

# Cellular sequence insertion in HIV-1 reverse transcriptase confers resistance to RTIs and partly compensates for replication deficits of RTI mutations

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## Background

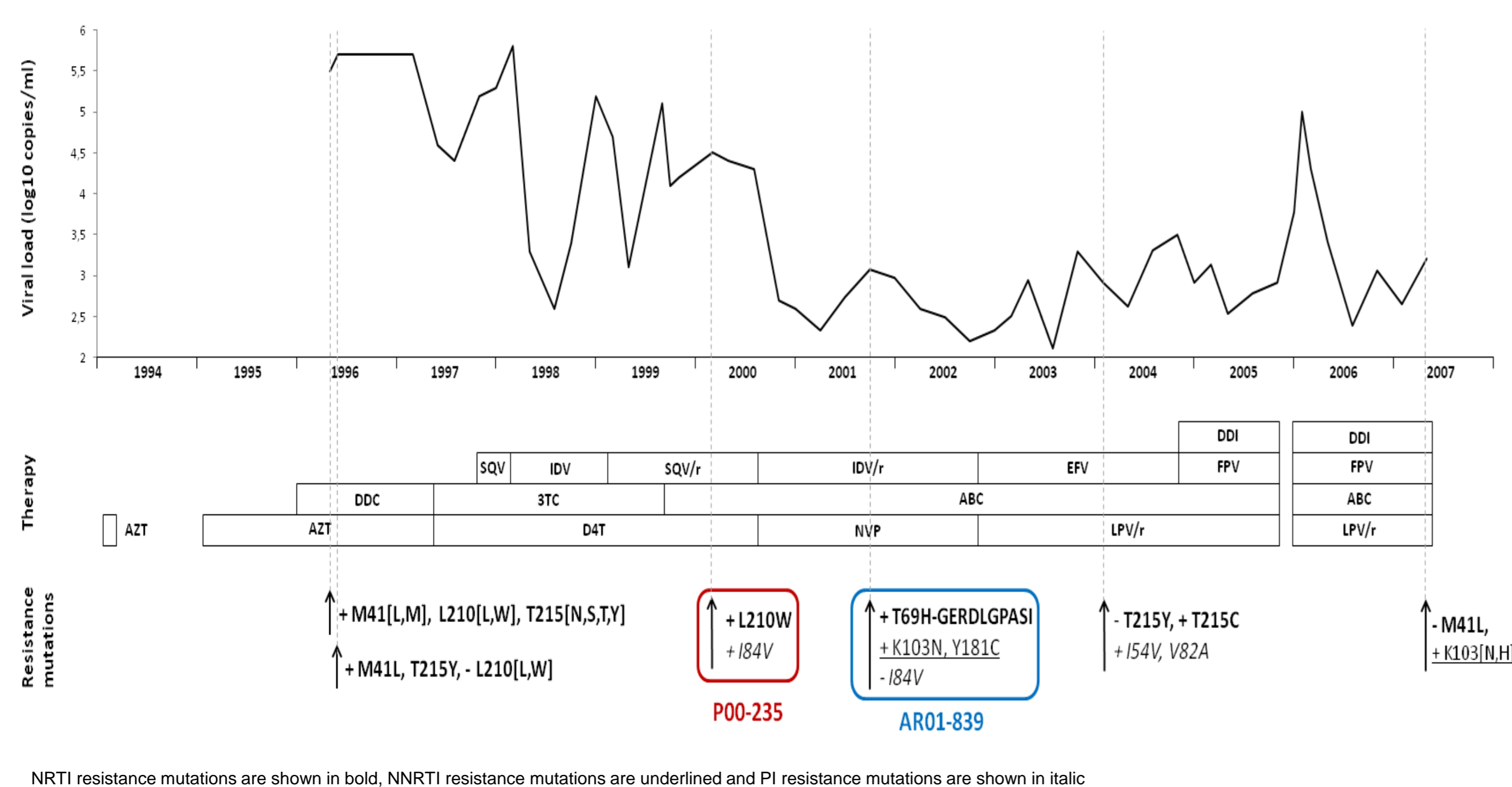
High-level HIV-1 resistance to antiviral drugs generally arises by stepwise accumulation of mutations, rather than deletions or insertions. Insertions within the RT finger subdomain have occasionally been observed and are often the result of duplication of nearby nucleotides. Within the background of TAMs, they confer NRTI multi-drug resistance. The aim of this study was to investigate the origin of a 10-amino-acid insert within RT from a HIV-1 patient failing antiviral therapy and to characterize the genotypic and phenotypic viral profile.

