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HOW MUTATIONS IN THE HIV-1 3'-POLYPURINE TRACT CONFER DOLUTEGRAVIR RESISTANCE

José G. Dekker, Bep Klaver, Ben Berkhout, Atze T. Das
Laboratory of Experimental Virology, Department of Medical Microbiology and Infection Prevention, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

BACKGROUND

The integrase strand-transfer inhibitor (INSTI) dolutegravir (DTG) is a popular drug of combinatorial antiretroviral therapy for HIV-infected individuals. Resistance to DTG is usually associated with mutations in the integrase protein, but a previous in vitro HIV selection study identified a mutation in the 3'-polypurine tract (PPT) that reduced DTG sensitivity[1], and mutation of the PPT sequence motif was also observed in a patient who failed on DTG monotherapy[2]. We predicted that such PPT mutations may affect the reverse transcription process[3], in particular the start site of second-strand DNA synthesis that determines the 5' end of the viral DNA product that is the template for integration. We here set out to identify other PPT mutations that cause DTG resistance and to determine the molecular mechanism of PPT-mediated resistance.

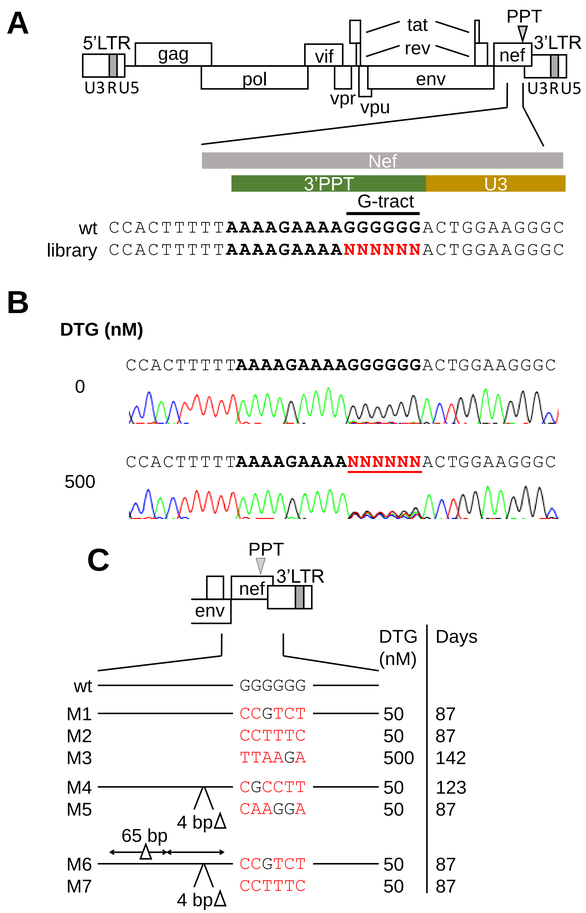


Figure 1: (A) HIV library with a randomized 3'PPT. (B) PPT sequence observed upon culturing in C8166 cells with 0 or 500 nM DTG. (C) Individual sequences detected in cultures with DTG.

Several PPT mutations can confer DTG resistance. PPT–inactivation does not restore integration in the presence of DTG, but triggers the production of non-integrating 1-LTR circles and may allow integration-independent virus replication.

METHODS

We produced a library of HIV LAI genomes with a randomized PPT G-tract and selected virus variants that replicate in the presence of DTG in T cell lines. DTG resistance was demonstrated in single-cycle infection and virus replication experiments. Integrated and non-integrated viral DNA products were analyzed to investigate the effect of PPT-mutations on reverse transcription and integration.

RESULTS

Introduction of the HIV library in C8166 T cells in the presence of DTG resulted in the selection of viruses with different mutations in the PPT (Fig. 1). Replication of the M6 and M7 PPT mutants was compared with that of wild-type LAI in competition experiments and analyzed by PCR with primer combinations that detect all viral DNA forms (PPT PCR; Fig. 2A), integrated viral DNA (Alu PCR; Fig. 2A) and non-integrated 1-LTR circles (Fig. 2B). The PPT mutants replicate less efficient than wild-type LAI without DTG, but they demonstrate improved replication with DTG (Fig. 2C). While the wild-type virus integrates efficiently without DTG and not with DTG, the PPT-mutants do not integrate efficiently in either condition (Fig. 2D). Surprisingly, the PPT mutations stimulate production of 1-LTR circles in infected cells (Fig. 2E, day 1 after infection).

