

Incidence and severity of immune-related hepatitis after dual checkpoint therapy is linked to younger age independent of herpes virus immunity

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Research Article

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Abstract

Background and Aims:

Dual immune checkpoint blockade (ICB) therapy can result in immune-related-adverse events (irAE) such as ICB-hepatitis. An expansion of effector-memory (TEM) CD4 T cells associated with antiviral immunity against *herpesviridae* was implicated in ICB-hepatitis. Notably, these memory subsets are frequently associated with age. Here, we sought to understand baseline patient, immune and viral biomarkers associated with the development of ICB-hepatitis to identify currently lacking baseline predictors and test if an expansion of TEM or positive serology against *herpesviridae* can predict ICB-hepatitis.

Methods

A discovery (n = 39) and validation cohort (n = 67) of patients with advanced melanoma undergoing anti-PD-1&anti-CTLA4 combination therapy (total n = 106) were analyzed for baseline clinical characteristics, occurrence of irAE and oncological outcomes alongside serological status for CMV, EBV and HSV. Immune populations were profiled by high-parametric flow cytometry (n = 29).

Results

ICB-hepatitis occurred in 59% of patients within 100days; 35.9% developed severe (CTCAE 3–4) hepatitis. Incidence of ICB-hepatitis was higher in the younger (< 55y: 85.7%) compared to older (> = 55y: 27.8%) age group (p = 0.0003), occurred earlier in younger patients (p < 0.0001). The association of younger age with ICB-Hepatitis was also observed in the validation cohort (p = 0.0486). Incidence of ICB-hepatitis was also associated with additional non-hepatic irAE (p = 0.018), but neither positive IgG serostatus for CMV, EBV or HSV nor TEM subsets despite an association of T cell subsets with age.

Conclusion

Younger age more accurately predicts ICB-hepatitis after anti-PD-1&anti-CTLA4 checkpoint therapy at baseline compared to herpes virus serology or TEM subsets. Younger patients should be carefully monitored for the development of ICB-hepatitis.

Background

Combination immune checkpoint blockade (ICB) immunotherapy with anti-PD-1 & anti-CTLA4 is now in widespread use for unresectable/metastatic melanoma, non-small cell lung cancer (NSCLC) with TPS > = 1%, pleural mesothelioma and is currently under intensive evaluation in other oncological indication. The high clinical efficacy of combination immunotherapy however comes at the cost of a higher incidence of immune-related adverse events (irAE). In clinical trials for malignant melanoma, 30–55% patients treated

with combinational therapy had severe CTCAE grade 3–4 adverse events [1–5], which limits continuation of therapy, and, in some cases, may lead to significant harm and death [6]. Hepatitis is one of the most common irAE causing severe (CTCAE 3–4) toxicity in anti-PD-1 & anti-CTLA4 therapy with incidence rates reported up to 33% [1, 3, 7]. Immune-related hepatitis (ICB-hepatitis) is diagnosed during checkpoint blockade therapy based on changes in Alanine-Aminotransferase (ALT), Aspartate-Aminotransferase (AST) and other indices of liver function following exclusion of alternative etiologies of hepatitis[8]. Management strategies range from close observation to immunosuppressive therapy depending on CTCAE grading [9].

However, despite emerging evidence of dynamic changes in immune cell function in ICB-hepatitis [10], there is currently a lack of precise mechanistic understanding of the pathogenesis of this new disease entity, leading to the lack of effective prophylactic management and patient-tailored surveillance strategies. Recently, a potential association between baseline immune responses and the occurrence of severe irAE and ICB-hepatitis was reported. Lozano et al. described a link between activated CD4 + effector memory T cell (T_{EM}) populations and the development of severe adverse events after anti-PD-1/combinational blockade therapy [11]. Hutchinson et al. reported an enrichment of CMV-associated T_{EM} CD4 populations in the peripheral blood of patients who further developed hepatitis in their cohort [12], instigating a provocative suggestion whether introduction of selective antivirals against *Herpesviridae* might be beneficial in the prevention or therapy of checkpoint-related immune hepatitis.

Exposure to *Herpesviridae* as evidenced by seroprevalence against CMV, EBV or HSV 1–2 is increasing with age [13]. However, many changes in T cell populations, such as the reduction of naïve T cells and accumulation of T_{EM} or T_{EMRA} cells are associated with aging, and age together with CMV infection have been identified as major variables associated with expansion of TEM cells, including in cohorts of monozygotic twins [14, 15]. Thus, age and CMV infection may both contribute to expansion of T_{EM} CD4 responses and affect the incidence ICB-hepatitis. In this study we therefore sought to understand the role of age, gender and baseline herpes virus immunity in a prospectively recruited discovery and retrospective validation cohort of stage III/IV melanoma patients treated with anti-PD-1 & anti-CTLA4 combination therapy reflecting real-world patient cohorts at a tertiary academic medical center.

Our data from n = 106 stage III/IV melanoma patients who received combinational ICB therapy with anti-PD-1 and anti-CTLA4 identifies age, but not underlying herpes virus immunity or peripheral TEM subsets as the major variable associated with the risk for immune-checkpoint associated hepatitis.

