Nivolumab and anti-HCV activity, a case report

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Laura Wuyts[a], Annelies Janssens[c], Luisa Vonghia[a,b], Peter Michielsen[a], Jo Raskin[c], Ann Driessen[d], Stijn Van Hees[a,b], Sven Francque[a,b], Thomas Vanwolleghem[a,b]

[a] Department of Gastroenterology and Hepatology, Antwerp University Hospital (UZA), B-2650 Antwerp, Belgium; [b]Translational Sciences in Inflammation and Immunology (TWI2N), Faculty of Medicine and Health Sciences, University of Antwerp, B-2610 Wilrijk (Antwerp), Belgium; [c]Department of Thoracic Oncology, Antwerp University Hospital (UZA), B-2650 Antwerp, Belgium; [d]Department of Pathology, Antwerp University Hospital (UZA), B-2650 Antwerp, Belgium; .

Correspondence:

Dr. Laura Wuyts

Department of Gastroenterology and Hepatology

Antwerp University Hospital (UZA)

Wilrijkstraat 10

B-2650 Antwerp, Belgium

Tel: +32 38213323

E-mail: laura.wuyts@uza.be

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Exhaustion of antigen-specific T-cells in order to escape immune destruction is frequently seen in chronic viral infection and different types of cancer. Blockade of overexpressed negative co-stimulatory pathways, a process known as immune checkpoint modulation, is a promising novel therapy that could improve the treatment of liver diseases with features of T cell exhaustion. We present a case of a 54-year-old hepatitis C virus (HCV) positive patient with an acute flare of hepatitis during nivolumab treatment for a stage IV lung carcinoma, an anti programmed death-1 (PD-1)-immunotherapy. Retrospective testing of HCV RNA documented infection more than 6 months ago. Nivolumab treatment was associated with a Alanine Aminotransferase (ALT) flare reaching a peak value of 663 U/L, along with bilirubin levels of 0.74 mg/dL and no signs of coagulopathy. The assumption of a nivolumab-associated autoimmune hepatitis led to the interruption of the immune checkpoint inhibitor treatment. However, a subsequent 1-log decrease of HCV RNA load was noticed, which raised the possibility of an immune reconstitution against the HCV-infected hepatocytes with cell lysis. Liver biopsy specimen demonstrated no evidence for autoimmune liver disease or fibrosis. Clinical evolution was favourable and serum transaminases returned to normal levels and HCV RNA load increased to baseline values following nivolumab cessation. The current case suggests an anti HCV activity of anti PD-1 treatment in the setting of concomitant HCV viraemia and lung carcinoma.

Introduction

Exhaustion of antigen-specific T cells has frequently been described in different types of cancer and chronic viral infections due to continuous triggering with (non-self) antigens. Exhausted T cells display negative costimulatory molecules on their surface, such as programmed death-1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), referred to as immune checkpoints. This makes immune checkpoints attractive therapeutic targets for both cancer as well as chronic viral infections. The effect of immune checkpoint inhibitors on different cancer types has been well studied. As early as 2010, reports have shown that anti PD-1 antibody treatments can be potent immuno-oncological agents in patients with metastatic melanoma [1].

We present a case of a 54-year-old man chronically infected with hepatitis C virus (HCV), developing an hepatitis flare under nivolumab therapy, used for treatment of a stage IV lung carcinoma.

Case

Our patient, a 54-year-old man, presented with an acute hepatitis flare associated with nivolumab treatment, a PD1-inhibitor. He was diagnosed with stage IV lung carcinoma approximately two years before (adenocarcinoma cT3 N2 M1b with metastasis to his 4th rib). First-line chemotherapy with carboplatin/pemetrexed was discontinued after only one cycle due to subjective intolerance. In the following months he received antalgic radiotherapy on the bone metastasis of the 4th rib (5x4Gy). Five months before starting anti PD1-therapy, he endured recurrent abdominal pain, suspicious for metastasis of the kidney for which he received a Celiac plexus block.

Chronic medication consisted of denosumab, pregabalin, fentanyl patch, oxycodone hydrochloride, calcium and vitamin D supplements, acetaminophen upon need and quetiapine.

His social history was notable for repeatedly travelling to the Philippines, including several episodes of unprotected sexual intercourse and a tattoo some years ago in a possibly unhygienic setting.

