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| Journal Name:            | <a href="#">Journal of Cancer and Tumor International</a>                                                                                                                   |
| Manuscript Number:       | <b>Ms_JCTI_27025</b>                                                                                                                                                        |
| Title of the Manuscript: | <b>Circulating Prostate Gland Cells in Benign Prostatic Hyperplasia and Adenocarcinoma in Patients of African Heritage: diagnosis by liquid biopsy. Preliminary Results</b> |
| Type of the Article      | <b>Original Research Article</b>                                                                                                                                            |

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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**PART 1: Review Comments**

|                                           | Reviewer's comment                                                                                                                                                                                                                    | Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
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| <b>Compulsory</b><br>REVISION<br>comments | <p>1. Introductory basis of the study missing:<br/>Rewrite with proper references supporting this study</p> <p>2. The study at the end is unable to suggest any specific marker for differentiating BPH and ACP by this approach.</p> | <p><b>1Under the abstract</b>, section aim has been modified to read "To propagate in vitro circulating prostatic cells from patients of African heritage suffering from benign prostatic hyperplasia and adenocarcinoma of the prostate in order to facilitate the diagnosis of the two diseases <b>by a liquid biopsy platform</b>."</p> <p>The diagnosis of both BPH and ACP is confirmed by examination of biopsy material. There is urgent need to develop a less traumatic approach to the diagnosis of the two diseases, preferably by liquid biopsy."</p> <p>2.This is a preliminary report on circulating prostatic cells from patients with BPH and ACP. Use of specific markers to identify cells that cause ACP and those which cause BPH will be initiated soon. This brief report will inform researchers in ACP and BPH that a platform for the in vitro cultivation of prostatic cells by liquid biopsy is has been developed and is available for studies intended to</p> |