Methods

Patient recruitment:

Melanoma patients treated with anti-PD-1 & anti-CTLA4 combinational therapy from 01/2016-09/2021 at the University Medical Center Freiburg, Dpt. of Dermatology were prospectively included in the discovery cohort (n = 40). A total of 111 patients were identified in clinical records. The remaining (n = 71) patients

were retrospectively evaluated in the validation cohort (see Supplemental Fig. 2). All included patients had baseline ALT and AST levels below 2xULN and underwent screening for Hepatitis B and Hepatitis C Virus infection. Evaluation of hepatitis was based on ALT, AST and bilirubin evaluations according to CTCAE 5.0. Other adverse events were identified by retrospective evaluation of clinical records. Patients with hepatitis of other etiology were subsequently excluded from the analysis, this affected 1 patient in the discovery cohort was excluded from analysis due to alternative cause of hepatitis (acute HEV infection). 4 patients in the validation cohort were excluded from analysis due to untraceable clinical data and lost to follow-up after therapy initiation. Tumor response was evaluated by radiographic evaluation as per clinical pathways 9–12 weeks from commencement of treatment. Progression (PD) was defined by radiographic disease progression or clinically unequivocal rapid disease progression necessitating cessation of ICB treatment. Tumor regression was determined by radiographic total (CR) or partial (PR) regression of tumor sites. Stable disease (SD) was defined by unchanged radiographic diagnosis. Patients without radiographic evaluation were noted not evaluable (NE). Objective response rate (ORR) was calculated as $CR + PR / (\text{total patients} - NE)$; Disease control rate (DCR) was calculated as $CR + PR + SD / (\text{total patients} - NE)$. Tumor progression-free survival (PFS) was determined from therapy initiation until the date of tumor progression. Patients that switched therapy before tumor progression were censored at time of therapy switch.

Human Samples:

For patients in the discovery cohort, baseline blood was obtained on the day of therapy initiation. Plasma was isolated from EDTA tubes after 10 minutes of centrifugation at 1000g and stored at -20C until use. PBMCs were isolated by density gradient centrifugation and stored at -80C until use. For patients in validation cohort that did not have serology results for CMV, EBV and HSV prior to this study, leftover serum was used for identification of IgG positivity. Leftover serum was from the screening for HBV, HCV and HIV serology before therapy initiation during routine clinical management at the Institute of Virology, University Medical Center Freiburg.

Ex vivo flow cytometry:

Cells were thawed and counted. $1-2 \times 10^6$ cells were used for flow cytometry. Surface staining was performed in a total volume of 50 μ l antibody master mix at RT for 15 minutes and washed twice before acquiring on BD LSR Fortessa. For intracellular staining, cells were permeabilized with FoxP3/Transcription Factor Staining Buffer Set (eBioscience) on ice for 30 minutes and washed twice with FoxP3 permeabilization buffer (eBioscience), followed by intracellular staining in a total volume of 50 μ l antibody master mix on ice for 30 minutes. Cells were fixed with 2% PFA until measurement. Samples were then acquired and recorded on BD LSRFortessa™. For gating strategies see Supplemental Fig. 5.

Statistical analysis:

Statistical analysis was performed with Graphpad version 9.0. As indicated in figure legends, data were analyzed using two-tailed Mann–Whitney test, two-tailed chi-square test, Fisher’s exact test, Kruskal–Wallis test, log-rank survival analysis, receiver-operator characteristic (ROC) analysis or pairwise Pearson correlation.

Results

High incidence of hepatitis after PD-1 & CTLA-4 combination checkpoint therapy in melanoma.

We first evaluated the incidence of hepatitis and other immune-related adverse events (irAE) in a prospectively recruited discovery cohort of $n = 39$ patients after initiation of anti-PD-1 & anti-CTLA4 therapy due to advanced melanoma over a period of 100 days. 87.2% of patients (34/39) developed one or more irAE (Fig. 1A). While 28.2% of patients developed a single adverse event, 15.4% developed 2 types of adverse events and 17.9% and 25.6% of patients developed 3 or more types of adverse events. Specifically, we observed a high incidence of hepatitis irAE (59%) in our cohort (Fig. 1B). While 2.6% of patients developed mild hepatitis (grade 1), 20.5% developed moderate hepatitis (grade 2) and 25.6% and 10.3% developed severe grade 3 and 4 hepatitis, respectively, requiring immunosuppressive therapy and treatment pause or discontinuation (Fig. 1C). There was no grade 5 toxicity. In sum, we observed a relatively high rate of hepatitis incidence in the first 100 days after anti-PD-1 & CTLA-4 treatment initiation for advanced melanoma.

Hepatitis onset is associated with the development of additional irAE and age but not gender or treatment response.

