



## Letter

## In response to the Letter to the Editor by Romach et al. re our publication “Dolutegravir in pregnant mice is associated with increased rates of fetal defects at therapeutic but not at suprathreshold levels”



H. Mohan<sup>a</sup>, M. Guzman Lenis<sup>a</sup>, E.Y. Laurette<sup>a</sup>, O. Tejada<sup>a</sup>, T. Sanghvi<sup>a</sup>, K.-Y. Leung<sup>b</sup>, L.S. Cahill<sup>c,d</sup>, J.G. Sled<sup>c,e,f</sup>, P. Delgado-Olguin<sup>f,g,h</sup>, N.D.E. Greene<sup>b</sup>, A.J. Copp<sup>b</sup>, L. Serghides<sup>a,i,j,\*</sup>

<sup>a</sup> Toronto General Hospital Research Institute, University Health Network, Toronto, Canada

<sup>b</sup> Developmental Biology & Cancer Department, UCL Great Ormond Street Institute of Child Health, University College London, London, UK

<sup>c</sup> Mouse Imaging Centre, The Hospital for Sick Children, Toronto, Ontario, Canada

<sup>d</sup> Department of Chemistry, Memorial University of Newfoundland, St John's, Newfoundland and Labrador, Canada

<sup>e</sup> Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

<sup>f</sup> Translational Medicine, The Hospital for Sick Children, Toronto, Ontario M5G 0A4, Canada

<sup>g</sup> Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

<sup>h</sup> Heart & Stroke Richard Lewar Centre of Excellence, Toronto, Ontario M5S 3H2, Canada

<sup>i</sup> Department of Immunology and Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada

<sup>j</sup> Women's College Research Institute, Women's College Hospital, Toronto, Ontario, Canada

## ARTICLE INFO

## Article History:

Received 22 March 2021

Accepted 24 March 2021

Available online xxx

We appreciate the comments of Romach et al. on our recent publication [1].

In response to the comments on our study design, our objective when we designed our study was not to repeat pre-clinical studies performed as part of the regulatory approval process for dolutegravir (DTG), but to emulate the clinical scenario as closely as possible. Our study design was motivated directly by the Tsepamo study findings of higher incidence of neural tube defects (NTDs) in pregnant women receiving a DTG-based regimen from conception [2]. We do not agree that this is a sub-optimal study design for our research question.

Pregnant women living with HIV receiving a DTG-based regimen take their medication as one or more pills orally. Fetal defects in these women are compared to rates in the general population, i.e. women not receiving DTG-based antiretroviral therapy (ART). In this real-world scenario, the “treated” group would not be receiving DTG as a single drug, but in combination with a dual nucleos(t)ide reverse transcriptase inhibitor (NRTI), such as the one used in our study – tenofovir (TDF)/emtricitabine (FTC). TDF/FTC is a very commonly used NRTI combination that has not been associated with higher incidence of NTDs in the many years of widespread use by pregnant

women living with HIV. Further, the “control” group would not be taking inactive excipients. To best replicate this clinically relevant scenario we selected to crush the actual pills that pregnant women receive, to administer DTG with TDF/FTC – the most commonly used NRTI backbone in the Tsepamo study, and to administer all drugs orally. We chose a dose that yielded DTG C<sub>max</sub> concentrations similar to those seen in pregnant women (3000 ng/ml). Further, as the higher rates of NTDs were observed in women who received DTG-based ART from conception, we selected to treat our animals for the entire duration of pregnancy (unlike what was performed in the studies by Stanislaus et al. [3]). Our control group was handled identically to the treated group (i.e. gavaged daily with equal amounts of water) but did not receive any drug – to best model the clinical comparator group of pregnant women not taking DTG-based ART while controlling for effects of gavage on the pregnancy.

For our dose-response experiments we chose to administer DTG at a 5x dose which yielded a 4-fold increase in C<sub>max</sub> levels (12,000ng/ml). We chose to not increase the dose of TDF/FTC – thus only DTG was increased in our higher dose treatment arm. To our surprise we observed a non-monotonic dose response, with higher rates of defects observed at the 1x-dose of DTG-based ART vs. at the 5x-dose. While Romach et al. express concern that “the explanation for the non-dose responsive developmental defects are contrary to the established principles of teratology”, our findings speak to the importance of testing under conditions that mimic the clinical conditions and not extrapolating from foundational work. We were also surprised by our findings but are not willing to alter the interpretation of our data to fit “established principles of teratology”. We performed a highly powered study, including a much larger number of animals than traditionally used in pre-clinical teratogenic studies, and we observed higher rates of defects in our lower dose treatment arm compared to our higher dose treatment arm. There are several

DOI of original article: <http://dx.doi.org/10.1016/j.ebiom.2021.103335>, <http://dx.doi.org/10.1016/j.ebiom.2020.103167>.

\* Corresponding author at: 101 College Street, 10-359, Toronto, Ontario, M5G 1L7, Canada.

E-mail address: [lena.serghides@utoronto.ca](mailto:lena.serghides@utoronto.ca) (L. Serghides).

<https://doi.org/10.1016/j.ebiom.2021.103334>

2352-3964/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

examples in the literature of non-monotonic dose responses on fetal defects, as noted in the Discussion of our paper [1], and our observations add to that literature. It is of interest that a non-monotonic dose response was also observed for the effects of DTG on folate binding to folate receptor 1 in the presence of human serum albumin and/or calcium in the study by Cabrera and colleagues [4]. We think that it would be unscientific to dismiss our findings of a relationship between therapeutic levels of DTG-based ART and an increase in fetal defects simply because it does not fit the expectation of a classic dose response. We would also like to note that the one NTD observed in the rabbit fetotoxicity study by Stanislaus et al. [3] was observed in the lowest DTG dose treatment arm.

Unfortunately, we cannot comment on the study by Posobiec et al. [5] as this was only presented as a conference abstract. We encourage publication of these embryo culture experiments so these data can be added to the available literature on DTG and fetal defects.

On the suggestion by Romach et al. that a minimal effect on folate would somehow rule out a role for DTG-based ART in NTDs, we disagree. Not all NTDs are sensitive to folate status, and NTDs that are non-responsive to folic acid supplementation have been identified. Further, even small effects on maternal folates – which are under homeostatic control – may raise a concern.

We welcome discussion of our findings and challenge of our conclusions – this is the nature of science and peer review. However, we stand by our study design and by our conclusion that our findings provide support for DTG usage in pregnancy being associated with a small increase risk of NTDs.

## Contributors

LS and HM drafted the letter. All authors reviewed and edited the letter.

## Declaration of Competing Interest

The authors have no competing interests. AJC acted as consultant for ViiV Healthcare Limited, with fees going to support his research program. LS received personal support for participating in a ViiV organized Think Tank.

## References

- [1] Mohan H, et al. Dolutegravir in pregnant mice is associated with increased rates of fetal defects at therapeutic but not at supratherapeutic levels. *EBioMedicine* 2021;63:103167.
- [2] Zash R, et al. Neural-tube defects and antiretroviral treatment regimens in Botswana. *N Engl J Med* 2019;381:827–40.
- [3] Stanislaus DJ, Posobiec LM, Laffan SB, Solomon HM, Ziejewski MZ, Romach EH. Absence of developmental and reproductive toxicity in animals exposed to dolutegravir. *Birth Defects Res* 2020;112:245–61.
- [4] Cabrera RM, et al. The antagonism of folate receptor by dolutegravir: developmental toxicity reduction by supplemental folic acid. *AIDS* 2019;33:1967–76.
- [5] Posobiec LM, Chapman SP, Murzyn S, Rendemonti JE, Stanislaus DJ, Romach B. Using whole embryo culture to address risk to patients taking dolutegravir. *Teratology Society 59th Annual Meeting Birth Defects Res* 2019;111:490.