HIV-1 Resistance Mutations to Integrase Inhibitors Impair Both Integration and Reverse Transcription

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Pauline Ratouit [1], Vincent Guiraud [1], Isabelle Malet [1], Cathia Soulie [1], Jerome Denis [2], Ronan Legrand [2], Elisa Teyssou [1], Anne-Genevieve Marcelin [1], **Vincent Calvez** [1].

[1] Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpitaux Universitaires Pitié Salpêtrière – Charles Foix, Laboratoire de Virologie, F-75013 Paris, France.
[2] AP-HP, Hôpitaux Universitaires Pitié Salpêtrière – Charles Foix, Laboratoire de Biochimie Endocrinienne et Oncologie, F-75013 Paris, France.

BACKGROUND

Reverse transcription and integration are key steps of the Human Immunodeficiency Virus type 1 (HIV-1) replication, performed respectively by the viral enzymes 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. The use of integrase strand transfer inhibitors (INSTIs) has led to the emergence of resistant viral mutants, occurring mostly in the integrase gene. Most of them exhibit an impaired integration compared to wild-type (WT) viruses. However, their impacts on reverse transcription efficiency remain unclear.

Our objective was to determine the impact of three of the main INSTI-associated resistance mutation profiles, R263K, N155H and G140S/Q148H on reverse transcription and kinetics of integration.

METHODS

- In-vitro infections with wild-type and INSTI resistant viruses R263K, N155H and G140S/Q148H, produced by site-directed mutagenesis.
- Early and late reverse transcription products were measured by quantitative digital-droplet PCR.
- Integrated HIV DNA was separated from unintegrated using agarose gel migration.
- Kinetic of integration was analyzed using quantitative PCR on HIV DNA integrated forms at 24- and 72-hours post infection.

- **Integrase inhibitor 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, on a time dependent manner.

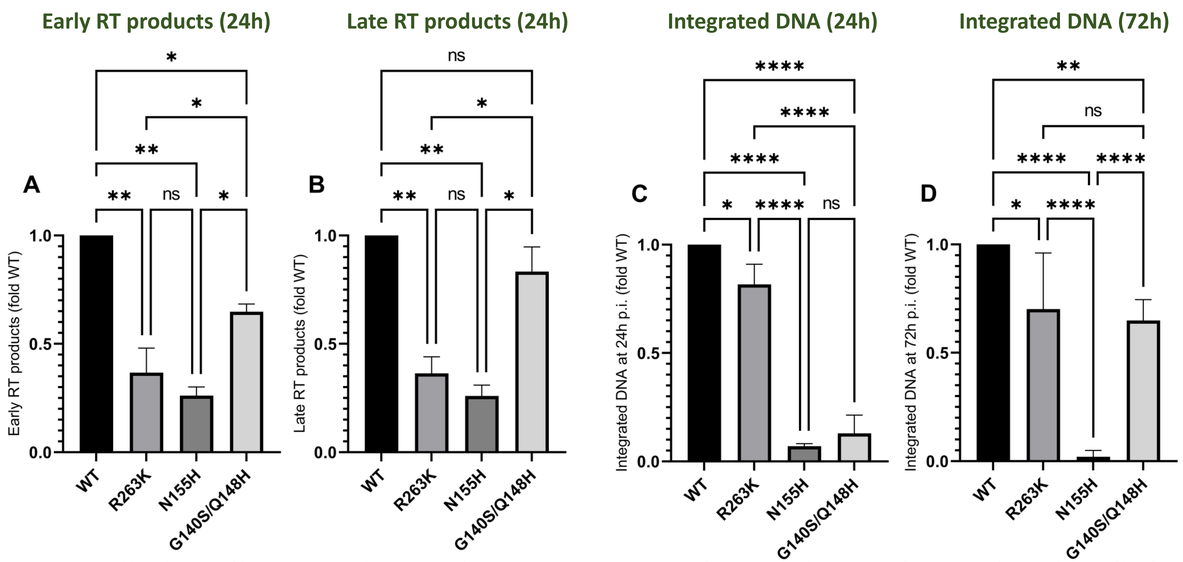

Figure: Effect of INSTI associated resistance on HIV reverse transcription (A, B) and integration (C, D)

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.

RESULTS

- **R263K** mutation had a major effect on reverse transcription and a weaker impact on integration.
- **N155H** mutation strongly affected reverse transcription (Figure 1A and B) and integration.
- The **G140S/Q148H** double mutant profile was associated with a weak impact on reverse transcription and a more important impact on integration compared to WT.

CONCLUSIONS

- INSTI resistance mutations altered integration efficiency but also the reverse transcription step.
- This phenomenon could be explained by the cross-talk between these two enzymes during HIV-1 replication.
- These observations might contribute to explain the loss of fitness observed in INSTI resistant mutants during virological failure
- These findings might contribute to explain the *in vivo* 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.

ADDITIONAL KEY INFORMATION

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Author Contact information: Vincent.calvez@aphp.fr