We next aimed to understand if development of hepatitis was associated with the development of other irAE, response to treatment, gender or age. Our discovery cohort consisted of patients with an age distribution between 19–73 years, a male dominance (71.8%) and ORR of 61.5% after 3 months, reflecting the real-life setting in our tertiary clinical centre (Supplemental Fig. 1). As shown in Fig. 2A, there was no association of hepatitis incidence with the oncologic response after three months ($p = 0.7397$) nor gender ($p > 0.99$) (Supplemental Table 1). Patients with hepatitis had significantly higher co-incidence of skin, thyroid, gastrointestinal or hypophysal irAE ($p = 0.018$, Fig. 2B). Development of irAE was significantly associated with age, with patients over 55 years old exhibiting higher incidence for top five frequent irAE compared to younger patients under 55 (Fig. 2C). These data indicate that age might be a determining correlate of the onset of hepatitis and other irAE in our cohort.

Younger age is associated with incidence and severity of hepatic irAE in the discovery cohort

We next analyzed the connection between age and hepatitis incidence in more detail. Of note, 85.7% of patients under age 55 had hepatitis during the first 100 days of therapy, while only 27.8% of patients older than 55 years had hepatitis during our observation period (Fig. 3A). The significantly higher

incidence of hepatitis in younger patients was also connected to hepatitis-free survival, an earlier onset and higher severity of hepatitis in younger patients (Fig. 3B, 3C) in the discovery cohort.

Younger age is associated with incidence and severity of hepatic irAE in the validation cohort

To exclude the possibility that patient characteristics other than age might have dictated the association with irAE in our discovery cohort, we sought to validate these findings in a retrospective analysis of all patients treated at our tertiary center during the recruitment period to address recruitment bias. The validation cohort consisted of $n = 67$ patients (see methods section for inclusion/exclusion criteria and Supplemental Fig. 2). We noted several differences in the composition of the validation cohort, namely reduced incidence of irAE, including hepatitis (Supplemental Table 2), potentially connected to a shift towards older age (median age 54 years vs. 60 years in discovery and validation cohort, respectively), reduced ORR (61.5% vs. 28.6%, respectively) while gender distribution was similar (Supplemental Table 2). Importantly, however, we also observed a significant association of younger age with hepatitis-free survival, severity and earlier onset in the validation cohort (Fig. 4). Together, these data support age as a validated risk factor for the development of hepatitis in the first 100 days after anti-PD-1 & anti-CTLA4 therapy.

Higher baseline liver function tests in patients that develop hepatitis

Interestingly, patients who developed hepatitis within 100 days from therapy initiation had small however significantly higher liver transaminase levels at baseline (Supplemental Fig. 3A). This association was observed despite the majority of patients ($n = 92$, 86.8%) having transaminase levels within the normal range. ROC analysis showed a slight predictive role of both baseline AST and ALT values for ICB-induced hepatitis (Supplemental Fig. 3B). Our study also included few patients ($n = 14$, 13.2%) that had elevated liver transaminases already at baseline (up to 2xULN). However, these few patients with elevated liver transaminases (above the ULN) at baseline did not show a significantly higher incidence of developing further hepatitis during therapy in comparison to those with normal liver transaminase levels (Supplemental Fig. 3C). In sum, these data suggest that preexisting mild liver inflammation can be associated with the onset of ICB hepatitis.

Baseline herpes virus serology is not significantly linked to hepatitis onset

The immune system is exposed to multiple antigens over time and immunological changes associated with herpes virus infections are connected to age [16]. A previous report described baseline antiviral T cell immunity to herpes virus infections as a potential driver of hepatic irAE [17]. We wondered if this association would explain the age-associated differences in the incidence of hepatitis in our cohort, since higher hepatitis incidence was observed in younger patients who would be predicted to have lower

immune memory to herpes virus infections. Herpes virus serology for CMV, EBV and HSV was determined and analyzed with respect to hepatitis incidence. However, we did not observe an association of herpes virus serology with hepatitis-free survival in our observation and validation cohorts (Supplemental Fig. 4). Interestingly, in pooled analysis of both cohorts, positive serology for CMV at baseline showed a non-significant trend ($p = 0.0767$) towards lower hepatitis-free survival (Fig. 5A). CMV status was not connected to baseline liver transaminases (Supplemental Fig. 3D). Since the effect of CMV positivity on hepatitis incidence was not significant, we wondered if it might be masked by different age groups. Subgroup analysis according to CMV serostatus positivity and age (cutoff 55 years) showed however again only non-significant trends towards lower hepatitis free incidence (Fig. 5B). In sum, we did not observe a significant difference in hepatitis-free survival connected to herpes-virus immunity in our cohorts.

T cell immunity is altered in older patients but not predictive for hepatitis incidence

Preexisting T cell memory is discussed to underlie immune-mediated toxicity after checkpoint therapy and would fit to a model where the immune checkpoints targeted contribute to attenuation of autoimmunity in a physiological setting. We therefore performed a detailed analysis of CD8 and CD4 T cell subsets in the discovery cohort and analyzed potential associations of these immune populations (Fig. 6, Supplemental Figs. 5 and 6) with age and the incidence of hepatitis. As expected [16], we observed a reduction of naïve CD8 + T cells in older patients while memory populations expanded (Supplemental Fig. 6A and B). However, we observed no difference between these age-associated T cell populations and viral hepatitis (Supplemental Fig. 6C). An association of an expanded CD4 + effector memory T cell (TEM) population with ICB-hepatitis was reported earlier [17]. However, we did not observe different frequencies of CD4 + TEM or CD8 + TEMRA cells in patients who subsequently developed hepatitis after anti-PD-1 & anti-CTLA-4 therapy (Fig. 6A), and there was no difference in the T cell subset distribution between patients which did or did not develop hepatitis according to CMV serology status (Fig. 6B). These data indicate that differences in baseline T cell differentiation subsets are not directly linked to the onset of hepatitis after anti-PD-1 & anti-CTLA-4 checkpoint therapy.

