

Case report

A rare case of hepatic vanishing bile duct syndrome occurring after combination therapy with nivolumab and cabozantinib in a patient with renal carcinoma

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Abstract: Tyrosine kinase inhibitors (TKI) and immune checkpoint inhibitors (ICI) significantly improve the outcomes of patients with advanced clear cell renal cell carcinoma (ccRCC), but high-grade toxicities can occur, particularly during combination therapy. Herein, we report a patient with advanced metastatic ccRCC, who developed grade 4 cholestasis during combined therapy with nivolumab and cabozantinib. After the exclusion of common disorders associated with cholestasis and a failure of corticosteroids (CS), a liver biopsy was performed that demonstrated severe ductopenia. Consequently, a diagnosis of vanishing bile duct syndrome related to TKI and ICI administration was made, resulting in CS discontinuation and ursodeoxycholic acid administration. After a 7-months follow-up, liver tests had returned to normal values. Immunological studies revealed that our patient had developed robust T-cells and macrophages infiltrates in his lung metastasis, as well as in skin and liver tissues at the onset of toxicities. At the same time, peripheral blood immunophenotyping revealed significant changes in T-cell subsets suggesting their potential role in the pathophysiology of the disease.

Keywords: Checkpoint inhibitors; Immune related adverse events; Cholestasis; Severe ductopenia; Vanishing bile duct syndrome

1. Introduction

During the last decade, targeted therapies such as the new-generation tyrosine kinase inhibitors (TKI) and immune checkpoint inhibitors (ICI), have transformed the treatment of clear cell renal cell carcinoma (ccRCC) in the advanced stages of the disease[1-5]. Despite the excellent results observed with these drugs, a significant proportion of patients never benefits from treatment and most initial responders will ultimately develop progressive disease.

Another drawback is represented by the burden of added toxicity associated with combination strategy. Regarding overlapping toxicities, all vital organs can be affected, including the liver. In clinical trials, hepatitis was observed in up to 50% of patients during TKI treatment, but was generally transient and low grade, self-resolving without treatment discontinuation[6]. Severe hepatotoxicity also varies according to the VEGFR-TKI molecules (e.g. pazopanib, sunitinib)[6]. The management of such toxicities is often difficult, partially owing to a poor understanding of their underlying pathogenesis, particularly in the setting of dual therapy due to the overlapping spectra of toxicity[6, 7]. Safety

data related to the concomitant use of ICI and TKI are issued from early phases of clinical trials or case reports, mostly corresponding to manageable toxicities which resolved after treatment discontinuation and/or administration of low dose corticosteroids (CS)[8].

Herein, we report a rare case of vanishing bile duct syndrome (VBDS) that arose after concomitant treatment with cabozantinib and nivolumab in the setting of an advanced metastatic ccRCC. A detailed assessment of the peripheral blood and tissue immune responses is then presented.

2. Case Report

A 58-year-old man with a history of mRCC was hospitalized in the setting of a pruritic cutaneous rash, grade IV cholestasis (total bilirubin: 28 mg/dL, including 25 mg/dL conjugated; alkaline phosphatase: 805 UI/L) and grade IV hepatitis (aspartate aminotransferase: 694 UI/L; alanine aminotransferase: 1137 UI/L). This occurred one month after the introduction of a TKI inhibitor (cabozantinib, 40 mg/day) to his current treatment with nivolumab (3 mg/kg/3 weeks) started in the context of metastatic progression 8 months before. There were no recent medication changes except the introduction of cabozantinib. Physical examination revealed a diffuse maculopapular skin eruption and conjunctival icterus, without any other sign of hepatic failure.

Blood analyses rapidly ruled out infectious, metabolic and autoimmune diseases. Ultrasound imaging and liver MRI excluded hepatic metastasis and obstructive biliary disease. A first liver biopsy was performed and showed images of centrilobular necrosis and cholangitis with poor mixed inflammation consisting of eosinophils and lymphocytes, consistent with a drug reaction (Figure 1, A-B). According to the presumed ICI-related adverse event (irAE) and/or TKI toxicity, nivolumab and cabozantinib were discontinued, and high dose CS were started (methylprednisolone, 2 mg/kg/d). Seven days later, no improvement in serum bilirubinaemia was observed, prompting the addition of oral mycophenolate mofetil (MMF, 1g/d) to CS according to ESMO guidelines [9]. Four weeks after dual immunosuppressive therapy and the lack of improvement in liver tests, CS were tapered to minimize side effects and a second biopsy was performed.

