Title	Complete response to pembrolizumab in advanced hepatocellular carcinoma with microsatellite instability
Author(s)	Ando, Yuwa; Yamauchi, Masami; Suehiro, Yosuke; Yamaoka, Kenji; Kosaka, Yumi; Fuji, Yasutomo; Uchikawa, Shinsuke; Kodama, Kenichiro; Morio, Kei; Fujino, Hatsue; Nakahara, Takashi; Ono, Atsushi; Murakami, Eisuke; Kawaoka, Tomokazu; Takahashi, Shoichi; Tsuge, Masataka; Hiramatsu, Akira; Imamura, Michio; Chayama, Kazuaki; Aikata, Hiroshi
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Relation	


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Complete Response to Pembrolizumab in Advanced Hepatocellular Carcinoma with Microsatellite Instability: A Case Report.

Yuwa Ando[1], Masami Yamauchi[1], Yosuke Suehiro[1], Kenji Yamaoka[1], Yumi Kosaka[1], Yasutoshi Fuji[2], Shinsuke Uchikawa[1], Kenichiro Kodama[1], Kei Morio[1], Hatsue Fujino[1], Takashi Nakahara[1], Atsushi Ono[1], Eisuke Murakami[1], Tomokazu Kawaoka[1], Shoichi Takahashi[1], Masataka Tsuge[1], Akira Hiramatsu[1], Michio Imamura[1], Kazuaki Chayama[1], Hiroshi Aikata[1]

[1] Department of Gastroenterology and Metabolism, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan.

2. Department of Clinical oncology, Hiroshima Prefectural Hospital, Hiroshima, Japan

Corresponding author: Hiroshi Aikata

Department of Gastroenterology and Metabolism, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734-8551, Japan. Telephone: +81-82-257-5192, Fax: +81-82-257-5194,

E-mail: aikata@hiroshima-u.ac.jp

Abstract

Hepatocellular carcinoma (HCC) has limited systemic treatment options and a poor prognosis. The immune checkpoint inhibitor pembrolizumab was recently approved for treatment of solid tumors with microsatellite instability (MSI). However, its clinical utility for the management of HCC remains to be clarified. Here, we present a case of unresectable HCC with MSI that showed an impressive response to pembrolizumab treatment.

A 64-year-old man with chronic HCV infection was diagnosed with a large HCC. His severe liver dysfunction and poor performance status prevented any treatment option other than sorafenib. However, sorafenib failed after a few days due to rapid progression of the tumor. Based on the finding of MSI in a biopsy specimen, immunotherapy using pembrolizumab was initiated. A dramatic improvement in his general condition and a reduction in tumor size were observed after the initiation of pembrolizumab treatment. Among a cohort of 50 consecutive patients with advanced HCC who were refractory to standard systemic therapy, MSI was found only in the present case.

Immune checkpoint blockade therapy induced prominent anti-tumor effects in HCC with MSI. Screening for defects in DNA mismatch repair function may be warranted in HCC patients despite the low frequency of MSI.

Keywords: hepatocellular carcinoma, mismatch repair deficiency, MSI-high, immune checkpoint blockade, PD-L1

Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide, and the prognosis of unresectable HCC is considerably poor [1, 2]. Although the multikinase inhibitor sorafenib has been widely used as first-line chemotherapy, its objective response rate is low (2%), with a complete response rate of 0% observed in two randomized controlled trials of patients with advanced HCC [3, 4]. Lenvatinib was recently approved as a systemic treatment for HCC, and it showed a higher response rate (40%) in patients with unresectable HCC [5]. However, its toxicity often leads to early termination of the treatment and a reduced benefit among patients with impaired hepatic reserve function.

Pembrolizumab is a humanized IgG4 monoclonal antibody that inhibits the programmed cell death 1 (PD-1) protein, activating a host immune response against tumor cells [6]. In pivotal phase 2 studies, a prominent antitumor effect of pembrolizumab was recognized in patients with solid tumors exhibiting microsatellite instability (MSI) [7]. Consequently, pembrolizumab was quickly approved in many countries for the treatment of unresectable or metastatic solid MSI tumors that progressed after prior systemic treatment and are poor candidates for other treatment options. Since the clinical trials contributing to the approval of pembrolizumab did not include a wide variety of tumors, its practical use as a systemic treatment for HCC remains unknown. Furthermore, the incidence of MSI in unresectable tumors has not been thoroughly clarified.

Here, we describe an impressive case of MSI HCC that exhibited a dramatic response to pembrolizumab.

Case Report

The patient was a 64-year-old male with a medical history of diabetes. His family history included lung cancer in his mother and diabetes in his father. Although he was diagnosed with chronic HCV infection at the age of 40 years, he refused to receive any antiviral treatment. In December 2018, he presented to his family physician with weight loss and intermittent abdominal pain that had persisted for 3 months. After the detection of a large hepatic mass by computed tomography (CT), he was referred to our hospital for further diagnosis and management.

