CTC Technologies Meeting: Minutes on Discussion Points

1. In situ RNA expression of CTCs and machine learning approaches for cell classification (*Amin El-Heliebi, Medical University of Graz*)

Galatea Kallergi:

- Have you compared the in-situ RNA and protein expression? What are your positive and negative controls to ensure you do not count non-specific dots?

Amin El-Heliebi:

- In prostate cancer for example, we work with well-established cell lines as controls for *AR* and *ARV7* expression. We also performed ddPCR and compared the results to those of our method. Furthermore, we have performed antibody staining in parallel with our in-situ approach. It is possible, but for some proteins of interest (e.g. ARV7) no suitable antibodies have been established so far, thus limiting the possibility of direct comparison. However, using tissue as a control, we see localization of our probes exactly where it would be expected. Also, the use of dual colouring (at different ends of the fluorescence spectrum) increases our sensitivity as an event is only counted if both colours give a signal.

Sigfried Hauch (*Qiagen*):

- Is this technology also applicable to immune repertoire testing, meaning that you could see the RNA setup for example in T-cells?

Amin El-Heliebi:

- What you saw here was special transcriptomics with cell populations. Generally, it would be feasible to target subclones of T-cells, however, we have not tested it yet.

Rui Neves:

- Is the multiplexing possible through differently coloured padlocks recognizing different sequences of the RNA transcripts? Or are they targeting the same sequence?

Amin El-Heliebi:

- It is a little more complex. We have a padlock in the backbone of a padlock ensuring two colours on the same target. However, we usually use several padlocks on one transcript – recognizing different sequences.

Rui Neves:

- And the amplification is not to complicated despite the close proximity

Amin El-Heliebi:

- The technology is very stringent. Sometime we see blots/background, this is dealt with by digital background correction.

Rui Neves:

- Does the training set for the machine learning approach need to be set up new for each project?

Amin El-Heliebi:

- Yes, mostly. We require enough cells, enough biological replicates, the closer to the patient sample the better. This is a bottleneck of supervised training and machine learning – the need

for enough pictures. We are currently thinking about using synthetic data to train the supervised machine learning.

Rui Neves:

- Have you picked the cells you have analysed and checked the quality of the DNA you tend to receive?

Amin El-Heliebi:

- No, subsequent molecular analysis via PCR or sequencing has not been done so far.

2. Utilization of CT(A)C: From therapy management towards screening (*Stefan Schuster, Datar Cancer Genetics*):

Klaus Pantel:

- Can you comment on the number of cells measured by your method under benign conditions/ in healthy donors versus actual cancer patients?

Stefan Schuster (*Datar Cancer Genetics*):

- This is under review in a publication now. When looking at asymptomatic individuals we are focussed on CTCs, not CTACs. We see CTACs in amount 2-3% of the population, dependent slightly on age. To make sure what we detect is actually clinically relevant we then go into the next steps, the short term culture and perform ICC. All together, we have a specificity of > 99% in the end. In the end, it is more a qualitative than quantitative outread.

Evi Lianidou:

- Have you compared your technology in a subset of samples with other technologies, such as the CellSearch? This would give us an idea of how it compares to what we are currently using in the labs.

Stefan Schuster (*Datar Cancer Genetics*):

- We have internally compared our approach to other machines, not the CellSearch as far as I am aware of. The outcomes were slightly different, despite being focussed on EpCAM and Cytokeratin. However, we were focussed more on the hallmarks of cancer and one of these is survival in conditions that other cells could not thrive in. We are currently going through the PMA (pre-market approval) with the FDA, which is very complicated.

Evi Lianidou:

- I personally don't question the validity of your technology. My question was more technical. In clinical chemistry for example if you want to establish a new marker you need to compare it to the gold standard.

Stefan Schuster (*Datar Cancer Genetics*):

- Yes, I completely understand. However, for cancer screening or chemosensitivity testing there is no "gold-standard" as not enough cells are typically found.

Eric Kaldijan (RareCyte):

- What type of cells are CTACs?

Stefan Schuster (*Datar Cancer Genetics*):

- Certain fibroblasts can survive in our approach and tend to associate with cancer cells. If you would like more information on the nature of these cells, I would be happy to bring you into contact with our lab director.

Galatea Kallergi:

- How have you decided on the period that the cells are cultured and when enough time has passed to ensure that no blood cells remain. Do you have a special medium for this?

Stefan Schuster (*Datar Cancer Genetics*):

- We have our own culture medium, which is patented. Of course we have tried several days but after having validated a few thousand samples, we are sure that what is left after 5 days usually has nothing to do with a normal cell. Tested from 1 up to 12 days, but 5 days makes most sense.

3. Cell-based liquid biopsy to assess protein biomarkers and cancer genomics (*Eric Kaldjian, RareCyte*)

Klaus Pantel:

- We should still keep ring experiments in mind in terms of standardization and harmonization of methods

4. Update on subgrouping by Evi Lianidou and Nikolas Stoecklein

Evi Lianidou:

- We have grouped together the members of ELBS according to technology in order to form smaller, more agile subgroups which can discuss potential ring-studies and potentially move towards ISO certification for the labs that are interested.
- Please check the list of technologies and make sure the information on your lab is up to date. If not, let us know
- How can we use this information to move towards ISO certification? External quality assessment (EQA) schemes do not exist for CTCs so far. However, ring studies can be taken into account in the certification process.
- Suggestion: Goal by end of this or next year to have some assays running with ISO for some markers and technologies. We will need to also check in with the clinical group to make sure we are focussing on markers that are highly important for the clinical setting.

Klaus Pantel:

- Please send us updates on your methods if anything has changed. The labs interested in ISO certification are welcome to contact us and we will support the respective labs getting together.