Exosomal RNA-based liquid biopsy detection of EML4-ALK in plasma from NSCLC patients

Presentation 2591

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1. Introduction

Molecular profiling to direct targeted therapy has revolutionized cancer treatment, particularly in the field of lung cancer. The EML4-ALK translocation is a predictive 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**).

Predominant Fusion Variants: EML4-ALK v1, v2, v3

EML4-ALK v1	EML4 exon 13	ALK exon 20
EML4-ALK v2	EML4 exon 20	ALK exon 20
EML4-ALK v3	EML4 exon 6	ALK exon 20

Figure 1: Fusion transcripts of three EML4-ALK variants in NSCLC

As the presence of these translocations determines both resistance to EGFR inhibitors and eligibility for treatment with FDA-approved ALK-kinase inhibitors, molecular testing for EML4-ALK is critical to the choice of therapy. Ongoing clinical trials and development of new ALK-kinase inhibitors for personalized treatment demand development of companion diagnostics.

Today's tissue-based diagnostics, such as fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC), are limited by the need for tissue, which frequently does not accurately reflect the current disease status. These current assays (FISH/ISH) are troubled by technical challenges of limited sensitivity and subjective interpretation. Therefore, assessing a biomarker from plasma would be a valuable alternative to tissue based testing and provide a straightforward option for identifying and monitoring EML4-ALK positive NSCLC patients.

Previously we demonstrated the technical feasibility to detect EML4-ALK fusion transcripts in 6 plasma samples from patients known to be positive by tissue FISH testing. Here, we present clinical performance characteristics in a larger cohort of samples demonstrating the application of our new diagnostic test **ExoDx™*** *Lung(ALK)*, analyzing the EML4-ALK transcript in plasma of NSCLC patients.

We determined the variant-specific expression profile of EML4-ALK fusion transcripts in a large cohort of NSCLC patients with high sensitivity and specificity.

The **ExoDx** *Lung(ALK)* technology represents a valuable diagnostic test for non-surgical treatment guidance and longitudinal monitoring of patients positive for EML4-ALK fusions.

2. EML4-ALK Detection

Current determination of EML4-ALK fusions (for frequency of variants, see **Figure 2**) 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 technology and to streamline the diagnostic procedure for NSCLC patients, Exosome Diagnostics developed the **ExoDx** *Lung(ALK)* assay to rapidly detect fusion transcripts in plasma (**Figure 3**).

Distribution of EML4-ALK Variants

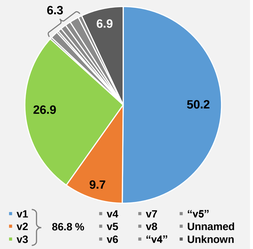


Figure 2: Frequency of EML4-ALK fusion variants in NSCLC patients. Adapted from Ou et al. The Oncologist 2012, 17.

ExoDx ***Lung(ALK)*** **Assay Workflow**

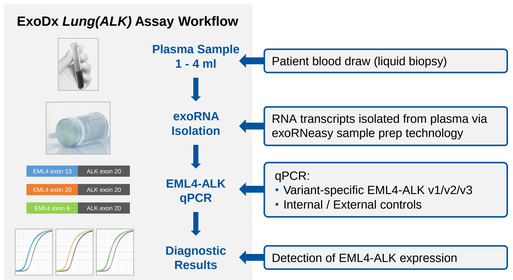


Figure 3: Assay workflow for detection of EML4-ALK from plasma using ***ExoDx*** *Lung(ALK)*.

3. Assay Performance on Clinical Data

Clinical Samples:
From a large cohort of plasma samples from patients with known ALK tissue status by FISH, we selected two groups of samples defined to be true positive and true negative samples based on clinical review:

- **ALK Positive** samples (N=8) were defined as samples with FISH ALK+ that were either not currently under treatment with ALK-inhibitor or had progressive disease at the time of blood draw. Circulating exoRNA signal was not expected to be detectable during response to treatment.
- **ALK Negative** samples (N=15) were defined as samples with FISH ALK- that were also EGFR+ or KRAS+. Due to the limited sensitivity of the FISH ALK assay, positivity for another driver mutation (mutually exclusive to ALK translocation) was considered a better standard for true ALK negative status.

Sample characteristics:
- All patients were NSCLC stage IV
- Median age = 51 years, 63% female
- Plasma volume analyzed: 0.9-1.5 mL

ExoDx ***Lung(ALK)*** **Clinical Sample Results**

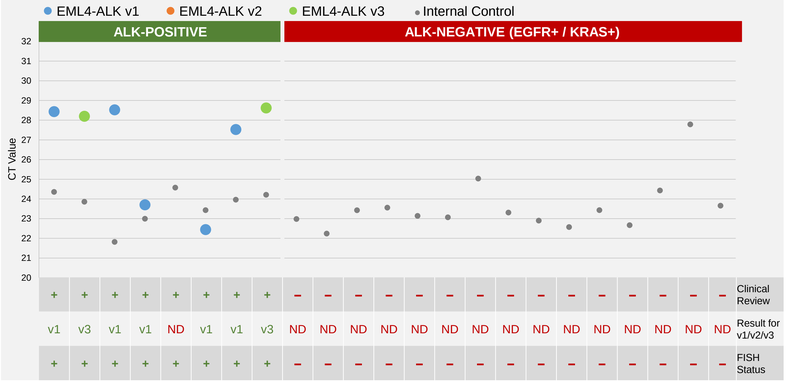


Figure 4: ExoDx *Lung(ALK)* analysis of tissue-correlated NSCLC plasma samples.

ALK status derived from plasma test analysis:
- 7 of 8 samples clinically reviewed as positive were defined for specific EML4-ALK fusion variant by **ExoDx** *Lung(ALK)*
- All the 15 samples clinically defined as true negative confirmed negative for EML4-ALK by **ExoDx** *Lung(ALK)*

Samples defined by Clinical Review			
Pos	**Neg**		
7	0	**Pos**	**ExoDx** *Lung(ALK)*
1	15	**Neg**	
Sensitivity [%]	**Specificity [%]**		
88%	**100%**		

Figure 5: Clinical performance of **ExoDx** *Lung(ALK)* on 24 NSCLC patient plasma samples

Assay performance derived from clinically reviewed plasma samples:
- Sensitivity of **ExoDx** *Lung(ALK)* was determined to be 88% based on a set of 23 patients
- Specificity of **ExoDx** *Lung(ALK)* was calculated to be 100% (no false positive detection)

4. Methods Comparison

Current Standard: FISH on Tissue

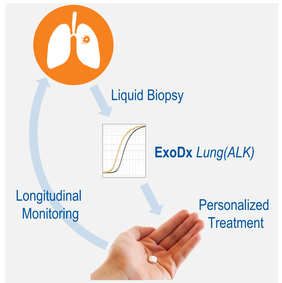
- FISH analysis has shown substantially reduced sensitivity of 43% compared to deep sequencing by NGS (Pekar, 2015)
- No variant-specific detection of ALK rearrangements
- Tissue biopsy sampling has increased risk of adverse events (19% of all patients); repeat biopsy is not intended (Lokhandwala, 2014)
- Restricted access to tissue sample in NSCLC (biopsy/tissue unavailable in 30% of patients)
- Tissue analysis impaired by >40% rejection rate of available sample biopsies due to low number of tumor cells, or due to assay artefacts (Conde, 2014 / Camidge, 2013)
- 20% inter-lab discordance; significant inter-operator discordance (Wallander, 2012)

ExoDx *Lung(ALK)*: Liquid Biopsy on Plasma

- Plasma-based assay overcomes the challenges of tissue sample scarcity and heterogeneity
- Reproducible PCR-based process
- Analysis of stable, high-quality exoRNA to detect EML4-ALK mutation with high sensitivity
- Detects distinct fusion transcripts (v1, v2, v3a,b,c) with high specificity; increasingly important for treatment selection
- Liquid biopsy enables non-invasive, longitudinal monitoring to determine molecular response to therapy and potential relapse

5. Conclusions

- Liquid biopsies, in contrast to tissue testing (FISH, IHC), represent a non-invasive and low-risk method for detecting the predictive biomarker EML4-ALK in plasma of NSCLC patients.
- The **ExoDx** *Lung(ALK)* test can be used both at baseline to help guiding treatment choice, and longitudinally to display patient progress during therapy.
- Here we demonstrate the capability of our diagnostic test to determine variant-specific expression of rare EML4-ALK fusion transcripts in low volumes of patient plasma.
- The initial clinical results suggest a high sensitivity and specificity of **ExoDx** *Lung(ALK)* in patients suspected to be positive for ALK translocations.
- The **ExoDx** *Lung(ALK)* test has been validated in the Exosome Diagnostics CLIA laboratory.



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