Investigation of Integrase Inhibitor Resistance Mutations in gp41 in Clinical Samples

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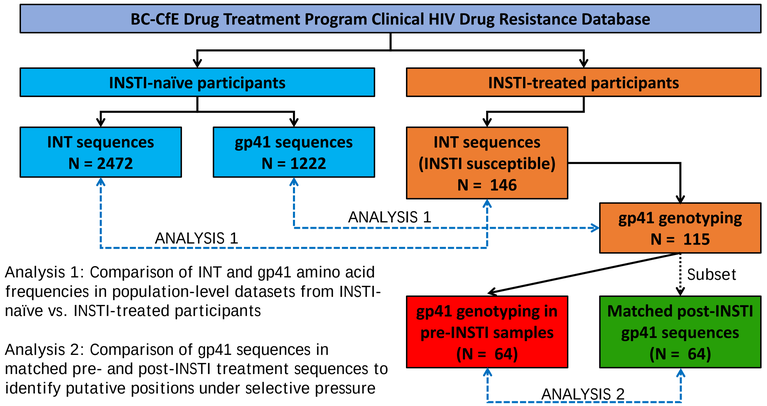
Background

- *In vitro* studies suggest that mutations conferring resistance to HIV Integrase Strand transfer inhibitors (INSTI) can occur outside integrase, including in the *env* gene
- It remains unclear whether these mutations arise frequently *in vivo*
- We sought to identify mutations in gp41 associated with exposure to INSTI *in vivo u*sing a database of clinically-derived HIV-1 sequences

Study Design and Methods

- Retrospective analysis of integrase (INT) and gp41 sequences collected during physician-requested resistance genotyping in BC-CfE Drug Treatment Program (DTP) participants with subtype B HIV-1
- Analysis 1: Population-level analysis of INT and gp41 amino acid frequencies in INSTI-naïve and INSTI-treated participants
  - The INSTI-treated group comprised 146 individuals who had been exposed to INSTI for ≥3 months, but had detectable plasma viremia, where INT resistance genotyping indicated that their virus was susceptible to all INSTI (Stanford HIVdb v8.8; score <15)
  - HIV-1 gp41 genotyping was performed on these same samples (115 successfully sequenced)
  - The INSTI-naïve comparison groups comprised independent datasets of subtype B INT (N=2472) and gp41 (N=1222) sequences from INSTI-naïve individuals who had undergone clinical resistance genotyping
  - We compared amino acid frequencies at all INT and gp41 codons between INSTI-naïve and INSTI-treated participants; Fisher's exact test was used to identify Amino Acids significantly over- or under-represented between groups. Multiple comparisons were addressed using the Benjamini-Hochberg method (q-values)
- Analysis 2: Identification of putative INSTI resistance mutations in gp41 selected by INSTI treatment
  - Pre-INSTI gp41 genotyping for INSTI-treated group: Archived plasma collected prior to INSTI exposure were identified for all 115 INSTI-treated participants described above
  - gp41 genotyping was successful in 64 pre-INSTI samples
  - Pre- and post-INSTI treatment sequences from each participant were compared; mutations putatively selected for ("gained") and against ("lost") during INSTI treatment were identified in each sequence pair
  - The frequency of mutations "gained" and "lost" during INSTI treatment was summarized

Participant selection, data collection and analysis



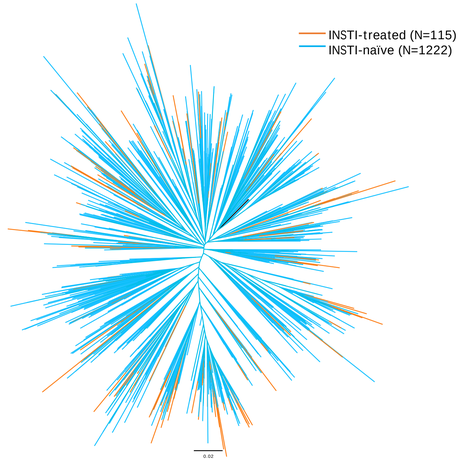
Analysis 1: Comparison of INT and gp41 amino acid frequencies in population-level datasets from INSTI-naïve vs. INSTI-treated participants

Analysis 2: Comparison of gp41 sequences in matched pre- and post-INSTI treatment sequences to identify putative positions under selective pressure

Participant demographic and clinical characteristics (Analysis 1)

Variable	INSTI-naïve (N=1222)	INSTI-treated (N=115)
Age (Years); Median [IQR]	38 [32.5 - 44.9]	51 [44.3 - 56.1]
Male Sex; N (%)	1064 (87%)	82 (71%)
Female Sex; N (%)	148 (12%)	33 (29%)
Unknown Sex; N (%)	10 (1%)	0 (0%)
Plasma Viral Load (log_{10} copies/mL); Median [IQR]	5.0 [4.5 - 5.3]	3.6 [2.9 - 4.5]
CD4 (cells/μL); Median [IQR]	290 [140 - 430]	350 [195 - 580]
raltegravir; N (%)	-	60 (52%)
elvitegravir; N (%)	-	34 (30%)
dolutegravir; N (%)	-	55 (48%)
Cumulative INSTI exposure (Months); Median [IQR]	-	32 [13 - 56]

