Author's response to reviews

Title: Gene expression profiling revealed novel mechanism of action of Taxotere and Furtulon in prostate cancer cells

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Author's response to reviews:

November 30, 2004

Iratxe Puebla
Assistant Editor
BioMed Central
Middlesex House
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Dear Iratxe Puebla:

Enclosed please find our revised manuscript entitled "Gene expression profiling revealed novel mechanism of action of Taxotere and Furtulon in prostate cancer cells" submitted for potential publication in the journal of BMC Cancer. Revision has been made according to the comments made by the reviewers. We will be looking forward for its acceptance and speedy publication.

Thank you very much for your consideration.

Sincerely yours,

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Point-by-point response to the reviewer's critiques

Dr. Rajvir Dahiya: We thank Dr. Dahiya for his valuable time in reviewing our manuscript and we sincerely appreciate the acknowledgement regarding the importance of our data. No response is required by him.

Dr. Lukas Bubendorf: We thank Dr. Bubendorf for his valuable time in reviewing our manuscript and we
sincerely appreciate the acknowledgement regarding the importance of our data. We have addressed and corrected all concerns raised by him.

Critique: It should be clarified to what extent there is overlap of the data between the two studies. Is the current study an extension of the previous study by including Furtulon and LNCaP?
Response: We have previously published the data regarding the alternation of gene expression profiles of Taxotere treated PC-3 and LNCaP cells (Li et al. Neoplasia 2004;6:158). This published article was focused on the investigation of molecular effects of Taxotere on microtubule, apoptosis, and cell cycle in prostate cancer cells. The current study is an extension of our previous study. The current manuscript is mainly focused on the investigation of the alternation of gene expression profiles in PC-3 and LNCaP cells with Taxotere and Furtulon combination treatment. We analyzed and reported the differences in the molecular effects of combination treatment compared to mono-treatment. We also compared the molecular effects between Taxotere and Furtulon treatments. The purpose of this extended study has been described in the section of "Introduction" (page 3, line 42-46).

Critique: Was there a mechanistic hypothesis or clinical data to suggest that Taxotere and Furtulon may have synergistic effects in prostate cancer?
Response: Taxotere or Capecitabine mono-treatment has shown improved objective response in metastatic prostate cancer (Ferrero et al. Oncology 2004;66:281; El-rays et al. Urology 2003;61:462). Clinical trials have also showed that the combination of Taxotere and Capecitabine resulted in improved objective response rate and overall survival without a significant increase in the treatment related adverse effects in metastatic breast cancer and advanced non-small cell lung carcinoma (Han et al. Cancer 2003;98:1918; McDonald et al. Int J Clin Pract 2003;57:530). Therefore, the combination treatment with Taxotere and Capecitabine may show improved response in prostate cancer. Mechanistically, Taxotere and Capecitabine exert their effects on the different points of biosynthetic processes. Taxotere impairs mitosis by stabilization of microtubules while Capecitabine is incorporated into DNA to inhibit normal bioprocess function of the cells after being converted to 5-FU. Thus, Taxotere and Furtulon may have synergistic effects in prostate cancer.

Critique: Since protein expression is critical, it would be worthwhile to supplement figure 2 with the corresponding Western Blots.
Response: We have conducted more Western Blot analysis on the genes that showed changes in combination treatment. We have added a new figure (Figure 3), and modified the sections of "Materials and Methods" and "Results" to include these data (page 5, line 27-29; page 6, line 35-36).

Critique: It should be clarified whether all genes with expression changes >2 are shown on the tables, or whether a selection of genes is presented. I wonder how the expression of prominent genes such as Ki67 and Bcl-2 responded to treatment. Although Ki67 has been analyzed by RT-PCR (table 1), no data are shown. It would at least be surprising and worth reporting how and to what degree the expression of Ki67 was affected by the treatment.
Response: In the Tables 2 and 3, we listed the genes which showed a >2 fold change in expression in at least one time point in both mono and combination treatment. In the Tables 4 and 5, we listed the genes which showed a >2 fold change in expression in at least one time point in combination treatment. We have added a description for Table 2 to 5 according to the reviewer's suggestion (page 15-19, Table 2-5). A >2 fold change of Ki-67 was only observed in Taxotere mono-treated prostate cancer cells. We have published the data previously, so we did not include it in the Table 2-3 and Figure 2. We did not observe any alternation in the Bcl-2 mRNA expression by microarray analysis.