A novel and accurate real-time PCR approach for simultaneous detection of multiple driver gene mutations in non-small cell lung cancer

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Background

Lung cancer is the leading cause of cancer death worldwide, and 80~85% of lung cancers are non-small cell lung cancers (NSCLC). Patients with NSCLC often harbor driver mutations in multiple oncogenes, including *EGFR*, *ALK*, *ROS1*, *BRAF*, *HER2*, *RET*, etc. The presence of gene alterations can impact the selection of and the response to targeted therapies. Testing of lung cancer for multi-gene mutations is important for identification of potentially efficacious targeted therapies. Therefore, identifying mutations in oncogenes and tailoring therapy accordingly are widely accepted in clinical cancer management. Faster detection of driver mutations enables quicker initiation of proper therapy.

A fast and sensitive real-time PCR based assay, named as AmoyDx Multi-Gene Mutations Detection Kit (ADx Multi-Gene assay) has been developed for detection of mutations in 9 driver genes in NSCLC at a time from a single tissue specimen. The ADx Multi-Gene assay is a one-shot test for simultaneous detection of most common hotspot alterations in 9 genes and developed by combining DNA-based mutation detection (for *EGFR*, *KRAS*, *BRAF*, *NRAS*, *HER2*, and *PIK3CA* genes) and mRNA-based fusion detection (for *ALK*, *ROS1* and *RET* genes). The multi-gene mutation reaction mixes have been already pre-loaded in a 12-tube PCR strip that was very convenient for users, and its one-step procedure allows quick turn-around time of less than 24 hours.

Materials and Methods

Specimen and DNA/RNA Extraction

The clinical study was conducted in three hospitals in China. A cohort of 1,015 formalin-fixed, paraffin-embedded (FFPE) tissue samples were collected from patients with NSCLC in 2015, and confirmed to be with a minimum 30% tumor cells.

Ten sections (5μM thickness) of tumor tissue samples were used for both DNA and RNA extraction by using AmoyDx FFPE DNA/RNA Kit (Amoy Diagnostics, Xiamen, China) according to the manufacturer's instructions. The quantity and quality of isolated DNA and RNA were determined with NanoDrop 1000/2000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

Multiple Gene Mutations Testing

The isolated DNA and RNA were tested for multiple genomic alterations by ADx Multi-Gene assay (Amoy Diagnostics, Xiamen, China). Experimental procedure and data analysis followed the manufacturer's instructions.

The qPCR assays for individual driver genes (for *EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *ALK/ROS1* alterations) which have been approved by China FDA, and next-generation sequencing (NGS) method (for *NRAS*, *RET*, *HER2* alterations) were used as reference to confirm the mutation status.

Statistical Analysis

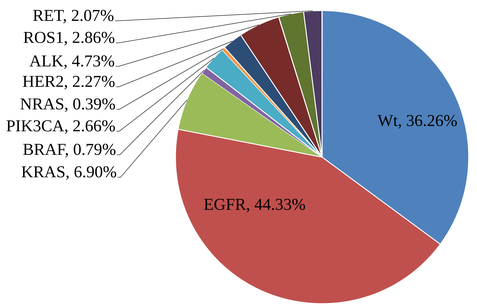
Fisher's exact test was implemented to assess the concordance between ADx Multi-Gene assay and reference assay results. The overall concordance rate was calculated as the sum of the positives and the negatives in both methods, divided by the total numbers.

Results

Multiple Gene Mutations and Frequency

In the cohort of NSCLC samples, 63.74% (647/1,015) were detected to show alterations by ADx Multi-Gene kit (Figure 1), 58 of which were found with co-existing mutations in two or three driver genes. The alteration positive rates were quite close to the reported frequencies in NSCLC. 632 positive samples were confirmed the mutation by Sanger sequencing or NGS.

Figure 1. Alteration frequency in total of 1015 samples (by ADx Multi-Gene assay)



* Wt: Wild-type

Comparison of Mutation Testing by ADx Multi-Gene Assay and Reference Assay

By reference assays, 63.35% (643/1,015) were detected as positive samples. The overall concordance rate of mutations determined with ADx Multi-Gene assay compared with reference was 96.45% (Table 1).

Table 1. Comparison of mutations between ADx Multi-Gene assay and reference

All （N=1,015）		Reference		Total
		Mutated	Wild-type	
ADx Multi-Gene Assay	Mutated	627	20	647
	Wild-type	16	352	368
Total		643	372	1,015
PPV		98% (95%CI: 95.99% ~98.57%)		
NPV		95% (95%CI: 91.82% ~ 96.69%)		
Overall concordance		96.45%		

The positive predictive value, negative predictive value and overall concordance rate between two methods by each gene are listed in Table 2.

Table 2. Concordance between ADx Multi-Gene assay and reference by each gene

Variant	PPV*	NPV*	Overall concordance
EGFR Mutation	97.97%	97.20%	97.54%
KRAS Mutation	95.77%	99.79%	99.51%
BRAF Mutation	100%	100%	100%
PIK3CA Mutation	96%	99.9%	99.8%
NRAS Mutation	100%	99.9%	99.9%
HER2 Mutation	100%	99.8%	99.8%
ALK Fusion	94%	100%	99.7%
ROS1 Fusion	100%	100%	100%
RET Fusion	100%	100%	100%

* PPV: positive predictive value, NPV: negative predictive value.

Conclusion

The ADx Multi-Gene assay provides accurate and efficient detection of mutations in multiple tumor driver genes simultaneously with high concordance rate of 96.45% compared with the reference assay.

References

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2. NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Version 5. 2018.

Presented at AMP 2018 Annual Meeting, November 1-3 2018, San Antonio, TX, USA

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