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Liver injury from cancer immunotherapy using monoclonal immune checkpoint inhibitors.

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Title Liver injury from cancer immunotherapy using monoclonal immune checkpoint inhibitors.

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Abbreviations
ALT, alanine aminotransferase; Alk P, alkaline phosphatase; AMA, anti-mitochondrial antibodies; ANA, anti-nuclear antibodies; anti-LKM, anti-liver-kidney microsomal antibodies; anti-LC1, anti-liver cytosol type1 antibodies; ASMA, anti-smooth muscles antibodies; anti-SLA, anti-soluble liver antigen antibodies; AST, aspartate aminotransferase; CTLA-4, cytotoxic T lymphocyte antigen 4; DILI, drug-induced liver injury; GGT, gamma-glutamyl transferase; INR, international normalized ratio; IRAEs, immune-related adverse events; PD-1, programmed cell death protein 1; PD-L1-2, programmed cell death ligands 1-2; ULN, upper limit of normal.

Keywords: immunotherapy; immune-related adverse events; immune checkpoints inhibitors

Conflict of interest

EDM: nothing to disclose; JMM: advisory board for BMS; BP: nothing to disclose; SC: lecture and consulting fees from AstraZeneka, BMS, MSD, Roche; CM: nothing to disclose; OL: personal fees from BMS, MSD, AstraZeneca, Genzyme, Janssen; BR: nothing to disclose; TMA: nothing to disclose; AC: nothing to disclose; SL: nothing to disclose; CR: discloses consultancy for BMS, GSK, Novartis, Amgen,
BACKGROUND & AIMS

Immunotherapy for metastatic cancer may be complicated by the onset of hepatic immune-related adverse events (IRAEs). This study compared hepatic IRAEs associated with anti-PD-1/PD-L1 and anti-CTLA-4 monoclonal antibodies (mAb).

METHODS

Among 536 patients treated with anti-PD-1/PD-L1 or CTLA-4 immunotherapies, 19 (3.5%) were referred to the liver unit for grade ≥ 3 hepatitis. Among 16 included patients: 9 received anti-PD-1/PD-L1 while 7 received anti-CTLA-4 mAb, in
monotherapy or in combination with anti-PD-1. Liver investigations included viral assays, autoimmune tests and liver biopsy, histological review and immunostaining of liver specimens.

RESULTS
Among 16 included patients 9 (56%) were female, median age 63 [33-84] years. Time between therapy initiation and hepatitis was 5 [1-49] weeks, median doses was 2 [1-36]. No patients developed hepatic failure. Histology related to anti-CTLA-4 mAb demonstrated granulomatous hepatitis including fibrin ring granulomas and central vein endothelitis. Histology related to anti-PD-1/PD-L1 mAb was characterized by lobular hepatitis. The management of hepatic IRAE was tailored according to the severity both biological and histological of liver injury: 6 patients improved spontaneously, 7 received oral corticosteroids at 0.5-1 mg/kg/day, 2 were maintained on 0.2 mg/kg/day corticosteroids and 1 patient required pulses and 2.5 mg/kg/day of corticosteroids, and the addition of a second immunosuppressive drug. In three patients, immunotherapy was reintroduced without recurrence of liver dysfunction.

CONCLUSIONS
Acute hepatitis due to immunotherapy for metastatic cancer is rare (3.5%) and in most cases not severe. Histological assessment can distinguish between anti-PD-1/PD-L1 and anti-CTLA-4 mAb toxicity. The severity of liver injury is helpful to tailoring patient management, which does not require systematically corticosteroid administration.

LAY SUMMARY
In patients receiving immunotherapy for metastatic cancer and developing immune-mediated hepatitis, liver biopsy is helpful for diagnosis and evaluation of the severity of liver injury. This study demonstrated the need for a management patient oriented, which could eventually avoid useless systemic corticosteroid treatment.

Characterization of liver injury caused by cancer immunotherapy using immune checkpoint inhibitors

Introduction

Immune-modulatory therapies have dramatically improved the survival of patients with metastatic tumors. During the development of cancer, the immune system becomes naturally “tolerant” towards cancer cells, which are seen as part of the “self”. This tolerance is maintained by immune checkpoint pathways that down-regulate immune functions, permitting cancer cells to evade immune attacks. Monoclonal antibodies (mAb) directed against regulatory immune checkpoint molecules that inhibit T-cell activation enhance this anti-tumor immunity. Ipilimumab, a human IgG1 mAb, blocks cytotoxic T-lymphocyte antigen 4 (CTLA-4). Pembrolizumab and nivolumab, humanized IgG4 kappa and human IgG4 mAb respectively, block the interaction between programmed cell death protein 1 (PD-1) and the two programmed cell death ligands PD-L1 and PD-L2, by selectively binding the PD-1 receptor. Durvalumab, a human IgG1 kappa mAb, targets the PD-L1.
By unbalancing the immune system, these new immunotherapies may give rise to immune-related adverse events (IRAEs) which mimic autoimmune conditions. The incidence of immune-related acute hepatitis of all grades is estimated to affect between 4% and 9% of patients treated with anti-CTLA-4 mAb, and 18% of patients treated with the combination of anti-PD-1 and anti-CTLA-4 mAb. Liver immune-related adverse events occur more rarely with anti-PD-1 mAb alone, with a reported incidence of 1% to 4% of patients.

The presentation of anti-CTLA-4-related hepatitis remains highly heterogeneous, with symptoms ranging from a mild rise in aspartate aminotransferase levels to the patient's death from fulminant liver failure, and pathological reports are scarce because liver biopsies are rarely performed. As with all IRAEs induced by cancer immunotherapy, clinical guidelines are based on the discontinuation of immunotherapy and the administration of corticosteroids (1-2 mg/kg). Very few data are available to date on liver IRAEs induced by anti-PD-1/PD-L1 mAb, and management recommendations are similar to those used for patients treated with anti-CTLA-4 mAb. Considering the implications of permanently discontinuing a potentially life-saving treatment, as well as the adverse effects of long duration high doses of corticosteroids, it seems essential to clarify the characteristics of these drug-induced liver injuries (DILI). We propose here a comprehensive clinical and pathological description of the hepatic IRAEs associated with immune checkpoint inhibitors, comparing the patterns observed with anti-PD-1/PD-L1 and anti-CTLA-4 treatments.

