EFFECT OF L74I POLYMORPHISM ON FITNESS OF HIV-1 SUBTYPE A6 RESISTANT TO CABOTEGRAVIR

Poster # 102

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ABSTRACT

Background: In phase 3 clinical trials of long-acting (LA) Cabotegravir (CAB) and rilpivirine (RPV), subtype A6 virus, characterized by the L74I IN polymorphism, was associated with an increased risk of virologic failure. We investigated the effect of L74I on the viral replication capacity of recombinant viruses carrying this in combination with various INSTI resistance mutations.

Methods: Plasmids carrying HIV-1 NL4- deleted in pol were co-transfected into 293T cells together with IN from HIV-1 A6 carrying 74L or I in combination with various INSTI resistance mutations including G118R, G140R, Q148H, Q148K, Q148R, N155H and R263K, and the double mutation G140R/Q148R to generate replication-competent recombinant viruses. Resulting virus stocks were titered on TZM-bl cells and replication capacity (RC) determined in the absence of and presence of CAB were performed in TZM-bl and MT-2 cells. Drug susceptibility to CAB was determined using a standard drug susceptibility testing protocol.

Results: Susceptibility to CAB of recombinant HIV-1 expressing the subtype A6 IN was similar whether L or I was present at position 74 (IC50=1.36 nM and 1.10 nM, respectively). Recombinant viruses with 74L or 74I showed similar replication capacity on TZM-bl and MT-2 cells in the absence and presence of CAB (2 nM). In the absence of CAB, viruses carrying 74I outcompeted 74L viruses in growth competition assays, demonstrating greater fitness of L74I in an A6 IN context. Recombinants carrying the L74I polymorphism had significantly higher replication capacity in TZM-bl and MT-2 cells when present together with the G118R, G140R, Q148R and R263K INSTI resistance mutations; no significant difference in replication was observed for the Q148H or K mutants. Surprisingly, the opposite effect was observed with respect to N155H mutant, in which case the 74L variant showed greater replication capacity than 74I. Double mutants carrying G140R in combination with Q148R replicated too poorly in the context of either 74L or I to allow formal assessment of replication capacity.

Conclusions: Presence of the L74I polymorphism conferred greater replication capacity to recombinant viruses expressing HIV-1 A6 IN when present together with INSTI resistance mutations at positions 118, 140, 148 and 263. This finding may explain, in part, the association of HIV-1 subtype A6 and virologic failure observed in clinical trials of CAB-LA in combination with RPV-LA.

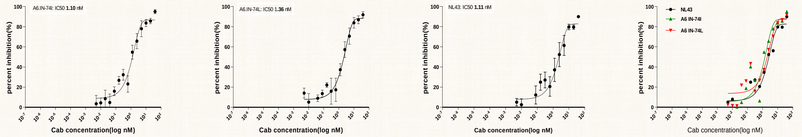
METHODS

- An HIV-1 IN gene based on the A6 consensus sequence (A6.IN) was synthesized by GENEWIZ, Inc and used to construct a plasmid containing HIV-1_{NL4-3} *pol* in which the IN-coding region was replaced by A6.IN.
- Major CAB resistance mutations in the A6.IN backbone were introduced by site-directed mutagenesis. Infectious recombinant viruses carrying the pol gene of interest were generated by co-transfection with a plasmid carrying an HIV-1_{NL4-3} backbone deleted in *pol* together with the PCR-amplified *pol* into 293T cells. Resulting virus stocks were titered on TZM-bl cells and equal amounts of infectious virus used to infect TZM-bl or MT-2 cells to determine CAB susceptibility, replication capacity and replication kinetics.
- Replication capacity (RC) in the absence or presence of 2 nM CAB was determined by quantifying virus production at **XX** days in TZM-bl and MT-2 cells by β-galactosidase assay or p24 ELISA, respectively. Relative replication capacity was expressed as the percent viral production compared to wildtype HIV-1_{NL4-3} virus
- Replication kinetics were determined in MT-2 cells by quantifying virus production over time by p24 ELISA.
- Viral fitness of recombinants carrying A6.IN.74I or A6.IN.74L were compared by growth competition assays as described (Hu Z, Kuritzkes DR. J Virol 2014; 88:9268-76). Cultures were inoculated with different proportions of the 74I and 74L variants. The proportion of the two variants over time was quantified by qPCR assay.

RESULTS

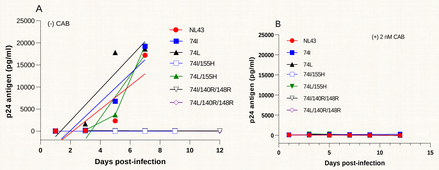
Recombinant HIV-A6.IN.74I/74L susceptibility to Cab

Figure 1. Susceptibility to CAB of HIV-1 recombinants carrying the A6.IN.74I and A6.IN.74L variants, respectively, was determined by a standard drug susceptibility assay in TZM-bl cells.



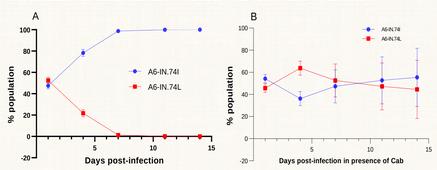
Replication Kinetics Assay

Figure 2. Replication kinetics of recombinant viruses 74I, 74L, 74I/155H, 74L/155H, 74I/140R/148R and 74L/140R/148R in absence and presence of 2 nM CAB.