On physical examination, he had a debilitating fever and serious abdominal discomfort that resulted in a poor performance status. Blood test results showed severe liver dysfunction indicating Child–Pugh class B and extremely elevated levels of des-γ-carboxy prothrombin (DCP) (Table 1). Contrast-enhanced CT revealed an invasive liver tumor 13 × 10 cm in size. 18-fluorodeoxyglucose positron emission tomography showed strong uptake in the main tumor and widespread invasion to the lymph nodes (Fig. 1). Percutaneous liver biopsy prior to treatment revealed moderately differentiated hepatocellular carcinoma (Fig. 2a).

Although the patient had Child-Pugh B liver function, he had advanced HCC with extra hepatic metastasis and vascular invasion. Therefore, we decided to administer sorafenib to this patient. In initiation of sorafenib to patients, strict informed consent was performed. However, after systemic treatment with sorafenib was started, his general condition continued to deteriorate. CT indicated progressive disease and a remarkably enlarged tumor. An additional biopsy showed an MSI-H tumor. Relatively high programmed death-ligand 1 (PD-L1) expression and the presence of $CD8^+$

lymphocytes in the main tumor were observed (Fig. 2b, 2c). Based on these findings, pembrolizumab treatment was initiated. The patient's physical condition and liver function improved immediately, and the tumor marker levels had recovered to within their normal ranges by the end of two treatment cycles. Imaging studies showed exceptional shrinkage of the tumor accompanied by decreased tumor vascularity and viability (Fig. 3). Furthermore, histopathological assessment of a tumor biopsy specimen obtained after four cycles of treatment showed extensive necrosis with no evidence of viable cancer cells (Fig. 4). The therapeutic effect was determined to be a complete response, as assessed by the modified Response Evaluation Criteria in Solid Tumors. The patient has been receiving pembrolizumab treatment for over 4 months, without any side effects or sign of recurrence.

Between January and June 2019, we investigated the prevalence of MSI in 50 consecutive HCC patients with unresectable disease that had progressed after standard chemotherapy. Using a companion diagnostic sequencing kit (polymerase chain reaction analysis of five microsatellite markers: BAT25, BAT26, NR21, NR24, and MONO27), we successfully performed the tests in biopsy specimens, even very small ones, obtained from liver tumors. As a result, MSI was found only in the present case (2%), in which all five markers showed slight shortening (Fig. 5).

Discussion

We describe a case of MSI-H HCC that showed a favorable response to pembrolizumab therapy. To our knowledge, no other report has addressed the MSI status or efficacy of immune checkpoint blockade therapy in advanced HCC.

We established the importance of determining the MSI status of tumors in a population with a substantially low incidence of MSI. T cells attack the tumor by recognizing tumor specific antigens. At the same time, T cells trigger the expression of PD-L1 molecules, and PD-L1 molecules bind to PD-1. Then, signals that negatively affect tumor immunity are delivered to cytotoxic T cells, reducing T cell activity and resulting in immune escape or tolerance [8]. The administration of PD-1 antibodies such as pembrolizumab demonstrate anti-tumor effect by unlocking this immune mechanism and restoring the ability of the immune system to attack tumor cells [9]. On the other hand, Le DT et al identified more potential new mutation-associated antigens in tumors of mismatch repair-deficient cancers compared to mismatch repair-proficient cancers [10]. As a result, in patients with MSI, it is considered that T cells are more likely to recognize tumor cells and anti-PD1 treatment is very effective. However, the prevalence of MSI was very rare, as expected. In general, mismatch repair deficiency is more common in early-stage cancers, and to date, the presence of MSI in late-stage HCC has not been reported [11]. Despite the low prevalence of MSI, the low cost and good benefit seen in patients suggest the necessity of MSI testing in poor HCC candidates for standard treatments.

Given the extraordinary response observed in our patient, mismatch repair function should be considered a biomarker guiding systemic treatment decisions for HCC. According to a previous report evaluating the efficacy and safety of pembrolizumab for HCC in a second-line setting [12], the objective response and complete response rates were only 18.3% and 2.2%, respectively. Determining the MSI status may help identify the patients who would benefit the most from this novel immunotherapy. Additionally, we assume two important factors enhanced the anti-tumor effect in the present case. First, prolonged inflammation in the liver caused by untreated hepatitis may facilitate the evolutionary process of MSI-related hepatocarcinogenesis. Hepatitis-associated DNA methylation influences a considerable number of oncogenes and tumor-suppressor genes [13]. Once methylation and gene silencing of mismatch repair genes occur, the tumor mutation burden may increase exponentially under persistent inflammatory conditions such as chronic hepatitis and may contribute to remarkable immunogenicity. Second, the present case initially had a distinct "hot" immune phenotype in terms of PD-L1 expression and immune cell infiltration. Less than 20% of all HCC patients are PD-L1 positive [14], and most HCC patients have a low amount of $CD8^+$ T-cell infiltration [15]. On the other hand, the relationship between the immunological condition and MSI status of a tumor has not been fully investigated in HCC. Accumulation of enough patients to obtain adequate power for genomic analyses and profiling of the tumor immune microenvironment is warranted for better understanding of the biology of MSI HCC.

