Supplementary Online Content

Lee JH, Long GV, Menzies AM, et al. Association between circulating tumor DNA and pseudoprogression in patients with metastatic melanoma treated with anti–programmed death 1 antibodies. *JAMA Oncol*. Published online February 8, 2018. doi:10.1001/jamaoncol.2017.5332

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Patient Inclusion and Exclusion Criteria

Patients who did not have restaging imaging or on therapy blood draws due to clinical disease progression were excluded. Patients with predominant brain metastases, which include patients with no or minimal extra-cranial disease, or those patients who had a discrepancy between intra- and extra-cranial response were excluded in view of recently published evidence [4]. In the previous study analysing longitudinal ctDNA in 105 patients, 8 out of 10 patients with RECIST PD at first re-staging CT imaging at week 12 had intracranial progression only and had extracranial disease control, defined as stable disease at worst.

eMethods 2. Disease Characteristics Assessment

Patient demographics and clinicopathologic features including Eastern Cooperative Oncology Group (ECOG) performance status, LDH levels at baseline and during treatment, mutation status and American Joint Committee on Cancer (AJCC) 7^{th} edition M stage were collected.

eMethods 3. Plasma Collection and Circulating Tumor DNA (ctDNA) Extraction and Quantification

Peripheral blood samples from patients were collected prospectively at baseline and at regular intervals during therapy, as previously described [4]. In brief, plasma samples were processed within 4 hours of collection. ctDNA was extracted from at least 1ml of plasma and digital droplet PCR subsequently performed.

eMethods 4. Circulating Tumor DNA (ctDNA) and Lactate Dehydrogenase (LDH) Profile Groups

A favorable ctDNA profile was defined as undetectable ctDNA at baseline, which remained undetectable during treatment or detectable ctDNA at baseline which became undetectable or decreased at least 10-fold within 12 weeks of treatment. An unfavorable ctDNA profile was defined as persistently detectable ctDNA which showed minimal change or increase in copy numbers during the 12 weeks of therapy.

Patients were also classified as ‘favorable’ and ‘unfavorable’ according to their LDH profile using the model proposed in a previous study. A favorable LDH profile was defined as normal LDH at baseline, a relative decrease of > 27.3% from baseline to on treatment, or an LDH > upper limit of normal (ULN) which became normal during treatment or had a relative decrease of > 27.3% from baseline to on-therapy. An unfavorable LDH profile was defined as LDH persistently > ULN or an increase of 39% (and > ULN) from baseline.

eMethods 5. Statistical Analysis

Patient characteristics and clinical parameters including LDH, disease volume and AJCC M stage were summarised by their response pseudoprogression and true progression. Frequencies and percentages by group are reported in Table 1. Landmark analysis was performed to explore the association of ctDNA and overall survival. Patient’s ctDNA profile was sequentially assessed at two landmark time points, 6 and 12 weeks respectively. Cox proportional hazard model was used to estimate hazard-ratios (HR) conditional to the ctDNA at each landmark time point and including only alive patients at that time. This statistical analysis was performed using R version 4.3.1.

