Reviewer’s report

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

Version: 0 Date: 24 Jul 2017

Reviewer: Robert J. Kemppainen

Reviewer's report:

General Comments: This report describes use of a novel small molecule ATR-101 in canine Cushing's disease. ATR-101 is a selective inhibitor of ACAT1 and as such reduces formation of cholesterol ester accumulation and storage in steroid secreting cells; in this case, targeting those in the adrenal cortex. The drug has been studied in healthy dogs and this reports extends examination of its effects to dogs with either pituitary dependent Cushing's disease or with functional adrenocortical tumors.

1. From the introduction, ATR-101 has differing effects depending on dose; that is reduction of cortisol and other adrenal steroids in circulation when administered at low doses to adrenal cell apoptosis when given at high doses. What is the ultimate goal, in the author's opinion, when using the drug to treat hyperadrenocorticism? Is it simply to reduce steroid secretion or is it to kill adrenal cells? This also relates to lines 341-354, the description of adrenal histology in the 3 dogs with adrenal tumors.

2. What is the relative tissue distribution of ACAT1? Specifically, is it present in hepatocytes? Could this explain the effects on liver enzymes?

Specific Comments:

1. Lines 164-166. What happened to dogs with pituitary disease after discontinuance of ATR-101? Did they start on trilostane?

2. Lines 222-229. Please provide citations for validations of the hormone assays.

3. Lines 300-310. Was there any difference in ACTH stimulated cortisol responses comparing dogs with pituitary disease versus those with adrenal tumors?

4. Line 332. Did the ACTH stimulated cortisol decline (as much) in the 2 dogs whose clinical signs were unchanged or the dog whose signs worsened? Or were they outliers?
5. Lines 443-444. "…biochemical parameters associated with liver function remained normal". What parameters are the authors referring to?

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

Yes

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

Yes

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

Yes

**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.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable

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