HIV-1 Subtype Influences the Pathways of Genotypic Resistance to Integrase Inhibitors

T Doyle[1], D Dunn[2], F Ceccherini Silberstein[3], C De Mendoza[4], F Garcia[5], E Smit[6], AG Marcelin[7], J Martinez-Picado[8], R Kaiser[9] and AM Geretti[10] on behalf of the CORONET Study Group

1. King's College London, UK; 2. MRC Clinical Trial Unit, London, UK.; 3. University of Tor Vergata, Rome, Italy; 4. Hospital Carlos III, Madrid, Spain; 5. San Cecilio Hospital, Granada, Spain; 6. Heart of England NHS Foundation Trust, Birmingham, UK; 7. Pitié-Salpêtrière Hospital, Paris, France. 8. AIDS Research Institute Caixa, Barcelona, Spain; 9. University of Cologne, Germany; 10. University of Liverpool, UK

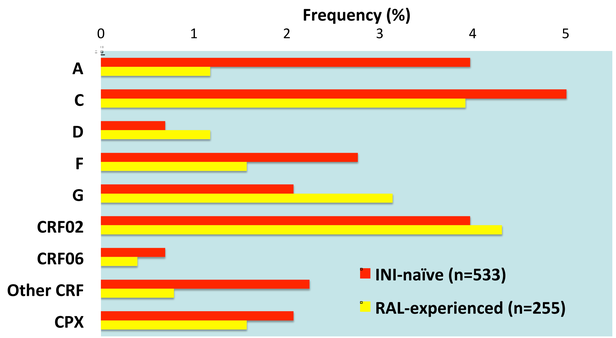
BACKGROUND

- The mutational profiles selected by integrase inhibitor (INI) therapy have been primarily defined for HIV-1 subtype B. In the global context, subtypes other than B account for most HIV-1 infections
- *CORONET* is a European network for collaborative research on integrase resistance that provides a central repository of integrase sequences produced as part of routine genotypic resistance testing at multiple collaborating centres. The overall aim is to characterise prevalence and patterns of INI resistance across Europe, with focus on the impact of HIV genetic heterogeneity

OBJECTIVES OF THE STUDY

To characterise patterns of genotypic INI resistance in relation to HIV-1 clade among INI-naïve and raltegravir (RAL)-experienced subjects

Figure 1. Non-B HIV-1 clades in INI-naïve and RAL-experience subjects



Other CRFs comprised CRF01, CRF05 and CRF35

RESULTS-1

- Integrase sequences were analysed from 533 INI-naïve subjects and 255 RAL recipients with viraemia. The most prevalent HIV-1 clades in the two groups were subtype B (75% and 82% respectively), subtype C (5% and 4%), and CRF02_AG (4% and 4%). Other non-B clades were highly diverse (**Figure 1**)

METHODS

- Integrase sequences and clinical data were collected from 9 European centres and 2 multi-centre repositories (UK HIV Drug Resistance Database; EuResist Database) in 2008-2011
- HIV-1 clades were assigned by submitting integrase sequences to the Stanford HIV Drug Resistance Database and the Rega HIV-1 Subtyping Tool. Discrepant results were resolved through the NCBI genotyping tool and by phylogenetic analysis with PhyML
- Major INI resistance-associated mutations (RAMs) comprised T66A/I/K, E92Q/G/V, F121Y, G140S/A/C, Y143H/R/C/K, S147G, Q148H/R/K, N155H/S/T. Minor INI RAMs comprised mutations at codons 51, 74, 95, 97, 114, 128, 138, 145, 146, 151, 153, 157, 163, 230, 263
- Cross-resistance to dolutegravir (DTG) was classified according to the definition used in the VIKING trials [1], modified to include the potential low-level resistance effects conferred by isolated Q148 mutations. DTG resistance categories were:
  - *No resistance*: absence of Q148H/R/K
  - *Low-level resistance*: Q148H/R/K in the absence of G140S/A/C, L74I or E138A/K/T
  - *Intermediate-level resistance*: Q148H/R/K with 1 of G140S/A/C, L74I or E138A/K/T
  - *High-level resistance*: Q148H/R/K with 2 or 3 of G140S/A/C, L74I, or E138A/K/T
- Fisher's Exact Test was used to compare amino acid frequencies across all integrase codons in INI-naïve vs. RAL-experienced subjects and to compare prevalence of INI RAMs in subtype B vs. non-B HIV-1 clades. Plasma HIV-1 RNA levels were compared between different subgroups by Wilcoxon rank-sum test. Analyses were performed using STATA v12.1

