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4D Lifetec AG - Independent Technology Assessment

4D Lifetest™

The startup 4D Lifetec has developed a liquid biopsy assay for the early detection of cancer based on the well-known technology of Single Cell Gel Electrophoresis (SCGE). This technology is fundamentally different from most other assays, since it can integrate the measurement of DNA damage and DNA repair capacity in living cells in one assay. Most other assays analyze only individual endpoints, e.g., DNA sequencing, quantification of RNA transcripts, proteins or DNA repair enzyme activity. SCGE-based assays can also be considered as "relatively cheap" compared to other, much more complex assays such as tumor mutational burden (TMB) by next generation exom sequencing (NGS) or microsatellite instability (MSI) assays. Many scientific publications for SCGE applications exist, mostly in the field of assessing genotoxicity in cell cultures or in human exposure. In addition, there are many publications describing the application of SCGE in connection with the detection of various solid tumors and also in blood cells of cancer patients. However, the diagnostic application of the technology has so far failed due to its low reliability.

With the novel 4D Lifetest™ the company has developed the technology platform of SCGE to a unique new level of standardization and accuracy. The new assay fundamentally enhances the given potential of SCGE in terms of sensitivity as demonstrated in the initial technology paper (Cassano J et al., A Novel Approach to Increase Robustness, Precision and High-Throughput Capacity of Single Cell Gel Electrophoresis, 2019 Aug 28 Altex doi:10.14573/altex.1906252). It therefore effectively opens the door for the diagnostic use of DNA Repair Capacity (DRC) as a reliable general biomarker. Although 4D Lifetest™ addresses DNA damage and repair, it is not a genetic test. This is advantageous because the assay does not fall into the legal framework of genetic assays and does not require special release by human genetic certified bodies. In addition, the test shows a high level of robustness and is "easy to use" in routine application as a liquid biopsy assay.

DNA Repair Capacity (DRC) as a novel biomarker for the early detection of cancer

In the ongoing clinical studies, 4D Lifetest™ is used to demonstrate DNA Repair Capacity (DRC) as a novel biomarker for the early detection of solid tumors in patients' blood before treatment. The clinical studies includes untreated lung, breast, prostate or colon cancer patients versus matched healthy controls. The cancer types included in the study are the most prevalent ones, and all of them form solid tumors.

The beauty of the approach is that DRC can be measured in a surrogate tissue, in peripheral blood mononuclear cells (PBMCs), instead of the tumor itself. Most alternative methods such as the ones mentioned above require either biopsy material from the tumor itself, which is invasive and often difficult to obtain. Alternatively, the use of circulating tumor cells or circulating tumor DNA (ctDNA) as Liquid Biopsies from peripheral blood suffers from major technical challenges. First of all, the amount of tumor cells or ctDNA in blood is available only at relatively low amounts and, secondly, their release into the blood stream can vary significantly. Overall, this might result in sensitivity problems when measuring circulating tumor cells or ctDNA.

Initial observations that DRC measured by SCGE can separate untreated cancer patients at early stages of different types of cancer from healthy matched controls already exist and have been published. However, to my knowledge no systematic approach has been applied to develop DRC as a minimally invasive, early detection biomarker for cancer due to the low level of reliability of the existing technologies.

Therefore, for the ongoing clinical study of 4D Lifetest™, the company first developed a sample collection protocol which allows the separation of the sampling timepoint from the analysis timepoint. This approach enables the test to fit into the routine diagnostic workflow of hospitals and allows the analysis of the patient

samples in a centralized lab. A challenge protocol was established and standardized. In this protocol PBMCs from patients or controls are treated with UV light in order to enhance the separation between them. Data so far are based on a limited number of prostate cancer and lung cancer patients plus healthy controls. Data are extremely promising since they indicate a robust detection of cancer patients based on the tested cases. Even more the outstanding increase of sensitivity of the biomarker detection of 4D Lifetest™ shown in the *in vitro* publication has been demonstrated now to be effective also *in vivo* measuring affected patients. However, the scientific hypothesis that surrogate tissue (PBMCs) of untreated cancer patients is systematically reflective of the presence of solid tumor still needs to be demonstrated for other types of cancer.

Inter-individual differences in the highly outbred human population is a known challenge for most of the biomarkers currently proposed for the use in liquid biopsy assays. This is also reported in the literature for DRC (Nagel Z D et al., Inter-individual variation in DNA repair capacity, DNA Repair, 2014 July, 19:199-213). Tumor type and stage, gender, life-style factors etc. may play a role in the future development of the assay. The company's clinical study design already considers these factors since they are reflected in the case report form (CRF).

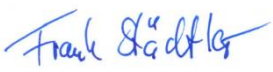
The current results of the clinical studies confirm already the clinical proof of concept for DRC as a robust biomarker using 4D Lifetest™. This is also evident from the fact that these results have already demonstrated a high flexibility in terms of a potential cut off definition. It will be important to support these initial results with a larger number of cases and controls. The envisaged numbers of patients, e.g., 50 patients for the initial validation and 250 patients for the planned LDT reimbursement, I regard as more than sufficient from a scientific and diagnostic development perspective.

In conclusion, the development of this highly innovative test procedure has made DRC accessible as a reliable and standardized biomarker for the early detection of cancer. The exceptional accuracy achieved through an innovative adaptation of a relatively simple technology has been standardized and published *in vitro*. The concept has now been successfully transferred to liquid biopsy diagnostics in first clinical studies and has proven its potential for prostate and lung cancer. For the statistical validation and the clinical routine application of this new test procedure, further patients and cancer types will certainly have to be tested. Nevertheless, it can already be said that with 4D Lifetest™ and DRC a general cancer biomarker has been established. This has the potential to significantly influence the use of liquid biopsies for the early detection of cancer.

The Author

Dr. Frank Städtler holds a PhD in molecular biology and acts as an independent consultant and a genomics and genetics biomarker expert. He worked over 25 years as a pharma R&D professional and biomarker scientist and holds a deep expertise in diagnostically, pre-clinical and clinically applied nucleic acid analytical technologies. In his professional career he worked for Novartis Pharma Development as well as the Novartis Institutes for BioMedical Research and the Roche Pharma AG in different leading positions as senior scientist.

For this Technology Assessment of the 4D Lifetest™, the company gave Dr. Frank Städtler full access to all relevant documents and results. He had the opportunity to discuss with its Senior Clinical Scientist Dr. Zeinab Barekati as well as other team members. The assessment also included a live demo of a part of the 4D Lifetest™ protocol, namely data acquisition, access to the eCRF forms for all cancers addressed, and access to the available clinical results including samples from cancer patients and matched controls.



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