Toxicity of HIV antiretrovirals belonging to the integrase inhibitor class in human embryonic stem cell models

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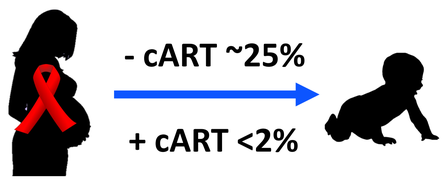
Background

- Women living with HIV give birth to ~1.5M infants each year
- ~80% of women living with HIV 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) dolutegravir (DTG) and raltegravir (RAL), have not been fully characterized in the context of pregnancy
- A recent study reported an early signal for increased neural tube defects in infants exposed to DTG from conception
- The neural tube forms within the first four weeks of pregnancy and any disruptions in the initial stages of embryonic and placental development could be detrimental, resulting in perturbed function of key tissues and organs

Objectives

To characterize and compare the dose-dependent effects of the InSTIs bictegravir (**BIC**), cabotegravir (**CAB**), dolutegravir (**DTG**), and raltegravir (**RAL**) on cultured human embryonic stem cells with respect to cellular health and pluripotency.

Methods

- CA1S and H9 human embryonic stem cells (hESCs) were cultured in three and six independent replicates, respectively
- CA1S hESCs were passaged and plated in media containing 0.1% DMSO (drug diluent) or one of four InSTIs, at doses ranging below and above pharmacologically relevant concentrations (1X C_{max}):
  - InSTIs tested: **BIC**, **CAB**, **DTG**, and **RAL**
  - Doses: 0.001, 0.01, 0.1, 0.15, 0.2, 0.25, 0.5, 1, 2, and 3X C_{max}
- Doses used for the H9 biological replicate experiments are highlighted above
- Cells were harvested at 3.5 days and assessed by:
  - Cell counts
  - Flow cytometry of:
    - Viability (DAPI)
    - Apoptosis (Annexin V)
    - Differentiation (Pluripotency markers SSEA-3 (early) and TRA-1-60 (late))
  - RT-qPCR of genes present in:
    - Pluripotent cells
    - Cells beginning to differentiate towards one of the three germ layers: mesoderm, endoderm, and ectoderm