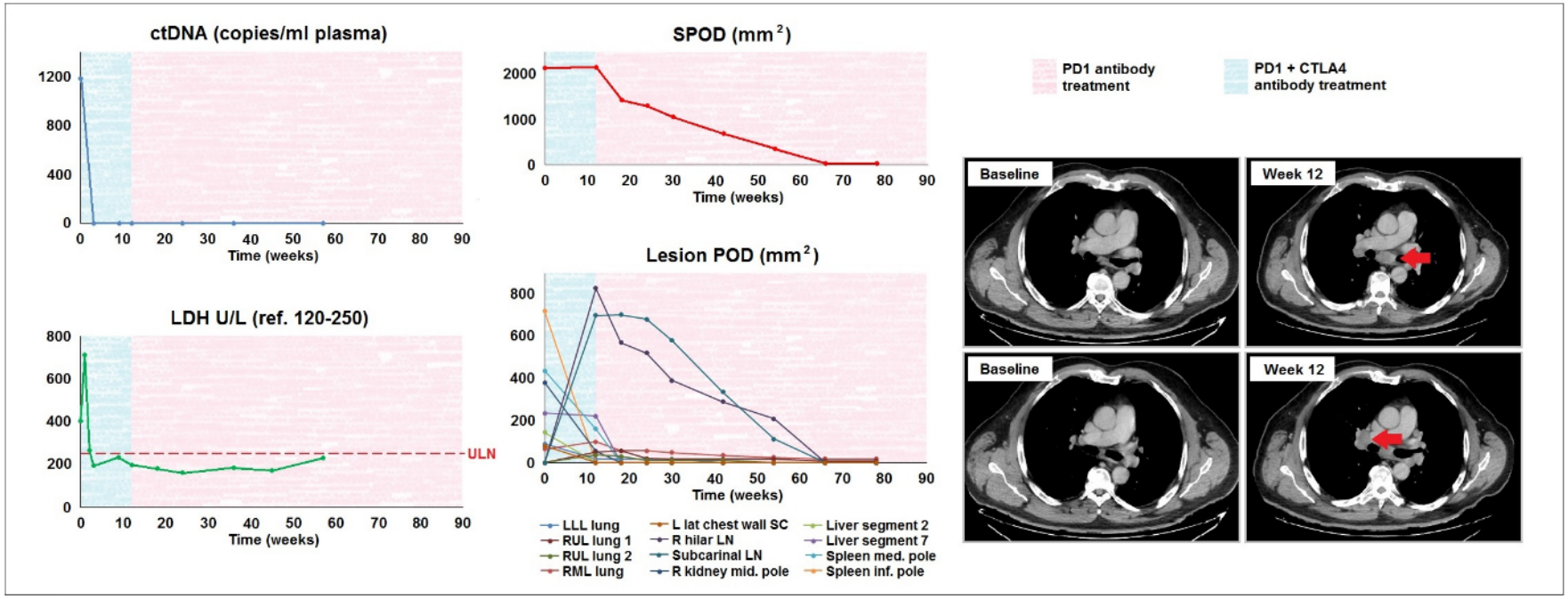
eTable. Patient and Disease Characteristics at Baseline

Characteristics	Pseudo-progressors (n = 9)	True progressors (n = 20)	Total (n = 29)
Age – no. [%]			
≤ 65	5 [56]	12 [60]	17 [59]
> 65	4 [44]	8 [40]	12 [41]
Sex – no. [%]			
Male	7 [78]	11 [55]	18 [62]
Female	2 [22]	9 [45]	11 [38]
ECOG – no. [%]			
0	5 [56]	8 [40]	13 [45]
1-2	4 [44]	12 [60]	16 [55]
AJCC tumor stage – no. [%]			
M1a or M1b	2 [22]	2 [10]	4 [14]
M1c	7 [78]	18 [90]	25 [86]
Mutation – no. [%]			
BRAF	6 [67]	12 [60]	18 [62]
NRAS	3 [33]	8 [40]	11 [38]
Treatment type – no. [%]			
Single agent PD-1	6 [67]	17 [85]	23 [79]
PD-1 + CTLA-4	3 [33]	3 [15]	6 [21]
LDH – no. [%]			
≤ 1x ULN	7 [78]	8 [40]	15 [52]
> 1x ULN	2 [22]	12 [60]	14 [48]

ECOG; Eastern Cooperative Oncology Group; AJCC, American Joint Committee on Cancer; PD1, programmed death 1; CTLA4, cytotoxic T-lymphocyte 4; LDH, lactate dehydrogenase; ULN, upper limit of normal.