He was referred to the outpatient hepatology clinics because of HCV seropositivity with an increase of gamma-glutamyltransferase (GGT) levels to 65 U/L and normal Alanine Aminotransferase (ALT) and bilirubin levels as lab parameters. HCV RNA turned out to be positive with a viral load of 6.41 log IU/mL, genotype 1b. Retrospective testing of HCV RNA documented exposure for at least 6 months (*Figure 1).* At the time of referral the patient had just been started on nivolumab 2 mg/kg every three weeks because of PDL-1 expression of 80% on the initial tumour biopsy. After the 8th cycle of nivolumab, an ALT flare was seen with a peak value of 663 U/L, along with bilirubin values of 0.74 mg/dL, GGT values of 100 U/L, a normal leukocyte count, and no signs of coagulopathy. The laboratory derangements were accompanied with a feeling of exhaustion, but no further clinical signs. Autoimmune serology showed an antinuclear factor (ANA)-positivity with a titre of 1:320 (nuclear, dotted pattern), other autoimmune serology markers were not significantly increased. Viral serology other than HCV was negative and there were no potential hepatotoxic causes.

The assumption of a nivolumab associated autoimmune hepatitis led to the interruption of the immune checkpoint inhibitor treatment for the duration of one month. However, a subsequent 1-log decrease of HCV RNA load was noticed, which raised the possibility of an immune reconstitution against the HCV-infected hepatocytes with cell lysis. On liver biopsy there was a combination of signs with a predominant

centrolobular inflammation and loss of hepatocytes, and periportal hepatitis, but no interfase hepatitis. Besides some MUM-1+ plasma cells, no other histological signs for autoimmune liver disease or fibrosis was found *(Figure 2).* Subsequently, ANA-titres declined to 1:160. Clinical evolution was favourable and serum transaminases returned to normal *(Figure 1).* To prevent progression of cancer metastasis, nivolumab was reintroduced at the same dose. After 3 further cycles, nivolumab (*i.e.* 12th cycle) had to be discontinued because of subjective intolerance. Liver tests remained stable *(Figure 1).*

Because of general deterioration, treatment was halted and palliative care was increased. The patient died due to disease progression one year after initiation of nivolumab therapy.

Discussion

Chronic hepatotropic viruses and tumoral cells of hepatocellular carcinoma (HCC) develop mechanisms to induce exhaustion of the antigen-specific T cells in order to escape immune destruction. Blockade of overexpressed negative co-stimulatory pathways, a process known as immune checkpoint modulation, is a promising novel therapy that could improve the treatment of liver diseases with features of T cell exhaustion [2,3].

Nivolumab, a PD-1 immune checkpoint inhibitor and a fully human IgG4 monoclonal antibody, is Food and Drug Administration (FDA)-approved for the treatment of several solid tumours, including lung carcinoma, and haematological malignancies [4]. The FDA previously granted orphan drug designation to nivolumab for the treatment of HCC [5], because of objective response rates of 10%-20% in advanced HCC patients [2,4]. Other PD-1 blockade agents are currently undergoing

clinical trials in advanced HCC patients [6]. Nivolumab is being evaluated in the Check Mate 459 phase 3 trial (NCT02576509) in comparison to sorafenib as a first-line treatment in patients with advanced HCC. This trial will likely provide more insights into the safety and efficacy of nivolumab in this setting.

Theoretically, this treatment may result in both antiviral and antitumour activity in a single patient. We here present a case of a 54-year-old man chronically infected with HCV, developing an hepatitis flare under nivolumab therapy for a stage IV lung carcinoma.

Differential diagnoses included drug-induced hepatitis, autoimmune hepatitis and an antiviral effect of checkpoint-inhibitor therapy. Histology was equivocal with signs of toxic hepatitis, but also periportal inflammation possibly related to the underlying HCV infection and plasma cell positivity, as a sign of auto-immune phenomena. Drug-induced hepatitis was however deemed less probable since quetiapine and acetaminophen were only taken in low doses and no other culprit medication was recently introduced, except for nivolumab.