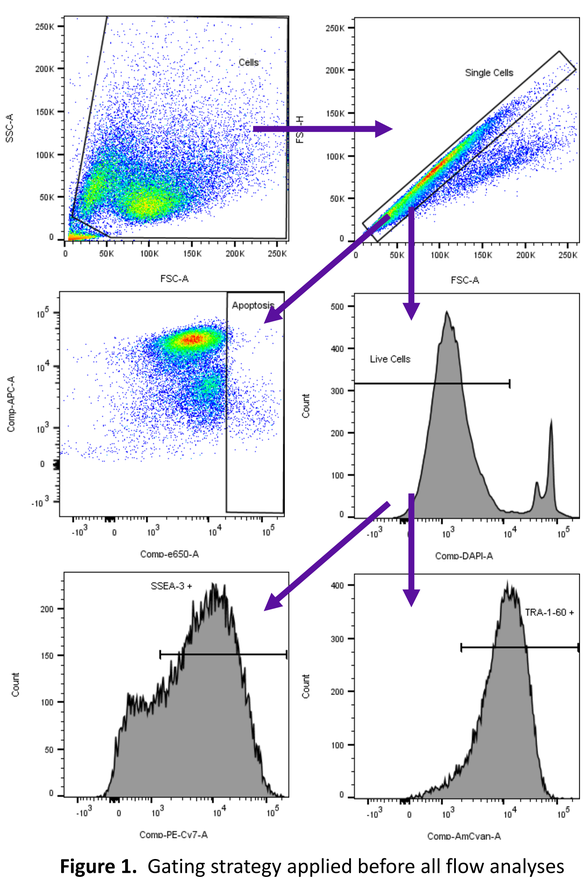


Figure 1. Gating strategy applied before all flow analyses

Results

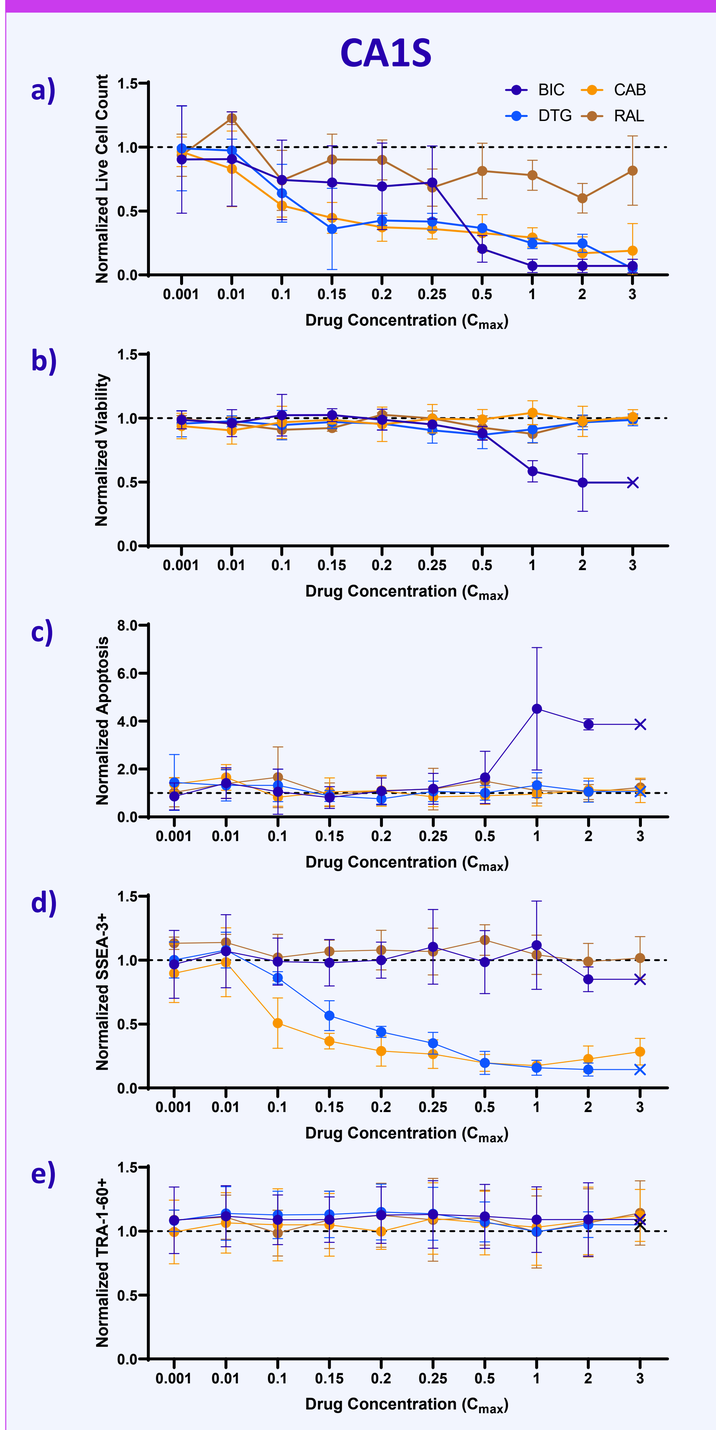


Figure 2. Live cell count (a), viability (b), apoptosis (c), SSEA-3+ (d), and TRA-1-60+ (e) normalized to 0.1% DMSO control in CA1S hESCs treated with four different InSTIs for 3.5 days (n=3), mean and SD presented.

- CA1S hESCs exposed to ≥0.1X C_{max} **BIC, CAB,** and **DTG** show decreased proliferation (Fig. 2a). **BIC** exposure at ≥0.5X C_{max} further decreased viability and increased apoptosis (Fig. 2b & 2c)
- CA1S hESCs exposed to ≥0.1X C_{max} **CAB** and **DTG** show decreased SSEA-3 expression (Fig. 2d)
- H9 hESCs exposed to ≥0.5X C_{max} **BIC, CAB,** and **DTG** show decreased proliferation (Fig. 3a). **BIC** and **DTG** exposure at 1X C_{max} further decreased viability and increased apoptosis (Fig. 3b & 3c)
- H9 hESCs exposed to ≥0.5X C_{max} **DTG** and **CAB** show decreased SSEA-3 and TRA-1-60 (Fig. 3d & 3e). Further, expression of early mesendoderm markers appear increased with exposure to 0.5X C_{max} **BIC**, **CAB**, and **DTG**

