



Case report

Rapid decrease of circulating tumor DNA predicted the treatment effect of nivolumab in a lung cancer patient within only 5 days



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ABSTRACT

A 77-year-old Japanese man presented to our hospital with a 1-month history of low back pain and was diagnosed as having stage IV *EGFR* mutation-positive lung adenocarcinoma. After treatment with *EGFR* tyrosine kinase inhibitor and cytotoxic chemotherapy, nivolumab was started as fourth-line therapy. Remarkable regression of the primary tumor was observed, suggesting high anti-tumor activity of nivolumab. We retrospectively investigated the change in circulating tumor DNA (ctDNA) variant allele fractions in serial plasma samples before and after the nivolumab therapy. Targeted sequencing analysis showed tumor-derived *TP53*^{R249S} and *EGFR*^{L858R} mutations detectable in plasma, and the timing of decrease was only 5 days, much earlier than the appearance of radiological changes. Overall, these results suggest that ctDNA might reflect tumor burden and might be a surrogate marker of the therapeutic efficacy of immune checkpoint therapy.

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1. Introduction

With the advent of immune-checkpoint therapy, prognosis has been improved in patients with advanced solid tumors [1–3]. Nivolumab is a human IgG4 monoclonal antibody and programmed death-1 (PD-1) immune-checkpoint inhibitor. However, pseudo-progression mimicking progression of the tumor can make the validity of continuing treatment uncertain for clinicians [4–8].

Circulating tumor DNA (ctDNA) is shed into the bloodstream from tumor cells through the processes of necrosis and apoptosis. Therefore, it is considered to be a snapshot of mutational profiles in tumor-derived DNA [9,10]. However, it remains unclear whether ctDNA level likewise predicts the effects of nivolumab treatment in lung cancer patients.

In this case, the ctDNA level remarkably decreased after an administration of nivolumab much faster than radiological remission of the tumor, suggesting that monitoring ctDNA changes may reflect the treatment effect in real time.

2. Case report

A 77-year-old Japanese man presented to our hospital with a 1-month history of low back pain. Past medical history was noncontributory and he had a smoking history of 28-pack years. Physical examination revealed swelling of the left inguinal lymph node. Magnetic resonance imaging (MRI) showed a mass with an irregular border and needle biopsy of the lymph node revealed metastatic adenocarcinoma. An enhanced computed tomography (CT) scan showed a 3-cm mass in the left lower lobe with left pleural effusion, intrapulmonary metastatic nodules, and low density areas in the liver. No metastasis was observed on brain MRI. Positron emission tomography-CT showed bone metastasis on L2, L4, L5 of the spine. The patient was clinically diagnosed with stage IV lung adenocarcinoma, and *EGFR*^{L858R} mutation was detected in the biopsy specimen. Gefitinib as a first-line chemotherapy was started but failed after 6 months. Next, carboplatin and pemetrexed as second-line therapy, and afatinib as a third-line therapy had been used until tumor progression was observed. Subsequently, we started nivolumab as a fourth-line therapy. Eight days after initiation of nivolumab, we were obliged to stop the treatment because a grade 3 rash had appeared. Sialyl SSEA-1 antigen (SLX), a tumor

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Abbreviations

PD-1	programmed death 1
PD-L1	PD-1 ligand 1
PD-L2	PD-1 ligand 2
ctDNA	circulating tumor DNA
MRI	magnetic resonance imaging
CT	computed tomography
SLX	Sialyl SSEA-1 antigen
FFPE	formalin-fixed paraffin-embedded
irRC	Immune-Related Response Criteria

marker in this patient, continued increasing up until day 35. However, CT findings on day 25 showed remarkable remission of the tumor (Fig. 1). After that, his performance status had worsened to ECOG 3, no more chemotherapy could be continued. On day 42,

he died of pneumonia.

As part of the genomic research project in our hospital, 4 serial plasma samples were collected from the patient before and after nivolumab treatment. Laser-Capture Microdissection was used for extracting tumor DNA from formalin-fixed paraffin-embedded (FFPE) tissues of the metastatic lymph node at diagnosis. To identify somatic mutations in these DNA samples, we performed targeted sequencing using lung cancer panel targeting whole exons of 53 genes and buffy coat DNA was used as control [11–13]. Emulsion PCR and sample loading into the PI chip v3 were performed on Ion Chef system, and next generation sequencing was performed on Ion Proton System (Thermo Fisher Scientific, Waltham, MA, USA). We considered as confident somatic mutations harboring over 20% variant allelic fraction in the FFPE tissue. Of these, 5 mutations, namely *CTNNB1*^{Q773X}, *TP53*^{R249S}, *EGFR*^{L858R}, *BRAF*^{E586K}, and *EGFR*^{Q1113E}, were detected as the confident somatic mutations. Next we monitored the ctDNA levels containing identical mutations in the tumor. Prior to the treatment with nivolumab, *TP53*^{R249S} and *EGFR*^{L858R} mutations were identified in plasma at allelic fractions 7%