Methods

Patients

A pharmacovigilance register was set up to focus on the adverse effects of anti-PD-1,
anti-PD-L1 and anti-CTLA-4 immunotherapy (the REISAMIC register) in 2013 at Institut Gustave Roussy, France. Since the beginning of the registry 1425 patients were treated with immune checkpoint inhibitors. However patients were not systematically identified and addressed to the hepatologist since the beginning of the registry. In order to evaluate more accurately the incidence of hepatic IRAEs the study period was limited from August 2015 to April 2017. All patients treated with immune checkpoint inhibitors for metastatic cancer, who developed grade ≥ 3 hepatitis, according to the Common Toxicity Criteria for Adverse Events (CTCAE) of the National Cancer Institute using version 4.03. (Supplementary Table 1), and addressed to the expert liver center, Centre Hepato-Biliaire at Hôpital Paul Brousse, France, were included in this observational study. Severity of liver injury was also classified according to the Drug-Induced Liver Injury Network (DILIN) 5 point scale (Supplementary Table 2). Informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution’s human research committee (Comité de Protection des Personnes – Ile-de-France VII).

Clinical characteristics that included age, gender, past medical history, risk factors for liver disease, concomitant medications and clinical symptoms were recorded. Biological data such as aspartate-aminotransferase (AST) (normal range: 5-55 IU/L), alanine-aminotransferase (ALT) (normal range: 5-55 IU/L), total bilirubin (normal range: <17 μmol/L), alkaline phosphatase (Alk P) (normal range: 30-100 IU/L), gamma-glutamtranspeptidase (GGT) (normal range: 10-45 IU/L), white blood cell count (normal range: 4.00-10.00 x10⁹/L), hemoglobin (normal range: 13-17 g/dL), platelet count (normal range: 150-400 x10⁹/L), prothrombin level (normal range: >70 %), international normalized ratio (INR) and renal function were also recorded. Blood
tests were performed per-protocol before each immunotherapy injection. All the patients included had a consultation with an expert hepatologist and undergone a comprehensive work-up that included anti-tissue antibodies (Ab) (anti-nuclear Ab, ANA; anti-smooth muscles Ab, ASMA, anti-mitochondrial Ab, AMA; anti-liver-kidney microsomal Ab, anti-LKM; anti-liver cytosol type1 Ab, anti-LC1 and anti-soluble liver antigen/liver pancreas antigen Ab, anti-SLA/LP), immunoglobulin G (IgG), measured at admission, serological tests for of hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV) and viral load of cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV) 1 and 2, human herpes virus (HHV) 6 and 8 and HEV. Caeruloplasmin, serum copper concentrations, serum iron concentrations, transferrin saturation levels, serum ferritin levels and urinary toxic substances were determined and urine and blood cultures tested. The causal relationship between the immunotherapy and hepatic IRAEs was assessed using the Roussel Uclaf Causality Assessment Method (RUCAM). The interpretation of the final score is as follows: 0 or less indicate that the drug is “excluded” as a cause; 1 to 2 that it is “unlikely”; 3 to 5 “possible”; 6 to 8 “probable”; and greater than 8, “highly probable”.

Immune checkpoint therapies

The patients received different immunotherapy regimens: a combination of anti-PD-1 nivolumab 3mg/kg + anti-CTLA-4 ipilimumab 1mg/kg intravenously every 3 weeks (4 courses) followed by nivolumab 3g/kg every two weeks; anti-CTLA-4 ipilimumab intravenously 10mg/kg every 3 weeks for 4 infusions; anti-PD-1/PD-L1 treatments (nivolumab intravenously 3 mg/kg every 2 weeks; pembrolizumab 2mg/kg or a flat dose of 200 mg every 3 weeks; durvalumab 20 mg/kg intravenously every 4 weeks).
Between August 2015 and April 2017, 536 patients were treated, 413 with anti-PD-1/PD-L1 mAb, 105 with anti-CTLA-4 mAb and 18 with the combination of anti-PD1 and anti-CTLA4 mAb. Nineteen patients developed grade ≥ 3 hepatitis according to CTCAE system (AST/ALT and/or GGT/Alk P > 5 times ULN and/or total bilirubin > 3 times ULN). Three patients were excluded. One patient was excluded because of HEV acute hepatitis diagnosis. This patient underwent anti-PD-1 therapy for Hodgkin’s lymphoma and presented with acute hepatitis 61 weeks after the initiation of immunotherapy. The course was rapidly and spontaneously favorable and the immunotherapy could be reintroduced. One patient was excluded because of tumor infiltration at histology and one patient because the liver biopsy was not performed due to the extent of liver metastases. Among the 16 included patients: eight were treated with an anti-PD-1 and one with an anti-PD-L1 mAb (9/413, 2%). Seven patients (7/123, 6%) received an anti-CTLA-4 (ipilimumab) including three patients (3/105, 6%) on monotherapy and four patients (4/18, 22%) in combination with nivolumab. Patients who received a combination anti-CTLA-4 and anti-PD-1 mAb were included in the anti-CTLA-4 group for common clinical and histological findings (Fig. 1).

**Histological evaluation**

All patients underwent a liver biopsy. Due to deteriorating liver tests despite corticosteroid treatment, a paired biopsy was performed in one patient (patient 5) with an interval of 16 days. Except one that was fixed in formaldehyde (patient 4), all liver biopsies were fixed in AFA (alcohol, formaldehyde and acetic acid) and paraffin embedded. Sections (4 μm) were then stained with Haematoxylin-Eosin-Saffron, Picro-Sirius and Perls.
The biopsies were reviewed by a single hepatic expert focusing on the following features: portal fibrosis according to the METAVIR classification (F0-F4), portal inflammation (0-3), interface hepatitis according to the METAVIR classification (0-3), lobular inflammation (0-3), type of inflammatory infiltrates, lobular necrosis (spotty or confluent, with percentage and distribution) and steatosis (percentage). A principal pattern of liver injury was defined for each patient.

Immunostaining for CD3, CD4, CD8, FOXP3, ETS-related antigen (ERG), PD1 and PDL-1 was performed in an automatic immunostainer (Bond III, LEICA) on de-paraffinized sections of the 16 biopsies. The antibodies, clones, dilutions and sources are listed in Supplementary Table 3.