Here, we report a patient with MSI-H advanced HCC that showed a complete response to pembrolizumab. We propose that the MSI status should be determined in

a broad spectrum of patients with HCC who are poor candidates for standard treatments.

Abbreviations

HCC: hepatocellular carcinoma; MSI: microsatellite instability; PD-1: programmed cell death 1; CT: computed tomography; DCP: des-γ-carboxy prothrombin; PD-L1: programmed death-ligand 1.

Acknowledgements

We would like to thank Dr. Arihiro who helped us with pathological data collection.

Table 1. Blood test results.

CBC		γGTP	505 IU/L	**Viral markers**	
Leukocyte count	9760/μL	Na	134 mEq/L	HBs antigen	(-)
Erythrocyte count	3.78×10^6/μL	K	3.7 mEq/L	HCV antibody	(+)
Hemoglobin	10.8 g/dL	Cl	102 mEq/L	HCV-RNA	4.8 logIU/mL
Hematocrit	32.5%	TP	8.4 g/dL	genotype	1b
Platelet count	314×10^3/μL	Albumin	2.5 g/dL	**Liver function**	
Blood coagulation test		BUN	20.8 mg/dL	ICG-R	14.2%
Prothrombin time	81%	Cr	1.01 mg/dL	Child–Pugh score	7
PT-INR	1.13	CRP	8.74 mg/dL	Child–Pugh grade	B
Blood chemistry		NH3	33 μmol/L		
Total bilirubin	1.2 mg/dL	HbA1c	5.3%		
AST	74 IU/L	**Tumor markers**			
ALT	80 IU/L	AFP	1.3 ng/mL		
LDH	274 IU/L	AFP-L3	<0.5%		
ALP	1516 IU/L	DCP	16052 mAU/mL		

BUN, blood urea nitrogen; TP, total protein; PT-INR, prothrombin time-international normalized ratio; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; CRP, C-reactive protein; LDH, lactate dehydrogenase; ICG-R, indocyanine green retention

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Figure legends

Figure 1. Contrast-enhanced CT findings. (a) The tumor (13 cm in diameter) showed early-phase enhancement in the portal region of the liver. (b) The equilibrium phase of CT showed that the tumor was slightly hypodense. (c, d) The tumor had invaded the left portal vein and inferior vena cava. (e) Positron emission tomography/CT showed a maximum standardized uptake value of 6.8 in the main tumor and of 3.4 in the para-aortic lymph node metastasis.

Figure 2. Histopathological findings in the liver tumor. (a) The tumor was moderately differentiated hepatocellular carcinoma (hematoxylin and eosin staining, ×200). (b) Immunohistochemical examination revealed positive PD-L1 expression in a portion (5%) of the tumor cells (×200). (c) Diffuse tumor infiltration by $CD8^+$ lymphocytes was evident (×200).

Figure 3. Clinical course. Over the course of the treatment, changes were evident in DCP level and in the arterial phase of contrast-enhanced CT images. Despite having started sorafenib therapy, the tumor size and tumor marker levels increased. However, both showed dramatic improvements upon initiation of pembrolizumab treatment. DCP, des-γ-carboxy prothrombin; PS, performance status.

Figure 4. Histopathological findings after pembrolizumab therapy. (a) Hematoxylin and eosin staining showed extensive necrosis and no cancer cells (×100). (b) $CD8^+$ lymphocyte infiltration persisted around the necrotic tumor tissue (×200). (c) Immunostaining of Foxp3 showed almost no regulatory T cells in the tumor (×200).

Figure 5. Polymerase chain reaction analysis of five microsatellite markers. In all markers, the waveform peak of tumor tissue was shifted to the left compared to that of normal tissue.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent to publish

Written informed consent was obtained from the guardians of the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

