



# Changes outside integrase in a patient failing on dolutegravir maintenance monotherapy points to a new resistance mechanism

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## Introduction

In our randomized DTG maintenance monotherapy study (DOMONO study) and its pilot study, virological failure (VF) was observed in ten patients with good drug levels and compliance. Integrase strand transfer inhibitor-resistance associated mutations (INSTI-RAMs) could be identified in four of these patients. Mechanisms of failure in the other patients remain unclear. Integrase complex assembly and subsequent strand transfer catalysis are mediated by specific interactions between integrase and bases at the end of the viral long terminal repeat (LTR). *In vitro* mutations in the 4 terminal nucleotides of the LTR (Dicker et al, 2007) and mutations in the 3'PPT (Malet et al, 2017) have been shown to confer resistance to (INSTIs).

## Objective

To investigate whether changes in the integrase binding regions rather than the integrase itself could explain virological failure.

## Results

We analyzed additional amino acid substitutions in HIV-1 integrase at time of VF relative to baseline, pre-cART for six out of ten patients that failed without any known INSTI-RAMs. Most of these substitutions were at positions that are less conserved with a higher frequency rate of mutation. For two patients (Pat 3 and Pat 6), amino acid comparisons were not possible as the integrase sequence at VF could not be determined. Therefore, we cannot exclude the presence of INSTI-RAMs in these patients.

In one patient (Pat 10), Time to VF was 24 weeks (Fig.1). No INSTI-RAMs were present. Two substitutions (IV72V and LI101I) could be identified when baseline, pre-cART and VF sequences of the integrase gene were compared. These substitutions occur at positions with >25% frequency rate of mutation. We also found naturally occurring polymorphisms; S17N, S39C, K156N, K173R and V201I (Fig. 2)