Remarkably, this second biopsy revealed a severe ductopenia with limited immune infiltrate within liver parenchyma, suggesting vanishing bile duct syndrome diagnosis (Figure 2). Accordingly, immunosuppressors were slowly tapered and ursodeoxycholic acid (UDCA) was started (5 mg/kg/d, with a progressive increase to 15 mg/kg/d). After 3 months, a slow decrease in serum conjugated bilirubin level was observed until its normalization at 7 months.

3. Discussion

There has been significant progress in the treatment of patients with mRCC, especially with the recent approval of TKI and immunotherapy combination as first-line treatment. In 2019, the FDA approved pembrolizumab (anti-PD-1) plus axitinib therapy, the combo demonstrating survival advantages compared to sunitinib[10]. Since then, the combination of avelumab (anti-PD-L1) with axitinib has also been compared to sunitinib and finally approved (survival data are still immature; follow-up for the final analysis is ongoing)[11]. Despite their efficacy, the major concerns associated with these dual therapies are the potential cumulated and/or overlapping toxicities.

(Case)

Here we report an exceptional hepatic manifestation, namely a vanishing bile duct syndrome, in a patient receiving cabozantinib and nivolumab for metastatic progression of a mRCC. VBDS is a rare condition in which patients develop chronic cholestasis associated with ductopenia, defined as loss of interlobular bile ducts in >50% of portal areas

on histological examination[12]. Causes of ductopenia are diverse and include drugs (e.g., antibiotics, anticonvulsants), immune disorders (e.g., primary biliary cholangitis), infectious diseases (EBV, CMV) and haematological malignancies (Hodgkin lymphoma)[12]. To our knowledge, this case represents the first VBDS occurring during VEGFR-TKI/ICI combination to date, although Zhong and al. reported the onset of a fatal VBDS 25 days after pazopanib introduction, directly succeeding pembrolizumab therapy[13]. In our case, the recent initiation of cabozantinib suggests its potential causative role although grade IV cholestasis seems rare and often explained by other factors during VEGFR-TKI therapy (e.g., hepatic insufficiency associated with hepatocellular carcinoma)[6, 14, 15].

On the other hand, ICI have been associated with various types of liver impairment, including marked cholestatic hepatitis with recent case reports describing steroid and MMF therapy-resistant disease where the biliary tract seems the major target of injury, even if hepatocellular damages are also present[16, 17]. Moreover, three cases of VBDS occurred in patients receiving ICI alone while no reports of TKI-related VBDS could be retrieved from the literature [7, 18, 19]. Table 1 summarizes the main clinical features of those patients. Briefly, VBDS was always associated with pembrolizumab use, appeared after an a single injection in two cases and induced a mean elevation of conjugated bilirubinemia of 26,3 mg/dL. The ICI was discontinued in all patients, and CS, MMF and UDCA were used in two cases while the remaining patient declined any therapy. Surprisingly, this last patient normalized his hepatic tests after 16 weeks, while another required CS and UDCA to achieve the same outcome. The third patient was ultimately treated by plasmapheresis and died 26 days after cholestasis onset. These observations, including ours, suggest that ductal loss leading to VBDS could explain why immunosuppressive therapy could be ineffective once ductal damages appear. In our case, whether cabozantinib had enhanced the immune response especially towards ductal tissue or impaired cellular regeneration after immune destruction is unknown, but could explain the timescale between VEGFR-TKI initiation and cholestasis occurrence.

(Biomarkers)

Given the unique features of this case and the absence of any relevant pathophysiologic mechanism underlying VBDS during ICI/VEGFR-TKI therapy, we decided to assess local immune responses occurring in our patient at the onset of adverse events. First, we studied tissue immune markers in lung metastasis at disease progression, and in skin and liver tissues at the onset of irAEs using multiplex immunohistochemistry (mIHC) on Formalin-fixed and paraffin-embedded (FFPE) tissue blocks coming from biopsies from lung metastasis (January 2019), skin eruption (November 2019), and liver at the onset of the irAES (November 2019 and January 2020). Description tissue immunophenotyping using mIHC is presented in Supplementary Figure S1.