Statistical analysis

Continuous variables were expressed by medians and ranges, categorical variables were expressed by number of patients and percentages. Parametric and nonparametric tests were performed when appropriate. The Mann-Whitney test was employed for continuous variables. The Chi-squared test with Fisher’s correction was employed for categorical variables. A p-value <0.05 was considered to be statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY IBM Corp.

Results

Clinical characteristics

In the sixteen patients with histologically proven immune-related hepatitis, the median age was 63 [33-84] years and nine of them were female (56%). One patient had previous risk factors for liver disease consecutive to metabolic syndrome. Two patients had hepatic tumor metastases at the initiation of immune checkpoint
inhibitors. According to the RUCAM scale, the relationship between acute hepatitis and immune checkpoint inhibitors was classified as highly probable in 14 patients (87.5%) as all the other causes of hepatitis were excluded and the advanced pharmacovigilance survey did not identify any other medications or drugs with potential hepatic toxicity. The relationship was classified as probable in 2 patients (12.5%).

Overall population and single patient characteristics are depicted in Table 1 and 2. Overall, five patients (31%) (patients 5, 6, 7, 12, 14) had previously been exposed to another immunotherapy, either anti-PD-1 or anti-CTLA-4 mAb. In particular three patients with acute liver injury after receiving anti-CTLA-4 mAb had previously received an anti-PD-1 mAb with an interval of 4 weeks between the two treatments, while two patients treated with anti-PD-1 mAb had previously received an anti-CTLA-4 mAb with an interval of three and seven months between the two treatments, respectively.

The hepatitis occurred after a median duration of 5 [1-49] weeks since therapy initiation, and after a median of 2 [1-36] doses, six patients received only one dose, among them three received a combination of anti-CTLA-4 and anti-PD-1 mAb and three an anti-PD-1/anti-PD-L1 mAb. Six patients (38%) experienced fever at the onset of hepatitis, five receiving anti-CTL-4 alone or in combination with anti-PD-1 and one receiving anti-PD-1 mAb. A concomitant cutaneous diffuse maculopapular rash was observed in 5 patients (31%): three on anti-CTLA-4 mAb (two monotherapy and one on the combination with anti-PD-1) and two on anti-PD-1 mAb. Four patients (25%) remained strictly asymptomatic and liver tests impairment was found during a per-protocol routine blood test. A previous extra-hepatic immune-related adverse event was identified in 6 patients (38%), including one with a granulomatous pneumonitis, which had been resolved with corticosteroids (patient 11), one with
hypophysitis (patient 12), two with hyperthyroiditis (patients 3 and 9), two with pancreatitis (patients 14 and 16) one with bronchitis (patient 16). No previous hepatic adverse events were reported.

Laboratory test results

Laboratory test before immunotherapy was within the normal range for all but one patient (patient 4) who had GGT at 104 IU/L. Peak levels of laboratory tests included a median ALT of 460 [266-3137] IU/L, AST 437 [147-2289] IU/L, GGT 317 [39-1252] IU/L, Alk P 309 [53-768] IU/L and bilirubin 18 [6-324] μmol/L (Table 1). The R ratios were usually with mixed profile (hepatocellular and cholestatic) at onset, the median ratio being 4 (0-26) and with hepatocellular profile at peak, the median ratio being 6 [1-15]. One patient had a PT nadir at 45% with an INR of 1.91 and high bilirubin, with a normal factor V value. The PT normalized rapidly with the improvement in bilirubin levels. In all other patients, the PT, INR and FV values remained normal.

Autoantibodies were negative or present in low titer only. ANA was present in half of patients but generally in low titer (1:80 in 7 patients) with speckled patter and ASMA was detected in 3 patients (all 1:80), but all without anti-F actin activity positivity. The other autoantibodies tested were negative. The median serum Immunoglobulin IgG level was within the normal range at 9 [6-18] g/dL. EBV-DNA was positive in two patients (3.74 log and 3.57 log), but this was not considered to be clinically significant.

Comparison between anti-CTLA-4 and anti-PD-1/anti-PD-L1 recipients (Table 1)
Patients on anti-PD-1/PD-L1 mAb were older when compared to those receiving anti-CTLA-4 mAb or combination therapy (median age 69 [52-84] years versus 52 [33-65] years, p=0.029). Hepatitis occurred at a median of 14 [2-49] weeks after the initiation of anti-PD-1/PD-L1 treatment, versus a median of 3 [1-7] weeks with anti-CTLA-4 alone or in combination with an anti-PD-1 (p=0.019). Most of the patients who received the anti-CTLA4 mAb presented with fever at the time of hepatitis compared with patients who received anti-PD-1/PD-L1 mAb (p=0.034). A comparison of AST, ALT, GGT, Alk P and IgG values did not reveal any statistically significant difference between patients receiving anti-CTLA-4 versus anti-PD-1/PD-L1 mAb.

**Histological features**

**Histological overview (Table 3).** All sixteen patients underwent initial liver biopsies at the diagnosis of immune-related hepatitis, which revealed active hepatitis with different degrees of severity and different histological patterns of liver parenchyma. The biopsy was performed within 48-120 hours since the rise of liver tests. At the time liver biopsy was performed two patients were already on corticosteroid therapy for the treatment of acute hepatitis and two other patients were on 0.2mg/kg of corticosteroids due to non hepatic IRAEs. In all cases, the inflammatory infiltrates mainly consisted of activated lymphocytes and histiocytes with only very few or no plasma cells. In one case (patient 4), the portal inflammatory infiltrate contained numerous eosinophilic polynuclear cells, knowing that it was the only biopsy fixed in formaldehyde. More than half of the biopsies showed bile duct injury, with lymphocytic cholangitis and/or ductal dystrophy. Eight (50%) patients presented with mild portal fibrosis, despite one of them had cholangiocarcinoma, this finding suggested a possible trend of acute hepatitis towards chronicity.
**Anti-CTLA-4 hepatitis pattern.** Five biopsies from patients with immune-related hepatitis due to anti-CTLA-4 showed a specific pattern of granulomatous hepatitis associated with severe lobular necrotic and inflammatory activity. Granulomas composed of epithelioid cells without multinucleated giant cells were poorly delimited. Fibrin deposits were associated with histiocytic infiltrate in five patients, and in two patients (patient 5 and 7) numerous fibrin-ring granulomas with central lipid vacuoles were observed (Fig. 2A and 2B). Necrosis was either spotty or confluent, and mainly seen in centrilobular areas. Central vein endothelitis was present in all the seven cases (Fig. 2C and 2D).

