Plasma-based diagnostics for detection of EML4-ALK fusion transcripts in NSCLC patients


1. Introduction
The EML4-ALK translocation is a predictive driver mutation in non-small cell lung cancer (NSCLC). EML4-ALK translocations comprise several variants, the clinical majority of which are v1, v2, and v3 (Figure 2). As presence of these translocations determine both resistance to EGFR inhibitors and drugability with FDA-approved ALK kinase inhibitors, molecular profiling of the respective fusion transcripts is a critical prerequisite to therapy. Ongoing clinical trials and development of new ALK inhibitors for personalized tissue biopsies and fine-needle aspirates – techniques that rapidly detect fusion transcripts via a single blood draw. This liquid biopsy diagnostic provides valuable benefits for non-surgical treatment guidance and longitudinal monitoring of EML4-ALK positive patients.

2. EXO501a Work Flow
EXO501a, which leverages our proprietary spin column-based method (EXO50), we were able to consistently and reproducibly isolate sufficient amounts of high-quality exosomal RNA from a few ml of NSCLC patient plasma for analysis and quantification of EML4-ALK fusions (Figure 2).

3. Patient Data
EXO501a was validated on NSCLC patients (see Figure 3 for exemplary results). As a proof of concept, we analyzed tissue-correlated plasma samples for presence of EML4-ALK v1/v2/v3, respectively.

Additionally, positive plasma samples were confirmed by qPCR for increased ALK expression. In a cohort of 20 patients, no false positive samples were detected; true positive concordance will be determined on an increased number of defined patient samples.

4. Assay Performance
We evaluated the reproducibility and sensitivity of EXO501a for each variant of EML4-ALK applying synthetic reference RNA spiked into healthy patient plasma at the RT step (Figure 4).

Limit of detection (LOD) was determined as 2.5 copies per reaction. Assay specificity was identified as 100% for variant-specific detection of EML4-ALK. Efficiency of qPCR is ranging between 92-100% (data not shown here).

5. Comparison of Downstream Analytics
Using total RNA of an EML4-ALK v1 expressing cell line, we compared EXO501a with two commercially available tests for EML4-ALK detection: Amoy Diagnostics and Qiagen (Figure 5). Monitoring the LOD, we observed superior performance of EXO501a over the competitors for EML4-ALK v1-specific analysis.

6. Conclusions
Liquid biopsy, in contrast to tissue, represents a non-invasive and less-risk method to detect the predictive biomarker EML4-ALK in plasma of NSCLC patients at baseline and to monitor longitudinally during therapy.

Here, we demonstrated proof of concept data for detection of EML4-ALK with high specificity for individual fusion variants from the plasma of NSCLC patients on exosomal RNA.

The qPCR-based liquid biopsy assay’s performance on cellular RNA exceeds that of alternative test kits.

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