Liver biopsies were taken before (PRE) and on (ON) corticosteroid treatment (Figure 3, A). We measured the relative amount (percentage in total cells) of CD4+, CD8+ T cells, CD20+ B cells, FOXP3+ regulatory T cells (Treg), and CD68+ macrophages within the stroma and the epithelium area of each tissue. All tissues showed immune cell infiltration including CD4+, CD8+ T cells, and macrophages, both in the stroma and epithelium areas (Figure 3, B). B cells and Treg were less present. Interestingly, T cell infiltration in liver was reduced by corticosteroid treatment, both CD4+ (15% and 3% of total cells in the stroma, respectively) and CD8+ (27% and 7% of total cells in the stroma, respectively) T cells. CD68+ macrophages were not impacted by corticosteroid treatment in liver.

In line with these observations, robust T-cell infiltration, activation, and clonal expansion were already described in cardiac and skeletal muscle of two patients with melanoma in whom fatal myocarditis developed after treatment with ipilimumab and nivolumab[20]. Further studies of T cells reactivity within tumor microenvironment are needed to elucidate their contribution in toxicities.

Metastatic ccRCC patients treated with nivolumab from the CheckMate-010 trial revealed that high percentage of CD8+ tumor infiltrating lymphocytes (TIL) that are PD-

1+TIM-3-LAG-3- within tumor microenvironment (assessed by mIHC) correlated with high levels of T-cell activation, including cytolytic activity, effector T-cells and IFN γ response, and was associated with longer median immune-related PFS and higher odds ratio rate on nivolumab [21]. However, CyTOF analyses on clinical samples from melanoma patients treated with ICI didn't show any association between the frequency of PD-1 expression on either CD4+ or CD8+ T cells with clinical benefit[22]. In the current study, the presence of PD1+ T cells were not assessed by mIHC, however increase of PD1+ CD4+ and CD8+ T cell frequencies was observed in the PB on CS therapy at the onset of toxicities. Further analyses are required to understand whether the expression of PD-1 reflects T cell exhaustion/dysfunction or activation of anti-tumor response and whether these cells could be responsible for hepatic duct injury in this setting of ICI/TKI-induced VBDS.

4. Conclusions

Given overlapping hepatic toxicities of TKI and ICI, severe liver impairment can occur with a delayed management because of the poor understanding of the underlying pathogenesis. Although data concerning toxicity and management of each drug used as monotherapy are more and more available, the current lack of knowledge regarding potential toxicity of their combined administration should prompt thorough evaluation including liver biopsy, case reporting and translational research. Particularly, the occurrence of immunosuppressor-resistant cholestatic hepatitis should raise suspicion for vanishing bile duct syndrome in patients treated with TKI/ICI bitherapy.

Author Contributions: AG, FL, JC, MLF and TG managed the patient. AL was the expert consultant in hepatology. MGG performed the histopathological diagnosis. SG and KWG performed the immunological analysis. JC, JK, KG, MLF and SG wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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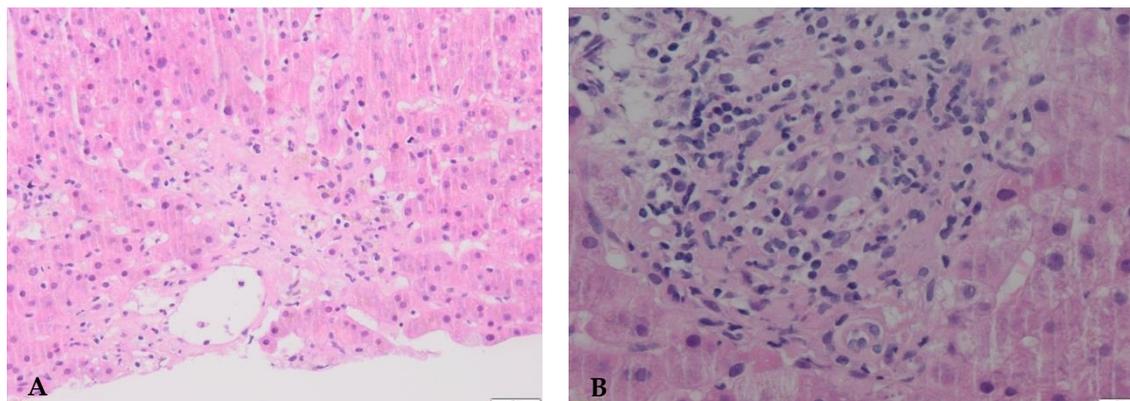


Figure 1. First liver biopsy performed at presentation. Hematoxylin and eosin-stained sections. A: Liver parenchyma is characterized by centrilobular necrosis, sometimes confluent. B: Immune infiltrate predominates around portal areas and is mainly composed of lymphocytes and rare eosinophils.