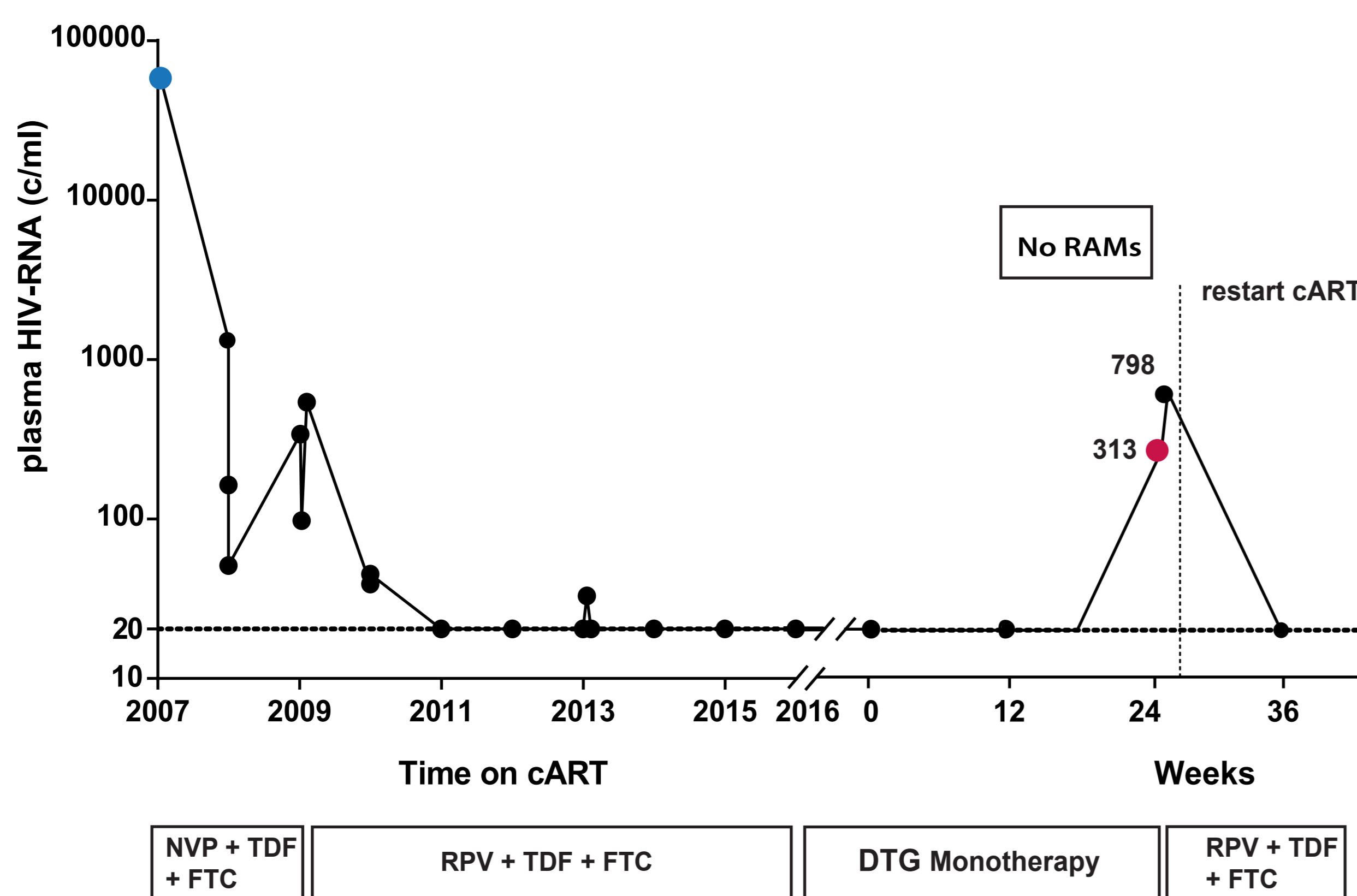


Fig.1 HIV-RNA kinetics for Patient 10 (DTG-10) in response to induction and maintenance therapy. The coloured dots mark the sampling points for RNA isolation, cDNA synthesis and sequencing. NVP = nevirapine. TDF = Tenofovir Disoproxil Fumarate. FTC = emtricitabine. RPV = rilpivirine. DTG = dolutegravir.

