Development of Hepatocellular Carcinoma During Nivolumab Treatment for Recurrent Non-Small Cell Lung Cancer: A Case Report

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Nivolumab, a monoclonal antibody targeting programmed cell death 1 (PD-1), is the standard second-line therapy for advanced non-small cell lung cancer (NSCLC). In the current immunotherapy era, it is often difficult to evaluate the therapeutic effect, disease progression, and pseudo-enlargement of the tumor or the emergence of another etiology. In the present report, we describe a 79-year-old patient with hepatocellular carcinoma (HCC) newly detected during nivolumab treatment for recurrent NSCLC. When the patient was 73 years old, he had suffered from NSCLC and received concurrent chemoradiotherapy comprising cisplatin and docetaxel, achieving a complete response. Six years after the chemoradiotherapy, the patient had multiple lung and hepatic lesions. We thus started the treatment with nivolumab for recurrent NSCLC. All those lesions responded to nivolumab over nine cycles. By contrast, a lesion was newly detected in the medial segment of left hepatic lobe, liver segment 4 (S4), and was gradually getting larger, as judged by computed tomographic scan. Liver biopsy revealed the growing lesion to be a well-differentiated HCC. Consequently, the patient was treated with radiofrequency ablation to HCC, while nivolumab treatment was continued for NSCLC. Immunohistochemical analysis of the HCC specimens revealed nuclear accumulation of β-catenin compared with normal liver cells and undetectable expression of program death ligand 1 (PD-L1). Such expression profiles of β-catenin and PD-L1 in HCC may be responsible for the resistance against nivolumab treatment. Immunohistochemical features of the biopsy specimens may be predictive of the effectiveness of the immunotherapy in HCC.

Introduction

Nivolumab, a monoclonal antibody targeting programmed cell death 1 (PD-1), is now the standard drug for various cancers, including metastatic melanoma (Weber et al. 2015), non-small-cell lung cancer (NSCLC) (Borghaei et al. 2015; Brahmer et al. 2015), squamous cell carcinoma of the head and neck (Kiyota et al. 2017), classical Hodgkin lymphoma (Younes et al. 2016), urothelial carcinoma (Sharma et al. 2016; Kang et al. 2017), and renal cell carcinoma (Motzer et al. 2015). The results of clinical trials of nivolumab for each type of cancer are different, and various clinical trials for other cancers are ongoing. In addition, the response to nivolumab is sometimes known to be unique. For instance, target lesions become larger at first, but they start to shrink during nivolumab treatment. This phenomenon is referred to as pseudoprogression and is not experienced with cytotoxic or existing molecular targeted agents, including tyrosine kinase inhibitors (Wolchok et al. 2009). Oncologists are therefore facing difficulties in estimating the therapeutic effects of nivolumab when the lesions are growing. Here, we present a case of hepatocellular carcinoma (HCC) newly detected during nivolumab treatment for recurrent NSCLC.

