Author’s response to reviews

Title: Transcriptomic signature of the chemopreventive Bexarotene (Rexinoid LGD1069) on mammary gland from three transgenic mouse mammary cancer models.

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Author’s response to reviews: see over
Dear Editor,

Please find enclosed the revised electronic version of our manuscript entitled “Transcriptomic signature of Bexarotene (Rexinoid LGD1069) on mammary gland from three transgenic mouse mammary cancer models” (Ms.N°3202285352019889) by Abba et al., which we are submitting for your consideration for publication in *BMC Medical Genomics*.

We have modified the manuscript according to the reviewers’ suggestions. Attached you will find a point-by-point response to all concerns and comments expressed by the reviewers. We thank you and the reviewers for the time invested in reviewing our work. We hope you will find this revised version of the manuscript acceptable for publication in *BMC Medical Genomics*.

Thank you very much for your consideration.

Sincerely,

Dr. Martín C. Abba, PhD
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Response to Reviewer N°1:
Reviewer's report: This manuscript was previously submitted to Breast Cancer Research, I being one of the reviewers from the original submission my comments were already presented to the authors.

1) Reviewer: The authors have addressed my comments and included means to access the raw data. However, they were unable to properly address my concern towards the biological relevance of the data. It is obvious from the results that one of the animal models has a different response to the bexarotene treatment. This observation must be discussed in the manuscript because it goes against the search of a common answer to bexarotene.

1) Author response: We have now included in the manuscript (Result and Discussion section) a paragraph related to the biological relevance of the data. The response to this point and modification of the text basically reads as follow:

“In other words, it appears that a better correlation was observed between MMTV-ErbB2 with the other two models, than between p53-null and C3(1)-SV40 tag transgenic mouse mammary gland models. These data suggest that mammary tumors derived from different primary oncogenic pathways could respond differently to the same chemoprevention agent. In addition, these results indicates that transcripts modulated by bexarotene in the MMTV-ErbB2 mammary gland share almost all the common features profiles among the transgenic mouse models analyzed. As mentioned above, we have previously shown that LGD1069 suppress mammary tumor development in the MMTV-ErbB2, p53-null and C3(1)-SV40 tag transgenic mouse mammary gland models [6,7]. Interestingly, the specific response of these three transgenic mouse mammary models to bexarotene treatment varies with the genetic background assessed. For instance, the bexarotene treatment is much more effective against MMTV-ErbB2 induced mammary tumors than against C3(1)-SV40 or p53-null mammary tumors [Medina et al., unpublished]. In the MMTV-c-neu mammary gland, LGD1069 reduced tumor incidence by 75% and lengthened median tumor latency from 234 days to over 420 days [7]. However, in the p53-null and C3(1)-SV40 mammary gland where p53 or p53/Rb activities are affected respectively, bexarotene treatment showed modest chemoprevention activity. Both these molecules exert primary functions downstream of the CDKs, one locus of LGD1069 activity. In this sense, human breast cancer is a complex disease caused by dysregulation of many different oncogenes, tumor suppressor genes and growth factor pathways. The MMTV-ErbB2, p53-null and C3(1)-SV40 tag mouse mammary gland cancer models are valuable tools for the elucidation of the mechanisms of mammary tumorigenesis [3]. However, it is important to recognize that no one model can represent all the different forms of human breast cancer.”

2) Reviewer: Also, I still think the sentence in the abstract ?? revealed that 89 genes were commonly dysregulated in more that one of the transgenic mammary models? is misleading and confusing. There are only 9 genes common to the three models. I suggest the authors remove the word common and have the sentence like: ??revealed that 89 genes were dysregulated among the three transgenic mouse mammary models. From these 9 were common to the three models.?
2) **Author response**: The abstract was modified according to the reviewer’s suggestion, and now the text reads as follow:

“Analysis of gene expression changes induced by bexarotene in mammary gland revealed that 89 genes were dysregulated among the three transgenic mouse mammary models. From these 9 genes were common to the three models studied.”

**Response to Reviewer N°2:**

Reviewer's report: The manuscript by Abba reports the analyses by SAGE technology of gene expression changes induced in the mammary glands of three transgenic mammary cancer models models by treatment with the rexinoid, bexarotene. While a total of 711 expression changes were observed in analyzing all of the models, comparative analyses revealed that 89 genes were commonly dysregulated in more than one of the transgenic mouse mammary models. The authors provide discussion of how the gene expression changes are related to metabolic function, signal transduction, protein metabolism and cell proliferation/differentiation and apoptosis. Analyses such as these are important for further identifying mechanisms of actions of chemopreventive and therapeutic agents.

1) **Reviewer**: The manuscript, however, lacks validation of these findings for any of the genes that are highlighted in the discussion and are important for confirming the conclusions of the authors. Such validation, at least at the RNA level and preferably at the protein level, would strengthen the manuscript and conclusions.

1) **Author response**: We thank the reviewer for these very excellent suggestions for further experimentation. However, we would wish to emphasize that our primary goal for this study was to characterize the global gene expression changes induced by bexarotene on mammary gland from three transgenic mouse mammary cancer models, thus creating a foundation for future studies designed to elucidate the precise mode of action of this compound in the breast. We consider that SAGE analysis is equivalent to Northern analysis with the further advantage of being quantitative. This technology is proven and supported by a myriad of publications. It is not inferior to conventional microarrays and the reality is that the currently most global gene expression studies, most genomic studies, are published in most journals without additional gene expression validations other than those provided by the original methodology, we don’t understand why we should be subjected to a different standard. We have performed validations in several previous publications using SAGE and in our experience the results are extremely reliable (see http://sciencepark.mdanderson.org/ggeg). This study represents a very significant amount of work and DNA sequencing 360,000 tags (60,000 tags per library and equivalent to more than 5 Megabases of sequencing) and monitoring the behavior of more than 20,000 transcrips.