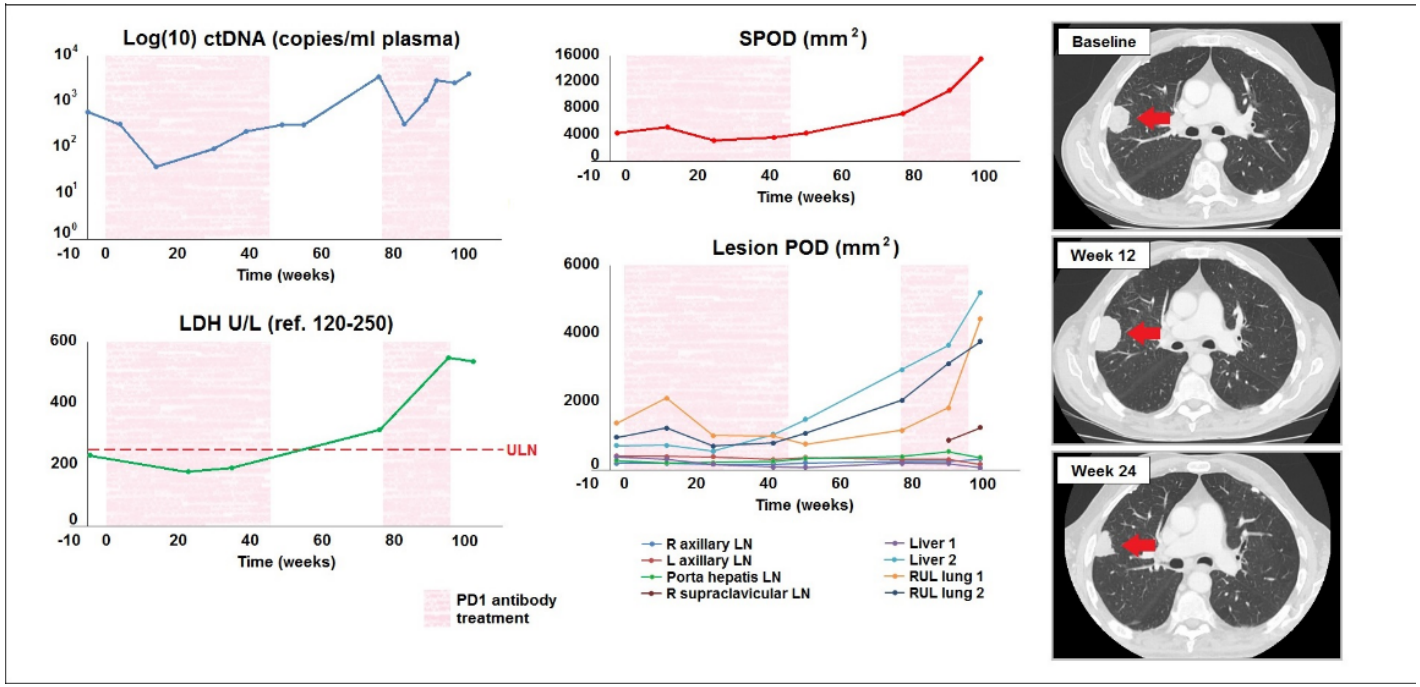
eFigure 1. Changes in Circulating Tumor DNA (ctDNA), Lactate Dehydrogenase (LDH), and Disease Volume With Treatment in Patient 1

This patient presented with rapidly progressive $BRAF^{V600K}$ mutant metastatic melanoma involving the lung, multiple lesions in the liver and spleen, kidney, lymph node and subcutaneous disease. A combination of pembrolizumab and ipilimumab was administered, and despite tumor shrinkage in most of the pre-existing lesions, this patient developed a new right hilar and subcarinal lymph node on first restaging at week 12 (Figure 3a). Baseline ctDNA which was markedly elevated at 2960 copies per ml of plasma became undetectable after 2 cycles of treatment at week 6, and remained undetectable throughout treatment. The two new lymph nodes subsequently underwent an objective response, and at week 66, the patient had a complete response.