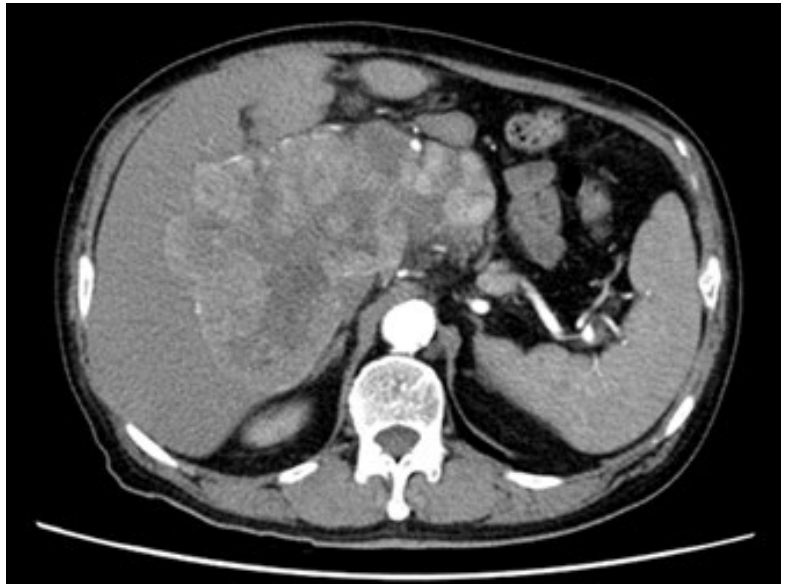
Authors' contributions

YA took the lead in drafting the manuscript. MY and HA conducted a literature review and participated in drafting the manuscript. YS, KY, YK, YF, SU, KK, KM, HF, TN, AO, EM, TK, ST, MT, MI, KC reviewed the literature and critically reviewed the

manuscript. HA provided supervision, participated in the literature review and in drafting the manuscript. All authors read and approved the final manuscript.

Figure 1

(a)



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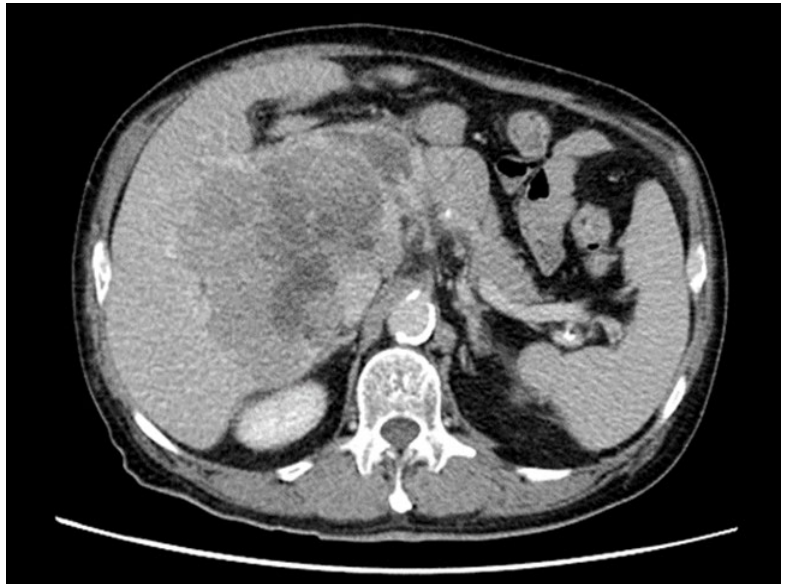

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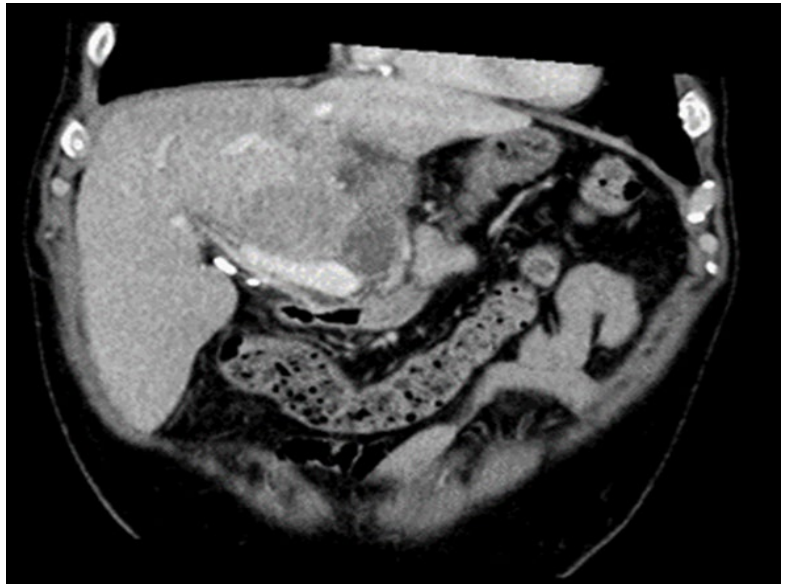

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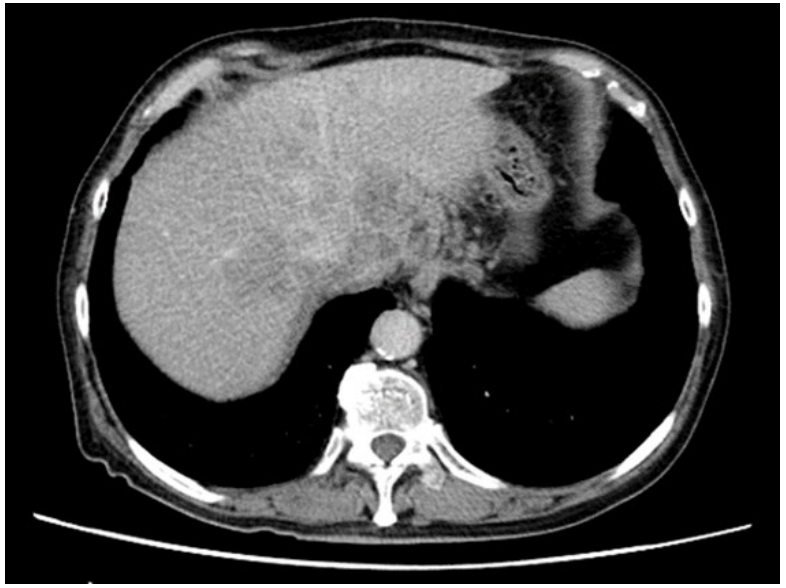

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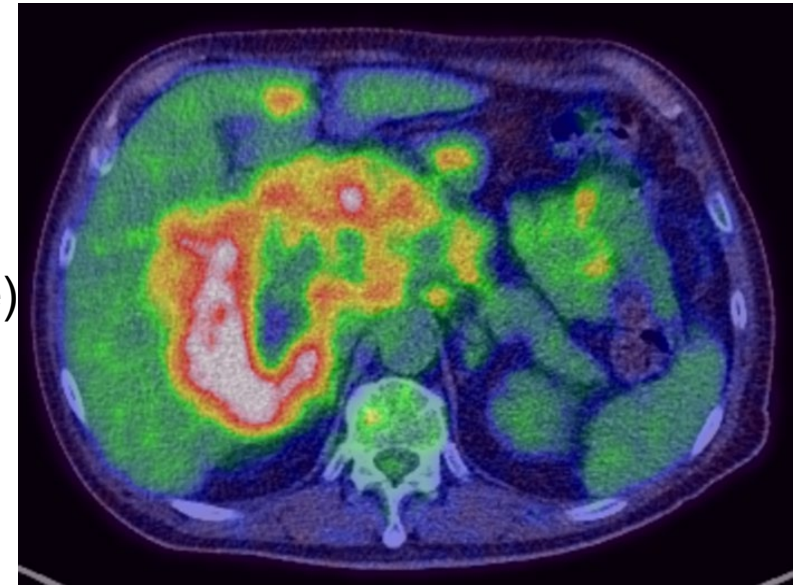
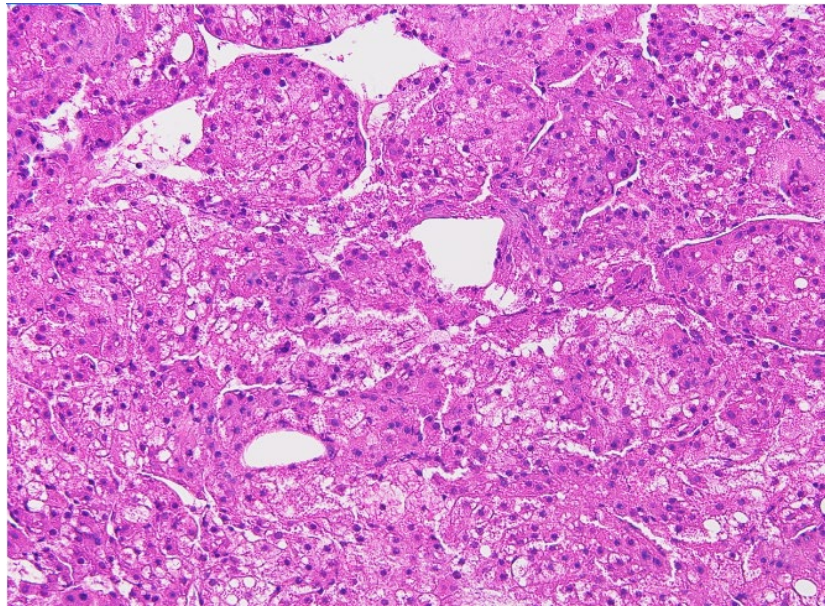


Figure 2

(a)



