Reviewer’s report

Title: Resistance gene expression determines the in vitro chemosensitivity of non-small cell lung cancer (NSCLC)

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Reviewer: Paul Boutros

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Glaysher and colleagues have performed an important study evaluating the ability of mRNA expression levels to predict cell-culture based chemosensitivity analysis. This is an important area of research because, as the authors cite, patients are often refractory to one chemotherapeutic regimen but respond to another. Optimization of therapy is one of the major areas of personalized medicine actively being investigating. Therefore this paper is timely and appropriate in its subject matter. The authors employed a 96-gene TaqMan-based PCR system and looked at 49 samples. While their data generation appears sound, there are several issues regarding data analysis and interpretation that need to be resolved.

Major Compulsory Revisions

1. The drug sensitivity analysis relies on calculation of the "ATP-TCA IndexSUM", which is described as a "natural logarithmic index" and as "600-Sum(Inh200….Inh6.25)". Given that this equation is fundamental to the work, it's surprising that it isn't explained or motivated by the authors.

2. The authors discuss using 4 house-keeping genes in the introduction, but in the methods they describe using only one (PBGD). If the author's had four HK genes then rigorous explanations of why three were excluded need to be given. If the results are strongly sensitive to the choice of house-keeping gene then that casts doubt on the reliability of the mRNA abundance data.

3. The statistical analysis described in the last paragraph of page 14 may or may not be appropriate. From the descriptions given, though, I can't determine what has been done. My best guess is that ATP-TCA results were treated as a continuous dependent variable and that genes were sequentially added one by one according to their univariate correlations (Pearson's?). It's unclear if my presumptions are correct, if an intercept term was included, and what a "probability of F > 0.1" means. This latter issue is troublesome, as it's unclear if the authors are using an overall F-statistic for variable inclusion or not. Further there doesn't seem to be any control for model over-fitting on a held-out fraction of samples. Clarification of these issues is essential.

4. The authors devote a paragraph in the discussion to synergy/additivity in drug-treatments, but surprisingly have not evaluated synergy/anergy/additivity.

5. The per-patient curves in Figure 1 are very hard to interpret. Some sort of summary curves, for example means with 90% and 99% confidence-intervals,
would be a significant improvement.

6. Several samples were used for multiple drug tests. What is the correlation in the activity of one drug to the next across patients? A correlation-matrix with treatments along each axis would be appropriate here. This is essential to determine if the authors are truly identifying differences in agent-specific activity rather than just subsets of patients that are generally chemo-resistant or chemo-sensitive. The author's discuss these issues extensively in the intro & discussion with regard to clinical samples and literature data, so it's a surprise they did not perform the relatively simple analysis to address this issue.

7. Most critically, no raw data appears to be deposited in a public database. This study involves 49 samples x 96 data-points per sample = 4704 data-points, and thus is of sufficient scope to be deposited in GEO or a similar database. Surprisingly it was difficult to even locate a list of the genes assessed in this experiment. Without this being addressed the dataset generated by the authors would not be useful to the community as a whole.

8. Similarly it's surprising that a table giving the univariate analysis (correlations) between each gene and each chemo treatment was not given, at least as supplementary data. The authors describe the results of this kind of analysis in the text, so it should be presented for the readers to assess.

Minor Essential Revisions

1. Page 3: "data from other tumor types suggests that these tests correlate relatively well with outcome" -- the references provided only support this for ovarian cancer, so this statement is too broad.

2. Page 3: "This means that there is currently no direct correlation of clinical outcomes with ATP-TCA results" -- ATP-TCA has not yet been defined in the paper.

3. Page 3: "there is good evidence to suggest that low passage cell line" -- this is very reasonable, but references to this good evidence are needed.

4. Page 6: "but several suggest that this is likely to be of importance" -- missing word, "several studies" is presumably what is intended here.

5. Page 14: "gene expression ratio (GER) was calculated as Ln(" -- the natural logarithm should be in lower-case (i.e., "ln")

6. Page 14: "an on-line multiple regression sample size calculator" -- the URL provided did not work at the time of review.

7. Table 1: what does the column "Chemotherapy Series" mean here?

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests