Reviewer’s report

Title: ATR-101, a selective ACAT1 inhibitor, decreases ACTH-stimulated cortisol concentrations in dogs with naturally occurring Cushing's syndrome

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Reviewer: J.A. (Jan) Mol

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The manuscript of Langlois et al states that they provide proof of concept for ATR-101 as a novel agent for the treatment of endocrine disorders like Cushing's syndrome in humans by testing this compound in dogs with spontaneous Cushing's syndrome. However some serious problems arise in the paper.

1. Diagnosis

For the diagnosis hyperadrenocorticism either a LDDST or an ACTH-stimulation test was done. According to the consensus statement of the ACVIM the LDDST is the method of choice and an ACTH stimulation test is of less use. For a proof of concept therefore only dogs with an appropriate LDDST should be used.

2. Selection of animals

Against the background of a possible suppressive effect of ATR-101 that is only seen after ACTH stimulation it is advisable to select only dogs with pituitary-dependent hyperadrenocorticism. The measurement of plasma ACTH concentrations are a prerequisite, but assays for human ACTH have to be validated properly for the dog as the sequence of the C-terminal part of ACTH in dogs is different from humans and can result in false low values. At least a good reference to validation studies should be given. Dog number 9 with metastasized prostate cancer should be totally excluded from the study.

3. Estimation of the effect

The effect of ATR-101 should be present under basal conditions will it be therapeutically helpful. The conclusions of the current paper rely completely on an effect of ATR-101 on ACTH-stimulated plasma cortisol concentrations between day 0 and the other days. No effect is seen on basal plasma cortisol concentrations. As the authors also discus measurement of cortisol
secretion over time, such as accumulation in the urine, is advised. A suitable control group is missing making it possible that the repeated ACTH stimulation test causes the diminished cortisol response. As the distribution of plasma cortisol concentrations maybe not normal non-parametric statistics should be used and p-values corrected for multiple testing.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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