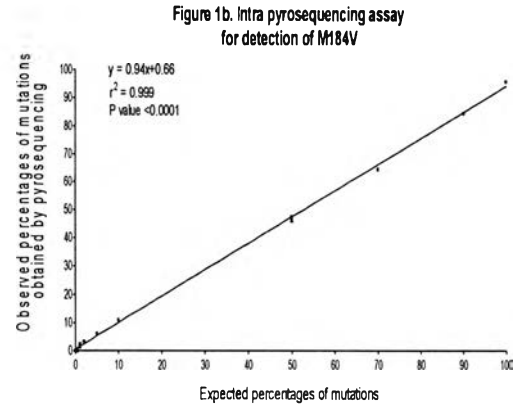
CHAPTER IV

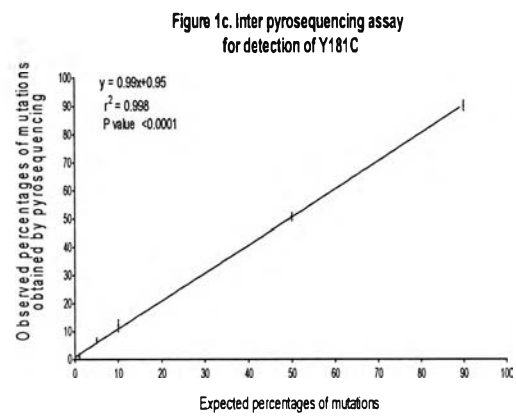
RESULTS

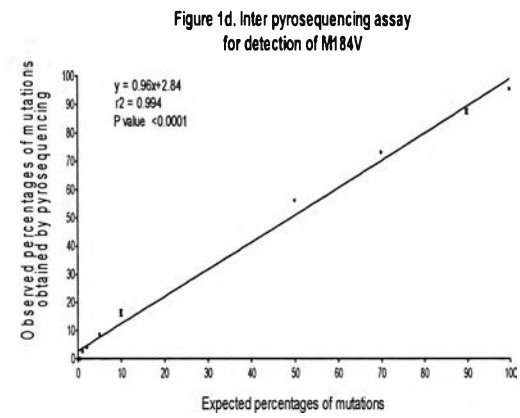
4.1. Pyrosequencing validation

The threshold value for the detection of each PSQ assay was identified as above the mean plus 3 standard deviation (SD) of the percentages of mutations found on WT plasmid templates in 44 and 25 independent measurements for Y181C and M184V, respectively. The threshold values were 0.85% and 1.19% for Y181C and M184V, respectively. To evaluate the accuracy and reproducibility of the assay, serial dilutions of each mutant and WT plasmids were prepared and quantified by corresponding PSQ assays (Figure 15). Intra assay variation was analyzed by triplicate determinants of the same dilution of a given mixture of mutant and WT plasmid. Inter assay variation was identified by measuring 3 different aliquots of each prepared mixture in 3 independent experiments performed on 3 different days [155]. Results of intra and inter assays of PSQ assay for Y181C and M184V showed good linear correlations between the real percentages of mutant variants in the mixture and the percentages detected by PSQ with all correlation coefficients (r^2) greater than 0.99. The lowest level of accurate detection of both mutations was 1% which corresponds to the thresholds for the detection assays (Figure 15).









Percentage of mutation dilutions (%)		90	50	10	5	1
Intra assay (Mean ± SD*)	Y181C	91.57 ± 0.25	56.63 ± 0.5	14.47 ± 1.26	8.50 ± 0.44	2.33 ± 0.21
	M184V	84.53 ± 0.15	46.83 ± 1.11	10.93 ± 0.58	6.04 ± 0.32	1.97 ± 0.85
Inter assay (Mean ± SD)	Y181C	90.34 ± 1.8	50.84 ± 1.5	12 ± 2.17	6.73 ± 1.11	1.67 ± 0.94
	M184V	87.6 ± 0.82	56.17 ± 0.54	16.33 ± 1.11	8.63 ± 0.42	2.7 ± 0.1

Figure 15. Pyrosequencing assay on plasmids.

DNA standard samples were prepared by mixing plasmids harboring mutant and WT sequences according indicated percentages. These expected proportions were quantified by validated pyrosequencing assay. Each point on the fitted lines was equal to mean ±SD obtained in 3 independent determinations. SDs were too small to be seen on graphs at some points. *SD, standard deviation.

4.2. Minority Y181C and M184V variants in people recently infected with HIV-1 and naïve to antiretroviral treatment

We assessed 104 samples from homosexual men recently infected with HIV-1 and naïve to ARV therapy with no Y181C and M184V found by standard genotypic sequencing. Their demographic characteristics were summarized in Table 5. All of these subjects were men with a mean age of 24 years (standard deviation [SD] = 5 years), infected with HIV-1 subtype CRF01_AE with a mean duration of infection of 1 year (SD=0.6 year. The median of plasma HIV-1 RNA and CD4+ T lymphocyte count were 48,987 copies/ml (IQR=21,607-13,987) and 343 cells/ mm^3 (IQR=273-501), respectively. For Y181C detection, PSQ assay was successfully performed for all 104 samples with one sample (0.96%) quantified (Figure 16) at the frequency of 2.9 ± 0.9 %. For M184V detection, 101 samples (97%) were successfully pyrosequenced and among them, minority mutant variants were detected in three (3%) (Figure16) with frequencies of 2.8 ± 0.5 %, 3.4 ± 0.5 %, and 4.2 ± 0.2%.