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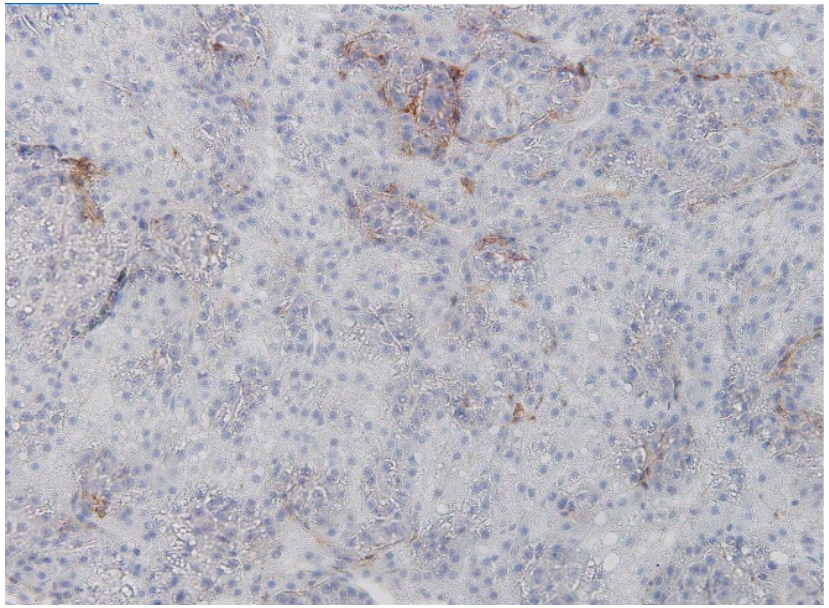

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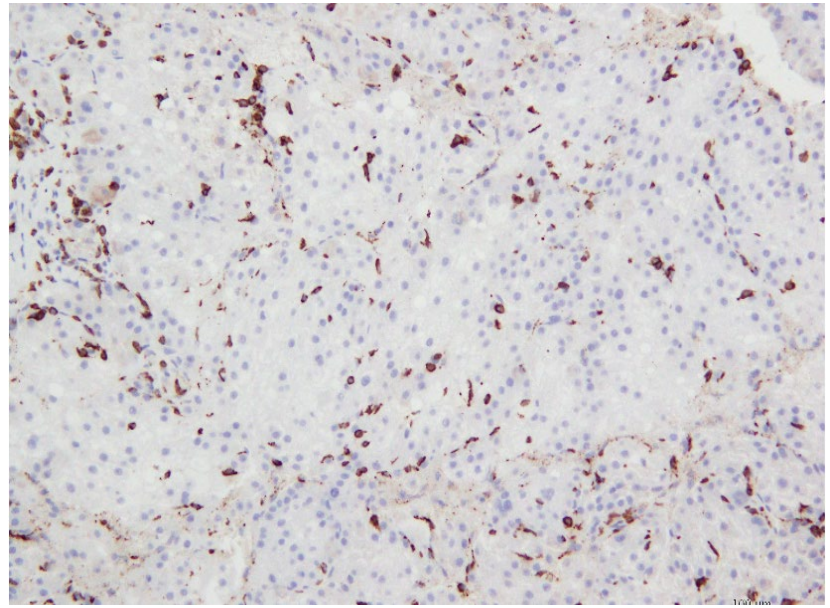
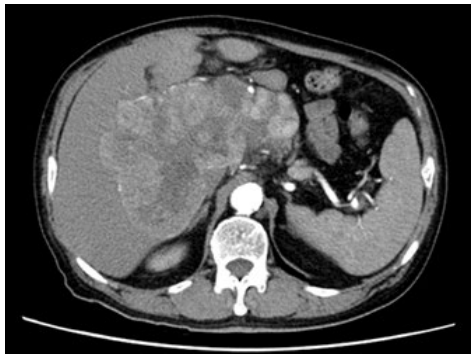
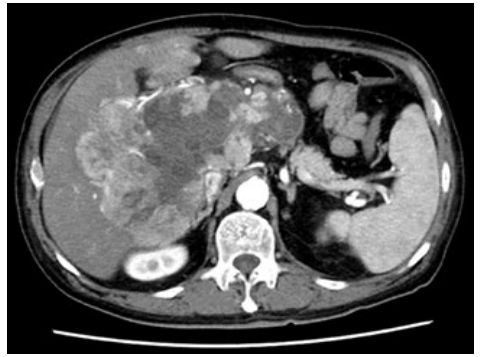
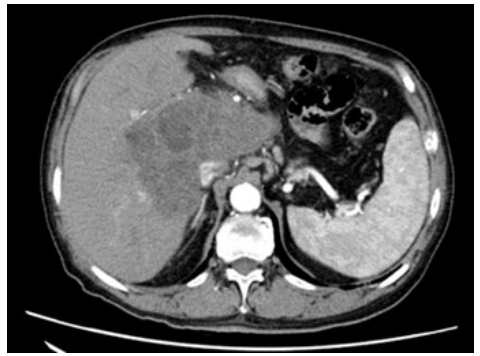


