Assessing the Cellular and Mitochondrial Effects of Integrase Inhibitors in a Human Embryonic Stem Cell Model

CS1.04

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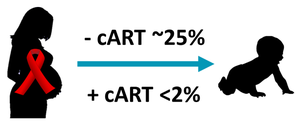
Background

- Women living with HIV give birth to ~1.5M infants each year
- ~80% of HIV+ women receive combination antiretroviral therapy (cART) during pregnancy, reducing vertical transmission rates from ~25% to <2%



- The safety of antiretrovirals (ARVs), such as newer integrase inhibitors (InSTIs) have not been fully characterized in pregnancy
- Many ARVs affect mitochondria and can lead to mitochondrial dysfunction, which could impact embryo development

Objective

To characterize and compare the effects of different cART regimens on cultured human embryonic stem cells with respect to cellular and mitochondrial health, as well as pluripotency

Methods

- CA1S, a human embryonic stem cell (hESC) line adapted for cell culture screening
- CA1S hESCs were cultured in the presence of 0.1% DMSO or 1X C_{max} of the following regimens:
  - Dolutegravir (DTG), raltegravir (RAL), bictegravir (BIC), cobicistat-boosted elvitegravir (EVG/COBI), or efavirenz (EFV) on TDF/FTC
  - DTG, RAL, BIC, EVG/COBI, or rilpivirine (RPV) on TAF/FTC;
  - DTG, RAL, or ritonavir-boosted darunavir (DRVr) on ABC/3TC;
  - Cabotegravir (CAB) and RPV
- After three days of cART exposure, cells were harvested and assessed via flow cytometry of:
  - Cell health markers (viability (DAPI) and apoptosis (Annexin V))
  - Mitochondrial characteristics (mass, intermembrane potential (MMP), and reactive oxygen species (ROS))
  - Pluripotency markers (SSEA-3 and TRA-1-60)
- Data were collected for n=5 independent experiments
- Regimens were grouped according to base ARV and compared to DMSO control using paired t-tests with Bonferroni correction

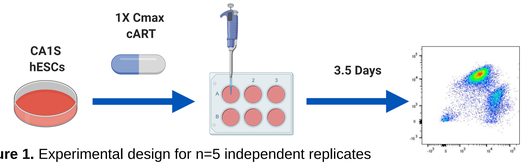


Figure 1. Experimental design for n=5 independent replicates

Dolutegravir or **bictegravir** appear toxic and **dolutegravir** or **cabotegravir** induce differentiation in cultured **human embryonic stem cells**

Results

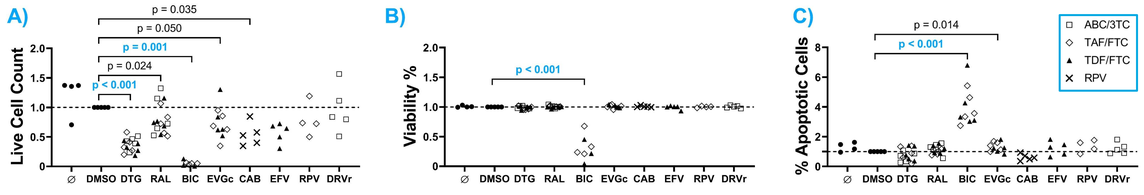


Figure 2. Cell proliferation (A), viability (B), and apoptosis (C) following exposure to 1X C_{max} of one of the 14 cART regimens normalized to corresponding DMSO controls (dashed lines). Each data symbol represents a single replicate on one of four backbones (shape). P-values determined using paired t-test, values in blue represent significance after Bonferroni correction.

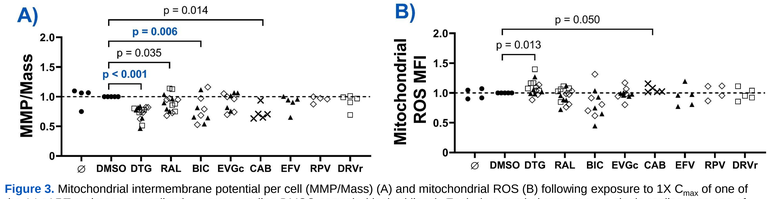


Figure 3. Mitochondrial intermembrane potential per cell (MMP/Mass) (A) and mitochondrial ROS (B) following exposure to 1X C_{max} of one of the 14 cART regimens normalized to corresponding DMSO controls (dashed lines). Each data symbol represents a single replicate on one of four backbones (shape). P-values determined using paired t-test, values in blue represent significance after Bonferroni correction.

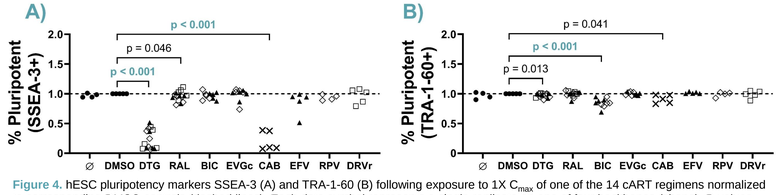


Figure 4. hESC pluripotency markers SSEA-3 (A) and TRA-1-60 (B) following exposure to 1X C_{max} of one of the 14 cART regimens normalized to corresponding DMSO controls (dashed lines). Each data symbol represents a single replicate on one of four backbones (shape). P-values determined using paired t-test, values in blue represent significance after Bonferroni correction.

*Cells exposed to **DTG** and **BIC** have decreased cell counts*

*Cells exposed to **BIC** have decreased viability and increased apoptosis*

*Cells exposed to **DTG** and **BIC** have decreased MMP/Mass*

*Cells exposed to **DTG** & **CAB** have decreased expression of SSEA-3*

Conclusions

- Exposure to cART containing **DTG** or **BIC**:
  - Reduced cell counts 3-fold ($p \leq 0.001$)
  - Reduced mitochondrial intermembrane potential ($p \leq 0.006$)

  compared to controls
- Exposure to cART containing **BIC**:
  - Decreased viability 3-fold ($p < 0.001$)
  - Increase total % apoptosis 3-fold ($p < 0.001$)

  compared to controls
- Exposure to regimens containing **DTG** or **CAB**:
  - Decreased SSEA-3 expression >80% ($p < 0.001$)

  compared to controls
- There were no significant effects after Bonferroni adjustment detected for the backbones, RAL, EVG/COBI, EFV, RPV, or DRVr

These data indicate that exposure to some cART regimens at pharmacological concentrations especially DTG or BIC, appear toxic to cultured hESCs

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Significance

Given the widespread use and overall favourability of **DTG** and other newer InSTIs among women of reproductive age, it is imperative to further elucidate their short and long-term safety in the context of pregnancy and embryonic development

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