Case Presentation

The 73-year-old Japanese man had been treated with concurrent chemoradiotherapy comprising cisplatin (40 mg/m^2) and docetaxel (40 mg/m^2) as the first-line treatment for NSCLC, achieving complete response (Fig. 1). However,
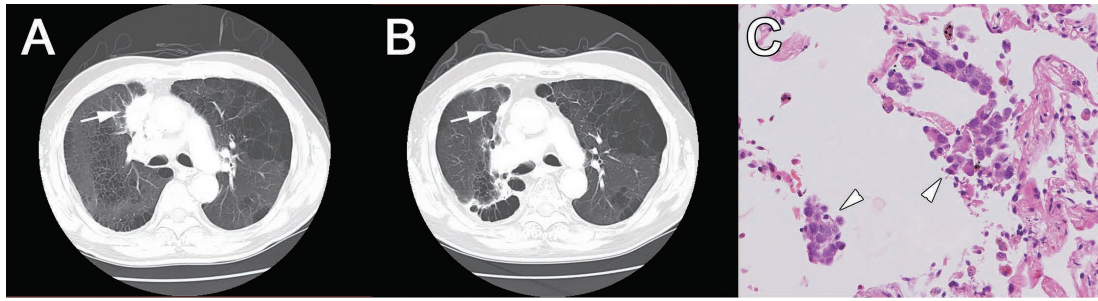
Fig. 1. Computed tomography images before and after chemoradiotherapy.
Computed tomography images of a 73-year-old patient on initial admission (A) and after treatment with chemoradiotherapy (B). The primary lesion (arrow) shrank after chemoradiotherapy. Transbronchial lung biopsy stained with hematoxylin and eosin showing non-small cell lung carcinoma (arrowhead) (C).
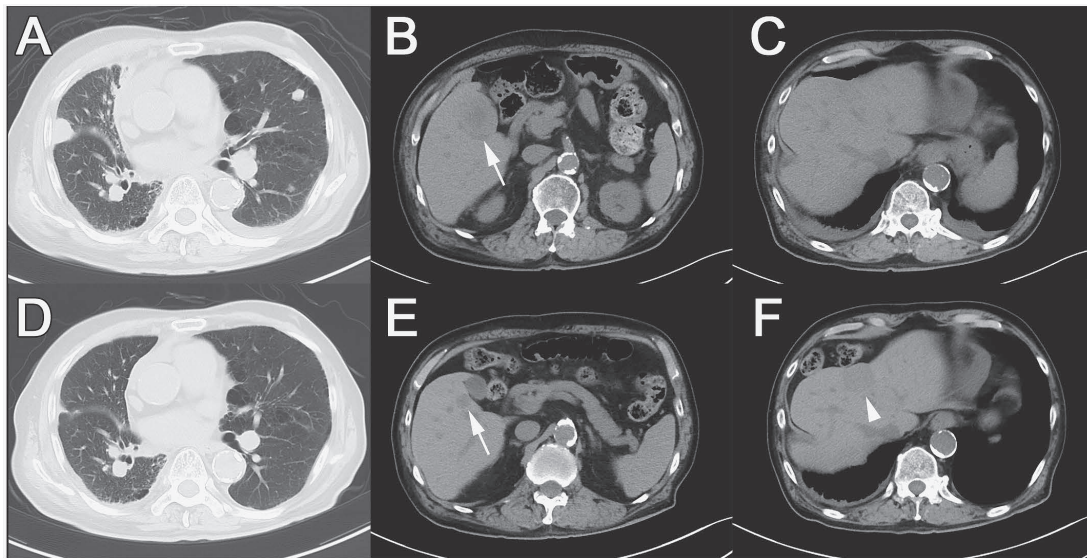

Fig. 2. Computed tomography images before and after nivolumab treatment.
Computed tomography images of a 79-year-old patient with recurrent non-small cell lung cancer before treatment with nivolumab (A, B, C) and after nine cycles (D, E, F). The lung tumors (A, D) and the mass in the anteroinferior segment of right hepatic lobe, liver segment 5 (S5), (B, E) were shrunk (arrow), while the mass in the medial segment of left hepatic lobe, liver segment 4 (S4), was growing (arrowhead) (C, F). Note that no mass was detectable in S4 before nivolumab treatment (C).

we were unable to make the histological diagnosis of lung cancer with transbronchial lung biopsy due to the limited amount of tissue specimens.

Six years later, multiple pulmonary tumors and liver metastases in the anterior segment of right hepatic lobe appeared (Fig. 2A, B), and thus the patient, aged 79 years, was treated with nivolumab at 3 mg/kg every 2 weeks as the second-line treatment for recurrent NSCLC. Computed tomography was performed without contrast, as the patient had chronic kidney disease. All lung lesions and multiple liver masses rapidly responded to nivolumab, and after nine cycles, most lesions were diminished in size or disappeared (Fig. 2D, E). By contrast, a mass was newly detected in the medial segment of the left lobe, liver segment 4 (S4); the tumors grew from an undetectable size to 28 × 26 mm (Fig. 2C, F). We then biopsied the growing mass in S4 to evaluate for disease progression, pseudoprogression, or other etiologies. The immunohistochemical analysis of the biopsy specimens demonstrated pathologically well-differentiated HCC with nuclear accumulation of β-catenin and undetectable levels of programmed cell death 1 ligand (PD-L1) (Fig. 3). No fibrosis was observed in the liver tissue. The patient had a past history of hepatitis B virus (HBV) infection, and HBV infection was resolved; HBs-antigen, HBs-antibody, and HBV-DNA were negative, whereas HBc-antibody was positive. There was no elevation in serum levels of alpha-fetoprotein and protein induced by vitamin K absence or antagonist-II (PIVKA-II). Consequently, a single lesion of HCC was treated with radiofrequency ablation (RFA), while nivolumab treatment for NSCLC is continued.

