HIV-1 resistance mutations to integrase inhibitors impair both integration and reverse transcription steps

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Highlights
- INSTIs resistant mutations affect both reverse transcription and integration steps.
- R263K mutation impaired mostly the reverse transcription.
- N155H was associated with a drastic decrease of both steps.
- G140S/Q148H impaired only integration.

Reverse transcription and integration are key steps of the Human Immunodeficiency Virus type 1 (HIV-1) replication, respectively performed by the viral enzyme's reverse transcriptase (RT) and integrase (IN). Interactions between these two enzymes are critical: IN improves both reverse transcription early steps and processivity, while the RT enhances the integrase strand transfer activity [1]. The use of integrase strand transfer inhibitors (INSTIs) has led to emergence of resistant viral mutants, occurring mostly in the integrase gene. The most common resistance mutation patterns are R263K, N155H and G140S/Q148H [1]. Most of them exhibit an impaired integration compared to wild-type (WT) viruses [2]. However, their impacts on reverse transcription efficiency remain unclear.

The purpose of this study was to evaluate the impact of three INSTI resistance-associated mutation profiles, R263K, N155H and G140S/Q148H, on both reverse transcription and integration.

HIV-1 mutants were produced from the pNL4-3 plasmid using the QuikChange II XL kit (Agilent) and the following primers: R263K-F [5'-GACATAAAAGTAGTGCCAAGAAAAAAAGCAAAGATCATCAGGGAT-3'], R263K-R [5'-ATCCCTGATGATCTTTGCTTTTTTTCTTGGCACTACTTTTATGTC-3'], N155H-F [5'-CCAAAGT CAAGGAGTAATAGAATCTATGCATAAAGAATTAAAGAAAATTATAGGACA-3'], N155H-R [5'TGTCCTATAATTTTCTTTAATTCTTTATGCATAGATTCTATTACTCCTTGACTTTGG-3'], G140S-F1 [5'-GGCGGGGATCAAGCAGGAATTTAGTATTCCCTACAATCC-3'], G140S-R1 [5'-GGATTGTAGGGAATACTAAATTCCTGCTTGATCCCCGCC-3'], G140S/Q148H-F2 [5'-CATTCC CTACAATCCCCAAAGTCATGGAGTAATAGAATCTA-3'] and G140S/Q148H-R2 [5'-TAGATT CTATTACTCCATGACTTTGGGGATTGTAGGGAATG-3']. HIV-1 virus stocks were prepared using 293T cells transfected with GeneJuice® Transfection Reagent (Sigma). Supernatants were filtered through a 0.45 µm-pore-size-filter. Residual plasmids were eliminated through ultracentrifugation (50,000 x g for 2h) and treatment with DNase (ThermoFisher). HIV-1 p24 antigen was quantified using VIDAS p24 II kit (BioMerieux) and supernatants were stored at -80°C. SupT1 cells were infected with 120 ng of p24 antigen per 10^6 cells corresponding to a multiplicity of infection of 0.3. Cells were harvested at different times post infection (p.i.) and frozen at -80°C. Total cell DNA was extracted using the Monarch High Molecular Weight DNA for Cells & Blood (New England Biolabs).

Early (minus-strand strong-stop DNA) and late (double-strand DNA) stages of reverse transcription were quantified as previously described [3], using the digital droplet Supermix for Probes (no dUTP) on a Qx200 platform (BioRad). Genomic DNA, including integrated HIV DNA, was separated from unintegrated HIV DNA using agarose gel migration [4]. Generic HIV DNA Cell kit (Biocentric) was used to quantify HIV-DNA on the LightCycler480 (Roche) platform, and results were normalized using the albumin gene.

Normality was assessed using the Shapiro-Wilk test. Multiple comparisons were performed using One-way ANOVA and Tukey tests using GraphPad Prism 9.0.0.121.