Figure 3







800 mg/day

Sorafenib

Pembrolizumab 200 mg

ECOG PS	1	2	1	0	
Child-Pugh score	7			6	5

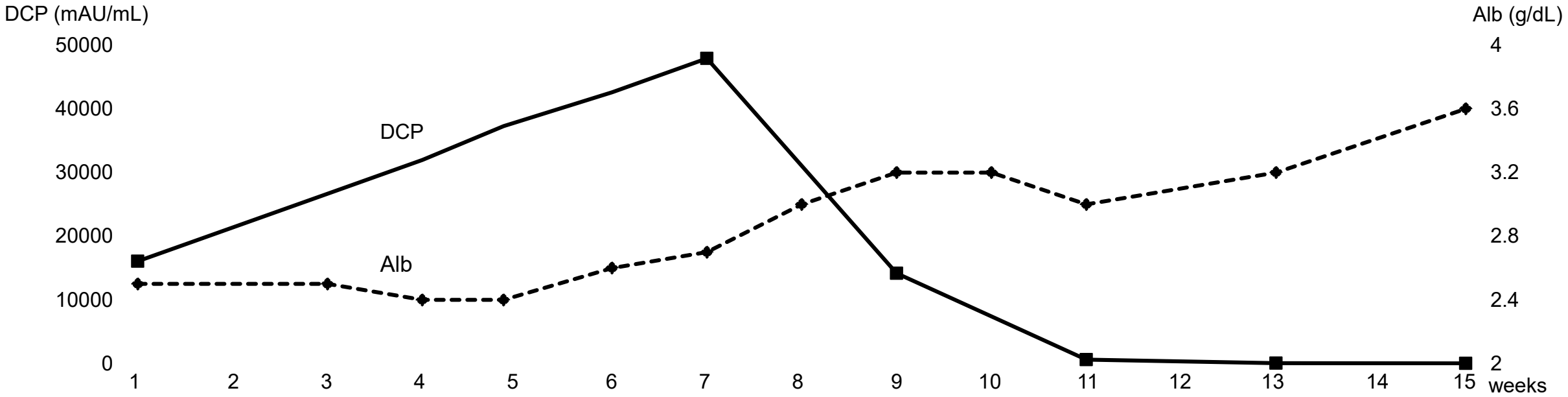
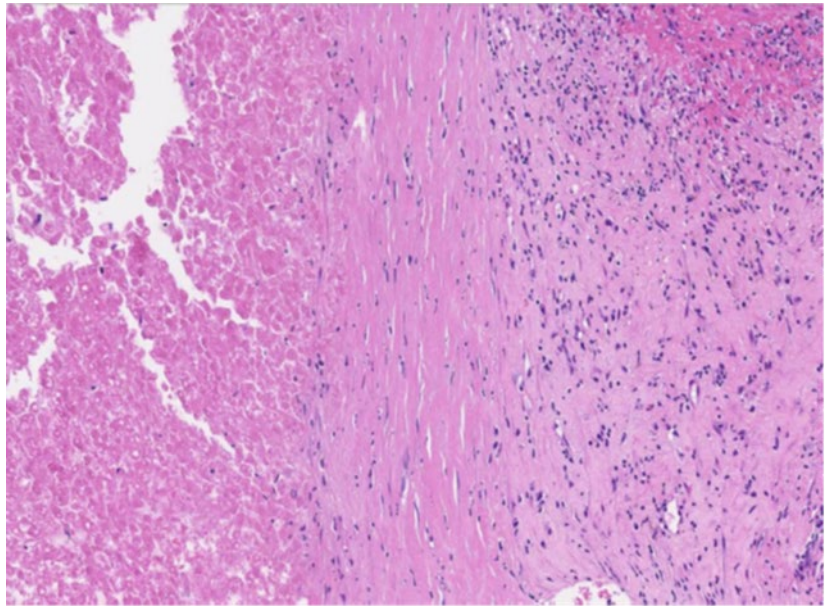


Figure 4

(a)



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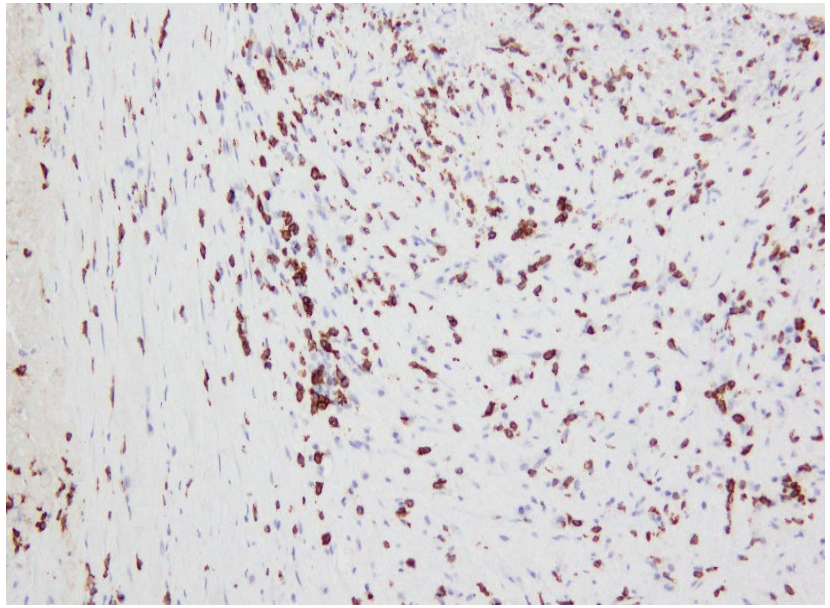

