



Case Report

Hepatocellular Carcinoma Tumor Progression and Severe Liver Injury after Anti-PD-1 Administration to a Liver-Transplanted Patient

Ben-Ari Z^{1,7}, Ben-Haim G², Loewenthal R⁴, Inbar Y⁵, Tzaref K¹, Stienlauf S², Pikarsky E⁸, Pappo O⁶, Shacham-Shmueli E^{3*}

¹Liver Disease Center, Ramat Gan, Israel

²Department of Internal Medicine, Sheba Medical Center, Ramat Gan, Israel

³Institute of Oncology, Sheba Medical Center, Ramat Gan, Israel

⁴Tissue Typing Laboratory, Sheba Medical Center, Ramat Gan, Israel

⁵Institute of Imaging, Sheba Medical Center, Ramat Gan, Israel

⁶Department of Pathology, Sheba Medical Center, Ramat Gan, Israel

⁷Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

⁸Department of Pathology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

***Corresponding author:** Shacham Shmueli Einat, Institute of Oncology, Sheba Medical Center, Derech Sheba 1 St. Ramat Gan, 5265601, Israel. Tel: +972-35307180; Fax: +972-35307190; Email: Einat.ShachamShmueli@sheba.health.gov.il

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Abstract

Checkpoint molecules are receiving increasingly recognition as new targets for cancer immunotherapies. Although antibodies against the programmed death 1 (PD-1) receptor have elicited tumor regression in multiple cancer types, these therapies were not been tested in patients treated on long-term immunosuppressive regimens. Moreover, the clinical benefit of anti-PD-1 therapy can be hampered by immune-related adverse events, including hepatitis caused by dysregulation of host immune system. However, options for therapy are very limited for patients with a history of organ transplantation and recurrent HCC. Here we describe, for the first time, administration of an anti-PD-1 antibody (nivolumab) to a liver transplant recipient presenting with recurrent lung hepatocellular carcinoma metastases. Non-resolving severe hepatitis developed 7 weeks after nivolumab administration, highlighted by a fulminant course leading to patient death. No evidence of graft rejection or of antibody-mediated rejection was observed in liver histology, yet, grade 4 immune-mediated hepatitis, displaying positive hepatic PD-1 and PD-1L immunostaining was shown.

Conclusions: The co-administration of immunosuppressive agents to prevent graft rejection is suggested to have impacted the efficacy of the immune checkpoint inhibitors in our patient, leading to tumor progression and fulminant hepatitis. This case advocates for further investigation of the safety and efficacy of cancer immunotherapies in solid organ transplant recipients.

Keywords: Anti-PD-1; Hepatocellular carcinoma; Fulminant hepatic failure; Liver-transplantation

Introduction

Immune checkpoints are critical modulators in the immune system that either enhance a signal (co-stimulatory molecules) or attenuate a signal (co-inhibitory molecules). It is now clear that tumors modulate immune checkpoints as one of the mechanisms employed to escape immune surveillance [1]. The US Food and

Drug Administration (FDA)-approved the Programmed Death 1 (PD-1) immune checkpoint pathway inhibitor, nivolumab, a fully human IgG4 monoclonal antibody indicated for the treatment of patients with unresectable or metastatic melanoma and squamous cell lung carcinoma [2]. However, immune checkpoint blockade has recently emerged as a promising therapeutic approach for various other malignancies including Hepatocellular Carcinoma (HCC) [3]. Interim results of an ongoing Phase I dose-escalation study involving nivolumab treatment of advanced HCC patients

with or without chronic viral hepatitis demonstrated an estimated 62% survival rate in evaluable patients (n=47) at 12 months [3].