Every INSTI resistance associated mutation profile induced a decrease of early reverse transcription products (Fig 1A). The G140S/Q148H mutant was associated with a 1.5-fold reduction compared to WT, while the R263K and N155H mutants exhibited a 2.7 and 3.8-fold decrease, respectively (Fig 1A). Late products of the reverse transcription were less altered with G140S/Q148H mutant, with a no significant 1.2-fold reduction compared to the WT (Fig 1B). R263K and N155H mutations had the same significant decrease than described for early products compared to wild type.

At 24h p. i., N155H and G140S/Q148H mutants had a significantly important decrease with respectively a 14- and 8-fold reduction compared to WT. For R263K mutant, this decrease was less important, but still significantly compared to WT (Fig 1C). At 72h p.i., integration of all mutants was also altered (Fig 1D). We observed a lightly decline for R263K mutant compared to the value at 24h p. i.. N155H mutation was associated with a 50-fold reduction of integrated DNA compared with WT. The G140S/Q148H mutant exhibited a 5-fold increase compared with 24h, without reaching the WT level. For this mutant, the integration might start with a delay.

This study described the negative impact on two early steps of HIV-1 replication in viruses carrying INSTI resistance mutations, by studying reverse transcription products and kinetics of integrated DNA. This impact was dependent on the type of INSTI resistance mutation profiles. R263K had a major effect on reverse transcription and a weaker impact on integration. N155H mutation strongly affected both steps. Finally, the G140S/Q148H double mutation profile was associated with a weak impact on reverse transcription and a more important impact on integration compared to WT. This mutation impacted mostly the integration step on a time-dependent manner.

These results could be explained by the important interactions between RT and IN [1]. They were also consistent with the fact that mutant viruses have a lower fitness than WT [5] expressed here by a less efficient reverse transcription or/and integration. It might also partially explain *in vivo* the switch observed during INSTI associated virological failure episodes, with first a viral population harboring a N155H mutation, followed by the selection of G140S/Q148H double mutant [2].

In conclusion, INSTI resistance mutations had varying impacts on reverse transcription and integration in resistance pattern manner. These observations might contribute to explain the loss of fitness observed in INSTI resistant mutants during virological failure.

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Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

[1] Tekeste SS, Wilkinson TA, Weiner EM, Xu X, Miller JT, Le Grice SFJ, et al. Interaction between Reverse Transcriptase and Integrase Is Required for Reverse Transcription during HIV-1 Replication. J Virol 2015;89:12058–69. https://doi.org/10.1128/JVI.01471-15.

[2] Delelis O, Malet I, Na L, Tchertanov L, Calvez V, Marcelin A-G, et al. The G140S mutation in HIV integrases from raltegravir-resistant patients rescues catalytic defect due to the resistance Q148H mutation. Nucleic Acids Res 2009;37:1193–201. https://doi.org/10.1093/nar/gkn1050.

[3] Mbisa JL, Delviks-Frankenberry KA, Thomas JA, Gorelick RJ, Pathak VK. Real-time PCR analysis of HIV-1 replication post-entry events. Methods Mol Biol 2009;485:55–72. https://doi.org/10.1007/978-1-59745-170-3_5.

[4] Koelsch KK, Liu L, Haubrich R, May S, Havlir D, Günthard HF, et al. Dynamics of total, linear nonintegrated, and integrated HIV-1 DNA in vivo and in vitro. J Infect Dis 2008;197:411–9. https://doi.org/10.1086/525283.

[5] Hu Z, Kuritzkes DR. Effect of raltegravir resistance mutations in HIV-1 integrase on viral fitness. J Acquir Immune Defic Syndr 2010;55:148–55. https://doi.org/10.1097/QAI.0b013e3181e9a87a.