Growth Competition Assay

Figure 3. Growth competition assays comparing the fitness of recombinants carrying A6.IN.74I vs A6.IN.74L in the absence (A) or presence (B) of 2 nM CAB. The data shown here from 1:1 ratio inoculation of recombinant viruses 74I.A6.IN and 74L.A6.IN.



CONCLUSIONS

- Susceptibility to CAB of recombinant HIV-1 expressing the subtype A6 IN was similar whether L or I was present at position 74.
- 74L or 74I variants showed similar replication capacity on TZM-bl and MT-2 cells in the absence and presence of 2 nM CAB. In the absence of CAB, viruses carrying 74I outcompeted 74L variants in growth competition assays, suggesting greater fitness of L74I in an A6 IN context.
- Presence of the L74I polymorphism conferred greater replication capacity to recombinant viruses expressing HIV-1 A6 IN when present together with INSTI resistance mutations at positions 118, 140, 148 and 263. This finding may explain, in part, the association of HIV-1 subtype A6 and virologic failure observed in clinical trials of CAB-LA in combination with RPV-LA.
- Surprisingly, the opposite effect was observed with respect to N155H mutant, in which case the 74L variant showed greater replication capacity than 74I. Further analysis and clinical correlation are needed to understand the significance of this finding.

Replication capacity assay in TZM-bl and MT2 cells (1)

Figure 4. Replication capacity of HIV-recombinants carrying 74I, 74L, 74I/155H, 74L/155H, 74I/140R/148R or 74L/140R/148R substitutions in A6.IN was determined in TZM-bl cells (A, B) or in MT-2 cells (C, D) in absence (A, C) and presence (B, D) of 2 nM CAB. The y-axis shows the viral production as assessed by β-galactosidase assay (A,B) or p24 ELISA (C,D). Data shown are the means ± S.D. of triplicate experiments. Table 1 shows the replication capacity of the variants as compared to wild-type HIV-1_{NL4-3} in the absence or presence of 2 nM CAB.

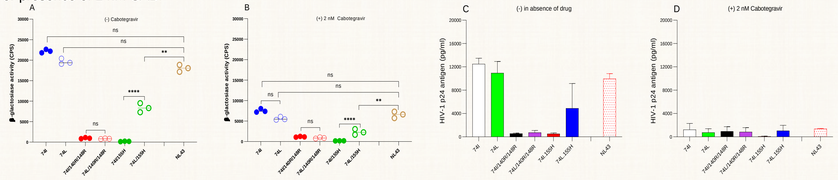


Table 1. Relative replication capacity of the mutant in absence and presence of 2 nM Cab in TZM-bl cells and in MT2 cells (1)

Relative RC	***In TZM-bl***	NL43	74I	74L	74I/155H	74L/155H	74I/140R/148R	74L/140R/148R	***In MT=2***	NL43	74I	74L	74I/155H	74L/155H	74I/140R/148R	74L/140R/148R
(-) Cab		100.0	123.2	108.9	0.9	46.4	5.4	4.6		100.0	125.5	110.0	5.0	73.6	5.6	7.2
2 nM Cab		100.0	115.0	85.6	2.7	35.4	17.7	13.4		100.0	134	81.3	7.5	113.9	102.2	92.2

Replication capacity assay in TZM-bl cells (2)

Figure 5.. Replication capacity (RC) assays in TZM-bl cells of HIV-1 recombinants carrying 74I or 74L together with the G118R, G140R, Q148R, N155H and R263K INSTI resistance mutations in A6.IN in absence (A) or presence of 2 nM (B) or 4 nM (C) CAB. The y-axis shows the viral production as assessed by β-galactosidase assay. Data shown are the means ± S.D. of triplicate experiments (p* < 0.05, p** < 0.005, p*** or p**** < 0.001). Table 2 shows the replication capacity of the variants as compared to wild-type HIV-1NL4-3 in the absence or presence of 2 nM CAB.

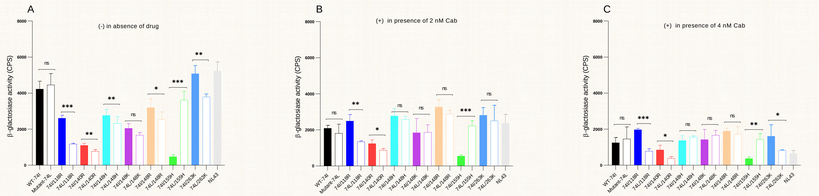


Table 2. Relative replication capacity of the mutant in absence and presence of 2 nM Cab in TZM-bl cells (2)

Relative RC	NL43	74I	74L	74I/118R	74L/118R	74I/140R	74L/140R	74I/148H	74L/148H	74I/148K	74L/148K	74I/148R	74L/148R	74I/155H	74L/155H	74I/263K	74L/263K
(-) Cab	100.0	80.8	85.3	**49.9**	**22.5**	21	14.7	**52.8**	**44.4**	39.2	32	**60.9**	**48.6**	9	**69.1**	**97.1**	72.5
2 nM Cab	100.0	88.1	76.1	**104.8**	**56.8**	52.1	37.1	**116.8**	**108.5**	77.6	78.7	**137.8**	**121.1**	22.8	**93.6**	**118.6**	105.4

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