Dolutegravir-containing HIV therapies appear to increase cellular apoptosis and mitochondrial toxicities in human PBMCs

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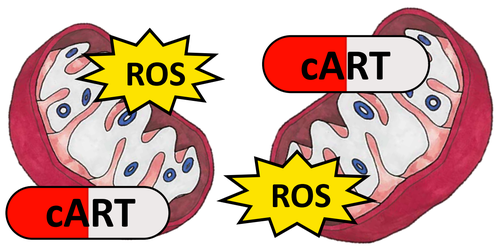
Introduction

- Certain cART regimens are associated with mitochondrial and cellular toxicity

Oxidative stress | **Abnormal mitochondrial morphology and/or mitochondrial mass** | **Altered mitochondrial DNA (mtDNA) levels and/or function** | **mtDNA damage/mutations** | **Apoptosis and/or mitophagy**



- The toxicity and long term safety of Integrase Inhibitors (InSTIs) are relatively unknown
- In an *ex vivo* study done in CD4+ T cells, Dolutegravir (DTG) has been shown to:
  - impair mitochondrial respiration
  - increase levels of mitochondrial reactive oxygen species (ROS)
  - reduce overall immune response

Objective

- To characterize cART-related mitochondrial and cellular toxicity, and to compare the effects of different InSTIs, specifically DTG and Raltegravir (RAL), in human PBMCs

Hypothesis

- DTG-containing cART regimens increase mitochondrial ROS and apoptosis in human PBMCs.

Methods

- **PBMC isolation:** PBMCs were isolated from healthy donors using Ficoll-Paque
  - n = 6 biological replicates, 3 independent experiments
  - Cells were seeded at 10^6 cells/ml and incubated for 2 hours at 37ºC, 5.0% CO_2 prior to treatment
- **6-day cART exposure:** Cells were cultured in media containing anti-CD3/CD28 proliferating agent, IL-2 survival factor, and pharmacological concentrations (1 C_{max}) of the following cART regimens:

1. ABC/3TC/DTG
2. TAF/FTC/DTG
3. TDF/FTC/DTG
4. TDF/FTC/RAL
5. TDF/FTC/EFV
6. ABC/3TC/LPVr
7. ABC/3TC/DRVr
8. Untreated control
9. 0.1% DMSO drug solvent control

Nucleoside Reverse Transcriptase Inhibitors (NRTI)
ABC – Abacavir
TAF – Tenofovir Alafenamide
TDF – Tenofovir Disoproxil Fumarate
3TC – Lamivudine
FTC – Emtricitabine

InSTIs
DTG – Dolutegravir
RAL - Raltegravir

Non-NRTI (NNRTI)
EFV - Efavirenz

Protease Inhibitors (PI)
LPVr – Ritonavir-boosted Lopinavir
DRVr – Ritonavir-boosted Darunavir

- Cells treated with 0.1% DMSO were cultured in triplicate
- **Flow cytometry:** Markers of mitochondrial and cellular toxicity were quantified among live cells using the following probes:

Mitochondrial mass → Mito Green Tracker
Mitochondrial Intermembrane Potential (MMP) → Mito Deep Red Tracker
Mitochondrial ROS → Mito SOX
Apoptotic cells → Annexin V
Viability → DAPI

- All measurements and median fluorescence intensities (MFI) were normalized to the 0.1% DMSO condition