Activation of the immune system by immune checkpoint inhibitors may also lead to immune responses directed at normal tissue and consequently induce autoimmune adverse events. These most commonly affect the skin (rash, xerosis, pruritus), the gastrointestinal tract (colitis, diarrhoea), the liver (hepatitis), and endocrine organs (hypophysitis, thyroiditis). Any organ may be affected [1]. On the other hand, data from prospective studies suggest that the safety profile of nivolumab is manageable in chronically HCV infected patients [2,4]. In phase I-II studies of

nivolumab for HCC, a total of 262 patients were included, of which 48 in a dose escalation phase (0.1-10 mg/kg/2 weeks) and 24 in a dose expansion phase (3 mg/kg/2weeks). Three (6%) patients had treatment-related serious adverse events, two of which a grade ≥ 3 hepatitis, as described by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE): cytolysis and/or cholestasis more than 5 times, bilirubin more than 3 times upper limit of normal (ULN) [2,4]. In a phase III study with pembrolizumab, a humanized IgG4 kappa monoclonal anti PD1 antibody, a grade 3/4 hepatoxicity was seen in 4% of patients [7].

Autoimmune serologies were not disclosed in the three previously mentioned studies, nor were liver biopsies performed in order to detect a nivolumab-associated autoimmune hepatitis. In an observational study of 536 patients treated with anti PD1 or CTLA-4 immunotherapies, serological and histopathological examinations of checkpoint inhibitor hepatotoxicity were performed and a total of 3.5% of patients experienced a grade ≥ 3 hepatitis. Autoantibodies were either negative or present in low titres (1:80) [8]. In our patient, some plasma cells were identified on liver histology, but the absence of interfase hepatitis, the centrolobular inflammation and hepatocyte cell loss, do not favour a diagnosis of auto-immune related hepatitis. Furthermore, except for an ANA-titre of 1/320, no other autoimmune markers were found to be elevated.

A chronic HCV infection was retrospectively diagnosed after initiation of nivolumab. Theoretically a hepatitis caused by viral reactivation seemed possible but was considered unlikely: viral flares of HCV are not known to occur during any immune modulatory therapy. It is important to note that some cases of hepatitis B virus (HBV) reactivation due to immune checkpoint inhibitors have been reported [9-11]. Indeed, immune control for HBV differs from HCV and HBV flares have been

described during treatment with B cell depleting agents, chemotherapeutics or monoclonal antibodies against Tumour Necrosis Factor (TNF)-α [10].

Host induced flares, characterised by reinforced immune responses against viral antigens with concomitant cell lysis, are more likely in this context. Evidence of antiviral effects of immune checkpoint inhibitor treatment in chronic HCV is accumulating. However, the association with ALT increase is not always reported. A single dose of nivolumab was tested in 54 chronic HCV infected patients, many of whom had undergone interpheron (IFN)-α treatment. Five patients (9.2%) who received BMS-936558 (0.1 or 10 mg/kg) and one placebo patient achieved a reduction in HCV RNA ≥ 0.5-log10 IU/mL on at least 2 consecutive visits; 3 (5.6%) (10 mg/kg) achieved a > 4-log10 reduction. Two patients (3.7%) (10 mg/kg) achieved HCV RNA below the lower limit of quantitation (25 IU/mL), one of whom remained RNA-undetectable one year post-study. In all 5 patients there were ALT increases preceding the viral load reduction [12-13].

The antiviral effect of repetitive treatment with nivolumab has also been studied. In phase I-II studies of nivolumab for HCC no viral reactivation or worsening of viraemia were observed in 51 patients with HBV (of note, patients with HBV infection were required to be receiving effective antiviral therapy and to have a viral load less than 100 IU/mL at screening) and 50 HCV infected patients. No patient achieved a sustained virological response for more than 24 weeks. Some patients had, however, transient reductions in HCV RNA, which was not further specified. The presence of an accompanying concurrent or preceding ALT increase was also not reported in this study [2,4]. In a retrospective analysis of HBV or HCV-infected patients (n=14 and n=14 resp.) receiving anti PD-1 immune checkpoint inhibitor for various solid tumours, a > 1-

log decrease of HCV RNA load was observed in 5/28 patients (all of which were receiving concurrent antiviral therapy) and none of them experienced an acute hepatitis [15]. In a phase III study pembrolizumab, another anti PD-1 immune checkpoint inhibitor in clinical development, was administered to 413 sorafenib-refractory advanced HCC patients and no viral reactivation or flare were observed in 101 HBV or 64 HCV patients. Whether there were any viral load decreases was not mentioned [6-7]. A prospective case series of HCV-infected patients with solid tumours receiving immune checkpoint inhibitors reported a hepatitis flare (ALT increase $\geq$ 3 times ULN) in 3 out of 4 cases, but neither HCV reactivation (HCV RNA $\geq$ 1 log10 IU/mL over baseline), nor HCV-associated hepatitis (HCV reactivation and hepatitis flare) was described. The presence of HCV RNA decreases was not mentioned [16].

