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

A Joyce1, N Ndemb2, R Goodall3, M Chirara4, D Gibb3, C Gilks5, J Hakim4, K Kityo6, A McCormick1, David Dunn7 on behalf of the DART Virology Group.

1 UCL, London, UK; 2 MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda; 3 Med Res Council Clin Trials Unit, London, UK; 4 University of Zimbabwe, Harare; 5 Imperial College London, UK; 6 Joint Clin Res Ctr, Kampala, Uganda.

RESULTS

STIs were compared with continuous therapy (CT) in an “opened” resource-limited setting. Since TDF has a longer half-life than other NRTIs (approx 12-15hrs in plasma), there is a risk of resistance following repeated 12 week periods on or off therapy. CD4 cell counts and plasma was stored 8 weeks into each STI and 8 weeks back on therapy to examine the full effect of ART re-introduction.

CD4 counts and viral load testing CD4 counts and viral load were measured 8 weeks after ART initiation were randomized to CT (n=405) or STI (n=408) with repeated 12 week periods on or off therapy.

We were particularly interested in detection of K65R minority species in rebounding virus, which is associated with resistance to Tenofovir. In addition to K617V minority species, which is selected by Lamivudine.

**K65R minority species was not detectable in any of the patients genotyped by pyrosequencing.** cDNA generated by RT-PCR was amplified using the following primers:

K65R forward: 5'CAA AAA TTG GGC CTG AAA ATC CAT A 3' and reverse: 5'AGT ATT TGC CAT AAA GA 3' (all other HIV-1 subtypes except C)

The PCR reactions were performed using Pyro Gold SQA reagent kit and a PSQ assay analyser (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:

K65R: 5'AGT ATT TGC CAT AAA GA 3' (C subtype).

Conclusions

- K65R minority species was not detectable in any of the patients genotypically or quantitatively using an ABI capillary sequencer, which has a 2% limit of sensitivity. The upper 95% confidence limit for risk of resistance per cycle was 4.1%.

**K65R minority species was not detectable in any of the patients genotyped by pyrosequencing.**

**Conclusions**

- The level of viral rebound and re-suppression was similar across successive STIs: Mean HIV-1 RNA ranged from: 4.5-7.4 log_{10} copies/ml off treatment to 2.3-2.6 log_{10} copies/ml on treatment.

- Minority and population sequencing was performed on 76 of these samples.

- The PCR reaction conditions were as follows: Initial denaturation at 94°C for 1 minute, followed by 30 cycles of 94°C for 30 secs, 57°C for 30 secs, and 72°C for 30 secs, with a final extension at 72°C for 2 minutes.

- The Pyrosequencing reaction was performed using Pyro Gold SQA reagent kit and a PSQ assay analyser (Biotage, Uppsala, Sweden) according to the manufacturer's instructions.

- Single stranded DNA derived from biotinylated PCR amplicons served as a template in the pyrosequencing reaction along with primer:

K65R: 5'AGT ATT TGC CAT AAA GA 3' (all other HIV-1 subtypes except C).

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