Age as a potential predictor for therapy-induced hepatitis but not for tumor response

We next tested other possible clinical, oncological (BRAF/NRAS status, stage, LDH levels, presence of metastasis), virological or hepatological characteristics of our patient cohorts that might contribute to ICB-hepatitis during dural checkpoint therapy in the pooled cohort (Supplemental Table 3). In addition to age, AST and ALT levels, interestingly, this analysis also indicated a significant association of anti-HBs positivity with ICB-hepatitis, while anti-HBc or HBsAg were not significantly associated, in line with a status post HBV vaccination. We interpreted this finding as an age-dependent cohort effect due to wider introduction of HBV vaccination in younger patients. Since age was the most significant variable

associated with ICB-hepatitis, we next tested if age could be used as a predictor of the development of ICB-Hepatitis using receiver operating characteristic (ROC) curve analysis. Indeed, age was a relatively reliable discriminator of patients who developed or escaped therapy-induced hepatitis (n = 106, AUROC = 0.7455, $p < 0.0001$) (Fig. 7A). Specifically, for an age cut-off of 55 years, ROC analysis indicated specificity of 66.67% and sensitivity of 75% respectively in the pooled analysis). We did not observe a similar predictive role for herpes virus serostatus (data not shown). Interestingly, this predictive function of age was not observed with respect to tumor response in the comparable time frame (Fig. 7B). Moreover, presence of any irAE (including non-hepatitis irAE) was also not associated with improved survival in our cohort (Supplemental Fig. 7). This data suggests that the immunological mechanisms behind successful anti-tumor responses and hepatic adverse events are not necessarily connected. In sum, our data highlights age as a predictor of ICB-hepatitis.

Discussion

In this work we analyzed baseline clinical, immune and virological variables as potential predictors of anti-PD-1 & anti-CTLA-4 combination therapy associated ICB-hepatitis in patients with stage III/IV melanoma. We identified age as the major clinical variable associated with the incidence, early onset and severity of immune hepatitis in our prospectively recruited discovery and retrospective validation cohort independent of treatment efficacy. Of note, preexisting antiviral immunity against herpes virus infections did not significantly associate with the incidence of hepatitis. Moreover, differences in effector memory T cell subsets at baseline in our discovery cohort were associated with age but not with the risk for developing ICB-hepatitis. Our data therefore highlights younger age as the major clinical risk factor ICB-hepatitis in combination therapy and does not support close surveillance or prophylactic antiviral treatment strategies based solely on immunological and virological screening.

One of the main barriers for successful anti-PD1 & anti-CTLA4 therapy are severe adverse events occurring in particular during combinational therapy cycles [6]. The efficacy of anti-PD-1 & anti-CTLA blockade is thought to largely depend on the disinhibition of tumor-specific T cell populations controlled by the PD-1 and CTLA4 immune checkpoints for enhanced proliferation and tumor cytotoxicity. However, checkpoint blockade induced T cell activation may not be strictly confined to tumor-reactive repertoires and “off-target” activation can potentially contribute to immune-related adverse events, a concept that is supported by recent studies revealing enriched activated/cytotoxic T cell populations in the tissue site of adverse events [10–12, 17, 18]. In particular, bystander activation of T cells leading to hepatitis can occur independent of antigen recognition [19] in the context of an inflammatory cytokine milieu [20].

Hutchison et al. recently suggested a role of CMV-related T cell immune response in triggering therapy induced hepatitis by demonstrating enrichment of a CMV-associated CD4 TEM population in the periphery of patients who later developed hepatitis [17]. It has to be noted however, that their study did not show direct evidence of CMV presence in the liver in patients tested (CMV immunostaining and PCR negative), despite individual treatment decisions with antivirals as prophylaxis or in addition to immunosuppressive therapy. Our study used a related approach to profile immune responses and

baseline patient characteristics but did not identify the reported relationship of hepatitis incidence connected to CD4 TEM cells. Further, serological IgG positivity at baseline against CMV, EBV or HSV did also not significantly correlate with hepatitis incidence. We wondered if these discrepant results in our prospectively recruited discovery cohort as well as the validation cohort could be due to differences in the patient cohorts.

Patients with preexisting mild levels of hepatitis could have other mild forms of underlying liver diseases, but potentially also herpes virus-related inflammation. A sub-analysis by Hutchinson et al. who included patients with elevated liver transaminases at baseline, did not find an association of this baseline status with the incidence of hepatic irAE after therapy [17]. Similarly, our cohort included patients with predominantly normal liver function tests at baseline but also potentially mild hepatitis (ALT levels < 2 ULN according to clinical guidelines allowing these mild elevations for ICB therapy). Here, we did not observe a connection between baseline transaminase levels and CMV serostatus. However, patients that developed ICB-hepatitis had mildly higher transaminase levels at baseline in our cohort. This baseline transaminase elevation at the cohort level however occurred frequently below the ULN (Supplemental Fig. 3). Thus, while this observation points to a higher degree of underlying liver inflammation in patients that subsequently develop hepatitis, it also poses a challenge for identifying them based on liver function tests.