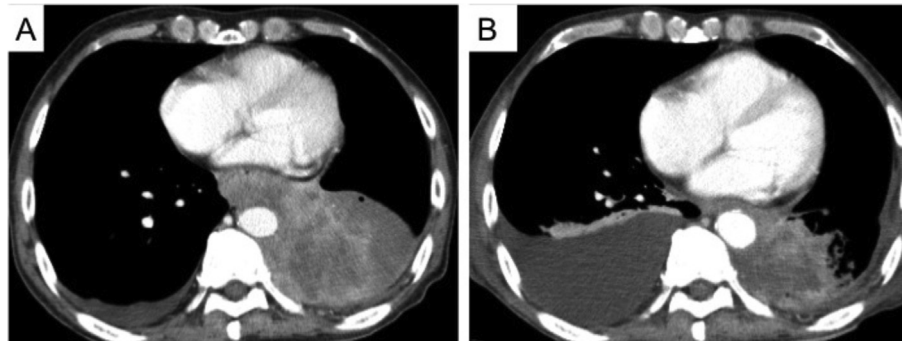


Fig. 1. Radiological change after the treatment of nivolumab. (A) CT scan shows a large tumor mass in the left lower lung, and mediastinal lymphadenopathy. (B) Thirty-five days after the initiation of nivolumab, CT scan shows apparent regression of the tumor. Increase in the contralateral pleural effusion was also seen, which seems to be a transient immunologic reaction known as pseudo progression.

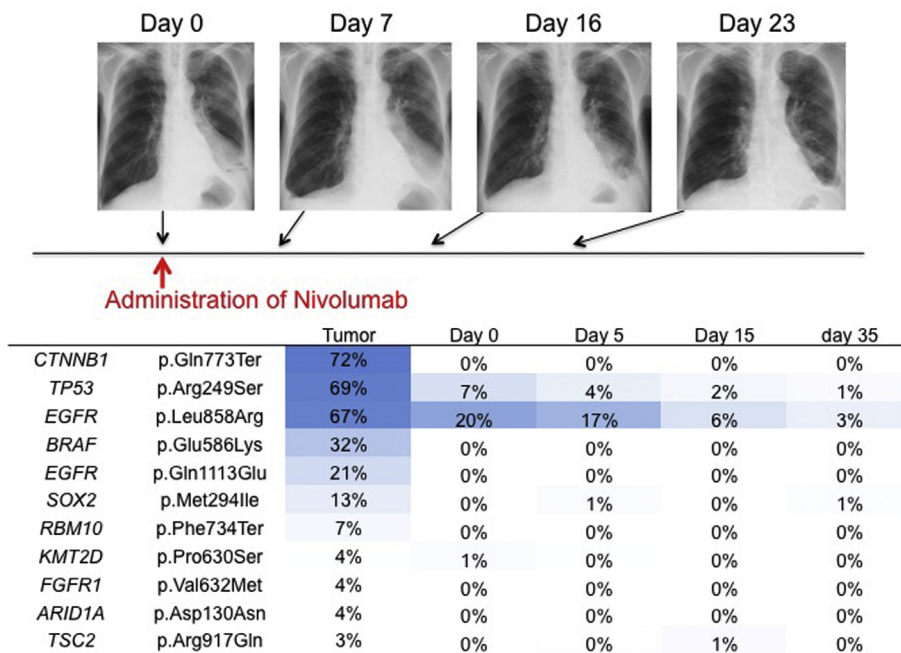


Fig. 2. Comparison of the changes between X-ray evaluation, tumor marker, and the levels of ctDNA. Chest X-ray shows regression of the shadow 16 days after nivolumab treatment. Heat map shows 11 somatic mutations in the tissue obtained by needle lymph node biopsy before starting first-line treatment. Among them, *TP53* and *EGFR*^{L858R} were evaluated in the plasma samples as ctDNA. These ctDNA had rapidly decreased in only 5 days.

and 20%, respectively. Notably, only 5 days after nivolumab administration, the levels of ctDNA harboring *TP53*^{R249S} and *EGFR*^{L858R} mutations had quickly decreased, suggesting that a rapid treatment response could be assessed by ctDNA levels. In addition, ctDNA levels had decreased over time and on day 15, *TP53*^{R249S} mutation was finally almost undetectable. These changes had preceded and correlated with the radiological changes (Fig. 2).