The sequential paired biopsy performed in patient 5, who underwent initial therapy with anti-PD-1 antibody for 17 months, without any liver enzyme modification and then developed acute hepatitis 7 weeks after initiating anti-CTLA-4 therapy, revealed the disappearance of fibrin-ring granulomas and an evolution towards active chronic hepatitis with portal fibrosis and severe periportal activity (METAVIR equivalent: A3F1). The interval between the withdrawal of anti-PD-1 and the beginning of anti-CTLA-4 was 1 months. Anti-PD-1 immunostaining was negative in the initial biopsy but some positive lymphocytes were seen in the second biopsy. Whereas there was no bile duct injury on the first biopsy, lymphocytic cholangitis and ductal dystrophy appeared on the second biopsy. However no bile duct loss was observed.

**Anti-PD-1/PD-L1 hepatitis pattern.** The histological pattern in the nine liver biopsies from patients receiving anti-PD-1/PD-L1 agents alone was more heterogeneous. Lesions of active hepatitis with spotty or confluent necrosis and mild to moderate periportal activity were not associated with granulomatous inflammation (Fig. 2E and 2F). Only two of the nine biopsies showed microgranulomatous clusters, without fibrin deposit. Portal fibrosis was present in five patients. In patient 11, the F3 fibrosis
was linked to a chronic biliary obstruction due to hilar cholangiocarcinoma. Central vein endothelitis was present in only 2 cases.

Immunostaining revealed different proportions of CD4 and CD8 lymphocytes between patients who did or did not receive anti-CTLA-4 mAb. In patients treated with anti-CTLA-4 mAb, inflammatory infiltrates in portal tracts, and even more in lobules, were mainly represented by CD8 lymphocytes. In patients treated with anti-PD-1/PD-L1 mAb, portal infiltrates consisted of CD4 and CD8 lymphocytes in equal proportions, whereas lobular infiltrates showed a slight predominance of CD8 lymphocytes. FOXP3 positive lymphocytes were rarely detected in liver biopsies except for patient 2 who received a combined therapy and no difference was observed between patients treated with anti-CTLA-4 and patients treated with anti-PD-1/PD-L1 alone. Immunostaining for ERG highlighted alterations of sinusoidal endothelial cells, particularly those in close contact with granulomas in patients treated with anti-CTLA-4 mAb, but not in patients treated with either anti-PD-1/PD-L1 mAb alone.

Management and outcomes

The management was specific for each patient based on the clinical course and histological features. Six patients (38%) did not receive any corticosteroid therapy and experienced a spontaneous improvement in liver tests, one treated with anti-CTLA-4 mAb and five treated with anti-PD-1 mAb. Patient 1 is still in complete remission, without re-introduction of immunotherapy. Three patients (patients 7, 10 and 12) were re-challenged with an anti-PD-1 mAb, one patient died of tumor progression (patient 7), one experienced a transient recurrence of a grade 1 elevation of liver enzymes (patient 10) and no liver recurrence of adverse events in
the second (patient 12). Two patients (patients 3 and 11) were on 0.2mg/kg of corticosteroids (10 mg/day) before the acute hepatitis and the dose was not increased. Two patients (patients 6 and 9) were treated with 0.5 mg/kg corticosteroids and five patients (patients 2, 4, 7, 8 and 15) with 1 mg/kg. Three patients treated with 1mg/kg of corticosteroids were jaundiced (bilirubin > 42 µmol/L / 2.5 mg/dL). All patients treated with 1mg/kg of corticosteroids showed a more severe histological damage. In all, but one, liver histology showed a grade 3 inflammation in at least one zone and presented with central endothelitis. Immunotherapy was not reintroduced in these seven patients. Only one patient (patient 5) with severe histological lesions required high doses of corticosteroids administered in boluses and then up to 2.5 mg/kg, as well as an additional immunoospressive agent (mychophenolate mofetil). In this patient, the corticosteroids were slowly tapered over a year to reach a maintenance dose of 5mg/day, obtaining a normalization of liver tests. Anti-cancer immunotherapy was permanently discontinued. Two patients showed a rise of liver tests after weaning of corticosteroids, one due to hepatic metastases progression and one without defined causes and spontaneous resolution (long-term effect of the immunotherapy was hypothesized). Comparing patients who underwent corticosteroid therapy and patients who did not, in treated patients there was a non significant trend for lower interval time between immunotherapy and acute hepatitis, higher cytolysis and cholestasis, in particular higher bilirubin.

Discussion

To date there has been no large-scale review of patients with DILI due to the major classes of immune checkpoint inhibitors. This study examined a comprehensive
serological, chronological and histopathological data set of patients with checkpoint inhibitors hepatotoxicity. Despite the heterogeneous presentation of this type of DILI, patients receiving anti-CTLA4 mAb had a similar presentation and a distinct histological patterns compared to patients receiving anti-PD-1/PD-L1 mAb. Furthermore, this study demonstrated the need for a management patient oriented, which, unlike current guidelines, could eventually avoid useless systemic corticosteroid treatment. Based on these findings, we propose an algorithm for the diagnosis and management of immune-mediated hepatitis due to checkpoint inhibitors (Fig. 3).

Acute grade ≥ 3 hepatitis according to CTCAE system (cytolysis and/or cholestasis > 5 times, bilirubin > 3 times ULN) was diagnosed in only 3.5% of patients who received immunotherapy for metastatic cancer. These data confirm the rarity of this DILI, which has been reported in 1% to 8% of patients during treatment with immune checkpoint inhibitors. Due to the lack of specific biomarkers, the diagnosis of DILI remains an exclusion diagnosis. Of the 19 patients concerned, one was excluded from the study because of a positive viral load for HEV, which is a rare cause of acute hepatitis. Therefore an extensive work-up is mandatory in order to exclude any other cause of liver injury. This work-up must also be highly accurate before any decision is made to administer high doses of corticosteroids, which might futile or even harmful.