Ethics approval and consent to participate
The case report was waivered by the Ethics Committee of Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital. The clinical information presented in this case report was obtained from Tokyo
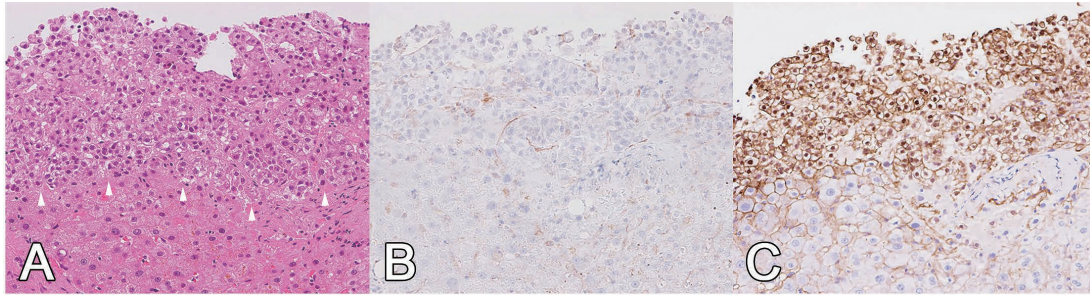
Fig. 3. Histopathological findings of the mass in the medial segment of left hepatic lobe.
The biopsy specimens of the mass in S4 stained with hematoxylin and eosin demonstrates well-differentiated hepato-cellular carcinoma (above arrowheads) (magnification × 200) (A). On immunostaining, the tumor cells are negative for membranous PD-L1 expression (magnification × 200) (B) and positive for nuclear β-catenin expression (magnification × 200) (C).

Metropolitan Cancer and Infectious Disease Center Komagome Hospital's medical records.

Consent for publication
        Written informed consent was obtained from the patient for publication of this case report.

Discussion

        Unconventional responses to immune check point inhibitors (ICIs), such as monoclonal antibodies against PD-1 and PD-L1, are often experienced (Chiou and Burotto 2015). In the present case, multiple pulmonary lesions and hepatic metastases decreased in size at first; however, the lesion in S4 grew during the nivolumab treatment. Based on immune-related Response Evaluation Criteria in Solid Tumors guidelines, this apparent progression of NSCLC mandated local treatment as long as all the other lesions were well controlled with nivolumab (de Vin et al. 2014). There are possibilities accounting for the growing solitary lesion: resistance to nivolumab, pseudoprogression, or a new lesion. Pseudoprogression is rare, but leads to a misled in precise assessment of therapeutic effects. We therefore performed hepatic biopsy to confirm the pathological diagnosis before RFA and found it to be HCC. It is important that diagnosis should be confirmed before local treatment is initiated.

