Response to reviewers:

Thank you for your efforts in reviewing our manuscript, and for your consideration of our revised manuscript for publication in PLOS One.

Reviewer comment:

*The major issue is the extremely small cohort of patients in which this study was based. Please validate your findings in silico, using data from published independent cohorts of cancer patients.*

Although our *in silico* analysis of The Cancer Genome Atlas cancer data (TCGA approximately 10,000 patients) may seem small by modern genome-wide association analysis standards (where cohorts can be an order of magnitude higher), the TCGA is the largest cohort of cancer patients ever systematically studied with genomic analysis. As our work uses RNA expression as a quantitative trait (a.k.a eQTL study), our analytical approach absolutely requires a cohesive body of both genetics (mutation and copy number) and RNA-seq transcriptome profiling data. For the cancer field, it is a singular achievement to have data of this scale. In the field, dozens of papers have been published (including many in the references) solely based on TCGA information.

We have spent a significant amount of time over the past few months evaluating the only other cancer data set that might rival TCGA in terms of scale, the International Cancer Genome Consortium (ICGC) effort. The data from this effort is not as well organized as TCGA, and the data generated is primarily analysis of mutation. Some patient samples in ICGC have RNA-seq gene expression and/or copy number variation data available. We assembled and integrated all available expression, mutation, and copy number data and analyzed the overlap of genetic and RNA expression across patients (necessary for the analysis). We concluded that the ICGC data set is insufficient to serve as an appropriately sized independent cohort. The number of ICGC samples with combined information were in the hundreds at best, often much less. For example, the total number of samples with combined gene expression and mutation data, across the dozens of cohorts in ICGC, totaled only 257 samples. This included 101 ovarian, 15 breast cancer, 23 malignant lymphoma samples, etc. Perhaps the ovarian cohort could be compared with our TCGA analysis of mutation, but we did not observe any significant association of mutations in TCGA ovarian cancer in our analysis, so for ovarian there are no results to compare to.

Since our manuscript was submitted, an analysis of a clinical melanoma data set was published in *Cell* by the Sharma group at M.D. Anderson ([Cell.](https://www.ncbi.nlm.nih.gov/pubmed/27667683) 2016 Oct 6;167(2):397-404.e9. doi: 10.1016/j.cell.2016.08.069). They concluded that clinical response to immuno-therapy could be predicted by a signature of genetic changes in interferon pathway genes. Roughly half of the genes the Sharma group used in their paper to predict response are part of the chromosome 9p region that we identified as in association with immune levels, as the major thesis of our manuscript. We have included both this reference and a discussion of it in the revision.

The other major thesis of our manuscript concerned markers of regulatory T cells (Tregs) in tumors. Although one would not have selected it based on analysis of peripheral blood lymphocytes, our analysis of correlation structure within tumors concluded that the chemokine receptor CCR8 is the best selective marker for Tregs in tumors other than the FOXP3 transcription factor. Since our submission to PLOS, the Rudensky group at Sloan-Kettering has published a paper in Immunity (<http://dx.doi.org/10.1016/j.immuni.2016.10.032)> that identifies CCR8 as highly and selectively expressed by Tregs in tumors, and they propose that it will be a good target for Treg-depleting therapies. We’ve modified our manuscript to incorporate this information.

In summary, there’s currently no other cancer cohort in existence of the scale of TCGA to compare to in the field of oncology. Thus, we cannot perform a statistically powered test of our results in an independent cancer cohort. However, smaller clinical studies have now confirmed the relevance of chromosome 9p alterations to clinical outcomes in immune checkpoint therapies. Finally, our observation of CCR8-specific expression by regulatory T cells has also been confirmed. The cancer research community looks forward to a time when we have additional confirmatory data sets of the same magnitude as TCGA. When created, they may also be RNA-seq of single cells, which will be very exciting.

Reviewer comment:

*Reviewer also asked for improvements in the way data are presented.*

We have significantly edited the results section to elaborate on details of immune signature generation and other points. We redrafted the text and added figures to support these changes.

We appreciated the reviewer’s comments about the complexity of the original Manhattan plot, which tried to summarize information across all cohorts and all immune signatures. We have redesigned the figure, using a more conventional bar chart for copy number associations, while also limiting the data to a single tumor type and single cellular signature. We think this is a major improvement, and hope you agree.

Thank you for your consideration,

Nathan Siemers, Ph.D.