It is interesting to note that three of the patients of this series who developed anti-CTLA-4-induced acute hepatitis had previously been exposed to anti-PD-1 therapy (pembrolizumab), with only a short period elapsing between receiving the two immunotherapies. Whether the immune system activation induced by anti-PD-1 therapy had enhanced the immune response and should be considered as a risk factor for the onset of hepatitis still needs to be determined. A study compared
sequential treatment in two cohorts. In the first one, patients were treated by nivolumab for 3 months followed by ipilimumab and in the second cohort, the opposite. The tolerance was better for patients firstly treated by ipilimumab. Adverse events observed under ipilimumab in first line were not worsened by nivolumab, whereas low toxicity under nivolumab front line was dramatically worsened by ipilimumab in second line\textsuperscript{21}. Four patients of this study had the combination therapy of anti-CTLA-4 and anti-PD-1 mAb. Indeed, it has been reported that the combination of ipilimumab and nivolumab is associated with a greater risk of developing high-grade hepatic IRAEs\textsuperscript{22,23}.

Important elements in this study are the histological findings. Five patients who received anti-CTLA-4 as monotherapy or in combination with anti-PD-1 mAb displayed a specific pattern of granulomatous hepatitis with fibrin deposition and central vein endothelitis. Granulomatous hepatitis had also been reported in six patients with metastatic melanoma who had received vemurafenib, a protein kinase inhibitor, and ipilimumab\textsuperscript{24}. Similar poorly formed granulomatous inflammation has been described in a patient who underwent treatment with vemurafenib alone\textsuperscript{25}. In other organs, such as the mediastinum, central nervous system, lung or spleen, sarcoid-like granulomas have been reported with ipilimumab\textsuperscript{26,27,28}.

Acute hepatitis with fibrin ring granulomas was recently described in two patients treated with a combination of ipilimumab and nivolumab\textsuperscript{29}. The presence of fibrin exudates associated with the inflammatory infiltrates suggested destruction of the sinusoidal wall. Indeed, immunostaining for ERG demonstrated endothelial cell alterations only in the case of granulomatous hepatitis related to anti-CTLA-4 therapy.

Another interesting finding was lobular hepatitis with necrosis that was either spotty or confluent. This observation was in line with another study which had described
lobular hepatitis and grade 3 hepatitis accompanied by lymphocytic and histiocytic infiltrates, in patients treated with anti-CTLA-4 mAb. In patients who received anti-PD-1/PD-L1 immunotherapy, the liver damage was more heterogeneous, involving lobular and periportal activity. Micro-granulomatous lesions without fibrin depositions were observed in two patients. Only one published case report has described histological features of liver toxicity induced by anti-PD-1 mAb which highlighted the presence of several foci of lobular inflammation and necrosis, with mild fibrosis also being present in the centrilobular zone.

Immunostaining revealed that both the periportal and lobular inflammatory infiltration seen in patients treated with anti-CTLA-4 was generated by CD8 lymphocytes, in line with what had been reported by Johancille et al. In patients treated with anti-PD-1/anti-PD-L1 mAb, the inflammatory infiltrate consisted of both CD4 and CD8 lymphocytes. Although regulatory T cells (Treg) identified by FOXP3 staining constitutionally express CTLA-4, the number of FOXP3 Treg in liver parenchyma was not different in patients treated with anti-CTLA-4 or anti-PD-1/anti-PD-L1.

It should be noted that liver injury due to immune checkpoint inhibitors is not typical of what is seen in autoimmune hepatitis as the characteristic features of autoimmune hepatitis such as plasma cell infiltration, severe interface hepatitis, piecemeal necrosis and rosette formation are lacking. It should thus be referred to as immune-mediated hepatitis rather than autoimmune-like hepatitis.

Secondly, the histological assessment was informative regarding the severity of liver damage, which can provide crucial elements that will drive the therapeutic decision. This finding highlights the need for the precise grading and staging of histological features in such patients.

Thirdly, our findings suggest a risk that this acute hepatitis related to immune checkpoint inhibitors may rapidly evolve towards chronicity. One of our patients
(patient 5) underwent two liver biopsies due to a second peak of transaminase levels during corticosteroid therapy. The second biopsy showed the development of stage 1 fibrosis, absent from the first biopsy, and portal inflammation had deteriorated from grade 1 to grade 3.

The observations of this study can challenge the present recommendations concerning the management of immune toxicity. Indeed, spontaneous improvement in liver test results can occur following the suspension of immunotherapy without any corticosteroid administration and low doses of corticosteroids may be sufficient to control the disease. The current recommendation for grade 3 hepatitis, according to the CTCAE system, is to introduce corticosteroid therapy at a dose of 1-2 mg/kg/day \(^{15}\) or 2-4 mg/kg/day \(^{32}\). Patients are usually treated without a prior histological assessment \(^{16}\). The decision to initiate the corticosteroid treatment, and the choice of the dose, should be based on the presence of jaundice (bilirubin > 2.5mg/dL) and/or liver failure (INR \(\geq 1.5\)) and the severity of histological liver damage (activity grade of 3). Patients in our study without grade 3 hepatitis and no severe histological damage could be probably improved without corticosteroid therapy. The addition of a second immunosuppression drug \(^{12}\) should be considered in patients not improving with high doses of corticosteroids. In the event of corticosteroid intolerance, the use of antithymocyte globulin therapy may constitute an effective therapeutic option \(^{33}\).

Whether high doses of corticosteroids have an impact on tumor response remains unclear \(^{34}\). One study demonstrated that the duration of tumor response was not affected by the use of high-dose corticosteroids to treat immune-related toxicities \(^{35}\). This observation was confirmed by another study during which treatment failure and survival were no worse in patients requiring corticosteroid therapy when compared to patients not treated with corticosteroids \(^{36}\). By contrast, in a pooled analysis of treatment with nivolumab, patients who experienced any grade 3 or 4 toxicity had a
slightly worse response rate \(^{37}\).