In sum, in this work, we could not confirm a clinically relevant role of virus serology or T_{EM} CD4 T cell populations in patients who later developed hepatitis as previously reported. In contrast, our clinical data revealed a strong predisposition of younger patients to develop hepatitis during therapy, while no such link was observed with tumor response. This data also suggests that immunological mechanisms responsible for successful tumor suppression and incidence of immune mediated hepatitis are not necessarily coupled. It is further exemplified by 2 responders (1 reached CR in 3 months and the other in 6 months) in our discovery cohort that were both exempted from any type of adverse events. This disassociation between tumor response and adverse events necessitates further in-depth research to understand the underlying immunological mechanisms accounting for the respective biological events and their relationship to different age groups. Our data shows that younger patients are at higher risk for developing immune-related hepatitis after combination of anti-PD-1 & anti-CTLA4 therapy and should be closely monitored to allow rapid identification and treatment of this side effect when it occurs.

Conclusions

Taken together, our work highlights younger age but not TEM expansion or herpes virus immunity as a clinically relevant predictive factor for the onset of anti-PD-1 & anti-CTLA4 related immune hepatitis. These findings have implications for the monitoring of patients at risk for developing checkpoint hepatitis during immunotherapy.

Abbreviations

cCBI: combinational checkpoint blockade immunotherapy

irAE: immune-related adverse events

Tem: effector memory T cells

Temra: CD45RA positive effector memory T cells

CR: complete response

PR: partial response

SD: stable disease

PD: progressive disease

NE: not evaluable

ORR: objective response

DCR: disease control rate

PFS: tumor progression-free survival

Declarations

Ethics approval: Study participants were recruited with approval of the Institutional Review Boards and biomaterials were collected after informed consent (Ethics committee of the Albert-Ludwigs-University, Freiburg, #474/14 & #22/1074). The study was performed in agreement with the principles expressed in the Declaration of Helsinki (2013).

Consent for publication: Not applicable.

Data availability: Data relevant to the study are included in the article or uploaded as online supplemental information. Flow cytometry data is available by the authors via <https://flowrepository.org> upon reasonable request.

Competing interests: FM served as consultant and/or has received honoraria from Novartis, Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Pierre Fabre, Sanofi Genzyme and travel support from Novartis, Sunpharma and Bristol-Myers Squibb. DRS served as consultant and/or has received honoraria from Roche and Bristol-Myers Squibb and travel support from Sunpharma and Sanofi, with no relation to the submitted work.

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Authors' contributions: Conceptualization – D.R., F.M., B.B.; Patient recruitment and clinical data: D.R., S.L., R.D., N.B. Methodology – Z.Z., D.R., D.H.; Investigation – Experiments and data acquisition Z.Z., D.H. ; Formal analysis – Z.Z.; Resources – R.T., B.B; Writing – Original Draft –B.B.; Writing – Review & Feedback – Z.Z., D.R., D.P, F.M.; Visualization – Z.Z.; Supervision F.M., B.B. ; Funding Acquisition – B.B.

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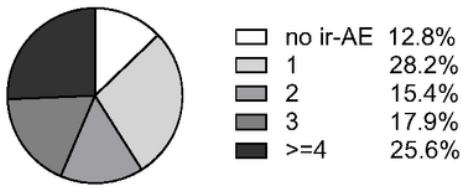
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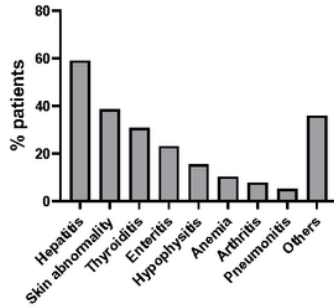
Figures

Figure 1

A ir-AEs (n=39)



B



C Hepatitis (n=39)

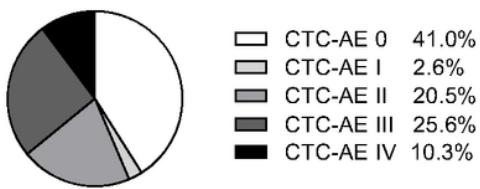


Figure 1

Incidence of immune-related adverse events (irAE) in the first 100 days after initiation of therapy.

(A) Pie chart depicting the percentage of patients who developed single or multiple types of irAE in the first 100d after therapy initiation. (B) Bar plot depicting the percentage of patients who developed certain types of adverse events as indicated. (C) Pie chart depicting the percentage of patients who escaped hepatitis or developed hepatitis of indicated severity in the first 100 days post therapy.

