Reviewer's report

**Title:** Cell type- and Tumor zone-specific expression of pVEGFR-1 and its Ligands influence Colon cancer Metastasis

**Version:** 2  **Date:** 12 January 2015

**Reviewer:** Elisa Dama

**Reviewer's report:**

An interesting study investigating the expression profile of the total and phosphorylated form of VEGFR-1 and its ligands in colon cancer (CC) tissue and the relevance of this receptor for metastasis.

However, several points regarding study design, data analysis and presentation should be clarified and amended to ameliorate this manuscript before publication.

I. Major Compulsory Revisions

I.a Material and methods

- 86 subjects with sporadic CC were included in this study. All patients underwent surgery between 1998 and 2003. The authors should clarify whether the sample size was planned (i.e., statistical power was calculated) or based on tissue availability. The authors should describe in details inclusion/exclusion criteria for tissue analysis.

- The authors state that immunostaining reactions of each sample were evaluated independently by two authors without knowledge of the metastatic status. However, there is a lack of description of methods used to consider/combine these two independent measures. No concordance measure is provided.

- The intensity of the tumoral staining was scored on a semiquantitative scale from 0 to 2 (0: no staining, 1: weak staining, 2: strong staining). For the statistical analysis, the intensity of the tumoral staining was grouped in two levels (score=0 vs score=1,2) for all biomolecule but PIGF.

  PIGF staining was grouped in the following two levels: score=0,1 vs score=2. The authors should justify this approach for PIGF and provide also results based on the other method (score=0 vs score=1,2).

  The authors should justify why a scoring system based on 3 classes was used and only 2 classes were considered in the analysis.

- p<0.05 was considered to be statistically significant. All p-values larger than this cut-off were reported as “NS”, not significant. It would be helpful to have all p-values (even when not significant) reported to better understand the results.
I.b Results
- Table 1 and text.
Statistical association was evaluated for PIGF staining by considering two levels (score=0,1 vs score=2). However, CC % is reported for score=0 vs score=1,2. See also comment on the scoring system used.

Table 2 and text.
Overall correlations between expression of VEGFR-1 and ligands are reported with stratification by tumor center and tumor budding. It could be interesting to see described in this table not only the overall correlation but also correlations stratified by N0/M0, N+ and M+ CC.

The r coefficients reported in tables and text indicate weak/moderate correlations (r ranging from 0.3 to 0.5). However, the p-values for r coefficients reported are below the significance cut-off of 0.05. The authors should discuss the impact of sample size on the significance p-value reported. Since only a moderate/weak correlation was observed, the author should also understate the comment on the observed affinity.

II. Minor Essential Revisions
II.a Material and methods
The correlations between expression of VEGFR-1 and ligands were assessed with the spearman rank test. R coefficient is rounded to 3 decimal places. Rounding to 1 or 2 decimal places is more than enough for this statistics.

II.b Results
Table 3 and text.
P-value on difference in distribution should be reported.

Table 4 and text.
No match between percentage reported in table and text for:
Tumor budding - pVEGFR-1 Tyr1213 – CC% (64% in table; 62% in text)

Table 6 and text.
Please check the percentages reported in table and text for:
“None of the CC showed VEGF-B immunopositivity. VEGFR-1 and pVEGFR-1 revealed immunoreactivity in different frequencies, ranging from 48% to 73% intratumorally and from 57% to 89% along the invasive front.” Are the percentages reported in text an average of the values reported in tables?

“The only significant difference was observed in the tumor border, where in 92% of the non-metastatic CC inflammatory cells were VEGFR-1 positive whereas only 68% of the cases with distant metastasis had a positive immunoreaction (p=0.02, Figure 1E).” The authors reported only the p-value for difference
between 92% and 68%. To better understand the results, the authors should report all p-values for difference in distribution in table 6.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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