Dolutegravir-containing HIV combination antiretroviral therapy induces reversible alterations to mitochondrial morphology *in vitro*

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Background

Despite successful suppression of HIV viremia, combination antiretroviral therapy (**cART**) drug **toxicity** remains a concern. Various regimens can impair mitochondrial health and function, yet mechanisms remain unclear.

Mitochondrial morphology is a strong indicator of mitochondrial and cellular health; more **fragmented** morphological phenotypes are linked to **altered respiration, apoptosis**, and **mitophagy**. Here, we describe a proof-of-concept study using a recently developed deep learning tool to quantitatively assess mitochondrial morphology alterations following exposure to clinically relevant cART regimens *in vitro*. We focus on **dolutegravir** (**DTG**), a widely used antiretroviral.

Methods

- Lung fibroblasts (WI-38) were **exposed** to seven cART regimens (three including DTG) at pharmacological concentrations (1X C_{max})
- The following regimens were investigated: TDF/FTC/EFV, TDF/FTC/**DTG**, TDF/FTC/RAL, TAF/FTC/**DTG**, ABC/3TC/DRV/r, ABC/3TC/LPV/r and ABC/3TC/**DTG**
- 9-day exposure period was followed by **untreated recovery** for 6 days
- Cells were stained with MitoTracker Deep Red probe, and images acquired by fluorescence microscopy after exposure and recovery
- **The MitoSegNet tool** which produces a segmentation model for unbiased identification of mitochondria was finetuned using hand-traced mitochondrial images from two annotators.

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

Figure 1. Improved MitoSegNet performance after finetuning. (**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.

Results

Between 1296-5795 mitochondrial objects were identified for each treatment.

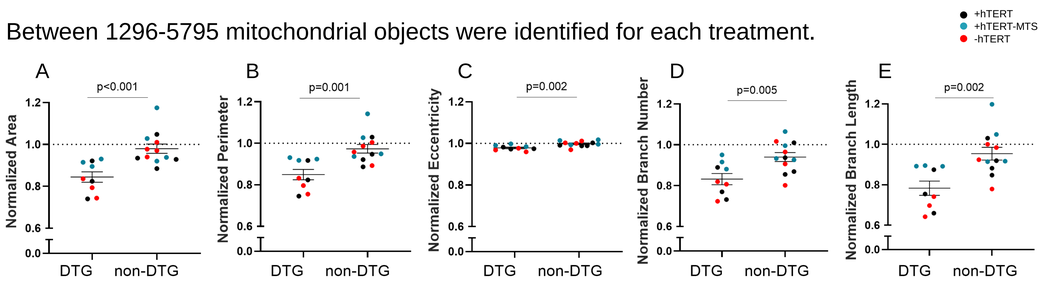


Figure 2. Cells exposed to DTG-containing regimens show altered morphology compared to non-DTG regimens. (A-E) Mitochondrial morphology metrics after 9-day exposure for a single independent experiment, shown with mean and SEM. All data are normalized to corresponding 0.1% DMSO drug-vehicle controls (dashed lines). Statistical significance was assessed by unpaired t-tests. N=9 and 12 for DTG- and non-DTG containing regimens.

Mitochondrial networks after DTG exposure exhibited reduced area, perimeter, eccentricity, branch number, and branch length.

