Early-stage diagnosis of lung cancer with liquid biopsy test based on peripheral blood cells

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Background

Liquid biopsy using peripheral blood sampling is a minimally invasive approach that permits investigating cancer diseases in a cost-effective and patient-friendly manner. Several liquid biopsy assays, mostly targeting circulating tumor cells or ctDNA, have been introduced into clinical practice but their applicability for early stages is limited due to low levels of circulating markers in the blood.

Peripheral blood mononuclear cells (PBMCs) are floating in large quantities in the blood and have been extensively used in biomonitoring studies. Here, we establish a novel non-genomic liquid biopsy approach that allows the detection of early-stage lung cancer with PBMCs serving as a surrogate marker.
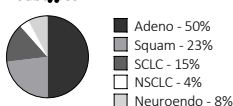
Method

57 subjects presenting with either a diagnosis of lung cancer or healthy donors were enrolled in the observational study. All lung cancer subtypes were included. Blood samples were collected before any kind of treatment through venipuncture. Extracted PBMCs were tested using the 4D Lifetest[TM] assay, an algorithm-based, high-performance electrophoresis approach, which measures the level of DNA damage at the single-cell level. The level of DNA damage was assessed both at the baseline level and after minimal induction of DNA damage by UV, and DNA damage sensitivity (DDS) was calculated.

Table 1. Patient characteristics

	Lung cancer	Healthy Ctrl
Total	26	31
Age, Median (IQR)	69	47
Gender, n (%)		
female	58	55
male	42	45

Subtypes
- Adeno - 50%
- Squam - 23%
- SCLC - 15%
- NSCLC - 4%
- Neuroendo - 8%

Clinical Stage
- I - 12%
- II - 19%
- III - 34%
- IV - 35%

Lifetest[TM] work flow

Step I — Isolation and cryo-preservation of PBMCs
Step II — Loading of Lifeplate[TM] / UV radiation of cells
Step III — Single cell electrophoresis in Lifetank[TM]
Step IV — Data acquisition / Data analysis and report
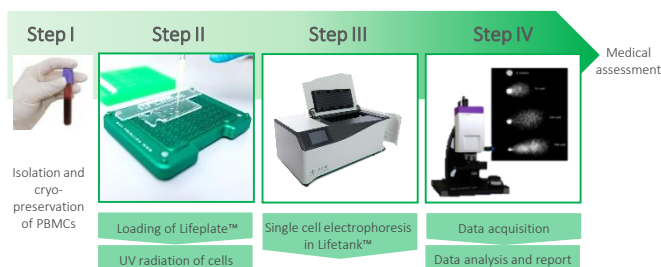Medical assessment

Figure 1. DNA damage sensitivity (DDS) assay workflow. Lifeplates[TM] are designed in a 12 or 96 spot format. Per patient two spots are loaded. Single cell electrophoresis is performed using the Lifetank[TM] allowing simultaneous processing of six 12 or 96 spot Lifeplates[TM], respectively.

Results

High sensitivity for early detection

In total, 26 patients with lung cancer grouped into clinical stages I/II (n=8) or III/IV (n=18) and 31 healthy donors were recruited, and the DDS was determined. Evaluation of DDS comparing non-cancer with early-stage lung cancer I/II samples resulted in >95% sensitivity at 97% specificity (95% CI: 83.8% to 99.8%). For later stages III/IV, we observed a sensitivity of 94%. Across all stages, the sensitivity reached >95%. With regard to tumor types, the assay did not significantly discriminate between lung cancer subtypes, thus serving as a broad test for lung cancer diagnosis.

Detection of early stage lung cancer with high sensitivity (specificity >95%)
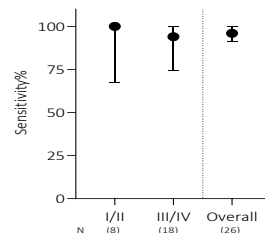
Figure 2. Sensitivity at specificity >95% (95% CI: 89-100) with error bars representing SD.
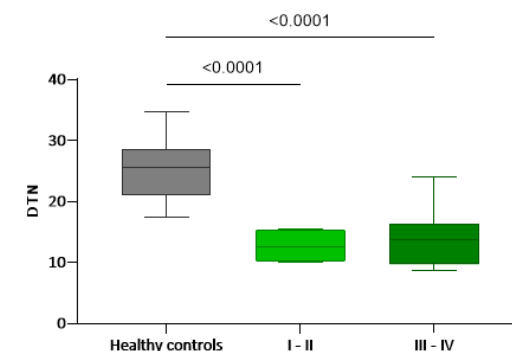
Detection of Lung cancer

Figure 3. The graph illustrates the DTN (%DNA tail after UVB exposure normalized with baseline values) of samples derived from 26 healthy donors compared to 26 lung cancer patients (UICC stage 0-II: 31%, stage III-IV: 79%). Patient samples with >80% viability were analyzed. Box plots display the 25-75% percentile and whiskers represent the minimum and maximum of the values. Statistical significance was calculated with ordinary one-way ANOVA and Tukey's multiple comparisons. Age and gender differences were not found.

Conclusion

Results indicate that changes in DNA damage sensitivity (DDS) is a common feature amongst cancer patients and can be used to distinguish early and advanced stage lung cancer from healthy donor samples. The DDS biomarker assay has the potential to overcome the limitations of conventional liquid biopsy approaches for early-stage cancer detection.