Exosome-based Detection of *EGFR* T790M in Plasma from Non-Small Cell Lung Cancer Patients

Poster/Abstract #: LB-B03

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Introduction:

T790M is a point mutation that arises in about 60% of non-small cell lung cancer (NSCLC) patients that are treated with first-generation EGFR tyrosine kinase inhibitors (TKIs)[1]. Early detection of this mutation, currently done by direct biopsy of the tumor tissue, is important to guide patient treatment. However, obtaining tissue biopsies is challenging and not feasible in up to 49% of the patients[2].

The rapid development of DNA-based liquid biopsies led to the approval by the Food and Drug Administration (FDA) of the first liquid biopsy companion diagnostic with the cobas® EGFR Mutation Test version 2, where T790M mutation status in plasma was specifically indicated as an aid for use of osimertinib (TAGRISSO).

In biofluids, the source from which cell-free DNA (cfDNA) is unclear, but it is likely to be released through cell death mechanisms (necrosis and apoptosis). In contrast, exosomes and other extracellular vesicles contain both RNA and DNA and are actively from living cells[3]. As coding mutations such as T790M can be found in both cfDNA and exosomal RNA/DNA (exoNA), combining these two sources of nucleic acids have the potential to increase sensitivity (Figure 1). To this end, an exosome-based test was developed and clinically validated.

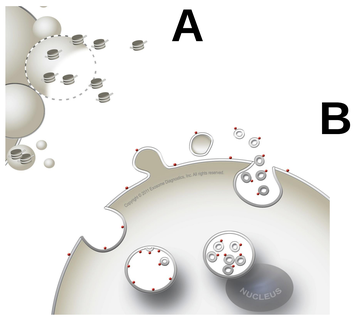

Figure 1: Schematic representation of the release of nucleic acids into the extracellular matrix. (A) Associated to nucleosomes or (B) inside of extracellular vesicles.

Methods:

The test consists of four steps: co-isolation of exoNA and cfDNA in a single step using a cGMP manufactured isolation kit (ExoLution plus™, Exosome Diagnostics, Waltham MA); reverse transcription (RT); pre-amplification with a wild type allele-specific blocker; and a triplex TaqMan-based quantitative PCR (qPCR) step that employs an allele-specific amplification strategy (Figure 2) (Exosome Diagnostics, Waltham, MA).

Test was analytically and clinically validated on 210 clinical samples in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory, following published guidelines.

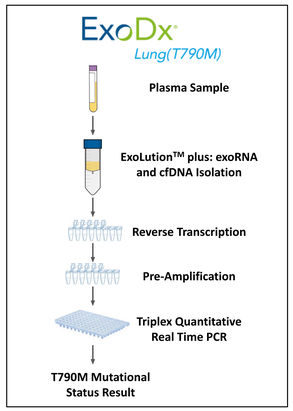

Figure 2: Left panel: Plasma collection kit and exoNA and cfDNA extraction kit from Exosome Diagnostics. Right panel: Schematic representation of assay workflow.

Results:

Of the 210 clinical samples, 105 subjects are randomly selected for training in a cancer stratified manner to keep the ratio of cancer stages constant.
The remaining 105 samples are used as validation set. Bootstrapping was used on the training data to estimate the optimal cut-off for disease discrimination between EGFR T790M-positive and –negative samples.

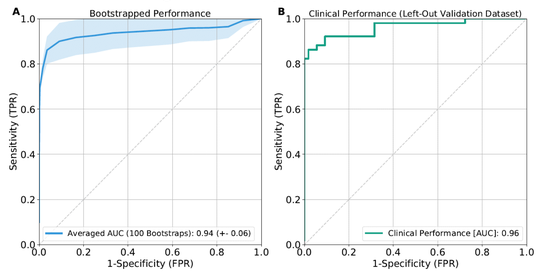

Figure 3: The x-axes show 1-Specificity or the False Positive Rate (FPR), and y-axes show the sensitivity or True Positive Rate (TPR). (A) Performance of training cohort. (B) Performance of the validation cohort.

The clinical performance on the validation set has an AUC of 96%, with a sensitivity of 92% and specificity of 89% (Table 1).
Our test achieves a sensitivity of 88% (14/16) for patients with confined thoracic disease (stages M0/M1a) and a sensitivity of 94% (20/32) for patients with M1b. Also, all three patients with unknown disease stage (MX) were detected.

A

		Plasma results using cfDNA and exosomal NA		Total number
		+	-	
Tissue result	+	47	4	51
	-	6	48	54

B

M stage	Sensitivity
M0/M1a	14/16 (88%)
M1b	30/32 (94%)
MX	3/3 (100%)

Table 1. Validation cohort performance. (A) Confusion matrix that correlates T790M results in tissue with plasma (exoNA and cfDNA). The sensitivity and specificity is 92% and 89%, respectively. **(B)** Sensitivity of the plasma test with cfDNA and exosomal RNA based on M status.

Conclusions:

- **This is the first CLIA validated test that uses exosomal derived nucleic acids (exoNA) in combination to cfDNA.**
- **This study included one of the largest clinical sample sets (n=210). In this sample set, the test shows high concordance to tissue biopsy, thus avoiding unnecessary reflex testing to tissue biopsy.**
- **This test demonstrates a better performance than other commercially available reference assays that analyze the cfDNA fraction alone (cobas® EGFR Mutation Test v2, BEAMing).**
- **Expanded panel that includes EGFR Exon19 deletions and L858R is under development and near completion.**

References:

1. Wang, S. et al. J Hem Oncol 9: 34, 2016
2. Lokhandwala T. Clin Lung Cancer 18:e27-e34, 2017
3. Skog, J. et al. Nat Cell Biol 10:1470-6, 2008

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