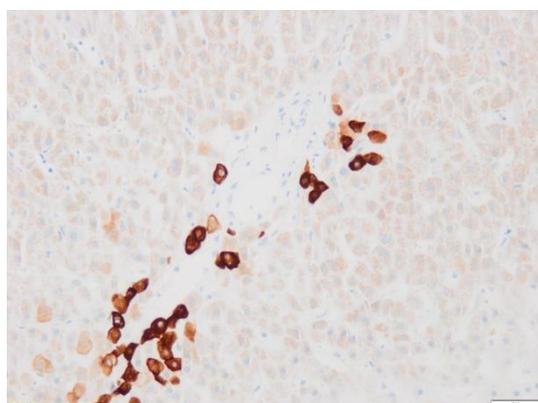
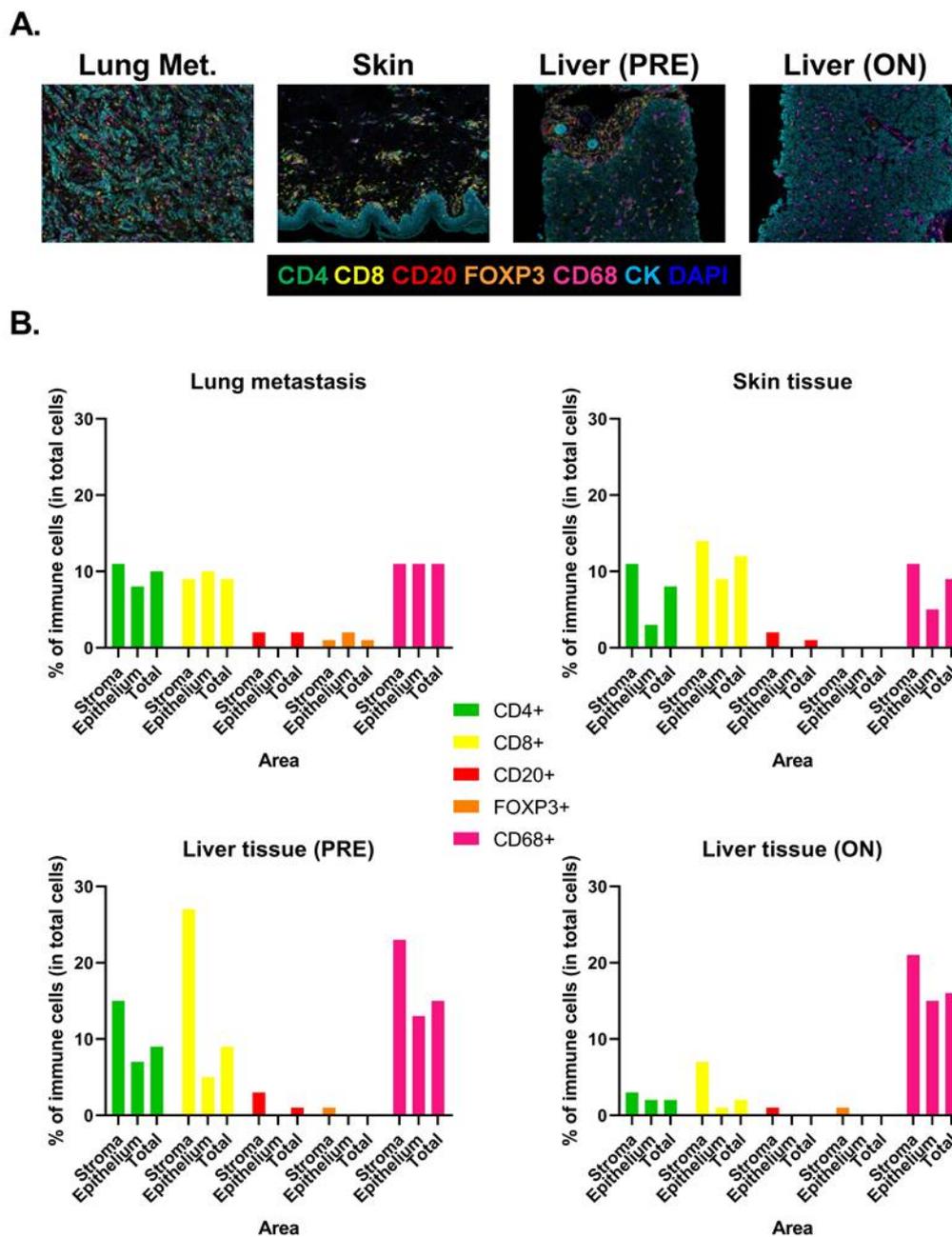


Figure 2. Second liver biopsy performed 5 weeks after cholestasis onset. Immunohistochemical staining with anti-cytokeratin-7 (CK7) antibody. Large interlobular bile ducts are absent as anti-CK7 staining only reveal ductular reaction. These findings are suggestive of ductopenia.