3. Discussion

Here, we encountered a case of lung adenocarcinoma with rapid decrease of ctDNA predicting the anti-tumor activity of nivolumab. The changes in ctDNA levels had occurred much faster than the changes in radiological findings. Previous reports showed that ctDNA can be a sensitive index of tumor burden due to its short half-life (about 2 hours) [14,15]. Lipson et al. in a series of 3 cases of melanoma treated with nivolumab reported that the changes in ctDNA level correlated with the therapeutic effect [16]. Imamura et al. also presented cases of lung cancer treated with an EGFR tyrosine kinase inhibitor, and found the same tendency [17]. Similarly, changes in ctDNA levels may also predict the effect of nivolumab in lung cancer. This is particularly meaningful in immune checkpoint therapy because it helps to ascertain whether the treatment is effective or not quite early in the clinical course. In immune checkpoint therapy, we sometimes encounter the situations in which tumor size temporarily increases despite the high anti-tumor activity. This pseudoprogression makes the outcome of treatment unpredictable for clinicians [4–8]. Hence, monitoring ctDNA levels might play an important role and help to avoid missing the opportunity to treat these patients with pseudoprogression, or the chance to change therapy in non-responding patients in the early period.

It is also meaningful that this ctDNA response was shown within only 1 week. “Only 1 week” is faster than the timing of second administration of nivolumab. Accordingly, if rapid change of ctDNA may be useful for predicting efficacy, we may decide whether to continue the treatment only by the first administration of nivolumab. This may also serve to reduce medical care expenditure.

In conclusion, this is the first case report on monitoring the initial changes in ctDNA levels after administration of immune checkpoint therapy in lung cancer. Although further analysis is needed in more patients, ctDNA is expected to be a surrogate marker for evaluating the therapeutic effect of immune checkpoint inhibitors for clinical application.

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References

- [1] J.R. Brahmer, C.G. Drake, I. Wollner, et al., Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates, *J. Clin. Oncol.* 28 (2010) 3167–3175.
- [2] C. Wang, K.B. Thudium, M. Han, et al., In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates, *Cancer Immunol. Res.* 2 (2014) 846–856.
- [3] S.L. Topalian, F.S. Hodi, J.R. Brahmer, et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, *N. Engl. J. Med.* 366 (2012) 2443–2454.
- [4] E.J. Lipson, W.H. Sharfman, C.G. Drake, et al., Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody, *Clin. Cancer Res.* 19 (2013) 462–468.
- [5] J.R. Brahmer, S.S. Tykodi, L.Q. Chow, et al., Safety and activity of anti-PD-L1 antibody in patients with advanced cancer, *N. Engl. J. Med.* 366 (2012) 2455–2465.
- [6] F.S. Hodi, S.J. O'Day, D.F. McDermott, et al., Improved survival with ipilimumab in patients with metastatic melanoma, *N. Engl. J. Med.* 363 (2010) 711–723.
- [7] S.L. Topalian, M. Sznol, D.F. McDermott, et al., Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab, *J. Clin. Oncol.* 32 (2014) 1020–1030.
- [8] A. Ribas, B. Chmielowski, J.A. Glaspy, Do we need a different set of response assessment criteria for tumor immunotherapy? *Clin. Cancer Res.* 15 (2009) 7116–7118.
- [9] E. Heitzer, P. Ulz, J.B. Geigl, Circulating tumor DNA as a liquid biopsy for cancer, *Clin. Chem.* 61 (2015) 112–123.
- [10] L.A. Diaz Jr., A. Bardelli, Liquid biopsies: genotyping circulating tumor DNA, *J. Clin. Oncol.* 32 (2014) 579–586.
- [11] Cancer Genome Atlas Research Network, Comprehensive genomic characterization of squamous cell lung cancers, *Nature* 489 (2012) 519–525.
- [12] Cancer Genome Atlas Research Network, Comprehensive molecular profiling of lung adenocarcinoma, *Nature* 511 (2014) 543–550.
- [13] T. Goto, Y. Hirotsu, T. Oyama, et al., Analysis of tumor-derived DNA in plasma and bone marrow fluid in lung cancer patients, *Med. Oncol.* 33 (2016) 29, <http://dx.doi.org/10.1007/s12032-016-0744-x>.
- [14] K. Kato, J. Uchida, Y. Kukita, et al., Numerical indices based on circulating tumor DNA for the evaluation of therapeutic response and disease progression in lung cancer patients, *Sci. Rep.* 6 (2016) 29093.
- [15] F. Diehl, K. Schmidt, M.A. Choti, et al., Circulating mutant DNA to assess tumor dynamics, *Nat. Med.* 14 (2008) 985–990.
- [16] E.J. Lipson, V.E. Velculescu, T.S. Pritchard, et al., Circulating tumor DNA analysis as a real-time method for monitoring tumor burden in melanoma patients undergoing treatment with immune checkpoint blockade, *J. Immunother. Cancer* 2 (2014) 42.
- [17] F. Imamura, J. Uchida, Y. Kukita, et al., Monitoring of treatment responses and clonal evolution of tumor cells by circulating tumor DNA of heterogeneous mutant EGFR genes in lung cancer, *Lung Cancer* 94 (2016) 68–73.