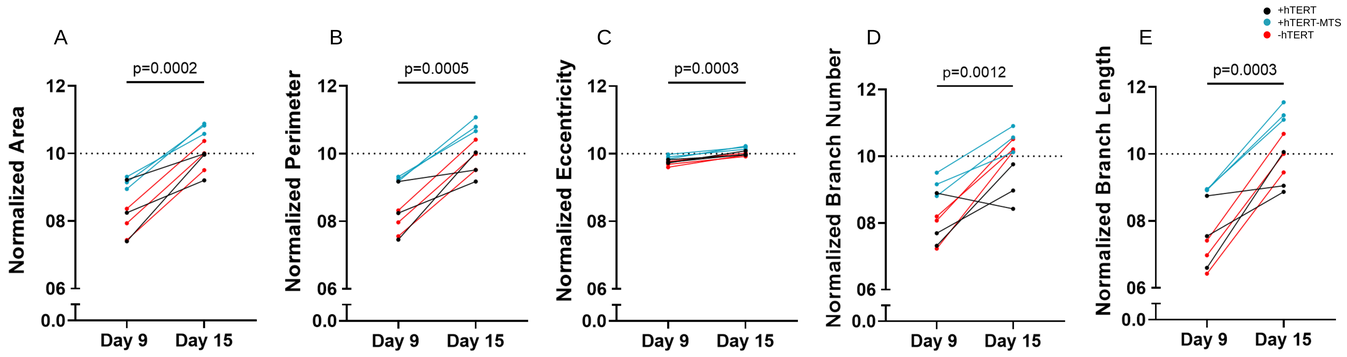
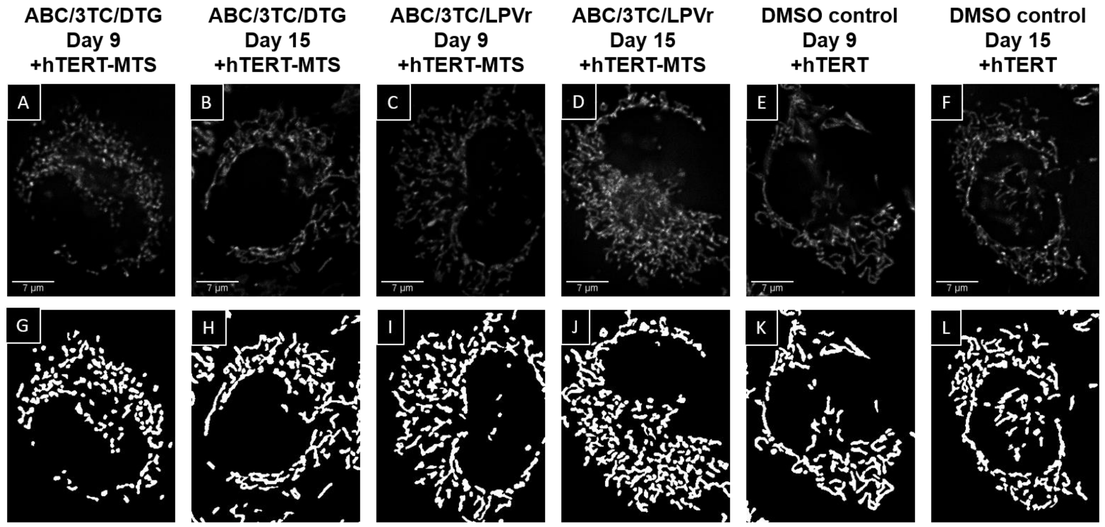


Figure 3. DTG-containing cART induced toxicities are reversible and show return to control levels for all morphological metrics assessed between the end of exposure (9 days) and 6-day recovery period (A-E) All data are normalized to corresponding 0.1% DMSO drug-vehicle controls (dashed lines). Statistical significance was assessed by paired t-tests. n = 9 pairs.

All altered morphological metrics returned to control levels after the recovery period and became comparable to the DMSO control.

The effect of the other regimen components warrants further investigation.



M

Area (px)	91.5	119	119.4	110.9	122.8	130.7
Perimeter (px)	43.8	56.5	54.3	52.7	59.2	61.2
Eccentricity	0.8	0.84	0.82	0.82	0.84	0.83
Number of branches	1.7	2.1	1.9	2.2	2.3	2.5
Total branch length (px)	15.8	22.7	20.9	20.6	24.8	25.4

Figure 4. Paired representative images of (A-F) raw and (G-L) processed mitochondrial networks after 9-days of cART exposure and 6-days of recovery, with (M) corresponding quantitative morphological metrics. Cell exposed to ABC/3TC/DTG appears more fragmented post-exposure compared to cell exposed to ABC/3TC/LPVr and DMSO control. Both cells look similar to 0.1% DMSO vehicle control post recovery. Scale bar is 7 µm. TDF – tenofovir disoproxil fumarate, FTC – emtricitabine, EFV – efavirenz, **DTG** – dolutegravir, RAL – raltegravir, TAF – tenofovir alafenamide, ABC – abacavir, 3TC – lamivudine, DRV/r – darunavir, LPV/r – lopinavir.

Conclusions

- **DTG** induces a more **fragmented mitochondrial network** *in vitro*
- Although observed effect was **reversible**, its potential health implications are **unclear** given that HIV therapy is lifelong and without interruption
- **DTG**-induced **mitochondrial toxicity** should be explored further.

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