Table 5. Demographic characteristics of 104 Thai homosexual men recently infected with HIV-1 and naïve to antiretroviral treatment from cohort of TASER-S* with no Y181C and M184V mutations found by conventional genotyping

	(n=104)
Age (years) [mean (SD)]	24 (5.3)
Duration of infection (years) [mean (SD)]	1 (0.6)
CD4 lymphocyte count (cells/mm3) [median (IQR)]	343 (273-501)
HIV-1 RNA (copies/ml) [median (IQR)]	48,987 (21,607-139,877)
Clade CRF01_AE [n (percentage)]	104 (100%)

* TASER-S, surveillance of transmitted HIV drug resistance, TREAT Asia Studies to Evaluate Resistance; IQR, interquartile range.

4.3. Minority Y181C and M184V variants in patients who experienced first-line antiretroviral treatment failure

The characteristics of 22 Thai patients who failed first-line HAART and standard sequencing showed no detectable Y181C and M184V were described in Table 6. Two patients harbored K103N and one of these patients also had TAMs (M41L, L210W, T215Y) (data not shown). Seventy three percent of patients received regimen containing d4T, 3TC, NVP in a fixed-dose combination pill (GPO VIR® S). The median of duration on therapy was 3.3 years (interquartile range (IQR) =2-4.1). At the time of virological failure, the median of viral load was 12,150 copies/ml (IQR=4,320-36,950) and CD4+ T cell count was 272 cells/mm^3 (IQR=115-360).

Table 6. Characteristics of 22 Thai patients infected with HIV-1 who failed first-line antiretroviral therapy with no Y181C and M184V mutations found by conventional genotyping at time of failure

Total	n=22
Age (years) [median (IQR)]	33 (30.3-38.4)
Gender [number (%)]	
Male:	8 (36.4)
Female	14 (63.6)
Subtype CRF01_AE [n (percentage)]	22 (100%)
Duration on ART (years) [median (IQR)]	3.3 (2-4.1)
HIV-1 RNA (copies/ml) [median (IQR)]	12,150 (4,320-36,950)
CD4+ T cell count, cells/mm^3 [median (IQR)]	272 (115-360)
Regimen at time of failure [number (%)]	
d4T+ 3TC+ NVP	16 (72.7%)
AZT+ 3TC+ NVP	2 (9.1%)
3TC+EFV	2 (9.1%)
Others	2 (9.1%)

IQR, interquartile range; ART, antiretroviral therapy; d4T, stavudine; 3TC,lamivudine ; AZT, zidovudine; NVP, nevirapine; EFV, efavirenz.

PSQ assays were successfully done in all 22 selected samples. One sample (4.5%) was found harboring 8.4 ± 0.4 % M184V variants and no minority Y181C mutation was

identified (Figure 16). This sample was subjected to standard clonal analysis to confirm the presence of minority M184V mutants. The sequencing results showed that two M184V variants were detected in 23 (8.7%) randomly selected bacterial colonies containing cloning pGEM®-T Easy Vector with correct ligated RT amplicons. This patient was a woman aged 37 years, on a regimen composed of d4T, 3TC, and NVP for 47 months before experiencing a failure with viral load of 36,800 copies/ml and CD4+T cell count 300 cells/mm^3. After switching to a new regimen composed of 3TC, TDF, and LPV/RTV, the patient achieved viral load re-suppression below 50 copies/ml and CD4+T cell count increased to 448 cells/mm^3. Currently, she has been on the successful therapy for 1.8 years.

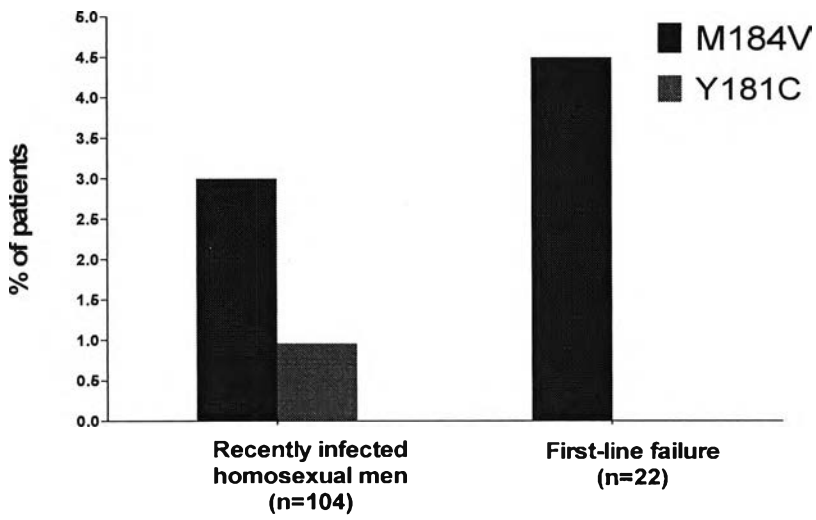


Figure 16. Proportions of patients with neither M184V nor Y181C found by conventional genotyping harbored M184V and/or Y181C minority strains in their plasma.