References: 1. Castagna A, et al. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. JID 2014; 2. Nguyen HL, et al. Genetic barrier to the development of resistance to integrase inhibitors in HIV-1 subtypes CRF01_AE and B. Intervirology 2012; 3. Maiga AI, et al. Genetic barriers for integrase inhibitor drug resistance in HIV type-1 B and CRF02_AG subtypes. Antivir Ther 2009; 4. Brenner BG, et al. Subtype diversity associated with the development of HIV-1 resistance to integrase inhibitors. J Med Virol 2011. 5. Turrizianni O, et al. Short communication: analysis of the integrase gene from HIV type 1-positive patients living in a rural area of West Cameroon. AIDS Res Hum Retroviruses 2012; 6. Fourati S, et al. Cross-resistance to elvitegravir and dolutegravir in 502 patients failing on raltegravir: a French national study of raltegravir experienced HIV-1-infected patients. J Antimicrob Chemother 2015

geretti@liverpool.ac.uk

RESULTS-2

INI-NAÏVE SUBJECTS (n=533)

- Plasma HIV-1 RNA load at time of sequencing was median 21,476 cps/ml (IQR 2,298-113,308). No patient was found to carry major INI RAMs

INI-EXPERIENCED SUBJECTS (n=255)

- Plasma HIV-1 RNA load at time of sequencing was median 3,523 cps/ml (IQR 555-27,793). 113/255 (44.3%) patients showed ≥1 major INI RAM
- In RAL recipients with vs. those without major INI RAMs median viral load was 3,420 cps/ml (IQR 1,195-20,899) vs. 3,834 cps/ml (IQR 376-32,342), respectively (p=0.30)
- The most common major INI RAMs were N155H (57, 22.4%), G140S (33, 12.9%), Q148H (28, 11%), Q148R (15, 5.9%), E92Q (9, 3.5%), and Y143R (9, 3.5%) (**Table 1**). Preferential associations comprised N155H with E92Q; Y143H/R/C with T97A; and Q148H/R/K with G140S/A

Table 1. Major INI RAMs detected in RAL recipients with viraemia

RAMs n (%)[a]						Pattern
E92Q	G140S/A	Y143R/C/H	S147G	Q148H/R/K	N155H	n (%)[b]
9 (3.5)	37 (14.5)	14 (5.5)	1 (0.4)	44 (20)	57 (22.4)	
					■	45 (17.6)
	■			■		35 (13.7)
		■				12 (4.7)
■					■	8 (3.1)
				■		6 (2.4)
		■			■	2 (0.8)
			■	■		1 (0.4)
				■	■	1 (0.4)
	■			■	■	1 (0.4)
			■	■	■	1 (0.4)
■						1 (0.4)

[a]113/255 (44.3%) RAL recipients had ≥1 major INI RAM. Mutations observed: E92Q (n=9); G140S (n=33), G140A (n=4); Y143R (n=9), Y143C (n=4), Y143H (n=1); Q148H (n=28), Q148R (n=15), Q148K (n=1); and N155H (n=57). [b]Shaded boxes within each row indicate mutations present together in each pattern

- **Tabke 2** shows minor INI RAMs occurring at a frequency >2% in RAL recipients. L74M, T97A, E138K, V151I, G163R were significantly associated with RAL exposure

Table 2. Minor integrase mutations in RAL recipients with viraemia

Mutation	Frequency n (%) RAL-naïve (n=533)	RAL-experienced (n=255)	*P value*	Positive association with major INI RAMs
74I	35 (6.6)	13 (5.1)	*0.53*	
74M	3 (0.6)	8 (3.1)	*0.007*	
97A	8 (1.5)	20 (7.8)	*<0.001*	Y143R/C/H
138K	0 (0.0)	9 (3.5)	*<0.001*	Q148H/R/K
151I	10 (1.9)	25 (9.8)	*<0.001*	N155H
157Q	11 (2.1)	6 (2.4)	*0.80*	
163R	3 (0.6)	10 (3.9)	*0.001*	
163E	29 (5.4)	7 (2.8)	*0.10*	
230N	51 (9.7)	17 (7.2)	*0.22*	

NOVEL MUTATIONS

- 4 (1.6%) RAL recipients (subtype B) had non-classic substitutions at major integrase resistance codons: a) E92A, detected with N155H and T97A (viral load 3,170 cps/ml); b) S147I alone (477 cps/ml); c) N155D alone (140 cps/ml); and d) N155Q alone (111 cps/ml). By comparing sequences from INI-naïve and RAL-experienced patients, 3 positions were newly identified as being subjected to selective pressure at codons 159 (p=0.009), 161 (p=0.004), and 170 (p=0.009); mutations occurred in subtype B (K159Q/R, I161L/M/N/T/V, E170A/G) and CRF02_AG (I161L/T) and commonly alongside major INI RAMs

