1st CTC Technologies Meeting 2024: Minutes on Discussion Points

WG LEADS: Evi Lianidou, Nikolas Stoecklein, Yong-Jie Lu

KEY TAKE AWAYS

- The first application for COST-funding, which covers primarily travel for academic partners with focus on young investigators was unfortunately not approved. However, we received very good feedback and will re-submit the application in fall of this year

MEETING MINUTES: QUESTION TO THE SPEAKERS

1st Presentation by **Zahra Eslami Samarin:**

- **Nikolas Stoecklein:** The effects that you see, clearly come from the thrombocytes. Can you distinguish between real interactions of them with the cells or are they just releasing their cargo within your cell culture medium? Did you see platelets on the cancer cells?
  Zahra Eslami Samarin: I think this is really an important point to consider. However, for us it wasn't the main aim of the study, therefore we did not explicitly check this. We did observe direct interaction and close connection in cell culture via microscopy, therefore I would say the effects are a combination of both cargo and interaction. Taking into consider existing literature on this, I personally would lean towards the interpretation that the cargo has higher influence that the direct interaction.
- **Yong-Jie Lu:** The majority of researchers appear to believe that the cancer cells need direct contact with platelets. But from your data, do they have better potential for metastasis than those not directly co-cultured?
  Zahra Eslami Samarin: Good question. My first question was the direct contact between CTC and platelets and to understand how a platelet becomes a tumor-educated platelet. However it is a great idea for future studies.
  Yong-Jie Lu: You looked at the prognostic factor of PDL1 expression on CTCs. Did you happen to have any patients in your cohort that received immunotherapy? Is there any predictive information available?
  Zahra Eslami Samarin: Yes, in our study some patients were under treatment, some were not. In this setting we compared the different markers, such as CTC, ctDNA, and EVs to see whether one or more would give us prognostic information in this heterogeneous patient group. Actually this is why we have set up another clinical trail in context of immunotherapy and its almost finished. In this trial all patients were receiving immunotherapy we have a measurement at diagnosis and follow-up. Therefore, we hope to be able to answer this question soon.

2nd Presentation by **Clotilde Costa**:

- **Yong-Jie Lu:** Have you explored CDK4 and CDK6 expression singularly vs. combined on how good the predictive value is?
  Clotilde Costa: It's good, I don't remember exactly but its near 0,8.
- **Evi Lianidou:** Concerning STAT3: As far as I know STAT3 should be phosphorylated in order to be active. Through mRNA analysis and gene expression you don't discriminate whether it is in fact phosphorylated or not. Despite that you show interesting results. So what do you suggest?
  Clotilde Costa: Now we are focusing on trying to decipher the implication of STAT3 in this response or sensibility of the CTK4 inhibitors. So we want to know if STAT3 is activated or not. Indeed we also try to perform some proteomic analysis from CTC, but we just started on that and its very complicated. But I would like to collect some data on that in the future, because we know that we have our mRNA but it's not the activation of the pathway. So it's something we are trying to work on now.
- **Yong-Jie Lu:** Is it possible to do the immunofluorescence to check the CTCs for that expression?
  Clotilde Costa: Yes its possible. But at the moment we don't have CTCs fixed to do so. So we have to wait to collect those patients.
- **Klaus Pantel**: I was just wondering, if you compare the CTC fraction and the normal PBMC with your PCR. In the CTC fraction you usually also have a majority of PBMCs and a minority of tumor cells. I'm just wondering how you can be sure that your signal is from the CTCs?
  Clotilde Costa: We did the same analysis from the CTC and the PBMC fraction and then we normalized to use the value that we get from the PBMCs. With this we are left with the genes that are expressed solely in the CTCs. We discard all genes that we are unsure about and that are contained in the PBMC fraction. It is possible that we lose some of the information this way, but to make sure that what we see orginiates from the CTCs, we do it this way.
- **Nikolas Stoecklein:** You also did the CellSearch assay in the sub-group. Was there a correlation between your signal positivity and the CellSearch positivity?
  Clotilde Costa: In this work there were very small amounts of samples. I think 12 that were directly compared. But in similar works in breast cancer and prostate cancer were we have the gene expression and the CellSearch data, there we see an association. The keratin and E-cadherin expression for example was higher in those that have CTC detection by CellSearch.

