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 1). As presence of these translocations determine both resistance to EGFR inhibitors and druggability 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 treatment demand development of robust diagnostics. Current determination of EML4-ALK fusions relies on tissue biopsies and fine-needle aspirates — techniques constrained by surgical complications, availability of tissue, and sample heterogeneity. To address the shortcomings of current tissue-based molecular profiling and to streamline the diagnostic procedures for NSCLC patients, Exosome Diagnostics has developed a plasma-based assay, EXO501a, to rapidly detect fusion transcripts via a simple blood draw. This liquid biopsy diagnostic has the potential to provide valuable benefits for non-surgical treatment guidance and longitudinal monitoring of EML4-ALK positive patients.

2. EXO501a Work Flow
Using 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 29 patients, no false positive samples were detected, true positive concordance was 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).

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 Qigian (Figure 5). Monitoring the LOD, we observed superior performance of EXO501a over the competitors for EML4-ALK v1-specific analysis.

6. Conclusions
• Liquid biopsies, in contrast to tissue, represent a non-invasive and low-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
• The qPCR-based liquid biopsy assay’s performance on cellular and exosomal RNA of alternative last kits.
• Further work is ongoing in a larger series of patients to demonstrate clinical utility of this approach compared to FISH, the gold standard.