Results

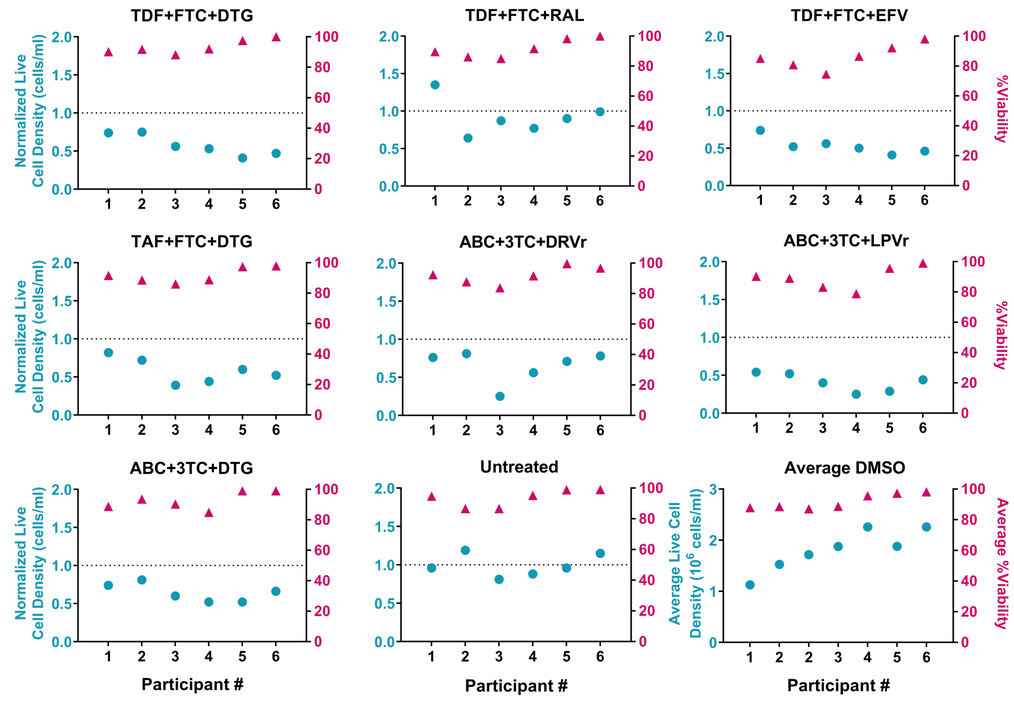


Figure 1. Live cell density and viability following 6-day exposure to 1X C_{max} cART regimens for all participants, normalized to corresponding DMSO controls (dashed horizontal lines).

- Cell viability over the treatment phase was largely **unaffected**, with the **median cell viability** across all regimens remaining **above 85%**
- **Live cell density** in RAL-containing cART was relatively **unaffected** compared to DTG on the same TDF+FTC backbone
- **Live cell density** in non-RAL treatment conditions were lower, indicating reduced cell proliferation

