SHORT COMMUNICATION

Circulating Tumour DNA Reflects Tumour Burden Independently of Adverse Events Caused by Systemic Therapies for Melanoma

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Significant advances in the field of melanoma therapy have been made in recent years. For example, immune checkpoint inhibitors (anti-PD-1 and anti-CTLA-4 antibodies) and small molecule-targeted therapies (BRAF and MEK inhibitors) offer remarkable long-term benefits. However, these agents often cause severe adverse events (AEs). Thus, a reliable biomarker that reflects tumour status is required in order to provide appropriate treatment. Although computed tomography (CT) is useful for evaluating tumour status, frequent imaging is not feasible. Serum lactate dehydrogenase (LDH) is often used as a surrogate marker of tumour status; indeed, changes in LDH levels are associated with tumour burden (1). However, LDH levels can increase in response to liver dysfunction and interstitial pneumonia (2, 3). Furthermore, LDH levels are frequently affected by therapy-induced AEs. Circulating tumour DNA (ctDNA), which is released from tumour cells into the peripheral blood, is a novel biomarker of tumour status (4) as it harbours the same genetic alterations present in the tumour. Also, the amount of ctDNA correlates strongly with tumour burden in cancer patients, including those with melanoma (5). Here, we show that melanoma therapies induced severe AEs, which increase LDH levels, but do not affect ctDNA levels. This study examined the utility of *BRAF*^{V600E} ctDNA as a tool for monitoring melanoma.

CASE REPORT

Two patients with *BRAF*^{V600E}-mutated melanoma who had metastatic disease were examined. They provided written informed consent for the use of their peripheral blood and resected tumour tissues. Cell-free DNA was extracted from peripheral blood from patients with melanoma, and ctDNA was measured by droplet digital polymerase chain reaction (QX200 ddPCR system; BIO-RAD, Hercules, CA, USA), as described previously (5). Each AE was evaluated using Common Terminology Criteria for Adverse Events (version 4.0). The study was approved by the ethics committee of Shinshu University School of Medicine and conducted according to Institutional Review Board guidelines.

Patient 1, a 28-year-old woman diagnosed with *BRAF*^{V600E}-mutated melanoma on the lateral chest, presented with multiple metastases and was treated with ipilimumab (3 mg/kg every 3 weeks) (i-Day 0; **Fig. 1**A). Although *BRAF*^{V600E} ctDNA was detected in plasma DNA on i-Day -5 (540 copies/ml), ipilimumab administration decreased the levels of *BRAF*^{V600E} ctDNA to undetectable levels by i-Day 98. On i-Day 77, the tumour response was classified as stable disease (SD) according to the Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.1) assessment. However, grade 3 liver dysfunction occurred after the third administration of ipilimumab (i-Day 49); therefore, the fourth administration was stopped. *BRAF*^{V600E} ctDNA levels increased on i-Day 161 (72 copies/ml) and again on i-Day 189 (285 copies/ml). A CT scan on i-Day 245 revealed progressive disease (PD). LDH levels (normal range, <230 IU/l) increased with the onset of ipilimumab-related liver dysfunction (i-Day 98; LDH, 350 IU/l); however, levels normalized as liver function improved (i-Day 217; LDH, 259 IU/ml), a finding that was inconsistent with clinical and imaging evaluation of the melanoma. Treatment was switched to dabrafenib (300 mg/day) plus trametinib (2 mg/day) on dt-Day 0 (i-Day 252). A computed tomography (CT) scan revealed that the tumour had not grown; the tumour response was classified as SD on dt-Day 62. The level of *BRAF*^{V600E} ctDNA fell substantially after 2 weeks of combination therapy (dt-Day 14, 0 copies/ml). However, AEs (grade 2 high fever, grade 3 liver dysfunction, and grade 2 erythema nodosum) meant that dabrafenib and trametinib had to be tapered before withdrawal on dt-Day 49. A CT scan on dt-Day 91 revealed enlargement of the tumour, along with a rebound in *BRAF*^{V600E} ctDNA levels (dt-Day 105, 540 copies/ml). However, LDH levels appeared to be associated with liver dysfunction rather than with melanoma. Thereafter (p-Day 0 [dt-Day 140]), treatment was switched to pembrolizumab (2 mg/kg every 3 weeks). The patient then achieved SD (p-Days 105 and 189). RECIST status correlated with both ctDNA and LDH levels. Thus, *BRAF*^{V600E}