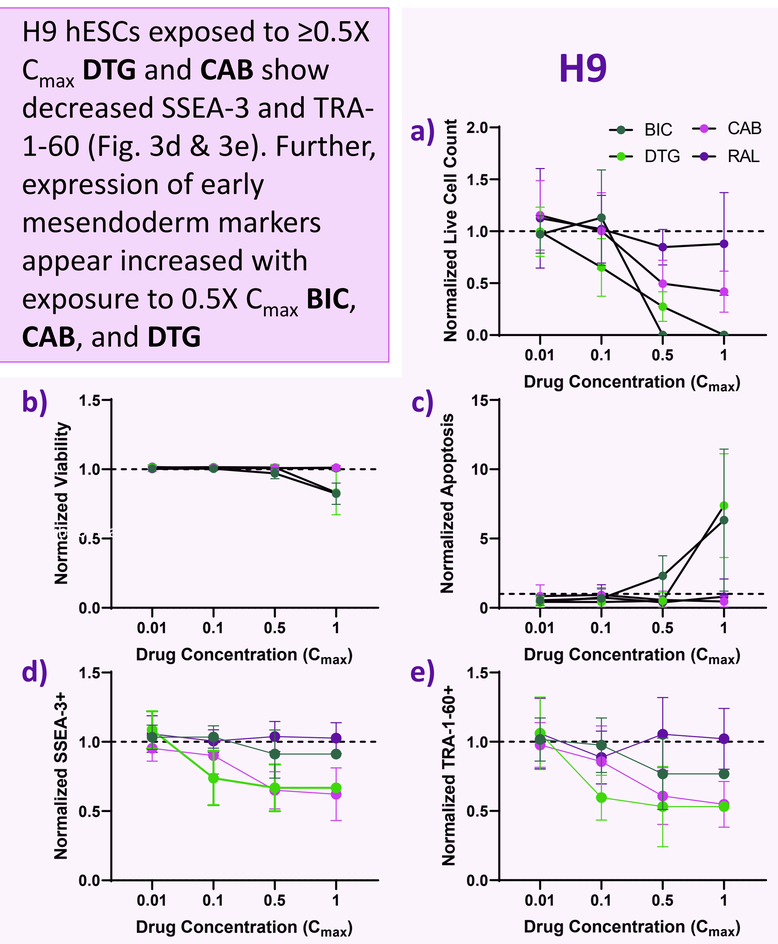


Figure 3. Live cell count (a), viability (b), apoptosis (c), SSEA-3+ (d), and TRA-1-60+ (e) normalized to 0.1% DMSO control in H9 hESCs treated with four different InSTIs for 3.5 days (n=6), mean and 95% confidence interval presented.

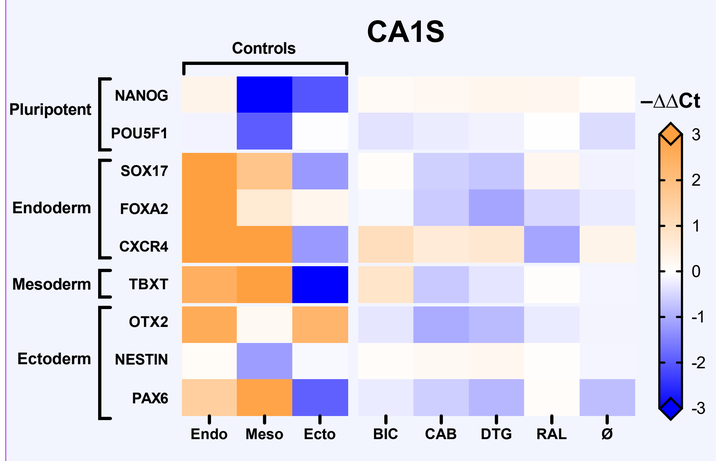


Figure 4. Heatmap showing the expression patterns of pluripotency and early germ layer lineage markers in CA1S hESCs after treatment with 4 different InSTIs at 0.5X C_{max} for 3.5 days (n=3: drug treatments; n=1 Endo, Meso, Ecto controls).

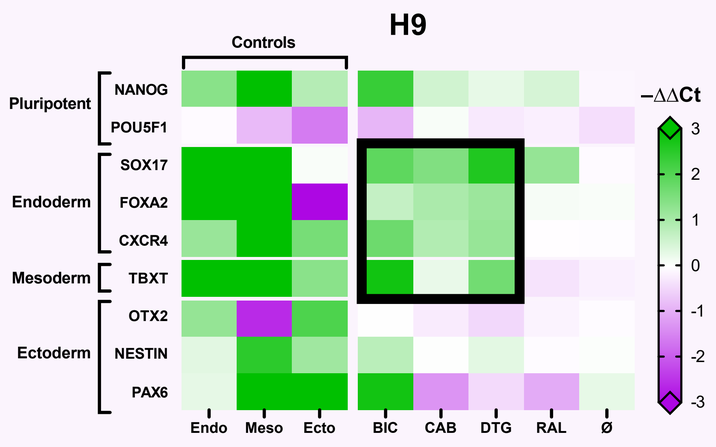


Figure 5. Heatmap showing the expression patterns of pluripotency and early germ layer lineage markers in H9 hESCs after treatment with 4 different InSTIs at 0.5X C_{max} for 3.5 days (n=3: drug treatment; n=1 Endo, Meso, Ecto). The black square indicates increased mesendoderm gene expression with exposure to BIC, CAB, and DTG.

Conclusions

Exposure to **BIC**, **CAB**, and **DTG** appears to induce cytotoxicity and differentiation in hESCs, even at sub-clinical concentrations. Further investigation is needed to a) identify whether longer exposure to the drugs will result in further differentiation and b) if so, towards which germ layer.

Significance

Given the increasing worldwide use of InSTIs in first line cART regimens, including by women who are of reproductive age or pregnant, it is imperative to elucidate their long-term safety in the context of pregnancy.

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