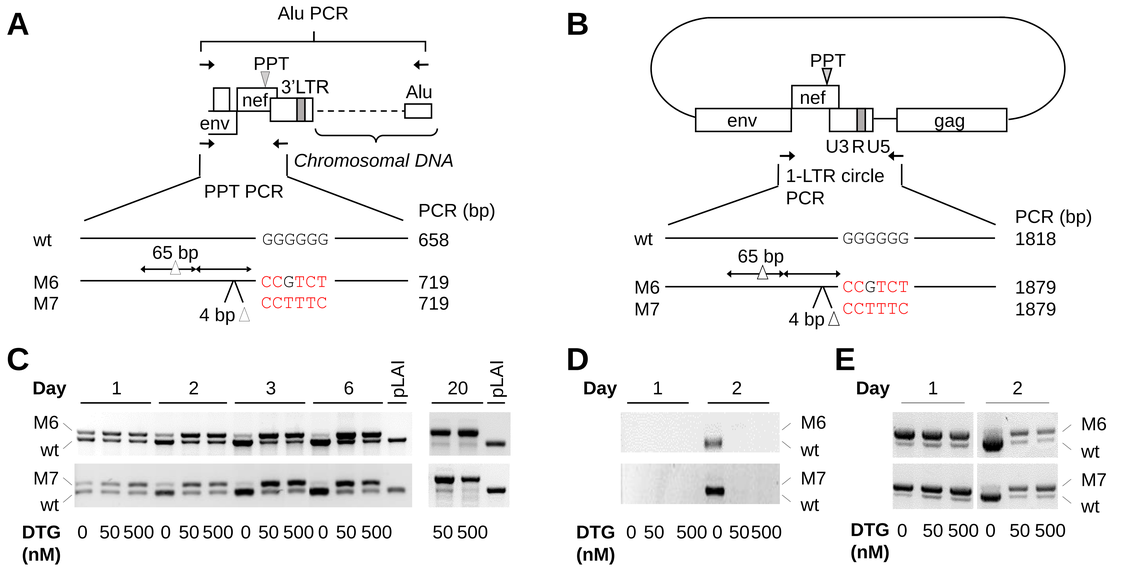


Figure 2: (A) PCR analyses to analyze viral DNA production (PPT PCR) and integration (Alu PCR followed by PPT PCR) upon simultaneous infection of C8166 cells with wild-type and PPT-mutated virus. M6 and M7 carry a nef-duplication, leading to an extended PCR product. (B) PCR analysis to analyze viral 1-LTR circle production (C-E) Agarose gel analysis of PPT PCR (C), Alu PCR products (D) and 1-LTR circle DNA (E).

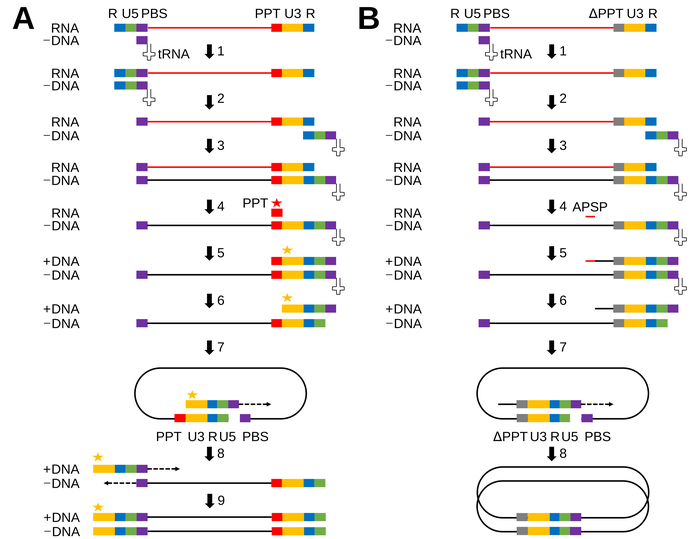


Figure 3: Reverse transcription of wild-type HIV (A) and PPT-mutants (B). APSP = alternative positive strand primer

CONCLUSIONS

Resistance to anti-viral drugs, including INSTIs, is normally caused by specific mutations in the targeted gene product. Our results demonstrate that a variety of mutations in the PPT motif can confer DTG resistance in C8166 cells, that is in the absence of integrase mutations. We demonstrate that such PPT-inactivating mutations do not restore integration, but rather stimulate the production of 1-LTR circles, an intermediate of HIV reverse transcription (Fig. 3).

Intriguingly, C8166 cells express the HTLV-1 Tax protein that was recently shown to stimulate episomal (2-LTR circle) replication of an integrase-deficient HIV variant[4]. In a parallel study, we documented that Tax also stimulates replication of PPT-mutated viruses[5].

Taken together, these results indicate that the PPT mutations may trigger integration-independent HIV replication, thereby circumventing DTG-mediated inhibition.

Further analysis of the detailed replication mechanism of the PPT-mutated viruses and the impact on viral fitness is important for a complete understanding of this potent and very popular drug class.

REFERENCES

1. Malet, I., et al. *mBio. (2017) 10.1128/mBio.00922-17,* **2**. Wijting, I.E.A., et al. *J. Infect. Dis.(2018) 10.1093/infdis/jiy176,* **3**. Das, A.T. and B. Berkhout. *mBio. (2018) 10.1128/mBio.00006-18,* **4**. Irwan, I.D., et al., *mBio. (2020) 10.1128/mBio.01038-20,* **5**. Dekker, J.G., et al. *AAC. (2021) 10.1128/AAC.01027-21*

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