4.4. Minority N155H drug resistance in circulating RNA and archived proviral DNA in heavily treated patients with raltegravir-based salvage failure

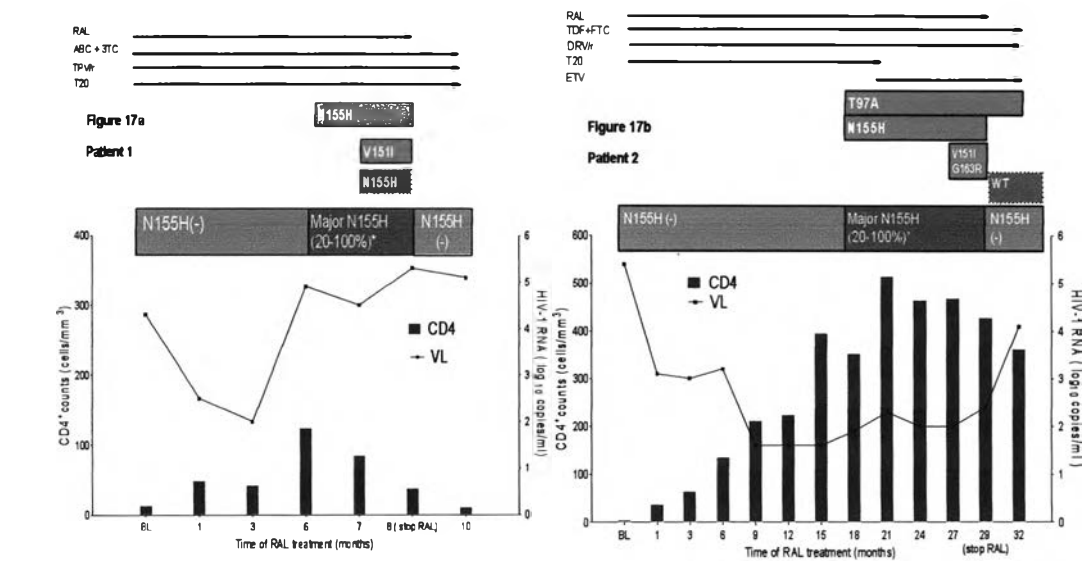
All 5 patients have been infected with HIV-1 for more than 15 years and experienced a long treatment history with multiple classes of ARV agents and also multiple treatment

failures. At the start of RAL-based regimen, they harbored multiple drug resistance mutations to NRTIs, NNRTIs and PIs according to a large number of failing therapies (Table 7).

A reduction in plasma HIV-1 RNA level was observed in all 5 patients after initiating a RAL- containing regimen (Figure 17). After 1 month, a markedly dropped viral load was seen in patient 3 (3.2 logs), patient 2 (2.3 logs), patients 1 and 5 (1.8 logs) and patient 4 achieved 0.7 logs reduction of HIV-1 viral load after 4 months. At the same time, a significant increase in $CD4^+$ T cell count was observed in all except patient 4. Notably, in patient 2, $CD4^+$ T cell count increased from 4 to 514 cells/mm^3. However, none of the patients had ever achieved viral suppression (at the viral load threshold of 50 copies/ml) and a virological rebound occurred in all cases. Virological rebound was associated with the emergence of RAL-associated mutations detected by bulk sequencing: N155H mutation in patients 1, 2 and 3, and mutations Q148H + G140S in patients 4 and 5 (Figure 17). In plasma samples from patient 1, the primary mutation N155H was selected at month 6 and the secondary mutation V151I at month 7. Both mutations were detected in circulating viruses until RAL interruption. In HIV-1 DNA, the N155H mutation was detected at month 7 and no V151I mutation was identified. After RAL withdrawal the N155H mutant was no longer found in plasma and whole blood samples. In plasma samples from patient 2, the primary N155H and secondary T97A mutations appeared together at month 18 followed by the selection of the secondary mutations V151I and G163R at month 27. At month 29, RAL therapy was stopped leading to the disappearance of all mutations in plasma samples except the mutation T97A. No mutation in cellular HIV-1 DNA was detected after RAL withdrawal. In plasma samples from patient 3, after 2 months under RAL pressure, the N155H mutant was firstly selected and then 2 months later it was replaced by the double mutant Q148H + G140S. A similar switch in resistance pathway was observed in cellular HIV-1 DNA. Both mutations were still present in plasma 2 months after RAL stop. In plasma samples from patient 4, mutations Q148H and G140S were selected together at month 4 and maintained throughout the duration of RAL

failure. The mutations Q148H + G140S also emerged in plasma viruses from patient 5 at month 1, and were later accompanied by additional secondary mutations E138A/K and Y143S. After RAL discontinuation, these mutations went undetected in plasma and whole blood samples.

No minority N155H-mutated variant was found by AS-PCR in both plasma and whole blood samples collected at baseline in all 5 patients. During virological failure, among 3 patients displaying the N155H resistance pathway (patients 1 to 3), the mutation N155H was found in a range from 20% to 100% of plasma viral population by AS-PCR between month 6 in patient 1 and month 18 in patient 2 until RAL withdrawal. In patient 3 exhibiting a switch to the Q148H resistance pathway, the N155H mutant was detected at 26 ± 2.6% of plasma viral population at month 2 and gradually declined to 0.4 ± 0.21% at month 4. In this patient, a similar but more rapid reduction in the proportion of the N155H mutant was observed in cellular HIV-1 DNA quantified by AS-PCR (from 100% to 0.48 ± 0.17% after 2 months). In these 3 patients, after RAL therapy was discontinued, the N155H mutant was no longer detected by AS-PCR in both plasma and whole blood samples. In patients 4 and 5 displaying the Q148H resistance pathway, no minority N155H-mutated variant was detected by AS-PCR during RAL failure in both viral RNA and DNA.



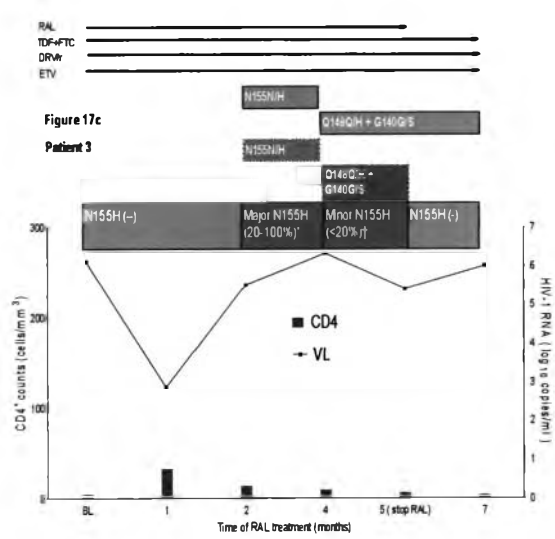


Figure 17. Raltegravir resistance patterns in 5 patients failing raltegravir salvage therapy.

Plasma viral load (lines), CD4+T cell count (black bars), and regimen (arrows). Pink rectangles show durations of mutations detected by bulk sequencing in viral RNA; Green rectangles show durations of mutations detected by bulk sequencing in viral DNA; Blue rectangles show durations of the N155H mutation detected by AS-PCR. *major N155H defined as the N155H variants detected at major levels (20-100% of total viral population) by AS-PCR; †minor N155H defined as the N155H variants detected at minor levels (< 20% of total viral population) by AS-PCR. 3TC, lamivudine; ABC, abacavir; ATV, atazanavir DRV r, darunavir ritonavir; ETV, etravirine; FTC, emtricitabine;LPV r, lopinavir ritonavir; T20, enfuvirtide; TDF, tenofovir; TPV r, tipranavir ritonavir; RAL, raltegravir; AS-PCR, allele specific PCR; wt, wild-type.

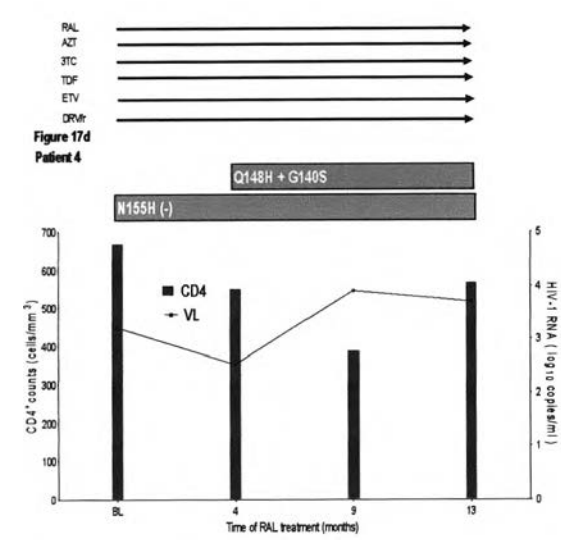


Table 7. Therapeutic and resistance characteristics of 5 heavily treated patients failing raltegravir salvage therapy

Patient	Years on ART	Previous failing regimen	RAL-based salvage therapy	NRTI mutations*	NNRTI mutations*	PI mutations*
1	19	ABC 3TC ATV/r	ABC 3TC TPV/r T20	M41L, D67N, M184V, T215Y, K219Q	None	L10I, L33F, M46L, G48V, L63P, A71T, V77I, V82A, L90M
2	16	TDF FTC	TDF FTC DRV/r T20, ETV	M41L, D67N, M184V, L210W, T215Y, K219N	Y181C	L10V, L33F, M36I, M46I, G48V, L63P, A71V, V77I, V82T, L90M
3	15	FPV TPV/r	TDF FTC DRV/r ETV	M41L, M184V, L210W, T215Y, K219N	Y181C, G190A	L10I, L33F, M46I, I47V, I54M, L63P, A71T, G73T, V77I, V82F, L90M
4	19	ABC 3TC DDI	AZT 3TC TDF ETV DRV/r	M41L, D67N, K70R, L74V, M184V, L210W, T215Y, K219E	L100I, K103N	L10F, K20R, V32I, L33F, M46I, I47V, I54M, L63P, A71V, V82A, I84V
5	15	TDF FTC AZT ETV DRV/r	TDF FTC ETV ATV DRV/r LPV/r	M41L, D67N, M184V, L210W, T215Y, K219E	K101E, V106I, Y181C, G190S	L10I, V11I, K20R, V32I, L33F, M46I, I47V, I54L, L63P, A71V, G73S, V82T, L90M

ART, antiretroviral therapy; RAL, raltegravir; NRTI, nucleoside reverse transcriptase inhibitor, NNRTI, non nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; ABC, abacavir; 3TC, lamivudine; ATV/r, atazanavir/ritonavir; TPV/r, tipranavir/ritonavir; TDF, tenofovir; FTC, emtricitabine; DRV/r, darunavir/ritonavir; T20, enfuvirtide; FPV, fosamprenavir; ETV, etravirine; DDI, didanosine; AZT, zidovudine.

* Mutations detected at the initiation of RAL-based regimens following the International AIDS Society-USA (IAS-USA) drug resistance mutations list 2010.