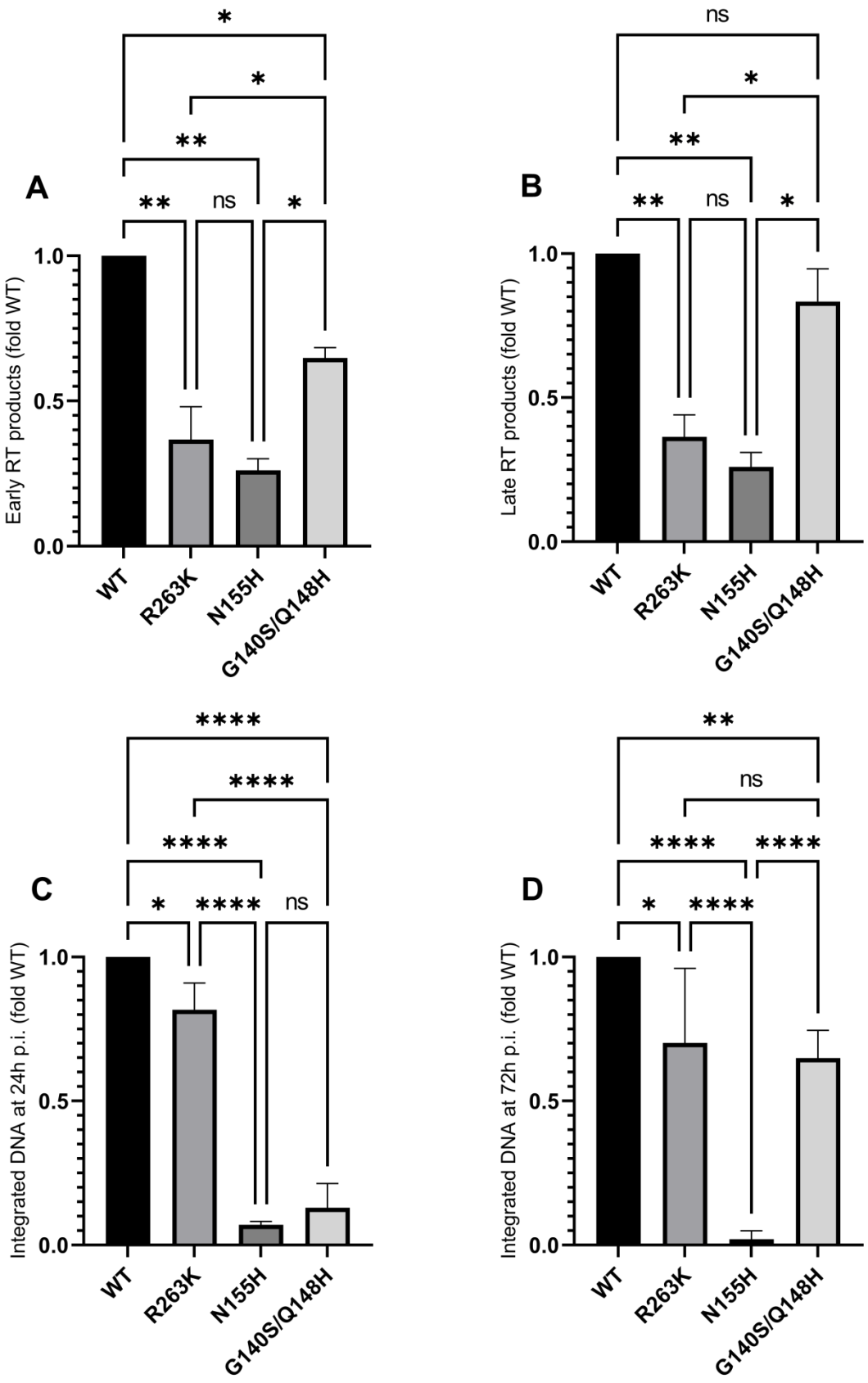

Fig 1. Effect of INSTI associated resistance on HIV reverse transcription (A, B) and integration (C, D) steps.

A. Quantification of early reverse transcription products at 24h p. i. for WT, R263K, N155H and G140S/Q148H. **B.** Quantification of late reverse transcription products at 24h p. i. for WT, R263K, N155H and G140S/Q148H. **C.** Quantification of integrated DNA at 24h p. i. for WT, R263K, N155H and G140S/Q148H. **D.** Quantification of integrated DNA at 72h p. i. for WT, R263K, N155H and G140S/Q148H. * $p<0.05$; ** $p<0.01$; **** $p<0.0001$; ns (no significant). Results are expressed as a fold change relative to WT.