Author's response to reviews

Title: Exploiting high-throughput cell line drug screening studies to identify candidate therapeutic agents in head and neck cancer

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Response to Reviewers’ Comments

Exploiting high-throughput cell line drug screening studies to identify candidate therapeutic agents in head and neck cancer

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Comments – Reviewer 1

• The authors should refer to a recent, complimentary study by Lui et al (Cancer Discov. 2013 Jul;3(7):761-9) where they describe the presence of PI3K pathway mutations in 30% of HNSCC tumors and the selective activity of BEZ-235 for PIK3CA-mutant HNSCC cell lines and patient-derived tumor xenografts and how PIK3CA mutation status may serve as a useful biomarker for treatment selection in HNSCC. Could the authors compare this with their findings where NVP-BEZ235 was not associated with a response in HNSCC?

Our Response: We have carefully reviewed the paper by Lui et al. and added their interesting findings to our discussion of PIK3CA inhibitors (lines 307-311). However, after reviewing our analysis, we verified that there was indeed no difference in response to NVP-BEZ235 found. This may be explained by the fact that only 13 HNSCC cell lines were tested with this drug by Garnett et al. in this setting. Perhaps with an expanded HNSCC cell line database, the effect observed by Lui et al. would be captured.

• Interestingly, the EGFR inhibitor erlotinib did not yield a significant response in HNSCC cell lines. Perhaps the authors could discuss the potential reasons for this inconsistency?

Our Response: We agree with the reviewers’ suggestion and have added information with respect to potential explanation(s) as to why Erlotinib did not yield a significant response in HSNCC cell lines (lines 335-338). We have added a discussion highlighting the importance of drug selection in cancer therapy as it is often observed that drugs targeting the same protein can display varied potencies based upon the drug’s specific interactions with its target as well as how the drug is metabolized (lines 281-284).

• The authors highlight the presence of additional mutations in the HNSCC cell lines that are not observed in the primary tumors but do not describe these observations in detail and should comment on their potential relevance. In particular, the top-ranking mutated gene, NCAM1, appears to have a potential functional significance as it is known to signal through FAK, a direct target of PF-562271, which may contribute to its activity in the PIK3CA-mutant HNSCC cell lines.
**Our Response:** We have included a paragraph describing this extremely interesting signaling target and appreciate the connection between NCAM1 and PF-562271 being brought to our attention (lines 263-270). However, after carefully reviewing our data, we believe that this mutation is either the result of immortalized cell line generation or a germ-line polymorphism as it occurs in almost all samples. Matched normal tissue from the original patients would have also been sequenced and compared with the cell line results so that only somatic mutations could be reported. As nearly all of the acquired mutations are identical, we feel it is much more likely that these variants are an artifact of tissue culture.

**Comments – Reviewer 2**

- The authors need to clearly elaborate on the limitations of their analysis as far as applications of their findings to drug development and patient care.

**Our Response:** We acknowledge the lack of limitations in regards to eventual patient care and have added a section expanding on the limitations of our analysis in this respect (lines 284-288). Such limitations include the inability to distinguish germline mutations from acquired mutations due to lack of match-normal samples and the shortcomings of cell lines as cancer models including lack of a 3D environment, lack of an immune system and lack of drug delivery issues (lines 290-297). We argue that cell line screening should be a first pass of anti-cancer agent efficacy before embarking on expensive xenograft studies or even more expensive clinical trials (lines 321-329).

- The authors need to give a brief overview of the current targeted agents under investigation in SCCHN in the introduction as well as allude to this in the discussion.

**Our Response:** This discussion has now been expanded in both the introduction and the discussion sections to include information on the current targeted agents used in HNSCC including PI3K, EGFR, mTOR, pRB, VEGFR etc (lines 85-97, 318-320 [PI3K], 331-333 [EGFR]).

- The authors need to refer to recent information from ASCO 2014 reporting NGS data in actual patient samples with SCCHN. These reports identified NOTCH among other as a commonly found aberration in SCCHN samples both HPV positive and negative. The authors need to reconcile this with the findings from these recent reports in the discussion section.

**Our Response:** This paragraph has now been adjusted to include emerging data from the The Cancer Genome Atlas. This information is publically available via
cBioPortal and further expands upon the information and conclusions made from the ASCO 2014 presentation in relation to HNSCC (lines 245-252).