These findings all support the potential antiviral benefit derived from immunotherapy in treating chronic HCV infections, although seldom persisting viral control is obtained [4, 12-15, 17]. In our patient a 1-log decrease of HCV RNA load under nivolumab treatment was observed by retrospective analysis of viral loads, which returned to baseline after nivolumab withdrawal. HCV RNA generally varies < 0.5 log10 IU/mL in patients with established HCV infection and spontaneous remissions are rare [13], reflecting the possible relationship of the observed reductions in viral load in our patient to nivolumab treatment.

Whether the observed ALT flare is due to this increased anti HCV response is difficult to ascertain. Indeed, most of the reported HCV reductions during anti PD-1 immune checkpoint inhibitor treatment, were not associated with a steep ALT increase. One of the factors involved is probably the variable number of HCV-infected hepatocytes in chronically infected patients, that ranges from 1%-54% [18]. Reinforced

immune responses that target half of the liver would probably lead to more pronounced ALT flares, compared to only 1%. Finally, we cannot exclude that separate from an anti HCV response, a direct anti PD1 hepatotoxicity in our patient resulted in recruitment of immune cells, with secondary antiviral activity through bystander effects [19].

In conclusion, in our patient the combination of high ALT levels with a transient 1-log drop in HCV RNA load may be ascribed to antiviral immune reconstitution during nivolumab treatment. The potential role of immune checkpoint inhibitors in the management of chronic viral infections remains unclear and is to be further investigated.

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Conflicts of interest: none.

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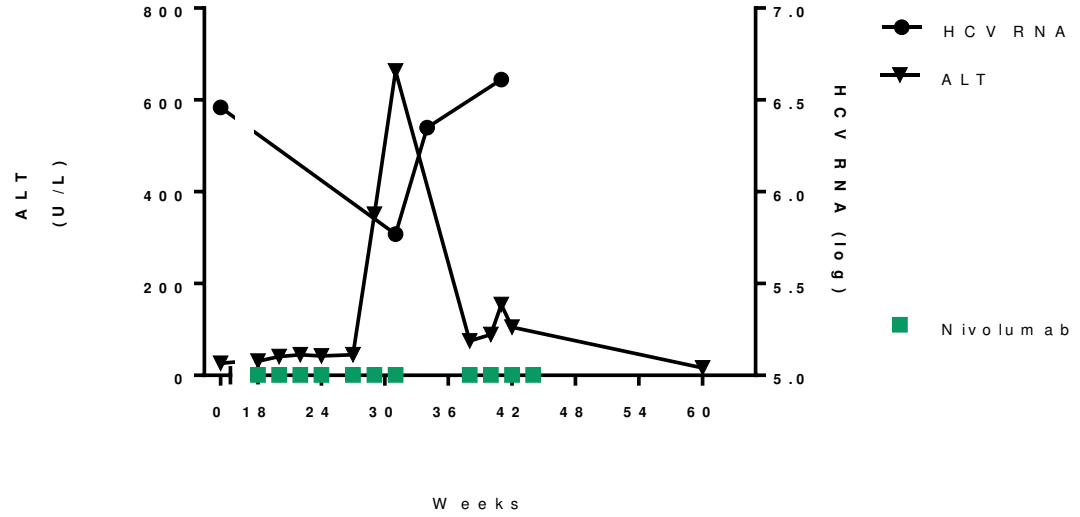
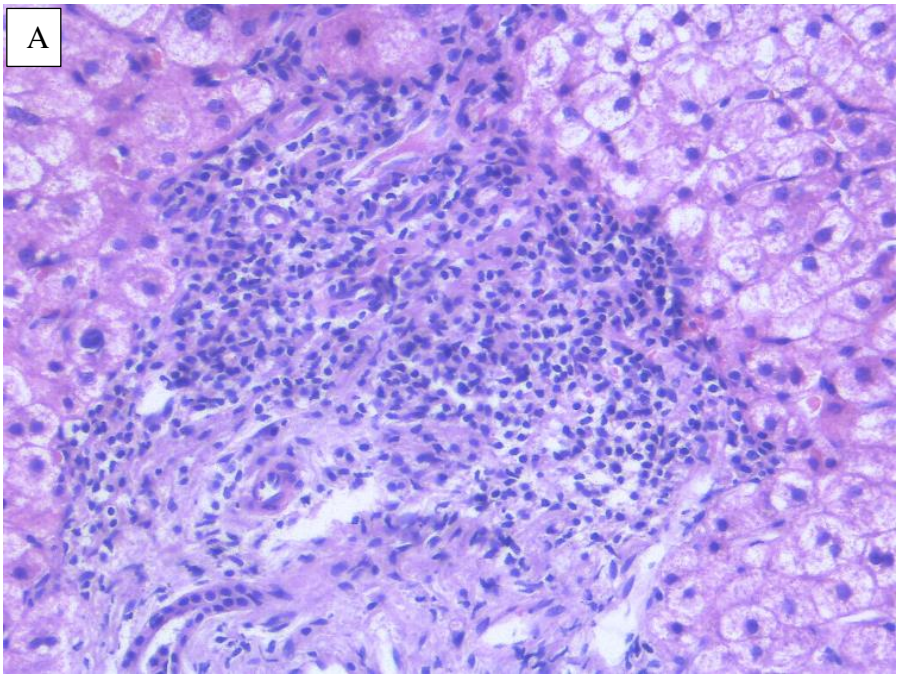
Figure 1.

