CROI-0271: Vulnerable targets in HIV-1 Pol for attenuation-based vaccine design

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Introduction

CD8+ T cell mediated escape mutations in Gag can reduce viral replication and alter HIV-1 disease progression, but less is known about immune-mediated attenuation in other HIV-1 proteins. Identification of viral mutations that compromise HIV's ability to replicate may aid rational vaccine design.

Here, we aimed to investigate whether specific amino acid mutations are associated with altered RT-integrase fitness and its impact on the clinical course of HIV infection, using a large population of individuals infected with HIV-1 subtype C, the most prevalent subtype world-wide.

Methods

We generated 487 recombinant viruses encoding RT-integrase sequences from individuals with chronic (n = 406) and recent (n = 81) HIV-1 subtype C infection and measured their *in vitro* RC using a GFP-reporter T-cell assay.

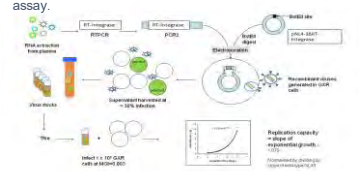


Figure 1: **Measurement of replication capacity.**
Summary of methods for measuring viral replication capacity using an HIV inducible green fluorescent protein reporter T-cell line.

Phylogenetic analysis

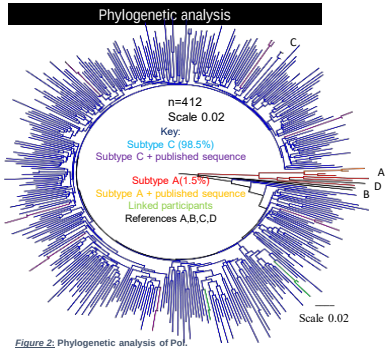


Figure 2: **Phylogenetic analysis of Pol.**
Maximum likelihood tree showing the phylogenetic relatedness of 412 Pol

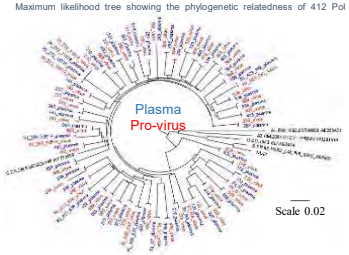


Figure 3: **Phylogenetic relatedness of plasma and recombinant viral sequences of Pol.** The prefix "RI" indicates sequences derived from recently infected individuals, with the rest derived from chronically infected individuals. Blue indicates the bulk plasma sequences while red indicates the recombinant virus sequences.

Pol-driven RC is clinically relevant

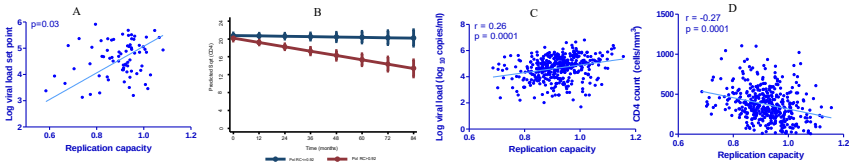


Figure 4: **Correlation of RT-integrase RC with markers of disease progression**
The RC of the recombinant viruses encoding RT-integrase derived from individuals with recent infection correlate positively with subsequent viral load set point (A), and higher RC corresponds to a faster rate of CD4 decline (GEE model, p<0.001) (B). The RC of viruses from chronic infection correlates positively with the plasma log viral load (C) and negatively with the CD4 count (D).

Amino acid variants associated with altered RC

Table 1: Codon by codon analysis of Pol RT(A) and Integrase (B) HIV-1 subtype C sequences from recent and chronic infection cohorts combined, p<0.005, q<0.4.

Pol (RT) Codon	AA	Median Replication with aa	Median Replication without aa	# viruses with aa	# viruses without aa	P-value	q-value	A-list epitope*
V8	V	0.92	0.98	468	14	0.006	0.19	IL8 (B*40:01)[#]
V8	I	0.98	0.92	14	468	0.006	0.19	
E22	K	0.92	1.01	473	6	0.005	0.19	GL9 (B*08)
E22	R	1.01	0.92	6	473	0.005	0.19	
A36	E	0.94	0.92	123	336	0.003	0.19	AK11 (A*02:01/A*03:01)
T48	T	0.92	0.95	458	22	0.021	0.37	Flank AK11 (A*02:01/A*03:01)
A158	A	0.92	0.88	453	25	0.001	0.19	LA10 (B*54:01); SM9 (B*07); AK9 (A*03:01/A*11:01)[#]
A158	S	0.88	0.92	25	453	0.001	0.19	
A200	E	0.98	0.92	9	467	0.021	0.37	Flank IL9 (B*40:01)
V241	V	0.92	0.82	479	5	0.005	0.19	Flank IW9 (B*57:01)
V241	I	0.82	0.92	5	479	0.005	0.19	
I257	I	0.92	0.82	475	7	0.018	0.35	Flank IW9 (B*57:01) and LY12 (B*15:01)
I257	V	0.80	0.92	5	477	0.008	0.22	
P272	P	0.93	0.91	350	128	0.002	0.19	QR9 (A*03:01); YL9 (B*42:01)[#]
P272	K	0.84	0.92	5	473	0.013	0.28	
K311	K	0.92	0.89	458	17	0.012	0.28	IY10 – (A*02:01, B*15:01, C*12:02)
M357	R	0.93	0.92	171	299	0.014	0.30	RK11 – (A*33:02, A*03:01)

