Reviewer's report

Title: Transcriptional profiling elucidates metronomic cyclophosphamide-activated, innate immune-dependent regression of brain tumor xenografts

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Reviewer: Giannoula Klement

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The authors have shown in a number of previous publications that Metronomic chemotherapy induces a dramatic, innate immune cell-dependent regression of cancer. In this manuscript they use one of their therapeutic regimen of cyclophosphamide treatment on a six-day repeating schedule to induce U251 glioblastoma xenografts regressions. The authors then scrutinize genome-wide transcriptional profiles of untreated and metronomic cyclophosphamide-treated human U251 glioblastoma xenografts at two treatment time points to identify differentially expressed genes.

The authors analyzed treated and untreated glioblastoma xenografts on human and mouse microarrays at 12 and 18 days, to identify factors that contribute to the observed immune effects. The authors found that in human xenografts, genes whose expression was decreased at both cyclophosphamide-treated time points were associated with extracellular signal, cell adhesion, skeletal system and blood vessel development, and extracellular matrix genes.

While this is a valid approach to find biological signals, it is difficult to assign cause/effect relationship, and the authors quite appropriately use this approach to confirm/support their previously published work on the effects of metronomic chemotherapy on tumor microenvironment. They are careful not to over interpret the findings.

This is also true for their Upstream Regulator analysis to identify putative ‘master regulators’ of complex gene expression changes induced by metronomic CPA treatment. Using DAVID analysis the authors identified functional gene ontology clusters significantly enriched in the sets of U251 tumor cell-expressed genes at both cyclophosphamide treatment points. More specifically, the authors identified several interferon signaling network genes as upstream regulators of immune response genes, supporting their earlier work. They were able to show that the inhibition of these upstream regulators, which primarily have pro-tumor functions, was consistent with the therapeutic effectiveness of metronomic CPA in this glioma model.

In mouse microarrays, mouse genes up-regulated in both tumor models included genes involved in immune response, lysosome, regulation of cytokine production, lectin/ carbohydrate binding, cytokine receptor interaction, induction of
programmed cell death, leukocyte activation, and regulation of immune effector process. The authors again identified genes involved in activation of the complement immune response.

The identification of responsive cytokines, chemokine and immune regulatory genes linked to the immune response, as well as several immunosuppressive factors that may contribute to tumor escape, is very important for a successful implementation of this work into clinic. Very few scientists and even fewer clinicians are familiar with the many varied mechanisms in which metronomic chemotherapy works and this study sets out numerous pathways for future investigations. Authors rightfully suggest that the work may lead to discovery of biomarkers to evaluate therapeutic efficacy and to optimization of treatment schedules.

The paper is well written, and the analysis is thorough. I would recommend it for publication.