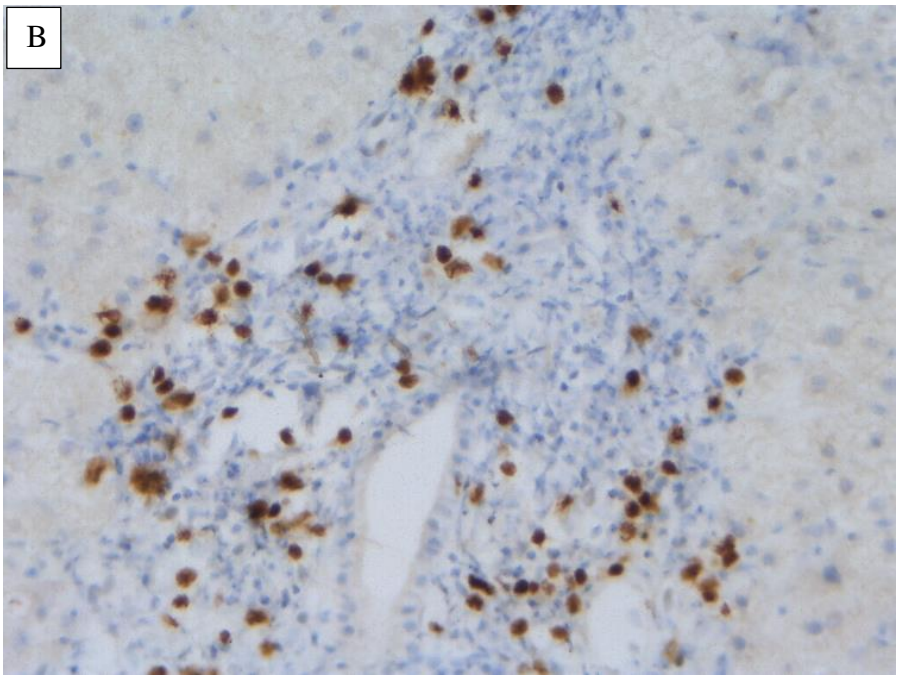


HCV RNA and ALT changes in our patient alongside nivolumab administrations. In our patient preceding high ALT levels are associated with a transient 1-log HCV RNA load decrease suggestive of a host induced flare under nivolumab treatment.

Figure 2.



A: Liver biopsy demonstrating absence of fibrosis and a dense lymphoid infiltrate within the portal fields. Plasma cells are seen in Haematoxylin and eosin stain.



B: Immunohistochemical staining for Multiple Myeloma Oncogene 1 (MUM-1) confirming the presence of plasma cells.