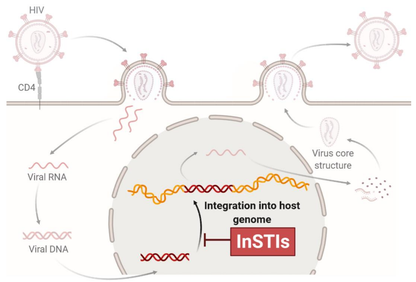
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Several InSTI Affect Immune Cell Mitochondrial, Proliferation and Apoptosis Ex Vivo

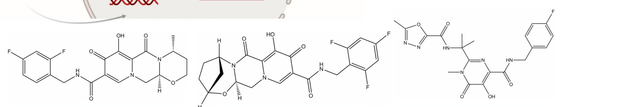
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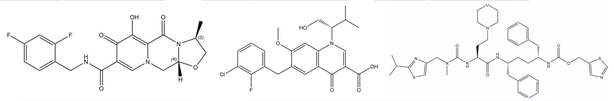
Introduction



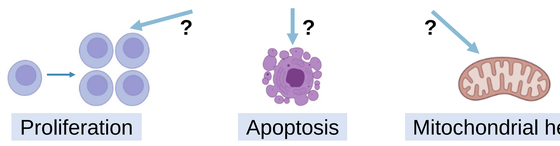
HIV Integrase Strand Transfer Inhibitors (InSTIs) toxicities are unclear



Dolutegravir (DTG) | Bictegravir (BIC) | Raltegravir (RAL)



Cabotegravir (CAB) | Elvitegravir+Cobicistat (EVG/COBI)



Proliferation | Apoptosis | Mitochondrial health

Aim

Characterize the effect of InSTI exposure *ex vivo* on immune cell mitochondrial health, activation, and proliferation on peripheral mononucleated cells (PBMCs) from healthy donors.

Methods



Isolate PBMCs from healthy volunteers



Activate with anti-CD3/CD28

Single InSTI exposure at pharmacological concentration (1xC_{max}) in 0.1% DMSO

Culture at 37°C, 5% CO_2 for 6 days



Flow cytometry

1. Mitochondrial mass
2. Mitochondrial intermembrane potential (mt potential)
3. Mitochondrial reactive oxygen species (mt ROS)
4. Apoptosis
5. Proliferation (Division Index)
6. Cell activation (CD69+)
7. Cell differentiation (CD27, CD45RA)

BIC or EVG/COBI inhibit proliferation and DTG, CAB, BIC or EVG/COBI affect mitochondria parameters while RAL exerts no effects in peripheral blood mononuclear cells

Results

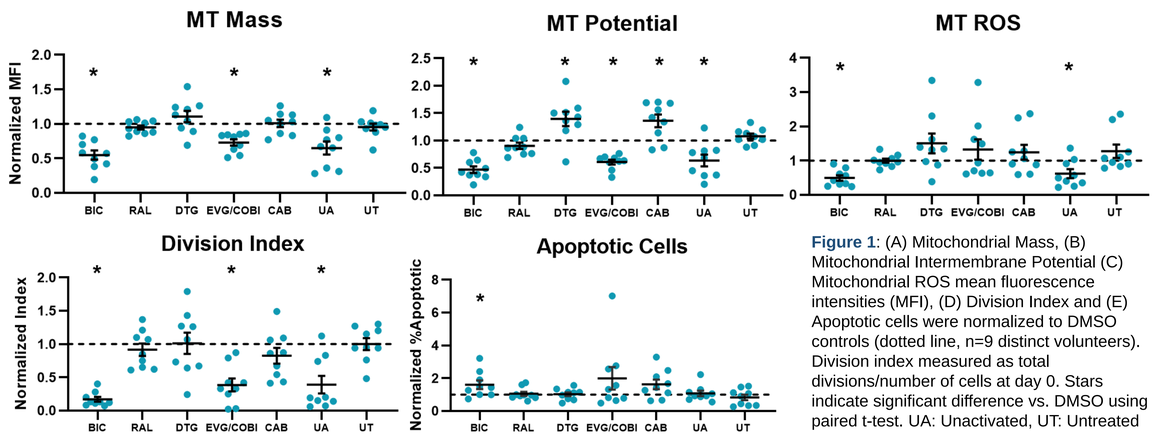


Figure 1: (A) Mitochondrial Mass, (B) Mitochondrial Intermembrane Potential (C) Mitochondrial ROS mean fluorescence intensities (MFI), (D) Division Index and (E) Apoptotic cells were normalized to DMSO controls (dotted line, n=9 distinct volunteers). Division index measured as total divisions/number of cells at day 0. Stars indicate significant difference vs. DMSO using paired t-test. UA: Unactivated, UT: Untreated

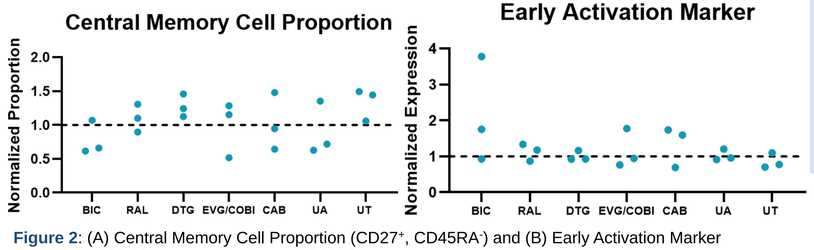


Figure 2: (A) Central Memory Cell Proportion ($CD27^+$, $CD45RA^-$) and (B) Early Activation Marker Expression ($CD69^+$) were normalized to DMSO controls (dotted line, n=3 distinct volunteers). UA: Unactivated, UT: Untreated

To further investigate the changes observed with mitochondria, apoptosis and proliferation, we investigated markers of immune activation

Conclusions

Activated cultured PBMC exposed to 1xC_{max} InSTI:

BIC

- Reduced mitochondrial mass, intermembrane potential and ROS by two-fold ($p<0.001$)
- Reduced proliferation by five-fold ($p<0.001$)
- Appears to elevate early activation marker expression and decrease proportion of CD4 central memory cells

EVG/COBI

- Reduced mitochondrial intermembrane potential and proliferation two-fold ($p<0.001$)

DTG and **CAB**

- Increase mitochondrial intermembrane potential by half-fold ($p<0.045$)

RAL

- No significant effects

Significance

- HIV treatment is lifelong, and these data clearly show that InSTIs can affect PBMC mitochondria
- The effects of **BIC** *ex vivo* suggest a potential underlying metabolic mechanism which could hinder immune responses
- This highlights the importance of further investigation of InSTIs as they could exert long-term immunological consequences that may not be detected in clinical trials
- RAL, a first generation InSTI appears to have no effect, a reassuring finding that warrants further clinical investigation

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CIHR IRSC

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