        Studies for checkpoint inhibitors for HCC are ongoing. The Checkmate040 study indicated a response rate of 15% to 20% and disease control rate of 58% to 64% in patients with HCC treated with nivolumab (El-Khoueiry et al. 2017). The KEYNOTE-224 study also revealed that pembrolizumab, a PD-1 inhibitor, has an objective response rate of 17% (Zhu et al. 2018). Although the KEYNOTE-224 study implied the relationship between the efficacy of pembrolizumab treatment and the PD-L1 expression in the tumor as well as NSCLC tumor cells (Borghaei et al. 2015), the Checkmate040 study showed that PD-L1 expression in the HCC was not associated with the response rate (El-Khoueiry et al. 2017). On the contrary, aberrant activation of β-catenin signaling, which is associated with onco-genesis, has a negative effect on anti-tumor immunity in metastatic melanoma (Spranger et al. 2015). Previous studies have implied that nuclear accumulation of β-catenin detected via immunohistochemistry is associated with β-catenin gene mutations in HCC (Terris et al. 1999; Zucman-Rossi et al. 2007). In addition, the abnormality of Wnt/β-catenin signaling in the HCC is related to poor survival in immunotherapy (Harding et al. 2018). In our case, the HCC was positive for nuclear expression of β-catenin and negative for PD-L1 expression, as judged by immuno-histochemistry staining. Although there is no confirmatory evidence of a relationship between immunohistochemical characteristics and therapeutic effect of anti-immune therapy in HCC, both β-catenin and PD-L1 status may account for its resistance to nivolumab. Immunohistochemistry for β-catenin may be predictive of the effectiveness of nivolumab in HCC, warranting further studies.

        In summary, the present case suggests that an unde-tectable cancer before nivolumab treatment potentially grows; therefore, biopsy is often required to select adequate treatment option if the cancer is indolent. Moreover, this case demonstrated positive for nuclear β-catenin and nega-tive for PD-L1 in the HCC. Resistance to nivolumab treat-ment might have been caused by abnormal β-catenin sig-nals, although the mechanism remains unclear. A detailed examination of the biopsy tissues, including immunohisto-chemistry, may be helpful in predicting the effectiveness of the immunotherapy.

Author Contributions

        K.N., Y.O., K.H. and J.K. acquired the clinical data and drafted the manuscript, read and approved the final manuscript. J.K. was responsible for pathological diagnosis.

Conflict of Interest

The authors declare no conflict of interest.

References

Borghaei, H., Paz-Ares, L., Horn, L., Spigel, D.R., Steins, M., Ready, N.E., Chow, L.Q., Vokes, E.E., Felip, E., Holgado, E., Barlesi, F., Kohlhaufl, M., Arrieta, O., Burgio, M.A., Fayette, J., et al. (2015) Nivolumab versus Docetaxel in advanced nonsquamous non-small-cell lung cancer. *N. Engl. J. Med.*, **373**, 1627-1639.

Brahmer, J., Reckamp, K.L., Baas, P., Crino, L., Eberhardt, W.E., Poddubskaya, E., Antonia, S., Pluzanski, A., Vokes, E.E., Holgado, E., Waterhouse, D., Ready, N., Gainor, J., Aren Frontera, O., Havel, L., et al. (2015) Nivolumab versus Docetaxel in advanced squamous-cell non-small-cell lung cancer. *N. Engl. J. Med.*, **373**, 123-135.

Chiou, V.L. & Burotto, M. (2015) Pseudoprogression and immune-related response in solid tumors. *J. Clin. Oncol.*, **33**, 3541-3543.

de Vin, T., Engels, B., Gevaert, T., Storme, G. & De Ridder, M. (2014) Stereotactic radiotherapy for oligometastatic cancer: a prognostic model for survival. *Ann. Oncol.*, **25**, 467-471.

El-Khoueiry, A.B., Sangro, B., Yau, T., Crocenzi, T.S., Kudo, M., Hsu, C., Kim, T.Y., Choo, S.P., Trojan, J., Welling, T.H.R., Meyer, T., Kang, Y.K., Yeo, W., Chopra, A., Anderson, J., et al. (2017) Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet*, **389**, 2492-2502.

Harding, J.J., Nandakumar, S., Armenia, J., Khalil, D.N., Albano, M., Ly, M., Shia, J., Hechtman, J.F., Kundra, R., El Dika, I., Do, R.K., Sun, Y., Kingham, T.P., D'Angelica, M.I., Berger, M.F., et al. (2018) Prospective genotyping of hepatocellular carcinoma: clinical implications of next generation sequencing for matching patients to targeted and immune therapies. *Clin. Cancer Res.*, **25**, 2116-2126.

