**Reviewer’s report**

**Title:** Sequencing and curation strategies for identifying candidate glioblastoma treatments

**Version:** 0  **Date:** 13 Dec 2018

**Reviewer:** Michael Berens

**Reviewer’s report:**

Summary

Frank et al describe consenting, collection, genomic analysis (whole genome sequencing and RNA Seq), gene-drug matching analytics and tumor board reporting to physicians for 30 GBM cases enrolled from 7 clinical sites in New York. Tumor (80X) and matched germline (40X) WGS were used to estimate chromosome, gene, and allele copy numbers, along with mutations, intronic and exonic splice variants; SNVs were assigned to Tiers 1 - 4 as previously published (citation #18 needs to be fully annotated). RNA Seq was used to support WGS variant calls as well as to report level of expression of each variant; by comparing against the gene expression of 169 GBM samples (TCGA), a modified z-score for each gene was calculated and used as a proxy of differential gene expression. Genomic reporting was compared between manual (expert) curation and Molecular Profiling Analysis (MPA) WGA (IBM Research proof-of-concept environment of Watson for Genomics) as published. MPA results were used to ascribe direct and indirect therapeutic options, selecting from molecularly-targeted therapies only. WGA updates with each addition of a new clinical sample, as all previous WGS and RNA Seq data of prior samples become the reference set against which z-scores were derived. WGS data were compared with findings reported on Targeted Panel findings for a subset of GBM cases. Time from sample receipt to tumor board averaged 4.5 months, with 1.9 months consumed in scheduling the tumor board after the pipeline analysis was completed. The results from 30 GBM cases uncovered 44 genes with targetable SNVs; all but one sample had CNVs that were considered targetable. Three (of 30) GBM cases sequenced led to a change in the therapeutic strategy in patient care.

Critique

The influence of tumor purity on sequencing analysis warrants comment. The influence on sample preservation method on sequencing analysis warrants comment.

The detailed molecular models of PIK3R1 and PIK3CA (Figure 1), indicating the SNVs detected seems a curious component of the manuscript. This figure is minimally referenced in the Results, and not commented on in the Discussion. Eliminate Figure 1.

The utility of including of reporting the 3 patients for whom two samples were sequenced is unclear. If these findings are to be included, the patients should be noted in Table 2, and the implications of the findings warrant comment in the Discussion.
Why were RNA Seq data available from only 26 samples? The source of the “fail” warrants notation. The correlation of VFA called by DNA and RNA sequencing (fig 3) is only minimally discussed. Did the correlation coefficient track better or worse for specific genes? No mention is made of the allele abundance from the RNA Seq analysis. The Splice Variant reporting (Figs 4 and 5) are of interest, and warrant elaboration in respect to driver events in oncogenesis and/or drug resistance. Would a more granular expansion upstream and downstream of some splice variants (transcriptional regulation, network pathway signaling, etc) warrant calling out for future improvements?

Which samples had "high mutation burden? Indicate these in Table 2.

The description of "…potentially synergistic combination therapy options in seven patients" warrants some explanation of the strength of the assignment of "synergy".

The genomic findings from four patients (NYGC-GBM7, NYGC-GBM17, NYGC-GBM25, and NYGC-GBM12) are reported in some detail. What was the reason to discuss these cases specifically in the Results section?

The description of the "Concordance of WGS/RNA Seq and panel-based diagnostic reports" is a strength of the manuscript. The results are succinctly and clearly described. Figure 6 is a helpful rendering of the comparison. Calling out the significance of the germline reference to make somatic SNV calls is done well.

The detailed rendering of the Concordance of Drug recommendations between NY-GGC and WGA" is a strength of the manuscript. Page 22 notes "(see Appendix)". but no appendix is provided. As cytotoxic therapy (temozolomide, specifically) remains a standard of care for GBM patients, and other traditional agents (platinum drugs, cell cycle disruptors, antimetabolites, etc), the limitations of the study using targeted therapeutics only warrant deeper discussion, and recommendation for how to improve the WGA and the NY-CCG.

The manuscript warrants a listing of strategies and risks to bridge the gap between the final (present) iteration of NY-GGC or WGA for patient treatment planning decisions and what would be a "routine" approach to using genomics as a mainline treatment resource. Especially, the enrollment of 30 patients by 7 institutes over 16 months seems to be an opportunity to discuss clinical adoption barriers, which the coauthors of the manuscript could articulate. A comment on the interval between resection to sample submission (shown as a median of 67 days) should be commented upon. This Discussion should also address what are the currently known challenges in regulatory approval for such technology.

Minor points

Page 16, line 7. …We also discovered a CHST11-PKP fusion. Should be: CHST11-PKP2 fusion
Reference #18 is incomplete.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

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