Institutional pitches:

1st pitch Melanie Janning:

- No questions from auditorium

2nd institutional pitch: Daan van den Broek:

- **Nikolas Stoecklein:** I was just wondering why you just use EpCAM as one marker for your flowcytometry?
  Daan von den Broek: Since we are using it only for lung cancer, these are mostly EpCAM positive tumors. So for that purpose it's working. But for melanoma you need to have a different application. I think the reason for it was that availability, cost-effectiveness and easy way using just your flowcytometer. And in validation studies it performed really well. So we also showed that doing molecular work on the CTCs and on cell-free part of the cerebral-spinal fluid, that we were really looking at tumor cells, so all the confirmations were there and then the EpCAM for the lung cancer is performing good.
- **Yong-Jie Lu:** Following that question I just wanted to ask: During the EMT process a lot of those CTC will loose the EpCAM marker. I was just wondering: will you miss some CTCs when they change from epithelial to mesenchymal?
  Daan von den Broek: In a clinical cohort from more than 80 patients we have 49% sensitivity and 100% specificity. So yes, you probably lose some, but it's really a small portion. And then when you look at the other methods of measuring laptomeningial metastasis they just perform less. They have a lower sensitivity than this essay. Could it be better? It might. Using a borader analysis than only EpCAM, we didn't look into that. But that should be investigated, wether this could really a significant improve the data as it would also making your essays more complicated and more expensive to use additional markers.

OPEN DISCUSSION: COST ACTION PROPOSAL

Nikolas Stoecklein: I wanted to inform you as a group, that we tried to get COST-funding to support younger investigators and so on to come to our meetings and this cost-network-funding seemed to be an opportunity. I submitted a proposal last October. We recently got the evaluation report back and unfortunately the proposal was not granted. However, the overall evaluation report was quite good. Out of 10 different items graded from 1-5 (and 5 is excellent), we got 4x excellent and 6x very good. We don't need to be excellent in all of them, but we have to push two or three of these items from a 4 to 5. The review panel gave quite good indicators on what should be improved for re-submission. These were more or less technical issues, one of them I already knew when we submitted it, was the proportion of the young investigators was too small. This is something that is of course easy to improve. And we will do so.
Another point was regarding the ICT-countries (inclusiveness countries). Here the criticism was that it was not so clear how people from the ICT-countries are going to be included in leading positions of the consortium. And I think that was the next mayor criticism that some of the aspects were for them not concrete enough so that they didn't rate that as an 'excellent'.
I think there are some technical aspects that need a better description, however I got the impression that it was a good first application with very good marks. To get founded I think we have to invest some more into planning and describing the future work of this consortium in order to be successful the next time.

So what I'm going to do is, reach out via e-mails you will also receive some from the cost-system so that we set up the consortium again. And then I think we have enough time until the 24th October, for the re-submission, so that we will have a competitive final document ready by then. The first step is to align the consortium, the call just opened one or two weeks ago, so it would be great to start with this. And then we can spend some more time with re-formatting the existing application. And I will maybe approach some of you with concrete questions. Thank you.

Yong-Jie Lu: Thank you Nik, for putting this together the application the first time and for doing this application. So apparently they all liked this proposal and just in some area we could expand and that's why we […?]
I think you did very well in putting all this things together, Nik.
Klaus Pantel: Absolutely, I can just also say that. I guess we just have to try again, Nik. Thanks for taking the effort and we will never give up!
Nikolas Stoecklein: Thank you. I think I will talk to you and then we will see what we concretely can improve.
Klaus Pantel: Yes, I think also implementation of research in clinical groups from Eastern Europe becoming more and more important. Also the European Union really sees that as a really important part of their future program, so I think that it would be good for ELBS to also reach out to partner in Eastern Europe. I just gave a presentation and meeting organized from a student group actually in Zagreb, Croatia, which was really nice. But I think we should really, as ELBS, probably think of that. That we actively invite the groups also from Eastern Europe to participate in our activities.