Figure 2

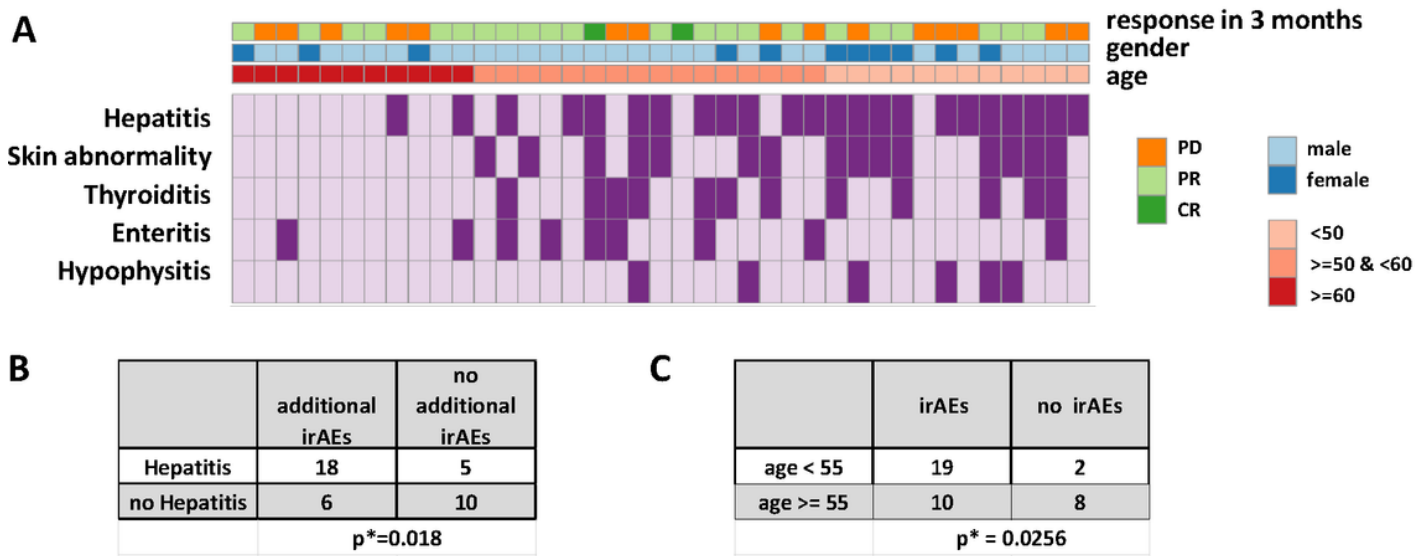


Figure 2

Hepatic irAE frequently co-occur with other irAE and are associated with age.

(A) Heatmap displaying the distribution of Hepatitis, skin abnormality, thyroiditis, enteritis and hypophysitis across tumor response, gender and age. Events are highlighted in dark purple. (B) Table showing the absolute number of patients who developed thyroiditis, hypophysitis, skin abnormality or enteritis grouped by the incidence of hepatitis. (C) Table showing number of patients with and without top 5 ir-AEs across age groups. Statistics were determined by Fisher's exact test in B and chi square test in C.

Figure 3

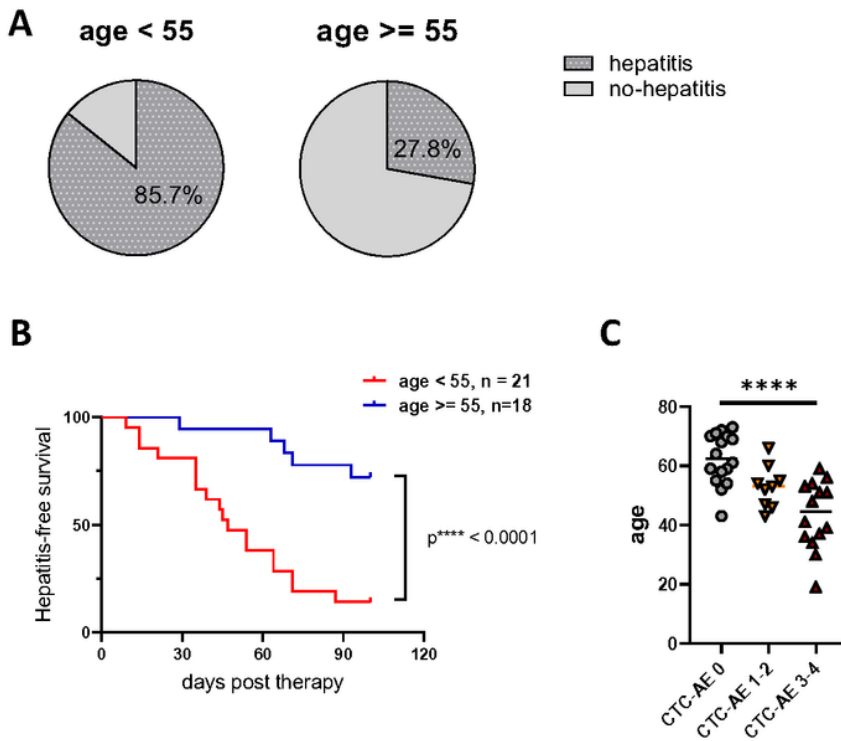


Figure 3

Age is associated with higher frequency, earlier onset and increased severity of therapy-induced hepatitis.