The clinical benefit of PD-1 inhibitors can be hampered by immune-related adverse events including immune-mediated hepatitis caused by dysregulation of the immune system. Hepatic toxicity often manifests as asymptomatic elevated levels of hepatic transaminases [4]. Moreover, PD-1 inhibitors were not tested within clinical trials in patients treated with long-term immunosuppressive medications. A recent publication by Lipson et al., [5] demonstrated that anti-PD-1 antibody (pembrolizumab) administration to a kidney transplant recipient with metastatic cutaneous squamous-cell carcinoma was associated with a robust antitumor response, but was accompanied by kidney allograft rejection [5]. Here, we report for the first time a case in which an anti-PD-1 antibody (nivulomab) was administered to a liver transplant recipient with recurrent HCC in lung.

Case

A 65-year-old man presenting primary sclerosing cholangitis and hepatocellular carcinoma (HCC) which was outside the Milan criteria (solitary tumor ≥ 8 cm), received a liver transplant from a deceased donor in December 2012 in China by patient selection. Thereafter, he received standard long-term immunosuppression therapy, including tacrolimus and certican. His graft function remains stable and normal. In November 2014, following detection of increased serum Alphafetoprotein (AFP) levels (from normal to 78.2micg/l), a lung metastasis, detected in the left upper lung by chest x-ray and CT, was diagnosed (Figure 1A). The lung biopsy collected under CT guidance was compatible with metastatic HCC. AFP staining was weakly positive (data not shown). PD-L1 and PD1 were not expressed on tumor cells and infiltrating immune cells. There was no evidence of HCC recurrence in the grafted liver. Immunosuppression therapy dosage was decreased (tacrolimus) and treatment with sorafenib (a multikinase inhibitor) 800mg was initiated in January 2015. However, the lung metastasis progressed, as observed in repeated chest CTs, and mediastinal bilateral hilar adenopathy developed. In addition, serum AFP levels continued to increase, reaching 225micg/l. Due to disease progression, sorafenib treatment was discontinued.

The possibility and availability of administrating immune checkpoint-blocking drugs was discussed, in light of the published results of the Phase I dose-escalation study of nivolumab in advanced HCC patients [3]. The risk of immune-related toxic effects associated with anti-PD-1 therapy, including liver allograft rejection [4,5], was clearly explained to the patient. Although there was no endorsement for the administration of anti PD-1 by our

local tumor board in the Sheba Medical Center, treatment with nivulomab was initiated in December 2015 in a private clinic, at a regimen of 80mg every 2 weeks. Following four cycles of treatment with nivulomab, serum AFP levels further increased to 1513micg/l, and a repeated chest CT clearly confirmed further disease progression, with new diffused metastatic bilateral lung nodules (Figure 1B). At the same time, that patient's serum liver enzymes and bilirubin levels increased considerably from their baseline normal levels (Figure 2) (alanine aminotransferase (ALT) 428U/ l, aspartate aminotransferase (AST) 210U/ l, bilirubin 7.3mg/dl, alkaline phosphatase (ALP) 702U/ l, GGT 1419U/ l, INR 1.4, albumin 4.0g/dl). Serology and PCR testing for HCV, HBV, HAV, CMV, EBV and herpes viruses were all negative. In addition, immunoserology for antinuclear a, anti-smooth-muscle, and anti-mitochondrial antibodies proved negative. Repeated abdominal CT, MRI, and Doppler ultrasound examinations excluded hepatic artery thrombosis, CBD and bile ducts dilatation and there was no evidence of HCC recurrence in the grafted liver. A liver biopsy (Figure 3A) demonstrated severe panlobular hepatitis with foci of confluent necrosis and prominent perivenular infiltrate, hepatocanalicular cholestasis, diffused sinusoidal and portal neutrophilic and lymphocytic infiltrates. Many foamy macrophages were noted. There was evidence neither of acute cellular rejection nor of humoral rejection. Immunohistochemistry staining for C4d was negative. Very few PD-1-positive T cells were noted (Figure 3B) however, membranous PD-L1 positivity was noted in hepatocytes (Figure 3C), endothelial cells and macrophages (Figure 3D). The infiltrating immune cells were predominantly CD8-positive findings consistent with an activated cytotoxic T-cells (Figure 3E). Evaluation of the patient's serum for antibodies to HLA class I and II antigens was negative. Liver histology findings were compatible with reported immune-related toxic effects associated with anti-PD-1. Nivulomab administration was discontinued and prompt treatment with three cycles of Intravenous (IV) methyl prednisolone 500mg, followed by the administration of prednisolone 60 mg daily was initiated, but no improvement was achieved. At the beginning of March 2016, the patient was admitted to our hospital due to severe weakness and fatigue, dyspnea, and jaundice. Laboratory findings peaked at WBC 17K, Hb 13.2 g/dl, PLT 106K, AST 968U/ l, ALT1618U/ l, total bilirubin 41mg/dl (direct 29.8mg/dl) (Figure 2), ALP 877U/ l and GGT 806U/ l albumin 2.9g/dl, INR 3.46, creatinine 3.6 mg/dl and urea 78 mg/dl. Multiple blood cultures tested sterile. Fluid resuscitation as well as the administration of IV methylprednisolone 125 mg daily and Cellcept 1000mg daily was not associated with any improvement, the patient continued to deteriorate rapidly and died on March 2016.