The reintroduction of immunotherapy following an episode of acute hepatitis is still debatable. It was shown here that in some cases, it was possible and safe to reintroduce immunotherapy without corticosteroid therapy. A report on two clinical cases described the re-administration of anti-PD-1 therapy together with the introduction of budesonide and ursodeoxycholic acid \(^{31}\). Liver-directed topical corticosteroids may represent an interesting approach because they interfere less with the anti-tumor activity of immune checkpoints inhibitors, but this modality also deserves further study.

The strengths of this study included the description of a multidisciplinary approach to this particular type of DILI, involving interplay between hepatologists, oncologists, pathologists and immunologists. To date there is no comparison between anti-PD-1/PD-L1 and anti-CTLA-4 induced liver injuries. Most of all, all subjects underwent a liver biopsy and for each biopsy characterization of lymphocyte subtypes was analyzed by means of an immunostaining assessment.

The number of patients remains limited. However, because of the considerable efficacy of immunotherapy, we can suppose that it will become increasingly administered in patients with metastatic cancer, and consequently that the number of immune-related liver injuries will also increase.

No predisposing immunological risk factors were identified that could foretell the risk of immunotherapy-induced hepatitis which is consistent with the fact that no biomarkers have yet been identified to predict the occurrence of immune related adverse events in patients receiving checkpoint inhibitors \(^{5}\).

In conclusion, acute hepatitis due to cancer immunotherapy is rare, and diagnosed in only 3.5% of treated patients. Two different histological patterns of immune-mediated hepatitis were identified: granulomatous hepatitis with fibrin deposits associated with
anti-CTLA-4 mAb, and lobular, non-granulomatous hepatitis associated with anti-PD-1/anti-PD-L1 mAb. The performance of a liver biopsy is of paramount importance as it provides information on the severity of liver injury and help guiding the choice of therapy, which can avoid corticosteroids. Nevertheless, management remains challenging and must be patient-oriented. Further studies will be necessary in order to elucidate the mechanisms underlying liver toxicities and to identify predictive and prognostic factors.

References


Figure legends

Fig. 1. Flowchart of study population

Fig. 2. Histologic patterns in patients treated with anti-CTLA4 versus anti-PD1 monoclonal antibodies.

First liver biopsy of patient 5 (who received anti-CTLA4 mAb)

(A) Centrilobular confluent necrosis with fibrin ring granulomas (HES x 100).

(B) Fibrin ring granuloma and sinusoidal inflammatory infiltrates composed of activated lymphocytes and histiocytes, without plasma cell (HES x 300).

Liver biopsy of patient 2 (who received anti-CTLA4 mAb)

(C) Endotheliitis of a centrilobular vein with perivenular and sub-endothelial infiltration by lymphocytes and histiocytes and focal disruption of the endothelium (HES x 40).
**D** Perivenular and sub-endothelial lymphocytes are CD8+ cytotoxic T lymphocytes (IHC CD8 x 40).

Liver biopsy of patient 8 (who received anti-PD1 mAb)

(E) Active hepatitis with mild periportal and moderate lobular activity (HES x 100).

(F) Lobular lymphocytes and histiocytes without plasma cell (HES x 300).

Fig. 3. Algorithm for the assessment and management of patients with acute hepatitis during immunotherapy for metastatic cancer
Highlights

- Acute hepatitis due to immune checkpoint inhibitors for metastatic cancer is rare
- Immune-mediated hepatitis diagnosis needs the exclusion of all causes of hepatitis
- Liver histology is paramount for diagnosis and severity evaluation of liver damage
- Management should be based on biological and histological severity of liver injury
- Immune-mediated hepatitis does not need systematic use of corticosteroids
Table 1. Characteristics of overall population with immune-mediated hepatitis induced by immunotherapy for metastatic cancer. Comparison between patients who received anti-CTLA-4 versus anti-PD-1/PDL-1 mAb.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=16</th>
<th>N=7 anti-CTLA4</th>
<th>N=9 Anti-PD1/PDL1</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63 [33-84]</td>
<td>52 [33-65]</td>
<td>69 [52-84]</td>
<td>0.029</td>
</tr>
<tr>
<td>Sex, female</td>
<td>9 (56)</td>
<td>5 (71)</td>
<td>4 (44)</td>
<td>0.357</td>
</tr>
<tr>
<td>Past history of immunotherapy</td>
<td>5 (31)</td>
<td>3 (43)</td>
<td>2 (22)</td>
<td>0.596</td>
</tr>
<tr>
<td>Interval time between immunotherapy and acute hepatitis, weeks</td>
<td>5 [1-49]</td>
<td>3 [1-7]</td>
<td>14 [2-49]</td>
<td>0.019</td>
</tr>
<tr>
<td>Number of doses</td>
<td>2 [1-36]</td>
<td>2 [1-2]</td>
<td>4 [1-36]</td>
<td>0.167</td>
</tr>
<tr>
<td>Previous extra-hepatic IRAEs</td>
<td>6 (38)</td>
<td>1 (14)</td>
<td>5 (56)</td>
<td>0.145</td>
</tr>
<tr>
<td>Fever at the time of hepatitis</td>
<td>6 (38)</td>
<td>5 (71)</td>
<td>1 (11)</td>
<td>0.034</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>437 [147-2289]</td>
<td>450 [147-2289]</td>
<td>424 [180-1387]</td>
<td>0.757</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>460 [266-3137]</td>
<td>491 [266-3137]</td>
<td>429 [305-2671]</td>
<td>1.000</td>
</tr>
<tr>
<td>Total bilirubin, µmol/L</td>
<td>18 [6-324]</td>
<td>24 [10-324]</td>
<td>18 [6-135]</td>
<td>0.426</td>
</tr>
<tr>
<td>Alk P</td>
<td>309 [53-768]</td>
<td>184 [71-768]</td>
<td>325 [53-699]</td>
<td>0.918</td>
</tr>
<tr>
<td>ANA ≥ 1:80</td>
<td>8 (50)</td>
<td>3 (43)</td>
<td>5 (55)</td>
<td>1.000</td>
</tr>
<tr>
<td>ASMA 1:80</td>
<td>3 (19)</td>
<td>-</td>
<td>3 (33)</td>
<td>0.212</td>
</tr>
</tbody>
</table>

IRAEs, immune-related adverse events; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk P, alkaline phosphatase; GGT, gamma-glutamyl transferase; ANA, anti-nuclear antibodies; ASMA, anti-smooth muscles antibodies; IgG, immunoglobulin G.

