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LOW LEVEL OF BASELINE RESISTANCE TO INTEGRASE INHIBITORS L731,988 AND L870,810 IN RANDOMLY SELECTED SUBTYPE B AND NON-B HIV-1 STRAINS

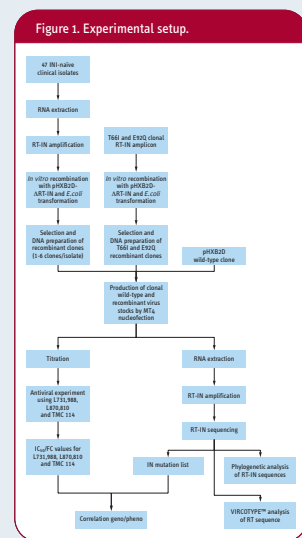
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BACKGROUND

HIV-1 integrase inhibitors (INIs) constitute a promising new class of anti-HIV drugs that act by blocking the integration of proviral DNA into the cellular genome. As INIs are currently in phase II and III clinical trials, they may soon be available in the clinic. The effect of naturally occurring polymorphisms in the IN gene on susceptibility to INIs L731,988 and L870,810 was investigated using a research phenotypic IN resistance assay.

METHODS



The experimental flow is shown in Fig. 1. Viral RNA was purified from 47 INI-naïve clinical isolates using the EasyMAG extraction platform (Biomérieux) and used to amplify the RT-RNaseH-IN region (2898 bp in HXBII, Genbank Acc Nr K03455, and referred to as RT-IN). After *in vitro* recombination of the RT-IN amplicons with an RT-RNaseH-IN-deleted HXB2D-based backbone, recombinant full-length HIV-genome clones were selected for Amara nucleofection into MT4 cells (1-6 clones/patient sample). Clonal recombinant virus stocks were tested in an antiviral experiment using INIs L731,988 and L870,810 and protease inhibitor TMC114. Wild-type HXB2D and amplicons containing the T661 or E92Q INI resistance-associated mutation were used as controls. The RT-IN region of the recombinant virus stocks was sequenced using ABI3730xl (Applied Biosystems). RT-IN sequences were aligned (ClustalW) and used for phylogenetic analysis using PHYLIP. RT-IN sequence-based clading was performed (BLAST) and finally, RT sequences were subjected to resistance testing using VircoTYPE™ analysis (version 4.1.00).

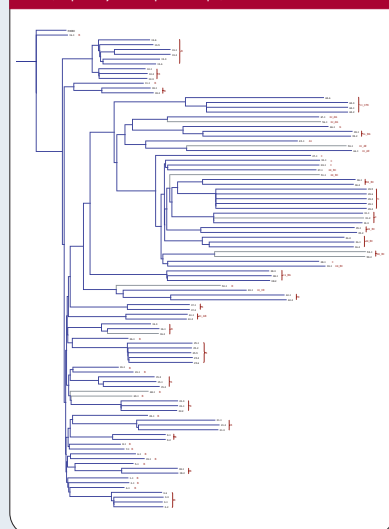
RESULTS

- From 47 patient samples, a total of 89 clonal recombinant virus stocks were selected for further genotypic and phenotypic analysis (Fig. 2 and Table 1).

Table 1. Overview of patient and clonal samples used in the analysis. The presence of RTI resistance (VircoTYPE™) is indicated by +. If applicable, the number of patient samples is shown between brackets.

clade	B	C	08_BC	14_BG	11_CPX	03_AB	D	10_CD	01_AE	A1	G	02_AG	total
n patients	26	6	5	2	1	1	1	1	1	1	1	1	47
n clones	51	11	9	5	4	2	2	1	1	1	1	1	89
RTI resistance	+(9)	+(2)	-	-	-	-	+	+	+	+	+	+	

Figure 2. RT-IN nucleotide-based phylogenetic analysis of clinical isolate-derived clonal recombinant virus stocks. Clones are numbered according to the patient sample they originate from and colored in blue or grey if phenotyping was successful or failed, respectively. For each patient sample, clades are indicated.