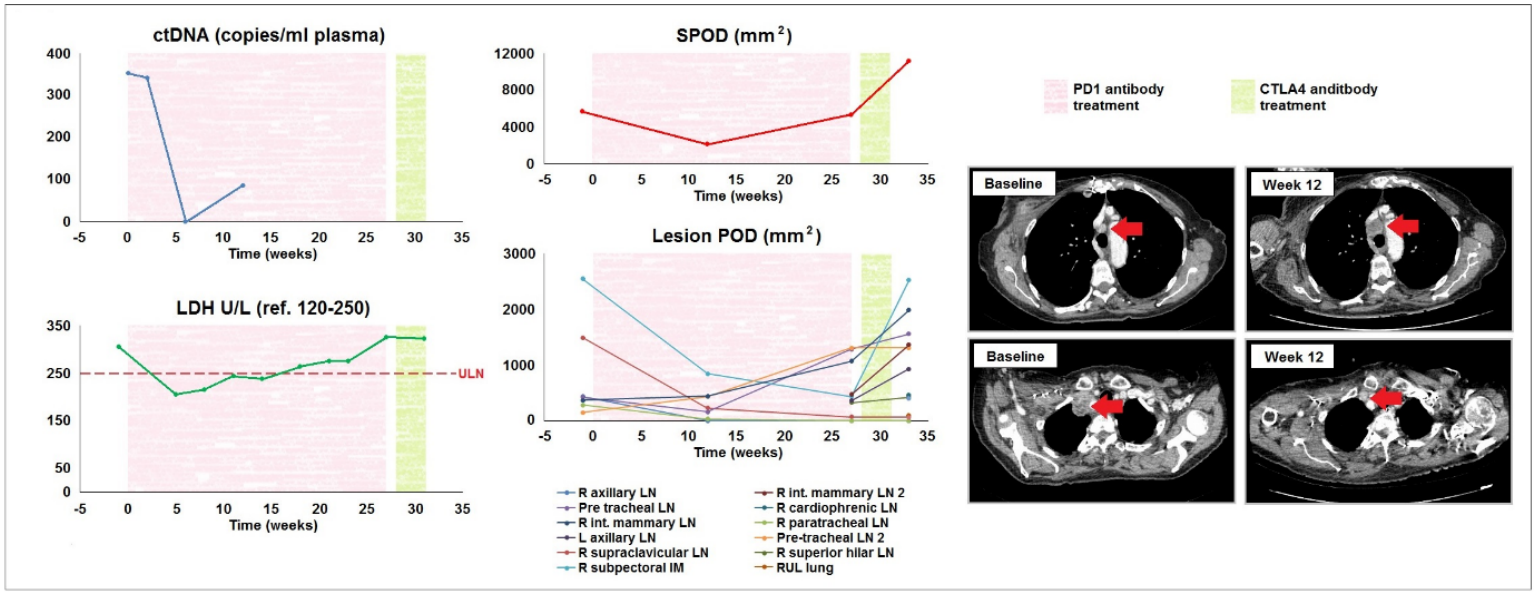
eFigure 2. Changes in Circulating Tumor DNA (ctDNA), Lactate Dehydrogenase (LDH), and Disease Volume With Treatment in Patient 2

This patient with pseudoprogression but a ctDNA level which remained persistently elevated presented with metastatic BRAF V600K melanoma. Following treatment failure to both MAPK inhibitors and ipilimumab, single agent pembrolizumab was commenced. At this stage, there was metastatic disease involving the axillary, abdominal and thoracic lymph nodes, lung and the liver. First restaging CT imaging at week 12 demonstrated stable disease in most lesions, with evidence of progression in both lung lesions [Figure 3b]. The ctDNA level was elevated at baseline (ctDNA = 590 copies per ml plasma) and decreased over ten-fold by week 12. There was subsequent response in both lung lesions, with no evidence of progression until week 50. Serum LDH remained normal, throughout this period, and did not reflect tumor responses. Treatment was ceased after 15 cycles of treatment due to severe synovitis involving the small joints of the hand, and corticosteroids were commenced to manage the symptoms. This patient eventually had true RECIST progression at week 80. Interestingly, ctDNA levels were on the upward trend months before any evidence of radiological progression, predicting eventual treatment failure.



eFigure 3. Changes in Circulating Tumor DNA (ctDNA), Lactate Dehydrogenase (LDH), and Disease Volume With Treatment in Patient 3

This patient had $BRAF^{V600K}$-mutant melanoma and developed metastatic disease in the lymph nodes and lung while on neo-adjuvant MAPK therapy as part of a clinical trial (NCT01972347). Despite a dramatic response on the first restaging CT scan following four cycles of pembrolizumab, there was a new pre-tracheal lymph node with evidence of central necrosis. The overall disease burden was reduced by 63%. The $BRAF^{V600K}$ ctDNA analysis predicted radiological treatment response, baseline ctDNA was elevated (352 copies/ml plasma) and zero-converting within 6 weeks. Interestingly, the ctDNA was detectable by week 12, and this was followed by a second restaging scan at week 27 confirming true progression, with further enlargement of the pre-tracheal lymph node and several new lymph node metastases. Treatment was changed to ipilimumab, which was ineffective, and the patient died from disease progression within 3 months following cessation of pembrolizumab.



SPOD, sum of product of diameters; POD, product of diameters; PD1, programmed death1; CTLA4, cytotoxic T-lymphocyte protein; LLL, left upper lobe, RUL, right upper lobe; RML, right middle lobe; lat, lateral; SC, subcutaneous; LN, lymph node; mid, middle; med, medial; inf, inferior; int, internal; IM, intramuscular.

eFigure 4. Overview of Serial LDH Results and Response in 29 Patients

Each column is an individual patient, demonstrating longitudinal LDH values (reference range 120-250 IU/L) up to week 12, represented as normal, >1x upper limit of normal (ULN) and >2x ULN. Patients were stratified in to favorable (n = 16) and unfavorable LDH groups (n = 13).