(A) Pie charts depicting the percentage of patients with therapy-induced hepatitis. (B) Kaplan-Meier analysis of hepatitis events in young patients (<55, n=21) and aged patients (>=55, n=18). (C) Dot plot describing the age of patients without hepatitis, or who developed mild hepatitis (CTC-AE 1-2) or severe hepatitis (CTC-AE 3-4) Statistics were performed by log-rank test(B) and Kruskal-Wallis test (C).

Figure 4

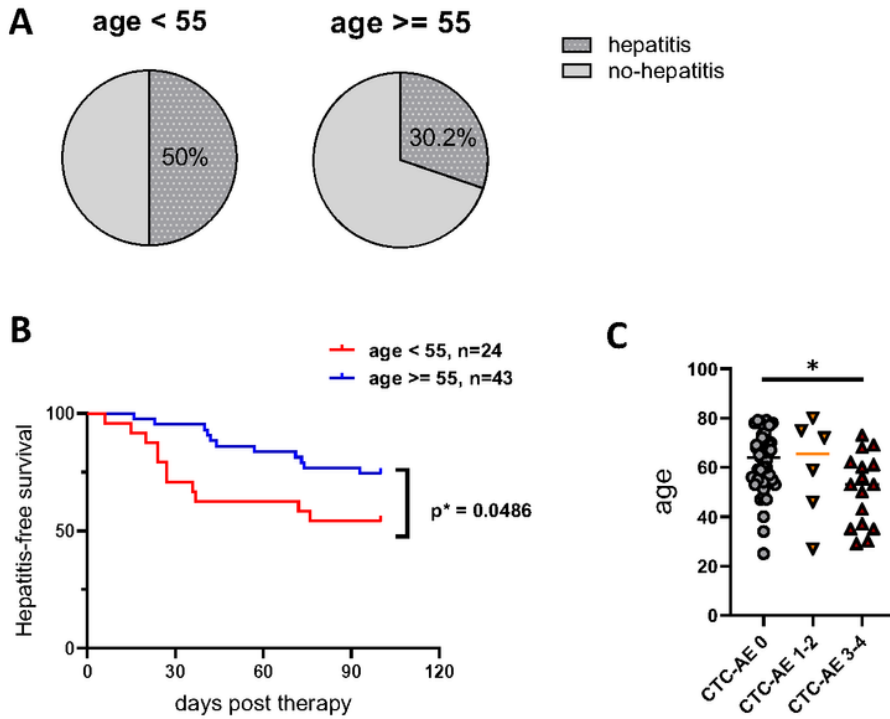


Figure 4

Validation of the age association with Immune-checkpoint-hepatitis in another cohort of 67 patients (validation cohort).

(A) Pie charts depicting the percentage of patients with therapy-induced hepatitis. (B) Kaplan-Meier analysis of hepatitis events in young patients (<55, n=24) and aged patients (>=55, n=43). (C) Dot plot describing the age of patients without hepatitis, or who developed mild hepatitis(CTC-AE 1-2) or severe hepatitis (CTC-AE 3-4) Statistics were performed by log-rank test (B) and Kruskal-Wallis test (C).

Figure 5

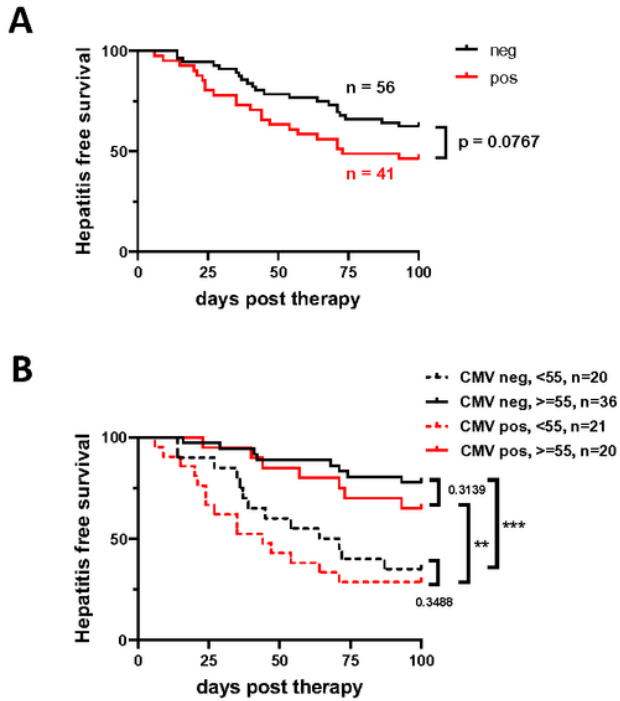


Figure 5

Incidence of Immune-checkpoint-hepatitis does not correlate with CMV serostatus.

(A) Kaplan-Meier analysis of hepatitis events in patients grouped by IgG positivity against CMV. (B) Kaplan-Meier analysis of hepatitis events in young (<55) and aged (>=55) patients with either negative or positive CMV IgG status.

Figure 6

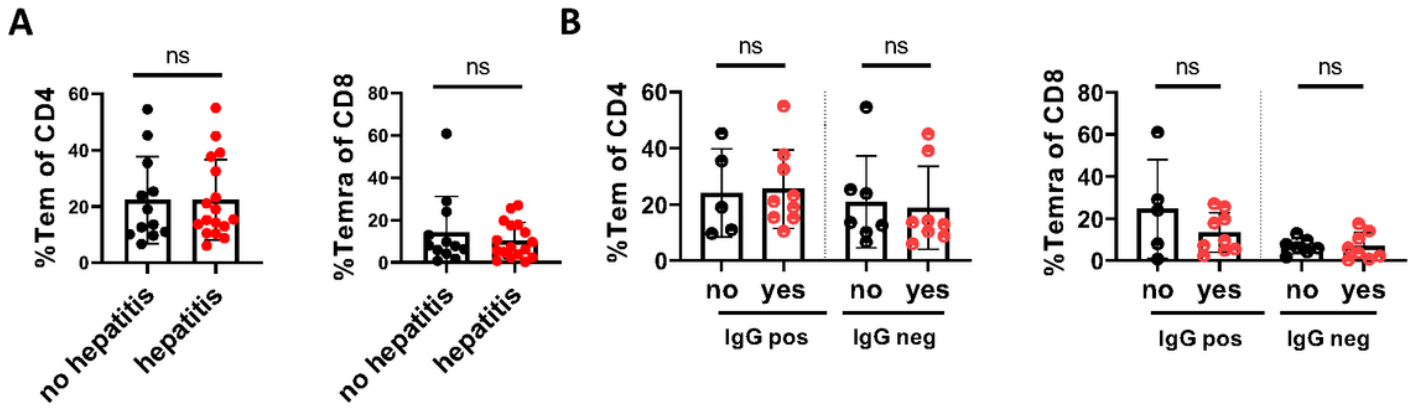


Figure 6

Frequency of effector memory T cells is not associated with ICB-hepatitis.

In a sub-cohort (n=29), T cell phenotypes at baseline were determined. For (A) %Tem(CCR7-CD45RA-) of CD4 (left) and Temra (CCR7-CD45RA+CD27-) of CD8 (right), no difference was observed between patients with or without Immune-checkpoint-hepatitis. (B) Patients were based on CMV-IgG serostatus (positive/negative), no difference of %Tem CD4 or %Temra CD8 was observed in patients with or without Immune-checkpoint hepatitis.

Figure 7

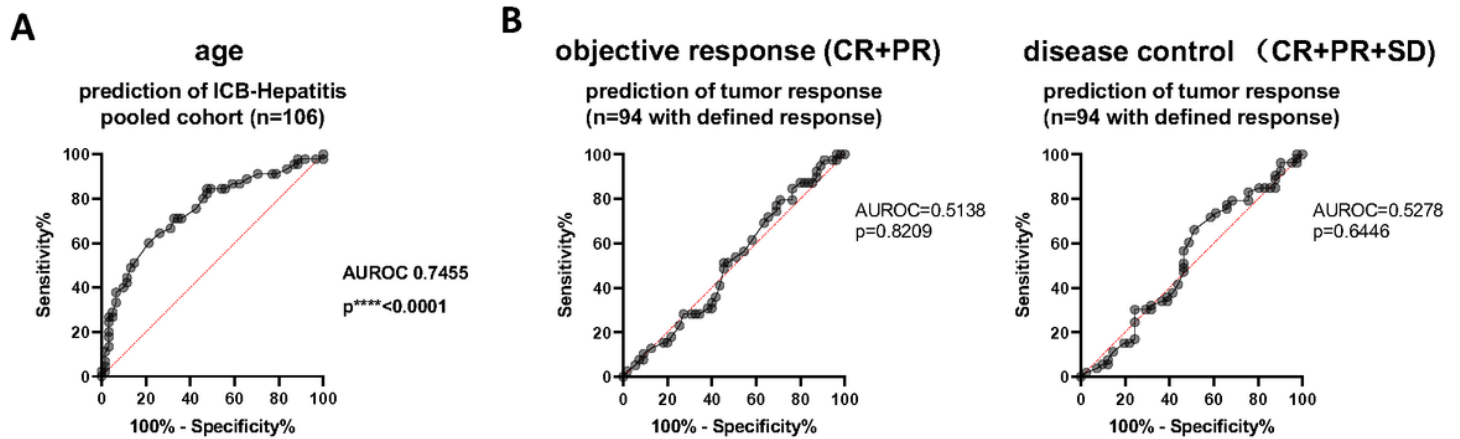


Figure 7

Age is a predictor for anti-PD-1/anti-CTLA4 therapy associated Immune-checkpoint hepatitis.

(A) ROC analysis using age as discriminator for therapy induced hepatitis the pooled cohort (n=106). (B) ROC analysis using age as discriminator for tumor response in 3 months in the total cohort of n=94 patients whose response was assessable. Treatment response was either defined by objective response (CR+PR) (left) or disease control (CR+PR+SD) (right)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [20220808SupplementalFigureLegends.docx](#)
- [20220930HepatitisIRAEsuppfigsandtablemerged.pdf](#)