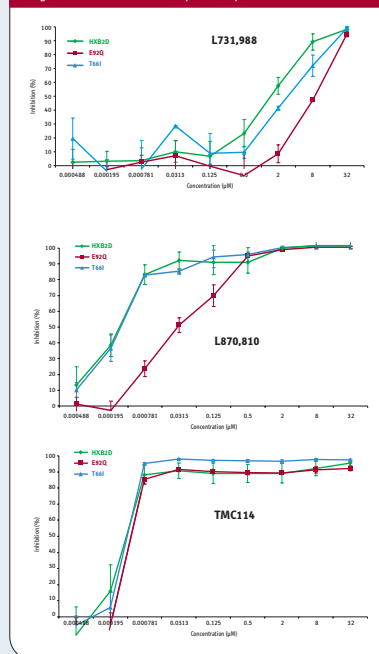
- Clade assignment based on RT-IN sequences indicated that 26 isolates were subtype B while 21 samples were non-B. The subtype B isolates contained 17 RTI inhibitor (RTI)-sensitive and 9 RTI-resistant samples while 13 RTI-sensitive and 8 RTI-resistant isolates were present in the non-B subtype group (VircoTYPE™ analysis).

- The IC₅₀s for HXB2D were 1.5 ± 0.3 μM for L731,988, 2.8 ± 0.6 nM for L870,810, and 3.9 ± 0.9 nM for TMC114 (Fig. 3). Phenotypic testing of E92Q and T661 mutants showed, respectively, a ~6-fold and a ~2.5-fold decrease in susceptibility to L731,988. Activity of L870,810 on E92Q was reduced by ~10-fold, while no fold change (FC) effect was observed on T661. Finally, E92Q and T661 mutants did not show decreased susceptibility to TMC114.

- Comparison of IC₅₀ and FC values of recombinant clones containing identical RT-IN or IN sequences showed the overall reproducibility of the antiviral experiments (Fig. 4).

- In general, patient plasma-derived recombinant virus stocks showed no baseline resistance to both INIs L731,988 and L870,810 (Fig. 4). Only 4 clones, derived from patient sample 13 (subtype B and RTI-sensitive), contained slightly increased FC values for L731,988.

Figure 3. Dose-dependent susceptibility of wild-type HXB2D and recombinant E92Q and T661 virus stocks to L731,988, L870,810 and TMC114. Data points represent the average ± standard deviation of 6 independent experiments.



- FC range of clade B samples was slightly broader than that of non-B subtype samples for L731,988 and L870,810 (Fig. 5). Further, the median FC of RTI-sensitive samples was slightly higher than the median FC of RTI-resistant samples for both L731,988 and L870,810.

- Sequence analysis of IN only revealed 39% variable positions (111 out of 288 amino acids, excluding variability at the 289 stop codon, Fig 4). None of the positions in the HHCC motif, the LEDGF/p75 interaction site, and the catalytic DDE triad were found to be polymorphic.

- Several IN polymorphisms known to be associated with INI resistance were detected: V72I, L74I, T97A, A128T, Q148H, V151I, K156N, E157Q, V165I, V201I, I203M, T206S and S230N (Fig. 4 and Table 2). However, these amino acid changes did not have a significant effect on susceptibility to L731,988 and L870,810 (Fig. 4).

Figure 5. FC distribution of patient plasma-derived recombinant clones towards L731,988, L870,810 and TMC114. Clones were classified according to clade and resistance to RTIs. Boxplots show the minimum FC, Q1 FC, median FC, Q3 FC and the maximum FC. O symbols indicate outliers (outside 1.5x interquartile range).

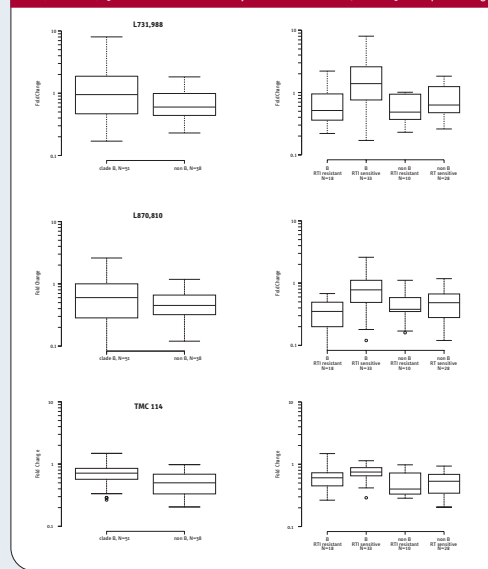


Figure 4. FC values and IN mutation list of patient plasma-derived recombinant clones. RT-based VircoTYPE™ results are indicated by + (presence of RTI resistance-associated mutations) or - (absence of RTI mutations). FC values represent the average ± standard deviation of 4 experiments. Four clones showing an increased FC for L731,988 are marked in orange. IN amino acids are aligned with the HXB2D reference sequence. The HHCC and DDE motifs are indicated in green and purple, respectively, while the LEDGF/p75 interaction site is marked in blue. The amino acids associated with INI resistance are marked in red. NR: no result.

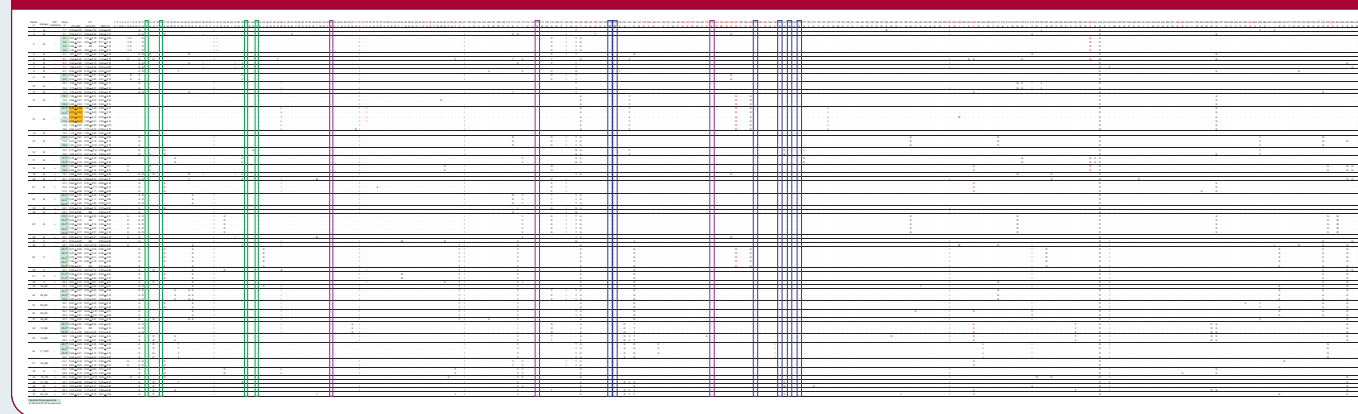


Table 2. Overview and prevalence of amino acid substitutions described to be associated with INI resistance.

	Resistance to INIs			n patients	n clones
	Natural polymorphism	Drug-associated			
H51Y		√		0	0
T66I	√	√		0	0
V72I	√	√		41	77
L74M/I	√	√		3 (I)	6
E92Q		√		0	0
T97A	√	√		2	2
F121Y		√		0	0
T125K		√		0	0
A128T		√		1	1
E138K		√		0	0
G140S/A		√		0	0
Y143R/C		√		0	0
Q146K		√		0	0
S147G		√		0	0
Q148K/R/H		√		1 (H)	1
V151I	√	√		1	1
S153Y/A		√		0	0
M154I	√	√		0	0
N155S/H		√		0	0
K156N	√	√		5	6
E157Q	√	√		3	14
K160D		√		0	0
G163R	√	√		0	0
V165I	√	√		1	4
V201I	√	√		33	48
I203M	√	√		2	2
T206S	√	√		11	18
S230R/N	√ (N)	√ (R)		4 (N)	9
V249I		√		0	0
R263K		√		0	0
C280Y		√		0	0

CONCLUSIONS

- The IC₅₀ values for wild-type HXB2D and FC values for E92Q and T66I IN mutant viruses were in concordance with earlier observations.
- Substantial genotypic variability was detected in clinical samples, but no significant FCs were observed.
- The range of INI FC values among subtype B versus non subtype B, and RTI-sensitive versus RTI-resistant isolates was similar.
- This assay contains the RT, RNaseH and IN genes, allowing the study of clinical significance of naturally occurring polymorphisms and NRTI-, NNRTI- and INI-selected variants.

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