## Material and methods

PR-RT and full-length HIV-1 genotypes were sequenced from patient plasma samples. Two consecutive patient samples were used for phenotypic characterization: the sample in which the insertion at position 69 was first observed (AR01-839) and the last samples without the insertion (P00-235). Replication competent viruses containing the patient-derived HIV-1 RT-region were generated by homologous recombination. The replication capacity and drug susceptibility towards NRTIs and NNRTIs were determined using a MT4 and U87.CD4.CXCR4.CCR5 cell line, respectively. Infection was determined as the percentage of EGFP-expressing cells as measured with flow cytometry.

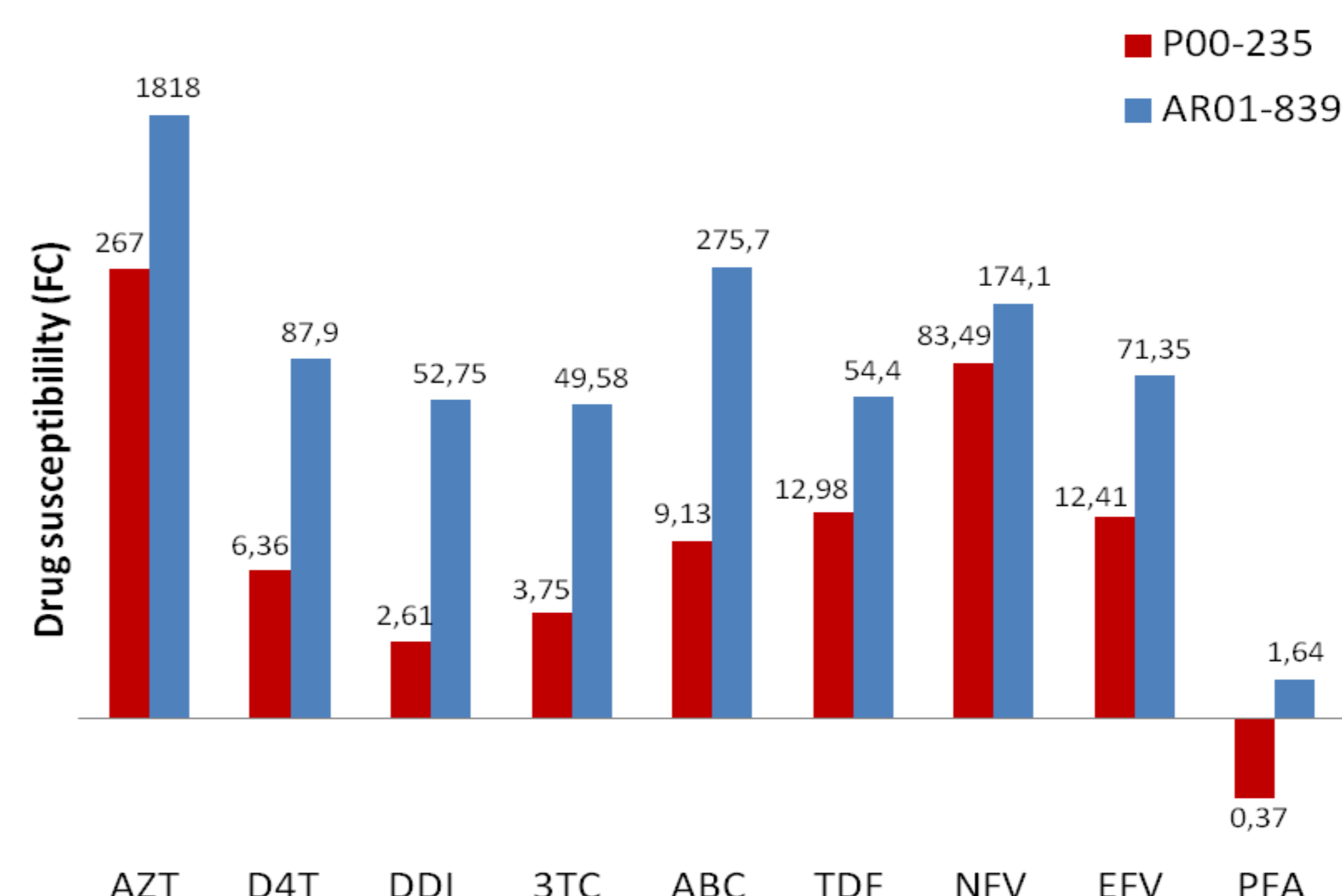
## Results

### Patient characteristics



The insertion T69H-GERDLGPASI was developed within an HIV-1 subtype A specific background already containing the NRTI resistance mutations M41L, L210W and T215Y (TAM1) during therapy containing abacavir, nevirapine and indinavir/r. Concurrently, the NNRTI mutations K103N and Y181C were detected for the first time.

### Drug susceptibility



Drug susceptibility testing revealed that the recombinant virus containing the insert and NNRTI mutations (AR01-839) displayed high-level resistance towards all NRTIs and NNRTIs (all fold changes > 50), significantly higher levels than the original virus displaying only M41L, L210W and T215Y (P00-235).

### Cellular insertion in HIV-1 reverse transcriptase

|               | 67  | 68  | 69  |   | 70  | 71  | 72  | Expect value |
|---------------|-----|-----|-----|---|-----|-----|-----|--------------|
| P00-235       | GAC | AGC | ACT |   | AAA | TGG | AGA | 1,00e-06     |
| AR01-839      | GAC | ACT | CAC | GGA GAA AGG GAC CTG GGC CCG GCC AGC ATT | AAA | TGG | AGA |              |
| CHR 17 ORF 68 | C   | ACT | CAC | GGA GGA AGG GAC CTG GGC CCG GCC AGC     |     |     |     |              |
|               | 67  | 68  | 69  |   | 70  | 71  | 72  |              |
| P00-235       | D   | S   | T   |   | K   | W   | R   |              |
| AR01-839      | D   | T   | H   | G E R D L G P A S I                     | K   | W   | R   |              |