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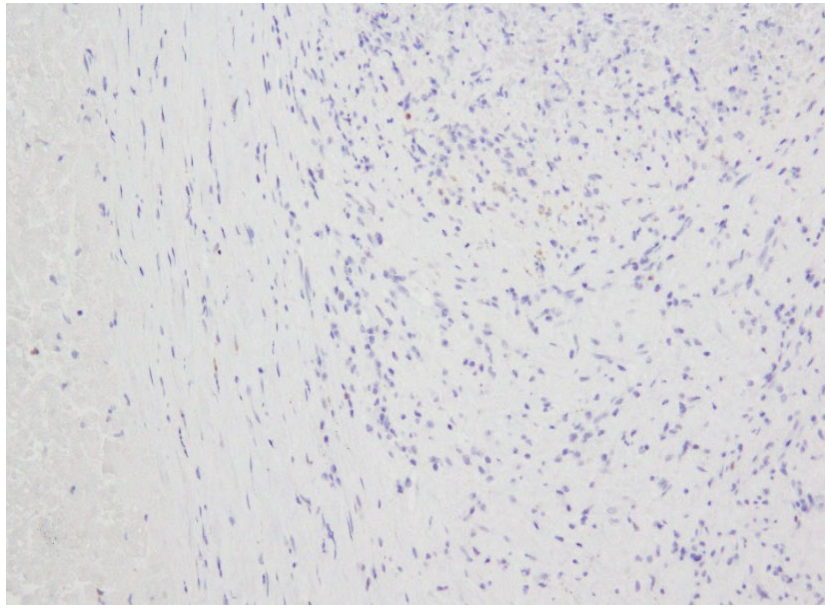
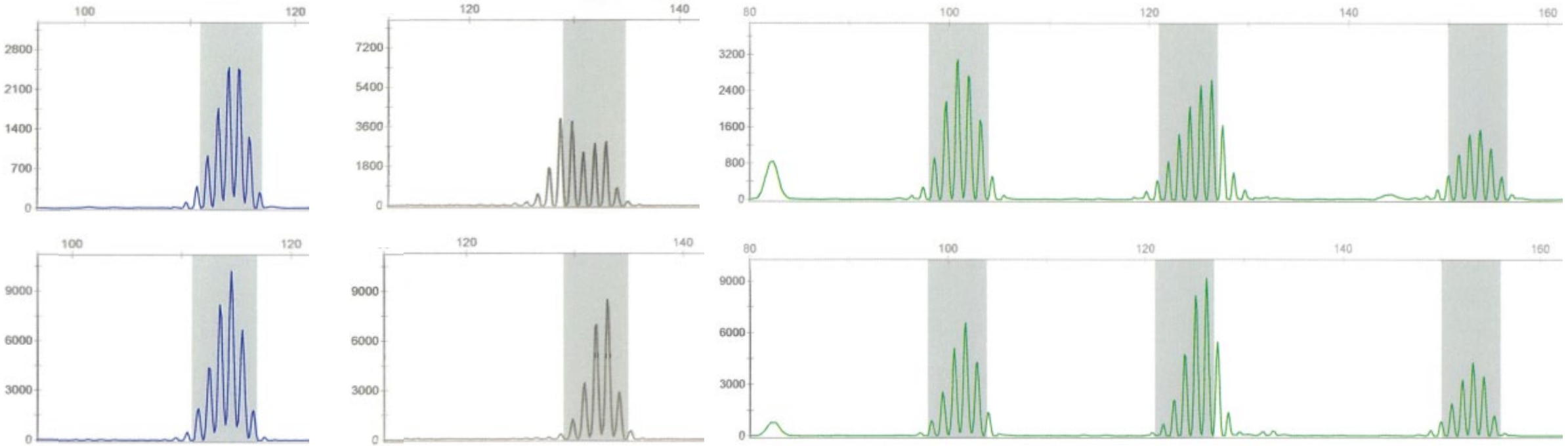


Figure 5

Tumor (Top)
Normal (Bottom)



BAT26

MSI(+)

NR24

MSI(+)

NR21

MSI(+)

BAT25

MSI(+)

MONO27

MSI(+)