Author's response to reviews

Title: Expression of the costimulatory molecule B7-H3 is associated with prolonged survival in human pancreatic cancer

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Author's response to reviews:

Dear Sir or Madam,

we would like to resubmit our manuscript entitled “Expression of the costimulatory molecule B7-H3 is associated with prolonged survival in human pancreatic cancer” for consideration for publication in BMC Cancer.

Our manuscript has been peer reviewed for BMC Cancer in February 2009. We would like to thank you for giving us the opportunity to revise our work.

We have been able to fully address all the reviewers’ concerns. We have now included a detailed point-by-point response to the reviewers’ concerns.

We think that the requested revisions have substantially strengthened our manuscript.

We are looking forward to a favorable decision.

With kind regards,

M. Loos

Title: “Expression of the costimulatory molecule B7-H3 is associated with prolonged survival in human pancreatic cancer”

Version: 1 Date: 25 February 2009
Reviewer: David Ramsden

Reviewer’s report: This paper contains some very interesting data on cellular factors associated with survival times in human pancreatic cancer. The basic conclusion of the authors is that expression of B7-H3 protein is associated with increased length of survival, which if confirmed in subsequent studies offers a potential novel treatment for a desperate condition. As such it would seem worthy of publication. Nevertheless the paper appears to have been written in a great hurry and consequently there are some specific points that require clarification before this should happen.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. Results:
   a) Figures 1 & 2. It is not specifically stated in either the main text or the legend as to what is the parameter that was assessed? B7-H3 protein or mRNA in Fig 2. I assume protein but given that both are referred to in the paper, it should be specified.
   
   To make clear whether B7-H3 protein or mRNA was assessed, we modified figure 1 and 2.
   
   In the revised version of the manuscript, figure 1 contains IHC photos showing the expression pattern and expression intensities of B7-H3 in pancreatic cancer.
   
   Figure 2 is now showing the correlation between B7-H3 protein expression levels and survival data.
   
   We also specified which parameter was assessed in the main text (results – page 11).

   b) If it is protein and this was based on immunohistochemical observation, what exactly is meant by a non-expressor and how many were non-expressors? I ask this because the information given in the histogram Fig 1 shows a quite tight error bar, which would be surprising if a reasonable number of tissues showed no expression. The histogram suggests that this is not a “yes or no” evaluation. The histogram in Fig. 1 should be replaced with a figure which shows the level of expression in individual tissues.
   
   If these are protein results and not the mRNA results, these latter are shown in a later figure. How do they correlate with survival?
   
   In the revised version of the manuscript, we did not use mRNA data of B7-H3 expression for correlation analyses with clinicopathological data, because mRNA expression levels might differ from actual protein levels due to posttranscriptional
changes.

Furthermore, we used a different scoring system to evaluate the intensity of B7-H3 stainings in pancreatic cancer to avoid any confusion. The new scoring system accounts for both the intensity and the area of cancer cells stained. It is explained in further detail in the revised “Methods” section (page 8).

c) Figure 3 is unnecessary. The data could easily be and should be incorporated into the main text.

Figure 3 originally displayed the mRNA expression levels of the cultured pancreatic cancer cell lines Panc-1, MiaPaCa-2, and SU86.86. In the revised version of the manuscript, we removed figure 3 and incorporated the expression data into the result section (page 12).

d) Figures 4 and 5. The main text, the legends and the labelling on the figures are confused.

In the original version of the manuscript, figure 4 was wrongly labelled as figure 5. Therefore, the main text, legends and labelling of figure 4 and 5 were confused. We corrected this mistake.

e) Figure 4 or 5 showing the FACS results - The legend offers no explanation as to what the colours mean. One can work this out, but one should not have to. Why were only two cell lines used when three are used immediately prior to this?

In the revised version of the manuscript, we modified figure legend 4 and exactly named every curve shown in this figure to avoid any confusion.

For FACS analysis of the effects of IL-4 and IFN-gamma on the expression of B7-H3 we used the two cultured pancreatic cancer cell lines with the highest baseline B7-H3 mRNA expression (Panc-1 and SU86.86).

f) Figure 4 or 5 showing the correlation results. The main text states that there are significant increases in CD4 and CD8 expression, data not shown. What is the basis of the comparisons/ How many controls; what is the extent of the increase? The data should be shown. There is no requirement of an extra figure, just put the data in the text.