In sample AR01-839 the insertion T69H-GERDLGPASI was first observed. As the insert was not the result of simple duplications of nearby nucleotides, the BLAST program was used to screen the autologous HIV-1 full length sequence and the sequences available at the NCBI databases. This analysis revealed that the insert did not originate from sequential template switches within HIV-1 but from the human chromosome 17 ORF 68 (33/34 nucleotide identity, E-value of 1,00e-06).

### Replication capacity

| Sample              | Replication capacity |       |
|---------------------|----------------------|-------|
| WT NL4.3            | k                    | 2,14  |
|                     | R <sup>2</sup>       | 0,940 |
| P00-235             | k                    | 0,74  |
|                     | R <sup>2</sup>       | 0,943 |
| AR01-839            | k                    | 1,87  |
|                     | R <sup>2</sup>       | 0,989 |
| P00-235 vs AR01-839 | p-value              | 0,012 |

The impaired replication capacity as observed for the original virus (34% of HIV-1 WT) was partly restored after acquisition of insert and NNRTI mutations (87% of HIV-1 WT).

## Conclusions

This study suggests that human sequence transduction of chromosome 17 within the  $\beta$ 3- $\beta$ 4 loop of HIV-1 RT can contribute to NRTI multi-drug resistance and to restoration of impaired replication capacity induced by TAM mutations. Surprisingly, the virus without insert already has phenotypic resistance to NNRTIs without any known primary NNRTI resistance mutations or previously administration of NNRTIs. A similar insertion resulting from the transductive copying of 37 nucleotides from human chromosome 17 also conferring multi-drug resistance was previously observed in a Japanese patient (Takebe and Telesnitsky, 2006).

## Acknowledgments

This work was supported by the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (grant G.0611.09N), by a grant from the Interuniversity Attraction Poles Programme, Belgian State, Belgian Science Policy (IUAP-VI P6/41), by the European Community's Seventh Framework Programme (FP7/2007-2013) under the project 'Collaborative HIV and anti-HIV Drug Resistance Network' (CHAIN, grant 223131) and by the AIDS Reference Laboratory of Leuven that receives support from the Belgian Ministry of Social Affairs through a fund within the Health Insurance System.