4.5. Genetic barrier to the development of resistance to INIs in HIV-1 subtypes B and CRF01_AE

All described 66 mutations conferring resistance to RAL, EVG and DTG at 41 amino acid positions in IN gene (51, 66, 68, 72, 74, 92, 95, 97,101, 114, 119, 121, 124,125, 128, 138, 140, 143, 145, 146, 147, 148, 151, 153, 154, 155, 156, 157, 160, 163, 165, 170, 193, 201, 203, 206, 230, 232, 249, 263, 280) were assessed in 144 nucleotide sequences (109 CRF01_AE and 35 B subtypes). Overall, the analysis of these 144 sequences at 41 studied amino acid positions showed 93 WT codons encoding for the expected WT amino acids in the HXB2 reference sequence. There were 23 polymorphisms with unknown significance at 12 amino acid positions (Q95H/S/A, L101V/P/F, S119P/G, A124N/S, T125A/V, V151E, M154T/L, K160R/T/Q/E, G163E, G193D, S230N, N232E) and 20 mutated amino acids at 19 amino acid positions (H51Y, L68I, V72I, L74I, T97A, L101I, S119R/G, A124T, M154I, K156N, E157Q, V156I, V165I, G193E, V201I, I203M, T206S, N232D, V249I). Among polymorphisms with unknown significance, T125A was highly frequent (98% in CRF01_AE group and 31% in subtype B group). Among mutated amino acids, the following DRAMs were found with a frequency of more than 10%: V72I (80% in subtype B group and 13% in subtype CRF01 group), L101I (15% in subtype CRF01_AE group), A124T (49% in subtype B group), K156N (17% in subtype B group), V165I (25% in subtype CRF01_AE group and 11% in subtype B group), V201I (99% in subtype CRF01_AE group and 31% in subtype B group), I203M (14% in subtype B group), T206S (17% in subtype B group), N232D (99% in subtype CRF01_AE group and 97% in subtype B group) (Table 8).

The predominant codon at each amino acid position (>69 % of the total sequences) was found in 41/41 (100%) and 38/41 (92.6%) of the studied positions in groups of subtype CRF01_AE and subtype B, respectively. In subtype B group, at position 119 motifs AGC, AGT, CCC, AGA, GGC, ACC accounted for 54%, 3%, 26%, 3%, 9%, 6%, at position 124

motifs GCT, AAT, AAC, TCA, AGT, ACT, ACC accounted for 20%, 23%, 2%, 3%,3%,46%, 3% and at position 125 motifs ACA, ACT, GCA, GCG, GTG accounted for 46%, 14%, 14%, 17%, 9%, respectively (Table 8).

All 93 WT and 23 unknown polymorphic codons at 41 amino acid positions were evaluated for genetic barrier. At each codon, the smallest number of ts and tv required to obtain all possible mutated codons encoding for 66 mutated substitutions conferring resistance to RAL, EVG, and DTG was calculated and the minimal score was determined by the sum of the number of ts and tv. The genetic barrier of each codon was identified by the minimal score of mutated codon(s) which allow(s) the smallest number of ts and tv. Among 41 studied amino acid positions in two subtype groups, 28 (68.3%) had similar predominant codons: 51, 66, 74, 95, 97, 119, 121, 138, 143, 145, 146, 147, 148, 151, 153, 154, 155, 157, 160, 165, 170, 193, 203, 206, 230, 249, 263, 280 leading to achieve the same calculated minimal score for genetic barrier at these positions. Of 13 amino acid positions with different predominant codons (68, 72, 92, 101, 114, 124, 125, 128, 140, 156, 163, 201, 232), six corresponding to seven DRAMs were found to have an impact on calculated genetic barrier: 1 vs 0 for V72I; 3.5 vs 2.5 for L101I; 1 vs 0 for A124T; 3.5 vs 2.5 for T125K; 5 vs 2.5 for G140C; 3.5 vs 1 for G140S; 0 vs 1 for V201I in CRF01_AE and B subtypes, respectively (Table 8, Figure18).

Table 8. Codon frequency and calculated genetic barrier corresponding to each codon in 66 mutations associated with raltegravir, elvitegravir, and dolutegravir resistance at 41 amino acid positions in integrase gene in HIV-1 subtypes B and CRF01_AE.

codon position	Substitution [a]	Wild type codon	Percentage (%) Subtype CRF01_AE (n=109)	Subtype B (n=35)	Codons of mutational resistance	Minimal score [b]
51	H51Y	CAT	100	89	TAT/C	1
		CAC	0	2		1
	Y51	TAT	0	9		0
66	T66A	ACA	97	94	GTC,GC C/A/G	1
		ACG	2	0		1
		ACT	1	0		2
		ACC	0	6		1
	T66K	ACA	97	94	AAA/G	2.5
		ACG	2	0		2.5
		ACT	1	0		5
		ACC	0	6		5
	T66I	ACA	97	94	ATT/C/A	1
		ACG	2	0		3.5
		ACT	1	0		1
		ACC	0	6		1
68	L68V	CTA	94	9	GTT/C/A /G	2.5
		TTA	5	89		2.5
		CTG	0	2		2.5
	I68V	ATA	1	0		1
	L68I	CTA	94	9	ATT/C/A	2.5
		TTA	5	89		2.5
		CTG	0	2		3.5
	I68	ATA	1	0		0
72	V72I	GTC	29	0	ATT/C/A	1
		GTT	5	20		1
		GTA	2	0		1
		GTG	1	0		2
	I72	ATC	12	0		0
		ATT	1	80		0
74	L74M	CTG	89	94	ATG	2.5
		TTG	6	0		2.5
		CTA	2	6		3.5
	I74M	ATA	3	0		1
	L74A	CTG	89	94	GTC,GC C/A/G	3.5
		TTG	6	0		3.5
		CTA	2	6		3.5
	I74A	ATA	3	0		1
	L74I	CTG	89	94	ATT/C/A	3.5
		TTG	6	0		3.5
		CTA	2	6		2.5
	I74	ATA	3	0		0
92	E92Q	GAA	98	23	CAA/G	2.5
		GAG	2	77		2.5
95	Q95K	CAG	84	71	AAA/G	2.5
		CAA	10	29		2.5
	H95K	CAT	3	0		5
	S95K	TCA	2	0		5
	A95K	GCA	1	0		4.5
97	T97A	ACA	100	97	GTC,GC C/A/G	1
	A97	GCA	0	3		0
101	L101I	CTG	83	0	ATT/C/A	3.5
		CTT	0	86		2.5
	I101	ATA	13	0		0
		ATC	1	0		0
	V101I	GTG	3	0		2
	P101I	CCT	0	3		3.5
	F101I	TTT	0	11		2.5
114	H114Y	CAC	95	3	TAT/C	1
		CAT	5	97		1
119	S119R	AGC	92	54	CGT/C/A/ G,AGA/G	2.5
		AGT	1	2		2.5
	P119R	CCC	3	26		2.5
	R119	AGA	2	3		0
	G119R	GGC	1	9		2.5
	T119R	ACC	1	6		5
	S119G	AGC	92	54	GGT/C/A/ G	2
		AGT	1	2		1
	P119G	CCC	3	26		5
	R119G	AGA	2	3		1
	G119	GGC	1	9		0
	T119G	ACC	1	6		3.5
121	F121I	TTC	96	91	ATT/C/A	2.5
		TTT	4	9		2.5
	F121Y	TTC	96	91	TAT/C	2.5
		TTT	4	9		2.5
124	A124T	GCT	85	20	ACT/C/A/ G	1
		GCA	2	0		1
	N124T	AAT	7	23		2.5
		AAC	0	2		2.5
	S124T	TCA	0	3		2.5
		AGT	0	3		2.5
	T124	ACT	6	46		0
		ACC	0	3		0
125	T125K	ACA	2	46	AAA/G	2.5
		ACT	0	14		5
	A125K	GCA	95	14		3.5
		GCT	1	0		6
		GCG	2	17		3.5
	V125K	GTG	0	9		3.5
128	A128T	GCA	100	0	ACT/C/A/ G	1
		GCC	0	86		1
		GCT	0	14		1
138	E138A	GAA	97	100	GTC,GCC/ A/G	2.5
		GAG	3	0		2.5
	E138K	GAA	97	100	AAA/G	1
		GAG	3	0		2
140	G140C	GGG	93	3	TGT,TTC	5
		GGA	7	3		5
		GGT	0	11		2.5
		GGC	0	83		3.5

Analysis of genetic barrier based on the minimal number of transitions (ts) and transversions (tv) required to obtain mutated codons conferring resistance to raltegravir, elvitegravir, and dolutegravir. [a] The substitution is in italic if it is different from the wild type amino acid of HXB2 and is underlined if it is expected mutant amino acid. [b] minimal scores are determined by the sum of the number of ts and tv for each codon with 1 ts scored as 1 and 1 tv scored as 2.5.

Table 8. Continued

codon position	Substitution *	Wild type codon	Percentage (%) Subtype CRF01_AE (n=109)	Subtype B (n=35)	Codons of mutational resistance	Minimal score **
140	G140A	GGC	93	3	GTC,GCC/AG	2.5
		GGA	7	3		2.5
		GGT	0	11		3.5
		GGC	0	83		2.5
	G140S	GGC	93	3	TCT/C/A/G, AGT/C	3.5
		GGA	7	3		3.5
		GGT	0	11		1
		GGC	0	83		1
143	Y143C	TAC	99	97	TGT,TTC	2.5
		TAT	1	3		1
	Y143H	TAC	99	97	CAT/C	1
		TAT	1	3		1
	Y143R	TAC	99	97	CGT/C/A/G, AGA/G	3.5
		TAT	1	3		2
	Y143G	TAC	99	97	GGT/C/A,GC G	3.5
		TAT	1	3		3.5
145	P145S	CCC	89	94	TCT/C/A/G, AGT/C	1
		CCT	9	6		1
		CCA	2	0		1
		CCG	0	0		1
146	Q146E	GAA	100	94	AAA/G	2.5
		CAG	0	6		2.5
	Q146P	CAA	0	94	CCT/C/A/G	2.5
		CAG	0	6		2.5
147	S147G	AGT	95	100	GGT/C/A/G	1
		AGC	5	0		1
148	Q148E	GAA	98	94	GAA/G	2.5
		CAG	2	6		2.5
	Q148G	CAA	98	94	GGT/C/A/G	3.5
		CAG	2	6		4.5
	Q148H	CAA	98	94	CAT/C	2.5
		CAG	2	6		2.5
	Q148K	CAA	98	94	AAA/G	2.5
		CAG	2	6		2.5
	Q148R	CAA	98	94	CGT/C/A/G, AGA/G	1
		CAG	2	6		1
151	V151I	GTA	91	89	ATT/C/A	1
		GTG	8	11		3.5
	E151I	GAA	1	0		3.5
	V151L	GTA	91	89	CTT/C/A/G,T TA/G	2.5
		GTG	8	11		2.5
	E151L	GAA	1	0		5
153	S153A	TCT	86	91	GTC,GCC/AG	3.5
		TCC	14	6		2.5
		TCA	0	3		2.5
	S153P	TCT	86	91	TTT/C	1
		TCC	14	6		1
		TCA	0	3		3.5
	S153Y	TCT	86	91	TAT/C	2.5
		TCC	14	6		2.5
		TCA	0	3		5
154	M154I	ATG	99	91	ATT/C/A	1
	T154I	ACC	1	0		1
	L154I	CTA	0	3		2.5
		TTA	0	3		1
	I154	ATA	0	3		0
155	N155H	AAT	97	97	CAT/C	2.5
		AAC	3	3		2.5
	N155S	AAT	97	97	TCT/C/A/G, AGT/C	1
		AAC	3	3		1
	N155T	AAT	97	97	ACT/C/A/G	2.5
		AAC	3	3		2.5

