Lack of Minority K65R Resistant Viral Populations Detected after Repeated Interruptions of Tenofovir DF/ Zidovudine/Lamivudine

Poster #681

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INTRODUCTION

Tenofovir (TDF) is being used increasingly as part of first-line therapy in resource-limited settings. Since TDF has a longer half-life than other NRTIs (approx 12-15hrs in plasma, see Figure 1), there is a risk of resistance following its cessation, due to toxicity, stockouts or as an agent to prevent mother-to-child-transmission (pMTCT).

We studied a subset of patients receiving ZDV/3TC/TDF within the DART trial who were allocated to the structured treatment interruption (STI) arm of a randomised substudy. This study presents both immunological data (ie: CD4 cell count) and virological data, regarding viral rebound and possible emergence of drug resistance in 18 patients who entered four consecutive Structured Treatment Interruptions (STIs) consisting of 12 weeks on and off treatment following continual first line therapy of CBV (ZDV/3TC) and TDF for 52 weeks (Figure 2).

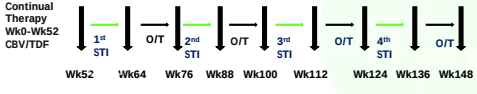
CD4 cell count and plasma was stored 8 weeks into each STI and 8 weeks back on treatment so that the wider group of patients next STIs could be deferred or ART restarted early after 8wks rather than12 weeks off treatment.

We were particularly interested in detection of K65R minority species in rebounding virus, which is associated with resistance to Tenofovir, in addition to M184V minority species, which is selected by Lamivudine .

Figure 1: Plasma and intracellular half-life of NRTIs

Drug	Plasma half -life (hrs)	Intracellular half-life (hrs)
Zidovudine (ZDV)	0.5-3.0	4.07
Abacavir (ABC)	0.8-1.5	18
Tenofovir (TDF)	12-15	150

Figure 2: Schematic diagram of STI protocol:



STI= Structured treatment Interruption O/T=On treatment.

METHODS

Study Design:

STI was compared with continuous therapy (CT) in an "opened" randomized trial (nested within DART, AIDS (2008) 22:p237-247) in two centres in Uganda (MRC/UVRI Uganda Research Unit on AIDS, Entebbe, and the Joint Clinical Research Centre, Kampala) and one in Zimbabwe (University of Zimbabwe, Harare).

813 participants with CD4 cell count ™ 300 cells/ml at 48 or 72 weeks after ART initiation were randomized to CT (n=405) or STI (n=408) with repeated 12 week periods on or off therapy.

249 participants were randomized to STI at week 48. In this substudy we examined 18 of these participants who were :

•From Ugandan sites

•Underwent 4 complete* 12-week cycles of STI before the study closure

•Made no changes to their ART treatment.

•* complete = without starting or stopping ART before 12 weeks.

CD4 counts and Viral load testing

CD4 counts and viral load were measured 8 weeks into each on/off cycle (see Figure 2). HIV-1 RNA was measured using the Roche ultrasensitive assay, on stored plasma, and was not performed in "real-time" .

Resistance testing (population and minority sequencing)

Plasma samples with HIV-1 RNA >1000 copies/ml were genotyped using RT-PCR and population sequencing of a contiguous region of *pol*, encompassing the entire of protease and codons 1-320 of RT using an ABI capillary sequencer.

K65R and M184V minority sequencing was performed using pyrosequencing. cDNA generated by RT-PCR was PCR amplified using the following primers:

K65R primers: Forward 5'CAA AAA TTG GGC CTG AAA ATC CAT A 3' and Reverse 5'ACT GAA AAA TAT GCA TCA CCC ACA TC 3' (biotinylated) .

M184V Primers : Forward 5'GGA ATT AGA TAT CAG TAC AAT GT3' and Reverse 5'CTC TAT GTT GCC CTA TTT CTA AGT CAG A 3' (biotinylated) .