INFLUENCE OF HIV-1 CLADE ON INTEGRASE MUTATION PROFILES

- Comparing subtype B with non-B clades, mutations at codon 148 and 140 occurred in 42 (20.1%) vs. 2 (4.3%) (p=0.009), and 36 (17.2%) vs. 1 (2.1%) (p=0.005), respectively (**Table 3**). G140S and Q148H were exclusively found in subtype B s. G140A occurred in 3 subtype B and 1 subtype G sequences; Q148R occurred in 13 subtype B, 1 subtype C, and 1 subtype G sequences
- E92Q was significantly less frequent in subtype B (4/209, 1.9%) than in non-B clades (5/46, 11%) (p=0.02), occurring in subtypes C (n=2), G (n=1), A (n=1), and CRF09 (n=1)
- In patients with ≥1 major RAMs, the viral load at time of sampling was median 3,535 cps/ml (IQR 1,472-25,704) vs. 1,820 (IQR 555-11,660) in subjects with B vs. non-B clades, respectively (p=0.33)
- Codon usage in integrase sequences of INI-naive patients is shown in **Table 4**. Sequences differed by clade in their propensity to acquire mutations at codon 140

Table 3 Major INI RAMs in RAL recipients with subtype B or non-B HIV-1 clades

RAM	Subtype B (n=209)	Non-B clades (n=46)
E92Q, n (%)	4 (1.9)	5 (10.8) [A, C, G, CRF09][a]
G140S, n (%)	33 (15.8)	0 (0)
G140A, n (%)	3 (1.4)	1 (2.2) [G][a]
Q148H, n (%)	28 (13.4)	0 (0)
Q148R, n (%)	13 (6.2)	2 (4.3) [C, G][a]
Q148K, n (%)	1 (0.5)	0 (0)

[a]Non-B clades with the mutation indicated in brackets

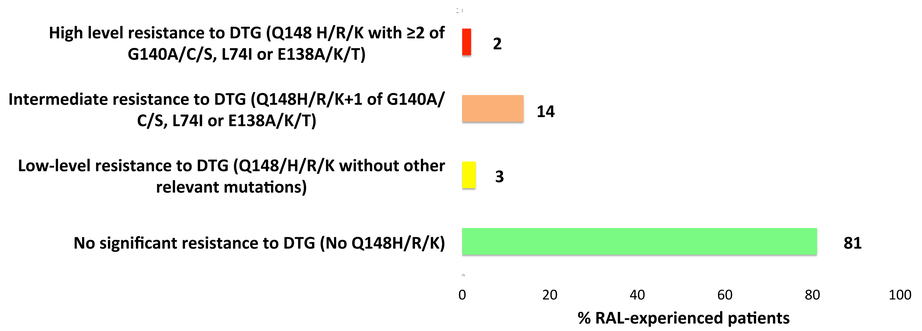
Table 4. Codon usage in INI-naïve subjects with subtype B or non B HIV-1 clades

Position	Subtype B (n=399) Triplet	n (%)	Non-B clades (n=134) Triplet	n (%)
92	GAG	248 (62.2)	GAG	7 (5.2)
	GAA	117 (29.3)	GAA	123 (91.8)
	GAR	34 (8.5)	GAR	3 (2.2)
140	GGC	331 (83.0)	GGA	84 (62.7)
	GGT	35 (8.7)	GGG	43 (32.1)
	GGY	18 (4.5)	GGR	7 (5.2)
	Other	15 (3.8)		
148	CAA	378 (94.7)	CAA	106 (79.1)
	CAG	13 (3.3)	CAG	27 (20.1)

DOLUTEGRAVIR CROSS-RESISTANCE

- Predicted DTG cross-resistance in RAL recipients is shown in **Figure 2**. Reflecting the lower prevalence of Q148H/R/K+G140A/S, DTG cross-resistance was significantly less common among subjects with non-B clades than in subjects with subtype B (2/46, 4.3% vs. 42/209, 20.1%; p=0.009)

Figure 2. Predicted cross resistance to DTG



CONCLUSIONS

- There exists a great diversity of HIV-1 strains circulating in Europe, largely reflecting introduction of African strains through immigration
- Major INI RAMs were not detected in INI-naïve subjects sampled in 2008-2011
- Sequence differences between HIV-1 clades influence the pathways of genotypic resistance to RAL, and prevalence of resulting cross-resistance to DTG
- Subtype B shows a higher propensity to develop G140/Q148 compared with prevalent non-B subtypes and thisis explained by different codon usage at the compensatory position G140
- These findings extend previous observations[2-6] and carry implications for the use of integrase inhibitors in regions with predominance of non-B subtypes