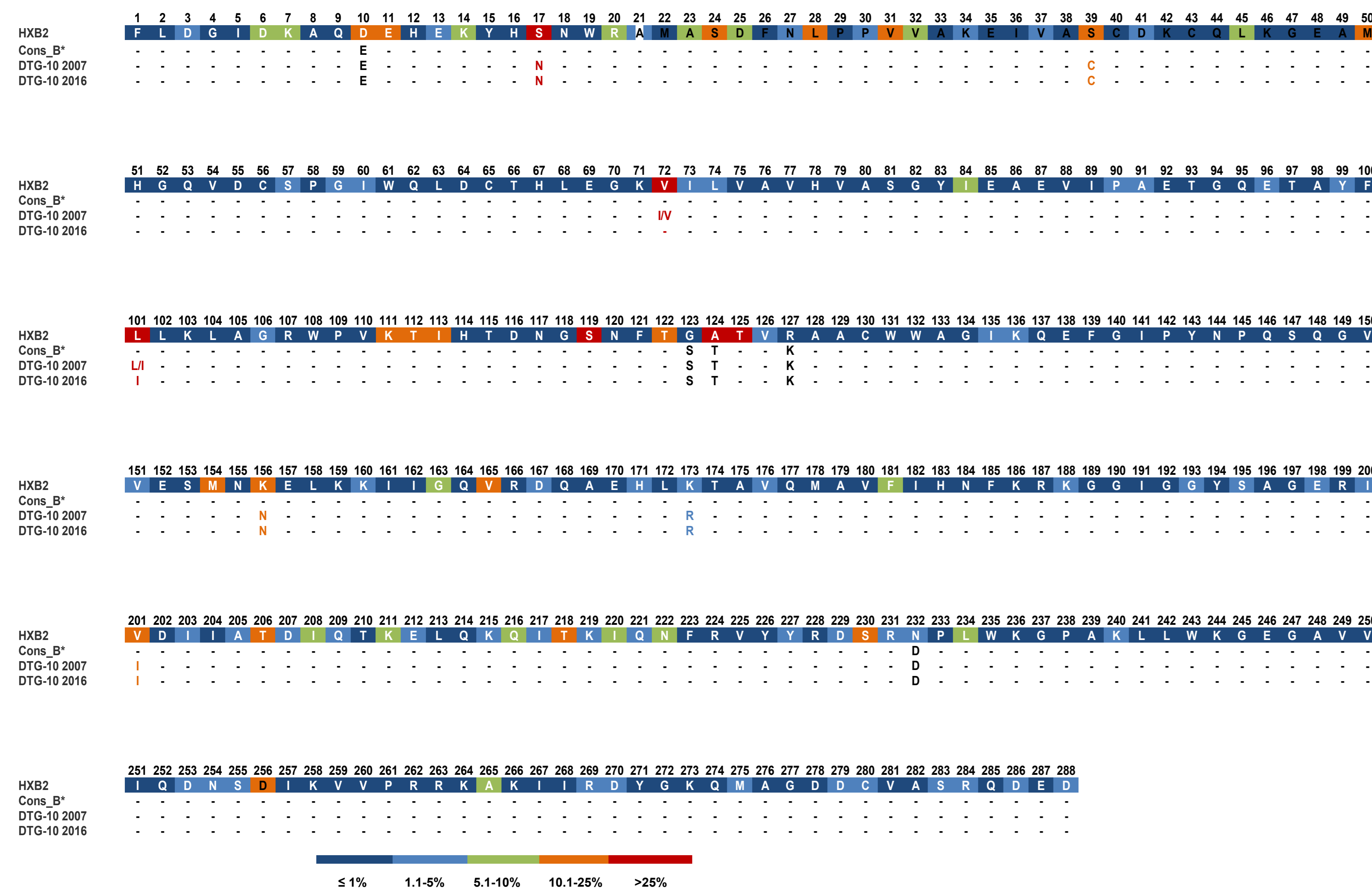


Fig.2 Amino acid substitutions in patient 10 HIV-1 integrase at time of VF relative to baseline. Substitutions are colored based on the frequency rate of mutations observed in plasma samples from 448 integrase inhibitor-naïve HIV-1 subtype B patients (consensus B sequence) (Ceccherini-Silberstein, et al. 2010). DTG-10-2007 is the sequence generated from HIV-RNA in plasma prior to cART initiation. DTG-10-2016 is the sequence generated from HIV-RNA in plasma at VF during DTG maintenance monotherapy. HXB2 is HIV-1 clade B reference sequence.

## Materials & Methods

For details on the DOMONO study, see poster by Wijting et al., EACS 2017, PE6/16.

Samples from ten patients with VF (HIV-RNA >200 c/ml) during DTG maintenance monotherapy were compared to baseline, pre-cART samples. All patients were infected with HIV-1 subtype B.

Viral RNA was isolated and synthesized into cDNA. Integrase and nef/LTR regions were then amplified and directly sequenced by Sanger method.

