CTC Technologies Meeting: Minutes on Discussion Points

1. Results of the Survey and Set-up of Ring Studies:

Evi Lianidou:

- Important to try to group labs that are doing similar kind of work. This will also help to set up ring studies with similar markers and cancer types allowing harmonization on what is currently being done.

Michael O'Brien (*ANGLE*):

- In strong support of this. It is very much a strength of the ELBS to do such a thing and bring credibility and strength to standards and technological results.

Evi Lianidou:

- We will form the groups and start thinking of how we can bring this information forwards.

Galatea Kallergi:

- These groups could also apply for joint funding in collaboration.

Evi Lianidou:

- Similar things have already been done within CANCER-ID and are now continued within ELBS. Potentially these efforts could be sponsored by ELBS partners at least in part.

Klaus Pantel:

- Currently in the process of setting up an initial ring experiment for a novel technology in which the provider is ensuring some financial support. Similarly, we could set up clusters of 5-6 sites testing different things, smaller groups are easier get started. The results will then be reported at these meeting.
- CTC ring experiments more complex that ctDNA because of shipment issues.
- We need to include companies into the planning to ensure we take flexibility of assays and protocols into account.
- Apart from industry, also academic groups can participate to validate an assay or marker as a collective.

Nikolas Stoecklein:

- This would be highly beneficial and enrich existing data
- These projects could then be driven by specific group leads.

Oddmund Nordgård:

- If everyone agrees, we could share the results of the survey to enable smaller groups to form.

Evi Lianidou:

- Coordination by a central group could be helpful in the beginning and we could offer this through ELBS.

Amin El-Heliebi:

- Regarding potential ring trials: It will be important also to exchange image data. A strength of CTCs is the added information on morphology, biomarker expression levels, cellular localization. Ring experiments with image analysis/image processing could be highly valuable.

Klaus Pantel:

- Today there is large interest of applying machine learning, AI approaches and other software to CTC detection. It would be sensible to include a group that is interested and knowledgeable in these studies. We should also try to include 1-2 of these people as part of our meetings.
- So far these analysis lead to: Yes/no/maybe CTC populations and much discussion on the "maybe" CTCs. With appropriate clinical follow-up data and AI capability, we could work on this issue.

Evi Lianidou:

- Can ACCEPT software be extended in other image galleries (confocal, etc.)?

Afroditi Nanou:

- It has not been tested with confocal images but in principle it should be possible. If you send the images to us we can test it.

Evi Lianidou

- Do EQAs exist from Menarini regarding image analysis for the CellSearch system?

Nicolò Manaresi (*Menarini Silicon Biosystems*):

- Will check whether a quality control scheme for image read out exists and will give feedback.

Nikolas Stoecklein:

- If you have concrete projects in mind please send it to Claudia to ensure we know to work on it

2. <u>The challenges of preserving sample integrity for optimal analysis (Roberta Carbone - Tethis):</u>

Roberta Carbone (*Tethis*):

- Could this approach be an alternative workflow? Shipping vials and cell slides instead of samples?

Nikolas Stoecklein:

- What is the slide capacity: how many ml of blood can be processed, how many white blood cells?

Roberta Carbone (*Tethis*):

- Usually, 10ml result in 20-30 slides. A monolayer consists of approx. 2,5 million cells per slide.

Evi Lianidou:

- The approach appears very similar to EPIC platform. They have an automatic imaging system downstream. How easy will it be to analyse so many slides?

Roberta Carbone (*Tethis*):

- Agree there are similarities to EPIC. However, we are standardizing pre-analytical step which allows samples to be collected in EDTA without shipment but placed at point of care.
- Ideally: 5-10 slides would be analysed semi-automatically and AI driven
- Microdissection of single cells and DNA analysis is possible (RNA analysis is not suitable)
- Future goal: bright-field based analysis determining CTCs according to their morphology and building an atlas of cells to identify "suspect" cells which would be confirmed by molecular analysis

3. <u>Is specificity more important than capture efficiency if sample size is no longer limiting</u>

Michiel Stevens:

- Currently with DLA only about 5% of sample is processed.
- Question: If you are developing new methods should you try to develop something that is very sensitive (high CTC count, high background) or very specific (lower CTC count, lower background)?

Nikolas Stoecklein:

- Sensitivity is more important. For some methods cellular background is not so relevant (if you have a clear target population). Also, methods are theoretically combinable (first high throughput methods such as FACS, then DEP-Array e.g.). Currently this is difficult as you have so little cells (low sensitivity). As soon as you have an overview of the situation and population of interest you can adapt the technology to be more specific taking into account the fact that you might lose some interesting cells.

Siegfried Hauch (*Qiagen*):

- From a clinical perspective, DLA has the potential to find rare events more abundantly, especially in the primary situation. The more tumor cells are recovered, the better.

Amin El-Heliebi:

- A major advantage of CTC vs. ctDNA is the possibility to perform functional tests. These could potentially be much more promising on DLA enriched CTC fractions (due to higher CTC count). The problem remains of how to handle the DLA product.

Michiel Stevens:

- It is also important to know whether cells remain viable after the processing.

Jaco Kraan:

- Whether sensitivity or specificity is more important could be determined by the clinical setting (early disease vs. metastatic disease).
- With high specificity it becomes important to know that recovery remains stable and reproducible also in patients.

Nikolas Stoecklein:

- Functional assays are extremely relevant, but more routine based the advantage of CTCs is in the direct visualization of targets for therapies (e.g. ARV7). The more CTCs are present, the easier it becomes to make robust conclusions. A decent number for diagnosis is about a 10 cell minimum. The DETECT studies are a perfect example of this

Nicolò Manaresi (*Menarini Silicon Biosystems*):

- In the DETECT studies a single HER2+ CTC was a successful criteria for inclusion. It will be important to identify more patients that are currently still assessed as CTC negative.
- CAVEAT: It is easy to be fooled when identifying a CTC, even when imaging by immunofluorescence. We need to still work on good definition of CTCs. Otherwise we run the risk of calling something a “CTC” that later on turn out not to be (no CNAs). These might represent cells (e.g. immune cells) worth exploring but not true tumor cells, which makes them ineligible for testing biomarkers for drug efficacy that work on the primary tumor for example.