Potential Interaction Between Dolutegravir and Folate Transporters/Receptor in Human Placenta

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Conflict of Interest Disclosure
I have no conflicts of interest.
Background

- Dolutegravir (DTG) is recommended by the World Health Organization as part of first/second-line antiretroviral therapy for adults living with HIV.
- Tsepamo study from Botswana reported an increased risk for neural tube defects (NTDs) in infants from mothers taking DTG during conception.

<table>
<thead>
<tr>
<th>Year (% with NTD)</th>
<th>Treatment</th>
<th>DTG from conception</th>
<th>Non-DTG from conception</th>
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<tbody>
<tr>
<td>2018</td>
<td></td>
<td>0.94% (n=426)</td>
<td>0.12% (n=11173)</td>
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<tr>
<td>2019</td>
<td></td>
<td>0.30% (n=1683)</td>
<td>0.10% (n=14792)</td>
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- Folates are critical for fetal development and folate deficiency is associated with an increased risk of NTDs.
- Placental folate transport is primarily mediated by folate receptor alpha (FRα), reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT).
- Recent study demonstrated that DTG acts as a partial antagonist of FRα and causes developmental toxicity in zebrafish.
- It is unclear whether DTG can interact with folate receptor/transporters in placenta cells and impair folate delivery to the human fetus, potentially leading to NTDs.
Hypothesis and Methods

**Hypothesis**: INSTIs, in particular, DTG, inhibit the functional expression of folate transporters and/or receptor in human and rodent placenta, and could impair adequate delivery of folates to the fetus resulting in a higher risk of fetal NTDs.

**Methods**

- Immunohistochemical staining was performed to localize RFC and PCFT in human first-trimester placenta tissues.
- First-trimester human trophoblast cell lines, HTR-8/SVneo and JAR, were treated with DTG (500-4000ng/ml) for 1-24 hours. qPCR and western blot analyses were performed to assess mRNA and protein expression of FRα, RFC and PCFT, respectively.
- Transport assays, using 50nM [³H]-methotrexate (MTX) and 50nM [³H]-folic acid (FA), were conducted to assess the functional activity of RFC and PCFT.
- Human first-trimester and term placenta tissues were examined for the mRNA and protein expression of FRα, RFC and PCFT by qPCR and western blot.
Results

**Figure 1.** Immunohistochemical localization of RFC (left) and PCFT (right) in human first-trimester placenta tissues (4 weeks). Blue arrowheads show syncytiotrophoblast. Both RFC and PCFT are highly expressed.

**Figure 2.** Relative mRNA expression of FRα (A), RFC (B), and PCFT (C) genes was determined in human placental tissues from non-infected donors, first-trimester (5-7 weeks) and term (38-40 weeks). Results are presented as mean relative mRNA expression ± SEM normalized to the housekeeping human Cyclophilin B gene (n=6). Relative levels of FRα (D), RFC (E), and PCFT (F) protein in human first-trimester and term placenta tissues, expression were determined by densitometric analyses. n=6

**Figure 3.** Effect of DTG treatment on RFC (A), and PCFT (B) mRNA expression and RFC (C) and PCFT (D) protein expression in HTR-8/SVneo cells. Results are expressed as percentage change normalized to vehicle (DMSO) control and reported as mean ± S.E.M. for n = 3 independent experiments. *, p < 0.05.
Results and Conclusion

**Conclusion:** RFC and PCFT functional expression is decreased by DTG treatment in first-trimester human placental cell lines, suggesting that DTG could potentially impair effective placental folate delivery to the fetus.

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