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|  | <p>3. This study will be more meaningful if cell specific markers are identified.</p> | <p>identify CTC. Our study did not enumerate circulating prostatic cells in ACP or BPH. Our study identified epithelial and stromal cells in BPH, ACP and in some apparently normal persons. This milieu of prostatic cells describedb here certainly will contain CTC. We will initiate studies to identify them.</p> <p>3.The identification of cell specific markers is a priority in this work and will be initiated soon.We are aware of CTC technologies including detection and characterrization of cancer cells: EpCAM, which is an epithelial cell molecule ; immunofluorescence (CK 8, 18, 19), CD 45 which is a leukocyte marker. It is our intention to employ different markers to identify CTC in our culture system (se Reference 7, Ignatidias and Dawson.</p> |
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| <p><b>Minor</b> REVISION comments</p> | <ol style="list-style-type: none"> <li>1. 'Fig. 4. Fifty prostatic acini that grew in suspension for 17 days from a patient with ACP.' Is not very clear to see prostatic acini</li> <li>2. In 'Fig. 7. A monocyte cell culture on day 21 of cultivation from an ACP patient' author has mentioned '<b>There is possibly a mesenchyme layer (M) on which the acini/epithelial cells (arrows) are situated</b>)' but it is hard to distinguish the dots that author wants to focus. A clear image is required.</li> <li>3. Fig. 8 does not provide information as author has mentioned in the explanation. No visible nuclei in H&amp;E stain of fibroblasts.</li> <li>4. line no 77 authenticity of verbal consent cite proper reference.</li> </ol> | <p>1.An acinus (or acini, Fig.4) has been described as a group peripherally arranged epithelial cells (thin arrows) which form a common duct (thick arrow) for collection of PSA. All cells in this image clearly portray this structure. The term "peripherally arranged" has been included in the legend for figure 4.</p> <p>The conspicuous circular/ tubular structure consisting of acini has been formed by a group of epithelial cells .</p> <p>2.The image consists of numerous dots of different size. The dots represent nuclei of epithelial cells ( arrows). This mimics the biopsy specimen of a patient with ACP whereby epithelial cells are supported by a mesenchymal cell type (Fig. 9).</p> |
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|  | <p>5. Line no 96 Lower urinary tract symptoms (LUTS)</p> <p>6. Explain the status of free and bound PSA level in plasma of both BPH and ACP patients.</p> | <p>3.Line 313: The explanation for Fig 8 has been modified to read “Fibroblast-like cells” as is portrayed in the figure and legend. Nuclei have been highlighted by black arrows.</p> <p>4.Line 77-79: A statement on authenticity and reference for verbal consent given is provided: as per Belmont report section on informed consent. The verbal consent was authenticated by an attending surgeon.”</p> <p>5.Line 104: The word ‘Syndrome’ has been modified to ‘Symptom’ (LUTS)</p> |
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|  | <p>7. ACP cells can be distinguished using different CD markers, it will also tell whether these are circulating cancer stem cells?</p> | <p>6.The amount of PSA in the blood is termed ‘free’ PSA. Total amount of PSA is free PSA plus PSA bound to other proteins. There is evidence that suggests a lower proportion of free PSA could be associated with more aggressive cancer, hence more clinically recognizable ACP (see National Cancer Institute, Prostate- specific Antigen (PSA, Test). Our study evaluated Total PSA. It did not measure and compare clinical severity in either BPH or ACP in terms of free plasma PSA levels. Nevertheless Table1 shows elevated plasma PSA levels in ACP patient 5 (8.4 .ng/ml) and patient 15, (11.0 ng/ml). Similar elevated plasma PSA levels for 2 ACP patients are shown in Table 2: (15ng/ml in patient 7 with corresponding monocyte culture media PSA level (38.54 ng/ml) and 14.3 ng/ml plasma PSA with corresponding high monocyte culture PSA (41.59ng/ml)for patent 8. Elevated plasma PSA in BPH patients was detected in patients 1,2,and 4 ( 12.2ng/ml, 8.6ng/ml 10.4ng/ml, respectively (Table 1) and in patients 17 and 18 (5.4ng/ml) and 9.2 ng/ml), respectively in Table 2. These intriguing findings call for more studies that will determine the clinical significance and severity of elevated plasma PSA vis-a-vis clinical severity of patients with BPH and PAC.</p> <p>7.We agree that ACP cells can be distinguished by</p> |
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|  |  | different CD markers. Our study provides a liquid biopsy platform for these future studies. |
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| <p><b><u>Optional/General</u></b><br/>comments</p> | <p>4. Basis of selection for 34 patients having prostatic complications.</p> <p>5. Recent Prevalence of BPH and ACP in African heritage is missing.</p> <p>6. How Epithelial and Stromal cells were identified after their isolation from BPH patients?</p> | <p>4.The 34 patients were consecutively enrolled on the basis of presenting with LUTS. LUTUS was the basis of selection.</p> <p>5.At our University recently it was shown that the top 3 cancer cases from August 2013 to December 2015 according to the Cancer Registry at KCMC were: cervical cancer (363), prostate cancer (268) and breast cancer (157) reference # 3 (Munishi et al.) cites prevalences of ACP. Also, Dr. J.W. Arends, visiting pathologist, 2008-2012, examined 85 prostate specimens at KCMC and found that 1-10% consisted of prostate adenocarcinoma (personal communication with MN, senior author).</p> <p>6. Yes, the characteristics of individual epithelial cells were noted after their propagation from plasma in cell culture from patients with BPH and ACP (see Fig. 6). In addition, please</p> |
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|  | <p>7. Few samples in the results are 'Not Done', reason?</p> <p>8. Zoom is mentioned to be 400x but images appears to be under 40x only. Have they considered Camera zoom as Eyepice (10x) zoom?</p> <p>9. Many images indicate <b>Patient Number</b>, whether author has performed experiment on a single patient only? Why?</p> <p>10. Is is possible to differentiate between BPH and ACP conditions for Liquid Biopsy? Explain.</p> | <p>see Reference 1. R. Veltri and R. Rodriguez The prostate: that the prostate forms acini and collecting ducts by arborization into the urethra. That is exactly what Fig. 4 shows.</p> <p>Also see same authors (Ref. 1) who list 4 types of <b>stromal cells</b>: as smooth muscles, fibroblasts, endothelial. In this study fibroblasts were identified by their filamentous morphology [see Wein: Campbell-Walsh Urology, 9<sup>th</sup> Ed. Section XV1- PROSTATE. Chapter 85... ].</p> <p>7.For some patients, insufficient sample was obtained to allow the full range of tests to be done. In other situations although sufficient sample was provided something went wrong in processing the sample. That is the reason for ND.</p> <p>8.The discrepancy has been addressed, images were off a digital microscope and only 40x magnification is valid. This has been corrected throughout the manuscript.</p> |
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|  | <p>11. quantification of AR presenting cells should be done in blood plasma by using some advanced techniques to support the data</p> <p>12. other prostatic molecular markers (western blotting techniques etc.) can be used for authentication and gene expression studies can be included in the present study</p> | <p>9. Wherever reference is made to patient number, it corresponds with data in Table 1 or 2. Experiments were done for all patients, but images provided are specific for some of those listed.</p> <p>10. There are specific markers that can be applied for identification of ACP. At the patient level cells detected in BPH patients are similar to those seen in ACP. The difference is that in ACP the epithelial cells are malignant and the identification of the malignant cells is a future study.</p> <p>11. Figure 5 represents cells that grew and stuck on coverslips. Apparently all have AR but the number differs from one cell to another. We would like to suggest that the acidic PSA secreted by the prostatic epithelial cells in the monocyte cytoplasm is deleterious to the cells and is probably killing them.</p> |
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|  |  | <p>12.This study identified circulating prostatic epithelial cells and fibromuscular cells in patients with BPH and ACP. We did not investigate whether cells identified from BPH are different from those in ACP patients. This study is on circulating prostatic and fibromuscular cells. The study opens ways of studying different cell types during BPH and ACP.</p> <p>We agree that other prostatic molecular markers are useful. We plan to include them in our future studies.</p> <p>-----</p> |
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