Dolutegravir Reversibly Induces Mitochondrial Fragmentation and Toxicity *In Vitro*

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Introduction

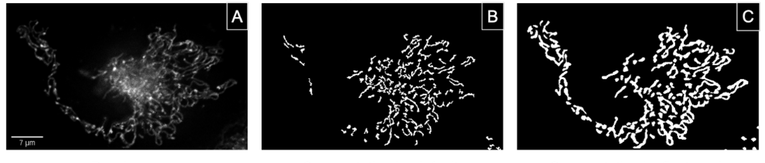
- HIV integrase inhibitors (InSTIs) such as dolutegravir (DTG) are increasingly used in first-line combination antiretroviral therapy (cART). **The toxicity and long-term safety of InSTIs are relatively unknown**
  - Early signal for increased neural-tube defects among Botswana children exposed to maternal DTG since conception
  - Increasing reports of weight gain with DTG — may have mitochondrial link given the role of mitochondria in metabolism
- Many older cART drugs cause cellular and mitochondrial toxicities — **deplete mitochondrial DNA (mtDNA), affect mitochondrial function, and increase mitochondrial reactive oxygen species (mtROS)**
- Catalytic unit of telomerase – human telomerase reverse transcriptase (**hTERT**) can **translocate to the mitochondria and protect against reactive oxygen species (ROS) and some mtDNA damage**

Objectives

- Quantify changes in mitochondrial health and morphology following exposure to current cART regimens, including those containing the InSTIs DTG and raltegravir (RAL), at pharmacological concentrations (1X Cmax)
- Determine whether any cART-associated toxicities are modulated by hTERT

Study Methodology

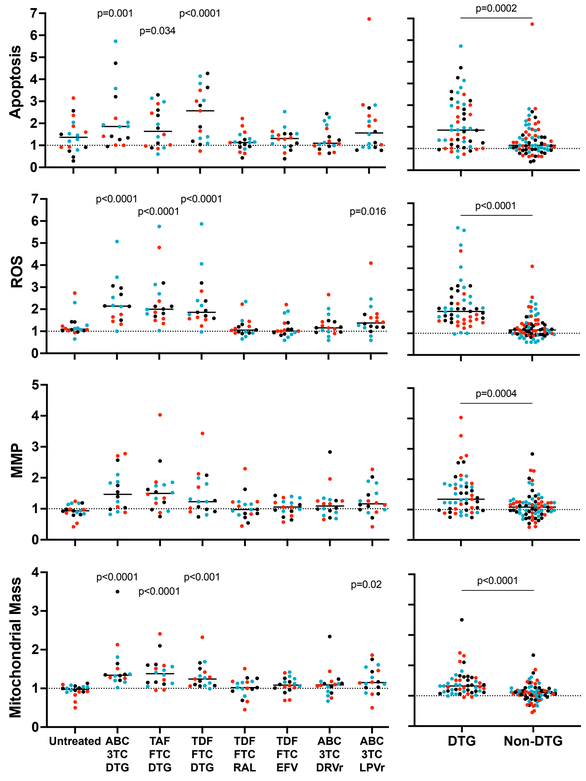
- Telomerase-independent human lung fibroblasts (WI-38 VA13) were transduced to express hTERT with and without its mitochondrial targeting signal (MTS).
- The three isogenic cell lines (–hTERT, +hTERT–MTS, and +hTERT) were exposed to cART regimens at 1X Cmax for nine days, followed by recovery in drug-free media for six days (n=6)
- After both exposure and recovery, mitochondrial mass, mitochondrial membrane potential (MMP), mtROS, and cellular apoptosis were quantified using flow cytometry
- Mitochondrial images were captured by fluorescence microscopy for one experiment, and the deep learning tool MitoSegNet was used to quantify differences in mitochondrial morphology.
- All measurements were normalized to their corresponding 0.1% DMSO drug solvent control (dashed lines in figures)



Raw image (TDF/FTC/DTG, -hTERT, day 15) | Segmentation by default MitoSegNet model | Segmentation by finetuned MitoSegNet model

Figure 1. Representative Image of mitochondrial identification using fine-tuned MitoSegNet (A) Raw 8-bit image. **(B)** Object identification by pretrained default model. **(C)** Improved object identification after fine tuning on 2 additional ground truths for 5 epochs. Scale bar is 7 µm.

Results



+ hTERT
+ hTERT - MTS
- hTERT

Figure 2. Apoptosis, mtROS, MMP and mitochondrial mass after 9-days of exposure to cART, normalized to DMSO. Individual cART regimens (left) and pooled comparisons of DTG- and non-DTG-containing regimens (right)

Medians for each treatment are shown by the black bars. Statistical significance was assessed by Kruskal-Wallis test with Dunn's multiple-comparisons test n=18 for all day 9 conditions except ABC/3TC/DTG (n=16), and TDF/FTC/DTF (n=17).