The PCR reaction conditions were as follows: Initial denaturation at 98ºC for 15 minutes, followed by 38 cycles of: 98ºC for 30 secs, 50ºC for 40 secs, 72ºC for 1 minute, followed by a final extension at 78ºC for 2 minutes.
The Pyrosequencing reaction was performed using Pyro Gold SQA reagent kit and a PSQ 96MA analyzer (Biotage, Uppsala, Sweden) according to the manufacturers instructions.
Single stranded DNA derived from biotinylated PCR amplicons, served as a template in the pyrosequencing reaction along with primer:

K65 : 5'AGT ATT TGC CAT AAA AA 3'(HIV-1 subtype C)
K65: 5'AGT ATT TGC CAT AAA GA 3' (all other HIV-1 subtypes except C)
M184: 5'AGA TCC TAC ATA CAA GTC ATC3'

RESULTS

Impact of STI on Viral load and CD4 cell count

CD4 counts and Viral load measurements were available for 160 (99%) and 153 (94%) of a possible 162 time points respectively.
Viral load and CD4 plots (Figure 3) showed the expected pattern of CD4 drop and viral load rise associated with an STI strategy.

At 48 weeks:

- median (IQR) CD4 was 394 (321-443) mm³.
- all patients had a viral load <400 RNA copies/ml (14/18 patients had <50 RNA copies/ml).

Impact of STI on emergence of resistance.

The level of viral rebound and re-suppression was similar across successive STIs:
Mean HIV-1 RNA ranged from:
4.5-4.7 log $_{10}$ copies/ml off -treatment
2.3-2.6 log $_{10}$ copies/ml on-treatment.
Overall, HIV-1 RNA was >1000 copies/ml in 82 samples, of which 69 were off treatment.

Minority and population sequencing was performed on 78 of these samples.

M184V was the only mutation detected, which was only present in one individual, at a frequency of 34% (on-treatment) and 13% (off-treatment) after the third STI. The minority assay had the potential to identify persistence of the M184V mutation ie: at wk120 and wk132 compared to conventional population sequencing which detected M184V only at wk120.

K65R minority species was not detectable in any of the patients genotyped by population or minority sequencing which like M184V minority sequencing assay, has a 2% limit of sensitivity . The upper 95% confidence limit for risk of resistance per cycle = 4.1%.

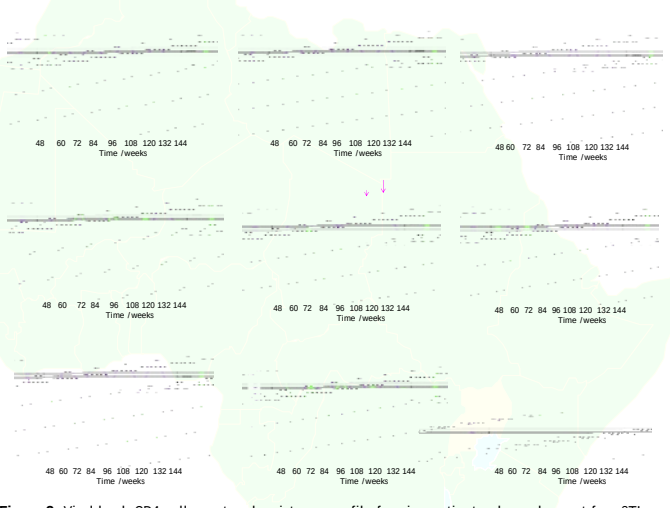


Figure 3: Viral load, CD4 cell count and resistance profile for nine patients who underwent four STIs.

CONCLUSIONS

•No evidence of K65R minority species in rebounding virus for patients whom repeatedly were stopping and restarting treatment with CBV and TDF.

• low risk of M184V mutation, although minority detection prior to emergence in majority population

•Low risk of resistance emerging upon stopping continual therapy

•potential problems associated with the possible emergence of resistance to TDF upon stopping continuous HAART appear not to be of concern in this instance.

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