Reviewer's report

Title: c-Met in esophageal squamous cell carcinoma: Independent prognostic factor and potential therapeutic target

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Reviewer: Jochen Lennerz

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Molecularly targeted therapy in esophageal squamous cell carcinoma (ESCC) is currently not standard of care; however, prior studies and current data are promising. The manuscript presented by Drs. Ozawa et al., now presents data that c-MET is an independent prognostic biomarker and the authors report that c-MET might be a therapeutic target in ESCC.

The data are an important contribution to the field and I appreciate the study design; in particular the combination of human- and cell-line data. There are however a few issues that should be addressed to justify the statements of efficacy of PFE-2341066 in ESCC.

I am happy to look at a revised version of the manuscript and appreciate the efforts of Drs. Ozawa et al.

Major points

1. How did the authors determine that the observed efficacy of PFE-2341066 is truly related to interference with MET? PFE-2341066 interferes with a variety of other molecules that converge (in part) on the same downstream signaling molecules.

2. Efficacy in one (of three cell lines) with a reduction of “proliferation” of ~15% is a weak basis for some of the rather drastic statements regarding ‘generalized’ efficacy of PFE-2341066 in ESCC. Reduction of proliferation by 15% means that 85% of the tumor cells continue to proliferate. Did the authors consider using other read-outs e.g. apoptosis?

3. The authors used three ESCC cell lines for some of their experiments; however, a direct comparison of efficacy between cell lines is not provided. Why do the authors focus on KYSE170?

4. The definitions of ‘cut-offs’ for high- vs. low- MET and HGF should be revised. Did the authors test other cutoffs (i.e. high as only 3+ etc..)? A clear definition is paramount because HGF clearly had an effect in the cell-line experiments and the authors report differences in the clinical phenotype of patients; however, there was no effect on survival. These findings are in contrast to other studies (Ref 34, 35) and the difference might be related to these cutoffs.

Minor points

1. Abstract: The statement that MET as a poor prognostic factor in ESCC is unexplored should be revised. Similarly the statement “a molecular targeted
therapy has not been fully developed” should be reworded.


3.1 Methods Line 109-113: “Relative immunointensity was evaluated and scored according to the following criteria: 0, completely negative; 1+, weak; 2+, moderate, and 3+, marked immunoreactivity. High c-Met group was defined as immunopositive cells with greater than 40% and a relative immunointensity of above 1+ as previously reported [30]. High HGF group was defined as immunopositive cells with greater than the median value and relative immunointensity of above in accordance with our previous report [31]. “ What do the authors mean with “Relative immunointensity”? relative to what normal epithelium (which is known to express MET; or relative to non-tumor/stroma/fibroblasts)?

3.2 The cut-off between low and high was apparently set as: 0/1+ vs. 2+/3+. How is the statement “immunopositive cells with greater than 40%” related to that? 40% of cells, 40% of one category? Similarly, High HGF was defined as “greater than the median value and relative immunointensity of above...”. I am not sure what that means?

Given that these definitions will drive application by other scientists – and the findings related to that are central to the current manuscript determination of these cutoffs have to be more clear (see major point 2).

4. Statistics: quantitation = quantification?

5. Statistics: which features/characteristics did the authors include in the multivariate model?

6.1 Results. Line 182-183 “c-Met status of carcinoma cells was significantly correlated with pathological stage” Does this mean that higher stages had higher “intensities” of MET immunoreactivity? If the authors truly tested correlation; what statistical method did they use?

6.2 Several other “correlations” are mentioned – the authors should specify/clarify this.

7 Discussion. The authors mention cancer-associated fibroblast; however, do not provided data on expression of HGF in this cellular compartment?

8 Statement “owing to the high frequency of alcohol consumption and smoking history” – these are not provided in Table 1.

9 The authors may want to discuss the efficacy of PF-2341066 in ESCC in comparison to other tumors because reduction of proliferation by ~15% (in one cell line!) is not compatible with findings in other tumors. Especially because
inhibition of cell proliferation by PF-2341066 was not detected without HGF pre-stimulation.

10 Figures Supplement: In “Additional file 1”, specifically the figure, the 5-year survival is displayed over time. Is this truly what is displayed or is it overall survival over a 5 year period?

12 What do the authors mean by cause-specific survival?

13 Additional file 2 – same as with Additional file 1 (minor point 10)

14 Additional file 3 – the authors likely “normalized” the starting amount of proliferation to 100; however, that is rarely the case. Please change axis label, normalize to 1, or provide absolute numbers.

15 Additional file 4; The axis labels in the figure are not self-explanatory. The legend of this figure mentions “correlation”. Now showing this figure is not absolutely necessary – however, if there was no correlation or rather statistically significant difference, the author should mention the test applied and the P-value. Showing the datapoints/distribution is optional.

16 Table3. Tumor size cutoff is at 48.7 mm – which is a strange value. How was this determined and what if size follows established criteria (AJCC/TNM)? How is this different from pT3/4 vs. pT1/2. Continuous testing? How was the cutoff determined?

17 Figure 1. The panel is ok; however for ease of the reader the authors should consider indicating their cut-off of c-MET (high) vs. c-MET (low) in the panel.

18 Figure 2. Please explain 5-year survival plotted against time after surgery? Is this overall survival? Please change label? What is cause-specific survival?

19 Figure 4. Axis labels (i.e., “percentage”) should be revised. The figure consists of 12 panel and the numbering is A-F. The authors may want to consider relabeling (for easier referral to the specific panel) or separate the figure.

20 Figure 4 data. The maximum effect of PF-2341066 on “proliferation” is a reduction to ~85% meaning 15% of cells are affected. The efficacy is mild (see major point 1).

21 The comparisons in the three “grey-bared” graphs on the right (D, E, F) show highly significant differences (i.e., ***) and presumably the comparison was made to the PF-2341066/+HGF bar; however, this is not clear.

22 Figure 4 legend. The authors should mention the cell line(s) used for these assays? Comparison of efficacy between three cell lines.

23 Grammar: e.g. “motility of ESCC cells were regulated”; or “considered pivotal in the ESCC patients.”

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests