Reviewer's report

Title: Lubiprostone ameliorates the cystic fibrosis mouse intestinal phenotype

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Reviewer: Hugo De Jonge

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In this study the authors have exploited previously developed and published methods to test a potential improvement of the intestinal phenotype of cystic fibrosis (CF) mice (Cftr-/-) by in vivo treatment with the putative ClC-2 activator and anti-constipation drug lubiprostone (once daily gavage of 0.1 ml emulsion in Peptamen; 10 µg/kg/day for 2 weeks). It is concluded that lubiprostone treatment has beneficial effects on some CF-relevant parameters (decreased bacterial overgrowth in the small intestine; reduced activation of mast cell genes in CF mice) but not on others (body weight gain, crypt width, mucus accumulation, small intestinal transit). Unexpectedly, lubiprostone promoted bacterial overgrowth in WT mice, and slightly enhanced rather than reduced mucus accumulation in the intestinal crypts of Cftr-/- mice, suggesting a Cftr-independent, stimulatory effect on mucus secretion in CF intestine.

Major Compulsory Revisions:

1. All Cftr-/- mice in this study are maintained on a liquid diet (Peptamen) rather than solid diet. Unfortunately, this choice did not allow the authors to investigate potential beneficial effects of lubiprostone on intestinal obstruction and survival, i.e. the most prominent intestinal phenotype of CF mice. If lubiprostone would be able to activate intestinal chloride and fluid secretion through a non-CFTR mediated pathway, possibly involving CIC2, one would expect to see a reduced obstruction and increased survival in lubiprostone-treated CF mice.

2. A very recent study performed too in Cftr-/- mice (Harmon GS et al 2010 Nature Med 16: 313-318) shows that PPAR-# signaling is defective in CF colon and that PPAR-# agonists (e.g. rosiglitazone) can induce bicarbonate secretion, reduce mucus retention, and promote survival of CF mice in a Cftr-independent fashion. In the present manuscript, stimulation of bicarbonate and mucus solubility and secretion rather than chloride secretion by lubiprostone, possibly triggered through EP4 receptors, is suggested as the most plausible mechanism by which lubiprostone may exert its anti-inflammatory and multiple other actions in the CF intestine. Both studies together raise the question whether lubiprostone, similar to 15-keto-PGE2, might additionally act as a PPAR-# agonist and could partially rescue the intestinal CF phenotype through this pathway. This possibility should be explored, or at least discussed in the manuscript.

3. Even if lubiprostone targets CFTR rather than apical ClC2 channels in the enterocyte (if they exist), it remains possible that the compound promotes luminal
hydration in Cftr-/- mice through an EP4/cAMP/PKA signaling pathway that is known to result in inhibition of NHE3 and NaCl/fluid absorption. Such a potential anti-absorptive action is ignored in the present manuscript.

4. Most experiments were apparently performed using total mouse intestine, despite the fact that intestinal functions may vary considerably along the length of the intestine. For example, the bacterial overgrowth and mucus retention from goblet cells is expected to be more prominent in the ileum as compared with jejunum. Were the measurements of crypt width (as an estimate of mucus accumulation) performed in ileum, jejunum, or in both segments? Idem for the microarrays?

Minor Essential Revisions:

1. p. 14, l. 10: The finding that lubiprostone can activate PGE2 receptors in the intestine is confirmed in ref. 10, showing inhibition of lubiprostone-induced anion secretion by a specific EP4 antagonist. Therefore this reference should be mentioned in addition to ref. 23.

2. p. 17, l. 13-14: A crucial finding reported in ref. 10 is that lubiprostone not only failed to induce intestinal chloride secretion in Cftr-/- mice but also in the ileal and rectal epithelium from human CF patients. This finding should be quoted in the Discussion.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.