AST, normal range: 5-55 IU/L; ALT, normal range: 5-55 IU/L; total bilirubin, normal range: <17 µmol/L; Alk P, normal range: 30-100 IU/L; GGT, normal range: 10-45 IU/L.
Table 2a. Principal characteristics of patients experiencing hepatic immune-related adverse events as a function of the immunotherapy received: anti-CTLA-4 or combo anti-CTLA-4 plus anti-PD-1 mAb.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender, age (years)</th>
<th>Tumor type and sites of metastases</th>
<th>Previous immune checkpoint Inhibitor exposure</th>
<th>Molecule(s) related to the hepatic IRAE</th>
<th>Time between immuno Therapy/ hepatitis (weeks)</th>
<th>Clinical symptoms</th>
<th>Serum Auto-antibody</th>
<th>Serum IgG level g/L</th>
<th>Therapy for hepatic IRAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 40</td>
<td>Melanoma, cutaneous</td>
<td>No</td>
<td>Combo Ipilimumab + Nivolumab</td>
<td>2</td>
<td>Fever 39°C</td>
<td>ANA and ASMA negatives</td>
<td>10</td>
<td>No therapy</td>
</tr>
<tr>
<td>2</td>
<td>F, 33</td>
<td>Melanoma, lung and pancreatic</td>
<td>No</td>
<td>Combo Ipilimumab + Nivolumab</td>
<td>5</td>
<td>Fever 39.3°C and rash</td>
<td>ANA and ASMA negatives</td>
<td>8</td>
<td>Steroids 1 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>M, 65</td>
<td>Melanoma, lymph node</td>
<td>No</td>
<td>Combo Ipilimumab + Nivolumab</td>
<td>2</td>
<td>No symptoms</td>
<td>ANA and ASMA negatives</td>
<td>9</td>
<td>Steroids maintenance 0.2 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>F, 64</td>
<td>Melanoma, hepatic and lung</td>
<td>No</td>
<td>Combo Ipilimumab + Nivolumab</td>
<td>3</td>
<td>Jaundice</td>
<td>ANA 1:320 and ASMA negatives</td>
<td>10</td>
<td>Steroids 1 mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>F, 63</td>
<td>Melanoma, loco-regional (Pembrolizumab)</td>
<td>Yes</td>
<td>Ipilimumab</td>
<td>7</td>
<td>Fever 40°C, rash jaundice arthralgia</td>
<td>ANA and ASMA negatives</td>
<td>6</td>
<td>Steroids 2.5 mg/kg IV + MMF</td>
</tr>
<tr>
<td>6</td>
<td>F; 48</td>
<td>Melanoma, cutaneous, lymph node and hepatic (Ipilimumab, subsequently pembrolizumab)</td>
<td>Yes</td>
<td>Ipilimumab</td>
<td>5</td>
<td>Fever 39.5°C, chills, and vomiting</td>
<td>ANA 1:80 and ASMA negatives</td>
<td>15</td>
<td>Steroids 0.5 mg/kg</td>
</tr>
<tr>
<td>7</td>
<td>F, 53</td>
<td>Melanoma, cerebral and lung</td>
<td>Yes (Pembrolizumab)</td>
<td>Ipilimumab</td>
<td>1</td>
<td>Fever 38°C and rash</td>
<td>ANA 1:80 and ASMA negatives</td>
<td>6</td>
<td>Steroids 1 mg/kg</td>
</tr>
</tbody>
</table>

*AST and ALT normal values = 5-55 UI/L
** ALP normal values = 30-100 UI/L; and GGT normal values = 10-45 UI/L/
2b. Principal characteristics of patients experiencing hepatic immune-related adverse events as a function of the immunotherapy received: anti-PD-1 or anti-PD-L1 mAb.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender, age (years)</th>
<th>Tumor type and sites of metastases</th>
<th>Previous immune checkpoint Inhibitor exposure</th>
<th>Molecule(s) related to the hepatic IRAE</th>
<th>Time between immunotherapy/hepatitis (weeks)</th>
<th>Clinical symptoms</th>
<th>Serum Auto-antibody</th>
<th>Serum IgG level g/L</th>
<th>Therapy for hepatic IRAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>F, 65</td>
<td>Bronchial carcinoma</td>
<td>No</td>
<td>Durvalumab</td>
<td>6</td>
<td>No symptoms</td>
<td>ANA 1:80 ASMA 1:80</td>
<td>13</td>
<td>Steroids 1 mg/kg</td>
</tr>
<tr>
<td>9</td>
<td>M; 52</td>
<td>Renal clear cell carcinoma, lymph node and lung</td>
<td>No</td>
<td>Nivolumab</td>
<td>14</td>
<td>Rhinitis and rash</td>
<td>ANA 1:80 ASMA 1:80</td>
<td>9</td>
<td>Steroids 0.5 mg/kg</td>
</tr>
<tr>
<td>10</td>
<td>M; 56</td>
<td>Bladder carcinoma, lymph node and lung</td>
<td>No</td>
<td>Pembrolizumab</td>
<td>4</td>
<td>Headache</td>
<td>ANA/ASMA negatives</td>
<td>18</td>
<td>No therapy</td>
</tr>
<tr>
<td>11</td>
<td>M, 61</td>
<td>Cholangiocarcinoma, lung and lymph node</td>
<td>No</td>
<td>Pembrolizumab</td>
<td>26</td>
<td>Fever 40°C, rash</td>
<td>ANA/ASMA negatives</td>
<td>17</td>
<td>Steroids maintenance 0.2 mg/kg</td>
</tr>
<tr>
<td>12</td>
<td>F, 69</td>
<td>Melanoma, cutaneous, lymph node and bone (Ipilimumab)</td>
<td>Yes</td>
<td>Pembrolizumab</td>
<td>40</td>
<td>No symptoms</td>
<td>ANA 1:80 ASMA negatives</td>
<td>7</td>
<td>No therapy</td>
</tr>
<tr>
<td>13</td>
<td>M, 84</td>
<td>Melanoma, cutaneous, lymph node</td>
<td>No</td>
<td>Pembrolizumab</td>
<td>49</td>
<td>Pruritus</td>
<td>ANA 1:80 ASMA negatives</td>
<td>9</td>
<td>No therapy</td>
</tr>
<tr>
<td>14</td>
<td>F, 78</td>
<td>Melanoma, lung, lymph node and bone (Ipilimumab subsequently Pembrolizumab)</td>
<td>Yes</td>
<td>Nivolumab</td>
<td>5</td>
<td>Pain</td>
<td>ANA/ASMA negatives</td>
<td>11</td>
<td>No therapy</td>
</tr>
<tr>
<td>15</td>
<td>F, 79</td>
<td>Melanoma, cutaneous</td>
<td>No</td>
<td>Nivolumab</td>
<td>4</td>
<td>Jaundice</td>
<td>ANA 1:80 ASMA negatives</td>
<td>9</td>
<td>Steroids 1 mg/kg</td>
</tr>
<tr>
<td>16</td>
<td>M, 76</td>
<td>Melanoma, cutaneous, lung</td>
<td>No</td>
<td>Pembrolizumab</td>
<td>15</td>
<td>No symptoms</td>
<td>ANA and ASMA negatives</td>
<td>9</td>
<td>No therapy</td>
</tr>
</tbody>
</table>
Table 3 Histological characteristics of each patient with acute hepatitis during to