codon position	Substitution *	Wild type codon	Percentage (%) Subtype CRF01_AE (n=109)	Subtype B (n=35)	Codons of mutational resistance	Minimal score **
156	K156N	AAG	80	0	AAT/C	2.5
		AAA	19	83		2.5
	N156	AAT	1	14		0
		AAC	0	3		0
157	E157Q	GAA	97	94	GAA/G	2.5
		GAG	2	6		2.5
	Q157	CAA	1	0		0
160	K160N	AAA	94	100	AAT/C	2.5
		AAG	1	0		2.5
	R160N	AGA	1	0		3.5
	T160N	ACA	1	0		5
	Q160N	CAA	2	0		5
	E160N	GAA	1	0		3.5
	K160D	AAA	94	100	GAT/C	3.5
		AAG	1	0		3.5
	R160D	AGA	1	0		4.5
	T160D	ACA	1	0		6
	Q160D	CAA	2	0		5
	E160D	GAA	1	0		2.5
163	G163K	GGG	92	9	AAA/G	2
		GGA	5	86		2
		GGT	0	5		4.5
	E163K	GAG	3	0		1
	G163R	GGG	92	9	CGT/C/A/G , AGA/G	3.5
		GGA	5	86		3.5
		GGT	0	5		2.5
	E163R	GAG	3	0		2
165	V165I	GTA	69	89	ATT/C/A	1
		GTG	5	0		3.5
		GTC	1	0		1
	I165	ATA	24	11		0
		ATC	0	0		0
		ATT	1	0		0
178	E178A	GAA	96	94	GCT/C/A/G	2.5
		GAG	4	6		2.5
193	G193E	GGG	95	77	GAA/G	1
		GGA	1	9		1
		GGT	0	11		3.5
	D193E	GAC	3	0		2.5
	E193	GAG	1	3		0
201	V201I	GTA	2	69	ATT/C/A	1
	I201	ATA	99	31		0
203	I203M	ATA	94	86	ATG	1
		ATC	1	0		2.5
	M203	ATG	5	14		0
206	T206A	ACA	94	80	TCT/C/A/G , AGT/C	2.5
		ACG	1	0		2.5
		ACC	0	1		2.5
	S206	TCA	6	17		0
230	R230K	AGC	100	91	CGT/C/A/G , AGA/G	2.5
	N230K	AAC	11	9		3.5
232	N232D	AAT	1	0	GAT/C	1
	D232	GAC	90	3		0
		GAT	9	94		0
	E232D	GAG	0	3		2.5
249	V249I	GTA	94	100	ATT/C/A	1
		GTG	4	0		2
		GTT	1	0		1
	I249	ATA	1	0		0
263	R263K	AGA	100	100	AAA/G	1
		AGG	0	0		1
280	C280Y	TGT	100	100	TAT/C	1