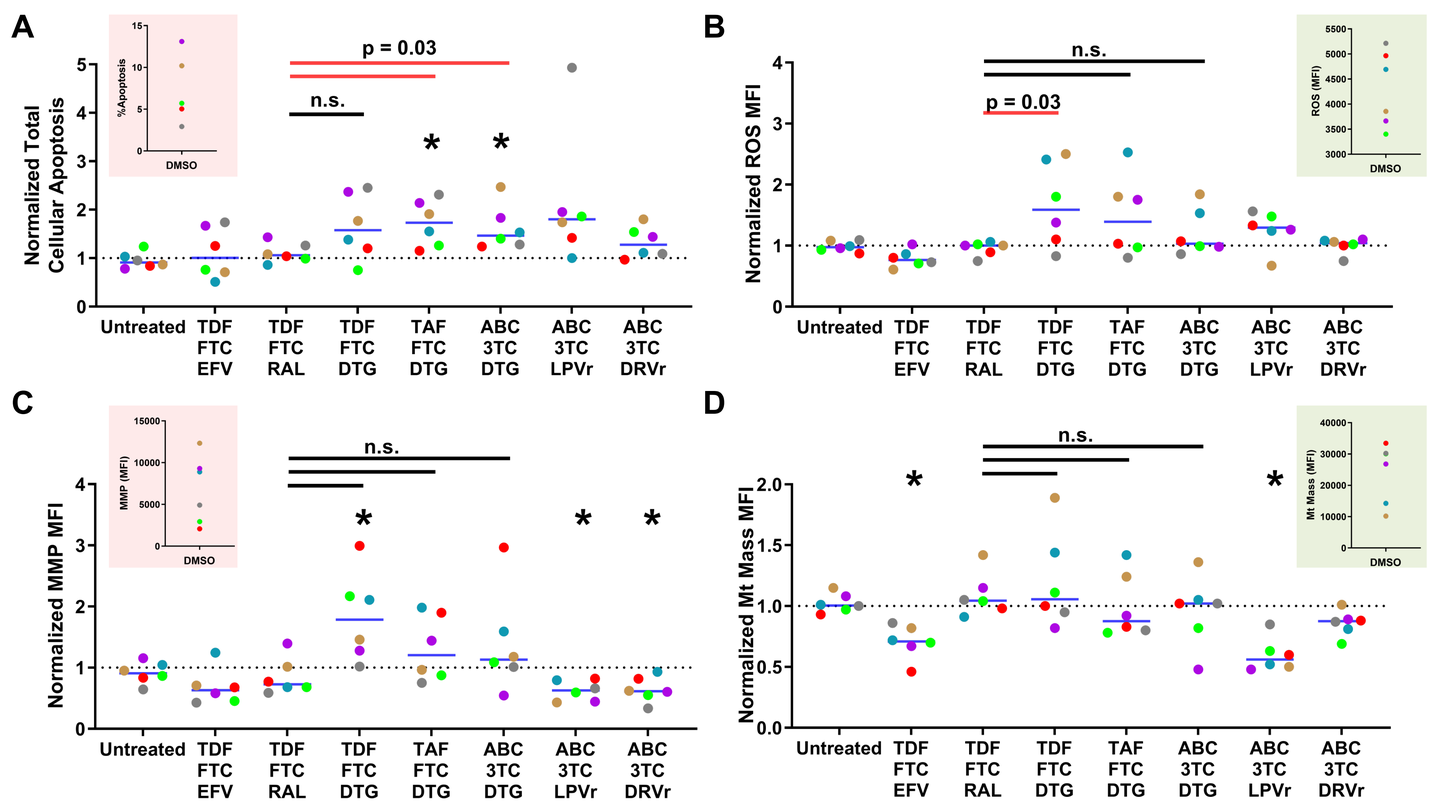


Figure 2. (A) Total apoptosis, (B) mitochondrial ROS, (C) MMP, (D) mitochondrial mass normalized to corresponding DMSO controls (dashed line) of each participant, represented by a unique color. Medians are shown and insets represent raw data of DMSO controls. Stars represent Wilcoxon signed-rank test p = 0.03 when compared to DMSO control. Comparisons between RAL and DTG-containing cART are shown in horizontal bars.

- Following 6-day *ex vivo* exposure, **DTG**-containing cART appeared to **increase apoptosis** (Fig. 2A), **mitochondrial ROS** (Fig. 2B) and **MMP** (Fig. 2C)
  - RAL on the same TDF+FTC backbone (Fig. 2A, 2B and 2C) did not affect mitochondrial and cellular health
  - **PI**-containing cART also seemed to **increase apoptosis** (Fig. 2A)
- RAL and DTG-containing cART did not affect mitochondrial mass (Fig. 2D)
  - **LPVr** and **EFV**-containing cART **reduced mitochondrial mass** (Fig. 2D)

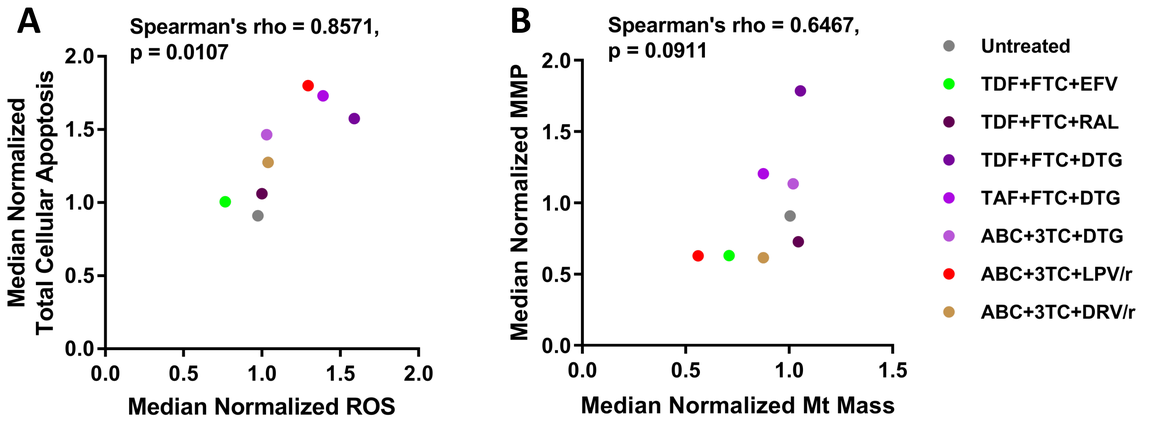


Figure 3. Relationship between (A) cellular apoptosis and mitochondrial ROS, and between (B) mitochondrial mass and MMP. Symbols represent the median of normalized values for each condition.

- **Positive correlation** between **apoptosis and ROS** (Fig. 3A), but not between mitochondrial mass and MMP (Fig. 3B)
- Cells treated with **DTG-based cART** regimens had **highest MMP, ROS and apoptosis**

Conclusions

- With the exception of RAL-containing regimens, cART exposure appears to reduce cell proliferation without any noticeable cytotoxic cell death during culture
- DTG-cART increased cellular apoptosis, mitochondrial ROS and MMP among live PBMCs
- This result highlights the importance of continued testing into the safety of DTG, a first-line regimen drug for many people living with HIV

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