<table>
<thead>
<tr>
<th>PTS</th>
<th>IMMUNOTHERAPY</th>
<th>PORTAL FIBROSIS 0-4</th>
<th>PORTAL ACTIVITY 0-3</th>
<th>PERI-PORTAL ACTIVITY 0-3</th>
<th>BILE DUCT INJURY 0-1</th>
<th>LOBULAR ACTIVITY 0-3</th>
<th>PLASMA CELL</th>
<th>GRANULOMAS</th>
<th>CENTRAL ENDOTHELITIS 0-2</th>
<th>PATTERN LIVER INJURY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>antiPD1 + CTLA4</td>
<td>F0 1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>+</td>
<td>central fibrin deposits</td>
<td>1</td>
<td>Granulomatous lobular hepatitis</td>
</tr>
<tr>
<td>2</td>
<td>anti-PD1 + CTLA4</td>
<td>F0 1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>+</td>
<td>minimal fibrin deposits</td>
<td>2</td>
<td>Granulomatous lobular hepatitis + fibrin deposits</td>
</tr>
<tr>
<td>3</td>
<td>anti-PD1 + CTLA4</td>
<td>F0 2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Subacute hepatitis + focal confluent necrosis</td>
</tr>
<tr>
<td>4</td>
<td>anti-PD1 + CTLA4</td>
<td>F1 3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Subacute hepatitis + periportal and lobular activity</td>
</tr>
<tr>
<td>5</td>
<td>anti-CTLA4</td>
<td>F0 1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>+</td>
<td>fibrin rings</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>anti-CTLA4</td>
<td>F1 3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Chronic hepatitis A3F1</td>
</tr>
<tr>
<td>6</td>
<td>anti-CTLA4</td>
<td>F0 1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>+</td>
<td>without fibrin deposits</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>anti-CTLA4</td>
<td>F1 2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>+</td>
<td>fibrin rings periportal&gt;lobular</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>anti-PDL1 + olaparib</td>
<td>F1 2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>anti-PD1</td>
<td>F0-F1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Chronic hepatitis A2F1</td>
</tr>
<tr>
<td>10</td>
<td>anti-PD1</td>
<td>F0 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>+</td>
<td>rares microgranulomas without fibrin</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>anti-PD1</td>
<td>F3 1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Acute hepatitis + submassive necrosis on fibrotic liver</td>
</tr>
<tr>
<td>12</td>
<td>anti-PD1</td>
<td>F0 1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Lobular hepatitis + nodular regenerative hyperplasia</td>
</tr>
<tr>
<td>13</td>
<td>anti-PD1</td>
<td>F2 1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Chronic hepatitis A2F3 + lobular necrosis</td>
</tr>
<tr>
<td>14</td>
<td>anti-PD1</td>
<td>F0 1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Slight lobular hepatitis</td>
</tr>
<tr>
<td>15</td>
<td>anti-PD1</td>
<td>F0 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Acute hepatitis + confluent and bridging necrosis</td>
</tr>
<tr>
<td>16</td>
<td>anti-PD1 +/-? (study)</td>
<td>F1 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>microgranulomas without fibrin</td>
<td>0</td>
</tr>
</tbody>
</table>
immunotherapy according to type of therapy: anti-CTLA-4 or anti-PD-1/anti-PD-L1.
patients on immunotherapy between 2015 and 2017  
N=536

Grade ≥ 3 hepatitis  
N=19

Immune-mediated hepatitis  
N=16

No liver histology  
N=1  
HEV infection  
N=1  
Hepatic tumor infiltration  
N=1

Recipients of anti-PD1/ PDL1  
N=9

No corticosteroids  
N=5  
Mantainance of 0.2 mg/kg/day  
N=1  
0.5/1 mg/kg/day  
N=3

Recipients of CTLA4 (+/- anti-PD1)  
N=7

No corticosteroids  
N=1  
Mantainance of 0.2 mg/kg/day  
N=1  
0.5/1 mg/kg/day  
N=4  
2 mg/kg/day + boluses + MMF  
N=1
Cytolysis and cholestasis grade ≥ 3 (AST/ALT/GGT/AlkP> 5 times ULN Bilirubin > 3 times ULN)

Rule out common causes of acute hepatitis or tumor liver invasion

Liver Biopsy *

Hepatic tumor infiltration

Immune-mediated hepatitis

Other findings

Based on severity of liver injury according to DILIN scale (Bilirubin ≥ 2.5 mg / dL and / or INR ≥ 1.5) and histology

Surveillance

Steroid therapy 0.5-1 mg / kg / day

Corticosteroids 2 mg / kg / day +/- 2nd immunosuppressive drug

* liver biopsy is not required in case of acute viral hepatitis
Immune check point inhibitors
Anti-PD1, anti-PDL1, anti-CTLA4

Based on biological and histological severity of liver injury

Surveillance or corticosteroid therapy

anti-CTLA4 antibodies

anti-PD1/PDL1 antibodies

Figure 2