- The method section needs to be much more detailed. The authors need to detail the methodology of their analysis.

**Our Response**: We appreciate the suggestion and have expanded the methods section to include a more detailed description of our methodology including the linear models used for both our one- and two-way ANOVA (lines 149-153, 160-166).

- In the discussion section there is practically no discussion of the limitations of such an analysis. The authors need to elaborate to a greater extent on the limitations of cell line analysis versus other types of analysis such as NGS of actual patient tumors as well as animal models such as PDX, as well as how they foresee the future of this research.

**Our Response**: A paragraph has been added about the limitations of our analysis in regards to cell line analysis as compared to responses in patient tumors and patient derived xenograft (lines 290-297). Ideally, the use of cell lines is a stepping-stone to drugs being testing in more complex models before implementation into clinical trials (an excellent example of this is PI3K inhibitor BYL719 on lines 321-329).

**Comments – Statistical Reviewer**

- Use either CNA or CNV as acronyms of copy number variant aberration (see page 7)

**Our Response**: We appreciate the suggestion and have changed all acronyms to CNV (e.g. line 126).

- Lines 173-174: please explain why identical cells treated with the same molecules display such diverse IC50. How many of the analyzed data (give %) are discordant?

**Our Response**: We have added an expanded explanation as to possible reasons why compounds targeting the same molecule display such varying effects (lines 281-284). In addition, we have added a new table (Table 2) that examines the 22 cases where identical cell lines were screened against the same compounds in both studies. When looking through the data, many (83%) of the IC50 values for sensitive (IC50<8 \(\mu\)M) cell lines were found to be similar (within 3\(\mu\)M). Examining resistant cell lines is more difficult as Barretina et al. only screened up to 8\(\mu\)M. However, if we compare scenarios where cell lines above 8\(\mu\)M are resistant, again we see many similarities between the two studies (Table 2).
• “We considered values greater than 2 and less than -2 as significant and reported them as amplifications and deletions, respectively.” A statistical and a clinical significance exist, please specify in this case “clinical significant”.

**Our Response:** These thresholds of copy number gain and loss reflect a gain of two extra copies and homozygous deletion respectively as reported by Barretina et al. We have altered this paragraph to reflect concerns of reported CNV (lines 137-140). This level of loss or gain is what’s reported in the cbioportal.org TCGA data and was found to decently correlate with the copy number changes reported by Garnett et al. (see Supplemental Table 1).

• “A LFDR < 0.05 was considered significant and a LFDR < 0.1 was considered a trend.” When using the word “Trend” you mean “trend towards statistical significance”, “Trend” has a specific statistical meaning and it is better to state “almost/approaching statistical significance”. However this wording is not statically sounding. (see also “Trap of trends to statistical significance”. BMJ 2014).

**Our Response:** We strongly agree with the reviewer’s suggestion that our wording be changed and have made the recommended changes (lines 157). The reading suggestion from BMJ was found to be extremely useful.

• The ANOVA tests the null hypothesis that samples in two or more groups are drawn from populations with the same mean values. The ANOVA produces an F-statistic, the ratio of the variance calculated among the means to the variance within the samples. When there are only two means to compare, the t-test and the F-test are equivalent; the relation between ANOVA and t is given by F = t². In supplement table 6 you report T-value in the table, but if you stated that you have applied ANOVA test you have to report F and not T or you state in methods that you applied a t-test.

**Our Response:** We appreciate the clarification and have added the necessary explanation to the methods section. In our analysis, a t-test was applied and this information has been added (lines 149). In addition, we have expanded our methods section to include more detail on the analyses used (149-153, 160-166).

• Please specify in table 2 footnotes that p-value is the p to test interaction, two-way-Anova provides three different p-values.

**Our Response:** We have made the recommended clarification in the footnotes of table 3 (as a new Table 2 has been added) as to which p value we are referring.

• “No correlation was observed for the 187 other PI3K inhibitors, GDC0941 and NVP-BEZ235, and PIK3CA mutation status (LFDR =1).” It is better to write “association” and not “correlation”.

**Our Response:**
Our Response: We agree with the reviewer’s suggestion and have changed the respective wording (line 219).