Maximum likelihood phylogeny of gp41 sequences from INSTI-naïve and INSTI-treated participants (Analysis 1)



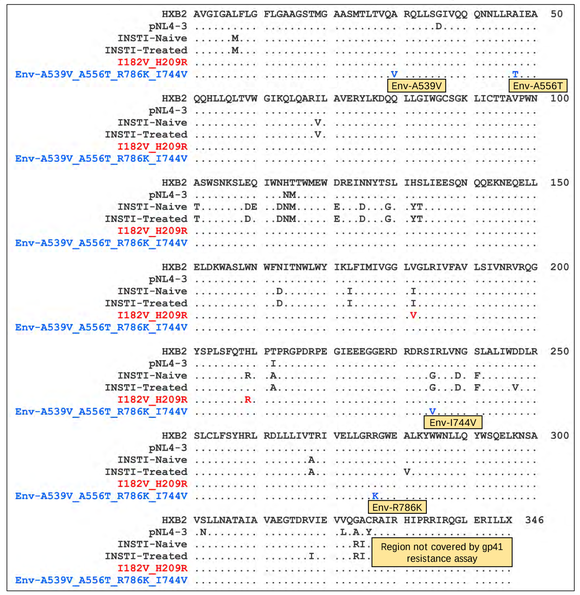
Phylogenetic tree of HIV subtype B gp41 sequences from INSTI-naïve (blue) and INSTI-treated (orange) participants. Sequences from both groups are dispersed throughout the tree and do not substantially cluster by group.

gp41 polymorphisms in INSTI-naïve and INSTI-treated participants (Analysis 1)

AA	AA frequency (%)		OR	p-value	q-value
	INSTI-naïve	INSTI-treated			
I182V	50.3%	28.7%	0.40	9.1×10^{-6}	0.0085
H209R	52.5%	33.9%	0.47	1.9×10^{-4}	0.086

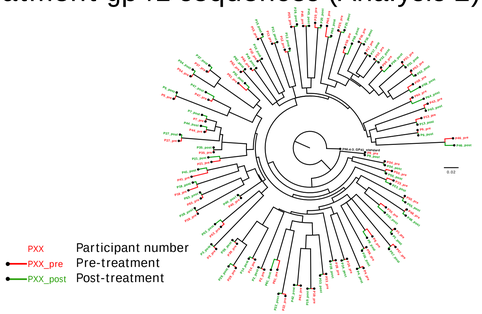
Amino acids with statistically significant (q<0.2) differences in frequency between INSTI-treated vs. -naïve individuals identified in Analysis 1. gp41 polymorphisms I182V and H209R were underrepresented in INSTI-treated compared to INSTI-naïve individuals. No significant differences in AA frequencies were observed in INT.

gp41 consensus sequences and residues putatively associated with INSTIs (Analysis 1)



gp41 amino acid alignment of HXB2, pNL4-3, and the consensus sequences of the INSTI-naïve and INSTI-treated groups. gp41 polymorphisms identified in Analysis 1 are in red (I182V_H209R). gp41 amino acids selected by INSTI *in vitro* (Env-A539V_A556T_R786K_I744V) identified in Van Duyne *et al. (PNAS, 2019)* are in blue. The gp41 resistance assay does not cover the final 20 amino acids of gp41.

Phylogeny relating pre- and post-INSTI treatment gp41 sequences (Analysis 2)



Phylogenetic tree of paired subtype B gp41 sequences collected before (red) and after (green) INSTI treatment. Pairing of pre- and post-therapy sequences from the same participants confirms that no obvious contamination or mix-ups occurred.

Putative sites in gp41 under selection during INSTI treatment (Analysis 2)

gp41 Codon	AA	Population frequency (%)	# gained	# lost
304	F	29.9%	6	5
122	R	71.4%	5	6
129	G	42.7%	5	3
157	N	25.7%	5	3
109	D	48.0%	4	4
182	V	50.3%	1	1
209	R	52.5%	1	1
30	V	0.15%	0	0
47	T	0.08%	0	0
235	V	8.8%	0	1
277	K	0.4%	0	1

Amino acids "gained" or "lost" in ≥4 participants while receiving INSTI-based therapy

Amino acids identified in Analysis 1

Env-A539V, A556T, R786K, I744V from Van Duyne *et al. (PNAS, 2019)*

Amino acids "gained" or "lost" in ≥4 participants in a comparison of gp41 sequences collected before and after INSTI treatment (grey shading). Sites under putative INSTI selection pressure were highly polymorphic in the INSTI-naïve population. Gain/loss of amino acids identified in Analysis 1 (pink shading) and by Van Duyne *et al* (orange shading) are also shown.

Conclusions

- gp41 substitutions previously associated with INSTI resistance *in vitro* were not enriched in INSTI-treated individuals vs. INSTI-naïve controls, nor were they observed to be selected during INSTI treatment
- Comparison of gp41 sequences from INSTI-naïve vs. INSTI-treated individuals identified two amino acids that were significantly underrepresented among the INSTI-treated group (Analysis 1), but these common polymorphisms were not frequently "lost" during INSTI treatment (Analysis 2)

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