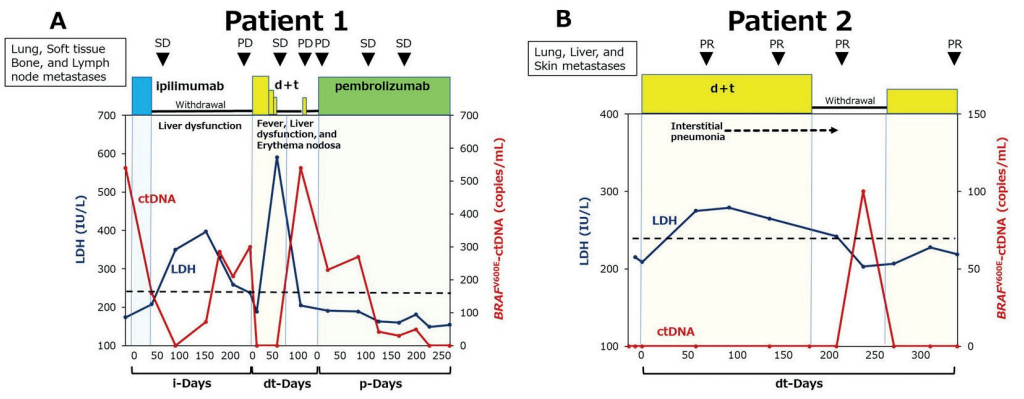

Fig. 1. Monitoring of circulating tumour DNA (ctDNA) and lactate dehydrogenase (LDH) levels, with clinical follow-up, in (A) patient 1 and (B) patient 2. Graphs show changes in *BRAF*^{V600E} mutant copy number and LDH levels. The upper limit of normal for LDH is 230 IU/l (dotted line). d: dabrafenib; t: trametinib; PR: partial response; SD: stable disease; PD: progressive disease.

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ctDNA levels reflected tumour burden more precisely than LDH levels, as the latter were affected by therapy-induced AEs.

Patient 2, a 66-year-old man diagnosed with *BRAF*^{V600E}-mutated melanoma on his chest, presented with multiple metastases and was treated with dabrafenib (300 mg/day) plus trametinib (2 mg/day) (dt-Day 0; Fig. 1B). CT scans revealed a marked reduction in tumour size, and tumour response was classified as a partial response (PR) after treatment initiation. However, treatment was withdrawn on dt-Day 189 due to drug-induced interstitial pneumonia; it occurred on dt-Day 70, and subsequently progressed on dt-Day 147 (grade 2). Although *BRAF*^{V600E} ctDNA was undetectable during treatment (dt-Days 0 to 217), levels increased suddenly on dt-Day 245 (100 copies/ml). Tumour relapse was diagnosed, therefore the patient resumed dabrafenib (300 mg/day) and trametinib (1.5 mg/day) at a reduced dose from dt-Day 273 after improving interstitial pneumonia (grade 1). CtDNA levels were again undetectable on dt-Day 277. However, LDH levels were above the upper limit of normal before withdrawal of dabrafenib and trametinib; this seemed to be related to interstitial pneumonia because LDH levels returned to normal after drug withdrawal and subsequent dose reduction.

DISCUSSION

This study examined the efficacy of ctDNA monitoring during treatment with immune checkpoint inhibitors and small molecule-targeted medicines. LDH is a useful marker for disease monitoring because elevations are frequently associated with a high disease burden and a poor prognosis (1). However, it can be difficult to determine whether LDH elevation is caused by therapy-related AEs. Immune checkpoint inhibitors and small molecule-targeted medicines can cause damage to various organs, resulting in increased LDH levels independent of tumour progression. Conversely, LDH levels can remain within the normal range despite tumour progression. CtDNA is also a useful marker of tumour load during treatment of patients with melanoma (5, 6). Here, we show that measuring ctDNA is a reliable tool for disease monitoring because ctDNA levels reflect tumour burden independently of therapy-related AEs. In addition, ctDNA levels in patient 2 fell 4 days after treatment resumption. Similar rapid responses were observed previously (7). Also, the short half-life (approximately 2 h) of ctDNA means that it is likely to reflect tumour status in real-time (8).

Despite the value of ctDNA as a biomarker, it has some limitations. CtDNA analysis often targets hotspot point mutations, such as *BRAF* and *NRAS*. Mutant *BRAF* (50–60%) and *NRAS* (15–25%) are common in Caucasians, and these mutations are usually seen in tumours on the trunk and extremities (9). However, *BRAF* and *NRAS* mutations are not as common in Asian populations (10, 11). Asian patients often have tumours at acral and mucosal sites, and these tumours show copy number alterations rather than point mutations. Further studies are warranted to evaluate the utility of ctDNA in cases of melanoma without hotspot point mutations.

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The authors have no conflicts of interest to declare.

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