Qeios

Peer Review

Review of: "Phosphatidylserine (PS)-Targeting Chimeric Interferon (IFN) Fusion Proteins for Anti-Tumour Applications"

Sara Zalba[1]

1. Pharmaceutical Sciences, Universidad de Navarra, Spain

I have read carefully the work entitled “Phosphatidylserine (PS)-Targeting Chimeric Interferon (IFN) Fusion Proteins for Anti-Tumour Applications”.

First, I would like to state that the therapeutic approach is extremely interesting and the results are impressive.

However, there are several important issues that need to be answered.

Major issues:

1- What is the composition of the short linker between both IFNs?

2- A scheme and deeper explanation of Gas-6 function, structure, and expression are required, as well as the role of carboxyglutamic acid, Warfarin, and vitamin K in the introduction and/or Materials and Methods. The supplementary Table 1 should be better commented on and explained.

3- Why is the graphical abstract located in the discussion section?

4- The origin of all cell lines and details such as cancer type or similar should be mentioned. All the used cell lines should be mentioned in the work and in the supplementary material, as well as the rationale behind their selection, with references and data if required (e.g., receptor expression).

5- The origin of vectors should be mentioned, and the structure shared. If already published, please refer to it.

6- Some in vitro details are missing, such as: How many cells were used for all assays? How long were the cells allowed to adhere before incubation?

doi.org/10.32388/SJO5A5

7- Some experimental procedures are not detailed properly or referred to, e.g., immunoblotting, TALON&sds page&comassie staining, IFN purification.

8- Please indicate the exact concentration of IFN in cell supernatants for antiviral assays and protection assays instead of dilution factors. Are the supernatants quantified?

9- Please include the number of animals per group.

10- Please explain in Materials and Methods the procedure to transfect E0771 and B16F10 cells with the vectors, probing the stability of transfection, levels of expression, and cell viability. VSV dosing is also missing. VSV-GFP is also mentioned somewhere... Please check.

11- Why did the authors choose this tumor volume formula?

12- Please indicate the real tumor size also or instead of relative tumor size.

13- Why did the authors choose 28 days as the end of the experiment?

14- The authors collect tumors, lungs, and spleens to perform further analysis, but no data is shown afterwards. Please explain.

15- What about the ascites, anemia, and other toxic effects? Please report how many animals suffered these side effects and the sacrificed animals' data based on ethical issues. What about the body weight data?

16- Cell surface binding assays require some context. The cytometry procedure and analysis should be detailed. The axis legends reported should be commented on or clarified.

17- RNA sequencing context is not properly explained in Materials and Methods. Although I agree with the rationale behind it, it is not enough to prove the immune-boosting effect as no in vivo immunological assay has been performed. Please be cautious when ensuring the immune effect of this approach.

18- Did the authors have information regarding the IFN amount obtained in tumors? Authors should compare the transfected cell lines with non-transfected and IFN intratumoral administration at a similar dose. Or a lower dose of IFN-transfected cells.

19- The statistical analysis did not include non-parametrical tests. Did the authors perform a normality test?

20- Please avoid repeating methodological details in the results section. Some information provided in this section should be relocated to Materials and Methods. Some details reported in figure legends should be relocated to Materials and Methods.

21- On page 9, do ARPE19 cells express both receptors also? Or is it a mistake? Why choose a human cell line instead of a rodent one?

22- Although the authors comment on the required dose to obtain an effect, 130 pg/mL for IFNβ and λ, the fusion protein showed activity at “very low amounts”. Please be specific with dose and dilutions.

23- What is the hypothesis behind the enhanced effect of the fusion protein compared to separate IFNs?

24- Some methodological details do not correspond between figure legends and methods, such as exposure times or cell numbers.

25- On page 12, the authors mention ectopic tumour assays. Which ones are they? Please indicate the previous works.

26- It is very difficult to prove the advantage of targeting the PS moiety when the tumour shrinkage obtained without targeting is already impressive. Hence, it seems that PS targeting cannot enhance the tumour effect. Please comment on that.

27- What are BMDMs and Jurkat cells assays, CHO assays? This is not mentioned or detailed in the materials and methods nor explained properly.

28- What is Mertk?

29- What is the advantage of the PS strategy described here compared with Annexin V or other approaches mentioned?

Minor issues:

1- What is HCC on page 4? Please check all the abbreviations in the document. Suggestion: including a list of abbreviations at the end of the work could help.

2- What is the method to test for mycoplasma?

3- Please include the origin of all products used in a materials list (e.g., LipoD293, VSV).

4- Please mention the devices and apparatus used for all the assays.

5- Supplementary material is not cited in the text in proper order; please check.

Declarations

Potential competing interests: No potential competing interests to declare.