Format for ANSWERING REVIEWERS

June 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10718-edited revision final.docx).

Title: Preclinical therapy of benign prostatic hyperplasia with neuropeptide hormone antagonists

Author: Petra Popovics, Andrew V. Schally, Norman L. Block and Ferenc G. Rick

Name of Journal: *World Journal of Clinical Urology*

ESPS Manuscript NO: 10718 (original invitation number: 02884434)

To address the criticism and comments of the Consultant, the following changes were made to the manuscript:

Reviewer 00505700

(1) *"The Core tip section describes the aims of the article and somewhat repeats the abstract. This section should focus exclusively on the Core tip of the article. In the Core Tip, 3rd line, "a decrease the level of" should be "a decrease in the level of"."*
The core tip has been rewritten accordingly to the following:

A new, effective treatment for BPH is critically needed. Present side effects of therapy include impotence, decreased libido, abnormal ejaculation, dizziness, weakness, blurred vision and insomnia. Preclinical data suggest that antagonists of neuropeptides GHRH, LHRH and GRP are effective in shrinking prostates in part by suppressing growth factors and inflammatory cytokines. Their effect is exerted through a decrease in levels of circulating hormones and also on a direct action on their respective prostatic receptors. These analogs seem to have the same clinical effects as the currently available BPH medical therapies but possess greater efficacy and have fewer or no side effects.

(2) *"Introduction: Overall, the introduction is well-written. However, rational of the article should be outlined clearly. Please describe in further details why the article focuses exclusively on neuropeptide hormones and their receptors. A brief description of the mechanistic link between neuropeptide hormones and increased epithelial and stromal cell number in BPH would be helpful."*
The following explanation has been added to the Introduction on page 6 line 4:

The utilization of these analogs in experimental BPH also improved our knowledge on the physiological role of neuropeptides and their receptors in the pathogenesis of BPH. The blockade of these receptors by specific antagonists inhibits the proliferation of stromal and epithelial cells and reduces the release of cytokines and growth factors[6, 20, 22, 24, 25] indicating the participation of the native neuropeptides in these processes. As new antagonistic analogs of neuropeptides have recently become available for clinical practice as well others are currently being developed for human trials, we felt that a review of recent findings related to their use in BPH is timely. This review therefore focuses exclusively on preclinical and clinical studies where neuropeptide antagonists were tested against BPH. Additionally, the use of somatostatin agonists is also

suggested based on previous findings in prostate cancer with the hope it will facilitate their experimental and clinical testing.

(3) "*Luteinizing hormone-releasing hormone antagonists: Please provide peer reviewed references for the statements below: "In addition, the expression of various proinflammatory cytokines and growth factors that have been implicated in the pathogenesis of BPH were found to be reduced following cetrorelix treatment (reference). A significant reduction in serum levels of DHT and LH was also observed. Interestingly, cetrorelix treatment reversed testosterone-induced morphological changes to resemble the histology of the normal prostate, including a decrease in epithelial height (reference). In addition, AR and 5a-reductase levels were reduced by cetrorelix (reference)."*
The missing references were inserted as requested

(4) "*Growth hormone-releasing hormone antagonists and their combination with luteinizing hormone-releasing hormone analogs: - It is stated: "GHRH is also secreted locally in the prostate, suggesting that it serves as an autocrine/paracrine regulator". Please describe whether GHRH is secreted by cancer cells line only or both cancer and non-cancer cells."*
As suggested by the Referee, this section has been completed on page 10 line 28:

GHRH is also secreted locally in normal and malignant prostate tissue, suggesting that it serves as an autocrine/paracrine regulator which process might be involved in the pathogenesis as well as the progression of prostate cancer[78, 23, 79].

(5) "*The mechanistic discussions rely heavily on findings in the rat model of BPH created by testosterone administration. Is this model reliable and clinically relevant? Limitations of the rat model should be discussed in further details."*
The section discussing the limitation of the rat BPH model has been expanded on page 9 line 26 with the addition of a new reference:

Testosterone-induced hyperplasia selectively appears in the ventral prostate lobe in rats that might be the result of the distinct anatomy of this model from humans[55]. Also, the efficacy of testosterone to induce prostatic hyperplasia varies among different rat strains[56]. In addition to the noted disadvantages of the model, only the proliferation of epithelial cells is triggered by the addition of testosterone[56], whereas stromal-epithelial interactions are believed to be crucial in the pathogenesis of BPH[57, 58].

(6) "*It is stated: "GHRH and LHRH antagonists administered together were also more effective in inducing apoptosis as measured by changes in the levels of Bcl-2, Bax, p53, NF-κβ and COX-2." The clinical relevance of these changes should be discussed."*
An explanation of the clinical relevance of these findings has been added on page 12 line 15:

The combination therapy therefore has a great prospect in reducing hyperplastic prostate volume by triggering apoptotic cell death. In addition, chronic inflammation has been linked to the development and worsening of BPH; COX-2 has been proposed to play a key role in this process[86]. Hence, coadministration of GHRH and LHRH antagonists may also improve clinical outcome by reducing the expression of inflammation-related proteins such as NF-κβ and COX-2[87].

(7) "*Does the role of GRPR in symptomatic benign prostate involve hyperplasia, smooth muscle contraction, or both? Please discuss."*
To clear this up, the following sentence has been added to the manuscript on page 13 line 17:

In the prostate, GRP and bombesin have been shown to display mitogenic activity, affect cell migration and induce contraction in bladder and left ventral prostate[95, 96].

(8) *"Potential use of somatostatin analogs: This section is written in a somewhat superficial manner and does not flow well with the rest of the article. Please detail the potential use of somatostatin analogs in BPH and provide peer reviewed references or remove this section from the article. "*
We extended this section by implementing clinical data with the hope that it becomes an important part of the review (page 15, line 14):

The inhibitory activity of somatostatin analog on the production of growth factors, IGF-I and IGF-II, is of particular interest since these powerful octapeptides have been linked to the pathogenesis of BPH[90].
Somatostatin analogs have also been tested clinically in patients with androgen-independent prostate cancer. A study by Maulard et al. showed improvement in PSA levels and achieved a reduction in bone pain[117]. A Phase-I study demonstrated the favorable toxicity profile of somatostatin analog lanreotide, and showed its inhibitory effect on plasma IGF-I levels. In contrast, no clinical improvement has been noted with this analog in advanced metastatic androgen-independent prostate cancer[118]. In a study by Berruti et al, lanreotide was also able to decrease plasma levels of IGF-I and of the prognostic marker, chromogranin-A, but had no effect on serum PSA levels in patients with advanced prostate cancer[119]. The poor or no inhibition of tumor growth to somatostatin analogs found in these clinical trials is thought to be due to differences in the receptor subtype-specific binding of the analogs. Consequently, the utilization of a non-receptor selective somatostatin analog has been suggested[120]. According to Cariaga-Martinez et al, whereas SSTR2 is expressed in benign prostatic hyperplasia, in most cases, it is repressed or absent in malignant prostate tissue[121]. Conversely, the profound expression of somatostatin receptors in non-malignant prostate tissue indicates the need for preclinical and clinical testing of its analogs in BPH.

Reviewer 00468214

(1) *"The term "prostatism" should be replaced by a standardized definition, since it refers to something not specific. LUTS? BPH?"*
The word "prostatism" was eliminated from the text.

Altogether, 10 references have been added to the manuscript, reference 31 was replaced and minor language polishing have been conducted.

We hope that this revised version of our manuscript will be deemed acceptable for publication in *World Journal of Clinical Urology.*

On behalf of all authors,

Sincerely yours,

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and
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