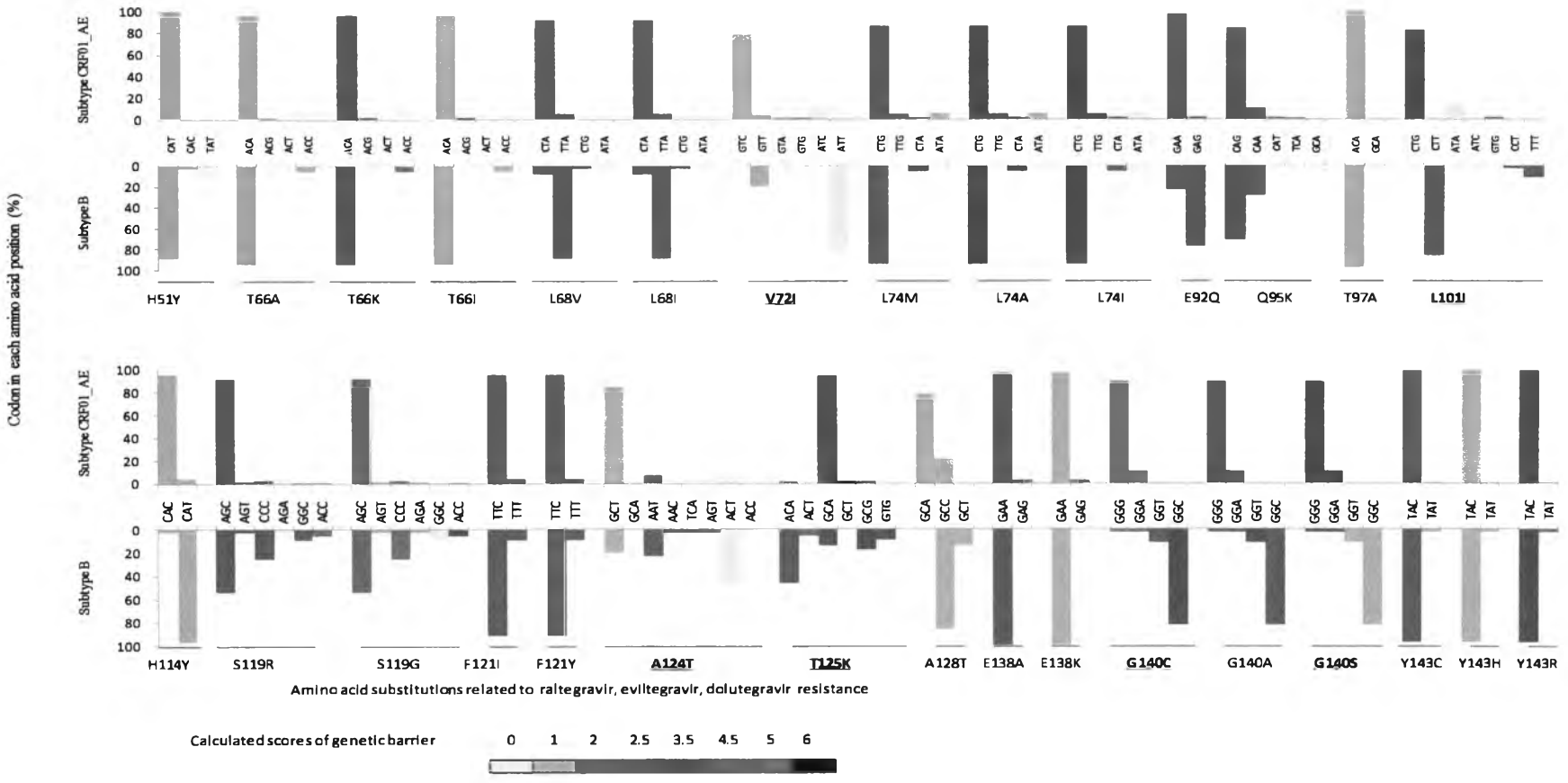


Figure 18. Comparison of genetic barriers for the development of mutations related to raltegravir, elvitegravir, and dolutegravir between HIV-1 subtype CRF01_AE and subtype B.

Upper bar indicates the prevalence of codon in each position associated to raltegravir, elvitegravir, and dolutegravir resistance mutations in HIV-1 subtype CRF 01_AE and lower bar shows that in subtype B. The minimal score of genetic barrier calculated for each codon is displayed in a color code. Mutations shown different calculated genetic barriers are bold and underlined.

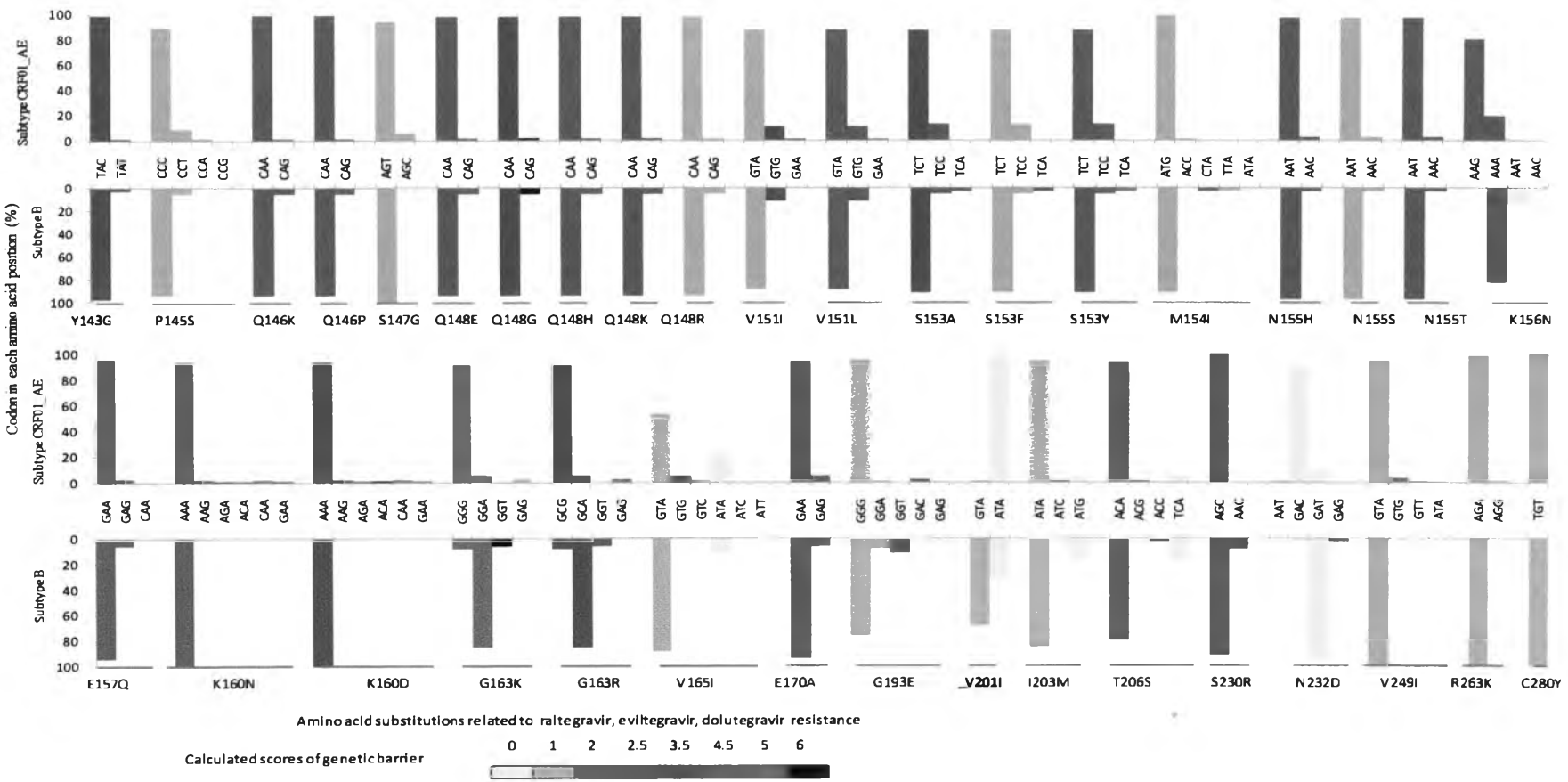


Figure 18 (continued). Comparison of genetic barriers for the development of mutations related to raltegravir, elvitegravir, and dolutegravir between HIV-1 subtype CRF01_AE and Subtype B. Upper bar indicates the prevalence of codon in each position associated to raltegravir, elvitegravir, and dolutegravir resistance mutations in HIV-1 subtype CRF 01_AE and lower bar shows that in subtype B. The minimal score of genetic barrier calculated for each codon is displayed in a color code. Mutations shown different calculated genetic barriers are bold and underlined