



Prediction of immune checkpoint blockade-related hepatitis in metastatic melanoma patients

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Summary

The introduction of clinical antibodies against programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) has revolutionized cancer treatment. Immune checkpoint blockade has enormous therapeutic potential and is widely prescribed for treating various cancers. However, immune-related adverse events in checkpoint blockade-treated patients are common and limit its clinical application. Despite efforts to understand the etiology of immune-related adverse events, the underlying cellular reactions remain elusive. Recently, our group identified a subset of patients with metastatic melanoma that are predisposed to hepatitis after combined PD-1 and CTLA-4 blockade. These patients are characterized by pre-treatment expansion of effector memory CD4⁺ T cells (T_{EM} cells) in blood. We attributed this expansion to chronic or recurrent subclinical immune responses against cytomegalovirus (CMV) infection. Accordingly, baseline expansion of T_{EM} cells is a reliable biomarker of hepatitis risk that identifies a subgroup of patients who might benefit from prophylactic CMV treatment with valganciclovir.

Clinical problem

With the advent of immunotherapy, treatment of metastatic melanoma has improved significantly. Dual checkpoint blockade with anti-PD-1 (Nivolumab) and anti-CTLA-4 antibody (Ipilimumab) is a standard, first-line treatment for unresectable metastatic melanoma. The clinical efficacy of this combined therapy is remarkable, with excellent response rates, progression-free survival, and overall survival [1–3]. Unfortunately, immune-related adverse events (irAEs) remain a serious constraint, affecting up to 96 % of treated patients [4]. Although rarely life-threatening, irAEs often require discontinuation of immunotherapy, multidisciplinary management, and the introduction of immunosuppression [5]. These limitations motivated us to find predictive biomarkers to guide avoidance or prevention strategies.

Immune-related adverse events affect virtually all organ systems, including the skin, liver, gastrointestinal tract, airways, endocrine tissues, and central nervous system. These reactions are triggered by disinhibition of T cells, which initiates inflammation. It is known that patients with pre-existing autoimmune diseases have an increased risk for both exacerbation of their autoimmune condition and for de novo

development of other irAEs [6–8]. There is also evidence that genetic influences also play a role in the pathophysiology of irAEs [9–11]. However, we still do not fully understand why certain reactions occur in some patients but not others.

Hypothesis

We hypothesized that an individual's risk of developing particular irAEs is pre-determined by immunological events that occurred before checkpoint blockade is administered. Hence, the goal of our study was to identify predisposing factors to guide alternative treatment strategies and preventive measures.

Research in clinical practice

In our observational trial, we investigated 89 patients with unresectable metastatic melanoma receiving combined Nivolumab and Ipilimumab therapy. To discover factors that predispose patients to immune-related hepatitis, we first examined routine clinical parameters, such as transaminases, bilirubin, lactate dehydrogenase (LDH), S100 β , C-reactive protein (CRP), seropositivity for hepatitis viruses and tumor burden, but these were uninformative [12]. Therefore, we

conducted a comprehensive immune phenotyping of peripheral blood leucocytes by flow cytometry from patients immediately before the start of therapy. This approach revealed an expansion of CD4⁺ effector memory T cells (CD4⁺ T_{EM}) in a subset of patients who later developed hepatitis. We observed that pre-therapy CD4⁺ T_{EM} expansion occurred most frequently in autumn and winter, leading us to speculate about the etiological involvement of seasonal pathogens. Screening for hepatotropic virus infections revealed a strong association between CD4⁺ T_{EM} frequency and CMV IgG serum levels. Moreover, CMV-reactive CD4⁺ T cells were significantly enriched in patients with elevated CD4⁺ T_{EM} [12].

These findings implied that CD4⁺ T_{EM} expansion was driven by subclinical reactivation of CMV before starting immunotherapy (Figure 1). Reactivation of latent viruses characteristically follows immune compromise and triggers T-cell immunity [13, 14]. Low-level and compartmentalized CMV reactivation can provoke extensive and life-threatening clinical manifestations [15]. However, we were unable to detect replicating virus in blood, saliva, or stool samples from patients with elevated CD4⁺ T_{EM} at baseline. Furthermore, CMV was undetectable in for-cause liver biopsies from two patients with elevated baseline CD4⁺ T_{EM} frequency who presented with treatment-refractory hepatitis after combined ipilimumab and nivolumab therapy. Consequently, we believe that CD4⁺ T_{EM} expansion must be sustained by chronic or recurrent, low-level CMV reactivation, possibly in the liver, lymphoid organs, or metastatic tumors.