Pol (Integrase)	AA	Median Replication with aa	Median Replication without aa	# viruses with aa	# viruses without aa	P-value	q-value	A-list epitope
I50	M	0.93	0.91	152	280	0.010	0.25	
I50	I	0.91	0.93	253	179	0.027	0.30	
I84	I	0.93	0.90	363	97	0.001	0.10	HA9 (B*54:01); IT10 (B*40:02)
I84	M	0.91	0.93	89	371	0.011	0.25	
Y100	Y	0.92	0.93	346	121	0.014	0.25	
Y100	F	0.93	0.92	118	349	0.027	0.30	
K111	K	0.92	0.97	469	11	0.022	0.28	Flank HF8 (C*05)
V112	I	0.92	0.89	445	26	0.036	0.36	Flank HF8 (C*05)
S119	S	0.92	0.93	403	75	0.015	0.25	HF8 (C*05), Flank SW10 (B*57)
Q136	K	0.88	0.92	28	368	0.028	0.33	IY9 (B*1503)[#]
I201	V	0.83	K311R [50]	K311E [52]	404	0.040	0.36	GI8 (B*4002)
I208	R	0.92	0.86	397	8	0.009	0.22	IK9 (A*11)
K211	K	0.93	0.89	414	59	0.005	0.24	
K269	R	0.91	0.93	197	238	0.016	0.25	RY9 (B*15:03), Flank VI9 (B*42)

RT-integrase: I257V, P272K, P272S, K311R and I208R. Integrase the non-consensus variants were at codons I201V. These are relatively uncommon polymorphisms (all <1.5% frequency in our dataset) occurring in or adjacent to optimally-described HLA-restricted CTL epitopes, and were associated with the lowest RC.

Confirmation of AA associated with altered RC

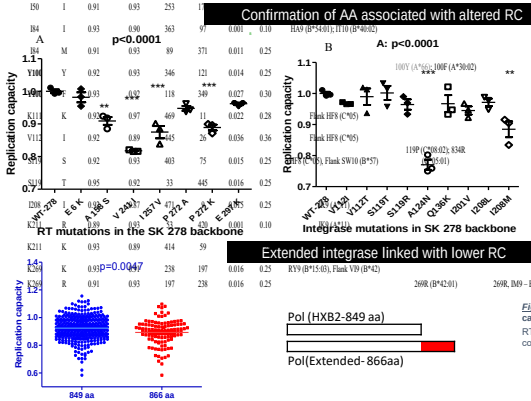


Figure 5: **Replication capacities of NL4-3 recombinant viruses encoding wild-type (WT) SK278 RT-integrase and SK278 with RT (A) and integrase mutations(B).** RT mutations A158S, V241I, I257V and P272K while integrase mutations 124N and 208M significantly reduced RC expressed relative to the SK278 wild-type.

Extended integrase linked with lower RC

Figure 6: **Length of RT-integrase sequence impacts replication capacity (RC).**
RT-integrase sequences of 866 amino acids (AA) had a lower RC compared to those of the same length as HXB2 (849 AA).

Phylogenetic analysis of extended integrase

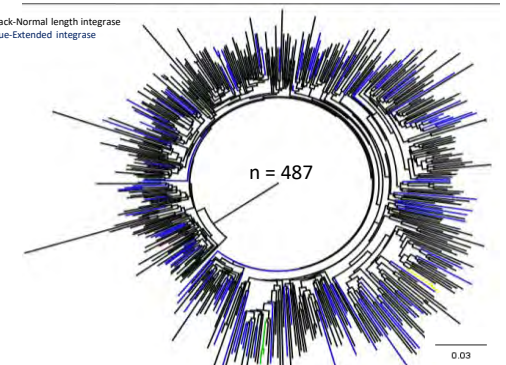


Figure 7: **Phylogenetic distribution of normal and extended integrase sequences of Pol.** Blue indicates the extended while red indicates the normal length integrase sequences. Yellow (normal length) and green (extended) highlights related/familial sequences.

Confirmed Low RC of extended NL4-3 integrase mutant

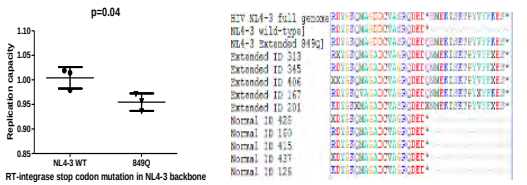


Figure 8: **Replication capacities of NL4-3 wild-type virus and the NL4-3 extended integrase mutant**
Replication capacities of wild-type NL4-3 virus and NL4-3 virus encoding extended integrase, conferred by the 849Q mutation. The extended integrase mutant displayed significantly lower RC compared to the wild-type (Student's t-test p =0.04).

Conclusion

Together, our data suggest that RT-integrase-driven RC is clinically relevant, and provide evidence that immune-driven selection of mutations in RT-integrase can compromise RC

- Identification of the specific RT-integrase amino acid variants that are significantly associated with altered viral RC, support a role for immune-driven mutations in Pol to modulate HIV replication
- RT-integrase variants in vital domains of the RT palm (158S) and RT thumb (241I and 257V) represent potential vulnerable targets for an attenuation-based vaccine.
- The mutagenesis experiments confirms the impact of the identified RT mutations on the vital process of viral reverse transcription
- Extended integrase is associated with decreased replication capacity

Acknowledgements

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