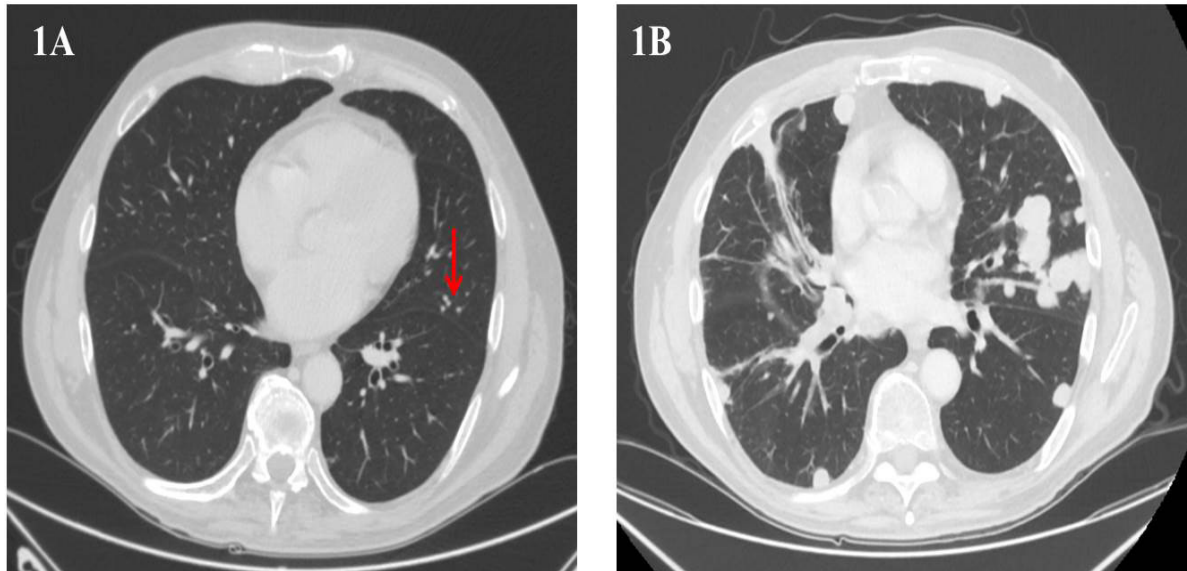


Figure 1: Chest CT. A. At diagnosis very small lesions ($\leq 6\text{mm}$) were noted in the upper left lung. B. Repeated chest CT. Diffuse metastatic lesions in both lungs (diameter 1.7-3.9cm) and large Mediastinal lymphadenopathy.