Considering only pre-treatment CD4⁺ T_{EM} frequency in CMV IgG⁺ patients, we are now able to predict hepatitis following combined ipilimumab and nivolumab therapy with a positive predictive value of 88.2 % and a negative predictive value of 82.6 %. Importantly, our predictive model opens new clinical possibilities to avoid or treat immune-mediated

hepatitis in our patients. We hypothesized that hepatitis is triggered in our patients by T cells responding to CMV reactivation in liver, as opposed to expanded CMV-specific T cells acting non-specifically following checkpoint blockade. This led us to treat two patients with elevated CD4⁺ T_{EM} frequency for steroid-refractory hepatitis using an anti-viral drug, valganciclovir. In both patients, we observed a rapid normalization of markers of liver damage after starting valganciclovir.

Building on these promising observations, we next investigated whether prophylactic treatment with valganciclovir could prevent immune-related hepatitis in four at-risk patients after combined PD-1 and CTLA-4 blockade. This approach showed great impact as none of the four patients developed liver inflammation. This treatment concept will now be examined within a clinical trial using a validated flow cytometry assay for immune phenotyping blood samples of melanoma patients [16].

Conclusions for clinical practice

Our study uncovered a novel mechanism responsible for immune-related hepatitis after checkpoint blockade. Being able to identify patients predisposed to hepatitis in clinical practice opens various possibilities for avoiding treatment-related hepatitis. Elsewhere, we have published our detailed methods and models to enable others to reproducibly implement our predictive model. We believe CMV prophylaxis with valganciclovir holds great promise for preventing and treating hepatitis in our patients. In the future, more detailed knowledge about the etiology of irAEs will lead to more personalized cancer therapies. The challenge for the field is now to design efficient and decisive clinical trials to assess the performance of predictive markers of irAEs and possible management strategies that stem from those predictions.

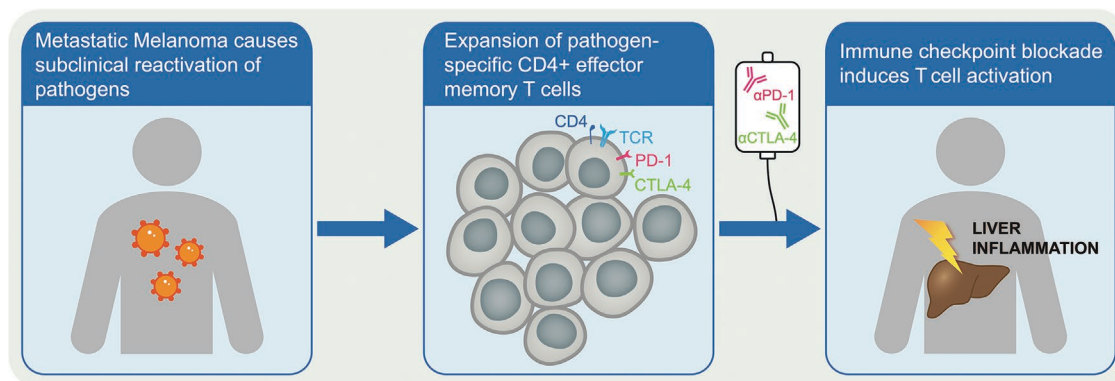


Figure 1 Subclinical expansion of pathogen-specific T cells before immune checkpoint blockade predisposes to hepatitis. Patients with unresectable metastatic melanoma experience a subclinical CMV reactivation. Low-level viral exposure drives expansion of specific CD4⁺ effector memory T cells, which contribute to liver inflammation after PD-1 and CTLA-4 blockade.

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Conflict of interest

SH received grants from BMS and served on the advisory board for MSD and BMS.

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