Kang, Y.K., Boku, N., Satoh, T., Ryu, M.H., Chao, Y., Kato, K., Chung, H.C., Chen, J.S., Muro, K., Kang, W.K., Yeh, K.H., Yoshikawa, T., Oh, S.C., Bai, L.Y., Tamura, T., et al. (2017) Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*, **390**, 2461-2471.

Kiyota, N., Hasegawa, Y., Takahashi, S., Yokota, T., Yen, C.J., Iwae, S., Shimizu, Y., Hong, R.L., Goto, M., Kang, J.H., Sum Kenneth Li, W., Ferris, R.L., Gillison, M., Namba, Y., Monga, M., et al. (2017) A randomized, open-label, Phase III clinical trial of nivolumab vs. therapy of investigator's choice in recurrent squamous cell carcinoma of the head and neck: a subanalysis of Asian patients versus the global population in checkmate 141. *Oral Oncol.*, **73**, 138-146.

Motzer, R.J., Escudier, B., McDermott, D.F., George, S., Hammers, H.J., Srinivas, S., Tykodi, S.S., Sosman, J.A., Procopio, G., Plimack, E.R., Castellano, D., Choueiri, T.K., Gurney, H., Donskov, F., Bono, P., et al. (2015) Nivolumab versus Evero-limus in advanced renal-cell carcinoma. *N. Engl. J. Med.*, **373**, 1803-1813.

Sharma, P., Callahan, M.K., Bono, P., Kim, J., Spiliopoulou, P., Calvo, E., Pillai, R.N., Ott, P.A., de Braud, F., Morse, M., Le, D.T., Jaeger, D., Chan, E., Harbison, C., Lin, C.S., et al. (2016) Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. *Lancet Oncol.*, **17**, 1590-1598.

Spranger, S., Bao, R. & Gajewski, T.F. (2015) Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature*, **523**, 231-235.

Terris, B., Pineau, P., Bregeaud, L., Valla, D., Belghiti, J., Tiollais, P., Degott, C. & Dejean, A. (1999) Close correlation between beta-catenin gene alterations and nuclear accumulation of the protein in human hepatocellular carcinomas. *Oncogene*, **18**, 6583-6588.

Weber, J.S., D'Angelo, S.P., Minor, D., Hodi, F.S., Gutzmer, R., Neyns, B., Hoeller, C., Khushalani, N.I., Miller, W.H., Lao, C.D., Linette, G.P., Thomas, L., Lorigan, P., Grossmann, K.F., Hassel, J.C., et al. (2015) Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.*, **16**, 375-384.

Wolchok, J.D., Hoos, A., O'Day, S., Weber, J.S., Hamid, O., Lebbe, C., Maio, M., Binder, M., Bohnsack, O., Nichol, G., Humphrey, R. & Hodi, F.S. (2009) Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin. Cancer Res.*, **15**, 7412-7420.

Younes, A., Santoro, A., Shipp, M., Zinzani, P.L., Timmerman, J.M., Ansell, S., Armand, P., Fanale, M., Ratanatharathorn, V., Kuruvilla, J., Cohen, J.B., Collins, G., Savage, K.J., Trneny, M., Kato, K., et al. (2016) Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. *Lancet Oncol.*, **17**, 1283-1294.

Zhu, A.X., Finn, R.S., Edeline, J., Cattan, S., Ogasawara, S., Palmer, D., Verslype, C., Zagonel, V., Fartoux, L., Vogel, A., Sarker, D., Verset, G., Chan, S.L., Knox, J., Daniele, B., et al. (2018) Pembrolizumab in patients with advanced hepatocel-lular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol.*, **19**, 940-952.

Zucman-Rossi, J., Benhamouche, S., Godard, C., Boyault, S., Grimber, G., Balabaud, C., Cunha, A.S., Bioulac-Sage, P. & Perret, C. (2007) Differential effects of inactivated Axin1 and activated beta-catenin mutations in human hepatocellular carcinomas. *Oncogene*, **26**, 774-780.