In the revised version of the manuscript, we included the increase of CD4 and CD8 mRNA expression in pancreatic cancer tissue sections in the main text (page 13,14). Furthermore, we used IHC to assess the distribution of tumor-infiltrating CD4+ and CD8+ T-cells in relation to the expression of B7-H3. The IHC data is now displayed in figure 5. Semi-quantitative analysis of the number of tumor-infiltrating CD8+ T cells in pancreatic cancer and the relationship with the level of B7-H3 protein expression is now shown in figure 6.
2. Discussion:

The first two pages are largely a statement of material that should go in the introduction if it has to be included. The important part of the discussion begins “Here we show...” The most significant statement in the discussion is that the authors have not elucidated the mechanism underlying the phenomena they observe.

The first two pages of the discussion included a short overview of the data shown in the manuscripts. We have thoroughly revised and shortened this section to avoid unnecessary data and redundancy.

Level of interest: An article of importance in its field

Quality of written English Not suitable for publication unless extensively edited

Language corrections of the manuscript have been thoroughly performed.

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

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

Reviewer: John P. Neoptolemos

Reviewer’s report: The authors investigated the expression of B7-H3 in human pancreatic cancer using qRT-PCR and immunohistochemistry (IHC) and correlated this with levels of CD4, CD8, IFN-gamma and IL-4 and with clinicopathological variables.

qRT-PCR showed increased B7-H3 expression in pancreas cancer tissues compare to normal tissues.

Major Compulsory Revisions are required for all of the work with the exception of qRT-PCR.

1. Results:

a) The quality of the IHC unfortunately is questionable. Six pancreas cancer specimens were negative and 24 were positive. The IHC in Fig 1b seems to show that all cells are stained. More convincing evidence is needed: several representative examples showing both negative and positive cases; normal
tissues; negative and positive controls.

Quality IHC needs to be shown for B7-H3.

In the original version of the manuscript, we investigated the expression of B7-H3 in 30 pancreatic cancer tissue specimens. In the revised version of the manuscript, we increased the number of investigated tissues to 68. We furthermore included high quality IHC pictures in figure 1 showing the different immunoreactivity intensities for B7-H3 in pancreatic cancer tissue.

b) The authors state that that no correlation could be observed between B7-H3 expression and age, gender and tumour stage and grade whilst it was associated with postoperative survival: median survival in patients with B7-H3 expression was 14 months and 9 months in patients without B7-H3 expression. Given the small number of negative cases [only six] it would not be possible to show correlations with age, gender and tumour stage and grade whilst the conclusion on postoperative survival must be unsafe.

More cases are needed to show correlation with survival.

We agree. Therefore, we have substantially increased the number of patients investigated in our study (from 30 to 68).

We were able to confirm our previous results.

c) Gene expression of IFN-gamma by qRT-PCR was significantly upregulated in pancreatic cancer tissues in comparison to normal pancreatic tissues and also correlated with B7-H3 expression but no data are shown.

In the revised version of the manuscript, we have included qRT-PCR data of IFN-gamma expression. Levels of IFN-gamma mRNA expression in pancreatic cancer as well as in normal pancreas are now shown in figure 3A. Moreover, we show the correlation of IFN-gamma expression with B7-H3 in figure 3B.

d) CD4 and CD8 mRNA expression was increased in pancreatic cancer tissue specimens (data not shown) and B7-H3 mRNA expression was correlated with CD4 and CD8 mRNA levels. The tissue was not microdissected however and without IHC of CD4 and CD8 it is difficult to draw any conclusions.

IHC for CD4 and CD8 is required.

We agree. Therefore, we have performed immunohistochemical stainings of consecutive slides for B7-H3, CD4, and CD8 in order to visualize the distribution of tumor-infiltrating CD4+ and CD8+ T cells and to determine correlations between tumor-associated B7-H3 expression levels and CD4+ or CD8+ T cells.

We could show a significant correlation between the level of tumor B7-H3
expression and the numbers of tumor-infiltrating CD8+ T cells.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English Needs some language corrections before being published

Language corrections of the manuscript have been thoroughly performed

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.

The authors acknowledge that the content of the manuscript is original and that it has not been published or accepted for publication, either in whole or in part, in any form, that no part of the manuscript is currently under consideration for publication elsewhere and that all of the authors are aware of and agree to the content of the paper and their being listed as an author on the paper. All individuals listed in acknowledgment declared their agreement. The authors also declare that there is no financial or ethical conflict of interest.