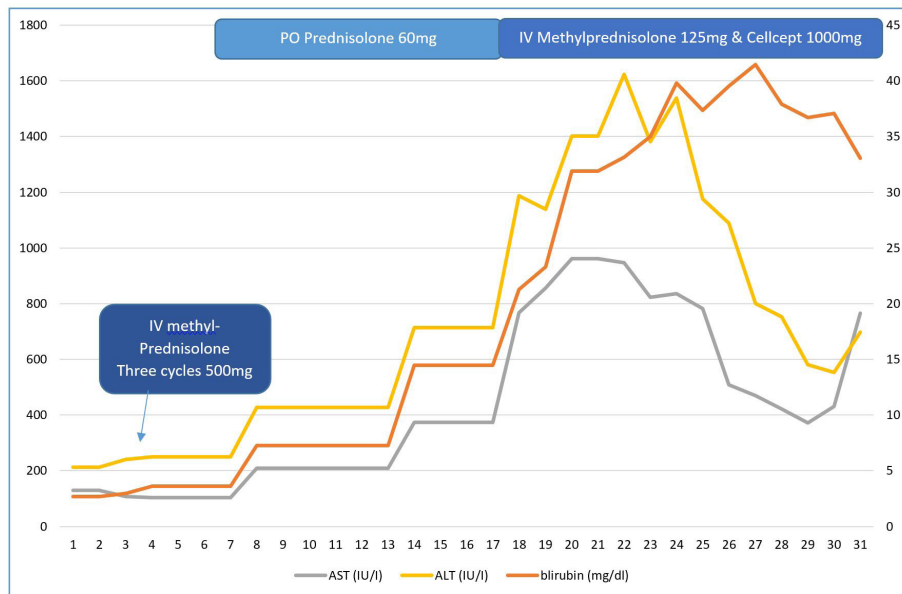


Figure 2: Patient management. Treatment with three cycles of IV 500mg methyl prednisolone, followed by administration of 60 mg prednisolone daily was initiated, with no improvement. Laboratory findings peaked at: AST 968U/L, ALT 1618U/L, total bilirubin 41 mg/dl (direct 29.8mg/dl). IV methylprednisolone 125 mg and Cellcept 1000mg were administered daily, but were not associated with any improvement.