9 days of DTG exposure:

- **Increased apoptosis, mitochondrial ROS, mass and MMP** compared to non-DTG (including RAL)-containing regimens (all p<0.001) (Figure 2)
- **Altered mitochondrial morphology** compared to DMSO controls (all p<0.005) (Figures 3-4)
- hTERT does **not appear to protect against DTG-induced stresses** (Figures 2-3 & 5)

Mitochondrial health and morphology alterations were reversed after 6 days of recovery for all metrics assessed between the end of exposure and recovery periods. (Figure 5)

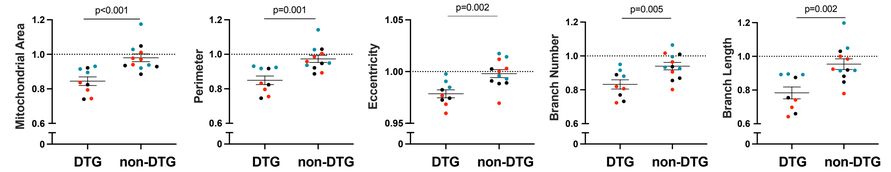


Figure 3. Pooled comparisons of DTG- and non-DTG-containing regimens by mitochondrial morphology metrics after 9-days of exposure. All data are normalized to DMSO. Mean and SEM are indicated by the black bars. Statistical significance was assessed by unpaired t-tests, n=9 and n=12 for DTG- and non-DTG containing regimens.

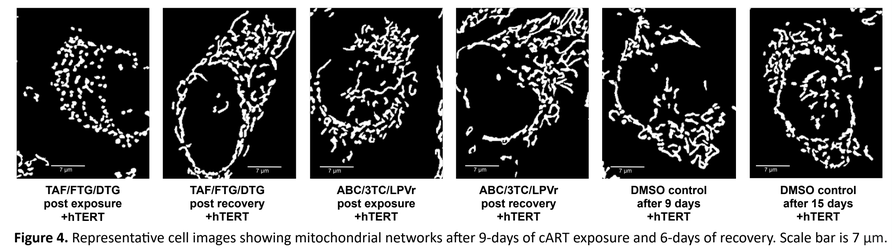


Figure 4. Representative cell images showing mitochondrial networks after 9-days of cART exposure and 6-days of recovery. Scale bar is 7 µm.

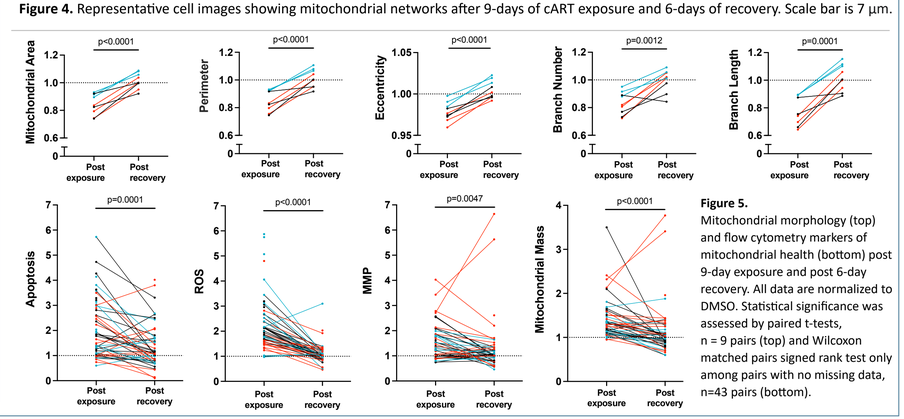


Figure 5. Mitochondrial morphology (top) and flow cytometry markers of mitochondrial health (bottom) post 9-day exposure and post 6-day recovery. All data are normalized to DMSO. Statistical significance was assessed by paired t-tests, n = 9 pairs (top) and Wilcoxon matched pairs signed rank test only among pairs with no missing data, n=43 pairs (bottom).

Conclusions

- DTG-induced alterations to mitochondrial morphology in human cells is a novel finding.
- Taken together, our results indicate that DTG-containing regimens induce mitochondrial toxicity and fragmentation of mitochondrial networks, something not seen with RAL, suggesting these effects are not common across all InSTIs and may be specific to DTG.
- hTERT did not seem to confer protection against DTG-mediated cellular stresses, although it may mitigate PI-associated ROS.
- It remains unclear whether fragmented mitochondria are a consequence or a cause, with respect to potential functional alterations such as impaired respiration and increased ROS formation. Further investigation is required to understand the mechanism of toxicity.
- These results highlight the importance of continued investigation into the safety of DTG and other second generation InSTIs.

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