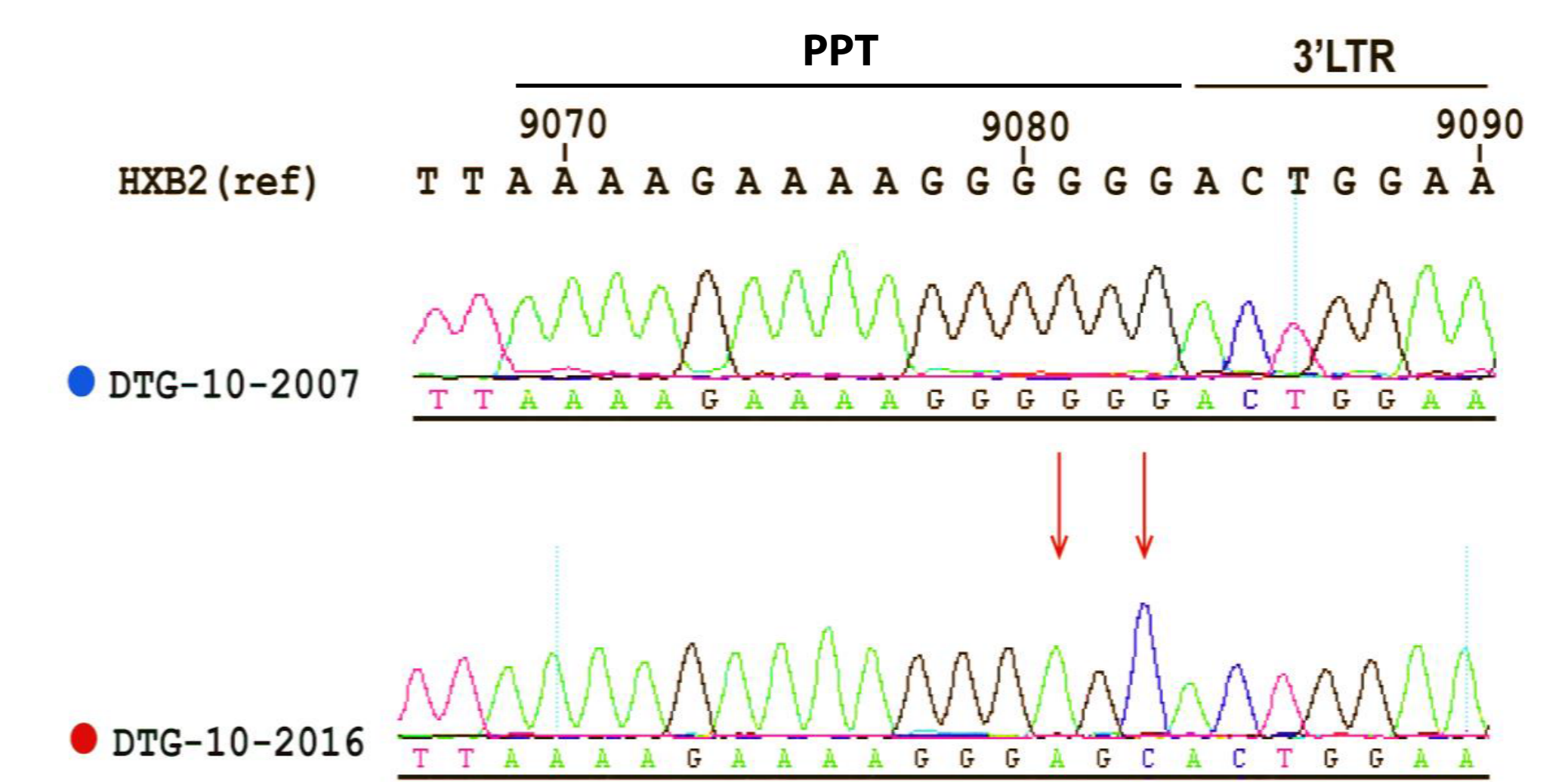
Sequences of the 3' PPT region of patient viruses at VF, relative to HXB2 reference strain sequence and 9053 Lai virus (Malet et al, 2017)

	3' polypurine tract (PPT)												LTR						
HXB2_ref	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	C	T	G
9053 Lai	A	A	A	A	G	A	A	A	A	G	C	A	G	T	-	A	C	T	G

Pat 1	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	C	T	G
Pat 3	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	C	T	G
Pat 4	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	C	T	G
Pat 6	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	C	T	G
Pat 8	A	A	A	A	G	A	A	A	A	G	G	G	G	G	G	A	C	T	G
Pat 10	A	A	A	A	G	A	A	A	A	G	G	G	A	G	C	A	C	T	G

In five patients, no changes were observed in the 4 terminal nucleotides of the 3' LTR and its extremity (15 nucleotides). In patient 10, two mutations emerged in a highly conserved G-stretch (GGGGGG to GGGAGC) of the 3'PPT. At the moment of VF, when the binding site changes were observed, plasma HIV-RNA level peaked to 798 copies/ml (Fig.1)

## Changes in the G-stretch of the 3'PPT in patient 10 at VF were not present at baseline



Sequence analysis 3'PPT. DTG-10-2007 is the sequence generated from HIV-RNA in plasma prior to cART initiation. DTG-10-2016 is the sequence generated from HIV-RNA in plasma at VF during DTG maintenance monotherapy. HXB2 is the sequence from the HXB2 reference strain.

## Conclusions

We identified a potential alternative INSTI-resistance mechanism *in vivo*. Changes in this fully conserved area have been shown to cause INSTI resistance *in vitro*. We postulate that under particular circumstances, altering the binding site may confer INSTI resistance. Further studies are required to determine the biochemical mechanism, phenotypic changes and frequency, and relevance in patients failing on INSTIs.

## References

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Dicker IB, Samanta HK, Li Z, Hong Y, Tian Y, Banville J, et al. Changes to the HIV long terminal repeat and to HIV integrase differentially impact HIV integrase assembly, activity, and the binding of strand transfer inhibitors. *J Biol Chem.* 2007;282:31186-96.Z  
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