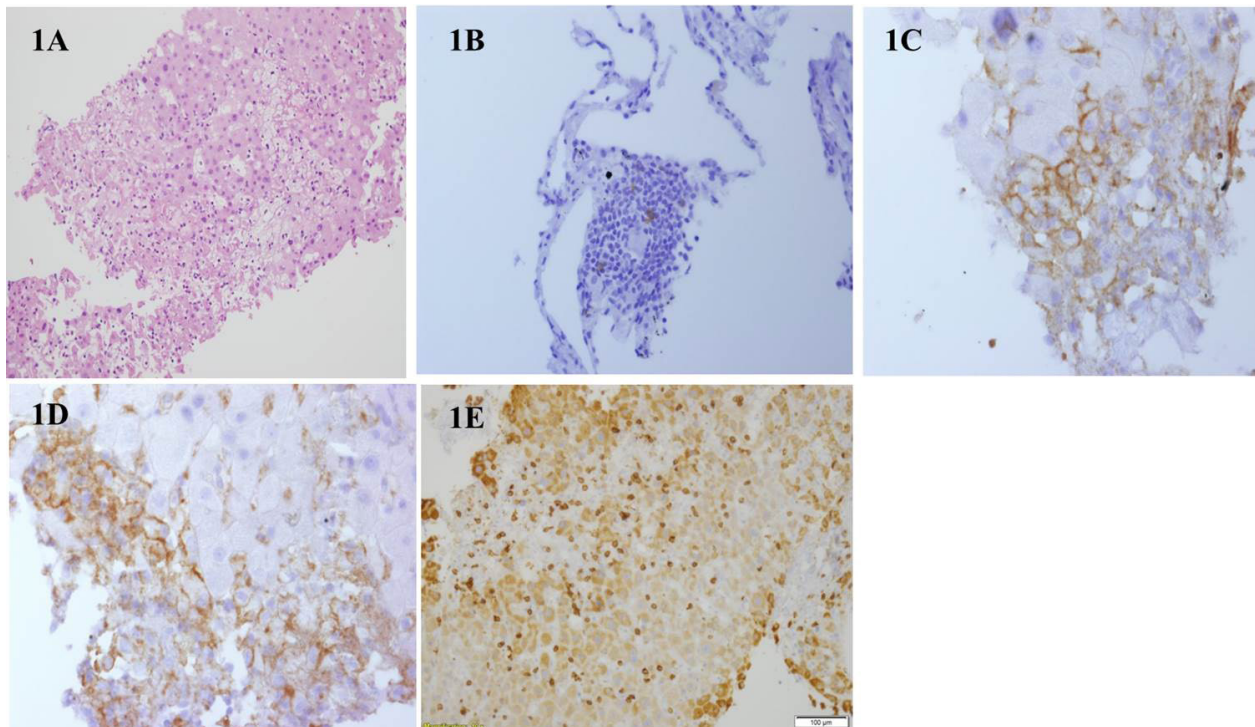


Figure 3: Liver histology findings. A. H&E staining. Severe panlobular hepatitis with foci of confluent necrosis and prominent perivenular infiltrate, hepatocanalicular cholestasis, diffuse sinusoidal and portal neutrophilic and lymphocytic infiltrates. Many foamy macrophages were noted. B-D. Immunostaining for PD-1 and PD-L1. Very few PD-1-positive T cells were noted (B), however membranous PD-L1 positivity was noted in hepatocytes (C), endothelial cells and macrophages (D). The infiltrating immune cells were predominantly CD8-positive consistent with an activated cytotoxic T-cells (E).

Discussion

Immune checkpoint blockade has recently emerged as a promising therapeutic approach for various malignancies, including HCC [1-3]. In a Phase I dose-escalation study of the efficacy of nivolumab in advanced HCC patients [3], an interim analysis demonstrated an estimated survival rate of 62% in evaluable patients (n=47) at 12 months. Durable partial responses and complete responses were detected in one out of five nivolumab-treated patients. It is of note that PD-1 and PD-L1 were not expressed in the HCC lung metastatic tissue, however, focal PDL1 expression in some tumors may be missed in small biopsy specimens, such as needle biopsies [6]. Nivolumab administration in our patient was associated with further disease progression. Moreover, the patient developed grade 4 immune-mediated hepatitis. Checkpoint drug-related immune-mediated hepatitis, which manifest as asymptomatic increased liver function tests, mainly AST, ALT and GGT, rarely bilirubin, must be distinguished from other etiologies of hepatic injury, such as recurrent HCC in the grafted liver, viral infections and effects of other medications [4], which were all ruled out in our patient. Biopsy of the liver showed severe panlobular hepatitis with foci of confluent necrosis and no

evidence of acute cellular or humoral rejection. The hepatic PD-L1 expression in endothelial cells, hepatocytes and macrophages might indicate that the previous depression of T-cells caused by PD-1/PD-L1 coupling was disrupted by the administration of nivolumab, thereby releasing T cells and thus inducing immune-mediated hepatitis. The median time to onset of hepatic drug-related adverse effects is highly variable, ranging from 25 weeks (range 4-31 weeks) in lung cancer patients, and 19 weeks (range 0.3-93 weeks) in patients treated with pembrolizumab, to 4 weeks (range 0.1-23 weeks) in melanoma patients treated with nivolumab [4]. The immune-mediated hepatitis in our patient developed within 7 weeks of nivolumab treatment. The frequency and severity of “adverse events of specific interest” with PD-1 inhibitors are 1-6% and mainly grade 1-2, respectively [4]. Severe liver adverse events (grade ≥ 3) occur in 0.2% of patients.

Histological findings of hepatitis related to ipilimumab, an immune checkpoint blocker targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4), were recently described [7]. Samples often indicated panlobular active hepatitis and zone 3 hepatitis that resembles autoimmune hepatitis however, findings were not specific in several cases.