Figure 3



- Figure 3:** Tissue immune infiltration at disease progression and at the onset of toxicities.
 - Representative fluorescent multiplexed IHC images of lung metastasis, skin tissue and liver tissues before (PRE) and on (ON) corticosteroid treatment for both first and second biopsy, and MMF only for the second biopsy. Tumor-infiltrating lymphocytes were revealed by CD20 (red), CD8 (yellow) and CD4 (green), FOXP3 (blue), CD68 (magenta), and tumor cells by panCK (cyan).
 - Quantification of immune cell as percent of total cells within the stroma, the epithelium, and total area of each tissue

Table 1. Published cases of vanishing bile duct syndromes occurring during immune checkpoint inhibitor and/or tyrosine kinase inhibitor therapy.

| Reference | Neoplasia | Suspected causal therapy | Time-to-onset | Peak c-bilirubin / ALAT | Signs of hepatic failure | Therapy | Issue |
|------------------------------|-----------|---|----------------|-------------------------|--------------------------|--------------------------------------|---|
| Doherty, 2017 (ref) | Melanoma | Pembrolizumab | 1 cycle (8d) | 23 mg/dL 1536 IU/L | Absent | CS 1m/kg, MMF, UDCA | Normalized c-bili under CS and UDCA therapy |
| Thorsteinsdottir, 2020 (ref) | Melanoma | Pembrolizumab | 12 cycles | 32 mg/dL 450 IU/L | Absent | CS 2mg/kg, MMF, UDCA, plasmapheresis | Death (d+26 after cholestasis onset) |
| Zhong, 2020 (ref) | mRCC | Pembrolizumab (previously) Pazopanib (ongoing) | 5 cycles (25d) | 22 mg/dL 800 IU/L | Absent | CS 2mg/kg, UDCA | Death (d+30 after cholestasis onset) |
| Masetti, 2021 (ref) | NSCLC | Pembrolizumab | 1 cycle (21d) | 24 mg/dL 818 IU/L | Absent | None (patient refusal) | Normalized c-bili after 16 weeks |
| Present case | mRCC | Nivolumab, Cabozantinib (both ongoing) | 6 cycles (28d) | 28 mg/dL 1137 IU/L | Absent | CS 2mg/kg, MMF, UDCA | Normalized c-bili after 28 weeks under UDCA therapy |

ase;

bilirubin; CS, corticosteroids; d, day; IU, international unit; MMF, mycophenolate mofetil; mRCC, metastatic renal cell carcinoma; NSCLC, non-small cell lung carcinoma; UDCA, ursodeoxycholic acid;

Abbreviations: 33

ALAT, alanine 34

aminotransfer- 35

c-bili, conjugated 36

37

38

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Supplementary material

Tissue immunophenotyping

Four-micrometer sections from full FFPE blocks were used for manual multiplex immunohistochemistry (mIHC) staining according to the manufacturer's instructions (Opal 7 Solid Tumor Immunology kit, Akoya Biosciences®).

The used antibodies were CD4 (80 ng/ml, 90 min, Akoya Biosciences®), CD8 (68 ng/ml, 90 min, Akoya Biosciences®), CD20 (45 ng/ml, 90 min, Akoya Biosciences®), FOXP3 (674 ng/ml, 90 min, Akoya Biosciences®), CD68 (16 ng/ml, 90 min, Akoya Biosciences®), and panCK (400 ng/ml, 90 min, Akoya Biosciences®).

Slides were mounted with Vectashield Hardset Antifade Mounting Medium (VectorLaboratories). Multiplexed slides were scanned on Vectra Polaris Automated Quantitative Pathology Imaging System (Akoya Biosciences®). InForm® Tissue Finder™ software was used in order to deconvolute the multispectral images, to segment tissue, and to segment and phenotype cells. The percentage of cells was calculated on full section except for lung metastasis for which 25 % of the section was multispectrally acquired. Digital quantification was then performed using PhenoptrReports (Akoya Biosciences®). GraphPad Prism8 software was used to graph individual datapoint.