Cancer stem cells are hypothesized to be a barrier in effective cancer treatment strategies. This theory has been extended to pancreatic ductal adenocarcinoma and targeting these cells may lead to possible cure for this deadly disease. The authors herein immunohistochemically stained 28 cases of pancreatic cancer for 6 proteins and correlated their findings with various clinicopathologic parameters. Furthermore, the cohort selected comprised of 17 patients treated with chemoradiation and another cohort without. While the results are interesting, there are a number of issues which remain unclear.

Major Compulsory Revisions:
1. The manuscript states the patients selected within their study have localized advanced pancreatic adenocarcinoma, however locally advanced disease is considered to be at least stage III disease. Per AJCC, stage III disease is clinically staged as T4, any N and M0. However, per Table 2, none of the patients had cT4 tumors. Thus, the term “locally advanced” should be omitted from the manuscript.

2. The selection criteria for patients enrolled in their study is ambiguous and should be clearly stated. The study enrollment was for a 10 year period and only enrolled 28 patients. It seems unlikely that only 28 patients were considered eligible during this time. If so why?

3. The authors evaluate their immunohistochemical stains by defining a positive score as an averaged score > median score for a specific antibody. How did the authors arrive at this method? If this is an accepted method for these antibodies, they should reference prior reports using this method. Previous studies the authors cite have used different semiquantitative methodologies and it may be best to refer to those.

4. Table 5, the only CSC marker considered significant was CXCR4 and per the authors correlated with liver metastases. The overall number of positive cases was 8, however, the table states 9 with a p-value of 0.498. This error may be significant considering the borderline p-value and will need to be reanalyzed and corrected. Whether this is statistically significant will effect the discussion within the manuscript.

5. The Discussion primarily restates the results of the study, rather than relating the data with previously published findings and possible future directions. In
addition, the authors assume their immunohistochemical results are directly related to cancer stem cells and do not consider a stem-cell independent correlation. Certainly, cancer stem cells are considered to be a minor population of cells, however the immunohistochemical stains are scored in a fashion where the majority of cells are immunoreactive. How do the authors explain these findings? The Discussion should be revised to reflect how their data adds or conflicts with published literature and alternative possibilities to the function of these proteins.

Minor Essential Revisions:

1. Within the Methods, immunohistochemical evaluation was performed by 2 independent observers. The text notes that discrepancies between observers were resolved using a conference microscope. It may be of interest to also enter how many discrepancies were there within the Methods.

2. Within Table 2, the histologic classification was considered indistinguishable for 1 case. The methods should reflect how histologic classification was done and why 1 case was consider indistinguishable. Furthermore, instead of indistinguishable, a better term may be ungradeable. Table 5 omits this case, why? This should be entered.

3. The Discussion primarily restates the results of the study, rather than relating the data with previously published findings and possible future directions. This should be revised so the reader understands the significance of their findings. In addition, the authors assume the immunohistochemical markers directly correlate with cancer stem cells when they have independent

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests