UNINTERRUPTED VIRAL DNA INVOLVED IN DOLUTEGRAVIR RESISTANCE

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Methods

The purpose of this study was to explore in vitro, escape mechanisms of HIV-1 virus to dolutegravir (DTG). Whereas resistance to the viral genome, RAL and EVO, are now very well known and involves several well characterized mutations selected in the integrase gene, it seems to be different with DTG. We previously selected a virus resistant to DTG with mutations located in the 3′-PPT region and not in the integrase gene.

In this study, we used quantitative PCR in order to characterize the viral genomes (total viral DNA, 2-LTR circles and integrated viral DNA) during the course of the infection. In the presence of a high and constant concentration of DTG we were not able to detect any integrated viral DNA in the case of infection with the 3′-PPT virus.

Fluorescence analysis.

• Infection of HEK293 cells with the WT or 3′-PPT mutant (+/− 3′-PPT DNA)

• During the course of the experiment, the viral DNA (total viral DNA, 2-LTR circles, 3′- PPT circles and integrated viral DNA) were quantified by quantitative PCR.

• For integrated viral DNA, in some experiments, the number of cycles of the first PCR was increased (32 instead of 30).

Results

SEQUENCING OF THE VIRUS SELECTED UNDER DTG

• Sequencing of the integrase gene

• The presence of mutations in the integrase gene of our previously selected virus using PCR suggested that the selected virus was replicating efficiently in the absence of DTG. No increase of 2-LTR circles was shown in samples from the 3′-PPT mutant where integrase is impaired.

• Quantitative PCR highlights an efficient replication as shown by an increase of total viral DNA.

• In the case of the 3′-PPT mutant, the expression of the viral DNA, which is very largely non-integrated, is 2 to 6 times higher (with or without DTG) than the expression of the non-integrated viral DNA from a WT infection in the presence of DTG.

CONCLUSION

• We described a mutant in the 3′-PPT region that replicated efficiently with or without DTG.

• Quantitative PCR highlights an efficient replication as shown by an increase of total viral DNA.

• In the course of the study, the expression of the viral DNA, which is very largely non-integrated, is 2 to 6 times higher (with or without DTG) than the expression of the non-integrated viral DNA from a WT infection in the presence of DTG.

REFERENCES
