CTC Technologies Meeting: Minutes on Discussion Points

1. Results of the Survey:

Evi Lianidou:

- We do not have the complete data available – please send us your surveys by the end of the coming month (end of October), so we can include them into the next meeting

2. Organization of the CTC technologies group:

Nikolas Stoecklein:

- ctDNA group will have its first meeting in the course of November
- Subgroups: We would like to facilitate the founding of smaller subgroups and support identifying interested members to participate. If you have specific interest, please let us know. (e.g. I would be open to initiating a diagnostic leukapheresis subgroup later this year or beginning of next year)
- Networking: Open channel for academia and industry to share updates, progresses, and issues. Please let us know if you would be interested in presenting something to the group, or if you have some talking points in mind that you would like to raise for discussion.

Klaus Pantel:

- Members could present surprising findings or preliminary study results that point towards issues in the field. This would support members in connecting on specific projects or problems to provide support in form of data, samples or problem solving ideas. We could open up about challenges and identify common issues, this may profit everyone.

Tim Pitfield (*Menarini Silicon Biosystems*):

- Should the scope here lie solely on CTCs or is there interest in including other biomarkers such as circulating endothelial cells and/or extracellular vesicles?

Nikolas Stoecklein:

- This is the CTC subgroup under the umbrella of the technology group. There will be specific subgroups catering to different biomarkers (e.g. ctDNA and exosomes) as the utilized technologies vastly differ. This is the current setup, which might be modified in the future.

Evi Lianidou:

- Already within the CTC subgroup we have a lot of different aspects to consider, as the questionnaire is showing us. If we want to group the members together to manage and standardize the technologies in a better way, we need to focus. We can combine the biomarkers a little later.

Leon Terstappen:

- Difficult to stick to original CTC definition. We should include everything that CTCs detect – we need flexibility. We should broaden our horizon to these additional fragments that can be detected in all technologies.

Catherine Alix-Panabières:

- We should definitely focus our efforts and also agree on a good definition of a CTC. However, in this scenario bio banking becomes very important to allow for the combination of different biomarkers in the actual clinical setting.

Klaus Pantel:

- We can extend the CTC technology group to other related, similar analytes in form of small subgroups. However, initially, we need to keep people together. There are also other existing groups focussing on EVs – we do not want to duplicate what others are already successfully doing (ISEV). We could reach out to them for additional information on things ongoing in the EV space.

3. <u>Discussion on development of CTC reference material</u>

Evi Lianidou:

- In combination LGC and Menarini could potentially provide a first CTC EQA for the CellSearch?

Tim Pitfield (*Menarini Silicon Biosystems*):

- The biggest challenge is the cost (panel plus Cellsearch run). The control budget shouldn't be higher than the actual testing budget.

Evi Lianidou:

- Control runs are a requirement for CLIA or ISO certifications. Running 3 samples per year is feasible. We could begin with the CellSearch and then move to other technologies.

Markus Sprenger Haussels (*Qiagen*):

- Will the developed standards also allow for RNA analysis? The combination of both the phenotype and transcription profiles of a tumor cell allowing for molecular read-outs would be beneficial.

Tim Pitfield (*Menarini Silicon Biosystems*):

- Menarini will release a new collection tube called "Cell rescue". It will allow for 120 h of sample stabilization and preserve intracellular RNA and DNA.

Krystina Nahlik (*LGC Clinical Diagnostics*):

- Will DNA analysis also be of interest for the cellular standards?

Nikolas Stoecklein:

- Yes, this is also important as many groups use methods that target DNA.

Evi Lianidou:

- We should continue this discussion in an upcoming meeting and ideally be able to test the novel preservative tubes developed by Menarini Silicon Biosystems as fast as possible

4. <u>Pre-analytical variables:</u>

Roberta Carbone (*Tethis*):

- Tethis focusses a lot on the pre-analytical aspects of sample integrity as it influences all further downstream analysis.

- Currently there are many different blood sample types used, different handling, and different shipment procedures. In literature conflicting results are described depending on the blood collection tubes and preservatives used. When looking at a fragile marker such as CTC, the question is will there be efforts by ELBS to standardize this? Which plans exist?

Yong-Jie:

- Agree. Pre-sample collection is very important especially for CTC and RNA analysis. My feeling is that RNA expression will significantly differ. We need to determine what time frame and which tube should be used for sample processing.

Klaus Pantel:

- I was recently contacted by a non ELBS member working on the development of RNA preserving tubes. This is an extremely important issue. Several parameters would need to be tested, ideally in form of a ring trial allowing comparison of e.g. 3 solutions after shipment to see what is still there. RNA analysis in CTCs is big advantage to the ctDNA field.

Michael O'Brien (*ANGLE*):

- We typically perform RNA analysis with EDTA blood samples. This poses restrictions on sample processing time. Ring trials for RNA tubes sounds like a good topic for the group.

Klaus Pantel:

- Another relevant aspect is the cell concentration for ring experiments. The performance of assays is highly dependent on cellular number. We therefore need controls with realistic sample CTC counts.

Krystina Nahlik (*LGC Clinical Diagnostics*):

- Would a linearity panel be helpful (1000, 100, 5 CTCs)?

Klaus Pantel:

- Down to 5 cells would be good starting point to test methods.

Nikolas Stoecklein:

- Question from the audience: Has ELBS considered to engange with eternal quality ensurance organisations (e.g. Niquas or GENQA)?

Evi Lianidou:

- In order for such organizations to initiate external QC for CTC analysis they require standards. This ist the 1st step. The 2nd step is to engage an external QC provider who is ISO certified to do this job.

Klaus Pantel:

- If you, as a member, have any ideas for presentations in future meetings, please let us know.