The standard algorithm for the management of hepatic checkpoint-related adverse effects involving grades 3-4 hepatotoxicity, includes the intravenous administration of high-dose glucocorticoids for 24 to 48 h, followed by an oral steroid with prednisone 1-2 mg/kg, tapered over no less than 30 days. If serum transaminase levels do not decrease within 48 h after initiation of systemic steroids, oral mycophenolate mofetil (Cellcept) 500 mg every 12 h is considered. Severe cases require permanent discontinuation of PD1 inhibitor treatment due to hepatic drug-related adverse events and might require prolonged immunosuppressive therapy [4]. Hospitalization is considered, as cases of death from liver failure have been reported in patients with this level of hepatotoxicity [8]. Our patient did not recover from the grade 4 immune-mediated hepatitis. Lipson et al., reported, for the first time, on two kidney transplanted patients with metastatic melanoma receiving immune checkpoint blockade therapy. Renal allografts from both patients appeared to have been unaffected by administration of ipilimumab (anti-CTLA-4) [9]. Both patients experienced an anti-tumor response to therapy. Similarly, allograft tolerance remained intact in two liver-transplanted patients who received ipilimumab for metastatic melanoma [10,11]. One [11] experienced increased AST and ALT levels (grade 3) and no hyperbilirubinemia in week 16 of treatment which resolved later. There was no evidence of graft rejection. In a recent report by Alhamad et al., [12] the use of pembrolizumab (anti-PD-1 antibody) in kidney transplant recipient with metastatic melanoma was associated with biopsy-proven rejection. Although treatment with methylprednisolone was initiated, kidney function was not recovered and the patient ended up on hemodialysis. Lipson et al., [5] has also demonstrated that anti-PD-1 antibody (pembrolizumab) administration to a kidney transplant recipient with metastatic cutaneous squamous-cell carcinoma was associated with a robust antitumor response along with kidney allograft rejection. Despite administration of high-dose glucocorticoids, the patient's transplanted kidney did not recover [5].

The administration of immune checkpoint inhibitors in organ transplant recipients might be associated with some adverse clinical impact. Immune checkpoint inhibitors require a competent T cell population to carry out their antitumor function, and co-administration of immunosuppressive agents to prevent graft rejection may impact the antitumoral efficacy of the immune checkpoint inhibitors. Our patient clearly experienced tumor progression while on nivolumab therapy. In addition, the ability of nivolumab to activate T cells specific for non-self antigens expressed by the allograft, might precipitate organ rejection. However, liver grafts are considered to be the least immunogenic organs for transplant and thus can sustain less aggressive immunosuppressive regimens; evidently, our patient did not develop acute cellular rejection. It is important to identify the patients who may tolerate a reduction of immunosuppression

as well as the use of immunomodulatory agents; for instance, increased length of time from transplantation may serve as a predictive factor for tolerance of immunosuppression withdrawal. Due to the conflicting reports highlighted above, further studies in a large patient cohort are warranted to study the potential effects of immune checkpoint inhibitors in immunocompromised patients. The risks surrounding graft rejection in the setting of immune activation with these agents must be carefully considered when making treatment decisions in this population. Our case shows that the antitumoral effect of PD-1 inhibitors might be compromised in the context of long-term immunosuppression in a patient with HCC and that it can be associated with severe and fatal hepatitis. Similarly, in kidney-transplanted patients with metastatic melanoma, the administration of anti-PD-1 was associated with a robust antitumor response, yet, the kidney allografts were rejected and the patients ended up on hemodialysis [5,12]. In contrast, administration of anti-CTLA-4 to kidney-transplanted patients was associated with an anti-tumor response without an effect on the allografts [9-11], suggesting that the CTLA-4 pathway might have an advantage over PD-1 pathway in the transplant setting, although the number of cases reported is very small.

The transplant community must be aware of the potential risk of immune-mediated hepatitis in liver transplant recipients considering use of anti-checkpoint inhibitor antibodies. Close monitoring of liver function and moderate reduction of immunosuppression are warranted. The benefits of tumor regression versus the potential risk of allograft rejection and allograft loss should be weighed carefully for each individual patient. Additionally, the utility of the reduction of immunosuppressive therapy and its relative contribution to the overall antitumor effect should be investigated.

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