Clinical implication of monitoring circulating tumor DNA in untreated non-small-cell lung cancer patients

Jun Hyeok Lim¹, Hyun-Tae Shin², Sekyung Oh³, Seung Jae Lee⁴, Jeong-Seon Ryu¹*

¹Division of Pulmonology, Department of Internal Medicine, Inha University Hospital, Incheon, South Korea
²Department of Dermatology, Inha University Hospital, Incheon, South Korea
³Department of Medical Sciences, Catholic Kwandong University College of Medicine, Incheon, South Korea
⁴DNA Link, Inc., Seoul, South Korea
Background

• Easily obtainable through liquid biopsy, circulating tumor DNA (ctDNA) has been of great interest to those who seek clinical-course monitoring of NSCLCs.

• Its utility has been investigated with intention to predict effect of targeted or immunological agents or surveil postoperative recurrence of tumor.
Elucidating the clinical course of such treatment-naïve patients provide us with valuable information on how NSCLC naturally progresses in relation to patient survival.

Genetic markers as carried by ctDNA would help reveal molecular paths that NSCLC would naturally follow in the absence of therapy. The information is believed to provide scientific basis for the current monitoring strategy using ctDNA under effective treatment or selective pressure against genetic evolution.

In this vein, we conducted longitudinal assessments of ctDNA obtained from treatment-naïve NSCLC patients and investigated their associations with the natural clinical course.
Methods – Patients and radiological evaluation

- Patients who had been histologically diagnosed as NSCLC between January 2008 and March 2017 were identified in the Inha Lung Cancer Cohort
- 153 patients did not take any anti-cancer treatment
- The treatment refusal was based on
  1. either severe comorbid diseases or more than three markings in the Eastern Cooperative Oncology Group performance status (n = 87)
  2. patients’ wish not to take treatment (n = 39)
  3. poor economic status (n = 18)
  4. unknown reasons (n = 9)
- Radiological evaluations were performed at the time of diagnosis and the ensuing follow-ups with the average of three-month intervals (range: 2–5).
- Tumor burden (TB) was measured with the sum of greatest diameters of target lesions with the standard of RECIST v1.1

Flowchart of patient selection
Methods – Plasma samples

- Thirty-seven serial plasma samples of the patients included in the study were drawn at the same time as the radiological evaluation.

- Cell-free DNA was extracted from the plasma samples using the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction.

- Targeted next-generation sequencing (NGS) was performed on all ctDNA samples using a panel of 113 genes. Several filtering steps were applied to sieve 4 out putative germline and false variants.

- A total of 66 variants were identified. Among these variants and some putative germline variants, 8 variants with sufficient amounts of DNA were validated with digital droplet PCR (ddPCR).
Results

Somatic mutation profiles of circulating tumor DNA and clinical characteristics

- The median duration of follow-ups was 298 days.
- ctDNA was detected in seven patients whose mutations were of heterogeneous (median ctDNA level: 9.1 haploid genome equivalents/mL [range: 0.8 – 108.5]; median VAF: 4.2% [range 0.1 – 21.9]).
- The results of validation ddPCR were comparable to those of NGS in the patients with detected ctDNA.
- In 7 patients (P1, P3, P6, P9, P10, P11, and P12), at least one mutation was detected in ctDNA whereas no mutation was detected in 5 patients (P2, P4, P5, P7, and P8).
- Tumor burden shows a tendency to increase in all the patients except P8.

Patient characteristics and circulating tumor DNA (ctDNA) detection

\(^{a}\)T1 to T4 denote a time point for plasma sampling and imaging tests.

ctDNA, circulating tumor DNA; VDT, volume doubling time; Br, brain; Lu, lung; Pl, pleura; Bo, bone; Al, abdominal lymph node; Cw, chest wall; Li, liver; Pc, pericardium; Kd, kidney; Pt, peritoneum; Sp, spleen; Aw, abdominal wall.
Results

• Four patients (P6, P9, P10, and P11) displayed a tendency of increasing ctDNA levels over time as TB increased.
• By contrast, other three patients (P1 with solely increased size of brain metastasis, P3 with decreasing necrosis of primary tumor, and P12 with pneumonia) did not.

Changes in circulating tumor DNA (ctDNA) level of mutated gene in longitudinal plasma samples for patients with detected ctDNA. Each circle indicates a time point for plasma sampling and imaging test. In P3, the size of the primary tumor slightly increased whereas the area with tumor necrosis decreased at the second follow-up period (black arrow). In P12, pneumonia was accompanied at diagnosis and the third follow-up period (red arrow).
Results

• The median value of VDT in the patients whose ctDNA was detected at diagnosis was 73 days. The patients whose ctDNA was not detected displayed a longer median VDT, 179 days (p = 0.039).

• In all the patients with detected ctDNA, new metastasis arose during follow-ups in the organs that had not had metastasis at the time of diagnosis. The patients lacking detectable ctDNA, however, did not show metastasis in a new organ (See Figure and Table on Slide 6).
Results

- Patients with detected ctDNA had a significantly worse OS than those without detected ctDNA (the median OS of 153 days versus 501 days, log-rank $p = 0.019$).

- Patients with high-level ctDNA had worse OS compared with those with low-level ctDNA (the median OS of 121 days versus 235 days, log-rank $p = 0.048$).

Kaplan-Meier curve for overall survival by (A) detection of circulating tumor DNA (ctDNA) and (B) ctDNA level.
Conclusion

• This study reveals that natural course could be monitored using ctDNA levels in NSCLC patients but various clinical factors such as brain metastasis, tumor necrosis or infection can affect the ctDNA levels.

• Our results also imply that detection of ctDNA at diagnosis can predict rapid tumor growth, development of new metastasis and poor survival of the NSCLC patients.

• This study indicates that natural course could be monitored or predicted with individually harbored somatic mutations in ctDNA of NSCLC patients.